QTL Mapping in Crop Improvement

Present Progress and Future Perspectives

Edited by
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Molecular breeding for the development of drought stress tolerance in soybean

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1. Molecular breeding for plant breeding

Food needs are considerably increasing due to the rapid growth of the world population and the food or feed industry. However, food producers seem unable to meet consumers' increasing and varied food needs. Breeding activities must be more intensive to solve the problem.

Plant breeding is a method that exploits the genetic potential of plants to maximize the expression of plants' genetic prospects under certain environmental conditions (Guzhov, 1989; Stoskopf et al., 1993). Plant breeding aims to maximize plants' genetic potentials by assembling new superior cultivars that are high-yield and high-quality, resistant to biotic and abiotic constraints (Shivanna and Sawhney, 1997; Mayo, 1980).

Although conventional breeding technology has proven successful in increasing crop production and feeding the current global population, conventional breeding has unavoidable limitations, particularly in terms of the time required to introgress the desired genes. Besides, the number of genotypes to handle, especially at the beginning of the selection, is vast, influencing the number of laborers required.

Advanced technology is necessary to overcome the limitations of conventional breeding techniques and accelerate the breeding program's ultimate goal. The breeding program is essential to overcome food problems in the future. The solution to the obstacles in conventional breeding is starting to get the discovery of molecular markers. The first-known molecular

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markers were protein markers genetically attributed as isozyme markers (Hunter and Markert, 1957).

Isozyme markers are usually used in plant genetic analysis, but the markers are still very limited in number. Besides, specific enzyme systems are affected by tissue development regulation; they only express a characteristic for particular tissues. These factors are the main obstacles to using isozyme markers in exploiting plants' genetic potentials (Hamrick and Gode, 1989).

The aim of plant breeding is improving the genetic traits of plants. This activity produces superior cultivars, known as plants with excellent characteristics that are beneficial to humans. In general, breeding consists of two inseparable stages, the formation of diversity and selection. Diversity formation is the primary activity that aims at obtaining diverse plant genetic material. Various ways make to form such a diversity: collection, introduction, crossing, mutation, polyploidization, transgenic, and genome editing. Selection is an activity of selecting plants with desired properties under the objectives of a plant breeding program. Selection is essential in plant breeding.

The basic concept in selecting is to carefully choose individual plants with the best traits from a set of existing plant populations. In the selection process, breeders take various desirable characteristics (such as seed size, fruit color, tuber weight, etc.) concerning their physical appearance. Unfortunately, the physical properties used as the basis of the selection are unstable. The expression of plants' physical properties results from gene arrangement influenced by various factors like environment and growth phases.

Plants with the same gene arrangement, for example, may have different physical appearances at different growth phases in different environments. Selection based on morphological characters is complicated to do with plant breeding activities. However, biotechnology's recent development allows breeders to use molecular markers to assist them in the selection process.

The use of DNA markers as a tool for selecting Marker-Assisted Selection (MAS) is more profitable than phenotypic selection. Selection with the molecular markers help plant genetic traits and are not influenced by environmental factors. As a result, plant breeding activities, regardless of extra cost to spend, become more precise, faster, and relatively more efficient.

Plant phenotypic-based selection has several weaknesses, it takes a long time to select, and it is not easy to choose precisely the target genes expressed in morphological or agronomic traits. Furthermore, such a conventional selection is not valid in the selection involving a large population. Finally, a gene-linking phenomenon between the desired and undesirable characteristics is difficult to separate during the crossing process.

Molecular marker refers to different sequences of Deoxyribose Nucleic Acid (DNA), which are the main elements that make up the genes. This difference can distinguish one individual from another. The basic concept of molecular markers is that one individual has a unique DNA sequence to another individual so that these distinctive features are used as markers. The advantage of DNA markers in making a selection is that they are stable, and not affected by the growing phases or environmental conditions. An individual gene arrangement will remain the same at various stages of growth and in different ecological conditions.

