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Research paper

The Expression of Drought Resistance GmDREB-1 Gene on Soybean (Glycine max L. Merr) as Result of EMS Mutation Induction (Etyl Methane Sulfonate)

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Abstract

Abiotic stresses, particularly drought, are harmful for the plant productivity. Mutagenesis is widely used as method that is potential to increase the variability of crop development. Mutagenic using EMS (Ethyl Methane Sulfonate) shows more effective results than using other mutagens. DREB1 gene is a subclass of DREB gene which is a transcription factor and it also serves as a key regulatory response of plants to drought stress. Mutation induction combines concentration treatment and immersion time in EMS. RNA isolation employs RNeasy Plant Mini Kit (Qiagen). GmDREB-1 expression is analyzed using Real Time PCR (Quantitative Real-time Polymerase Chain Reaction or Q-PCR). The analysis shows that mutation treatment with EMS using 0.07% concentration with a wide range of immersion time indicates that GmDREB-1 expression is higher than other treatments. The results of this study proved that mutation induction using EMS can be used as an alternative to plant breeding to obtain drought resistant soybean. Induction mutation useful for improvement genetic variability of plant.

Keywords: *GmDREB-1, Mutation Induction, EMS*

1. Introduction

Drought is harmful for crop productivity. Stress on the generative phase (age 51-75 days) can decrease crop yield up to 62%. The decrease in soil moisture content from 90% to 50% of available water will reduce the weight of seeds per plant respectively by 27% (Adisarwanto, 2006). Dry land in Indonesia is very wide and potential to increase agricultural production. According to Abdurachman et al (1997) in Subandi (2007), there are ± 13 million hectares of land employed for the development of soybeans, both paddy field and dry land today. In Sumatra, the dry land's width is about 5 million hectares and the neglected land is about 2.5 million hectares. Meanwhile, West Sumatra's dry land which is potential for the food crops development (including soybeans) is quite wide; approximately 590.450 ha is dominated by acid soils (Atman and Hosen, 2008).

Plants breeding using genetic mutation method which employs chemical and physical treatment followed by specific genotype selection has been a success for the food crops genetic development (Micke et al., 1985). Mutagenesis is widely used and this method is potential to increase the variability for plants development (Subuthi et al., 1991). Soybean mutations breeding identified in several mutant strains showed high percentage of germination and vitality (Rahman et al. 1994). Mutagenic using EMS (Ethyl Methane Sulfonate) showed more effective result than using other mutagens. EMS is known as having more power in generating useful mutation compared to gamma rays in rice (Kaul and Bhan, 1977), lentil (Solanki and Sharma, 1994), green beans (Singh, 2001), peas (Shah et al., 2008), and urdbean (Thilagavathi and Mullainathan, 2009). EMS treatment on pea (Girija and Dhanavel, 2009) and soybean (Khan and Tyagi 2010) suggests a more effective and efficient cause for mutations compared to gamma rays and a combination of both.

The main purpose of food crops breeding, particularly soybean, was to develop varieties with high yield potential shown by morphological and agronomic characters. The excellent character of soybean was also demonstrated with resistance to pests and diseases, also the quantity and quality of the seed. These breeding efforts focused more on minimizing the yield loss in less favorable conditions, drought, and maximizing crop yields in favorable conditions. This study employs Dering-1 variety, which is resistant to drought. Dering 1 Varieties were released in 2012, with average result of 1.95 t/ha, and the potential yield is 2.83 t/ha.

Some genes which are responsive to drought, high salinity and cold temperatures stress at transcription level (mRNA) have been reported (Ingram and Barterls, 1996; Shinozaki K, Shinozaki, 1997). Some mRNA, as genes' respond to drought stress, increase during stress condition. It is assumed that the genes expression is induced by water deficit in an environment.

DREB1 gene is a subclass of DREB genes which is a transcription factor and serves as a key regulatory response of plants on drought stress. Yamaguchi-Shinozaki and Shinozaki (1994) identify cis-acting dehydration-responsive element (DRE). The existence is influenced by COR78/RD29A promoter and is involved in response to drought, low temperature and salinity stress.

2. Materials and Method

Alat-alat yang digunakan dalam penelitian ini adalah mikropipet, mikro 22 R Hettich sentrifuge, waterbath, refrigerator, autoclave, microwave, plate chamber electrophoresis, sisir pembentuk sumur gel, power supply, vortex, spindown, Genesys 10 UV Spektrofofometer, Macro Vue-20 Hoefer UV transiluminator, gel doc, PCR (Master Cycler Gradient Eppendorf). Real time PCR Roche LightCycler Fast Start..

