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DEVELOPMENT OF LECTIN GENE-BASED MARKERS ASSOCIATED WITH ANTICANCER BIOACTIVE COMPOUND IN RODENT TUBER MUTANT (*TYPHONIUM FLAGELLIFORME*)

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SUMMARY

Rodent tuber (*Typhonium flagelliforme* Lodd.) has anticancer bioactive compounds, including beta-sitosterol, stigmasterol, octadecanoic, and hexadecenoic acids. Its mutant plants showed a higher cytotoxic effect on breast cancer cells than its wild-type plants. In rodent tuber mutants, the gene-based molecular markers associated with anticancer compounds have not reached identification. Designing the primer pairs at SNP sites observed in rodent tuber mutant lectin genes led to the latest study developing SNAP molecular markers. The exon part region of previous lectin gene sequences at 500 bp long also underwent analysis. In such a sequence, a three-point mutation helped analyze the amino acid transformation. A successful design of a pair of primers based on non-synonymous SNP sites was specifically for SNP sites that cause variations in amino acids. A non-synonymous SNP at the base of 183 bp changes threonine to arginine. Lec183 distinguished rodent tuber mutant plants better from their wild type in the amplification. The Lec183 marker detected a lectin gene SNP's G allele and analyzed rodent tuber plants, which produced an amplification that determined the G allele level. The Lec183 is an effective marker for selecting rodent tuber-lectin mutation sites in large populations. This way helped obtain the rodent tubers with the highest anticancer bioactive compound more precisely and rapidly.

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Keywords: Rodent tuber (*Typhonium flagelliforme* Lodd.), lectin gene, anticancer bioactive compound, specific primer design, SNAP, allele-specific marker

Key findings: Specific molecular markers designed with gene-based markers associated with anticancer bioactive compounds in rodent tuber (*Typhonium flagelliforme* Lodd.) plants are yet for study. Through primer lec183, the specific alleles in lectin genes can help differentiate the mutant and wild-type plants. The SNAP marker based on the lectin gene sequence could probably improve the accuracy of the selection of lectin anticancer compounds in rodent tuber mutant plants.

INTRODUCTION

Rodent tuber (*Typhonium flagelliforme* Lodd.), a medicinal plant, possesses potential anticancer properties. In past studies, a reported analysis on rodent tuber plants indicated the presence of bioactive compounds, including stigmasterol (Sianipar *et al.,* 2016). As a result of inducing apoptosis and cell death, this bioactive compound also inhibits breast cancer cell propagation. Previous studies reported that rodent tuber plants' development is ongoing and, with time, may gain better anticancer potential (Sianipar *et al*., 2015a, b, 2020b; Sianipar and Purnamaningsih, 2018a). Through involving gamma-ray irradiation, several studies have produced superior mutants of rodent tuber with higher cytotoxic effects against cancer cells compared to wild types (Sianipar *et al*., 2017; Sianipar and Purnamaningsih, 2018a; Sianipar *et al*., 2019). According to Sianipar *et al*. (2022a), the Bogor rodent tuber accession with four clones emerged superior to mutants with high anticancer content (HAC).

The Bogor rodent tuber accession showed a 19.113 mg/mL biological anticancer activity against breast cancer cells (Sianipar *et al*., 2019). By fraction with ethyl acetate, the mutant plants exhibited biological activity (1.06 mg/mL) against breast cancer cells (Sianipar *et al*. 2020b). Bogor accessions, viz., KB 6-3-3-6 and KB 6-1-2, have received the Plant Variety Protection (PVT) rights from the Ministry of Agriculture of the Republic of Indonesia (Sianipar *et al*., 2020c). Previously, several bioactive compounds in rodent tuber mutant plants have occurred, such as hexadecenoic acid ethyl ester, stigmasterol, squalene, and octacaine compounds (Purnamaningsih *et al.*, 2018). Moreover, several rodent tuber mutant clones possessing anticancer bioactive compounds have undergone molecular analysis using Random Amplified Polymorphic DNA (RAPD) molecular markers (Sianipar *et al*., 2015a, 2017; Sianipar and Purnamaningsih, 2018b). However, the RAPD markers' application could not distinctly differentiate the rodent tuber mutant from the wild-type genotypes. Therefore, in the prevailing study, the differences in nucleotide sequences of lectin genes present in the rodent tuber mutant plants and the wild type incurred scrutiny to develop specific molecular markers related to the anticancer compound.

In the genome, SNPs replace one base. SNP markers often aid in identifying specific traits or determining lineages and populations (Emanuelli *et al*., 2013; Inghelandt *et al*., 2010). SNP markers associated with anticancer genes can benefit medicinal plant selection (Wen-Ru *et al*., 2020; Muflikhati *et al*., 2023). Several plants have successfully obtained specific gene-based molecular markers (SNAP markers), such as *Phalaenopsis orchids* based on the *Pto* gene (Sukma *et al*., 2021), *Theobroma cacao* L. developed on the *Catalase-1* gene (Tarigan *et al*., 2021), and rice based on the rice-eating-quality coding gene (Lestari and Koh, 2013). One nucleotide polymorphism (SNP) has appeared in the *GmNARK* gene, controlling autoregulation of nodulation in the wild-type cultivar Sinpaldalkong 2 and its super-nodulating mutant, SS2-2 (Kim *et al*., 2005). SS2-2 was the only sample that showed a band using the A-specific primer pair, whereas Sinpaldalkong 2 was the only sample that produced a band using the T-specific primer pair (Kim *et al*., 2005). Supernodulation genotyping used both complementary PCRs applying allele-specific primer pairs.

The successful comparison of rodent tuber mutant and wild-type plants identified three-point mutations in a 500 bp of the lectin gene (Sianipar *et al*., 2022a). This information convinces us to develop specific molecular markers at the mutation sites associated with anticancer compound production at the lectin gene. SNAP markers can also help determine cultivars of medicinal plants with desired traits, such as anticancer activity. This study aimed to develop and investigate a molecular marker for SNAP based on SNP sites that detect mutant lectin genes. This marker will differentiate the specific alleles of rodent tuber mutant and wild-type plants. Therefore, these markers can also identify genes involved in anticancer compound biosynthesis.