DNA markers can be an excellent tool in the selection process. The various DNA markers such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Inter-SSR (ISSR), Amplified Fragment Length Polymorphism (AFLP), transposon-based markers,

and Single Nucleotide Polymorphism (SNP) have provided significant results in improving the properties of various crop commodities such as yield, quality, resistance to drought stress, salinity, and pest attacks.

Molecular markers have continued to develop in various ways. They are inseparable from the development of Next-Generation Sequencing (NGS) technology, enabling fast and inexpensive plant genome tracing, bioinformatics, and comfortable and affordable molecular detection techniques. Based on its superiority, DNA markers are potential tools, the selection process of which increases the efficiency and effectiveness to improve plant traits through plant breeding (https://breeding.faperta.ugm.ac.id/2019/07/23/seleksi-berbantuan-marka-molekuler-dalam-pemuliaan-tanaman).

1.1 DNA markers

In line with DNA marker-based technology development, three types of DNA markers have been found recently, with all their advantages and disadvantages. The three types of DNA markers are: (1) markers based on DNA hybridization, such as Restriction Fragment Length Polymorphism (RFLP); (2) markers based on Polymerase Chain Reactions (PCR) using nucleotide sequences as primers, such as RAPD, and AFLP; and (3) markers based on PCR using primers that incorporate specific complementary sequences in the target DNA, such as Sequence Tagged Sites (STS), Sequence Characterized Amplified Regions (SCARs), SSRs or microsatellites (microsatellites), and SNPs.

1.2 RFLP (restriction fragment length polymorphism)

RFLP analysis is a Southern hybridization procedure (Bostein et al., 1980). Hybridization of DNA cloned for DNA fragments in a sample of a restriction enzyme that cuts use for DNA variation or polymorphism. RFLP markers are a combination of specific enzyme probes. The primary source of probes for mapping RFLPs in plants are cDNA and *Pst*I clones of genome clones (Tanskley et al., 1982). This marker is codominant, so it is perfect for comparative genome mapping.

However, RFLP markers, when used as a selection tool, have some constraints: (1) In some species, the level of DNA polymorphism is very deficient; (2) It is energy- and time-consuming; (3) the required quantity and quality of the DNA are very high; (4) the hybridization procedure is complicated, thus complicating the automation; and (5) It requires probe libraries for previously unexplored plant species (Prasanna, 2002).

1.3 RAPD (random amplification of polymorphic DNA)

The working principle of RAPD markers uses the differences of PCR amplification in DNA samples from short oligonucleotide sequences, which are genetically the dominant marker group (Williams et al., 1990; Welsh and McClelland, 1990). RAPD primers are random and usually 10 nucleotides in length. The number of PCR amplification products is directly related to the number and orientation of sequences complementary to primers in the plant genome.

The use of RAPD markers, to some extent, is quite simple because it requires a small quantity of DNA, cost-effective, easy to learn, and easy to get. The weaknesses are that the degree

of reproducibility is different across laboratories or experimental results in the same laboratory. It is susceptible to variations in DNA concentrations, and it requires primary concentration and temperature cycle conditions at the time of testing. Besides, RAPD markers are dominant and cannot display homologous DNA sequences between fragments of nearly the same size (Riedy et al., 1992).

1.4 SCARs (sequence characterized amplified regions)

The SCAR and STS markers are PCR-based markers obtained by sequencing RFLP, RAPD, and AFLP fragments or genes with the recognized sizes. SCAR primers are 18–25 nucleotides in length. The reproducibility and usability of SCAR markers are much higher than those of RAPD markers. Although the SCAR markers are genetically dominant, they can be converted into codominant markers by truncation using restriction enzymes.

STS markers are used in genetic mapping. They are codominant, and they can produce stable, repeated amplification. The STS technique is quickly adopted and accepted in terms of automation. Its limitations have not been found much because of the polymorphic STS markers in the cultivated plants.

