Research Article

RAPD Analysis for Genetic Variability Detection of Mutant Soybean (*Glycine max* (L.) Merr)

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ABSTRACT
This study aimed to detect and evaluate the genetic mutation from mutagenized soybean by RAPD markers. Soybean seeds of "Grobogan" variety were treated with two different concentrations of EMS (0.5% and 1%) and three incubation times (4, 6 and 8 h). DNA whole-genome was isolated from young leaf seedling with the Qiagen DNeasy Plant Mini Kit. Twenty OPA primers (OPA-1 to OPA-20) were used for DNA amplification. The results showed that EMS treatments successfully generated genetic variation in soybean, which indicated by high values of PIC, EMR, and MI. RAPD primers that effective to detect the mutation were OPA-2, OPA-07, OPA-10, OPA-11, OPA-12, OPA-13, OPA-14, OPA-15, OPA-16, OPA-18 and OPA-20. The band expression of those primers was exhibited a stronger intensity along with increasing of EMS concentration and incubation time used in this study. Treatment of 0.5% EMS in 6 hours incubation was successfully generated soybean mutants with the lowest genetic similarity compared to the wild-type. Thus, this study provides a new approach to generate genetic variability in soybean and has the potential to improve for soybean breeding program.

Keywords: ethyl methane sulphonate, genetic mutation, RAPD, soybean.

INTRODUCTION

Soybean is one of the major foods crops commodities and can be considered as the cheapest source of protein in Indonesia (Setiawan, 2013). The productivity of soybean in Indonesia was still low and less profit if compared to rice and corn. This may be due to the lack of development and attention of soybean seed industry, land optimization, pricing policy for locals, exporters, and importers in the market (Kristanti *et al.* 2017). Furthermore, more than 60% of Indonesia's soybean consumption is still needed to be imported during 2012 – 2016 (Pusdatin, 2015; Wulandari, 2016). Therefore, The Indonesian government in 2015 has optimized the soybean farmland to enhance domestic soybean production (Suherman, 2014). However, about 40% of the soybean farmland is considered as suboptimal and drylands (Balitbangtan, 2005; Wulandari, 2017).

Optimization of dry land for soybean farming has many challenges, especially the limited availability of soybean genotypes which tolerate the drought stress. Increasing the variability and adaptablity of soybean by mutation breeding, and followed by selection may become one of the methods to solve the problem. Mutation breeding has been used in recent years as an alternative technique in generating new variability and development of crop species and varieties (Khan and Tyagi, 2013). One of the soybean varieties that have high yielding is Grobagan. Furthermore, Grobogan varieties have early maturity (76 days) and larger pod (Krisdiana, 2013)

Genetic mutations can be induced by chemical mutagenic agents using some mutation reagents (Oladosu *et al.* 2016) and physic mutagens by gamma rays, x-rays, and ultraviolet (Soeranto *et al.* 2001;

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Aisyah *et al.* 2005; Sreeju *et al.* 2011). Genetic mutations in soybean crops have been shown to increase the germination rate, viability, survival, yield, quality contributing characters such as pod setting, branching, protein, and oil content, also resistant to drought and diseases (Khan and Tyagi, 2013).

Ethyl Methane Sulfonate (EMS) is an alkylating chemical mutagen that has been shown effectively causes a high frequency of gene mutation. At low concentration, EMS generates G/C to C/G or G/C to T/A transversion, or A/T to G/C transition by pairing errors of 7-ethylguanine and 3-ethyladenine, respectively. Based on codon usage, EMS may also induce nonsense and missense mutations (Behera et al. 2012). If compared to other mutagens, genetic mutations induced by EMS were proven to be more effective than gamma rays in rice, lentil, mungbean (Singh et al. 2001), pea (Shah et al. 2009) and urad bean (Thilagavathi and Mullainathan, 2009). In addition, mutations using EMS in pea (Girija et al. 2013) and soybean (Khan and Tyagi, 2010) also caused mutations higher than the combination of gamma rays and EMS.

