

Determination of Lectin Genes in Superior Mutant of Rodent Tuber Bogor Accession (*Typhonium flagelliforme*) Based on PCR Amplification

Nesti Fronika Sianipar^{1,2,*} Reflinur Reflinur³ Muhammad Dzulkifly Ashan³

Khoirunnisa Assidqi^{1,2} Dwityantari Widyaningrum¹ Ragapadmi Purnamaningsih³

¹Food Technology Department, Faculty of Engineering, Bina Nusantara University, 11480 Jakarta, Indonesia

²Research Interest Group Food Biotechnology, Bina Nusantara University, 11480 Jakarta, Indonesia

³Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (BB-Biogen), 16111 Bogor, West Java, Indonesia

*Corresponding author. Email: nsianipar@binus.edu

ABSTRACT

Rodent tuber (*Typhonium flagelliforme*) is one of the Indonesian herbs that has not yet been developed as an anticancer drug. The local name rodent tuber plant has produced superior mutant strains with higher levels and bioactive compounds. Compared to the mother plant, GCMS analysis detected four new bioactive compounds. A superior mutant rodent tuber ethanol extract was tested in vitro against MCF7 (breast) cancer cells. In previous studies, extracts from different rodent tuber varieties showed cytotoxic effects on MCF7 cancer cells at much lower IC₅₀ doses and up to 10-fold potency than mother plants. The development of the lineage of the rodent tuber plant Bogor has morphological and molecular marker characteristics. However, it is unclear whether the activity of the lectin gene is associated with Bogor's accession. The purpose of this study was to validate specific primer amplifications associated with lectin gene characteristics in the Bogor lineage of rodent tubers. This technique uses PCR amplification with specific primers that encode the lectin gene. All mutants and control plants were successfully amplified in sizes in the 500 bp range. The PCR products continued to be sequenced and showed significant results with differences in DNA sequences (from the 5' end reading) in the three regions 59 bp, 183 bp, and 211 bp. It was confirmed that the superior mutant rodent tuber plant with high anti-cancer compound had different sequences in the control and the mutant plant with low anti-cancer compound. Based on the results, further sequence analysis studies should be conducted on the sequence differences between the rodent tuber control plant and the superior mutant plant. Information about lectin compounds in the Bogor lineage of rodent tubers can be used as the basis for developing healthcare products, especially cancer treatments.

Keywords: Anticancer, Lectin gene, Specific primer, Superior mutant rodent tuber.

1. INTRODUCTION

Typhonium flagelliforme is a rodent tuber known as a herbaceous plant in Indonesia. This plant has been studied and compared with several other types (about 40 species) as a medicinal plant and distributed in Asia and Australia [1]. Rodent tuber is widespread in Indonesia, but most of it is not cultivated, so it is possible to have variations in the compounds of secondary metabolites

among these plants. Potential developments in rodent tuber plants have been carried out, which are more focused on testing plants under various environmental conditions. These conditions can certainly affect the metabolism and content of secondary metabolites in plant cells. This potential is supported by the presence of one of the bioactive ingredients that are continuously expressed, namely the lectin gene [2]. The herbal medicinal product developed based on the lectin gene as

a bioactive constituent is *Viscum album* extract. This product has been clinically tested and used in cancer treatment via intravenous injection [3].

The rodent tuber (*Typhonium divaricatum*L.) from China has potential as an anticancer drug. Tubers contain lectins that can inhibit the growth of cancer cells [3]. Information on rodent tubertubertuber plants that carry lectins has not yet been studied in comparison to other species of lectins, such as legume lectins. The process of lectin biosynthesis in plants is the same as general protein synthesis. However, it was distinguished by the migration of lectins to storage tissues such as vacuoles after the biosynthetic process. For this reason, this lectin is used as a protein reservoir in plants [4]. Lectins are also classified as secondary metabolites because they also serve as seed protection against microorganisms, pathogens, herbivores, or predators [5].