MATERIALS AND METHODS

Plant genetic material

Plant specimens for this study came from the collection of Bina Nusantara University, Jakarta, Indonesia, in 2021. Molecular analysis ensued on young fresh leaves' tissue from each sample. The genetic material used in this study comprised two clones of *Typhonium flagelliforme* Lodd. The mutant plants surfaced by irradiating with gamma rays at a dose of 6 Gy (Sianipar *et al*., 2018). One Bogor accession of mutant rodent tuber plants and wild type became samples for amplification analysis using SNAP lectin markers.

Plant sequence material

Rodent tubers wild type (KB-control_61F) and four mutant clones were trials to derive lectin gene sequences. The bioactive compounds, with sequence lengths around 500 bp, reached classification as high anticancer (KB-6-2-5-3_61F and BM-8-2_61F) and low anticancer (KB-6-1-1-2_61F and KB-6-2-6-3_61F) categories (Sianipar *et al*., 2022a). The sequence helped verify the lectin gene sequence, SNP analysis, and SNAP marker design. The detection of an SNP site arose in the lectin gene sequence, and the lectin SNAP marker analyzed the amplification of the rodent tuber mutant and wild type of Bogor accession.

DNA isolation

Total genomic DNA isolation from young fresh leaves' tissues of the rodent tuber mutant plant with the wild type progressed according to the CTAB-based extraction method, as described by Calderón-Cortés *et al*. (2010). Crushing the leaves with a mortar and liquid nitrogen proceeded with the mixing with 1000 µL of extract buffer. Mixtures placed in 2 mL microtubes ensued for incubation in a water bath for 45 min and homogenized every 15 min. The next step was the addition of 800 µL of chloroform isoamyl alcohol solution in the tube. Following homogenization, the sample's centrifugation took 15 min at 13,500 rpm. After transferring 400 µL of supernatant to a 1.5 µL tube, 500 µL of cold isopropanol solution and 0.1x volume of sodium acetate supernatant followed. With the tube firmly closed, DNA strands underwent examination. Supernatant collection with a micropipette followed after the samples' incubation for 10 min at room temperature. Mixing the TE buffer with the remaining DNA pellets continued at room temperature. Following RNase addition was the slow mixing of 1/100 volume of solution, and 30 min of incubation transpired at 37 °C. In diluting the sodium acetate solution, adding a volume of 1/10 of the DNA solution continued careful stirring. The samples incurred treatment with 600 µL of 95% ethanol solution to precipitate DNA. Incubation at -20 °C for 45 min followed the slow merging of these samples. At 13,500 rpm for 10 min, MiniSpin plus (EU-IVD) (Eppendorf, UK) was used to centrifuge the solution to remove the supernatant. A 200 µL solution of TE buffer was added to the dried DNA after it had dried. An agarose gel with a 1.4% concentration of DNA aids the analysis of the isolated DNA samples. The quantity and quality of the isolated DNA attained subsequent measuring using a Nanodrop Spectrophotometer 2000 (Thermo Scientific TM, USA).

Characterization of lectin gene sequences and amino acid translation

The lectin gene sequence of rodent tuber from a previous study's investigation (Sianipar *et al*., 2022a) underwent comparison with other lectin gene sequences available in the National Center for Biotechnology Information (NCBI) database to determine its similarity using the Geneious software version 8.1.6 (Biomatters, Ltd). Exporting the sequence alignment results of lectin genes into MEGA software version 11 constructed a phylogenetic tree. The constructed phylogenetic tree showed a maximum likelihood approach and 1000 bootstrap iterations. Identification commenced to assess the similarity of lectin genes in rodent tuber with lectin genes in other plants in the database. The SNP sites incurred analysis for amino acid changes. A synonymous mutation occurs at an SNP site where changes in amino acids have transpired, and a non-synonymous mutation occurs at a site where no alterations happened with amino acids.

SNAP primer design

SNAP markers were designed for SNP sites with an alternative bi-allelic allele and causing amino acid changes using the WebSnapper software (http://ausubellab.mgh.harvard.edu). A PCR product size with a range of 325–375 bp, primer concentration of 5 µM, primer size range of 20–30 bp, and primer melting temperature of 55 °C–65 °C were used in designing the SNAP primer at 113 bp (C/T). At 183 bp (G/C), the SNAP primer design parameters include a PCR product size with a range of 225–275 bp, primer concentration of 5 µM, primer size range of 20–30 bp, and primer melting temperature range of 62 °C–70 °C. Each primer set incurred probing to determine whether at least three primers were evident - two forward primers (reference and alternative) and one reverse primer. Using PCR amplification helped genotype a single SNP locus. The first reaction used a pair of reference (Ref) and reverse (Rev) primers, with a pair of alternate (Alt) and reverse (Rev) primers employed in the second reaction.

The SNAP marker attained a URL assignment for each SNP site on the WebSNAPPER page. The selection of a standard genotype depended on the alleles that distinguish the mutant and wild-type plants. A wild-type allele served as a reference, and the mutant allele as an alternative. The SNAP primers were designed for the SNP position already identified in the lectin gene (Table 1). This resulted in the development of two unique forward primers designed to detect reference and alternative SNPs. In PCR analysis, one of the two forward primers received pairing with a reverse primer.

Table 1. Analysis of SNP sites in the lectin gene of rodent tuber mutant and wild type plants.

Gene	SNP position	SNP variations	Type of change in amino acids	Changes in amino acid residues
	59	[AAC/GGC]	Non-synonymous	Asparagine-Glycine
Lectin	183	[ACG/AGG]	Non-synonymous	Threonine-Arginine
	211	[CCA/CCG]	Synonymous	Proline

Amplification and visualization of the SNAP marker on SNP in lectin gene

SNAP markers sustained modification to identify the rodent tuber mutants and wild-type plants. Each sample had a prepared total reaction volume of 10 µL containing 2-5 ng of 2 µL DNA template, 1x MyTaqTM HS Red Mix (Bioline, UK, as much as 5 µL; primers with each 0.5 µL), and ddH2O. Following PCR protocol, Palm-Cycler Thermal Cycler CG1-96 (Corbett, USA) helped perform the following PCR profile: 94 °C denaturation for 4 min, 94 °C denaturation for 30 s, 63 °C annealing for 30 s for lec183 primer, 65 °C annealing for 20 s for lec113 primer, followed by 72 °C extension for 30 s. A final 5-minute extension cycle proceeded at 72 °C to conclude the PCR

reaction. After electrophoresis on a 2% agarose gel in 1x TBE buffer (Tris Borate EDTA) for 85 min, the DNA products received staining with Florosafe DNA stain (1st BASE) and attained visualization using UV fluorescence gels (Syngene, USA). In addition to detecting specific alleles, the amplified PCR products detected rodent tuber mutant and wild-type plants. Utilizing the PCR reaction amplified the lectin gene mutation, with the resulting fragments separated using electrophoresis. Amplification results in visuals showing bands indicating the presence of different alleles in mutants and wild-type clones. The bands allowed for comparing the alleles between the mutants and wild-type clones. The bands also provided a quantitative measure of the alleles present. This information helped to analyze the impact of the mutations on the phenotype of the clones.

