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Research Article

Detection of *nptII* Gene and 35SCaMV Promoter in Tomatoes (*Solanum lycopersicum* L.)

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ABSTRACT

The detection of *nptII* (kanamycin resistance) as a transgenic marker gene and 35SCaMV as promoter in tomatoes has been carried out. DNA from tomatoes samples was isolated using *PureLink™ plant total DNA purification kit.* The purity of DNA samples was estimated using UV-Vis spectrophotometry at 260 nm and 280 nm. They gave an absorbance ratio (A_{260}/A_{280}) of 1.74-1.79 which indicated its purities. The quality of the DNA was confirmed by a clear and thick band, as analyzed in 0.8% agarose gel electrophoresis. In order to identify the transgenic tomatoes, a 786-bp fragment of the *nptII* gene and a 86-bp fragment of 35SCaMV promoter were amplified using *polymerase chain reaction* (PCR). PCR reaction was prepared at optimum condition, namely annealing temperature at 56°C and 55°C for *nptII* gene and 35SCaMV promoter, respectively and 300 ng of DNA template. The PCR results were visualized on 2% agarose gel electrophoresis. The results showed that one of three tomatoes (code ST2) contains 35SCaMV promoter and no tomatoes contain *nptII* gene, indicating that ST2 is transgenic tomato.

Keywords: tomatoes, PCR, nptII, 35SCaMV

1. Introduction

Tomatoes, a fruit crop cultivated at tropical and subtropical regions is known for its nutritional benefits and medicinal applications. It is an excellent source of many nutrients and secondary metabolites that are important for human health such as folate, potassium, vitamins C and E, flavonoids, chlorophyll, carotene, and lycopene (Dorais *et al*, 2008; Beecher, 1998).

The big problem in the world, especially in Indonesia, is that the farmers lost many yields because of insect. Many years ago, this problem was solved using pesticide and herbicide that have many negative effects to many things. However, right now, people have modified crop plants in order to reduce the use of pesticides and herbicides. In addition, some farmers used genetic modification also in order to improve the quality of food and to increase the agriculture yield (Pretty, 2001; Uzogara, 2000; Davies, 2007)

Several methods have been developed to modify crops. The common method is by introducing foreign genes into plant cells and the subsequent regeneration of transgenic plants (Dobhal *et al*, 2010). Beside the gene of interest, a selectable marker gene is also necessary to be introduced into plant cells. The gene selection allows the transgenic cell to proliferate in the presence of antibiotic or herbicide, thus ensuring convenient and easy selection of the few transformed cells in large populations of untransformed cells. *Neomycin phosphotransferase* (*nptII*) gene is the gene that encodes the enzyme having the able to inactivate kanamycin. The resistance to kanamycin is one of the most frequently used selection markers for obtaining transgenic plants (Nap *et al*, 1992; Anklam *et al*, 2002; Sundar and

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Sakthivel, 2008). In order for the transgene to be expressed effectively in its new host, it needs to be controlled by promoter sequence; the commonly used is 35S-*Cauliflower Mosaic Virus* (35SCaMV) promoter. It is a promoter with strong expression capacity and constitutive nature for dicotyl plant. This promoter is relatively common used in expressing the targeted genes in the main organs of transgenic plants, including fruits, leaves, stems and roots (Anklam *et al*, 2002; Mawgood *et al*, 2010)

There are numerous methods to identify the product of the transgenes, such as, by identifying the *nptII* gene or 35SCaMV promoter (Holst-Jensen, 2009; Gryadunov *et al,* 2011). In this research, there are three steps were done: 1) isolation DNA from plant, 2) PCR test to detect foreign DNA in transgenic plants and it uses primers that are designed to amplify DNA fragments and 3) visualization of the results using agarose gel electrophoresis.

This research has purpose to identify and find out which transgenic tomatoes are present by detecting the *nptII* gene and 35SCaMV promoter with the consideration that most of the genetically modified plants utilize *nptII* as selection gene and 35SCaMV as promoter.

2. Materials and Methods

2.1 Materials

The used samples are three tomatoes coded by ST1, ST2 and ST3. The PCR primers which are used for the identification of transgenic plant are (1) for *nptII* gene : Nfwd 5'-GAA-CAA-GAT-GGA-TTG-CAC-GC-3', Nrev 5'-GAA-GAA-CTC-GTC-AAG-AAG-GC-3' with the expected size of the product is 786 bp, and (2) for 35SCaMV promoter: Nfwd 5'TCTCCAAATGAAATGAACTTCC3', Nrev 5'TCCACT GACGTAAGG-GATGAC3' with the expected size of product 86 bp. DNA extraction buffer (100 mM Tris-HCl pH 8.5, 100 mM NaCl, 50 mM EDTA, 2% SDS), isopropanol, ethanol 70%, phosphate buffer, 2-methoxyethanol, RNAse, sodium asetate, water oil, agarose, ethidium bromide and TBE buffer were purchased from Merck. Puretaq ready to go PCR beads was from GE Health Care. *PureLink™ plant total DNA purification kit* that consists of resuspension buffer, precipitation buffer, binding buffer, wash buffer, elution buffer RNAse and SDS 20% was purchased from invitrogen.

