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Physiologic Characteristic of Transgenic Rice (Oryza Sativa L.) Overexpression SoSUT1 Gene

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Abstract. Sucrose is major transport form of carbohydrates that used in carbon assimilation in plants (produced by photosynthesis). *SoSUT1* is a gene that encodes a protein sucrose transporter (SUT), which facilitates the process of sucrose translocation from source to sink. In the previous researched, *SoSUT1* gene transformation in the rice plants *cv.* Inpari 14 is obtained in 20 events plant with positive containing the *SoSUT1* gene. This study was aimed to characterize whether the *SoSUT1* gene that has been transformed in the rice plants can be inherited in the next generation. The study was conducted by planting T1 and T2 rice seeds in media containing Hygromycin antibiotics and for further analysis using PCR analysis. The results from the selection of 20 events T1 plant, there were 11 events T2 plant that has been confirmed containing *SoSUT1* gene and from 11 events was obtained 9 events of T3 plant

INTRODUCTION

Food is one of the basic needs of the world's population that supports human activity. Rice (*Oryza sativa* L.) is the staple food in some countries and one of them is Indonesia. Many of the strategies used in improving domestic rice productivity, one of them are through development through biotechnology. Biotechnology development to increase crop productivity through genetic engineering has been widely practiced in rice plants (Rahmawati, 2006).Assembly of high yielding varieties of rice can be done by introducing specific genes (genes with expected traits) that have been cloned and inserted into a plant cell.

Previous research has obtained genetically modified rice products with *SoSUT1*overexspression that have confirmed the presence of genes. *SoSUT1* is a cloned gene of sugar cane, this gene encodes a protein sucrose transporter. Sucrose transporter protein is a protein that facilitates the process of translocation of sucrose from source to sink (Rae *et al*., 2005). The synthesis of sucrose in rice plants in the source tissue and will be transported to the sink network, used as a source of energy during plant growth and development. (Campbell *et al*., 2002). Overexpression *SoSUT1* can increase carbon flow in plant tissues such as stems, roots, flowers and seeds of plants (Sugiharto *et al.*, 2008). *SUT* gene overexpression on pea plants can also increase the rate of accumulation of sucrose in the storage organ (Rosche. E, *et al.*, 2002).

The success of previous studies still requires further research. Gilbert *et al.*, (2009) suggests that the first generation of genetically modified plants (T1) still have heterogeneous traits. So, it is necessary to examine the next generation to obtain stable genetic modified plants. In addition, according to Christou *et al*., (1992), one of the constraints in assembling transgenic plants is not inheriting the target genes in subsequent generations because the target genes are not integrated stably into the host chromosome. The successful transformation of the gene requires further testing and selection to obtain the overexpressed *SoSUT1*GMO rice crop according to the original purpose of gene transformation. The successful transformation and stability of transgene expression can be known by analyzing

the expression of genotype and transgenic plant phenotype. The expression of plant genotype was analyzed by detecting the presence of the transgene in transgenic plant organ cells.

This study aims to find out how the physiological and biochemical or molecular characteristics of genetically engineered rice overexpression *SoSUT1*, so it is known that the potential for increased next generation of transgenic rice crops.

MATERIAL AND METHODS

Plant Material

Seed of rice crops *cv*. Inpari 14 *SoSUT1*overexspression, consisting of 20 events (1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 18, 19, 20, 21, 22, 24, 25, 28 and 29) and 1 plant *cv*. Inpari 14 wild types as control.

Screening on Transforms Seed

Approximately 20 seeds of rice from each event in generation 2 (T2) and generation 3 (T3) were screened on each jar of MS media supplemented with 25 ppm hygromycin.

Following the rapid and robust selection method designed by Harrison *et al*. (2006), the seeds were left on selection medium in 4'C for 2 days and continued with placed the jars under light for 6 h at normal conditions. Then jars were kept in the dark for another 2 days before growing in the normal conditions with 16 h day/ 8 h night (in growth chamber). The seedling grown were observed for hygromycin-resistant phenotypes, plants that have passed the selection will be acclimatized and after 7 days in the planting move to the greenhouse.