RESULTS AND DISCUSSION

DNA quantification and sequence verification of lectin gene

The DNA extracted from rodent tuber (*Typhonium flagelliforme* Lodd.) leaves' verification used a spectrophotometer NanoDrop and an agarose gel electrophoresis. DNA quantity measurements analysis showed that wild-type rodent tuber and mutant samples had DNA concentrations of 10–15 ng/µL. The DNA bands obtained were reproducible and extremely clear. It demonstrates that the DNA extracted from rodent tuber mutant and wild type has better genomic DNA band integrity. DNA appeared to be optimal based on the results obtained. According to Sukma *et al*. (2021), DNA was optimal in a PCR mixture with 12.5 µL and 4 µL concentrations. Kim *et al*. (2012) recommended using 50 ng of template DNA in a PCR mixture for a reaction volume of 20 µL. SNP markers incur high recommendations for low-concentration samples. SNPs are a type of molecular marker that accurately detects minimum amounts of DNA. They are especially beneficial for analyzing rare alleles or mutations. SNPs can also help detect two or more alleles at a single locus.

BLAST analysis on the NCBI database, obtained from previous research, progressed on rodent tuber wild-type sequence (KB-kontrol_61F) (Sianipar *et al*., 2022a). In the database, several species have come out with lectin gene sequences similar to the sequence from *Typhonium flagelliforme*, *Pinellia ternata*, *Typhonium divaricatum*, and *Sauromatum venosum* (Table 2). Rodent tuber lectin gene sequences had the highest similarity values with partial CDS lectin genes of *T. flagelliforme* (with accession MK90484.1) and complete CDS mRNA lectin genes of *Pinellia ternata* (with accession AY191305.1), with individuality percentages of 95.49% and 89.92%, respectively. An assumption also stated that the highest similarity observed between these plants was because of their relationship to the same family as rodent tubers, Araceae. Multiple sequence alignment (MSA) analysis helped determine exon regions between these two plant accessions using the complete CDS mRNA lectin gene sequence of *Pinellia ternate* (Figure 1). Additionally, it has a high similarity (89.92%) to rodent tuber, and this was a complete CDS mRNA sequence, making it suitable for MSA analysis.

For multiple sequence alignment, the six lectin gene sequences selected included *Pinellia ternata* lectin accession AY191305.1, rodent tuber wild-type lectin accession KB-kontrol_61F, and four mutant clones procured from previous studies (Sianipar *et al*., 2022a), achieved a label as high anticancer (KB-6-2-5-3_61F and BM-8-2_61F) and low anticancer (KB-6-1-1-2_61F). The MSA findings revealed that all the rodent tuber lectin genes encode the exonic sequences. The MSA results further indicated that, in rodent tuber plants, the lectin gene contains one exon, indicating no gaps in all the aligned sequences. The promising results are highly analogous to the findings of Aleksandar *et al*. (2006), who discovered that the lectin gene did not contain introns in *Pisum sativum* L., *Victa* sp., and *Victa faba* L. plants.

This study successfully authenticated the gene sequence of rodent tuber lectin, as compared to a previous study performed by

Table 2. Similarity analysis between the lectin gene in rodent tuber mutants and the lectin gene in different plant accessions in the NCBI Genbank.

Accession	Gene name	Species name	E value	Max identity (%)
MK904841.1	*lectin,* partial CDS	*Typhonium flagelliforme*	1e-167	95.49
AY191305.1	*lectin* mRNA, complete CDS	*Pinellia ternata*	1e-132	89.92
EF194099.1	mannose-binding tuber *lectin* mRNA, complete CDS	*Typhonium divaricatum*	5e-131	90.03
KX132811.1	*lectin* mRNA, complete CDS	*Sauromatum venosum*	2e-119	88.14