The instruments for identification of transgenic plant were *thermal cycler* [MJ-Research (PTC-100TM0], electrophoresis [Bio Rad (Wide Minisub® Cell GT)] and spectrophotometer UV-Vis [Shimadzu UV-1700].

2.2 DNA Isolation

DNA was isolated from tomato with *PureLink™ plant total DNA purification kit* according to the manufacturer's recommendations. The extraction quality of nucleic acid was confirmed by 0.8% agarose gel electrophoresis. The nucleic acid was stained with ethidium bromide. The purity of DNA was estimated by measuring the absorbance values at 260 and 280 nm (A_{260} and A_{280}) using UV-Vis spectrophotometer (Shimadzu UV-1700).

2.3 DNA Amplification

Amplification of DNA was performed using conventional polymerase chain reaction (PCR). A-300 ng of DNA samples was subjected for PCR amplification. The amplification was performed with *puretag ready to go PCR beads* method. PCR mixtures consisted of reaction buffer (25µL), 300 ng of DNA template, *Taq* polymerase, as well as forward and reverse primers. Amplification involved 5 min at 95°C; subsequently, 35 cycles consisting 1 min at 95°C (denaturation), 1 min at 56°C (primer annealing) for *nptII* gene detection and 55°C for 35SCaMV promoter detection and 1 min at 72°C (primer extension) were performed, followed by a 5 min final extension step at 72°C. PCR was analysed on 2% agarose gel (Haitami, 2010).

2.4 Detection *nptII* gene and 35SCaMV promoter

A-2% agarose gel electrophoresis for 40 minutes at 100 volt was used to separate amplified PCR products and to characterize *nptII* gene and 35SCaMV promoter. A-100 bp DNA *ladder*, DNA plasmid *pRT101 and pCV002* as positive control for 35SCaMV and *nptII* respectively were also visualized together with samples. The gels were photographed under UV transilluminator.

3. Results and Discussion

3.1 The quality and quantity of DNA

In order to identify transgenic plants using PCR technique, the first step to be carried out is DNA isolation. The search for a more efficient methods for extracting DNA, in the mean of both high quality and high yield, has lead to the development of variety protocols, even the fundamentals of DNA extraction remain the same. DNA must be purified from cellular material in such manner to prevent degradation.

In this experiment, we procured three different samples of tomatoes. We have successfully isolated DNA from tomatoes according to procedure recommended by *PureLink™ plant total DNA purification kit*. The average isolated DNA using this protocol were 0.11, 0.12

and 0.17% DNA, respectively (Tabel 1). The purity of isolated DNA was evaluated using spectrophotometric measurements of DNA samples at 260 nm dan 280 nm. DNA samples gave an absorbance ratio (A_{260}/A_{280}) of 1.74-1.79 indicating its purity (Tabel 1).

Tabel 1. Quality and quantity assessment of isolated DNA from three different samples

No	Sampel	Total DNA %(w/w)	Rasio (A_{260}/A_{280})
1	ST1	0.11	1.79
2	ST2	0.12	1.75
3	ST3	0.17	1.74

In order to confirm the quality of the extracted DNA, a sample was run on an agarose gel, stained with ethidium bromide and visualised under UV light. Quality of DNA was confirmed with the presence of a highly resolved of high molecular weight of DNA band, which indicated good quality of DNA, as shown in Figure 1.

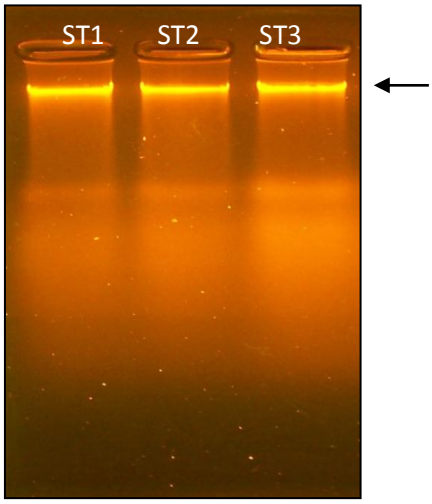

Fig 1. Profile of isolated DNA from three different tomatoes samples on 0.8% agarose gel Electrophoresis. (→) indicated the high molecular weight of total DNA fragments

3.2 Detection of *nptII* gene

The next process in this research is the identification of *nptII* gene (786 bp) existence as a transgenic marker in tomatoes using PCR technique. In this experiment, PCR amplification of isolated DNA was done using *nptII* primers. *pPCV002* DNA plasmid containing *nptII* was used as a positif control. *pPCV002* is a plant expression vector containing L_B (left border) and R_B (right border) of TDNA. The amplification process of DNA from tomatoes was performed using the optimum conditions in terms of DNA template and annealing temperature. The use of appropriate primer annealing temperature allows each primer to be annealed at the two edges of template DNA. For the sake of primer annealing temperature, the sufficient availability of DNA template is required in order to get a specific PCR product. In this experiment, 300ng of DNA template and 56°C of annealing temperature were used, as demonstrated in the previous work (Haitami, 2010). The amplified products were separated on a 2% agarose gel and visualized by ethidium bromide staining.

