ISBN: 978-602-9030-88-4

Revealing the Secrets of Life Through Protein and Peptide

PROCEEDING

INDONESIAN PROTEIN SOCIETY (IPS) INTERNATIONAL SEMINAR AND WORKSHOP 2014

October 29-30, 2014

Jember, Indonesia

Editors
Hardian Susilo Addy
Miswar
Jayus
Tri Agus Siswoyo
Maurice Ku
Toshiharu Hase

Membangun Generasi Menuju Insan Berprestasi

Center for Development of Advanced Sciences and Technology, University of Jember

Indonesia Protein Society (IPS)

Analysis AZF Gene Deletions in Infertile Men in Indonesia
Evi Hanizar, Aucky Hinting 51

Review: Phylogenetic Similarity based on amino acid sequence and Molecular characterization of 3-Phytase from *Klebsiella pneumonia* ASR1
Sajidan and Hailu Weldekiros Hailu 59

Overexpression Sucrose Transporter Protein (Sut) and Sucrose Content In Genetically Modified Product (Gmp) Sugarcane(*Saccharum officinarum* L.)
Parawita Dewanti, Purnama Okviandari, Nina Oktaria dan Bambang Sugiharto 67

Imunogenic Protein of Salivary Gland from *Anopheles sundaicus*
Yunita Armiyanti, Moh. Mirza Nuryady, Sugeng Setyo Utomo, Teguh Wahju Sardjono, Loeki Enggar Fitri, Kartika Senjarini 76

Biochemical Resistance Mechanism of Several Genotype of Soybean to Rust Diseases
Moh. Setyo Poerwoko, Endang Budi Trisusilowati dan Amarullah 82

Mineral Nitrogen in Soil of Sugarcane Plantation of PG Jatiroto
Ketut Anom Wijaya 89

Physical Properties of Gel and Edible Plastic from Whey and Tapioca In Various Ratio and pH Value
Triana Lindriati, Herlina, Ahmad Nafi 93

Specific sequence of Plasmodium falciparum DBL domains associated with severe malaria outcome
Erma Sulistyaningsih, Loeki Enggar Fitri, Thomas Loescher, Nicole Berens-Riha 102

Zinc Biofortification of Rice Using Fish Protein Hydrolysates Mixed With Zinc Sulfate.
Achmad Sjaifullah 107

The Potency of Protein Extracts fromCandida albicansas Bioreceptor on Immunosensor forDiagnosis of Candidiasis
Masfufatun, Noer Kumala and Afaf Baktir 111

Exploration of Lipase Enzyme from Soil through Metagenomic Approach
Sri Sumarsih,Afaf Baktir, Budi Putri Ayu Andina 117

Optimization pH of Enzymatic Hydrolysis of Endo-1,4-β-Xylanase for Xylooligosaccharides Production
Anak Agung Istri Ratnadewi, Andika Ade Kurniawan, Wuryanti Handayani 124

Transformation of Plasmid pET Endo-1,4-β-xilanase from *E. coli* TOP10 to *E. coli* BL21
Agung Budi Santoso, Eka Yuni Kurniawati, AA Istri Ratnadewi 130

Indonesian Protein Society
Proceeding of International Seminar and Workshop on Protein
Jember, October 29-30, 2014

Research Article

Overexpression Sucrose Transporter Protein (Sut) and Sucrose Content In Genetically Modified Product (Gmp) Sugarcane (*Saccharum officinarum* L.)

Parawita Dewanti[1,3], Purnama Okviandari[2,3], Nina Oktaria[2,3] dan Bambang Sugiharto[2,3]

[1]*Agriculture Faculty University of Jember,* [2]*Science and Technology Faculdulty University of Jember and* [3]*CDAST (Center for Development Advance of Sciences Technology) Universitas Jember*