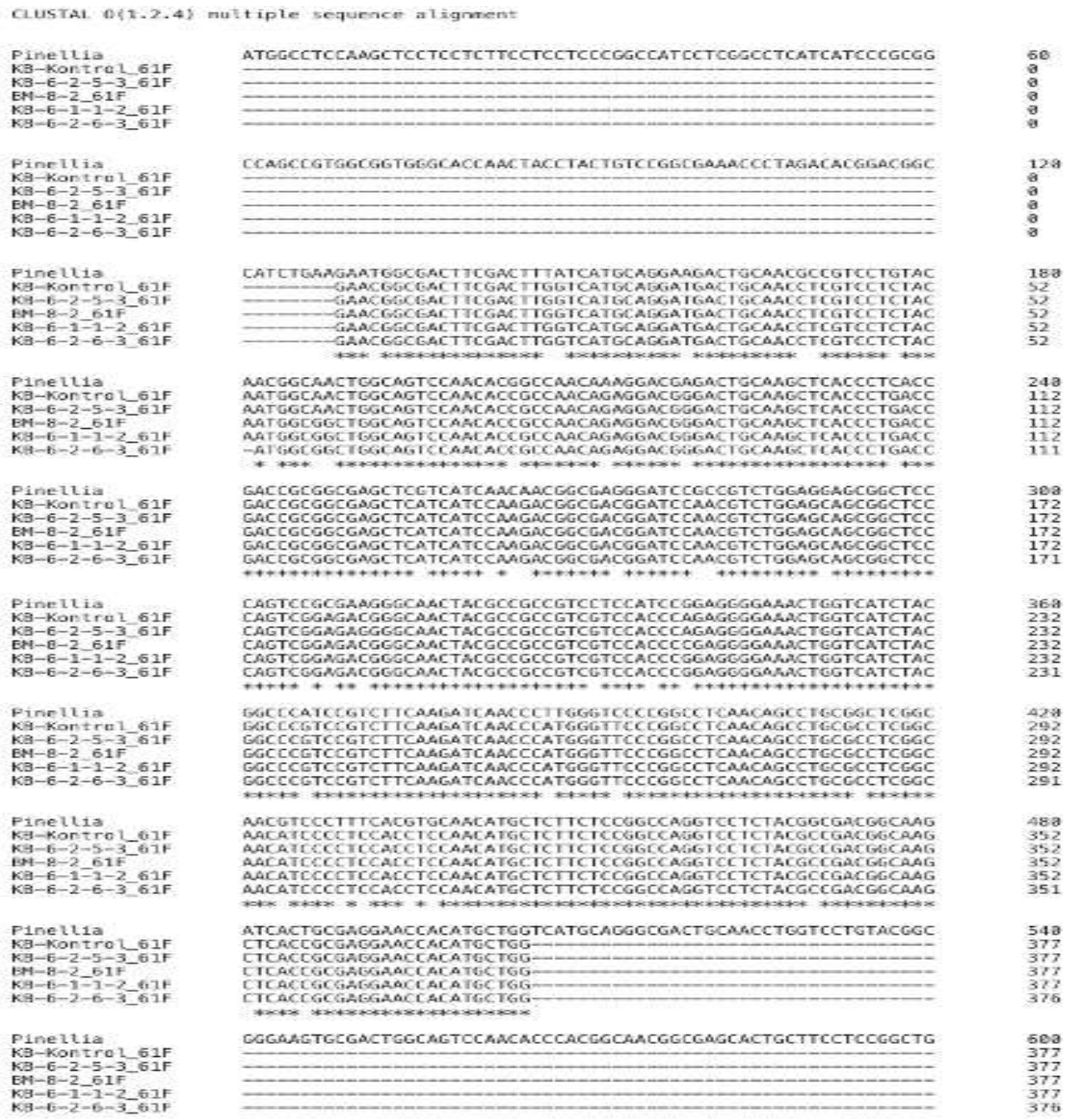

Figure 1. Multiple sequence alignment of lectin gene sequences in rodent tuber mutants and wild type with *Pinellia ternata* mRNA complete CDS lectin gene sequence.

Sianipar *et al*. (2022a). However, it remains a challenge for other researchers to develop more lectin information in the future (Hafez and Shati, 2016; Yassin *et al*., 2019; Sianipar *et al*., 2022a). Heuristic algorithms estimated the significance of BLAST matches between biological sequences. Analyzing the relationships among lectin gene sequences in other species with more lectin genes allows for obtaining these study results.

Characterization of rodent tuber lectin gene sequences

Pinellia ternata was the first plant identified with a lectin gene (Yao *et al*., 2003). A lectin gene with a size of 500 bp also had successful detection in the rodent tuber plant (Sianipar *et al*., 2022a). BLAST pairwise alignments in the NCBI database help construct a phylogenetic tree for the lectin gene (Figure 2). Based on the similarity to related genes in other plants, the phylogenetic tree led to grouping the individual rodent tuber plants (Chen *et al*., 2016).

Several lectin genes have close relations to those of *T. flagelliforme* (MK904841.1), *T. divaricatum* (EF194099.1) mannose-binding lectin mRNA, *Sauromatum venosum* (KX132811.1) lectin mRNA complete sequences, *Remusatia vivipara* (EU924066.1), *Arisaema lobatum* (AY557617.1), and *Pinellia ternata* (AY191305.1) (Table 2, Figure 2). The rodent tuber lectin gene sequence and *T. flagelliforme* lectin gene partial CDS have a distance of 0.05. It also reflects the variations between the query and NCBI database sequences (Kumar *et al*., 2016). Rodent tuber wild-type plants were responsible for the tiny difference in gene sequence relatedness. These species exhibited similar characteristics as both species belong to the family Araceae.

Amino acid transformation based on lectin gene SNP

Based on nucleotide bases that distinguish rodent tuber genotypes helped select the SNP sites. In rodent tuber mutants grouped into high anticancer, the SNP at position 183 bp has a C (cytosine) base, but, in rodent tuber wild-type genotypes, the SNP has a G (guanine). Sequencing results of the rodent tuber lectin gene were means to read the location of the SNP (Sianipar *et al*., 2022a).

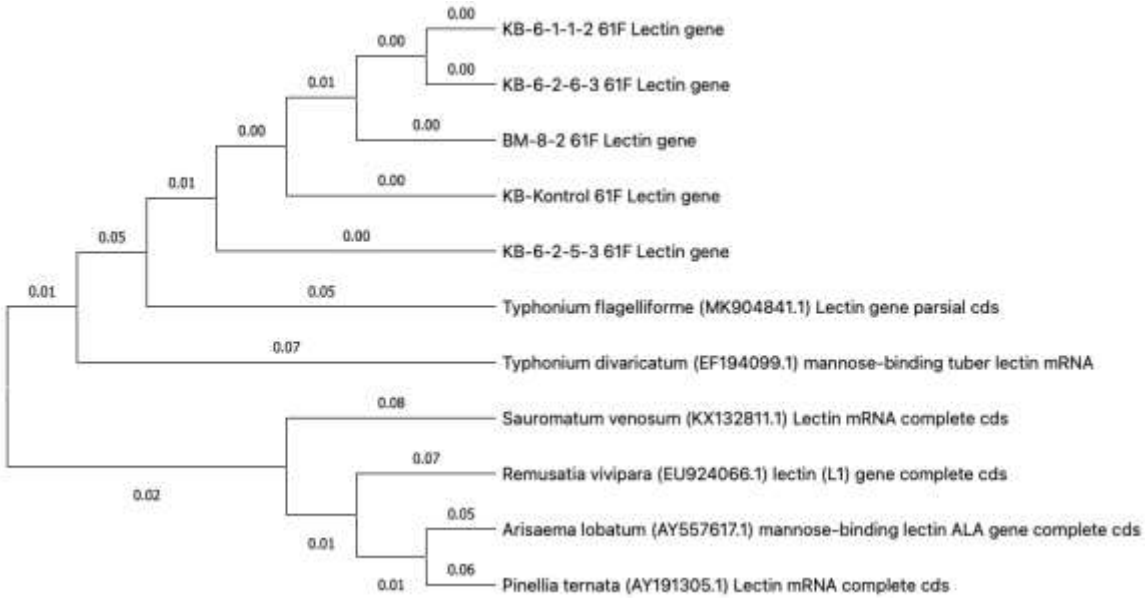

Figure 2. Analysis of the lectin gene phylogeny. Rodent tuber lectin gene sequences were aligned with the NCBI database using BLAST pairwise alignments (Sianipar *et al.,* 2022a).