Recently, the effective detection of induced genetic mutations is still difficult to be measured. Many laboratories could not afford it since it is a very lengthy task, and due to spatial and technical limitations. Mutation detection in plants can be performed through phenotypic and genetic approaches. However, mutation detection using the phenotypic approach has many limitations and generated non-fixed character (Garcia et al. 2016). Therefore, the utilization of the genetic approach becomes a more reliable method and suitable for early mutation detection in the plant. Some genetic approaches which can be used for mutation detection are Random Amplification Polymorphic DNA (RAPD) (Ashraf et al. 2007), Restriction Fragment Length Polymorphism (RFLP), Inter-Simple Sequence Repeat (ISSR) (Mishra et al. 2014), DNA sequences (Wahyudi et al. 2013; Nikmah et al. 2016), etc.

RAPD based detection of genetic polymorphism is a molecular marker that is based upon a variation of the primer annealing sites in polymerase chain reaction/PCR. Primers were modified by even a single nucleotide which produces different banding patterns to detect genetic polymorphisms (Behera et al. 2012). It is a genetic marker which commonly used for mutation detection (Ashraf et al. 2007) and genetic variation study (Sundari et al. 2017; Kawar et al. 2009). RAPD marker has proven to be more reproducible than another genetic marker such as ISSR and RFLP; and no need to know the background of the genome

being analyzed (Kumari and Thakur, 2014). The previous study proved that RAPD had been successfully used for mutation detection in rice (Ashraf et al. 2007), melon (Daryono et al. 2011), and sugarcane (Kawar et al. 2009).

In the present study, the seeds of soybean "Grobogan" variety were subjected to EMS-induced mutagenesis with combination treatments of different concentrations and incubation time to create variability. This study was aimed to detect and evaluate genetic mutation occurred in generated mutants seedlings of soybean, compared to the wild-type using RAPD marker. The finding of this study was expected to be useful in supporting the soybean breeding researches in Indonesia.

MATERIALS AND METHODS

Plant Material
Plant material used in this study was soybean "Grobogan" variety. It was obtained from Grobogan District, Central Java and released in 2008 by Grobogan District Local Government, Seed Control and Certification Service of Central Java and Central Java Province Local Government as superior soybean variety (Decreed 238/Kpts/SR.120/3/2008). The superiorities of soybean "Grobogan" variety is early matured (76 days), large seed size (about 18 g/100 seeds), and tolerant in fed rain dry land; with a potential yield up to 4.2 tonnes/ha (Rahajeng and Adie, 2013; Balitkabi, 2016; Balitbangtan, 2016).

Soybean mutation induction by EMS
A total of 40 Grobogan varieties soybean seeds were used in this study. About 30 soybean seeds were previously soaked in 40 ml of phosphate buffer 100 mM at 4 ℃ for 24 h. Then, it was added with new 40 ml phosphate buffer 100mM and treated with a combination of 2 variations of EMS concentration (0.5% and 1%) and 3 variations of incubation time (4, 6 and 8 h). The combination of treatments are as follows: 1) 5 seeds soaked with 0.5% EMS and incubated for 4 h; 2) 5 seeds soaked with 1% EMS and incubated for 4 h; 3) 5 seeds soaked with 0.5% EMS and incubated for 6 h; 4) 5 seeds were soaked with 1% EMS and incubated for 6 h; 5) 5 seeds soaked with 0.5% EMS and incubated for 8 h and 6) 5 seeds soaked with 1% EMS and incubated for 8 h. The 10 seeds left was soaked with aquadest for 24 hours as a control group.

After treatment, the EMS-induced mutant soybean seeds and control were sown on the germination tray. On 30th days after sown, each seedling was transplanted in a polybag containing growing media, i.e. sands, husks, and composts. The

Table 1. DNA sequences, GC content and annealing temperature of OPA primers used in this study

Primer	Sequences ('5-'3)	GC Content (%)	T_A (°C)
OPA-01	CAGGCCCTTC	70	36.4
OPA-02	TGCCGAGCTG	70	40.7
OPA-03	AGTCAGCCAC	60	35.0
OPA-04	AATCGGGCTG	60	35.1
OPA-05	AGGGGTCTTG	60	32.6
OPA-06	GGTCCCTGAC	70	35.2
OPA-07	GAAACGGGTG	60	33.2
OPA-08	GTGACGTAGG	60	31.1
OPA-09	GGGTAACGCC	70	37.4
OPA-10	GTGATCGCAG	60	33.1
OPA-11	CAATCGCCGT	60	36.7
OPA-12	TCGGCGATAG	60	34.0
OPA-13	CAGCACCCAC	70	37.7
OPA-14	TCTGTGCTGG	60	34.3
OPA-15	TTCCGAACCC	60	34.2
OPA-16	AGCCAGCGAA	60	38.3
OPA-17	GACCGCTTGT	60	35.7
OPA-18	AGGTGACCGT	60	36.2
OPA-19	CAAACGTCGG	60	34.2
OPA-20	GTTGCGATCC	60	33.5