Lectins have a general mechanism of inhibiting cancer cell apoptosis and cancer cell growth. As seen in legumes (ConA and ConBr), lectins can inhibit the growth of cancer cells such as HL60 and MOLT4 cells [6]. The biotechnology of rodent tuber plants has been in use since 2011. Based on studies Sianipar et al. [7-9] rodent tuber mutant plants have higher cytotoxic effects on cancer cells than the mother plants. This indicates the development of superior mutant plants of the anti-cancer compound of the lectin gene, which is associated with elevated levels of stigmasterol. Stigmasterol compounds as antioxidants can reduce Eric ascites cancer (EAC) by lipid peroxidation. A treated concentration of 20 g/ml stigmasterol may increase catalase in rat liver of EAC and inhibit the growth of uterine cancer cells by 50% [3]. This study served as a basis for explaining the linkage of lectin genes during biosynthetic processes in the Bogor's rodent tuber varieties. . Approximately seven mutant clones of Bogor rodent tubers have high information about anticancer drugs or superior mutants (HACs).

The bioactivity against cancer cells was about 19.113 µg/ml, whereas the ethyl acetate fractionated variant KB6336 plants showed about 1.06 µg/ml bioactivity against breast cancer cells (MCF7). [9,10] Based on this information, it can be efficiently, quickly, and accurately provide information on the differences between rodent tuber mutant plants, especially those with the lectin gene. The development of processed products from rodent tuber plants in the future is needed. The development of biotechnology selection methods using molecular markers is increasing in line with the needs of people in both food and health disciplines. The superior mutant rodent tuber plant Bogor lineage molecular markers were performed using RAPD markers and field morphological studies [9, 11]. (These results were obtained with highly anti-cancer compounds in several mutant strains of rodent tubers registered as superior mutant plants, including KB6336 and KB6112 in of the Ministry of Agriculture, Republic of Indonesia. However, the lectin

gene approach was not performed in a particularly mutant selection process due to the lack of available information on the sequence of rodent tuber mutant plant that carries the lectin gene. This study aimed to determine and select rodent tuber plants carrying the lectin gene in the superior superior mutant rodent tuber Bogor accession (*Typhonium flagelliforme*) based on PCR amplification and PCR analysis in both control plants.

2. MATERIALS AND METHODS

2.1. Genetic material

The plant material used in this study consisted of five accessions of superior mutant Bogor rodent tuber and control rodent tuber as a comparison (Table 1). The part of the plant used for molecular analysis is the fresh leaf.

Table 1. List of rodent tuber plant varieties used in this study

No.	Accession Name	Origin	Type of Rodent Tuber (Stigmasterol)
1.	KB Control	Binus Collection	Control plant
2.	KB 6-1-1-2	Binus Collection	Mutant with high anticancer content (HAC)
3.	KB 6-2-6-3	Binus Collection	Mutant with high anticancer content (HAC)
4.	KB 6-2-5-3	Binus Collection	Mutant with low anticancer content (LAC)
5.	BM 8-2	Binus Collection	Mutant with low anticancer content (LAC)

2.2. DNA isolation

The preparation of rodent tuber variants and control plant DNA was performed by CTAB (cetyltrimethylammonium bromide)-based extraction [12]. The samples used were four superior mutant clones of rodent tuber leaves, Bogor control, and rodent tuber. The leaves were beaten with a mortar, and 1000 liters of extraction buffer was added to liquid nitrogen. The leaf grind was placed in a 2 ml microtube, then placed in a

water bath for 45 min (homogenized every 15 min), followed by an 800 L chloroform-isoamyl alcohol solution in the tube. The solution was homogenized and centrifuged at a rate of 12,000 rpm for 15 min. The supernatant of 500 L was transferred into a 1.5 L tube, the solution was added with 500 L of cold isopropanol solution and 0.1X Vol of Sodium acetate (NaOAC). The supernatant slowly inverted. The tuber was tightly closed while observing the presence of DNA strands. The samples were then incubated at room temperature for 10 min. The supernatant was removed with a micropipette, and the DNA pellet was then added with 250 L of TE buffer at room temperature. RNase was added into 1/100 of the volume of the solution, mixed slowly, and then incubated for 30 min at 37 °C. NaOAC was added to the solution as much as 1/10 of the volume of the DNA solution and mixed slowly. Ethanol (cold) 95% solution was added as much as 600 L, the samples were then mixed slowly and incubated at 20 °C for 45 min to precipitate DNA. The solution was centrifuged at 13500 rpm for 10 min, discarded the supernatant, and dried the DNA pellet at room temperature (overnight). After the DNA dried, it was added 200 L of TE buffer and leave until the DNA dissolves.