Isolation of genomic DNA

Isolation of DNA was made with gentle grind the 0.5 grams of rice leaves using mortar by adding liquid nitrogen (Zheng *et al*, 1995).The powder was added to micro centrifuge tube and added 500 mL of extraction buffer, 30 mL of 20% SDS and β-mercaptoethanol 1,25µl then the samples were incubated at 65^0C for 10 minutes. Added 300 mL of 5M potassium acetate into the solution and then in the swirling and incubated again at -20^0C for 30 to 1 hour. Centrifuged at 12,000 rpm, at 4^0C for 10 minutes. Took the supernatant and transfered it into the new micro centrifuge tube then added 375 µl of isopropanol, swirling and centrifuged. Removedsupernatant and added 300 µl TE buffer and 300 µl PCI to the pellet, then centrifuged at 12,000 rpm for 10 min at 4^0C. Tooksupernatant again, moved to the new micro centrifuge tube and added chloroform (equal volume). Transferedsupernatant to micro centrifuge tube and then precipitated for 30 minutes to 1 hour at -20 ° C by adding Isopropanol and Sodium Acetate. Removed the supernatant, added 800 µl of ethanol 70% in the pellets and centrifuged at 12,000 rpm for 10 min at 4°C. Removedagain the supernatant and dried the pellets in a dry vacuum for 15 minutes. DNA was purified with 50 mL of TE + RNA-se 1µl buffer. The purified DNA was measured in the concentrations using the Nano Value Plus device (GE Healthcare) at 260 nm λ for use in PCR analysis.

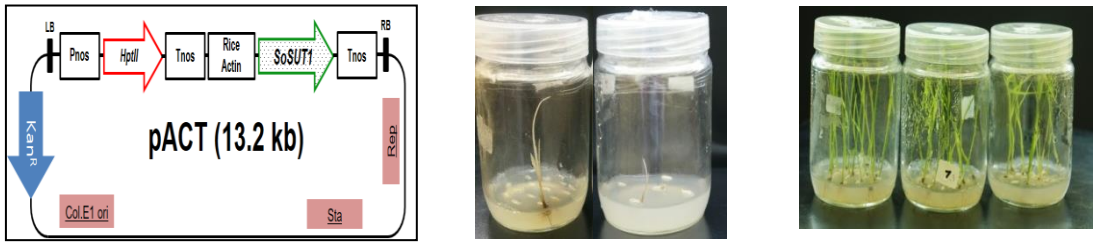
PCR Analysis

PCR analysis was performed using PCR kit from KAPA BIOSYSTEMS composed of 2X KAPA Taq Extra Hot Start DNA Polymerase, Taq Extra Buffer KAPA, dNTPs, $MgCl_2$ and stabilizer, and two inert tracking dyed. Total volume for each PCR reaction was 10 µl with PCR Master Mix 5 µl, 1µl primer (forward and reverse), 1µl genetic template and ddH_2O 2µl.

PCR was performed on 490 bp template size, using primer hptII F / R in sequence: primer hpt-F (5'-CCG CAA GGAATC GGT CAA TA-3 '), primer hpt-R (5'-CCC AAG CTG CAT CGA AA -3 '). The PCR process was performed for 40 cycles including pre-denaturation at 95 ° C for 3 min, denaturation at 95 ° C for 30 seconds, annealing at 58 ° C for 20 seconds, extending at 72 ° for 1 minute and extension at 72 ° C for 5 minutes. The results were analyzed by electrophoresis on 1% agarose gel that was containing with 3 µl ethidium bromide at 100 V for 25 minutes. The DNA marker was used 1 Kb Ladder marker of 2 µl to see the size of the DNA amplified. The electrophoresis results seen in the UV trans illuminator.

RESULT AND DISCUSSION

Plant seeds are planted in the selection medium. This aimed for selecting plants which is non-transformants using Hygromycin antibiotics. Hygromycin has toxicity to the plants, but the plants will be survived if thereare resistance gene to hygromycin such as overexpressing *SoSUT1* rice due to the presence of the hpt II gene in the plasmid pAct-*SoSUT1*. The plasmid constructs encoding the Hygromycin 3'phosphotransferase enzyme that was played a role in hydroxyl group phosphorylation on hygromycin antibiotics, which can make the hygromycin was inactive and toxic to the plants (Arago and Brasileiro, 2002).



