

# Utilization Of Molecular Marker Technology In Foxtail Millet (*Setaria italica* (L.) P. Beauv.) Breeding In Indonesia

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## Abstrak

Upaya peningkatan produktivitas dan kualitas jowar (*Setaria italica* (L.) P. Beauv.) dalam mendukung diversifikasi pangan di Indonesia dapat dilakukan melalui program pemuliaan tanaman dengan perakitan varietas unggul. Upaya mendapatkan varietas jowar unggul yang spesifik sesuai dengan keinginan, dibutuhkan ketersediaan plasma nutfah yang informatif, diantaranya melalui kegiatan karakterisasi. Pemanfaatan marka molekular merupakan metode yang saat ini telah terbukti dapat membantu mempercepat untuk mengintrogressi gen-gen mayor ke dalam kultivar elit. Pemanfaatan marka molekular sebagai alat bantu seleksi tanaman telah banyak dilakukan dengan tingkat akurasi yang lebih baik. Seleksi dengan marka molekular hanya didasarkan pada sifat genetik tanaman dan tidak dipengaruhi oleh faktor lingkungan, sehingga hasilnya lebih akurat dibanding seleksi berdasarkan morfologi. Uji molekular bervariasi dalam cara pelaksanaannya untuk mendapatkan data, baik tekniknya maupun tingkatan target data yang diinginkan, sesuai kemudahan pelaksanaan, ketersediaan sumber daya manusia, fasilitas dan dana. Marka molekular yang telah digunakan untuk tanaman jowar adalah RFLP, AFLP, RAPD, Transposon, EST-SSR, Mikrosatelit, dan ISSR.

**Kata kunci**—Jowar, marka molekular, pemuliaan tanaman

## Abstract

Efforts to increase the productivity and quality of foxtail millet (*Setaria italica* (L.) P. Beauv.) to supporting domestic food diversification in Indonesia can be done through plant breeding programs by assembling superior varieties. The effort to obtain superior millet varieties that are specific in accordance with farmer wishes, requires the availability of informative germplasm, which is delivered through characterization activities. The use of molecular markers is a method that now has been proven to help accelerate the introgression of major genes into elite cultivars. The use of molecular markers as a plant selection tool has been carried out with greater accuracy. Selection with molecular markers is only based on plant genetic traits and is not influenced by environmental factors, so the results are more accurate than selection based on morphology. Molecular tests have vary in the way they are implemented to obtain data, both the technique and the desired level of target data, according to the ease of implementation, availability of human resources, facilities and funds of money. The molecular markers that have been used for plant breeding of foxtail millet are RFLP, AFLP, RAPD, Transposon, SSR-Microsatelite, and ISSR.

**Keywords**—foxtail millet, molecular markers, plant breeding, Indonesia

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## 1. INTRODUCTION

Along with the rapid growth of population and the development of feed and food industries, the need for world food is increasing. However, in reality food producers are unable to meet increasing and diverse consumer needs. The efforts being made to answer these food problems are by intensifying breeding activities. Plant breeding is a method that explores the genetic potential of plants to maximize expression of the genetic potential of plants in certain environmental conditions. Plant breeding aims to maximize the genetic potential of plants through the assembly of high yielding and high-quality new varieties, resistant to biotic and abiotic constraints [1].

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is one of the non-rice food crops that has long been domesticated by the world community including Indonesia as an alternative food crop, mainly because of its ability to grow and adapt well to tropical, sub-tropical and temperate climates, such as in India, China, Asia, North Africa and America [2]. Foxtail millet is one type of cereal plant that has very good potential as an alternative food crop in terms of the nutritional value and growth capabilities in dry climates. Based on its nutritional content, this plant has good nutrition, including carbohydrates 63.2 g, protein 11.2 g, fat 4 g, and fiber 6.7 g [3]. Aside from being a source of carbohydrates, millet can be developed as a functional food source because it has a low glycemic index [4], high anti-oxidant content [5], even as anti-cancer potential [6].

One of the factors that must be known in assembling varieties to improve plant genetics is by identifying crossbred parents through characterization activities to obtain informative germplasm sources [7]. Information on genetic variability and kinship can be used as a reference in selecting crosses elders to get new genetic combinations with the quality and quantity of species through better artificial selection such as pest resistance, good adaptation and higher production results. Information on genetic variability also needs to be known as a basis for consideration in developing strategies for conservation, management and empowerment of plant genetic resources.