Corresponding e-mail : parawita@yahoo.co.id, Hp 08123463272

ABSTRACT

Sucrose transporter (SUT) is a protein that functions to translocate sucrose from source to sink. Protein activity is determined by the accumulation of sucrose in sugarcane stem. The level of sucrose accumulation is also influenced by the activity of sucrose phosphat synthase (SPS) and neutral invertase (NI) in conjunction with the synthesis and hydrolysis of sucrose. This aim of this study is to determine the sucrose transporter protein expression in Genetically Modified Product (GMP) of sugarcane.Genetically Modified Product GMP sugarcane events 1, 2, 3, 4, 5, 18, 20 and non GMP were used as materials. The analysis was performed by Western Blot method to observe the presence of SUT protein expression, stem sucrose content by resorcinol method to observe the translocation of sucrose, the SPS enzyme activity and NI that play a role in determining the accumulation of sucrose.The results showed that (1) an increase in SUT protein content in GMP plants events 2 and 18 with a protein band that is thicker than the non GMP plants, (2) SPS and invertase activity at all events GMP crop plants to increase compared to non GMP. Sucrose content of the stem at all events GMP plant to increase with age segment. The older age segments, the higher the sucrose content.

Keywords: sugarcane(Saccharum officinarum L.), geneticallymodifiedproduct(GMP), sucrosetransporterprotein(SUT), sucrose

INTRODUCTION

Sugarcane is a crop that can be grown in the tropics and sub-tropics. These plants are included in C4 plants that can accumulate sucrose in the stem (Bieleski, 2000; Bonnet et al., 2004). Sucrose is the major product of photosynthesis produced by carbon fixation that occurs in the leaves (Buchanan et al., 2000). Sucrose metabolism in leaves is influenced by several enzymes, such as sucrose phosphate synthase (SPS), sucrose synthase (Susy), and invertase (INV) (Hubbard et al., 1989).

SPS is a major enzyme in the biosynthesis of sucrose (Huber and Huber, 1996). SPS catalyzes the conversion of fructose-6-phosphate and UDP-glucose to form sucrose-6-phosphate, phosphate hereinafter contained in sucrose-6-phosphate is hydrolyzed by sucrose phosphate

phosphatase to produce sucrose and organic phosphate (Langenkamper et al., 2002). The amount of SPS activity can increase the accumulation of sucrose and growth of sugarcane (Sugiharto et al., 1997).

Sucrose is synthesized in the leaves, some will be hydrolyzed to produce energy. Invertase (INV) is an enzyme that acts to hydrolyze sucrose into glucose and fructose (Jin et al., 2009). Sugarcane has two types of invertase, which is neutral invertase (NI) and acid invertase (AI) that have different functions in the accumulation of sucrose (Gayler and Glasziou, 1972). According Miswar et al. (2007), NI activity in the leaves can affect to the accumulation of sucrose in the trunk.

MATERIAL AND METHODS

Plant materials.

Eventsof transgenic sugarcane plants 1, 2, 3, 4, 5, 18, 20 and non-transgenic var. BL from the CDAST (Center for Development Advance of Sciences Technology) Laboratory University of Jember. The first unfolded leaves samples were used for the protein and sucrose extraction.

Protein Extraction (Soluble proteins).

Eroded2grams of leaf samples by adding liquid nitrogen. Sample delicate leaves that have been given 6 ml of extraction buffer consisting of 50 mM MOPS/NaOH (pH 7.4), 10 mM MgCl 2, 1 mM EDTA, 2.5 mM DTT, 10 lm PMSF, and 10% PVP, and crushe dagain until homogeneous. The extractwas centrifuge dat a speed of 12,000 rpm, 4 °C, for 10 minutes. The supernatant was filteredby column chromatography *SephadekG-25 Plannacia*. Eluate which exitis used for the measurement of enzyme activity of SPS and neutralin vertaseand total soluble protein (TPT).

Protein Extraction (insoluble protein).

Eroded3grams of leaf samples by adding liquid nitrogen. Sample delicate leaves that have been given 15 ml of extraction buffer on sisting of 0.4 M sucrose, 75 mM MOPS/KOH, 5 mMEDTA/KOH, 5 mMEGTA/KOH, n 10 mM NaF, 5 mM β-mercapto and 5% PVP,thencentrifugedat a speed of9000rpm for10min4° Ctoobtaina supernatantand apellet(fraction 1). Obtained supernatant was centrifuged againat a speed of 14,000 rpm for 30 minute stoobtaina pellet (fraction 2). Pellet (fraction 2) was dissolved in 250 mL of solubilization buffer (100 mM Tris-HCl pH 7.5, 2% SDS, 1 mM EDTA, and 10 mM β-mercaptoethanol) and storedat- 80 °freezerfor SUT1 protein analysis by the method of Western Blot.

Measurement of Total Protein Dissolved (TPT).