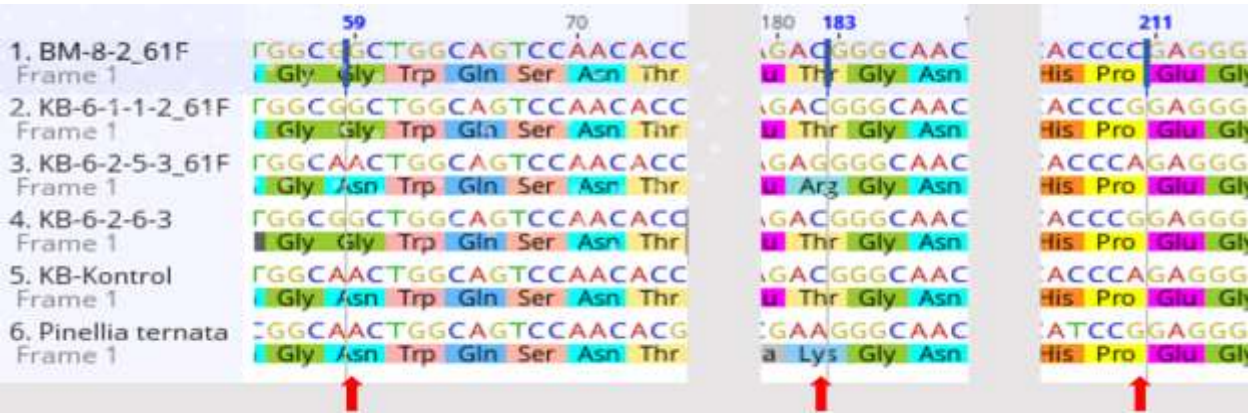

Figure 3. Amino acid translation of the lectin gene sequence of rodent tuber wild type, four rodent tuber mutant clones, and the complete CDS sequence of *Pinellia ternata*.

A sequence of four amino acids derived from rodent tuber wild type, the mutant clones with high and low cancer-fighting ability, and *Pinellia ternata* complete CDS sequence has progressed to execute amino acid transformation (Figure 3). Translating amino acids based on start codon alignment includes lectin sequences from *P. ternata*. The rodent tuber sequence revealed an unknown start codon position (ATG). Furthermore, the *P. ternata* lectin gene sequence used in sequence similarity has an amino acid-coding mRNA. The *P. ternata* lectin gene sequences were also beneficial for amino acid conversion analysis.

After adjustment of the start codon in the rodent tuber lectin gene sequence, the two-point mutations were visible (Figure 3). Synonymous and non-synonymous SNPs can be distinguishable by their amino acid changes. A synonymous SNP is a variation that does not alter the amino acid translation process, and a non-synonymous SNP changes the transformation process (Studer *et al*., 2013). A different amino acid residue was evident at position 183 of the SNP with C/G nucleotide variants. Mutant plants may express lectin genes differently when changes occur in amino acid composition at the 183 bp SNP.

In rodent tuber mutant and wild types, the identified lectin non-synonymous SNPs gene sequences occurred at 59 bp and 183 bp, respectively (Table 2). The base at 59 bp changed from AA to GG as part of a substitution at the GGC codon that codes for glycine due to point mutation, while codon AAC encodes asparagine. At 183 bp, the G base substitution at the AGG codon encodes arginine, and the C base at the ACG codon encodes threonine. It is also possible to substitute G codons for CCA codons at point mutation 211 bp by replacing CCA codons with G codons. However, based on these findings, there was no modification in amino acids at site 211 bp.

The present study also identified a non-synonymous SNP site at 183 bp that could become a primer design site. A bi-allelic SNP reached detection at this site from G to C (guanine to cytosine). A non-synonymous SNP replaced the threonine-arginine. As an amino acid, threonine has an uncharged R group (insoluble in water), while the amino acid arginine has a positive charge and contributes to the formation of proteins. The results suggested that rodent tuber mutants could contain different anticancer lectin compounds than their wild type. The final product of a protein can have influences from variations in the amino acids. Tarigan *et al*. (2021) reported that non-synonymous SNPs that change the amino acid leucine to phenylalanine could influence Theobroma cacao's phenotype. These variations may significantly impact the protein structure and its biological activity, which can result in function gain/loss. Furthermore, the effects of amino acid substitutions can also

help to provide insight into the potential functional consequences of variations in the genome.

SNAP primer design

The SNAP marker's usage detected mutants and wild-type alleles based on nucleotide base substitutions in the lectin gene. The molecular SNAP marker's construction continued from the SNP site identified in the lectin gene. The lectin gene SNAP primers' design utilized the allele distinguishing wild-type sequences at the guanine (G) base from the mutant sequences at the cytosine (C) base.

In rodent tuber mutants, the molecular markers for anticancer compounds (in this case, the lectin gene) have not reached identification. Genetic approaches like SNAP markers developed based on bi-allelic SNPs are highly relevant in identifying molecular markers associated with lectin properties. Kwong *et al*. (2016) emphasized the importance of eliminating indels and non-biallelic variants during the candidate selection of SNPs from discovered variants. These criteria would make it possible to select SNP sites suitable for various genetic analyses, including SNAP and evolutionary studies (Xu and Bai, 2015; Tarigan *et al*., 2021). Based on the SNP sites of the rodent tuber sequence (Sianipar *et al*., 2022a), the selection made for the flanking region of the SNP site serves as the primer design region, useful in amplification and determination of the target alleles (Islam *et al*., 2015).

Amplification of SNAP marker on SNP in lectin gene

In the presented study, the lectin gene-based SNAP primer's design succeeded, amplifying both rodent tuber mutant and wild-type plants. Primer lec113 (Forward_C: GACTGCAACCTCGTCCTCTAGAAC; Reverse: CATCACCAGCATGTGGTTCCT; Forward_T: GACTGCAACCTCGTCCTCTAGAAT) produced a specific band at a size of 351 bp, found consistent with the product size during primer production (Figure 4). Rodent tuber mutant and wild-type clones ably amplified the Reference and alternate alleles using these primers. These results indicated that the lec113 primer did not differentiate the rodent tuber mutant clones and wild types.

