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Detection of Transposon Gene in Lurik Peanuts (*Arachis hypogaea* var. *lurik* L.) with AhMITEs Analysis

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***Abstract*—Peanut (*Arachis hypogaea* L.) is one of the leading commodities in Indonesia that are consistently growing with high demand. However, its productivity in the current state is relatively few, thus causing dependency on imported products. Developing new varieties is one of the many solutions to these problems. Lurik peanuts are superior local varieties to any other peanuts in terms of productivity and disease resistance. Seed with the purple pattern is this cultivar's special characteristic and main attraction. This study aimed to identify and verify the activity of transposon genes in the seed pattern of Lurik peanuts. This research method was carried out by gene detection and sequencing analysis using PCR-AhMITEs (*Arachis hypogaea* Miniature Inverted Transposable Elements). The study used the Garuda variety as a comparison due to the absence of seed patterns, and it is a superior variety widely cultivated in Indonesia. Four types of primers used in this study were AhMITE1, AhTE0357, AhTE0391, and AhTE1317. The results revealed that the four primers had a linear relationship that could distinguish Lurik peanuts and Garuda peanuts based on the presence of transposon genes. The sequencing results confirmed that the detected genes were transposons from peanuts, located on chromosome 5 (Arahy.5), chromosome 9 (Arahy.9), chromosome 14 (Arahy.14), and chromosome 19 (Arahy.19). Based on the results of the study, the pattern on Lurik peanuts is an expression of the transposon gene activity.**

***Keywords*—Lurik peanuts; Garuda peanuts; *Arachis hypgaea*; transposon; AhMITEs.**

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I. INTRODUCTION

Peanut (A. *hypogaea* L.) is one of Indonesia's main essential food commodities, with a particularly high consumption level even though it imports much of the product to fulfill its consumption needs. According to recap data from the Ministry of Agriculture of the Republic of Indonesia, the growth of peanut harvested area in Indonesia within the last five years showed a 4.63% average decline estimated from the harvested area that fell by 30.03 thousand hectares (down 6.61%) accompanied by a decrease in productivity to 13.21 Ku/Ha (down 0.90%). One alternative to overcome this problem is using superior Lurik peanuts varieties [1]. Lurik variety is one of the solutions because of its high productivity and morphological characteristics that attract attention due to the pattern in the seed's unseen in other peanut varieties. This striated pattern is a transposon requiring further investigation (Figure 1) [1]. This study serves to verify that transposable elements (TEs) in peanuts play a role in forming the seed pattern.

Fig. 1 Morphological Characteristics of A. *hypogea.* Left side, Garuda Peanut; Right side, Lurik Peanut.

Transposable elements (TE) are segments of DNA that can move from one location to another in the genome. This sequence is often called the "jumping gene." Barbara McClintock first discovered the existence of TEs 1937-1945, and its exploration in the maize genome has been extensively explored [2], [3]. Although once described as "junk" DNA, recent evidence suggests that transposable elements (TE) have been a major factor in the evolution of various eukaryotic taxa [4], [5]. Transposons are vital contributors to the evolution and diversity of Angiosperm plants [6] that are widely distributed in the genome, and their polymorphic insertion allows the development of transposon-based markers.

Transposon-based markers have been developed and used for various genetic analyses and mapping [7]. In peanuts, the use of DNA transposon markers has long been proposed. One of these marker functions was developed to track the activity of Miniature Inverted Transposable Elements (MITEs) [8]. Like other transposons, MITE is embedded mainly in gene-rich regions, which could be why MITE influences gene expression and plays an important role in accelerating evolution. Previous research on transposon genes causes pattern variations in various peanuts. These genes are classified into AhMITEs, named after the plant subject *Arachis hypogaea* [9], [10]. Based on these references, transposon gene detection in this study will be carried out using a gene detection series through the AhMITEs primer group in Lurik peanuts and compared with the unpatterned Garuda variety.

II. Materials and Method

A. Lurik Pea Cultivation

The research was conducted from January to November 2020 and divided into two stages, namely, (1) lurik peanuts cultivation and (2) collection and analysis of molecular data. Cultivation was conducted in a greenhouse in Bokoharjo, Prambanan, Sleman, Yogyakarta. The distance of each planting hole was 30-40 cm, with a total of 4 x 50 meters wide. Harvesting was carried out after a feasibility test on the condition that the plants suitable for harvesting were hardened stems and fallen leaves.