2.3. DNA amplification

DNA amplification was performed using the access samples listed in Table 2. Each sample was PCR using two replications (encoded by a and b). The sample amplification process was performed with a total reaction of 20 L containing a 25 ng DNA template up to 2 L. 1x MyTaq red reaction buffer (Bioline, UK) 4 L; concentration 10 M 0.8 L, 0.16 L Using primers of My Taq HS Red DNA polymerase [5u/μL], sterile ddH₂O was added. Amplification was performed using the primer pair Primer Forward (Fbase61). GTAGGCACCAACTACCTGCTGT and vice versa (Rbase551); TTGGACTGCCACCCGTA CTT was edited based on a literature study of the NCBI lectin gene (ID: MK904840.1). The PCR reaction was performed on the PCR machine T100 thermal cycler T1 thermocycler (Biorad, USA) using the following PCR profile. The first denaturation was performed at 94 °C for 5 min, followed by a 35 cycle denaturation process at 94 °C. Annealing (binding step) primer for 1 min at 55 °C for 30 seconds and extension (base extension) for 2 min at 72 °C. The PCR reaction was completed with a final extension cycle (final stage of base extension) at 72 °C for 5 min. The PCR results were electrophoresed on a 2% agarose gel containing 0.5x TBE (Tris Borate EDTA) buffer at a voltage of 100 V for 35 min. The agarose gel was then stained with ethidium bromide and visualized under UV light using a UV transilluminator (Biorad, USA).

2.4. Sequencing

Sequence analysis of rodent tubers was performed on five rodent tuber samples such as control, HAC, and LAC. Sequencing is performed by the service provider First BASE Laboratory (Singapore). Sequencing data is output in the form of a Fasta format DNA sequence. This must be done by paired sequences and sequence alignment before the bioinformatics analysis process.

2.5. Bioinformatic analysis of differences in rodent tuber sequences

The results of further sequencing need to be seen the differences in the sequences of HAC, LAC, and control plants. The analysis process begins with the pairwise sequence between forward and reverse sequences with Bioedit software, then proceeds with sequence alignment with Clustel W software (<https://www.ebi.ac.uk/Tools/>) or the Bioedit software. The sequencing results were analyzed bioinformatically by comparing the sequences of the test samples. The similarity of the test sequences to the organisms' sequences is confirmed in the NCBI (National Centre for Biotechnology Information) database based on their genetic similarity [13].

3. RESULT AND DISCUSSION

3.1. PCR product amplification

Product amplification in the superior mutant rodent tuber plant, Bogor, was successfully amplified, indicated by the presence of DNA band fragments at a size of about 500 bp. Confirmation of the size can be seen in Figure 1.

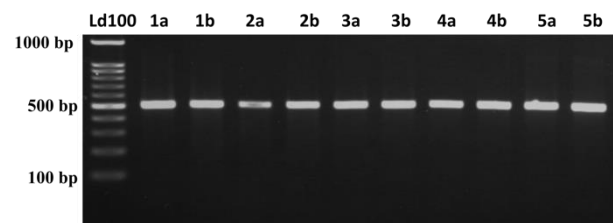


Figure 1 PCR visualization of lectin products in five test samples (each two replicate samples). Ld100: DNA base size marker 100 bp, 1a,b; Control plants, 2a,b; HAC (KB6112), 3a,b; HAC (K6253), 4a,b; LAC (KB 6263) and 5a,b; LAC (BM82).