(a) (b) (c)

FIGURE 1. (a). construct plasmid pACT-SoSUT1, (b). Symptoms of chlorosis, (c). Plant pass the selection

In figure b seen some rice plants that shownthe symptoms of the chlorosis. The Symptoms of the chlorosis are caused by the plant that does not have the *hptII* gene so the hygromycin becomes toxic. The hygromycin may inhibit plant growth by inhibiting the protein synthesis process present in the translocation of tRNA and mRNA that were associated with the elongation factors.The plants were not contained of the *hptII gene*, will be inhibited by its growth and its color has changedto white (albino) (Ee *et al*., 2014) and also the ultimately died (Gritz and Davies, 1983).

PCR analysis of the T2 and T3 generations were aimed to find out whether the *SoSUT1* gene that has been inserted in previous studies are still be presented in the genome of the next-generation. One of the obstacles in the assembly of transgenic crops is the lack of stable integration of the target genes within the host's chromosomes, thus causing them to be inherited from the next generation (Christou *et al*., 1992).

In the previous research, there were 20 events of T1 generation which is passed of the selection and PCR confirmation. In next-generation screening was obtained of 11 eventsfrom T2 generations and 9 events of T3 generation. The positive transgenic*SoSUT1*has been shown at 490 bp based from theDNA amplified with the pAct-*SoSUT1*plasmid as thepositive control.

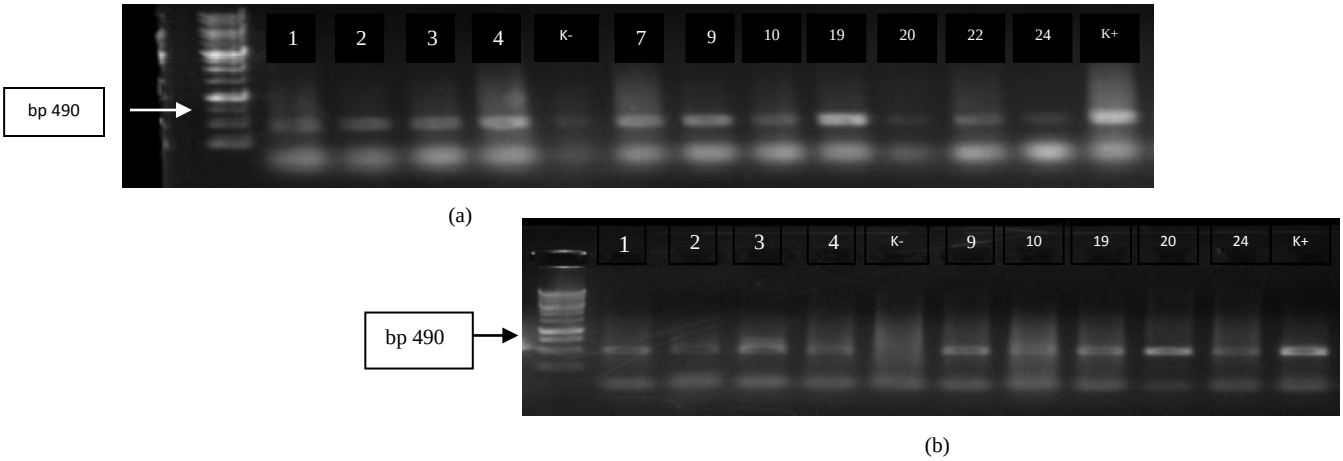


FIGURE 2. (a) Dna band in T2 generation, (b) Dna band in T3 generation

Based on Figure 2 (a) and 2 (b) the formation of the *hptII* DNA bands measuring 490 bp that the *SoSUT1* target genes which are in one T-DNA place with *hptII* as selectable markers have been inserted into the plant genome. Whereas in the negative plants did not form DNA bands at 490 bp due to the un-integration of the *SoSUT1* gene into the plant genome. The incident is suspected because the plant experienced chimera. Chimera is the spreader of target genes transformed unevenly across tissues (Yasmeen *et al*., 2009), chimeras may cause the target genes not to be inherited in the next generation, so in *SoSUT1*overgenerated genetic modified rice there are plants that do not contain the transformed genes

SUMMARY

Transformed plants require further testing to see if the genes inserted can be passed on to the next generation. As in the new stable SoSUT1 overexpressed rice in the 3rd generation (T3). But this study still requires more specific follow-up tests such as expression test of translational and transcription rates and research in terms of plant morphology.

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