The PCR amplification using primer lec183 (Forward_C: CAGCGGCTCCCAGTCGGAAAC; Reverse: GTGGTTCCTCGCGGTGAGCTTG; Forward_G: CAGCGGCTCCCAGTCGGTGAG) produced an amplicon with 208 bp, was also consistent with the product size during primer production (Figure 5). It was also possible to distinguish rodent tuber mutant plants from their wild type using this primer. Alternate alleles' amplification in the KB2 mutant clone (BM 8-8)

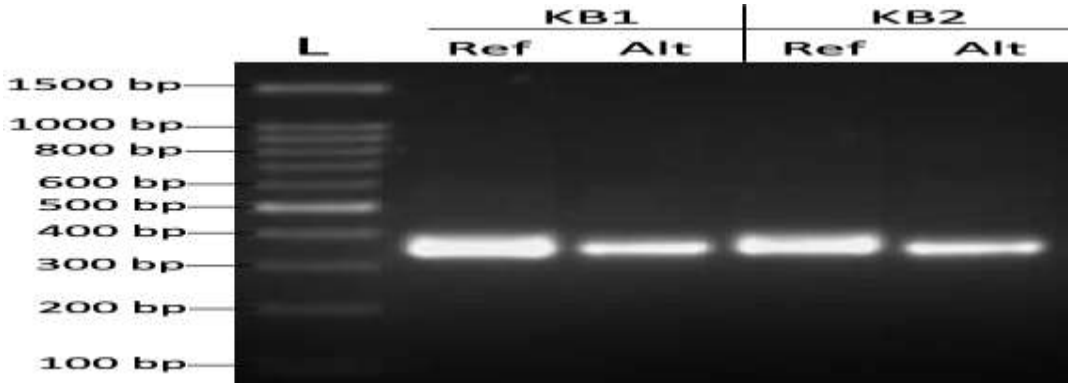

Figure 4. PCR amplification with lec113 primer. L: DNA ladder size. (KB1) KB Control; (KB2) BM 8-8. Ref: Reference. Alt: Alternate.

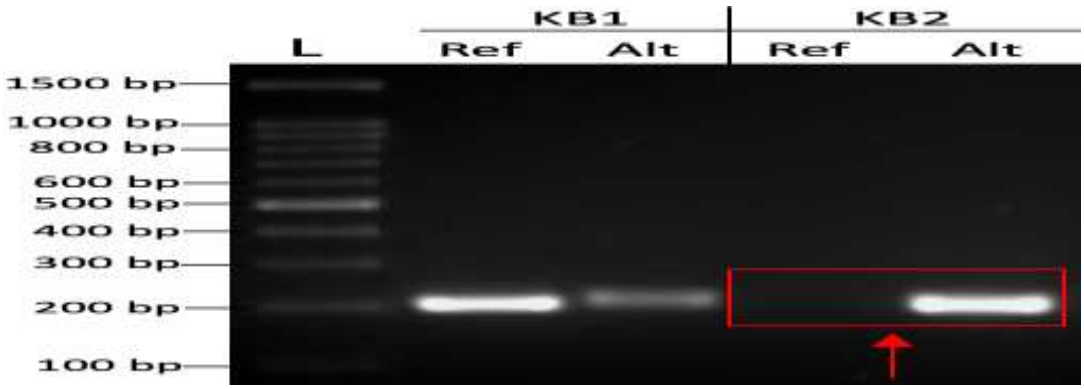

Figure 5. PCR amplification with lec183 primer. L: DNA ladder size. (KB1) KB Control; (KB2) BM 8-8. Ref: Reference. Alt: Alternate.

could occur; however, the reference alleles did not amplify (red arrow). These results revealed that the mutant clone has a different allele versus the wild types. In contrast to the wild type, where both reference and alternate alleles were present, the BM 8-8 clone has only one allele (guanine base), whereas the wild type has both bases (cytosine and guanine). Homozygous traits were also in BM 8-8 mutant clones but were also different from the wild type by having heterozygous traits.

The lec113 primer cannot distinguish the rodent tuber mutant and the wild-type plants. Lec113 primer also did not match the rodent tuber sequence at the 113th bp point, primarily due to its mismatch. Sianipar *et al*. (2018a) have also modified the mutant's properties by gamma-ray irradiation. Rodent tuber mutant and wild-type plants attained successful differentiation with the lec183 primer. This primer identified the BM 8-8 based on the alternate allele (guanine base). The lec183 primer causes this phenomenon because it matches the sequence of the mutant clone at 183 bp. A primer (lec183) resulted from the two types of primers designed to determine the clones of rodent tubers.

As reported in past studies, RAPD markers have detected variations in rodent tuber mutants (Laurent *et al*., 2015; Sianipar *et al*., 2022b). Based on the prevailing results, significant differences were visible between rodent tuber mutants and wild types. According to SNAP marker analysis, variations in rodent tuber mutant with wild type had reports based on lectin gene analysis. Variations in lectin gene nucleotide sequences lead to specific and codominant SNAP markers. The SNAP markers related to CAT1 genes have succeeded in identification in cocoa plants in association with an SNP at position 71 (C/T allele variation) (Tarigan *et al*., 2021). Sukma *et al*. (2017) have also studied the molecular mechanisms linking disease resistance to genes responsive to disease (Pto and Chitinase [Chi] genes) and developed the SNP-specific markers for screening resistant Phalaenopsis genotypes.

Based on various methods, SNPs have recently emerged as one of the most widely studied markers. Guajardo *et al*. (2020) investigated phylogenetic analysis, SNP distribution, and gene function prediction to identify the *Prunus* germplasm rootstocks by genotyping-by-sequencing (GBS). Several SNPs have also achieved identification in the exon region (Wu *et al*., 2020). An amplification procedure ensued with primers that originated from exons. Therefore, it is vital to note that the sequence originating in the exon region will significantly impact the transformation of amino acids, profoundly affecting the protein structure and its function.