Based on Figure 1, visualization of DNA band fragments is an essential step in seeing the match of primers that have been designed based on database information. Figure 1 shows the success of these primers attaching to the DNA strands of rodent tuber mutant and control plants. The fragments formed in the sample ranged from 500 bp to all accessions of mutant plants and control plants so that there was no visible variation using this primer. This condition indicates that the primer used

is a specific primer consistent in various plants. However, this condition can be continued with the sequencing analysis stage, which can be done by looking at the sequence of DNA bases in each test sample. The sequence of DNA bases can be seen as differences between the mutant and control plants. Based on this, the sample used for sequencing is the sample coded A for each representative of the accession. Burns et al. [14] reported that there are several interpretation techniques in analyzing PCR results when getting data that is still difficult to interpret, as shown in Figure 1. One of the existing techniques can be applied to compare the PCR product sequence with sequencing and specific primers on the sequence target. The technique is carried out direct the unique sequence of each sample is obtained.

3.2. Sequencing products

The product of the sequencing analysis is a product of DNA base sequences in the form of sequences of the Bogor rodent tuber mutant plant. The sequences have been successfully analyzed and can figure out in Figure 2.

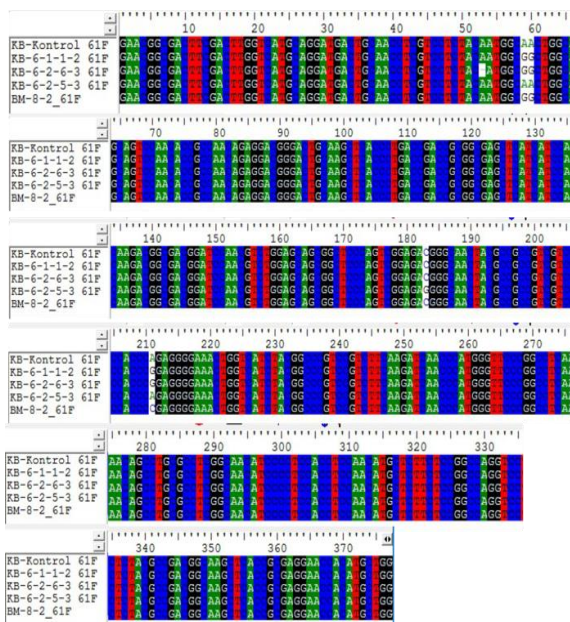


Figure 2 Sequencing data analysis of the superior mutant rodent tuber plant in Bogor processed using Bioedit software version 7.2.5 [15].

Based on the results sequence analysis results in Fig. 2, a comparison of the five Bogor rodent tuber mutant plants reveals that the DNA sequences vary. Each plant consisted of a control rodent tuber, HAC rodent tuber mutants (KB6112 and KB6263), and LAC rodent tuber mutants (KB6253 and BM82). The results of this analysis were confirmed to be correct based on the alignment of the forward sequence pair and the installation of the reverse sequence, which is not included in Figure 2. The analyzed sequence was aligned (alignment sequence), and the left and right ends (5' 3') were cut off during the

reading process. Based on data processing, the base length is about 375 bp and the test pattern has three different areas. This study found a missing sequence in sample KB6263 caused by a gap/insertion in another individual who caused an absence sequence. This is consistent with the statement by Frohme et al. [16], several factors can cause DNA sequence loss, including suboptimal binding process/gap closure at the end of the sequencing phase, gaps during the amplification process due to biological factors, and variations in the amplification process that prevented successful cloning, especially in the sequencing phase of each sequence. DNA sequences with base sizes of approximately 59 bp, 183 bp, and 211 bp distinguish between superior mutant plants with high anti-cancer compounds (HAC) and plants with low anti-cancer compounds and their controls.

The superior mutant plants were dominated by the guanine bases (59 bp and 211 bp) containing the high anti-cancer compounds compared to the control samples and the variants containing the low anti-cancer compounds. Based on this, the control plant KB6253 (LAC) had two guanine bases, and the mutant plants of HAC KB6112, KB6263, and the LAC BM82 had an adenine base at 59 bp. The base size of the control plant was 183 bp, and two HAC plants and one LAC plant (BM82) had the same base, cytosine, but guanine was used for the sample KB6253 plant. In addition, the HAC plant had a guanine base at 211 bp in both samples, while the control and LAC samples had different bases. This indicates that the mutant HAC plants in the three regions have similar sequences and consistency, while the controls and LACs have different sequences in each region.