Total soluble protein was determined by the Brad ford's method (1976). Standard proteins used Bovine Serum Albumin (BSA) with aprotein content of 0 mg, 5 mg, 15 mg, 30 mg, 50 mg. Brad for dreagent taken as many as 950 mL and added H_2O until the volumereaches 1 ml (as blank). Samples were taken 20 mL soluble protein, plus 30 mL of H_2O and 950 mL Reagent Bradford. Colors are formed from the mixture was measured with a spectrophotometerat a wavelength of 595 nm. Samplesin soluble proteins (SUT1) were taken 10 mL to measure total protein, then coupled with Brad for dreagentas much as 990 mL. Colors are formed from the mixture was measured with a spectrophotometerat a wavelength of 595 nm.

SPS enzyme activity measurements.

SPS activity was measured using a test solution with a composition of 86 mM MOPS / NaOH (pH 7.4), 26 mM MgCl 2 were taken as much as 40 mL. This solution was mixed with 10 mL of 0.1 mM fructose-6P, 10 mL of 0.1 mM Uridine Difosfo glucose (UDP), 10 mL of 0.1 mM glucose-6P and 50 mL sample of the enzyme. The mixture was incubated at 30 ° C for 0 minutes,

10 minutes, and 20 minutes. The enzyme reaction was stopped by the addition of 70 mL of 1 N NaOH at the specified time then samples were heated in boiling water for 10 minutes. After cold, samples coupled with a 0.1% resorcinol in 95% alcohol \ 250 mL and 750 mL of 30% HCl. Furthermore, the solution was incubated at 80 ° C for 8 minutes, then after cold the color formed were observed . The intensity of the color formed is the sucrose content, measured by a spectrophotometer at a wavelength of 520 nm. Absorbance obtained divided by the length of time of incubation to obtain the average absorbance per unit time. Furthermore, the average absorbance values entered on the standard equation of sucrose to obtain the value of the activity of SPS (g sucrose / min). SPS activity divided by the total soluble protein (TPT) is a specific activity of SPS (g sucrose / min / mg protein).

Measurement of Enzyme Activity Neutral Invertase.

Measurement of neutral invertase activity carried out using a solution containing 25 mM testers MOPS / NaOH pH 7.5 and 100 mM sucrose. This solution was taken as 450 mL and mixed with 50 mL of enzyme samples so that the total volume of 500 mL. The mixture was incubated at 30 ° C for 0 minutes, 15 minutes, and 30 minutes. The enzyme reaction was stopped by the addition of 1000 mL of dinitro salicylic acid (DNS) at the specified time, then heated in boiling water for 10 minutes. The intensity of the color formed indicates the amount of glucose formed and measured with a spectrophotometer at a wavelength of 560 nm. Absorbance obtained divided by the length of time of incubation to obtain the average absorbance per unit time. Furthermore, the average absorbance values entered on glucose standard equation to get the value of the activity of invertase (g glucose / min). Invertase activity divided by the value of total soluble protein (TPT) is a specific activity of invertase (g glucose / min / mg protein).

Western Blot Analysis.

Western blot analysis performed by the method of Towbin Deutscher (1990). Western blot analysis performed after the proteins are separated by molecular weight by SDS-PAGE (Sodium Dodecyl Sulphate Gel Polyacrilamide Eleskrophoresis). SDS-PAGE analysis carried out with a total protein concentration of 40 mg. Protein separation results by SDS-PAGE transferred to nitrocellulose membranes through the flow of electricity at 250 mA for 2 h, 4°C, then the membrane was washed with TBS (Tris Buffered Saline) 3 times each 5 minutes. After washing, proteins are undesirable, closed (blocking) by soaking in TBS with 0.5% skim milk for 30 minutes, then given primary antibody (antibody SUT1) and incubated on a shaker for one night. The next process is the membrane was washed again with TBS, and then given a secondary antibody and incubated for 1 hour. Before staining, the membrane was washed again with TBS and alkaline phosphate. Staining was done by administering 25 mL and 50 mL NBT BCIP were dissolved in 10 ml of alkaline phosphate.

Measurement of Sucrose Content of Leaves and Stems.