young EMS-induced mutants and wild-type soybean leaf on 60 days after transplant were taken for DNA extraction and genetic test in Molecular Biology Laboratory of State Islamic University of Maulana Malik Ibrahim, Malang.

DNA extraction

About 0.1 g of young soybean leaf was used for DNA extraction. DNA whole genome was extracted with the Qiagen DNeasy Plant Mini Kit (Promega™) following its manual instruction. DNA yields were confirmed qualitatively using electrophoresis separation on 1% agarose gels, stained with 2 µg/ml of Ethidium bromide (EtBr) in TBE buffer, then visualized under UV transilluminator. Geneon 100bp Plus Blue DNA ladder was used as a standard marker.

PCR RAPD

Twenty primers of OPA (Operon Technologies) were used for RAPD amplification. Total reaction volume for PCR RAPD was 10 ml containing 3 ml ddH2O, 5 ml PCR MasterMix Thermo scientific, 1ml of OPA primer (10 pmol) and 1 ml DNA template (5-25 ng). The PCR amplification was carried out in thermal cycler BioRad. One cycle of initial denaturation was conducted at 94 ℃ for 5 min, then followed by 45 cycles of denaturation for 30 sec at 94 ℃, annealing temperatures were varied each primer for 30 sec (Table 1), the extension for 90 seconds at 72 ℃ and terminated by final

extension for 7 min at 72 ℃.

Data analysis

The effectiveness of EMS to induce mutation in soybean seeds in this study was detected by polymorphism, clustering and distance method. The data were gathered based on the presence and the absence of amplification product bands, as shown on the agarose gel. It was scored as "1" for the present band and "0" for the absent band of each primer. The binary data matrix was then subjected to clustering and distance method using an unweighted pair group method with arithmetic mean (UPGMA) algorithm and Jaccard's coefficient similarity (Hammer et al. 2001) with PAST (Paleontological Statistics) software. The percentage of polymorphism was calculated by dividing the polymorphic bands shown by each primer with a total of scored bands x 100. The presence of the mutation was also checked with a qualitative method by comparing the band thickness of each primer.

Polymorphism information content (PIC), effective multiplex ratio (EMR) and marker index (MI), were also performed to evaluate the effectiveness of each primer used. PIC Value for each primer was calculated with the formula: $PIC_i = 2f_i(1-f_i)$, where PIC_i is the polymorphism information content of the primer i, f_i is the frequency of primer fragment that was present and $1-f_i$ is the frequency of primer fragment that was absent. EMR was calculated by using formula

Table 2. Analysis results of polymorphism and effectivity of RAPD primers used in this study

Primer	nB	nPB	PB (%)	PIC	EMR	MI
OPA-01	8	7	87.50	0.50	56	28.00
OPA-02	4	4	100.00	0.41	16	6.56
OPA-03	7	5	71.40	0.47	35	16.45
OPA-04	6	5	83.30	0.49	30	14.7
OPA-05	3	2	66.70	0.50	6	3.00
OPA-06	3	2	66.70	0.30	6	1.80
OPA-07	5	5	100.00	0.43	25	10.75
OPA-08	7	6	85.70	0.43	42	18.06
OPA-09	7	5	71.40	0.29	35	10.15
OPA-10	7	7	100.00	0.47	49	23.03
OPA-11	6	6	100.00	0.46	36	16.56
OPA-12	5	5	100.00	0.45	25	11.25
OPA-13	7	7	100.00	0.50	49	24.5
OPA-14	4	4	100.00	0.50	16	8.00
OPA-15	4	4	100.00	0.44	16	7.04
OPA-16	4	4	100.00	0.44	16	7.04
OPA-17	4	2	50.00	0.41	8	3.28
OPA-18	6	6	100.00	0.23	36	8.28
OPA-19	3	1	33.30	0.20	3	0.60
OPA-20	5	5	100.00	0.50	25	12.50
Total	**105**	**92**	**1716.07**	**8.42**	**530**	**231.55**
Average	**5.25**	**4.60**	**85.80**	**0.42**	**26.5**	**11.57**

Note: nB=Number of Band, nPB=Number of Polymorphic Band, PB%=percentage of Polymorphic Band, PIC=Polimorphic Information Content, EMR=Effective multiplex ratio, and MI=Marker index.

