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Research Article

FRAGMENT DNA 387BP GENE LECTIN OF SOYBEAN (*Glycne Max* L.) MERIIL

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ABSTRACT

Lectin gene is a housekeeping gene that can be used as a molecular marker soybean (Glycine max (L.) Meriil.). This study aimed to obtain the identity of the lectin gene molecular markers for breeding purposes. This descriptive study was performed using PCR amplification and identification of sequences using a lectin gene fragment sequencing techniques and phylogenetic search using Mega Tree programme. The results obtained are lectin gene fragment along 387bp used primer Leic Foward GCGGAAACTGTTTCTTTCAGCTGG and primer Leic Reverse CCGGAAAGTGTCAAACTCAACAGCG.

Keywords: *soybean, lectin gene, housekeeping gene, molecular marker*

INTRODUCTION

Protein, fats, and carbohydrates in soybeans has been studied several decades. Especially since the discovery of compounds isoflavones in soy could be expected to reduce the risk of cancer, heart disease, and osteoporosis, and also reduce the symptoms of menopause. Soybeans in Indonesia has a high protein content. (Ginting et al., 2009).

This study investigates soy DNA fragments (387bp) fragment of the lectin gene of soybean (*Glycine max*). Soybean lectin (SBL) specifically binds to terminal N-acetyl-D-galactosamine with greatest affinity and to a lesser extent with D-galactose. Lectins are carbohydrate-binding proteins or glycoproteins that occur widely in plants, animals and microorganisms (Vural, *et al.,* 2010). Protein lectins have been found mostly in seeds of Legumes (Hemalatha, *et al.,* 2011). Lectin gene is a housekeeping gene that can be used as a molecular marker soybean (Glycine max (L.) Meriil.). Lectin protein in soybean plants can be found in vegetative organs such as leaves, stems and roots. lectin protein content in vegetative organs is less than the levels in soybean seeds (Spilatro, SR, et. al, 1999). Differences in levels of protein lectins not only the location but also can be different for each variety of soybean (Fang, EF, et al., 2010).

PCR has already proved its worth as an analytical method for the detection of genetic organism material in seed or leaf, through its simplicity, specificity, and sensitivity in this study we isolated genomic DNA from soybean seed coat varieties detam-2 with DNA techniques. We use RIDE DNA technique modification that combines conventional DNA isolation techniques on animal tissues and DNA isolation technique with TriPure solution. We assume the isolation of DNA in the seed coat requires

special techniques and require a long time to lyse cell walls and cell membranes.

MATERIALS AND METHODS

DNA ISOLATION

Soya bean seeds soaked in water for 10-15 minutes, then take the skin of the seeds and weigh 0.3 grams. Next, prepare a mortar and pestle to soften the seed coat by means pounded, after fine inserted into microtube 1,5µL. Insert a 1 ml cell lysis DNA, Proteinase K 70µL, 80µL 20% SDS solution into the microtube. Then incubated for 2 hours. Insert a 7.4 ph STE 600µL into microtube, then the formation of pellet and supernatant portion. A total of 500µL supernatant was transferred to a new microtube, then put 1 ml Tripure. Samples were incubated for 5 min at room temperature (while inverted until homogeneous), then centrifuged at 10,000 rpm for 12µL. Formed supernatant and pellet, then take a supernatant. Add Tripure into supernatant, then added 100µL klroform cold, then incubated for 8 minutes, centrifuged 10,000 rpm for 13 minutes. Take the supernatant, and then is moved to mikrotube. Then added 500µL isopropanol. The next step, the sample divortex, then incubated at room temperature for 5-10 minutes. Then centrifuged 10,000 rpm for 10 minutes Discard the supernatant formed after the vortex, then add 1000µLdan cold 75% ethanol, and do Centrifuge 7500 rpm for 5 minutes. Discard the supernatant, then vacuum until dry and added as much as 50µL buffer Rehydration Solution.

PCR PRIMERS

Design primers Leic for amplification of regions of the soybean lectin gene. Leic Primer foward dan reverse use sequence nucleotide : Leic Foward 5'GCGGAAACTGTTCTTTCAGCTGG'3 (24bp), %GC : 50%. and primer Leic Reverse 5'CCGGA AAGTGTCAAACTCAACAGCG'3(25bp), %GC : 52% with size fragment PCR product 387bp.

Standard PCR Assays

Each amplification reaction contained 1 x reaction 5.5ul nuclease free water, 2ul DNA genom, 1ul Leic Primer foward, 1 ul Leic primer reverse and 12.5ul Kappa Taq Polymerase,were as follows: denaturation for 3 min at 94°C; 40 cycles of 30 s at 94°C, 30 s at 60°C, and 45s at 72°C; and a final extension o10 min at 72°C.

PCR Fragment Analysis

Analysis of amplified DNA fragments were electrophoresed on 2% agarose gels in 1 x xTAE buffer, and bands were visualized by ethidium bromide staining and UV transillumination.

RESULT

SUCCESSFUL DNA ISOLATED USING RIDE DNA TECHNIQUE MODIFICATION.