HAC plants can be significantly correlated with the lectin gene based on the sequence consistency of HAC plants using lectin-specific primers. Based on a study by Kellis et al. [17] the different sequences identification of yeast species associated with genes and their bioprocesses. The results of this study provide a robust and general approach for species comparative sequence analysis to identify functional elements. The evolutionary process of living organisms constantly optimizes genome sequences and tests the results by natural selection. The characterization should be characterized by a higher degree of conservation of the associated species. This approach has the advantage that rodent tuber plants receive a high degree of information on the physiological and agricultural differences between species. Recent studies have shown the LAC, and control plants. Heterogeneous conservation was previously used to identify these lectin genes that can be further developed for other plant species of high health and economic value.

The linkage of the lectin gene in rodent tuber mutant plants with superior HAC was successfully carried out,

indicated by the amplification of DNA band fragments at a size of 500 bp in all samples. This amplification was done by using specific SSR primers for the lectin gene. Furthermore, the sequencing analysis results were carried out to obtain differences in HAC, LAC, and control. These results succeeded to differentiate the sequences region of the plants. The first and second regions at positions 59 bp and 211 bp have a guanine base (G). The third region at 183 bp size has a cytosine base (C). The existence of this lectin compound is expected to be developed in the future in a rapid detection process with lectin primers that have a relationship with anticancer compounds in rodent tuber mutant plants.

AUTHORS' CONTRIBUTIONS

NFS, R designed the study. MDA carried out the laboratory work. MDA, KA analyzed the data. NFS, R, MDA, KA, RP wrote the manuscript. RP maintained the rodent tuber mutant plant varieties. All authors clarify and approve the final version of the manuscript.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Molecular Biology Laboratory, Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (BB Biogen), Bogor, West Java, Indonesia, for all the support materials and facilities. This research was funded by the Ministry of Education, Culture, Research, and Technology, the Republic of Indonesia, with contract No. 163/SP2H/LT/DRPM/2021.

REFERENCES

- [1] J.C. Wang, K.C. Yang, The genus *Typhonium* (Araceae) in Taiwan, *Botanical Bulletin of Academia Sinica*, vol. 37, 1996, pp. 159–163.
- [2] M. Singh, A. Kumar, R. Singh, K.D. Pandey, Endophytic bacteria: a new source of bioactive compounds, *3 Biotech*, vol. 7, 2017. DOI: <https://doi.org/10.1007/s13205-017-0942-z>
- [3] M. Alfarabi, S. Rosmalawati, M. Bintang, Miftahudin, E. Rofa'ani, Chaidir, Antiproliferation activity of tuber protein from *Typhonium flagelliforme* (Lodd.) Blume on MCF-7 cell line, *International Journal of Biosciences (IJB)*, vol. 6, 2015, pp. 52–60. DOI: <https://doi.org/10.12692/ijb/6.12.52-60>
- [4] Y. Luo, X. Xu, J. Liu, Y. Sun, Z. Liu, J. Liu, E. Van Damme, J. Balzarini, J. Bao, A novel mannose-binding tuber lectin from *Typhonium divaricatum* (L.) Decne (family Araceae) with antiviral activity against HSV-II and antiproliferative effect on human cancer cell lines, *Journal of Biochemistry & Molecular Biology*, vol. 40, 2007, pp. 358-367. DOI: <https://10.5483/bmbrep.2007.40.3.358>
- [5] H.P.S. Makkar, P. Siddhuraju, K.Becker, *Plant Secondary Metabolite: Methods in Molecular Biology*, New jersey (US): Humana Press, 2007, pp. 157-230.
- [6] G.V. Faheina-Martins, A.L. da Silveira, B.C. Cavalcanti, M.V. Ramos, M.O. Moraes, C. Pessoa, D.A.M. Araujo, Antiproliferative effects of lectins from *Canavalia ensiformis* and *Canavalia brasiliensis* in human leukemia cell lines, *Toxicology In Vitro*, vol. 26, 2012, pp. 1161-1169. DOI: <http://10.1016/j.tiv.2012.06.017>
- [7] N.F. Sianipar, R. Purnamaningsih, I. Darwati, D. Laurent, Chelen, The effects of gamma irradiation and somaclonal variation on morphology variation of mutant rodent tuber (*Typhonium flagelliforme* Lodd.) LINES, *KnE Life Sciences*, vol. 2, 2015, pp. 637-645. DOI: <https://doi.org/10.18502/cls.v2i1.236>
- [8] N.F. Sianipar, R. Purnamaningsih, D.L. Gumanti, Rosaria, M. Vidianty, Analysis of gamma-irradiated fourth-generation mutant of rodent tuber (*Typhonium flagelliforme* Lodd.) Based on morphology and rapid markers, *Jurnal Teknologi*, vol. 78, 2016, pp. 41–49. DOI: <https://doi.org/10.11113/jt.v78.8636>
- [9] N.F. Sianipar, Y.E. Hadisaputri, K. Assidqi, P. Simanjuntak, R.A. Purnamaningsih, A study of anticancer activity from the fractions of rodent tuber superior mutant extract (*Typhonium flagelliforme*) by presto blue assay method, *Rasayan Journal of Chemistry*, vol. 13, 2020, pp. 1992–1998. DOI: <https://doi.org/10.31788/RJC.2020.1335703>
- [10] N.F. Sianipar, K. Assidqi, R. Purnamaningsih, T. Herlina, In Vitro Cytotoxic Activity of Rodent Tuber Mutant Plant (*Typhonium flagelliforme* Lodd.) Against To Mcf-7 Breast Cancer Cell Line, *Asian Journal of Pharmaceutical and Clinical Research*, vol. 12, 2019, pp. 185–189. DOI: <https://doi.org/10.22159/ajpcr.2019.v12i3.29651>
- [11] N.F. Sianipar, R. Purnamaningsih, K. Assidqi, H.L.H. Spits Warnars, Genetic variation of somaclonal mutants from the 8th generation of Pekalongan accession rodent tuber (*Typhonium flagelliforme* Lodd.) based on RAPD-PCR analysis, in: *IOP Conference Series: Earth and Environmental Science*, vol. 195, 2018. DOI: <https://doi.org/10.1088/1755-1315/195/1/012054>
- [12] N. Calderón-Cortés, M. Quesada, H. Cano-Camacho, G.A. Zavala-Páramo, Simple and rapid method for DNA isolation from *Xylophagous*