EMR= $\eta.\beta$, where η is the total number of fragments per primer and β is the fraction of polymorphic fragments (Laurentin and Karlovsky, 2007). MI was calculated with formula MI= PIC x EMR (Varshney et al. 2007). Pearson correlation test was also performed using SPSS 16.0 subjected to the values of PIC, EMR, and MI.

RESULTS AND DISCUSSION

RAPD Profiles
RAPD is a technique based on the PCR method to identify genetic variation. RAPD has been used as an assessment intraspecific variation since 1990 (Kumari and Takur, 2014). Moreover, in recent years, RAPD was also used for genetic mapping, taxonomic study and even used to detect genetic mutation in the treated plant (Dhakshanamoorthy et al. 2014). Here, we used RAPD markers to assess genetic diversity among EMS-induced soybean mutants. This study will be useful as basic information for further soybean breeding programs since it will make it easy to select where the soybean which has high genetic variation can be used for crossbreeding.

About twenty OPA primers were selected for RAPD amplification to detect polymorphism and genetic diversity among EMS soybean mutants. In total, about 105 bands were detected, in which 92 bands were considered polymorphic. Each primer generated bands ranged from 3 to 8 bands, with an average of 5.25 bands (Table 2). The minimum of 3 bands was resulted by OPA-5, OPA-6, and OPA-19 primers, whereas the maximum of 8 bands was generated by OPA-01 (Figure 1). Polymorphism percentages were ranged from 33.30% to 100%, with an average of 85.80%. OPA-19 showed the lowest polymorphism percentage; it only generated 1 polymorphic band out of 3 total bands (Table 2). Whilst, about 11 primers showed 100% polymorphic band percentage i.e. OPA-2, OPA-07, OPA-10, OPA-11, OPA-12, OPA-13, OPA-14, OPA-15, OPA-16, OPA-18 and OPA-20.

The values of PIC, EMR and MI can be used to identify what primer is the most informative to detect polymorphism. PIC values were varied from 0.20 to 0.50, with an average of 0.42. The lowest PIC value was obtained by OPA-19, whereas the highest PIC value was generated by OPA-01, OPA-05, OPA-13, OPA-14 and OPA-20 (Table 2). EMR values
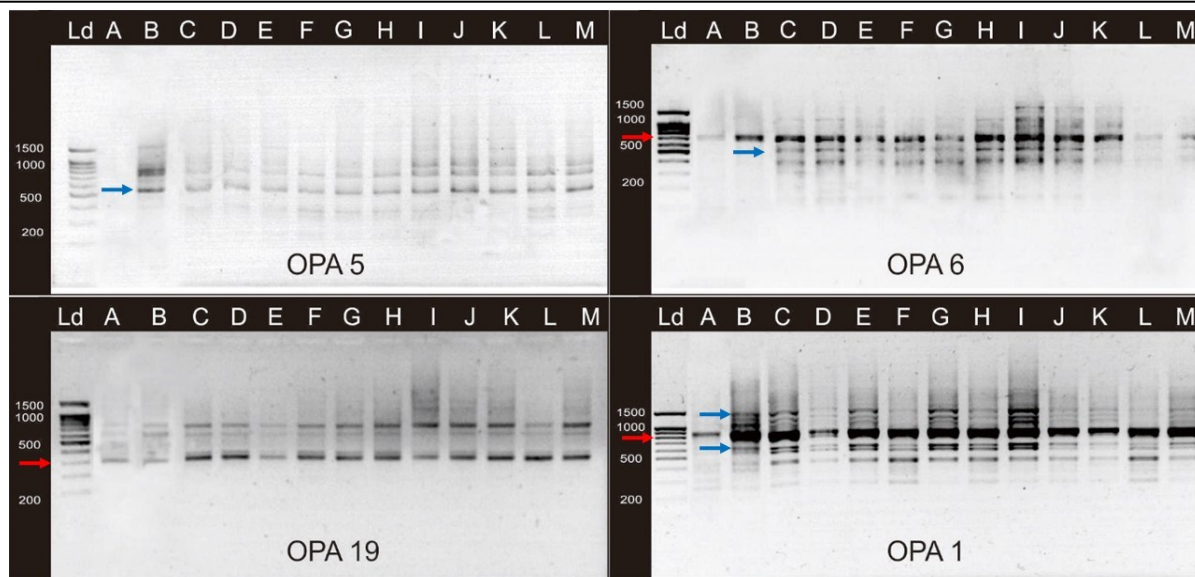
Figure 1. RAPD band profiles of the minimum bands (OPA 5, 6 and 19), a maximum band (OPA 1), the polymorphic band (blue arrow) and monomorphic band (red arrow). Ld: DNA Ladder, A & B: control/ wild-type, C & D: 0.5% EMS and 4 hours incubation, E & F: 1% EMS and 4 hours incubation, G & H: 0.5% EMS and 6 hours incubation, I & J: 1% EMS and 6 hours incubation, K & L: 0.5% EMS and 8 hours incubation, M: soaked with 1% EMS and incubated for 8 hours