1.5 SSR (simple sequence repeat)

Microsatellite markers are DNA sequences that are short and repeated in tandem with two to five nucleotide units scattered and covering the entire genome, especially in eukaryotic organisms. Microsatellites use genetic characterization and crop mapping, including maize, rice, grapes, soybeans, millet, wheat, and tomatoes (Gupta et al., 1996; Powell et al., 1996). PCR amplified the microsatellite's primary pairs based on the conservation of the flanking-region markers for a chromosome gene.

According to Powell et al. (1996), several considerations for the use of microsatellite markers in genetic studies include: (1) the markers' codominance and abundant genome location that evenly distributed, and it is many various alleles at the locus easily determined; (2) The test instrument has very high reproducibility and accuracy; (3) It is a reliable tool for genotype differentiation, evaluation of seed purity, mapping, and genotype selection of the desired character; and (4) it is used for population genetic studies and genetic diversity analysis. The weakness of this technique is that SSR markers are not available for all plant species, and designing a new primer is quite expensive and time-consuming.

1.6 AFLP (amplified fragment length polymorphism)

The AFLP marker is a type of marker developed based on selective amplification of the total genomic restriction DNA fragments with restricted endonuclease enzymes. The amplification separated uses electrophoresis, then visualized using autoradiography or silver staining (Vos et al., 1995). These markers are similar to the RAPD ones, but they have more specific primers and more numerous ribbons. The AFLP marker is categorized as a codominant marker, although often treated as the dominant marker. It is challenging to differentiate the band intensity between homozygous and heterozygous dominant.

The advantages of the AFLP technique, according to Vos et al. (1995), are that: (1) it does not require sequence information from the genome and the same oligonucleotide kit when

analyzed and applied to all plant species; (2) The amplification is stable, and the repetition rate and variability are very high; (3) It has high efficiency in locus mapping because once amplification can cover several loci; (4) It can be used for fingerprint analysis of all DNA regardless its complexity and origin; and (5) It can act as a bridge between the genetic map and the physical map of the chromosomes. The limitation of the AFLP technique is that it is complicated to apply. It requires more time, superior skills, and the procurement of equipment and materials is very expensive.

1.7 SNPs (single nucleotide polymorphism)

SNP markers are "third-generation markers." This marker is a point mutation where another nucleotide substitutes one nucleotide at a particular locus. SNPs are a more general type of differentiating sequences among alleles, codominant in nature, and signifying polymorphic markers from an inexhaustible source to use high resolution in genetic mapping of characters. The detection of SNP markers is codominant, based on primary amplification in the sequence information for specific genes. The SNP was a marking test on rice and maize, where the genomic information is quite complete (Philips and Vosil, 1994).

The advantage of the SNP technique is more comfortable to apply than the SSR or AFLP methods. They are more useful when several SNP loci are very close together to define haplotypes and the development of haplotype tags. The SNP technique's weakness is required sequence information for a gene as the target of analysis, and the procurement of tools and materials requires a very high cost (Doerge and Churchill, 1996).

1.8 Application of MAS in plant breeding

In the MAS context, DNA-based markers can be useful if used for three primary purposes: (1) proper identification of parental lines for the improvement of a character for specific purposes; (2) tracing favorable dominant or recessive alleles in each crosses generation, and (3) identification of target individuals according to the desired character among the segregated offspring, based on the allelic composition of the cross part or of the whole genome.

1.9 MAS for qualitative character improvement

Qualitative character refers to the expression of a target character controlled by one gene or genes that is/are fully responsible for the occurrence of phenotypic variations in the character. Introgression of specific genes (genomes) from the donor line to the recipient line through the backcross method can significantly improve the target character. The conventional selection uses the methods, but it takes a long time to introgress a single dominant, recessive gene (Hajiaqatabar et al., 2019).

1.10 MAS for qualitative character improvement

The most important agronomic plant characters are very complex and controlled by several genes. The disengagement of simple characters controlled by one or more significant genes results in the improved polygenic characters through MAS, which is very complicated.

The difficulty in manipulating quantitative characters associated with complex genetic characters is because their expression involves many genes while each gene affects the phenotypic appearance of small plants. Also, the interaction among genes (epistasis) is an inhibiting factor while manipulating quantitative characters. Thus, multiple genome locations and manipulating simultaneously have a real effect on a genome location in individual plants, although this is not easy to do.

