Detektion Soybean (Glycine Max L Merrill)
Transgenic GTS 40-3-2 Herbicide Resistant Active Based Glyphosate PCR using

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Abstract

Soybean (Glycine max L Merill) is one commodity that is used as food, raw materials and animal feed industry. Transgenic soy products is a way to detect soy and its derivatives such products need to be developed. This study aims to find ways to identify soybean GTS40-3-2 containing the resistance gene to the herbicide active ingredient glyphosate with using glyphosate herbicide active ingredient and the detection of the presence of the glyphosate tolerant genes in soybean GTS 40-3-2 (gene CP4) via PCR. Furthermore, DNA from leaves, roots, and the cotyledons of soybean imports in isolation and used as a template to identify the presence of glyphosate tolerant genes using PCR. Actin gene was used as an internal control. PCR can be performed using the template DNA concentration in the range of 13-39 to detect the CP4 gene with primer annealing temperature of 58 °C and 30 seconds extension. The existence of CP4 and actin genes can be detected together when starting with 5 cycles of PCR using specific primers 35S-CP4 area and followed 35 cycles of PCR with the addition of specific primers for actin gene. The result of the application note that 100% soybean imports from the four regions keep alive 7 days after spraying herbicide active ingredient glyphosate, while soybean Wilis entirely dead.

Keywords: Soybean, Transgenic, PCR,

I. INTRODUCTION

Soybean (Glycine max L Merill) is a commodity many benefits, it can be used as food, industrial raw materials and animal feed. Approximately 50% and 40% of soybeans available for foodstuffs processed into tempe and tofu, and the rest for
processing soy milk, soy sauce, bean sprouts and tauco (Ginting, 2009). Processed soy products is a source of vegetable protein which are relatively inexpensive and consumed by almost all walks of life in Indonesia. Thus, indirectly, soy also has a role in supporting food security and improve nutrition.

The Central Statistics Agency in 2015 stated that local soybean production amounted to 851.286 tonnes or 29% of total needs of Indonesia. The soybean production are Java, Sumatra, and Sulawesi (Supriyatna, 2015). Indonesia imported a total of 2,087,986 tons (71%), which originated from the United States (1.8 million tons), Argentina (73.037 tonnes), Uruguay (16.824 tonnes) and Brazil (13.550 tonnes) (Wong, 2015). Type soybeans are widely planted in the United States, Brazil and Argentina is a transgenic soybean with specifications resistant to glyphosate herbicide active ingredient (Cummins, 2012).

Glyphosate itself is a non-selective herbicide which has a wide spectrum of controllers and can control annuals or perennial weeds, broadleaf and used in the pre-growth rankings. (Ward et al., 2001). Glyphosate compound is absorbed through the leaves and transported into all the tissues of plants. How it works affect nucleic acids and protein synthesis (Sastroutomo, 1992). With transgenic plants containing genes for resistance to the herbicide glyphosate active ingredient in soy do not join it then die when the herbicide is applied to weeds around it, although herbicides are non-selective.

This study aims to find ways to identify soybean GTS 40-3-2 containing the resistance gene to the herbicide active ingredient glyphosate. The GTS 40-3-2 identity can be determined by looking at the ability to grow in the environment containing the herbicide glyphosate. At the molecular level, genes encoding GTS 40-3-2 tolerance to glyphosate can be detected by amplification of transgene contained by GTS 40-3-2.

II. RESEARCH TIME

This activity is carried out in the Laboratory of the Faculty of Agriculture, University of Islam Kadiri, in October 2015 through the month of December 2015

III. RESEARCH IMPLEMENTATION

3.1. Lethal dose of herbicide active ingredient glyphosate in soybean imports

Preparations made before planting soybean seed is sowed imports obtained from four different regions in Kediri. The medium used is ground in a polybag with the addition of NPK fertilizer as much as 10 g / polybag. Soybean seeds spread on the ground surface as much as 10 seeds per polybag, and soybeans in the respective regions are grown as much as 2 polybag. Seeds are planted and labeled for each horticultural with the numbers 1-20. Herbicide applications made when soybeans aged ± 14 days after planting or after growing 3 leaves. Some herbicide concentrations used topically on soybean leaves with a dose as follows:
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C: rub the leaves in water
Q: leaves smeared with glyphosate content of 4.8 g / l
X: leaves smeared with glyphosate content of 9.6 g / l
Y: leaves smeared with glyphosate content of 14.4 g / l

Observations were made after 6 days to look at the symptoms appear on the leaves smeared. If the leaves undergo necrotic it is assumed that these plants are not resistant to glyphosate

3.2. Selection of transgenic soybean herbicide active ingredient glyphosate

Selection of transgenic soybean is done by spraying the soybeans have been planted for ± 20 days and used as the control of local soybean varieties Wilis. Planting is done as determining the dose of herbicide above but soybean seeds spread as much as 15 seeds per polybag, and soybeans in the respective regions are planted as many as 3 polybag.