were ranged from 3 to 56, with an average of 26.5. OPA-03 had the highest EMR value (56), whereas OPA-19 had the lowest EMR value (3). A similar tendency was also observed for the MI value. The MI values were ranged from 0.6 to 28, with an average of 11.57. OPA-01 was observed as the highest MI value, while OPA-19 was observed as the lowest MI value (Table 2).

Polymorphism information content (PIC) was measured to evaluate the discriminatory power of the RAPD marker. PIC is determined by the ability of the primer to generate polymorphism in the population depending on their distribution frequency (Nagy et al. 2012). Thus, PIC is equivalent to genetic diversity. The maximum number of PIC for RAPD marker is 0.5 because of two alleles per locus are assumed in RAPD analysis. In this study, we found that the average PIC value was 0.421 and about 80 % of the primers have PIC more than 0.4. This fact indicated that there was genetic diversity in soybean mutant in this study caused by EMS treatment. Furthermore, the number of EMR and MI also resulted in high values. It was also indicated that EMS treatments were successfully induced high genetic variation in soybean mutants. The result of this study is similar and comparable to some previous studies in terms of mutation detection using the RAPD marker in Jatropha curcas (Dhakshanamoorthy et al. 2014), Vitis vinifera (Maia et al. 2009) and Vigna unguiculata (Kolade et al. 2016).

Some RAPD primers used in this study has detected polymorphisms among soybean mutants and wild-type. The appearance of new bands (soybean mutants) or disappearance of bands in wild-type were observed (Figure 1). The thickening band, along with EMS concentration and incubation time were also observed (Figure 2). The appearance of new bands in soybean mutants may be related to the changes in oligonucleotide priming site due to mutation, deletion and homolog recombination (Dhakshanamoorthy et al. 2014). Furthermore, Atienzar et al. (2010) revealed that only 10 % mutation of one locus could emerge a new band. Therefore, in this study, EMS has successfully induced mutations on soybeans indicated by the appearance of new bands in soybean mutants assessed with RAPD markers.