B. DNA Extraction

DNA extraction used the Nucleon-Phytopure Kit brand Illustra DNA Extraction Kit Phytopure TM RPN 1851, which consists of Reagent 1, Reagent 2, and Resin. The extraction procedure followed the protocol in the kit. The resulting DNA pellets were added with TE buffer and stored at -20°C.

C. Qualitative and Quantitative DNA Analysis

Qualitative analysis of the genome was carried out using the electrophoretic method. The running process used 0.8% agarose gene at 100 volts for 30 minutes. Good amplification results were indicated by the appearance of bands and the absence of smears. Quantitative analysis used a nanodrop spectrophotometer with wavelengths of 260 and 280. The purity level of DNA at an absorbance ratio of 260/280 nm had an optical density (OD) ratio ranging from 1.8 – 2.

D. DNA Amplification using AhMITE Primers

DNA amplification was carried out with the BOECO Thermal Cycler TC-PRO PCR machine. The amplification protocol used was pre-denaturation 95°C, denaturation 95°C, annealing 49-53°C, elongation 72°C, post-elongation 72°C, and endless 4°C. The four primers used are AhMITE1 (5'-AAGGTGGATACTACMATGAAGAT-3'), AhTE0357 (5'-GCCTCCCCCAGAAAAGTTATC-3';3'-GCCTCCCCCAGAAAAGTTATC-5'), AhTE0391 (5'-TTCCTTTTTCTGATGAAATATGAGAT-3'; 3'-GCCTTTGAAGATCTTTCACCC-5'), AhTE1317 (5'-AGAGCTACTATAAGATCGCGGGT-3'; 3'-GAGCACCAATTACACAAGTAGGC-5'). DNA amplification results were then visualized using the 2% agarose gel electrophoresis method.

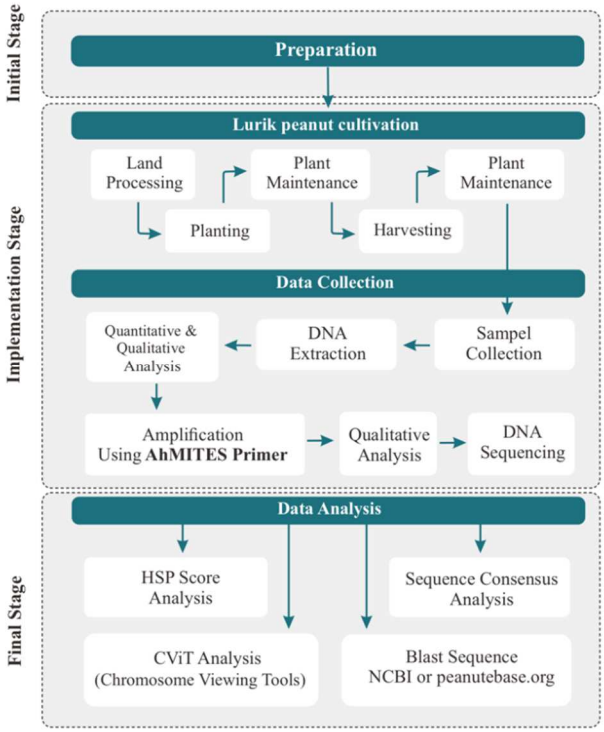

Fig. 2 Research Procedure.

E. DNA Sequencing

DNA amplification was carried out using the BOECO Thermal Cycler TC-PRO PCR machine. Five primers were used in this study, including AhTE0357, AhTE0391, AhTE1317, and AhMITE1. DNA amplification results were then visualized using the 2% agarose gel electrophoresis method.

F. Data Analysis

The sequencing results obtained were then edited using Genestudio software. The edits were then entered into the Basic Local Alignment Search Tool (BLAST) at NCBI or https://www.peanutbase.org/ regarding the *Arachis hypogaea* section. Analysis CViT (Chromosome Viewing Tools), to indicate the visualization of the location of the distribution of hit segments in the chromosomes. The green dot on each chromosome can identify the number of hits.

III. Results and Discussion

A. Transposon Gene Detection

The basis of this research was the number of mutation cases that conventional theory cannot explain. Transposable elements (TEs) play a role in such cases that can cause dysfunction, disease, and death. However, it also plays a role in adapting a population to environmental changes and encourages genetic variability [5].