In this case, the field test repositioning accurately characterizes the QTL effect by testing its stability in several different environments. Continuous evaluation of the interaction of Quantitative Trait Loci (QTL) with the environment (Q × E) is one of the significant limitations of MAS efficiency (Beavis, 2019). Epistasis interactions in different regions of the genome may also influence testing for QTL effects. If all the genome sites involved in the interaction do not merge in the selection scheme, QTL's effect on the selection process is biased.

In addition to developing QTL mapping, which requires testing in recent years, some barriers limit the efficient use of QTL mapping information on plant breeding via MAS. The most prominent obstacles, according to Tanskley and Nelson (1996) are as follows: (1) identification of a limited number of major players (QTLs) controlling for specific characters; (2) deficiency of experiments in QTL analysis, particularly in overestimating or underestimating the number and effects of QTL; (3) lack of general character in QTL validation (markers) associated with the application of different sets of breeding materials; (4) strength of QTL × E interactions; and (5) difficulty in evaluating epistatic effects with accuracy.

2. Plant breeding through mutation induction

Soybean production can be carried out in two ways: expanding the planting area and increasing the production by improving the cultivation system and superior varieties. The increasing genetic diversity through an introduction, hybridization, selection, biotechnology, and mutation. Mutation is a technique developed extensively to increase plant genetic diversity and acquire new genetic improvement plants. Mutation is a process where genes undergo changes or all kinds of genetic changes that cause phenotypic changes from one generation to the next (Ashadi, 2013). Mutations that occur can be inherited and can return to normal (epigenetics). Mutagens cause physical and chemical changes that can occur in the genome, chromosome, and DNA levels.

Mutations can occur naturally or intentionally induced for specific purposes for plant genetic repair. Natural mutations might happen due to the presence of sunlight or electrical energy such as lightning. The artificial mutations for plant breeding purposes to provide mutagens. Two groups of mutagens are mutants of physical mutagens and chemical mutagens. Physical mutagens include X-rays, gamma rays, and ultraviolet rays; whereas, chemical mutagens include Ethyl Methane Sulfonate, Diethyl Sulfate, Ethyl Amin, and Colchicine. The advantage of using gamma rays is that the dose is accurate, and the penetration of irradiation into cells is homogeneous. Meanwhile, the benefit of using chemical mutagens is that they have a high mutation rate and dominated point mutations (Savitri et al., 2013).

Mutations are divided into small gene mutations and chromosome mutations. Small mutations occur in the molecular arrangement of a gene or DNA while the gene locus are fixed. Mutations of this type can give rise to alleles. Meanwhile, large mutations refer to a change that occurs in the structure and arrangement of chromosomes. Gene mutations are commonly called point mutations. This mutation occurs due to changes in the base sequence of DNA or changes in the nucleotide of DNA.

The use of gamma rays has developed in various fields for human welfare, such as health, industry, food preservation, and agriculture. Gamma rays are electromagnetic waves that have a strong penetrating power. One source of gamma rays comes from 60 Co. Gamma rays' penetrating power is massive and used in plant breeding to create new genetic diversity in superior assembly varieties (Ashadi, 2013).

Plant breeding uses mutation techniques to obtain new traits from plants through changes in the parents' plant genetics after receiving gamma radiation at specific doses (Mugiono and Dewi, 2009). The advantages of mutation techniques, among others, are that one of the characteristics of an improving variety without changing other traits, they give rise to new traits that are not owned by the parent, they can separate gene linkages, and the method is complementary to other techniques, in conjunction with other techniques like hybridization and biotechnology (Nunoo et al., 2014).

3. Improvement of drought stress tolerance in soybean

The research of the drought-tolerant gene GmLEA-D11 based on PCR sequencing has the purpose identified of the gene drought tolerance. The conditions of plant response to adapt to drought by inducing particular genes (Zhu et al., 1997; Dure, 1993) include changes in gene expression related to drought tolerance. One of the drought tolerance genes is the LEA-D11 gene, which encodes dehydrin protein (Ingram and Barterls, 1996; Thomashow, 1999).