About 1 g crushed leaf samples were dissolved in 5 ml of MCW (Methanol Chloroform Water) and incubated at 60°C for 10 minutes. Samples centrifuged at 5000 rpm, 4°C, for 10 minutes. Supernatant were accommodated in a falcon, this treatment was repeated until the remaining pellets turned into white. Supernatant obtained was evaporated until the methanol chloroform evaporates, the remaining solution was used for the analysis of sucrose. Samples stem as much as 3 g of crushed and dissolved in 3 ml of distilled water. Sample centrifuged at 10,000 rpm, 4°C, for 10 minutes. Obtained supernatant was measured sucrose rod.

Tests carried out using the method Seliwanoff sucrose. Sucrose samples of leaves and stems of 50 mL was added 70 mL of 1 N NaOH, heated in boiling water for 10 minutes. Once cool, coupled with a 0.1% resorcinol in 95% alcohol as much as 250 mL and 750 mL of 30% HCl and

incubated at 80°C for 8 minutes. Color formed was measured with a spectrophotometer at a wavelength of 520 nm. Absorbance obtained calculated with standard formulas to obtain the concentration of sucrose.

RESULT AND DISCUSSION

SPS enzyme activity analysis results show that all gene overexpression transgenic sugarcane plants ScSUT1 SPS activity tend to increase compared to control plants. The highest SPS activity were obtained at 4 events with 1.296 g sucrose / min / mg proteinSPS activity value. High SPS activity was also present in the event 1 and 5 respectively 1.209 g sucrose / min / mg protein and 1.159 g sucrose / min / mg protein. The increase of SPS activity in other transgenic plants events tend to be lower than the event 4, 1, and 5, it means that SPS activityincreased more slightly than to control plants. The value of SPS activity at Event 2, 3, 18, and 20 respectively 1.116 g sucrose / min / mg protein, 1.015 g sucrose / min / mg protein, 1.070 g sucrose / min / mg protein, and 1.027 g sucrose / min / mg protein.

SPS enzyme is a key enzyme involved in the biosynthesis of sucrose in plants. SPS catalyzes the conversion of fructose-6-phosphate and UDP-glucose to form sucrose-6-phosphate, phosphate hereinafter contained in sucrose-6-phosphate is hydrolyzed by sucrose phosphate phosphatase to produce sucrose and organic phosphate (Langenkamper et al., 2002). According Miswar et al. (2007), the high activity of SPS will be able to produce the high sucrose.

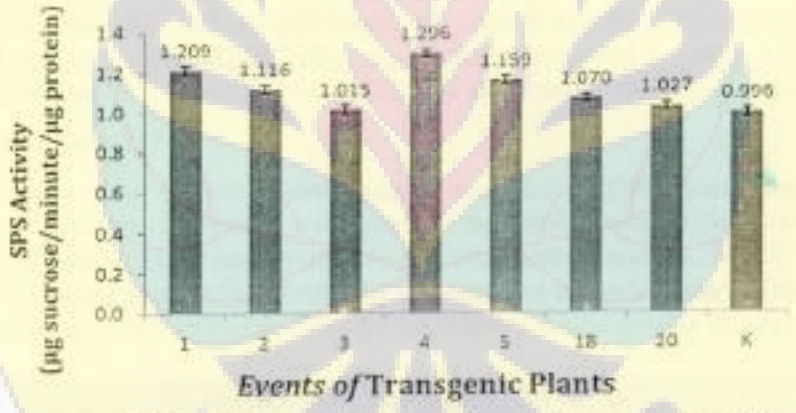

Figure 4.1 The value of SPS activity (Event 1, 2, 3, 4, 5, 18, and 20: transgenic plants; K: control plants).

Enzyme Activity Neutral Invertase (NI)

Remodel invertase hydrolysis of sucrose by reaction irreversible (irreversible) into glucose and fructose. Invertase analyzed were neutral invertase (NI). NI activity values showed the amount of glucose formed per unit time (g glucose / min / mg protein). From these results, it can be seen that the gene overexpression transgenic sugarcane plants ScSUT1 NI tend to have the higher activity than the control plants.