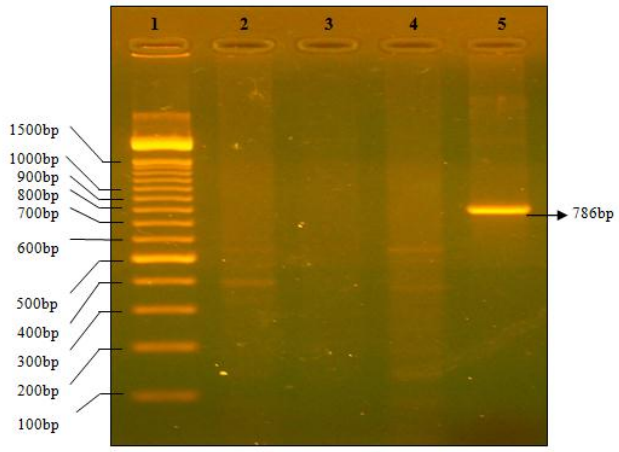

Fig 2. PCR amplification profile of *nptII* gene on tomato samples. Line 1: 100 bp DNA ladder; line 2-4: SP1, SP2, SP3, and line 5: *pPCV002* DNA as a positive control of *nptII* gene

Figure 2 showed typical results of the specific amplification at 786 bp DNA fragment of *nptII on the pPCV002* DNA plasmid used as a positif control (line 4). Furthermore, there are no amplification products appear in all evaluated tomatoes samples of SP1, SP2, and SP3 (line 1-3). It means that all of tomatoes samples do not contain *nptII* genes. However, it doesn't mean that these samples are not transgenic plants. Identification using other transgenic marker genes or promoters could also be performed. In this research, the identification of 35SCaMV as promoter was performed.

3.3 Detection of 35SCaMV promoter

The detection of 35SCaMV promoter (86 bp) in tomatoes was performed with the aid of PCR method. *pRT101* is used as positive control of 35SCaMV promoter. In this experiment, 300ng of DNA template and 55°C of annealing temperature was performed (Haitami, 2010). The visualization was done by agarose gel 2%, and the result obtained was shown in Figure 3.

The electrophoresis of PCR products using 35SCaMV primers showed a single 86 bp DNA fragment on ST2 (line3). This fragment was also found on the *pRT101* as a positive control (line 5). The other two samples, namely ST1 and ST3 (line 2, 4), the negative control (line 6) and primer (line 7) gave no amplification product of 35SCaMV.

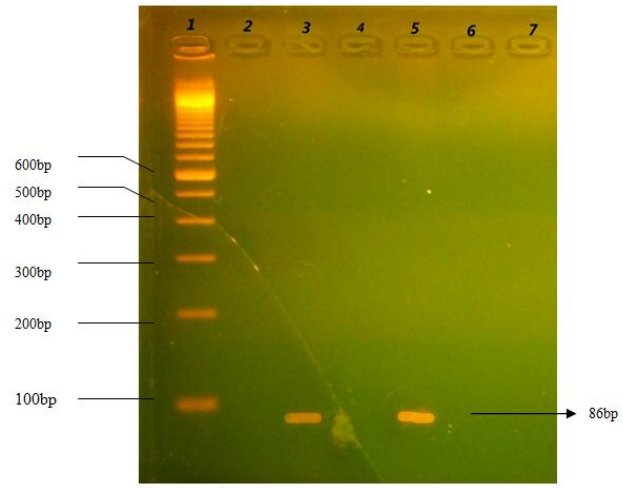

Fig 3. PCR amplification profile of 35SCaMV promoter on tomato samples. Line 1: 100 bp DNA ladder; line 2-4: SP1, SP2, SP3 tomato samples; line 5: *pRT101* as a positive control of 35SCaMV promoter; line 6: negative control; line 7: amplified primer without DNA sample

These results strongly indicated that ST2 contained a 35SCaMV promoter for expressing the gene target. Transgenic plants from tomatoes could be detected by the existence of 35SCaMV promoter, since no amplification product of *nptII*.

4. Conclusion

It can be concluded that one of three tomatoes (ST2) exhibited positive transgenic, as proven by the existence of 35SCaMV promoter. The kanamycin resistant (*nptII*) genes were not detected in all evaluated tomatoes samples.

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