Soybeans grown up to ± 20 days old then sprayed using a sprayer with glyphosate dose of 14.4 g / l. Observations were made on days 5 and 7 after the herbicide application.

3.3. The soybean DNA Isolation and DNA concentration measurements

Detection using PCR begins with the isolation of DNA from three parts of plants, roots, leaves and cotyledons. Roots obtained from seeds that in imbibition for 5 days and the cotyledons obtained after the seeds were grown for 3 days in the greenhouse. While the material used leaves, isolated from resistant plants after herbicide application (the result of the detection method of spray). The material selected is still fresh and clean and then after it was cut up and immediately wrapped in aluminum foil and then put in liquid nitrogen. The following chart summarizes the mechanisms of detection using PCR methods were optimized:

![Figure 1: Schematic detection using PCR](image-url)

Material Sampling

Isolation DNA

Check Genome (By Electropho Resis Gel)

Check Existence Of Gen Cp4 (PCR Trough Checks And Using Electro Phoresis Gel)

Check Existence Gene Endogenous And Cp4 (PCR Multiplex And Used Check Electrophoresis Gel)
3.4. Detection of soybean GTS 40-3-2 by PCR

The PCR reaction is used to detect DNA sequences that encode resistance to glyphosate consists of a promoter, specific genes which in this case is the CP4 EPSPS and terminator.

All components of the PCR prepared to melt it and then put in the ice except Taq polymerase enzyme. After all the components are added, the solution divortex then put in a PCR tube. Furthermore, the reactants are in the PCR with several PCR program. As an internal control is used soybean actin gene amplification using specific primers to actin soybean (Trisnaningrum, 2009).

IV. RESULT AND DISCUSSION

4.1. Lethal dose of herbicide active ingredient glyphosate in soybean imports

In the application of glyphosate herbicide active ingredient by means smeared on imported soybean leaves obtained the results shown in the following table:

<table>
<thead>
<tr>
<th>No.</th>
<th>code plants</th>
<th>Can not stand</th>
<th>The number of plants resistant to</th>
<th>number of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4,8 gr/l 9,6 gr/l 14,4 gr/l</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>K1</td>
<td>-</td>
<td>10 7 3</td>
<td>20</td>
</tr>
<tr>
<td>2.</td>
<td>K2</td>
<td>1</td>
<td>10 5 4</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>K3</td>
<td>-</td>
<td>13 6 1</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>K4</td>
<td>2</td>
<td>7 8 3</td>
<td>20</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td>3</td>
<td>40 26 11</td>
<td>80</td>
</tr>
</tbody>
</table>

From these results indicate that the leaves of soybean imports as much as 13.75% remained resistant to a dose of 14.4 g / l, so as to selection by spraying using the dose.

4.2. Selection of transgenic soybean herbicide active ingredient glyphosate

Application of glyphosate (14.4 g / L) in 20-day-old soybean plants showed high levels of resistance in soybean imports 100% compared to the local soybean variety Wilis. Soybeans are sensitive to glyphosate wither on the fifth day and died on the 7th day, the condition of the plant shown in the picture.
Table 2: Results of spraying herbicide active ingredient glyphosate (14.4 g / L) in the soybean crop was 20 days.

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant code</th>
<th>Crop conditions 5 days after spraying</th>
<th>Crop conditions 7 days after spraying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Life</td>
<td>Withered</td>
</tr>
<tr>
<td>1.</td>
<td>W (Wilis)</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>2.</td>
<td>K1</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>K2</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>K3</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>K4</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td>180</td>
<td>45</td>
</tr>
</tbody>
</table>
Figure 2: Results of glyphosate spraying herbicide active ingredient; A Soybean Wilis and soybean imports was 15 days before spraying; B Soybean after 7 days of glyphosate sprayed with a dose of 14.4 g/L; C Comparison of imported soybean and soybean Wilis sprayed after 5 days; D Comparison of imported soybean and soybean Wilis sprayed after 7 days.