The disappearance of regular bands was also detected by OPA 08 and OPA 10 primer (Figure 2). The disappearance of regular bands in soybean mutants may cause by DNA damage, modified bases, base oxidation, point mutation, and even chromosomal rearrangements induced by EMS (Dhakshanamoorthy et al. 2014). Taq polymerase can't bind DNA products due to some possible reasons including dissociation of the enzyme complex, polymerase blockage and disassociation of the enzyme complex, which may lead to the loss of the band (Atienzar and Jha, 2006). The appearance of new bands indicated the occurrence of mutations, whereas the disappearance of the band indicated the DNA damage (Dhakshanamoorthy et al. 2014). Both events were lead to genetic variations among soybean mutants compared to the wild-type.
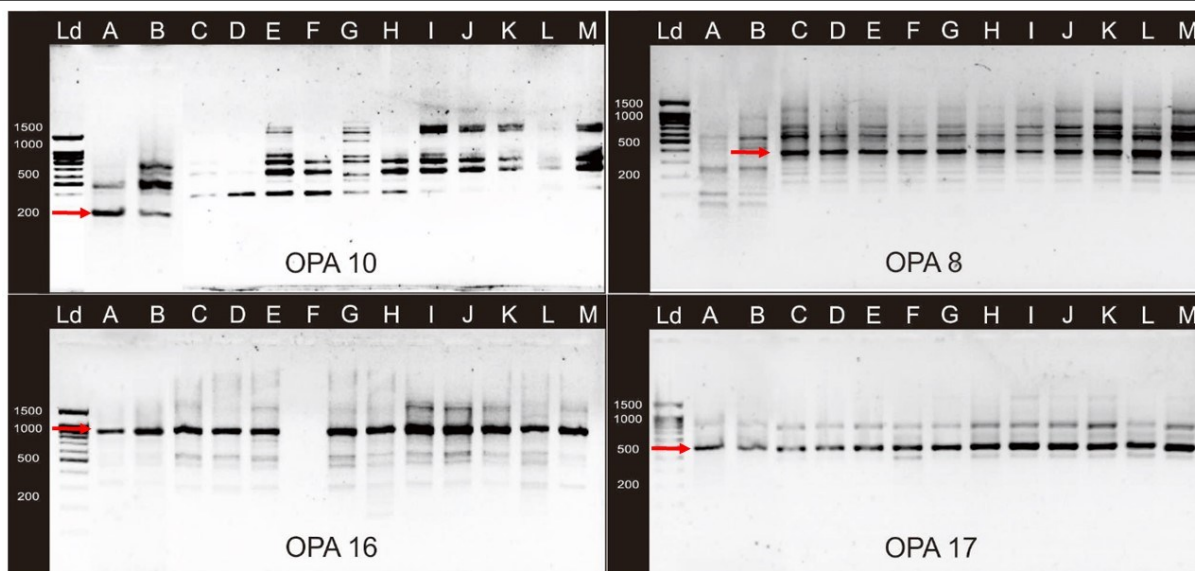
Figure 2. RAPD profiles of appearance of bands in soybean mutants (OPA 8), disappearance band in soybean mutant (OPA 10) and thickening bands along with the increasing of EMS concentration and incubation time (OPA 16 and 17). Ld: DNA Ladder, A & B: control/ wild-type, C & D: 0.5% EMS and 4 hours incubation, E & F: 1% EMS and 4 hours incubation, G & H: 0.5% EMS and 6 hours incubation, I & J: 1% EMS and 6 hours incubation, K & L: 0.5% EMS and 8 hours incubation, M: 1% EMS and 8 hours incubation.

Pearson correlation tests were performed to determine the relationships between pairs of continuous variables of PIC with EMR, PCI with MI, and EMR with MI. Result showed that there was a positive correlation between PIC with EMR (r = 0.308) but not significant (p= 0,187> 0,05). Whilst, PIC and MI had positive and significant correlation (r = 0,529; p= 0.016 <0.05). A positive and significant correlation (r= 0.948; p = 0,000 <0.05) were also found between EMR and MI.

In this study, we found a significant positive correlation between EMR and MI (r=0.948, p=0.05). It suggested that EMR, MI, and PIC are relevant to be used as markers for evaluation of genetic mapping and phylogenetic study (Dhakshanamoorthy et al. 2014). In addition, this study has also proved that RAPD marker is suitable for detection of genetic diversity in a breeding population (Biswas et al. 2010), as well as for mutation detection in plants (Dhakshanamoorthy et al. 2014; Kolade et al. 2016; Maia et al. 2009).

Qualitative tests based on band thickness showed that the band expression of some primers has resulted in stronger intensity with the increasing of EMS concentration and incubation time (Figure 2). The thickening bands were observed at 900 bp of OPA-16, and OPA-17 at 500 bp and 900 bp. Furthermore, the disappearance of the band in soybean mutants was observed by OPA 10 at 300bp and OPA 08 at 200bp (Figure 2).