The concentration of soybean genomic DNA obtained: 68.08 ug /ul and purity of the DNA of 1,619. purity DNA results are still contaminated by protein and phenol as Tripure solution in addition to isolating the DNA also can isolate RNA and proteins in a single reaction. But that does not mean there are no soybean genomic DNA. No soybean genomic DNA and results in the isolation of proteins and there is still a phenol solution covering absorbance values. Results of DNA purity value which little can be enhanced with PCR amplification methods. PCR amplification methods require 100ng / ul of genomic DNA. The concentration of soybean genomic DNA obtained 68.08 ug / ul, the volume of soya bean DNA used for PCR amplification techniques as much as 2 ul or 136.16 ug / ul.

Results of soya bean lectin gene amplification fragment 387 bp (Figure.1).

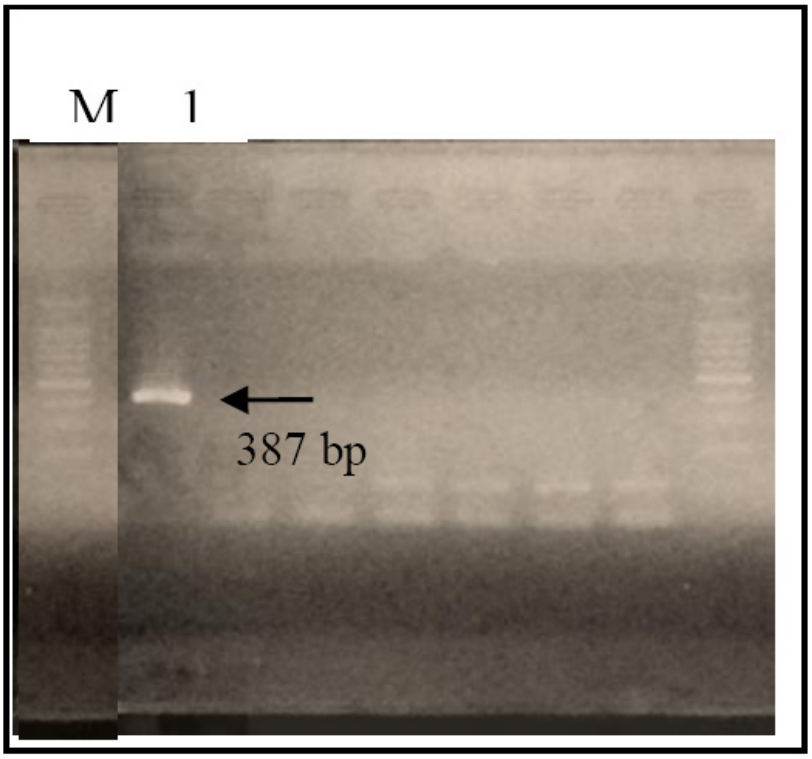

Figure 1. Fragments lectin gene of soybean Glycine max (L) Merr 387bp length using gel electrophoresis agarose 2%

SUCCESSFUL SEQUENCE LECTIN GENE OF SOYBEAN.

The results of lectin gene sequence shown in Figure 2.

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>150914-69_J22_D4_Leic-Forward.ab1      431
CAATCGTGCGAGCACCAAACATGATCCTCCAAGGAGACGCTATTGTGACC
TCCTCGGGAAAGTTACAACTCAATAAGGTTGACGAAAACGGCACCCCAAA
ACCCTCGTCTCTTGGTCGCGCCCTCTACTCCACCCCCATCCACATTTGGG
ACAAAGAAACCGGTAGCGTTGCCAGCTTCGCCGCTTCCTTCAACTTCACC
TTCTATGCCCCTGACACAAAAAGGCTTGCAGATGGGCTTGCCTTCTTTCT
CGCACCAATTGACACTAAGCCACAAACACATGCAGGTTATCTTGGTCTTT
TCAACGAAAACGAGTCTGGTGATCAAGTCGTCGCTGTTGATTAAACCTTT
TCCGGATTTTTTTTTTTTTTCATAGCCCTATTCCCCGCCCCTCCACCCGC
GCCCCTCCCTTCTTCGTTCCCCTTCTCACCC
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Figure 2. Lectin gene sequences using a primer leic_ foward

If results are combined and blast using NCBI Blast program resulting nucleotide sequence similarity of 99% with a predicted: Glycine max lectin-like (LOC100818710), mRNA (XM_003518752.2). This means Leic forward and reverse primer attaches to specific lectin gene from soybeans. Leic forward and reverse primer can be used to isolate the lectin gene in soybeans.

DISCUSSION

Research produces DNA that can be used as the identity of molecular lectin gene in the soybean Glycine max (L) Merr, and produce primers products foward and reverse for lectin gene of soybean Glycine max (L) Merr which

can be used as reference gene identification lectins in beans soy soybean varieties other. This research proved that the seed coat can be made of soybean genomic DNA isolation. The mature seed contains about 3% of the weight of it (Laija et al.,2010). The biological activities like anti-tumor, anti-proliferative, immune potentiating, antibacterial, antifungal, anti-insect, and antiviral activities have been found in lectins. Lectin compounds in black soya beans have been known to have hemagglutination activity, the enzyme reverse transcriptase inhibitor of HIV-1, antitumor and can bind to specific carbohydrate compounds that make up the cell membrane of bacteria and viruses (Fang, EF, et al., 2010). Consumption of lectins derived from soy can increase the activity of the pancreas to produce insulin in diabetics (Hemalatha, C., et al., 2011). Lectin proteins also recognize receptors on the tumor cells so that the lectin protein can also act as an agent of the target molecule tumor cell death (Fu, LL, et al., 2011). Lectin compounds have useful functions in the field of health.

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