



Increased activity of sugarcane sucrose-phosphate synthase in transgenic tomato in response to N-terminal truncation

Siti Nurul Afidah^{1,2}, Inyana Dwi Agustien², Parawita Dewanti³, Bambang Sugiharto^{1,2,4,*}

¹Biotechnology Master Study Program, University of Jember, Jember 68121, Indonesia

²Center for Development of Advanced Sciences and Technology (CDAST), University of Jember, Jember 68121, Indonesia

³Faculty of Agriculture, University of Jember, Jl. Kalimantan No. 37, Jember 68121, Indonesia

⁴Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember, Jember 68121, Indonesia

*Corresponding author: sugiharto.fmipa@unej.ac.id

SUBMITTED 23 September 2021 REVISÉD 21 November 2021 ACCEPTED 7 December 2021

ABSTRACT Sucrose-phosphate synthase (SPS) is a key enzyme catalyzing the formation of sucrose-6-phosphate through the transfer of uridine-diphosphate glucose (UDP-G) as a donor to fructose-6-phosphate (F6P) as an acceptor. Plant SPS consists of three main domains: N-terminal, glycosyltransferase, and C-terminal domains. Among these, the N-terminal domain is involved in regulating the allosteric activator glucose-6-phosphate (G6P). This study was directed toward determining the regulation and characterization of N-terminal truncated SPS in transgenic tomato. In this study, the N-terminal truncated mutant of sugarcane SPS (Δ N-SoSPS1) and full-length sugarcane SPS (FL-SoSPS1) were expressed into tomato plants to verify the functional role and importance of the N-terminal domain in plant SPS. Overexpression of Δ N-SoSPS1 led to an up to 3-fold increase in the specific activity of SPS compared to non-transformant plants (WT), while the specific activity of Δ N-SoSPS1 was higher than FL-SoSPS1 in transgenic tomato plants. Unlike WT and FL-SoSPS1, the Δ N-SoSPS1 mutant was not allosterically regulated by G6P. These results indicated that deletion of the N-terminal domain promotes the loss of allosteric activation by G6P and increases binding affinity between enzyme and substrate.

KEYWORDS sucrose-phosphate synthase; glucose-6-phosphate; sucrose synthesis; allosteric regulation; N-terminal domain

1. Introduction

Plant growth and development are closely related to carbohydrate metabolism in the form of sucrose. Allocation and accumulation of carbohydrate from source to sink organs provides the substrate for supporting plant growth. During photosynthesis, carbon dioxide (CO₂) is converted into glyceraldehyde-3-phosphate as a precursor for starch and sucrose biosynthesis. The starch is deposited and stored in the chloroplast, then breaks into sucrose at night (Osorio et al. 2014; Aluko et al. 2021).

Sucrose plays a crucial role as a carbon source from photosynthetic carbon assimilation and primary sugar transported form in higher plants. Sucrose is exported from leaves to various organs and is involved by many enzymes in the sucrose metabolism pathway. Sucrose phosphate synthase (SPS; EC 2.4.1.14) is a key enzyme catalyzing the formation of sucrose-6-phosphate through the transfer of uridine-diphosphate glucose (UDP-G) as a donor to fructose-6-phosphate (F6P) as an acceptor (Barker 2000). The activity of the SPS is regulated by a complex mechanism, including allosteric regulation and phosphorylation regulation (Huber and Hu-

ber 1996). This regulation is controlled by glucose-6-phosphate (G6P) and inorganic phosphate (Pi) as allosteric activators and inhibitors, respectively. Plant SPS is activated by a concentration-dependent manner up to 5 mM G6P (Doehlert and Huber 1983; Sawitri et al. 2016), while the recombinant SPS expressed in *Escherichia coli* is not allosterically regulated by G6P (Lunn et al. 2003; Sawitri et al. 2016). The previous study reported that the loss of allosteric property in recombinant plant SPS is caused by proteolytic cleavage at N-terminal region. Despite these reports, the expression of recombinant plant SPS in *E. coli* resulted in a shorter size compared to the full-size of plant SPS (Worrell et al. 1991; Sonnewald et al. 1993; Lunn et al. 1999).

The precise mechanism of SPS regulation during allosteric and phosphorylation-related processes remains unclear. Although the crystal structure of SPS from non-photosynthetic bacteria *Halothermothrix orenii* has been solved, the regulatory properties between *H. orenii* and plant SPSs are different. Plant SPS consists of three domains: glycosyltransferase domain, C-terminal domain, and N-terminal domain, whereas *H. orenii* SPS is composed of glycosyltransferase domain only (Teck

et al. 2008). The glycosyltransferase domain is essential for catalytic enzymes since it is associated with the substrate-binding region and shares a high similarity between prokaryotic and plant SPSs (Sawitri et al. 2017; Kurniah et al. 2021). Several reports suggest that the role of C-terminal domain resembles sucrose phosphate phosphatase (Cumino et al. 2002; Salerno and Curatti 2003; Castleden et al. 2004). However, the N-terminal domain was reported as an important region for allosteric regulation (Castleden et al. 2004). The plant SPS that was truncated its N-terminal domain could increase the activity up to 10 fold in *E. coli* (Sawitri et al. 2016). This shows that the N-terminal domain has a significant influence on regulating SPS activity in the plant. Previous studies reported that serine residue at position 158 in spinach SPS and 162 in *Zea mays* SPS were identified as a phosphorylation site (Huber et al. 1994; Toroser et al. 1999). The phosphorylation on this residue was reported to play an important role in the activation of spinach SPS.