4.3. Isolation of soybean DNA and DNA concentration measurements

Agarose gel electrophoresis was used to verify the DNA of the soybean crop was isolated using the CTAB. The verification results showing that DNA isolation using CTAB do.

![Agarose gel electrophoresis](image_url)

**Figure 3**: Genome DNA Soybean Wilis 1 and 2; imported soybean samples 1 and 2

Besides, agarose gel electrophoresis is also used to calculate the concentration of DNA. This calculation is performed by verification using different concentrations of DNA and comparing with λEcoRI/HindIII on agarose gel electrophoresis.

From these results indicate the test gel electrophoresis soybean genome with λEcoRI/HindIII produce every mL of template derived from the leaves 13 ng of DNA, while the cotyledons each mL sample containing 25 ng DNA template as shown in Figure 4B. So, from these results, it can be optimized amount of DNA is needed as a template PCR to detect the CP4 gene in soybean GTS 40-3-2.
**Figure 4**: Initial verification GTS 40-3-2 genome using agarose gel electrophoresis; A Genome leaves; B genome cotyledons

**Figure 5**: PCR results with different concentrations of template DNA

4.4. Detection of soybean GTS 40-3-2 by PCR

4.4.1. Types of samples (leaves, roots cotyledon).

Agarose gel electrophoresis of PCR CP4 results show that detection can be performed using the template DNA isolated from different plant parts are the leaves of plants aged 25-30 days, the roots of soybeans germinated after 5 days, and the cotyledons
were grown from seeds that planted after 3 days. This is shown in the figure below for the results of PCR leaves and roots, as well as on the image above for cotyledons. Presence of genes can be seen with the pitadengan size of 1,705 bp (primer length CP4) or indicated by the band well on the column leaves or roots of soybean imports.

**Figure 6**: Result PCR gen CP4 and DNA *template* in isolation from leaves and root

### 4.4.2. PCR

The GTS 40-3-2 detection using two different primer, initially using CP4 primer length so as to produce a copy of 1705 bp and CP4 short primer so as to produce a copy of 1285 bp.

### 4.4.3. PCR Multiplex

PCR multiplex PCR is a technique used to amplify two or more genes simultaneously. In the study, the program is used to amplify the gene that is resistant to the herbicide glyphosate CP4 and soybean actin gene. Some combinations of PCR reactions performed to obtain ideal conditions to obtain PCR products for the event GTS 40-3-2 and the actin genes simultaneously in a single reaction.

### 4.5. Lethal dose of herbicide active ingredient glyphosate in soybean imports

Observations made of necrotic symptoms on leaves due to application of glyphosate herbicide active ingredient in soybean imports were obtained from four different regions and with different dose ranges showed that only 3.25% of samples after the leaves are not smeared by the herbicide resistant, the rest experience level of resistance different that 50% hold until a dose of 4.8 g / l, 32.5% hold until a dose of 9.6%, and as much as 13.75% were resistant to doses of 14.4 g / l. So that the highest dose, ie 14.4 g / l is used for the selection using the spray method. The use of high
doses of this was done to confirm the results of the selection of imported soybeans are transgenic and non-transgenic. In addition, in accordance with the nature of the active ingredient glyphosate herbicides are systemic, then the use of high doses, can accelerate the onset of symptoms in plants are sprayed.

4.6. Selection of transgenic soybean herbicide active ingredient glyphosate

From the selection by spraying herbicide showed that soybean imports are genetically modified soybean event GTS 40-3-2. It is shown from the presenting symptoms in 7 days after spraying soybean, soybean imports are to stay alive while local soybean Wilis death. Soybeans GTS 40-3-2 event are not dying because they have CP4 EPSPS genes expressed in the chloroplast where EPSPS enzymes are and where over haul of aromatic amino acids. In the presence of the CP4 gene biosynthesis process is not hindered, while delivering the amino acids needed by plants. In contrast to what happens on the local soybean Wilis who suffered death after spraying, this happens because the soybean nontransgenic has EPSPS sensitive to glyphosate, so that when it is applied herbicide active ingredient glyphosate to it, then the process of biosynthesis of shikimate into amino acids phenylalanine, tyrosine, and tryptophan will experience delays or even stalled. Besides, the nature of the systemic herbicide glyphosate causes the translocation of glyphosate throughout the plant tissue so that most of the plant parts will be damaged and eventually die seven days after spraying.