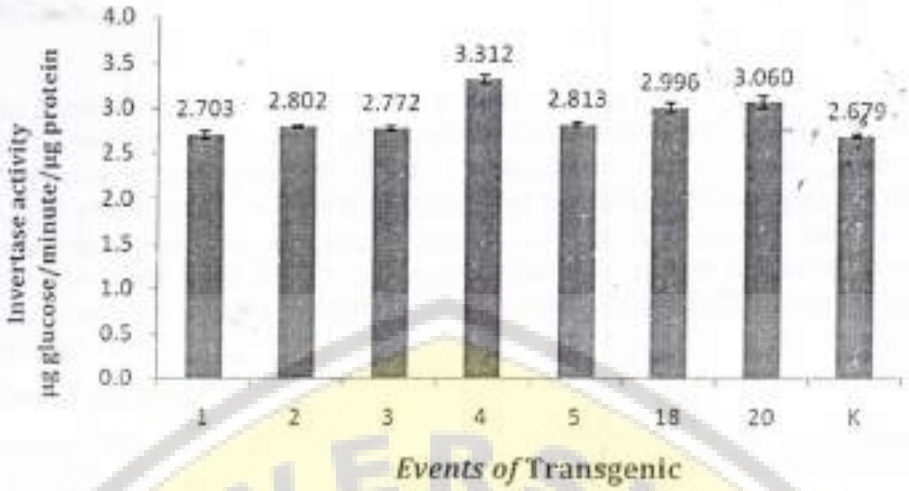

Figure 4.2 The value of NI activity (Event 1, 2, 3, 4, 5, 18, and 20: transgenic plants; K: control plants).

Based on Figure 4.2, the highest NI activity presented by Event 4 with a value 3,312 µg glucose/menit/µg protein, followed by Event 20 dan Event 18 with the value 3,060 µg glucose/menit/µg protein dan 2,996 µg glucose/menit/µg protein respectively. Transgenic plants overexpression SoSUT1 gene tend to have higher activity values than the control plants. NI activity will reduce the sucrose content in the leaves because NI will hydrolyze sucrose into glucose and fructose. The difference between SPS and NI activity will determine the amount of sucrose in leaves (Miswar et al., 2007). Partially hydrolyzed sucrose is used as an energy source in the leaves, and some will be transported in the form of sucrose to the storage tissue.

Content of Protein SUT1

Increased protein content in plants can be done through the overexpression of genes or DNA. SoSUT1 gene overexpression in sugarcane is expected to increase the protein content SUT1, in conjunction with increased translocation and accumulation of sucrose. SoSUT1 gene expression in the form of the protein can be detected by Western blot analysis. Western blot analysis can be used to detect the protein content through the thickness of the protein bands appear.

Figure 4.3 Western Blot of SUT1 Protein result (Event 1, 2, 3, 4, 5, 18, and 20: transgenic plants; K: control plants).

The results of Western blot analysis of protein SUT1 can be seen in Figure 4.3. Based on these images, SUT1 proteins can be detected in all gene overexpression transgenic sugarcane and sugarcane SoSUT1 control. From this result, not all gene overexpression transgenic sugarcane plants SoSUT1 increased compared to control plants. The increase of SUT1 protein content in overexpression transgenic sugarcane plants SoSUT1 genes was obtained atEvent 2 and Event 18. Increased protein content can be seen from the thickness of the protein bands appear. The thicker

the protein bands appear, the more the protein content. In the Event 2 and Event 18 showed thicker protein bands than the control plants, which means Event 2 and Event 18 having more SUT1 protein content than the control plants. Overexpression SoSUT1 gene caused increased protein content which can increase the transcription and translation. This suggests that the protein is integrated into the genome of sugarcane can be translated into proteins. Increased protein content SUT1 the Event 2 and Event 18 can be connected to the sucrose content of the stem. This relates to the function of proteins that play a role in the translocation SUT1 sucrose to the storage network (trunk).

SucroseContent ofLeaves

Sucrose content of the leaves is not only influenced by the level of sucrose synthesis in the leaves, but also the presence of invertase activity in the leaves that can hydrolyze sucrose. Sucrose content of the leaves was analyzed from transgenic plants and control plants.