- insects, *International Journal of Molecular Sciences*, vol. 11, 2010, pp. 5056–5064. DOI: <https://doi.org/10.3390/ijms11125056>
- [13] E.W. Sayers, J. Beck, J.R. Brister, E.E. Bolton, K. Canese, D.C. Comeau, K. Funk, A. Ketter, S. Kim, A. Kimchi, P.A. Kitts, A. Kuznetsov, S. Lathrop, Z. Lu, K. McGarvey, T.L. Madden, T.D. Murphy, N. O’Leary, L. Phan, V.A. Schneider, F. Thilbaud-Nissen, B.W. Trawick, K.D. Pruitt, J. Ostell, Database resources of the National Center for Biotechnology Information, *Nucleic Acids Research*, vol. 48, 2020, pp. D9-D16. DOI: <https://doi.org/10.1093/nar/gkz899>
- [14] M.J. Burns, H. Valdivia, N. Harris, Analysis and interpretation of data from real-time PCR trace detection methods using quantitation of GM soya as a model system, *Analytical & Bioanalytical Chemistry*, vol. 378, 2004, pp. 1616-1623. DOI: <https://doi.org/10.1007/s00216-003-2441-9>
- [15] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucleic Acids Symposium Series*, vol. 41, 1999, pp. 95-98.
- [16] M. Froome, A.A. Camargo, C. Czink, A.Y. Matsukuma, A.J.G. Simpson, J.D. Hoheisel, S. Verjovski-Almeida., Directed gap closure in large-scale sequencing projects, *Genome Research*, vol. 11, 2001, pp. 901–903. DOI: <https://doi.org/10.1101/gr.179401>
- [17] M. Kellis, N. Patterson, M. Endrizzi, B. Birren., E.S. Lander, Sequencing and comparison of yeast species to identify genes and regulatory elements, *Nature*, vol. 423, 2003, pp. 241–254. DOI: <https://doi.org/10.1038/nature01644>