The research results that the GmLEA-D11 gene sequences belonged to similar drought-tolerant varieties (Tanggamus, Nanti, Seulawah, and Tidar) and moderate tolerance (Wilis and Burangrang). However, the GmLEA-D11 gene sequence detected in the drought susceptible variety Detam-1 differs from the drought-tolerant and moderate varieties. This study's result indicates that the GmLEA-D11 gene sequence was successful as molecular markers, and they can vary drought susceptible from drought-tolerant (Arumingtyas and Savitri, 2013).

Drought is one of the environmental pressures that limit plant growth and productivity. Drought stress causes many changes, including biochemical changes such as the accumulation of osmolites and specific proteins involved in stress tolerance. One of the proteins playing an important role in the drought tolerance mechanism is dehydrin protein. This study aims to determine the protein profile and dehydrin accumulation in seven Indonesian soybean varieties: Tanggamus, Nanti, Seulawah, and Tidar (tolerant), Wilis and Burangrang (moderate), and Detam-1 (sensitive to drought stress). The drought stress adjusted soil moisture content to 25% below field capacity and compared with plants grown under normal conditions.

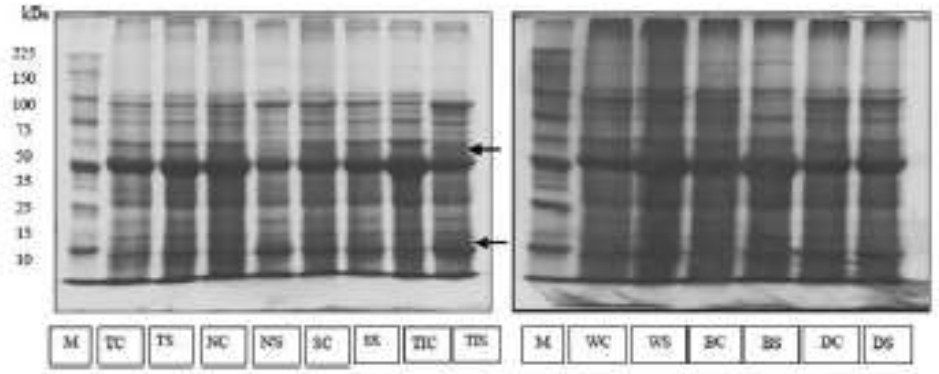

FIG. 1 The profile of protein isolated from soybean's leaves at 35 days after planting. *M*, marker; *TC*, Tanggamus control; *TS*, Tanggamus stress; *NC*, Nanti control; *NS*, Nanti stress; *SC*, Seulawah control; *SS*, Seulawah stress; *TC*, Tidar control; *TS*, Tidar stress; *WC*, Wilis control; *WS*, Wilis stress; *BC*, Burangrang control; *BS*, Burangrang stress; *DC*, Detam-1 control; *DS*, Detam-1 stress. *Arrows* show the new type of protein.

The SDS-PAGE electrophoresis results that the presence of new proteins with molecular weights of 13 and 52 kDa in the varieties of Tanggamus, Nanti, Seulawah, and Tidar (Fig. 1). Western blotting analysis for dehydrin showed that the amount of protein in the leaves of all varieties except Tanggamus decreased under drought stress conditions. The quantity of dehydrin protein in the tolerant varieties was higher than the amount of protein in medium varieties and was sensitive to drought (Arumingtyas and Savitri, 2013).

The research mutation in soybean aims to determine the effectiveness of chemical mutagens (EMS) and physical mutagens (gamma rays) in the induction of genetic diversity in soybeans. Mutation detection with molecular markers to characterize the genetic diversity of plants. This study uses four primers, the ISSR molecular marker. The primary PCR amplification results of ISSR2, ISSR3, and UBC888 showed 100% polymorphism. The mutation induction treatment using EMS chemical showed a higher level of polymorphism than a gamma-ray treatment (Fig. 2A–D; Savitri, 2018).