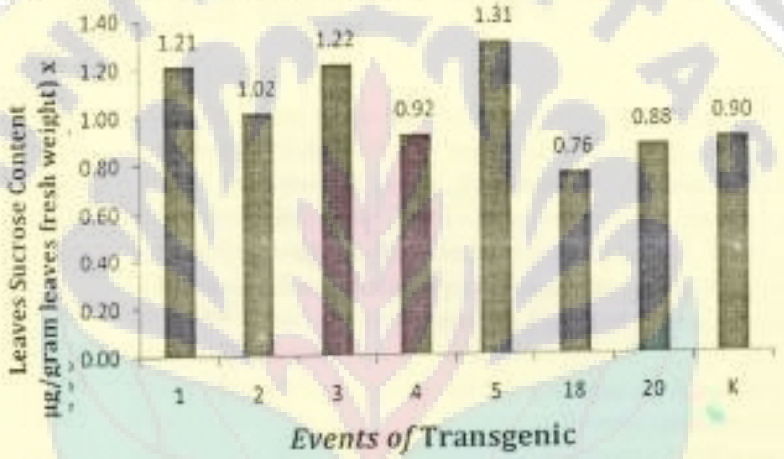

Figure 4.4 The value of leaves sucrose content (Event 1, 2, 3, 4, 5, 18, and 20: transgenic plants; K: control plants).

Leaf sucrose content value can be seen in Figure 4.4. The figure shows that the content of sucrose in most SoSUT1 gene overexpression transgenic plants tend to have increased compared to control plants. The content of sucrose in the leaves is affected by the SPS and invertase activity. The highest invertase activity was found in the Event 4 at the value 1,296 g sucrose / min / mg protein and 3,312 g of glucose / min / mg of protein, but the sucrose content of leaves in 4 events tend to be low, amounting to 9242.76 g / gram weight of the leaves. This shows that the high SPS activity, the high invertase activity, but the accumulated sucrose in the leaves were low.

The content of sucrose trunk (Nira)

Sucrose content of the trunk is the end result of the accumulation of sucrose. Sucrose content of the trunk is determined by the activity of protein transport sucrose (SUT1) that transport sucrose from the leaves toward the stem. Sucrose accumulation in sugarcane starts from the growth stem segments towards the stopped growth stem segments.

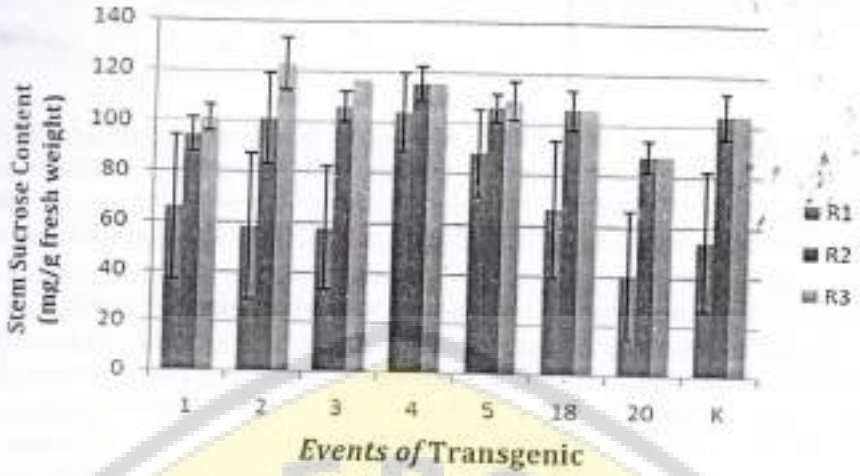

Figure 4.5 The average of sucrose trunk content (mg/ g fresh weight), R1: the average of sucrose content at the beginning 5 segments; R2: the average of sucrose content at the secondary 5 segments; R3: the average of sucrose content at the end (third) 5 segments; (Event 1, 2, 3, 4, 5, 18, and 20: transgenic plants; K: control plants).

The value of sucrose content in the trunk can be seen in figure 4.5. Based on these figure showed that stem sucrose content increased in the older section at all events SoSUT1 gene overexpression transgenic plants and control plants. The increase of stem sucrose content is proportional to the age of stem segmentsdirectly. The older age segments, the higher the content of sucrose. These results are consistent with studies conducted by Zhu et al., (1997), which states that the sucrose content will increaseof the older segmentbecause the younger segment requires more energy for growth. The energy provided by sucrose as a carbon source, so that the content of sucrose in the young segment will be lower.