The tools used in this research are micropipet, micro 22 R Hettich centrifuge, waterbath, refrigerator, autoclave, microwave, plate chamber electrophoresis, gel-forming combs, power supply, vortex, spindown, Genesys 10 UV Spectrophotometer, Macro Vue-20 Hoefer UV transiluminator, doc gel, PCR (Master Cycler Gradient Eppendorf). Real time PCR Roche LightCycler Fast Start The materials used in this research are Dering 1 varieties of soybean seeds obtained from RILET (Research Institute for Legumes and Tuber), Ethyl methanesulfonate (EMS). The material for DNA isolation is the isolation kit, primer specific real time PCR

Induction of Mutation with EMS, treatment of soybean seeds consists of 3 stages: Soaking the seeds with aquadest for 1 hour Soaking the seeds in EMS with Concentration: 0%, 0.05%, 0.5% and 1% for 4.6 and 8 hours at room temperature Flushing / washing of seeds that have been treated in water for 2 hours

Amplification with Real Time PCR, gene mutation in soybeans detection used Real Time PCR. DNA isolation was performed using a DNA isolation kit. The use of real-time PCR is done by creating a master mix then amplified by inserting the master mix into the real-time PCR tool.

The capillary tube containing the mixed master mix of DNA templates is inserted into real-time PCR that has been set with certain PCR protocols, then runs in real-time PCR.

Programme	**Analysis Model**	**Cycle**	**Segment**	**Temperature**	**Time**	**Fluorescenes Acquisition Mode**
Pre-incubation	None	1	1	95ºC	10 minute	None
Amplification	Qualification	45	Denaturation	95	10 sec	-
			Annealing	Tergantung dari primer	5-20 sec	-
			Extension	72	25 sec	Single
Melting Curve Analysis	Melting Curve	1	Denaturation	95	0 sec	-
			Annealing	65	15 sec	
			Melting	95	0 sec	Cont
Cooling	None	1	1	40	30 sec	-

3. Discussion and Conclusion

Induced mutation using EMS mutagenesis in appropriate treatment and RNA isolation is executed 35 days after planting. The young leaves, or leaf buds which are undeveloped yet were picked out and were treated with various concentrations of mutagens in drought conditions. The spectrophotometer measurement of total RNA concentration of Dering-1 variety is shown in Table

Table 1: Concentration of total RNA Dering-1 variety

Treatment	Concentration (ng / mL)	A260	A280	260/280	260/230
Control	397.9	3,606	2,437	1.47	0.43
The concentration of 0.03% + 4 hours	1139.5	11.107	7.28	1.2	0.72
The concentration of 0.03% + 6 hours	1124.5	30.192	19.56	1.54	0.97
The concentration of 0.03% + 8 hours	745.1	11.76	7.15	1.64	0.98
The concentration of 0.05% + 4 hours	649.0	25.201	14.269	1.77	1.20
The concentration of 0.05% + 6 hours	1184.8	15.154	9.656	1.7	0,71
The concentration of 0.05% + 8 hours	**1510.9**	20.024	12.376	1.62	0.93
The concentration of 0.07% + 4 hours	593.9	11.230	6.665	1.68	1.11
The concentration of 0.07% + 6 hours	855.7	13.258	7.249	1.83	0.86
The concentration of 0.07% + 8 hours	681.7	13.918	7,84	1.77	0.82

The concentration of total RNA were ranging from 397.9 (ng/µL) - 1510.9 (ng/µL). The concentration of total RNA showed the quality of the isolated RNA. RNA can be quantified using spectrophotometer with the wavelength of 260 nm and also be measured at wavelength of 280 nm to estimate the quality of the RNA. Pure RNA has the wave ratio of 260/280 nm which equals to 260/230 nm to 2.0. The low value is indicated by the protein which is shown in 280 nm or impurities in form of organic material (A230) of trizol and chloroform.

According to Farrell (2005), pure RNA isolation has the ratio of A260/A230 of 2.0 to 2.4. The value ratio of A260/A230 which is below or above the range of 2.0 to 2.4 can be caused by residual guanidine buffer or β-mercaptoethanol which were carried during the process of RNA isolation. The low ratio of A260/A230 also can indicate polysaccharides contamination (Rapley and Heptinstall 1998).

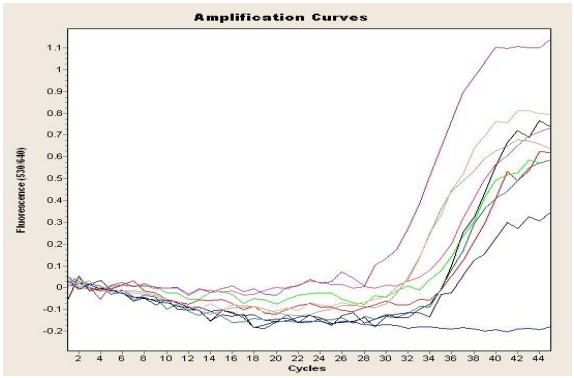

Fig.1: DREB gene expression (picomol) on EMS mutation induction treatment with a real-time PCR method that showed amplification curves

Fig.1 shows gene expression DREB1 (picomol) on EMS mutation induction treatment with a real-time PCR method that showed amplification curves. The average increase in the curve is in the cycle of 30. The analysis showed that EMS treatment at a concentration of 0.07% at some immersion time showed expression of Gm DREB1 which is higher than other treatments. The non treatment control of mutagen did not indicate gene expression. This was allegedly treated using EMS treatment at a concentration of 0:07% which were mutations point especially in genes. The intended target is GmDREB1. Whereas, mutation did allegedly not happen for the control without treatment so that the expression of target genes were not detected.