Identification of DNA markers is often used to search for plant productivity traits such as disease resistance and qualities that are useful in plant breeding. MITEs-specific markers have been used to evaluate and characterize resistance properties in mutant plants. These mutations cause plants to be more productive and more resistant to disease due to the automatic and random gene transfer commonly known as transposons. This study attempts to use Miniature Inverted Transposable Elements (MITEs) such as AhMITE1, AhTE0357, AhTE1317, and AhTE0391 to track the presence of TE activity that affects the color pattern on the skin of striated peanut seeds.

Based on Figure 3. All the AhMITE primers tested in this study (AhTE0357, AhTE0391, AhTE1317, and AhMITE1) showed amplified DNA bands. All amplified bands were found only in Lurik peanuts, not Garuda peanuts. The result indicates that there is transposon activity in the striated bean genome. The size of the amplified DNA fragments also varied, such as AhTE0357 (170 bp), AhTE0391 (352 bp), AhTE1317 (167 bp), and AhMITE1 (242 bp).

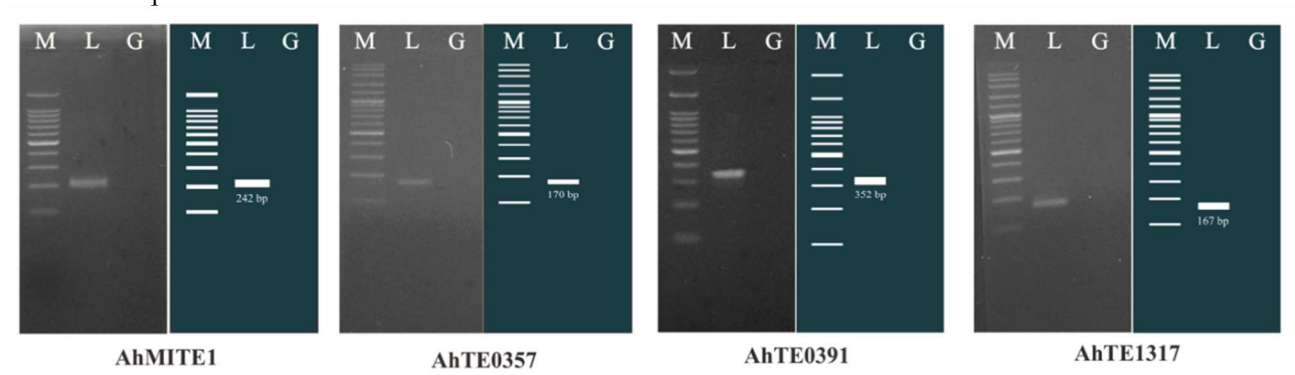

Fig. 3 Electrophoresis visualization of AhMITE primers. M= Marker 100bp (vivantis), L= Lurik Peanut, G= Garuda Peanut.

Furthermore, AhMITEs markers are used not only in expression analysis and identification of transposon activity but also to study genetic diversity among populations and groups and to identify peanut trait quality based on oleic acid content [11].

B. DNA Sequencing

The analysis of the sequencing results was carried out by aligning the sequences obtained with the database. Based on Figure 4, only the AhTE0391 markers perfectly match the database subject sequence.

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AhMITE1    Query:            40 ATCAAGCCCTACAATCTATCATCCTATG 67
                                |||||||||||||||||||||||||| |||
           Sbjct:      13787430 ATCAAGCCCTACAATCTATCATCCAATG 13787403

AhTE0357   Query:            12 CTTGTTTCTGCCTAGCTTATATATATATAA--GCTCTTTTCGCTCTTCTATACATATAAT 69
                                |||||||||||||||||||||||||||||||  |||| ||||||| |||||| ||||  ||
           Sbjct:     107623741 CTTGTTTCTGCCTAGCTTATATATATATAAAAGCTCATTTCGCT-TTCTATGCATACTAT 107623681

AhTE0391   Query:             2 AAATATGAGATATTACATTTCATACTTGACTTTATTGAAAAATGGAAAAAATATGTATGA 61
                                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
           Sbjct:     113753646 AAATATGAGATATTACATTTCATACTTGACTTTATTGAAAAATGGAAAAAATATGTATGA 113753705

AhTE1317   Query:             9 TGGTCAAGGTTATTAAGAGAAAA-TAATAGTATGCATAGAAAGCGAAATGAGCTTATATA 67
                                ||||||||||||||||||||||||| |||||||||||||||||||||||||||||||||||
           Sbjct:     129853556 TGGTCAAGGTTATTAAGAGAAAAATAATAGTATGCATAGAAAGCGAAATGAGCTTATATA 129853497
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Fig. 4 Sequencing results based on four AhMITE primers.