Abiotic stress, especially drought, significantly reduces plant productivity. Mutagenesis uses as a method that has the potential to increase genetic variability in plants. The DREB1 gene is a subclass of the DREB gene, which is a transcription factor and serves as a critical regulatory response of plants to drought stress.

This study aims to determine the expression of the DREB1 gene mutated by EMS mutagen. The method used was RNA isolation using the RNeasy Plant Mini Kit (Qiagen). The expression of GmDREB-1 was analyzed using Real-Time PCR (Quantitative Real-time Polymerase Chain Reaction or Q-PCR). The analysis showed that the mutation treatment with EMS using a concentration of 0.07% with an immersion time of 4.6 and 8 h, as shown in the expression of GmDREB-1, was higher than other treatments. This study proves that mutation induction using EMS uses as an alternative strategy for plant breeding to obtain drought-resistant soybeans (Savitri and Resmisari, 2019).

The chemical ethyl methanesulfonate (EMS) mutagen induction uses on soybeans. The mutation event results in the exchange of DNA bases, which leads to genotypic and

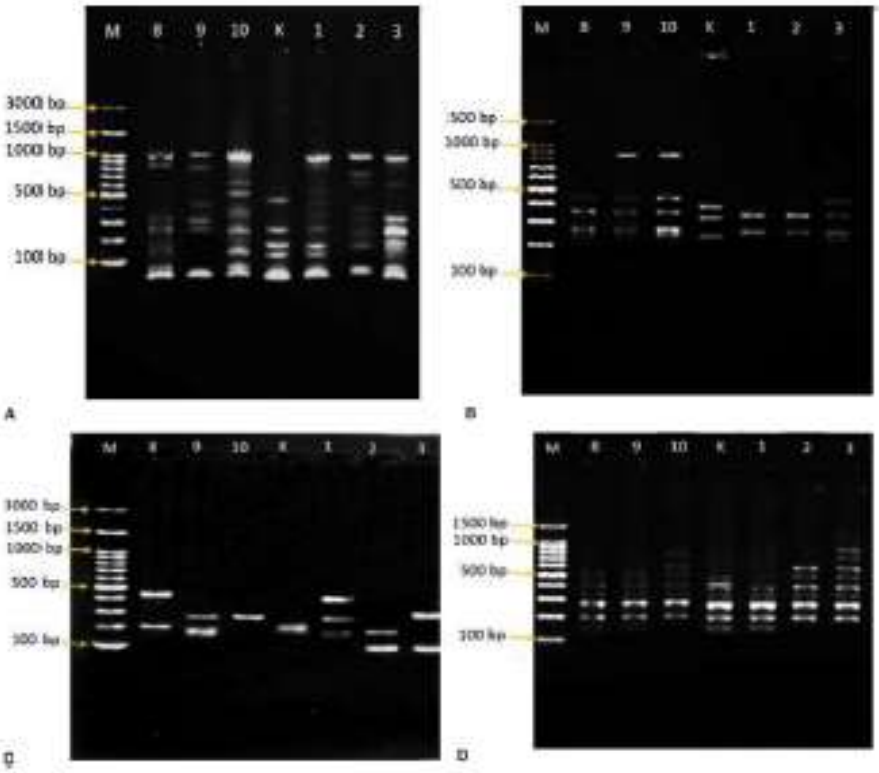

FIG. 2 (A) Amplification pattern of ISSR primers AGSCT/ISSR1 for soybean. 8: EMS 1% with 4h immersion; 9: EMS 1% with 6h immersion; 10: EMS 1% with 8h immersion. K—control, 1: 100Gy gamma rays; 2: 200Gy gamma rays; 3: 300Gy gamma rays, (B) Amplification pattern of ISSR primers CA8G/ISSR2 for soybean. 8: EMS 1% with 4h immersion; 9: EMS 1% with 6h immersion; 10: EMS 1% with 8h immersion, K—control 1: 100Gy gamma rays; 2: 200Gy gamma rays; 3: 300Gy gamma rays, (C) Amplification pattern of ISSR primers GT_7AC/ISSR3 for soybean. 8: EMS 1% with 4h immersion; 9: EMS 1% with 6h immersion; 10: EMS 1% with 8h immersion, K—control, 1: 100Gy gamma rays; 2: 200Gy gamma rays; 3: 300Gy gamma rays, (D) Amplification pattern of ISSR primers BDB $(CA)_7$/UBC888 for soybean. 8: EMS 1% with 4h immersion; 9: EMS 1% with 6h immersion; 10: EMS 1% with 8 h immersion, K—control, 1: 100Gy gamma rays; 2: 200Gy gamma rays; 3: 300Gy gamma rays.

phenotypic changes. Breeding mutations uses to obtain improved varieties. The DREB2 gene is a subclass of the DREB (dehydration-responsive element-binding protein) family of transcription factor genes. It serves to respond to and regulate gene expression during drought stress. The research about EMS mutagen aims to identify and characterize the GmDREB2 gene by various EMS mutation induction treatments. Mutation treatment is a combination of EMS concentration (0.05%, 0.50%, and 1.00%) and immersion time (4, 6, and 8h).