According to Sianipar *et al*. (2022a), gamma-ray irradiation caused variations due to point mutation in the lectin gene sequence, resulting in differences between rodent tuber mutants and wild types. Gene sequence mutations can affect amino acid translation and, ultimately, affect the final products of

compounds (Kimball and Jefferson, 2006). A point mutation discovery has surfaced at 183 bp in the rodent tuber mutant plants. The lec183 marker helped to differentiate it from wild-type plants. A significant increase in G levels appeared in rodent tuber mutants compared with wild-type plants. It suggests that the point mutation at 183 bp can significantly impact the production of anticancer compounds.

CONCLUSIONS

All the lectin gene sequences succeeded in detection and verification as part of the exon sequence. Based on the non-synonymous SNPs, a pair of SNAP primers' design was successful. With specific alleles of the lec183 primer pair, Forward_C: CAGCGGCTCCCAGTCGGAAAC; Reverse: GTGGTTCCTCGCGGTGAGCTTG; Forward_G: CAGCGGCTCTCCCAGTCGGTGAG, it was possible to distinguish the mutant and wild-type plants. The SNAP molecular marker lec183 detected the 183rd SNP of the lectin gene intended for detecting the G allele. A lec183 marker distinguishes rodent tuber mutants from the wild type based on the specific lectin gene. Overall, the SNAP marker lec183 could be advantageous for accurately identifying rodent tuber mutants. It would also provide a valuable tool for determining the mutant plants through specific lectin genes. Further research is necessary to confirm the role of this mutation in altering G allele levels and its potential implications for producing anticancer compounds. In addition, further studies could uncover the full range of effects that the point mutation at 183 bp may have on plant physiology.

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REFERENCES

Aleksandar M, Branko Ć, Katic S, Katic S, Đura K (2006). Importance of annual forage legumes in supplying plant proteins. *A Period Sci. Res. F. Veg. Crop*. 42(1): 91–103.

Calderón-Cortés N, Quesada M, Cano-Camacho H, Zavala-Páramo G (2010). A simple and rapid method for DNA isolation from xylophagous insects. *Int. J. Mol. Sci*. 11(12): 5056–5064. https://doi.org/ 10.3390/ ijms11125056.

Chen S, Deng LY, Bowman D, Shiau JJH, Wong TY, Madahian B, Lu HHS (2016). Phylogenetic tree construction using trinucleotide usage profile (TUP). *BMC Bioinformatics* 17(Suppl 13). http://dx.doi.org/10.1186/s12859-016-1222-3.

Emanuelli F, Lorenzi S, Grzeskowiak L, Catalano V, Stefanini M, Troggio M, Myles S, Martinez-Zapater JM, Zyprian E, Moreira M, Grando MS (2013). Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. *BMC Plant Biology*. 13(1): 1–17. doi:10.1186/1471-2229-13-39.

Guajardo V, Solís S, Almada R, Saski C, Gasic K, Moreno MÁ (2020). Genome-wide SNP identification in Prunus rootstocks germplasm collections using genotyping-by-sequencing: Phylogenetic analysis, distribution of SNPs and prediction of their effect on gene function. *Sci. Rep*. 10(1): 1–14. https://doi.org/10.1038/s41598-020-58271-5.

Hafez EE, Shati A (2016). The potential anticancer action of lectin extracted from *Pisum sativum* against human hepatocellular carcinoma cell lines. *Egypt J. Hosp. Med*. 65: 683–692. https://doi.org/10.12816/ 0033783.

Inghelandt DV, Melchinger AE, Lebreton C, Stich B (2010). Population structure and genetic diversity in a commercial maize breeding program assessed with SSR and SNP markers. *Theoretical and Applied Genetics*. 120(7): 1289–1299. doi:10.1007/s00122-009-1256-2.

Islam MS, Thyssen GN, Jenkins JN, Fang DD (2015). Detection, validation, and application of genotyping-by-sequencing based single nucleotide polymorphisms in upland cotton. *Plant Genome* 8(1). https://doi.org/ 10.3835/plantgenome2014.07.0034.

Kim JS, Jang HW, Kim JS, Kim HJ, Kim JH (2012). Molecular identification of *Schisandra chinensis* and its allied species using multiplex PCR based on SNPs. *Genes and Genomics* 34(3): 283–290. https://doi.org/10.1007/s13258-011-0201-3.

Kim MY, Van K, Lestari P, Moon JK, Lee SH (2005). SNP identification and SNAP marker development for GMNARK gene controlling supernodulation in soybean. *Theor. Appl. Genet.* 110: 1003-1010. https://doi.org/10.1007/ s00122-004-1887-2.

Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for Bigger Datasets. *Mol. Biol. Evol*. 33(7): 1870–1874. https://doi.org/10.1093/MOLBEV/MSW054.

Kwong QB, Teh CK, Ong AL, Heng HY, Lee HL, Mohamed M, Low JZ Bin, Apparow S, Chew FT, Mayes S (2016). Development and validation of a high-density SNP genotyping array for African oil palm. *Mol. Plant*. 9(8): 1132–1141. http://dx.doi.org/10.1016/j.molp.2016.04.010.

Laurent D, Sianipar NF, Chelen, Listiarini, Wantho A (2015). Analysis of genetic diversity of Indonesia rodent tuber (*Typhonium flagelliforme* Lodd.) cultivars based on RAPD marker. *KnE Life Sci*. 2(1):139. https://doi.org/10.18502/kls.v2i1.133.

Lestari PUJI, Koh HJ (2013). Development of new CAPS/dCAPS and SNAP markers for rice eating quality. *HAYATI Journal of Biosciences*. 20(1): 15–23. http://dx.doi.org/10.4308/hjb.20.1.15

Muflikhati Z, Sianipar NF, Syamsunarno MRAA, Anas (2023). Understanding of genes encoding bioactive compounds from potential medicinal plants in Indonesia as cancer cell inhibitors. *Biodiversitas*. 24(8): 4645–4660. doi:10.13057/biodiv/d240846.

Purnamaningsih R, Sianipar NF, Assidqi K, Spits Warnars HLH (2018). Genetic variation of somaclonal mutants from the 8th generation of Pekalongan accession rodent tuber (*Typhonium flagelliforme* lodd.) based on RAPD-PCR analysis. *IOP Conf. Ser. Earth Environ. Sci*. 195(1). https://doi.org/10.1088/1755-1315/195/1/012054.