Cluster analysis result
The genetic similarity among EMS-induced soybean

based on RAPD marker was ranged from 0.23 to 0.84 (Table 3). All of the soybean mutants were observed to have low similarity with the wild-type. Meanwhile, it has high similarity amongst mutants. The lowest genetic similarity was observed at a pair of wild-type (A) and soybean mutants of 0.5% EMS and 6 hours incubation (H), whereas, the highest genetic similarity was observed at a pair of soybean mutants of 1 % EMS and 6 hours incubation (J) and soybean mutants of 0.5% EMS and 8 hours incubation (K).

Clustering analysis was conducted to 11 EMS-induced soybean mutants and control/ wild-type, performed using Jaccard's algorithm. One soybean mutant (1 % EMS and 8 hours incubation) was not included in the clustering analysis because the seeds cannot grow caused by the toxicity of EMS. This condition was in accordance with the result of Li et al. (2017) that a high concentration of EMS showed reduced emergence and physiological damage, including inhibition of the main stem even failed to grow. The dendrogram of genetic relationship resulted in 2 major groups (Figure 3). The wild-type soybean became the first group, which act like an outgroup. The second group was consist of all soybean mutants, which can be separated into 3 subgroups based on EMS concentration and incubation time. The subgroup 1 was comprised of soybean mutants generated by 0.5 % EMS and 1 % EMS and incubated for 4 hours and 6 hours incubation. The subgroup 2 was consists of soybean mutants generated by 0.5 % EMS and 1% EMS for 6 hours and 8 hours incubation. The subgroup 3 was

Table 3. Jaccard's coefficient similarity among EMS induced soybean mutants and wild-type

	A	B	C	D	E	F	G	H	I	J	K	L	M
A	-												
B	0.63	-											
C	0.25	0.39	-										
D	0.31	0.42	0.77	-									
E	0.29	0.42	0.75	0.76	-								
F	0.32	0.37	0.67	0.69	0.74	-							
G	0.27	0.42	0.65	0.65	0.74	0.67	-						
H	0.23	0.36	0.76	0.77	0.73	0.70	0.59	-					
I	0.24	0.40	0.75	0.66	0.76	0.62	0.68	0.61	-				
J	0.30	0.40	0.76	0.79	0.75	0.69	0.69	0.67	0.80	-			
K	0.28	0.39	0.72	0.74	0.72	0.62	0.63	0.59	0.78	0.84	-		
L	0.38	0.42	0.63	0.67	0,63	0.79	0.65	0.62	0.57	0.65	0.60	-	
M	0.30	0.42	0.69	0.69	0.70	0.66	0.64	0.59	0.79	0.83	0.78	0.64	-

Note: A & B: control/ wild-type, C & D: 0.5% EMS and 4 hours incubation, E & F: 1% EMS and 4 hours incubation, G & H: 0.5% EMS and 6 hours incubation, I & J: 1% EMS and 6 hours incubation, K & L: 0.5% EMS and 8 hours incubation, M: 1% EMS and 8 hours incubation.

consists of the mixture of soybean mutants generated by 0.5 % EMS and 1 % EMS for 4 hours, 6 hours and 8 hours incubation.

The similarity index and clustering method were performed to ascertain the degree of genetic relationship among soybean mutants and wild-type. The lowest similarity index was observed by a pair of wild-type and soybean mutants from treatments of 0.5% EMS and incubated for 6 hours (similarity index = 0.23). It was indicated that those treatments generated soybean mutants with the highest genetic distance compare to the wild-type. Whilst, the highest similarity index was observed by soybean mutants with 1 % EMS and incubated for 6 hours and soybean mutants with 0.5 % EMS and incubated for 8 hours (similarity index = 0.59). It was indicated that both treatments resulted in quite similar soybean mutants with low genetic distance. Moreover, this study was proven that EMS treatments successfully resulted in the potential variation among soybean mutants compared to the wild-type. It was supported by dendrogram which showed different clustering between wild-type and soybean mutants, and also amongst soybean mutants in different concentrations of EMS and incubation time.

EMS is an effective mutagen which caused transitions of DNA bases G/C to A/T (Greene et al. 2003). Therefore, it was led to a mispairing of complementary bases and caused a high frequency of gene mutation (Behera et al. 2012). EMS has been widely used as genetic variability induction in several plants like Asteranctha longifolia (Behera et al. 2012), Vigna unguiculata (Girija et al. 2013), Cucumis sativus (Wang et al. 2014) and Jatropha curcas (Dhakshanamoorthy et al. 2014). In this study, EMS was also proven to induce genetic variability in the soybean plant.