The N-terminal domain truncation of sugarcane SPS called Δ N-SoSPS1 has been successfully expressed in *E. coli* and that activity was not affected by the presence of G6P because its phosphorylation site has been truncated (Sawitri et al. 2016). Therefore, in the absence of G6P activator, N-terminal truncated SPS remains functionally active. In this study, the cDNA of Δ N-SoSPS1 was transformed and expressed into tomato plants. This study was directed to determine the regulation and characterization of Δ N-SoSPS1 in transgenic tomato.

2. Materials and Methods

2.1. Binary vector construct

Two binary vector constructs were prepared by inserting N-terminal truncated and full-length SoSPS1 cDNA into pRI101AN plasmid (Takara, Shiga, Japan). To study effect of N-terminal truncation, the first construct was prepared by removing N-terminal domain (522 bp or 175 amino acids downstream of start codon) of full full-length SoSPS1 using PCR amplification with forward primer containing *NdeI* site (5'-GGTCCGCGCATATGGAGAAGAAGCTTTACATTGTG-3') and reverse primer containing *EcoRI* site (5'-CAGAATTCTCACATGCCGCTAGAAGTCTTGGAGA-3'). While second construct containing full-length SoSPS1 was prepared by amplification of the cDNA using a forward primer containing *NdeI* site at a position of the start codon (5'-GGTCCGCGCATATGGCCGGAACGAGTGGATCAAT-3') and forward primer with *EcoRI* site as above. The amplification of the cDNAs was digested with the corresponding restriction site and inserted into pRI101AN. The resulted constructs were named Δ N-SoSPS1 and FL-SoSPS1 for N-terminal truncated (Δ N) and full-length (FL) SoSPS1, respectively. A schematic diagram representing the construct of FL-SoSPS1 and Δ N-SoSPS1 in the binary plasmid of pRI101AN is shown in Figure 1.

The correct insertions were confirmed with restriction enzyme digestion and DNA sequencing.

2.2. Agrobacterium-mediated transformation for tomato

Tomato plant (*Lycopersicon esculentum* var. rampai) seed was surface sterilized using 70% alcohol and germinated in agar medium containing Murashige and Skoog (MS) salt under illumination. Apical shoots of tomato plants that have been grown in vitro for 14 days were collected and used as explants for transformation using *Agrobacterium tumefaciens* strain GV3101. The apical shoots were infected and co-cultivated with the *Agrobacterium* harboring either FL- or Δ N-SoSPS1 construct in the presence of 20 mg·L⁻¹ acetosyringone. The infected shoots were incubated in a selection MS medium containing cefotaxime (500 mg·L⁻¹ and kanamycin (50 mg·L⁻¹) under illumination for three weeks. After five successive cycles in the selection medium, surviving shoots were acclimated in a growth chamber and further cultivated in a greenhouse with luminous intensity estimates of 650 μ mol·m⁻²·s⁻¹ at the plant level. For molecular and biochemical analysis, tomato leaves were harvested and plunged in liquid nitrogen during the daytime. Tomato growth rate and the number of fruits were observed at the indicated time.

2.3. Genomic analysis

To confirm the transgene insertion of FL- or Δ N-SoSPS1, genomic DNA was isolated from the leaves of one-month-old transgenic and non-transgenic (wild type-WT) tomato plants grown in the greenhouse. Isolation of genomic DNA was conducted using a method as previously described (Apriasti et al. 2018). The transgene insertion was amplified by PCR using the genomic DNA and a pair of specific primers for detection of *nptII* gene (*nptII*-F: 5'-GTCATCTCACCTTGCTCCTGCC-3' and *nptII*-R: 5'-GTCGCTTGGTCGGTCATTTC-3'). The PCR reaction was performed in a T100 thermal cycler machine (Bio-Rad, Irvine, CA, USA) according to the method previously described (Anur et al. 2020). The PCR product was then separated in 1% agarose gel (w/v) and visualized with GelDoc (Major Science, Saratoga, California, USA).