In the Event 4, the sucrose content of the trunk on the first five sections tend to be higher than any other event, amounting to 104.13 mg / g wet weight. If connected with the process of synthesis and hydrolysis, Event 4 has a high SPS activity in the amount of 1.166 g sucrose / min / mg protein and invertase activity are quite high at 2,745 g glucose / min / mg protein. The high activity of invertase in leaf SPS and can reduce the amount of sucrose in the leaves. Event 4 has a lower content of sucrose leaves compared than the control, the amount of 2532.20 mg / g wet weight of the sample. Viewed from the side of the expression, gene SoSUT1 in events can be detected by Western blot analysis. Protein bands that emerged showed that SUT1 gene integrated into the genome of sugarcane can be translated into proteins, and can facilitate the transport of sucrose to the stem. Sucrose content of the rods in the event 4 tends to be high on the road is still young (five first segment), and it increases with age stem segments. This can be caused by sucrose accumulates in the young segment tends lot. Although partially hydrolyzed to a source of energy, but the Event 4 has a high SPS activity so that sucrose can continue to be fulfilled.

In the Event 5, SUT1 protein was not increased as indicated by the protein bands were thinner than the control. These results relate to the sucrose content of the stem, although not increase the protein content of SUT1, sucrose content in Event 5 tend to be high in the first five segments (youngsegments), and more increase together with age segment. This suggests that the sucrose transport activity can occur even though there was no increase in protein content SUT1. Basically plants already have sucrose gene transporter, but the presence of overexpression SoSUT1genes were expected can increase the SoSUT1 gene expression and increase the rate of sucrose content.

Based on the description above, the metabolism of sucrose in plants include the synthesis, hydrolysis, and translocation. Sucrose synthesis that occurs in the leaves is catalyzed by the enzyme Synthase Sucrose Phosphate (SPS). The high activity of SPS in the leaves, can increase the sucrose content in the leaves. Sucrose which has formed will be a source of carbon to produce the energy needed by the plants. The process to produce energy in the form of ATP can not directly involve the sucrose which is still in the form of a disaccharide, but it must be hydrolyzed to be monosaccharide sucrose, fructose and glucose. This hydrolysis process is catalyzed by the enzyme neutral invertase (NI) contained in the leaves. The difference between the synthesis and hydrolysis that occurs in the leaves will determine the sucrose content in the leaves. Sucrose in the leaves will be transported to the storage tissue, namely trunk. This translocation process mediated by sucrose transporter proteins (SUT1).

Tissue storage in sugarcane are in the trunk, so that the sucrose content of the trunk is the end result of the process of sucrose metabolism. The content of sucrose in the stem can be used as a food reserve. High sucrose content of the trunk can be obtained if high leaf SPS activity, whereas NI activity is low, and high protein content SUT1. Small difference between the synthesis and hydrolysis of sucrose causes the sucrose content in the leaves becomes high. It also must be supported by many SUT1 protein content in plants that can facilitate the process of translocation of sucrose to the storage network (trunk).

The results showed that all events SoSUT1 gene overexpression transgenic plants tend to increase the activity of SPS and NI. Based on Western blot analysis, all the events showed the expression of genes SoSUT1 characterized by the appearance of protein bands, but only event 2 and event 18 that increasing the protein content SUT1 because it has protein bands were thicker than the control plants. Increased protein content SUT1 not comparable with the increase in enzyme activity of SPS and NI. This is shown in event 2 and 18 who only have SPS activity of 1.116 g sucrose / min / mg protein and 1.070 g sucrose / min / mg protein, whereas NI activity of 2.802 g glucose / min / mg of protein and glucose 2.096 / min / g of protein. SPS and NI activity is highest in the event the value of 1.296 ug 4 with sucrose / min / mg of protein and glucose 3.312 / min / mg protein, but this event is not to increase the protein content SUT1 because protein ribbon thinner than the control plants. This suggests that the overexpression of genes in sugarcane SoSUT1has no effect to enzyme activity of SPS and NI. Sucrose content of the trunk at all events SoSUT1 gene overexpression transgenic plants tend to have increased compared to control plants.

CONCLUSION

The results showed that not all events gene overexpression transgenic plants SoSUT1 increased protein content SUT1, only Event 2 and 18 that increase the protein content SUT1 because it have thicker protein bands than the control plants. While the SPS and invertase activity at all events SoSUT1 gene overexpression transgenic plants tend to have increased compared the control plants. Sucrose content of the trunk at all events SoSUT1 gene overexpression transgenic plants also tend to increase with age segment. The older age segments, the higher content of sucrose.