Another method that can be used to increase the genetic variability of soybean is somatic embryogenesis (somatoclone). This method utilizes the polyethylene glycol (PEG) as an osmotic solution. Somotoclone method has been proven to derived new varieties of soybean that tolerance against drought stress (Sunaryo et al. 2016). However, this technology requires a high cost and advance tool that makes it difficult to be implemented by the farmer.

RAPD marker effectively detected variability and have been adopted in a population study, plant systematic (Arif et al. 2010) and plant breeding (Fei et al. 2014). Moreover, it has some other advantages such as it is applicable for anonymous genome, low DNA quantities and resulted in a high number of DNA fragments (Kumari and Thakur, 2014). However, it has some disadvantages regarding their low sensitivity and reproducible, which caused unstable results; the different study will result in a different outcome. In spite of the contrary argument regarding their usage for genetic diversity study,
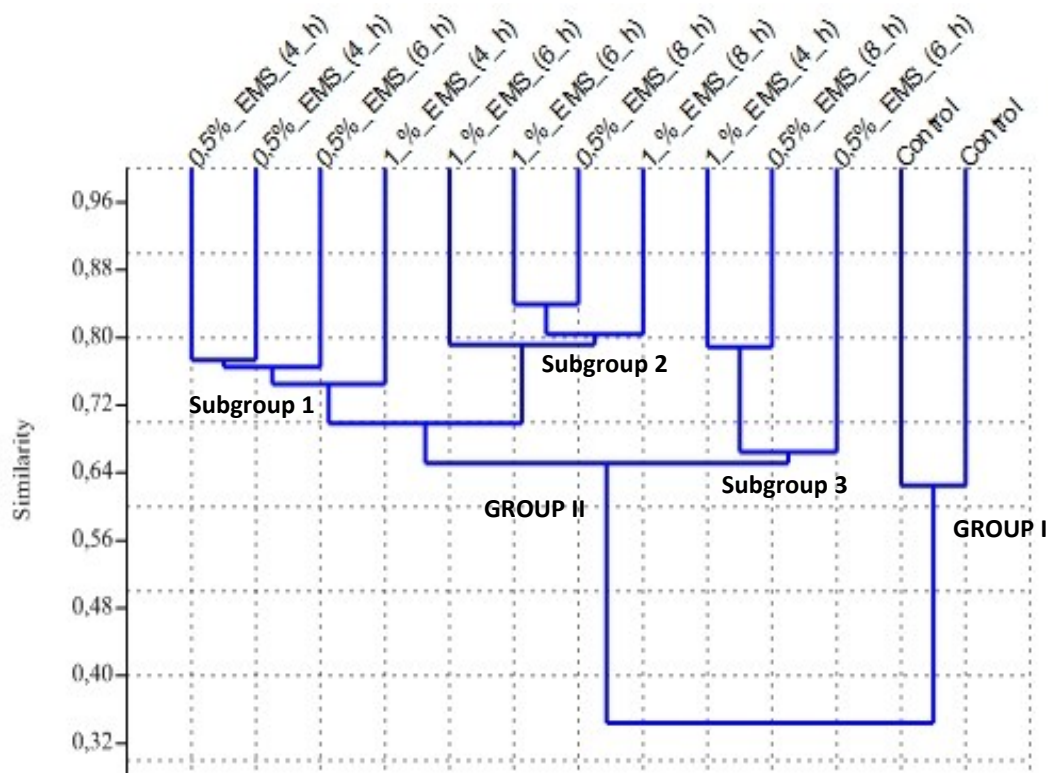
Figure 3. Dendrogram generated from Jaccard's coefficient similarity showing the genetic relationship of 11 EMS induced soybean mutants and control/ wild-type.

RAPD was still recommended and acceptable for detecting genetic variability than AFLP, ISSR and SSR (Sun and Wong, 2001).

CONCLUSION

EMS was an effective chemical mutagen to induce potential mutations in soybean. In this study, RAPD marker is still applicable and relevant to detect genetic mutations while also can be used as an alternative genetic approach. The occurrence of mutations in this study was shown by genetic variability, clustering and band appearance that all of them were obtained by RAPD marker.

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