Sianipar NF, Assidqi K, Abbas BS (2020a). The effects of subculture on the mutant plant regeneration of rodent tuber (*Typhonium flagelliforme*) in vitro mutagenesis using gamma-ray irradiation. *IOP Conf. Ser. Earth Environ. Sci*. 426(1). https://doi.org/10.1088/1755-1315/426/1/012180.

Sianipar NF, Assidqi K, Purnamaningsih R, Herlina T (2019). In vitro cytotoxic activity of rodent tuber mutant plant (*Typhonium flagelliforme* Lodd.) against to Mcf-7 breast cancer cell line. *Asian J. Pharm. Clin. Res*. 12(3): 185–189. https://doi.org/10.22159/ajpcr.2019.v12i3.29651.

Sianipar NF, Hadisaputri YE, Assidqi K, Simanjuntak P, Purnamaningsih R (2020b). A study of anticancer activity from the fractions of rodent tuber superior mutant extract (*Typhonium flagelliforme*) by prestoblue assay method. *Rasayan J. Chem*. 13(3): 1992–1998. https://doi.org/10.31788/RJC.2020.1335703.

Sianipar NF, Laurent D, Purnamaningsih R, Darwati I (2015a). Genetic variation of the first generation of rodent tuber (*Typhonium flagelliforme* Lodd.) mutants based on RAPD molecular markers. *HAYATI J. Biosci.* 22(2): 98–104. https://doi.org/10.4308/hjb.22.2.98.

Sianipar NF, Purnamanigsih R, Darwati I (2020c). Ministry of Agriculture of the Republic of Indonesia Plant Variety Protection No. 00490/PPVT/S/2020. 19 March.

Sianipar NF, Purnamaningsih R (2018a). Enhancement of the contents of anticancer bioactive compounds in mutant clones of rodent tuber (*Typhonium flagelliforme* Lodd.) based on GC-MS analysis. *Pertanika J Trop Agric Sci*. 41(1):305–320.

Sianipar NF, Purnamaningsih R (2018b). Molecular detection of putative mutant clones of rodent tuber (*Typhonium flagelliforme* Lodd.) CV. Pekalongan using RAPD markers. *Malaysian Appl. Biol*. 47(2):1–8.

Sianipar NF, Purnamaningsih R, Darwati I, Laurent DC (2015b). The effects of gamma irradiation and somaclonal variation on morphology variation of mutant rodent tuber (*Typhonium flagelliforme* Lodd.) lines. *KnE Life Sci*. 2(1):637. https://doi.org/10.18502/kls.v2i1.236.

Sianipar NF, Purnamaningsih R, Gumanti DL, Rosaria, Vidianty M (2016). Analysis of gamma irradiated fourth generation mutant of rodent tuber (*Typhonium flagelliforme* Lodd.) based on morphology and RAPD markers. *J. Teknol*. 78(5–6): 41–49. https://doi.org/10.11113/jt.v78.8636.

Sianipar NF, Purnamaningsih R, Gumanti DL, Rosaria, Vidianty M (2017). Analysis of gamma irradiated-third generation mutants of rodent tuber (*Typhonium flagelliforme* lodd.) based on morphology, RAPD, and GC-MS Markers. *Pertanika J. Trop. Agric. Sci*. 40(1): 185–202.

Sianipar NF, Reflinur R, Ashan MD, Assidqi K, Widyaningrum D, Purnamaningsih R (2022a). Determination of lectin genes in superior mutant of rodent tuber Bogor accession (*Typhonium flagelliforme*) based on PCR amplification. *Proceed. 7th Int. Conf. Biol. Sci. (ICBS 2021)*. 22. https://doi.org/10.2991/absr.k.220406.039.

Sianipar NF, Reflinur, Ashan MD, Assidqi K, Widyaningrum D, Purnamaningsih R (2022b). Direct verification of Bogor superior mutant (*Typhonium flagelliforme*) using RAPD marker techniques and sequencing analysis. *AIP Conf. Proceed*. 2554. https://doi.org/10.1063/5.0104057.

Studer RA, Dessailly BH, Orengo CA (2013). Residue mutations and their impact on protein structure and function: Detecting beneficial and pathogenic changes. *Biochem. J*. 449(3): 581–594. https://doi.org/10.1042/BJ20121221.

Sukma D, Elina J, Giyanto, Sudarsono (2017). Disease resistance breeding of *Phalaenopsis* spp. for tropical environment and molecular marker development for plant selection. *Acta Hortic*. 1167: 237–244. https://doi.org/10.17660/ActaHortic.2017.1167.36.

Sukma D, Elina J, Raynalta E, Aisyah S, Aziz S, Sudarsono S, Chan M (2021). Analysis of the genetic diversity of Phalaenopsis orchids with single nucleotide polymorphisms and SNAP markers derived from the Pto-gene. *SABRAO J. Breed. Genet.* 53(4): 620–631. https://doi.org/10.54910/SABRAO2021.53.4.6.

Tarigan R, Maharijaya A, Izzah NK (2021). SNAP markers derived from catalase-1 gene sequence used for black pod disease resistance in Cacao (*Theobroma cacao* L.). *SABRAO J. Breed. Genet*. 53(3): 510–526.

Wu WR, Cheng CS, Cheng QQ, Lao CC, Cui H, Tang ZY, Ouyang Y, Liu L, Zhou H (2020). Novel SNP markers on ginsenosides biosynthesis functional gene for authentication of ginseng herbs and commercial products. *Chinese Journal of Natural Medicines*. 18(10): 770–778.

Xu X, Bai G (2015). Whole-genome resequencing: Changing the paradigms of SNP detection, molecular mapping and gene discovery. *Mol. Breed*. 35(1). https://doi.org/10.1007/s11032-015-0240-6.

Yao JH, Zhao XY, Liao ZH, Lin J, Chen ZH, Chen F, Song J, Sun XF, Tang KX (2003). Cloning and molecular characterization of a novel lectin gene from *Pinellia ternata. Cell Res*. 13(4): 301–308. https://doi.org/10.1038/sj.cr.7290175.

Yassin A, El-deeb NM, Of C, Applications T, Elsaid FG, Shati AA (2019). Lectin from *Pisum fulvum* seeds as in vitro anticancer and apoptotic gene regulator. *Rev. Chim*. 70(4): 1490-1495. https://doi.org/ 10.37358/RC.19.4.7156.