REFERENCE

Bieleski, R. L. 2000. The Bigger Picture Phloem Seen Through Horticultural Eyes. Plant Physiol, 27: 615-624

Bonnet, G., Casu, R., Rae, A., Grof, C., Glassop, D., McIntyre, L., and Manners, J. 2004. Identification of Genes Contributing to High Sucrose Accumulation in Sugarcane. New Directions for a

Diverse Planet: Proceedings of the 4th International Crop Science Congress [on line] http ://www. cropscience. org. au/icsc2004/poster/3/2/1/1843_bonnett. htm.[7 April 2012].

Buchanan, B. B., Gruissem, W., and Jones, R. L. 2000. Biochemistry & Molecular Biology of Plants. United States of America: American Society of Plant Physiologists

Gayler, K. R. and K. T. Glasziou. 1972. Physiological Function of Acid and Neutral Invertase in Growth and Sugar Storage in Sugarcane. Plant Physiol, 27: 25-31.

Hubbard, N. L., S. C. Huber., and D. M. Pharr. 1990. Role of Sucrose Phosphate Synthase in Sucrose Biosynthesis in Ripening Bananas and Its Relationship to The Respiratory Climacteric. Plant Physiol, 94: 201-208.

Hubbard, N. L., Huber, S. C., and D. M. Pharr. 1989. Sucrose Phosphat Synthase and Acid Invertase at Determinant of Sucrose Concentration in Developing Musk Melon (*Cucumis melo*) Fruits. Plant Physiol, 91: 1529-1534

Hubbard, N. L., D. M. Pharr., and Huber, S. C. 1991. Sucrose Phosphate Synthase and Other Sucrose Metabolism Enzymes in Fruit of Various Species. Plant Physiol, 82: 191-196

Huber, S. C. and Huber, J. L. 1992. Role of Sucrose Phosphate Synthase in Sucrose Metabolism in Leaves. Plant Physiol, 99: 1275-1278

Huber, S. C. and Huber, J. L. 1996. Role and Regulation of Sucrose Phosphate Synthase in Higher Plants. Annu. Rev. Plant Physiol. Plant Mol. Biol, 47: 431-444

Jin, Y., An Ni, D., and Ruan, Y. L. 2009. Posttranslational Elevation of Cell Wall Invertase Activity by Silenciing Its Inhibitor in Tomato Delays Leaf Senescence and Increases seed Weight and Fruit Hexose Level. The Plant Cell, 21: 2072-2089.

Langenkamper, G., Fung, R. W. M., Newcomb, R. D., Atkinson, R. G., Gardner, R. C., and Macrae, E. A. 2002. Sucrose Phosphate Synthase Genes in Plants Belong to Three Different Families. Journal of Molecular Evolution, 54: 322-332

Miswar, Sugiharto, B., Handoyo, T., dan Made, S. A. 2007. Peranan Sucrose Phosphate Synthase (SPS) dan Acid Invertase (AI) Internoda Tebu (*Saccharum officinarum* L.) dalam Akumulasi Sukrosa. Agritrop, 26: 187-193

Sugiharto, B., Handoyo, T., dan Sumadi. 1997. Variabilitas Genetik Dalam Enzim Fotosintetik dan Enzim Metabolisme Sukrosa pada Beberapa Varietas Tebu (*Saccharum officinarum* L.). Zuriat, 7: 76-85

Sugiharto, B dan Safitri, H. 2011. A Comparison Study for Agrobacterium-mediated Transformation Method in Sugarcane (*Saccharum* spp L.). Jurnal Ilmu Dasar, 12: 140-147

Zhu, Y. L., E. Komor and P. H. Moore. 1997. Sucrose Accumulation in The Sugarcane Stem is Regulated by The Difference Between The Activities of Soluble Acid Invertase and Sucrose Phosphate Synthase. Plant Physiol, 115: 609-616

CERTIFICATE

given to

Dr. Ir. Parawita Dewanti, MP

has participated as a

Oral Presenter

in the International Seminar and Workshop
Indonesian Protein Society (IPS) 2014 on
"Revealing The Secret of Life Through Protein and Peptide"
October, 29-30 2014 at the University of Jember

Chairman of Organizing Committee

Dr. Ir. Jayus

Rector
University of Jember

Drs. Moh. Hasan, M.Sc., Ph.D