The method that can be used to analyze plant genetic variation is by comparing morphological characters. Each species has a specific and varied character so that it can be used as a basis for distinguishing and classifying a species. Morphological characters have the advantage of being easily visually observed in the field and more practical than other traits [8]. However, Prasetiyono et al. [9] revealed that selection based on morphological characters would have difficulties due to the influence of various environmental conditions. As a result, the selection pressure becomes uneven so that it can cause selection errors. Therefore, genetic identification using molecular markers is needed to complement and improve efficiency in analyzing genetic variation and kinship in food crops [10].

The use of DNA markers as a tool for assisting the Assisted Selection (MAS) selection is more advantageous compared to phenotypic selection. Selection with the help of molecular markers is based on plant genetic traits, not influenced by environmental factors. Thus, plant breeding activities are more precise, faster, and relatively more cost effective and time-consuming. Selection based on the phenotypic character of plants in the field has several disadvantages such as those summarized by Lamadji et al. [11], including (1) requiring considerable time, (2) difficulty choosing the right kind of gang that is the target of selection to be expressed in traits morphological

or agronomic characteristics, (3) the low frequency of desired individuals in a large population selection, and (4) the phenomenon of gene linkages between desirable traits and undesirable traits is difficult to separate when crossing.

Based on the description above, this paper discusses the DNA markers that are widely used by researchers for the repair of millet plants in Indonesia. The discussion will illustrate the prospects and challenges of using molecular markers in plant breeding.

## 2. RESEARCH METHODOLOGY

This study uses a systematic review method with analysis of synthesis techniques (meta-synthesis).

## 3. RESULT AND DISCUSSION

Based on the process of review the molecular markers technology for millet for application to breeding systems is developing in line with the increasing number of DNA markers, namely: a. *Restriction Fragment Length Polymorphism* (RFLP) [12]; [13]; [14]; b. *Amplified Fragment Length Polymorphism* (AFLP) [15]; [16]; [17]; c. *Randomly Amplified Polymorphic DNA* (RAPD) [18]; [19]; d. *Transposon* [20]; e. *SSR/Mikrosatellite* [21]; [22] -*Expressed Sequence Tag-Simple Sequence Repeat* (EST-SSR) [23]; [24]; and f. *Inter-Simple Sequence Repeats* (ISSR) [25]; [26].

### *Plant Breeding*

Plant breeding is a dynamic and sustainable activity. Its dynamism is reflected in the challenges and natural conditions of the environment that tend to change, for example pathogenic strains that are always evolving, tastes or preferences of consumers towards food that are also developing, therefore breeding activities will race in line with these changes. While its sustainability can be seen from its continuous activities, it continues from one stage to the next. Furthermore, breeding is a multidisciplinary applied science, using various other sciences, such as genetics, cytogenetic, agronomy, botany, physiology, pathology, entomology, molecular genetics, biochemistry, statistics [27], and bioinformatics. Whereas, from the method used, it is divided into two: conventional breeding approaches (for example through crossing, selection and mutation) and unconventional (gene cloning, molecular markers and gene transfer).

In general, the breeding process begins with (i) germplasm collection efforts as a source of diversity, (ii) identification and characterization, (iii) diversity induction, for example through crossing or by gene transfer, followed by (iv) selection process, (v) testing and evaluation, (vi) release, distribution and commercialization of varieties [28]. The crossing technique followed by the selection process is the most widely used technique in innovating new superior cultivars, then followed by introducing cultivars, techniques for inducing mutations and spontaneous mutations which also produce several new cultivars.

Natural genetic diversity is the source of genes for each plant breeding program. This variation can be utilized by making simple introductions and selection techniques or utilized in crossing programs to get new genetic combinations [29].

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### *Conventional Breeding Techniques*

Conventional plant breeding programs are usually selected for targeted characters based on phenotypic or morphological selection, both individually and plant populations. Determination of characteristics is crucial in the description of plants. The oldest and most commonly used characteristics are morphological and physiological properties, such as stem shape, leaf shape, disease resistance and others. Nuraida [30] revealed that the disadvantage of using this type is that the expression varies greatly with environmental conditions. The phenotype of a character is influenced not only by genetic factors, but also by environmental factors. Therefore selection of a character based on appearance / phenotype has many shortcomings, including giving inconsistent results, especially if the character is more influenced by environmental factors (low heritability) than genetic factors. Besides the inconsistent results, the time needed is also relatively long.

### *Plant Breeding Based on Molecular Markers*

The diversity of an organism at the species level in an ecosystem is a balance in the chain of life naturally. This is related to the value of the organism's heterozygosity in the population, the higher the value of the organism's heterozygosity, the better the reproduction rate so that it can survive in the face of stress or stress. Determination of the value of heterozygosity in an organism can be carried out molecularly so that the level of diversity in the population can be known. The molecular approach can also measure the extent to which the kinship relationship of a species is geographically separated. The expected implications are genotype changes at species level which can be used as superior brooders in plant breeding.