The average cycle of crossing point showing the curve starts to increase ranging from 29,56 to 33.468. The smaller crossing point suggested the greater quantity of DNA content. The high concentration curves showed higher gene expression.

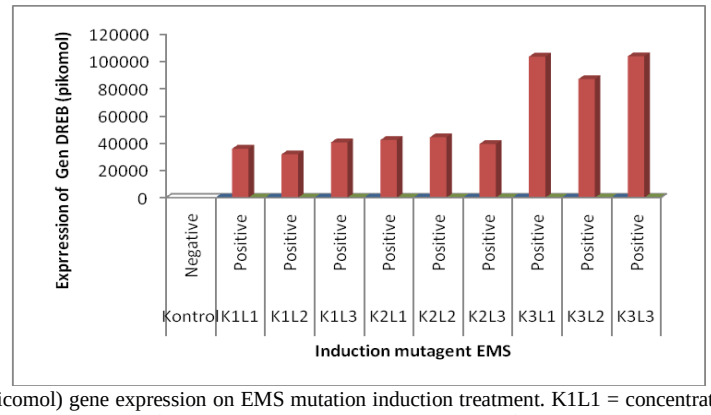

Fig. 2: Quantification of DREB (picomol) gene expression on EMS mutation induction treatment. K1L1 = concentration of 0.03% +4 hours, K1L2: concentration of 0.03% +6 hours, K1L3: concentration of 0.03% +8 hours, K2L1: concentration of 0.05% +4 hours, K2L2: concentration of 0.05% +6 hours, K2L3: concentration of 0.05% +8 hours, K3L1: concentration of 0.0 to 7% + 4 hours, K3L2: concentration of 0.0 to 7% + 6 hours, K3L3: concentration of 0.0 7% + 8 hours

Fig.2 shows gene expression DREB1 (picomol) on EMS mutation induction treatment with a real-time PCR method that showed amplification curves. The average increase in the curve is in the cycle of 30. The analysis showed that EMS treatment at 0.07% concentration at some immersion time showed expression of GmDREB1 which is higher than other treatments. Expression of GmDREB1 in EMS treatment at 0.07% concentration was about 9000-11.000 picomol.

Real-Time PCR (quantitative real time Polymerase Chain Reaction or Q-PCR) is an analysis method that was developed from the PCR reaction. This technique is used to amplify (multiply) as well as quantify (counting) the amount of the DNA molecule target of the amplification product. Real-time PCR enables the detection and quantification (as an absolute score from the DNA multiplication result or the relative number after being normalized for DNA inputs or added genes which function as normalizer) of specific sequences of analyzed DNA samples. The analysis using Real Time PCR enables the observation during the reaction, the presence of DNA amplification product can be observed in the chart that appears as a result of the accumulation of fluorescence of the probe (marker). On the Real Time PCR observations, the results no longer needed electrophoresis stage (Hoffmann et al., 2009). Compared to similar tools, Real-Time PCR is different because the results can be obtained quickly, it requires little sample of plant, and it can be used to search gene expression in transgenic plants as well as to detect GMOs (genetic modified organism).

On the real-time PCR, unlike the conventional PCR, detection phase and doubling of genetic material phase is carried out simultaneously. This offers several advantages: detection of PCR products is executed in the exponential phase so that the results were in the range area with high-precision results. In addition, the detection was done using a tracer with fluorescence marker. Tracer is a reagent that determines the specificity of the results. The using of fluorescence in the detection phase offers high sensitivity. Thus, real time PCR offers high sensitivity and pretty wide linearity range so that the result of the determination of the DNA or RNA content in the specimen becomes very accurate.

The analysis result shows that the treatment on the concentration of 0.07% in the several times of immersion shows the GmDREB-1 expression which is higher than other treatment, and control (without mutagen treatment) did not show any expression. Average cycle (crossing point) showed the curve increased around 29.56-33.468, the smaller the crossing point is, it is assumed that the DNA content is bigger in quantity. The curve's height shows higher concentration of gene expression measured.

Several studies of real-time PCR expression profile can be an expression from some genes around 20-50 genes from transcriptome in certain condition. The selected gene is perhaps an expression lane or signal chain, or the gene can be a member of an operon in responding certain environmental changes, or reflects disturbed circumstances or disease from organism, in which the expression can be used to diagnose and classify disease, and also to make prognosis.

When analyzing the expression, there were many approaches of genes reporter. The best one is to seek characteristics expression profile. It is a common approach in the study of microarray. Profiling expression by real-time PCR has many important advantages for expression, much better data quality, higher sensitivity, dynamic with a wider range of dynamicity, and all the genes that are irrelevant to the desired conditions and only contribute to the measurement. Meanwhile the cost for real time PCR measurement was way lower compared to microarray studies. One can observe a much larger number than the samples and do repetition with real-time PCR, which is the most important thing for the data statistical analysis (Kubista et al., 2006).

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