The results showed that the GmDREB2 sequence in the 1.00% EMS treatment with an immersion time of 8h showed the most significant changes, not only in the GmDREB2 gene sequence but also in the amino acid changes. The most significant change occurred in the M10 treatment, which had a 14-point mutation that changed the basic sequence. There are two

types of mutations in this treatment: 11 missense mutations and three nonsense mutations (Savitri and Fauziah, 2019). Mutation induction in soybean aims to develop high genetic diversity to develop breeding for superior varieties. Mutation induction can be done physically using gamma rays and chemically using EMS (Ethyl Methane Sulfonate) mutagens.

Black soybeans have a higher flavonoid content than yellow soybeans. Seed and habitus of planting black soybean are shown in Figs. 3 and 4. Flavonoids are phenolic compounds that have the potential to be used as antioxidants. Improving the quality of black soybean seeds can be done with mutation treatments that can increase flavonoids, antioxidant activity, and phenolic compounds. This study aims to determine the levels of flavonoids, antioxidant activity, and phenol of black soybean varieties Detam 3 using gamma rays treatment and Ethyl Methanesulphonate (EMS) induction. The doses used in this study were 1000 Gy gamma rays, 1% EMS, and a combination of gamma rays and EMS. Total phenolics was determined using the folin-ciocalteu method, expressed as gallic acid equivalent (GAE)/g of extract. The flavonoid content was determined by the $AlCl_3$ method, which is quercetin equivalent (QE)/gr extract. Antioxidant activity was determined using Ferric Reducing Antioxidant Power (FRAP), expressed as ascorbic acid equivalent (AAE)/gr extract. Mutagen treatment

FIG. 3 Black soybean (A) plant of black soybean variety Detam-1 (B) seed of black soybean variety Detam-1.

FIG. 4 Habitus black soybean variety Detam-3 mutant gamma-ray 1000 Gy dan/EMS 1%. T0U2 – control (seed soak in buffer phospat pH7 6h), T1U3 – control (without soaking), T2U90 – gamma rays 1000 Gy, T3U1 – EMS 1% soaking 6h, T4U12 – gamma rays 1000 Gy + EMS 1%.

producing the highest total flavonoids, phenols, and antioxidant activity was the 1000Gy gamma-ray treatment, namely, 185,748mg QE extract/g, 645,447 mg GAE extract/g, and 166,752 mg AAE extract/g (Figs. 5 and 6). This utility for further research is to develop black soybean varieties as a source of antioxidants.