4.7. The concentration of DNA template

DNA template is one of the important components in the PCR for which there are specific sequences as an object to do amplification, so often encountered difficulty in determining concentrations must be added for optimal PCR results obtained. If in the sample used for PCR contained no DNA template, then it seems when the results of PCR in the test through gel electrophoresis is a primer-dimer (primary bonds between each other), like the ribbon that looked at the negative control. In addition, the size of concentration also affect the results of PCR, the template concentration is too little can reduce the accuracy of the amplification process, so that in the end only a few results obtained copies desired or even none at all. Small concentration also increases the risk of contamination in the solution of DNA, can be the cause of freezing, adsorption, and degradation of chemical compounds and enzymes in PCR reactants. But if the template concentration is too high can increase the risk of the emergence of undesirable products such as the misprime. From the results of testing using PCR, showed that concentrations can produce CP4 gene copies of GTS 40-3-2 soybean genome most optimum is in the range of 13-39 ng, in addition to the concentration of primer dimer only appear at the bottom after doing separation by gel electrophoresis. This is in accordance with generally where the PCR result was obtained when the concentration is 5-50 ng of DNA in each reaction (Invitrogen Corp., 2006).
4.8. The GTS 40-3-2 detection using PCR

PCR is a method with high sensitivity to reproduce a new DNA strands in vitro and allows it generates a large number of specific DNA fragments with a length and a predetermined sequence of small amounts of DNA template (template). So far no one PCR program that can be applied to all conditions, so it needs a template for PCR optimization run. In addition, errors in running the PCR may lead to not obtaining the copy, the emergence of the tape which is not expected for their misprime, primer-dimer formation which competes with specific genes that are expected in the process amplifications, and mutations.

Detection using PCR for the presence of CP4 gene is performed using 3 parts of soybean GTS 40-3-2 different, ie leaves, roots and the cotyledons, derived from soya beans after diimbibisi for 5 days. Of the three showed the presence of the CP4 gene. This happens because the CP4 gene expression in soybean event GTS 40-3-2 is regulated by the enhanced 35S (E-35S) promoter of cauliflower mosaic virus or CaMV (GM Crop Database, 2012).

4.9. The PCR multiplex

Besides the single PCR CP4, to improve the accuracy in detecting the presence of the gene then the addition of actin gene detection through multiplex PCR. PCR multiplex PCR is a technique used to amplify two or more genes simultaneously. In the study, the program is used to amplify the gene that is resistant to the herbicide glyphosate CP4 and soybean actin gene. Soybean actin gene was used as specific genes that are owned by the soybean crop (endogenous gene) or as an internal control PCR. The use of internal control is important because to show that the PCR reactants used correctly there is a template to be amplified genes. Actin is one globular multifunctional protein that forms microfilaments and can be found in all eukaryotic cells. Actin has an important role in the cell, such as cell movement, cell division and cell differentiation, movement of cell organelles, as well as giving the cell shape (Domignesh, Roberto and Holmes, Kenneth C., 2014).

V. CONCLUSION

1. Detection using spray method, by direct application of herbicide on the plant showed that soybean obtained from four different regions in Kediri, 100% live either 5 days or 7 days after spraying. This is different from the local soybean crop varieties Wilis experiencing withered 100% after 5 days and then die on the seventh day after application of glyphosate herbicide active ingredient.

2. Source of DNA isolated as a PCR template can be obtained from some parts of the leaves, roots after germinated for 5 days, and from the cotyledons. Of the three can detect the presence of gene CP4, this is in accordance with the promoter who owned CP4 at 35 S that can be found in all parts of the plant. Besides the
preparation of the material can be performed faster by using cotyledons, because only wait 3 days after seeds sown.

3. Detection using PCR can be accomplished in several programs, program optimization results show that the use of 58˚ C annealing temperature and extension time of 40 seconds is a more effective program to detect the presence of CP4 gene contained in the genome of the soybean crop. As for detecting two genes are CP4 and actin gene as a marker gene soybean plants can be run with the programmed temperature 58˚C annealing and extension of 30 seconds, the cycle is run 5 times without primer actin, after 5 cycles, new actin primer is added and the cycle is repeated 35 times.

4. To obtain optimal detection results exact DNA concentration required anyway so when aplification can be obtained copies. From the results of research to obtain the optimum required amplicon DNA genome with 39ng 13ng- range

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