Molecular genetic approaches using DNA markers successfully form molecular markers capable of detecting genes and certain traits [31]. Genetic diversity based on agromorphological information to evaluate genotypic diversity is now felt to be inadequate. Breeding with the help of molecular markers is very useful to overcome the obstacles that often arise in conventional breeding, especially for qualitative character selection which is controlled by recessive genes and selection for quantitative characters [32]. Genetic information from a plant especially those related to a character is very important. For this reason, genetic identification with a molecular approach is needed in breeding activities, in order to obtain the right results in a short time [30].

The technology of molecular markers in millet plants develops in line with the increasing number of DNA markers, namely:

#### **a. RFLP**

RFLP markers are a technique to distinguish organisms based on the pattern of cutting DNA into restricted enzymes. The similarity of DNA fragmentation patterns can be used to distinguish species from one another. Restriction enzymes are enzymes that cut DNA in certain basic sequences according to their sequence of sequences, endonuclease enzymes produced by various prokaryotic organisms. Their natural function is to destroy invasion of foreign DNA molecules by recognizing and cutting the motives of certain DNA sequences, most of which consist of four, five, or six bases. Each enzyme has a special recognition sequence of palindromics, and bacteria usually protect their own DNA cut by methylation of cytosine or adenine residues in this sequence [14].

Cutting certain DNA molecules with certain restriction enzymes produces a set of fragments that can be reproduced in a well-defined length. Point mutations in the sequence of recognition and insertion or deletion between the two recognition sites produce a modified pattern of restriction fragments, and thus can inform polymorphisms between different genotypes [14].

These markers are codominant, have been developed and are very useful for detecting variations at the DNA level as well as mapping and characterizing genes from various plant species [32]. RFLP is one of the potential alternative methods that can help in field testing. However, RFLP is less desirable because it requires a lot of energy and time and a lower level of polymorphism compared to SSR markers [33]. There are several things that need to be considered in using molecular markers, among others, (1) providing a low level of polymorphism for some species (2) requires a lot of time and energy (3) requires a very large amount of DNA, clean and sometimes all plants are needed extraction process and (4) a probe library needs to be made in advance for plant species that have never been explored before [16].

#### **b. AFLP**

AFLP markers are based on selective applications of DNA fragments that are cut using retention enzymes. In this technique, DNA is cut into pieces using two different retention enzymes that will produce DNA fragments that have two different sticky ends. Different adapters that are specially designed short double strand DNA sequences are added to the different sticky ends. Then the primary pair specific to the two adapters is used to direct the amplification of these DNA fragments. For amplification, the primer used carries an additional base, generally 3 bases, so that only a small portion of the retention fragments are ligated with the amplified adapter. These markers are generally, codominant, where allele scores are based on the presence or absence of DNA bands. The advantage of this marker is that it does not require sequences of the genomes analyzed and the same oligonucleotide kit can be used for any plant species. In addition, this technology also produces a large number of polymorphic markers. However, AFLP is still relatively more complex which requires more time in its implementation and the costs required are relatively expensive [16];[17].

#### **c. RAPD**

RAPD markers only use a single short primer that is usually 10 bases that will hybridize with parts that complement the plant genome. The advantages of this technique are (1) the quantity of DNA needed is small, (2) saving the cost, (3) easy to learn, (4) the required primer has been commercialized so easily obtained [32]. Some conditions that must be considered to ensure reproducible RAPD amplification include (1) the concentration of genomic DNA must be accurately determined and the amount of DNA used must be uniform (2) RAPD sensitive to temperature profiles on PCR machines (3) the quality of DNA polymerase enzymes must be consistent and (4) piping errors must be as small as possible. Some things that are limiting the use of these markers are (1) reproducibility problems from the lab to the lab and even in the lab itself (2) fragments that have the same size do not necessarily have the same sequence and (3) these markers

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are dominant who cannot identify heterozygosity that must be considered when designing experiments [16].

#### d. Transposon

Transposons are sequences of DNA that can move from one place to another in the genome of a cell. This displacement is often also called Transposition. In the process, this transposition can cause mutations and changes in size from genomic DNA. Transposon is also commonly referred to as "*jumping genes*", and is one example of a mobile genetic element. Transposon was first discovered by Barbara McClintock by analyzing color variations in corn plants, more than 50 years ago [14].

Transposable elements are specific forms of genetic recombination that can move to certain genetic parts from one part of DNA to another [34]. At present a number of known and characterized transposons are many genetic components found in the eukaryotic cell genome. Transposon has an influence on genome structure and gene function in almost all organisms [35].