The suitability of the sequence is used to determine the HSP or High-scoring Segment Pair. HSP is a local alignment by determining a base sequence's highest score based on its alignment or similarity at a particular location and species. A BLAST "query" sequence is given as a single nucleotide character string or amino acid code, begins with a definition line, begins with the ">" symbol, and contains an identifier and descriptive information. The blast results from the sequences amplified by the four primers showed high similarity (Table 1).

TABLE I
HSP score and chromosome location of sequence

Primer	HSP (%)	Chromosome Location
AhMITE1	96.43	Arahy.14
AhTE0357	87.32	Arahy.05
AhTE0391	100	Arahy.09
AhTE1317	98,48	Arahy.19

C. Transposon Distribution in Lurik Peanuts

Transposable Element (TE) groups such as MITEs play an important role in adaptation and evolution, leading to the

diversification of plant species and insertion mainly in gene-rich regions. This could be why MITEs influence gene expression and play an important role in accelerating eukaryotic evolution [12] and genome organization [13]. TE plays an important role in improvising cultivated objects in plant breeding because it produces road, fast, and selectively responsive changes to the plant genome [14].

Miniature Inverted-Repeat Transposable Elements (MITEs) are a group of non-autonomous, widely distributed Class II transposons with high abundance in the plant genome [15]. The MITEs group comprised short DNA fragments (125 - 500 bp) with terminal inverted repeats (TIR; 10-15 bp), high AT content, and high copy number. MITEs also share target site preferences that distinguish MITEs from other groups [16]. High-copy number characters facilitate MITEs in evading genomic control, thereby influencing the expression of phenotypes more freely [13]. In addition, the high copy number character and its stable inheritance encourage the development of MITE-based molecular markers [17].

HSP score obtained a percentage of 87.32 - 100% of the four primers used (Table 1). The chromosome map was determined based on the highest HSP value in chromosomes containing transposon genes detected on chromosomes 5, 14, 9, and 19 (Figure 5). AhMITE1 was found at the location of the A. *hypogaea* chromosome on chromosome-14 (Direction 14). AhTE0357, with an HSP score of 87.32% is located on the 5th chromosome (Arahy.05). AhTE0391 is located on chromosome-9 (Arahy.09). Meanwhile, the AhTE1317 sequence is positioned on chromosome-19 (Arahy.19). This shows that there are differences in the distribution of transposon genes in the overall peanut DNA and the ability of transposable element genes to move locations even in an autonomous manner. This situation can be indicated as the cause of the pattern patterns on lurik peanuts will not be uniform.

Various primer groups of MITEs have been explored for their association with phenotypic characters (marker-trait association-MTA). 30 AhTE markers have been confirmed to affect MTA, and some are associated with multiple traits. For example, the markers AhTE0474 and AhTE0189 were strongly associated with total AOA (total antioxidant activity) and TPC (total polyphenol content). Multiple traits are exemplified by AhTE1277 which is associated with two nutritional traits (fat content and carbohydrate content) and four morphological traits (leaf length, leaf width, plant height, and nutshell percentage [8]. In Brassica napus L. species, six markers were based on MITE (MK05, MK19, MK23, MK24, MK39, and MK55) correlated with five traits, namely oil content, glucosinolate content, erucic acid content, the weight of thousand seeds (WTS) and plant height [18]. Marker associations have not been explored further, which is a consideration for future research and development of striated peanuts.