Research to determine the effectiveness of chemical combinations (EMS) and physical mutagens (gamma rays) in the induction of genetic diversity in black soybean varieties Detam 1 has been carried out. Mutation detection is carried out with molecular markers to characterize the genetic diversity of a plant. The mutation induction method used in this research is EMS treatment and gamma rays. This study used 5 ISSR (Inter Simple Sequence Repeat) molecular markers primers. The primers UBC 810, UBC 811, UBC 812, UBC 828, and K18 successfully amplified and showed genetic diversity in black soybeans treated with a combination of

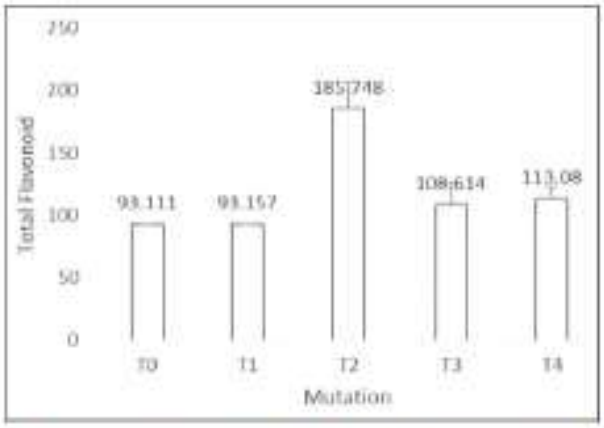

FIG. 5 Total flavonoid (mg QE/g ekstrak). T0—control (seed soak in buffer fosfat pH7 6h), T1—control (without soaking), T2—gamma rays 1000Gy, T3—EMS 1% soaking 6h, T4—gamma rays 1000 Gy+EMS 1% (Fauziah et al., 2020).

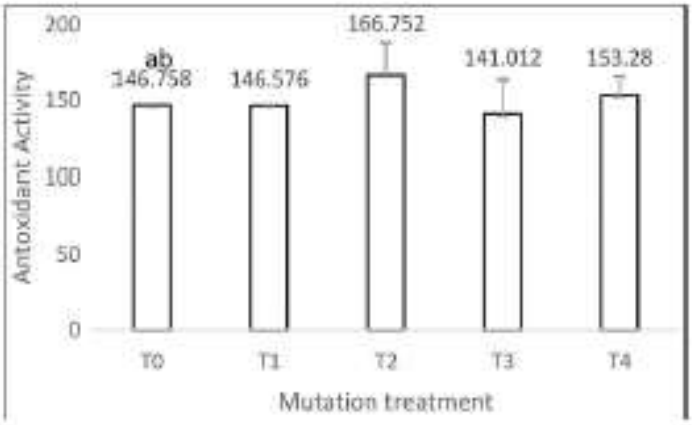

FIG. 6 Antioxidant activity (AAE QE/g ekstrak). T0—control (seed soak in buffer fosfat pH7 6), T1—control (without soaking), T2—gamma rays 1000Gy, T3—EMS 1% soaking 6h, T4—gamma rays 1000 Gy +EMS 1%.

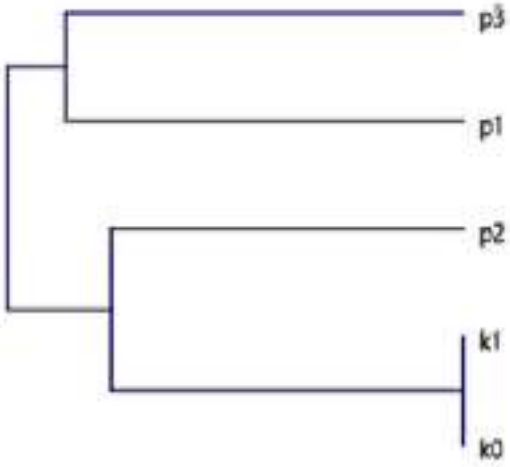

FIG. 7 Dendrogram of mutants from gamma rays and EMS induction. K0 and K1 = control, P1 = gamma rays irradiation, P2 = EMS, P3 = gamma rays and EMS.

gamma-ray irradiation and EMS. The results showed that gamma-ray irradiation produced a higher level of polymorphism than the control and EMS treatment, so it was suitable for the selected treatment candidates for further treatment (Savitri and Fauziah, 2020). The dendrogram result of mutation treatment in Fig. 7.

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