Multilocal application of transposons as molecular markers has great potential both genotyping, fingerprinting, and building genetic relationships between cultivars and wild types in breeding [36]. Transposable elements are divided into 2 classes, namely Retrotransposable elements that have a transposon mechanism through intermediate RNA so that when a retrotransposition occurs a new copy of transposon is created, while the other class is in the form of a transposon mechanism based on cut and paste and direct replication of DNA fragments. Retroposition is the creation of new copies with reverse transcript from RNA transcripts [37].

The existence of these two classes of transposons is widely distributed in eukaryotic organisms including plants and is currently being developed as a molecular marker for plant breeding by Roy et al. [38]. This is because the transposon element, in particular retrotransposon, produces a large number of polymorphism inserts due to the retrotransposition process thus creating many molecular markers. Many retrotransposon elements have been shown to be highly polymorphic for insertion sites in various plant species. Both of these transposon classes have been used as markers in barley plants (*Hordeum vulgare*) based on the technique of DNA fingerprinting (BARE-1) including markers *inter-retrotransposon amplified polymorphism* (IRAP) *retrotransposon microsatellite amplified polymorphism* (REMAP), and *inter-MITE polymorphism* (IMP).

#### e. SSR or Microsatellite

Microsatellites consist of DNA structures with motifs of 1-6 base pairs, repeating five times or more in tandem [39]. Some considerations for the use of SSR markers are (1) markers spread abundantly and evenly in the genome, very high variability (many alleles in the locus), the nature of codominant and genome loci can be known, (2) as a test that has very reproducibility and accuracy height, (3) is a very accurate tool for differentiating genotypes, evaluating seed purity, mapping and genotyping selection for desired characters, (4) population genetic studies and genetic diversity analysis. The weakness of this marker is that SSR is not available in all plant species, so designing a new primer requires a long time and costs are relatively expensive [40].

SSR technology has reproducibility, is fast in its implementation and costs more effectively than RFLPs markers [41]. The ease of SSR in amplification and detection of DNA fragments, as well as the high polymorphism that is produced causes this method to be ideal for use in studies with a large number of samples. In addition, SSR can be applied without damaging plant material because only a small amount is used in DNA extraction or can use other parts such as seeds or pollen [42].

Simple Sequence Repeat (SSR) is popularly used as a molecular marker because it is codominant. Microsatellite loci are also specific (one locus of each primary pair) with high polymorphic information content [43]. Microsatellites were amplified through PCR by using primary pairs that flank certain repetitive regions. The nucleotide sequence that flanks this repetitive part is used to design the primary pair. The amplification results were visualized through electrophoresis on polyacrylamide gel. These markers are codominant and highly reproducible [16].

#### f. ISSR

ISSR Marking (*Inter Simple Sequence Repeat*) is an area that lies between two regions of the SSR and is usually in the form of mono, di or trinucleotide. ISSR is a part of microsatellite that does not encode protein (*non coding region*). The ISSR mark corrects the shortcomings of the RAPD technique, where ISSR is more sensitive to detecting genetic diversity at a low level but relatively easy and as economical as the RAPD technique [44]. ISSR involves amplification of DNA segments that are at a distance that can be amplified between two identical repetitive microsatellite regions but with different directional orientations. ISSR markers are dominant markers, having a longer length (16-25 pb) longer than RAPD (10 pb) [45].

#### *Constraints*

Some genetic analysis techniques using molecular DNA markers have been available with all the disadvantages and advantages of each. The main consideration for choosing markers that will be used in genetic analysis activities is the genetic material that will be used, the type of genetic study, the objectives to be achieved, the availability of sufficient funds, and infrastructure. Some technologies are still relatively expensive and the availability of materials will greatly determine the smooth implementation. In addition, the level of polymorphism produced also needs to be considered because some molecular marking technologies still provide levels of polymorphism that are too low for the plants analyzed.

## 4. CONCLUSION

The use of molecular marker technology for foxtail millet breeding program in Indonesia is able to improve efficiency in assembling new plant varieties that are superior by maximizing yielding and high-quality plant genetic potential, resistant to biotic and abiotic obstacles. The use of molecular markers as a plant selection tool has been carried out with greater accuracy. Selection with molecular markers is only based on plant genetic traits and is not influenced by environmental factors, so the results are

more accurate than selection based on morphology. Molecular tests have vary in the way they are implemented to obtain data, both the technique and the desired level of target data, according to the ease of implementation, availability of human resources, facilities and funds of money. Molecular markers that are generally carried out on millet plants are using RFLP markers, AFLP, RAPD, Transposon, SSR-Microsatelite, and ISSR.

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