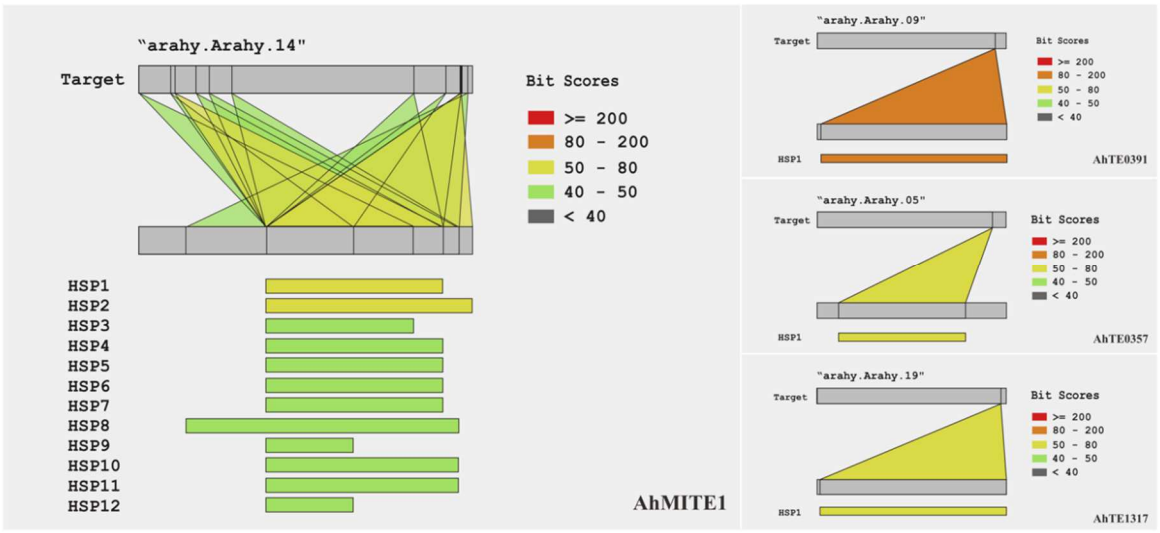

Fig. 5 HSP score and sequence primer location in the lurik peanut chromosome.

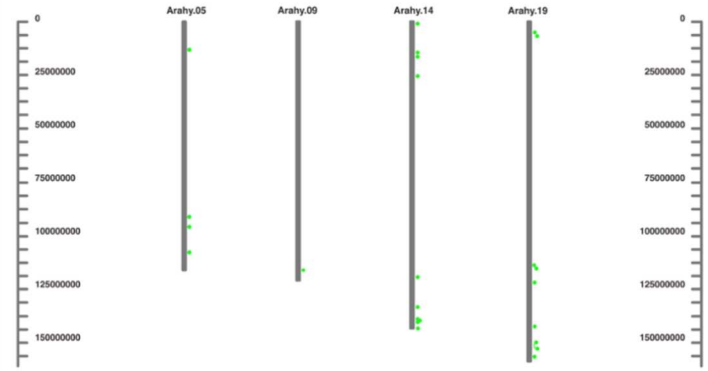

Fig. 6 CviT analysis of chromosomes 5, 9, 14 and 19.

Identification in the genomes of other plants, such as rice, has also been explored. Research in 2019 has successfully identified the presence of active MITE in rice. The active MITE is named miniature Jing (mJing) from the PIF/Harbinger superfamily. The evolutionary picture of rice between cultivated Asian (O. *sativa*), wild ancestor (O. *rufipogon*), and cultivated African (O. *glaberrima*) rice was due to mJing mobilization and variable copy number [19].

D. Chromosome Viewing Tools Analysis

Chromosome Viewing Tools (CViT) indicates a visualization of the location of the hit segment distribution in the chromosome. CViT helps visualize information across the entire genome. This method can be used to identify the density of repeating, duplication, or synteny segments and assess the grouping of a gene.

Based on Figure 6, the sequence of the consensus AhMITE1 primer found on chromosome 14 contained 10 hits (marked with a green dot). The ten hits were distributed at 283,901 bp to 142,674,125 bp. The consensus of the AhTE0357 primer located on the 5th chromosome found 4 hits distributed at 2,339,685 bp -128,238,618 bp. In the AhTE0391 primer, the HSP value from the majority query is on the 9th chromosome with one hit, which has a size of 115,678,432 bp. Based on the AhTE1317 primer sequence consensus, the highest hits were found as many as 11 segment hits distributed at positions 7,818,465 bp to 150,996,974 bp on the 19th chromosome.

CViT analysis can be used to visualize the whole genome and view the mapping of trait associations. However, because the targeted gene in this study is a transposon gene that can move autonomously, the results from CviT can only be used as a reference in predicting the location of segments in the whole genome. Allows the target gene of the transposon to change its position and location.

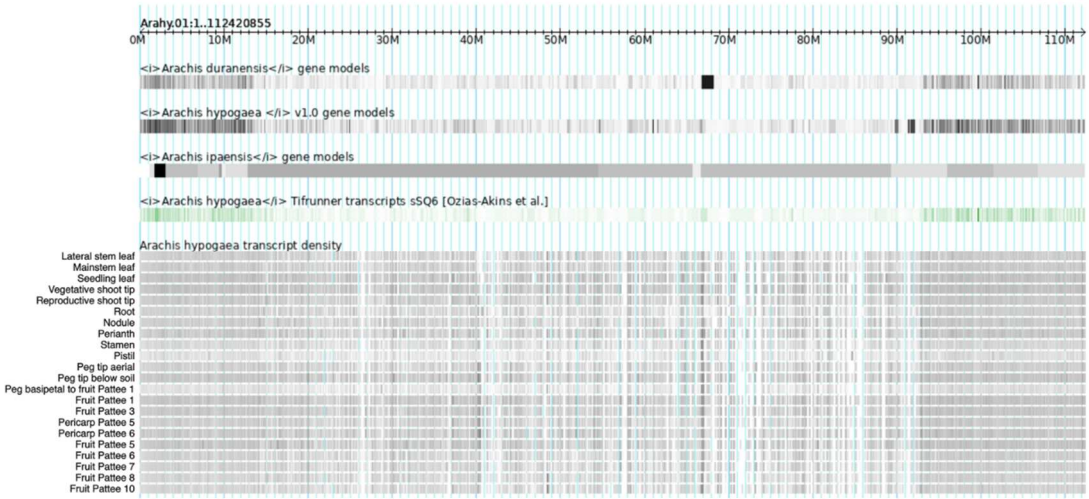

Fig. 7 Gene profile on the Arahy chromosome.19 peanut.

E. Gene Profile on Arachy Chromosome 19

The presence of the TE gene was explored based on the distribution of hits previously found on chromosome 19. The results show that at least 5 genes (Seed Pattee 5, 6, 7, 8, 10) on the chromosome affect the character of seeds in peanut plants (Figure 7). This strengthens the opinion that if the transposon activity is on the 19th chromosome, it is likely to affect the character of the plant's seeds.

The results of this gene profile analysis are very useful in the breeding process because by knowing a specific gene related to the location of the chromosome, an analysis of the success of the mutation can be carried out by observing the location of the gene being distributed that saves research time. Judging from various information related to transposons, transposable elements (TE) can transpose in the genome[20]. In the field of plant breeding that aims to improvise an agricultural product, TE has the potential to be an effective tool. Breeding approaches with TEs have also begun to be carried out, such as in conifers [21].

Most cultivated peanuts (*A. hypogaea* L.) are allotetraploids with AB genomes (2n = 4x = 40). It is the result of hybridization between the diploid A. *duranensis* (AA genome) and A. *ipaensis* (BB) which then underwent spontaneous chromosome duplication [22], [23]. However, the genomic in situ hybridization study explained that *A. hypogaea* originated from the ancestor of A. *monticola*[24]. Further investigations regarding the evolution of peanuts are currently being reviewed due to transposon activity [25]. Lurik peanuts with changes in the seed coat can be called mutants because the ancestors of peanuts A. *duranensis*and A. *ipaenensis* did not have any pattern on the seeds.

The previous use of AhMITEs transposon markers by [8] showed that activity of the transposon influenced the pattern on peanut seeds. Thus, this study's gene detection and sequencing process strengthens the opinion that TE is also inserted in the lurik peanut genome, which has a total genome size of 2.82 Gb of DNA. Peanut shell colour is highly correlated with total phenolic content. The colour has been proposed to be a biomarker for total polyphenol content and capacity and total anthocyanin. The use of the seed coat colour pattern as an indicator of the content of polyphenols and anthocyanins.

Flavonoids, a large group of anthocyanins, are important in maintaining physiological activity in peanuts. For example, it regulates the response to drought and oxidative stress [26]. Anthocyanin plays a role in staining the testa of lurik peanut seeds. The regulation may be caused by a group of flavonoid

biosynthetic pathway genes, including PAL, C4H, CHS, CHI, and homologue AtMYB111 [27]. The regulation of genes and accumulation of these metabolites allow the purple, pink and white staining of the peanut testa. Anthocyanins can benefit human health, such as antineoplastic, anticarcinogenic, antiatherogenic, antiviral, and anti-inflammatory effects, and increase immunity [28]–[30].

IV. Conclusion

Based on the data collected in this study, transposon genes are amplified in the *Arachis hypogaea* miniature inverted transposable elements (AhMITEs) group primers. The four primers (AhMITE1, AhTE0357, AhTE0391, and AhTE1371) have a linear relationship between Lurik and Garuda peanut in terms of the presence of the transposon gene. The blast results show the dominant location of the gene, although the gene may be randomly located on another chromosome of the peanuts.

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