2.4. Protein extraction

Protein was extracted from leaves of two months tomato transgenic lines and wild type (WT). One gram of frozen tomato leaves (1 g) were ground in liquid nitrogen, subsequently ground in three-time volumes (w/v) of cold extraction buffer containing 50 mM MOPS-NaOH (pH 7.5), 10 mM MgCl₂, 150 mM NaCl, 1 mM EDTA, 2.5 mM dithiothreitol (DTT), and 10% polyvinylpyrrolidone (PVPP). The homogenate was centrifuged at 12.000 g at 4 °C for 10 min, the supernatant (crude extract) was desalted using gel filtration of Sephadex G-25 (GE Healthcare, Piscataway, NJ, USA) that had been equilibrated with the extraction buffer. The desalted extract was then concentrated with centrifugal filters (Merck Millipore, Tullagreen, Carrigtwohill, Co. Cork, IRL). The concentrated enzyme was

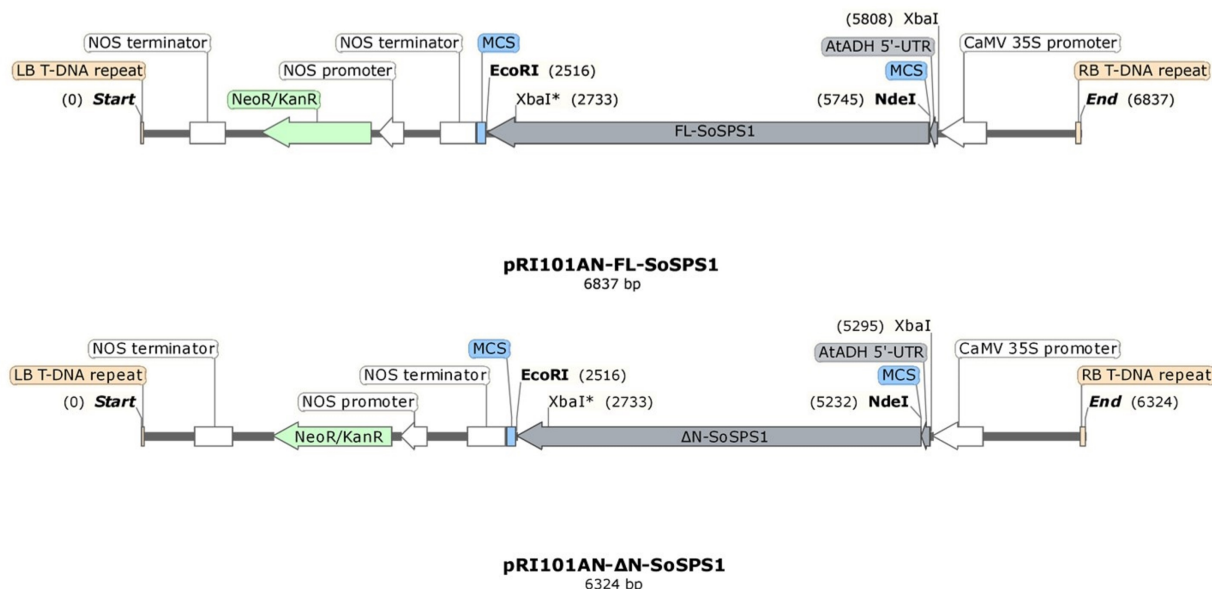


FIGURE 1 Schematic diagram representing the construct of FL-SoSPS1 (upper) and Δ N-SoSPS1 (lower) in the binary plasmid of pRI101AN. The targeted genes (FL-SoSPS1 and Δ N-SoSPS1) were inserted in *NdeI*-*EcoRI* site under promoter of CaMV35S and selectable marker of *nptII* (kanamycin resistant) gene. RB and LB, T-DNA right and left border, respectively. The vector map is constructed using SnapGene software.

stored at -80°C until used for enzyme assay and immunoblotting analysis. To determine the protein concentration, the Bradford protein assay was used to measure the total protein of tomato transgenic leaves (Bio-Rad, Des Plaines, IL, USA).

2.5. Enzyme assay

SPS activity was assayed in concentrated leaf extract by measuring the formation of sucrose-6-phosphate as previously described (Sawitri et al. 2016). For measurement at saturating substrate condition, assay mixture (70 μL) contained 50 mM MOPS-NaOH (pH 7.5), 20 mM MgCl_2 , 20 mM UDP-glucose (UDP-G), 20 mM fructose-6-phosphate (F6P). The reaction was initiated by adding 30 μL of concentrated leaf extract. The mixture was incubated at 30°C for 10 min, and the reaction was stopped by adding 70 μL of 1 M NaOH, followed by incubating at 95°C for 10 min. After that, 0.25 mL resorcinol (1% in 95% ethanol) and 0.75 mL of 30% HCl were added, and the mixture was incubated at 85°C for 8 min. The developed color was measured using a spectrophotometer microplate reader (Corona Electric, Ibaraki, Japan) at 520 nm. The limiting substrate assay mixture contained same assay mixture, except that either UDP-G and F6P concentration was lowered to 1 mM and 3 mM, respectively.

2.6. SDS-PAGE and immunoblot analysis

Crude extract protein was separated by sodium dodecyl sulfate (SDS) polyacrylamide-gel (10%) electrophoresis (PAGE) as previously described (Laemmli 1970). The gels were directly electroblotted onto Immobilon-P transfer membrane (Thermo Scientific, Rockford, IL, USA) using a semi-dry trans-blotter (Bio-Rad, Irvine, CA, USA) for immunodetection with the polyclonal antibodies

against SPS (Sawitri et al. 2016). Proteins reacted with the antibodies were visualized with a secondary antibody of goat anti-rabbit IgG Alkaline phosphatase conjugate (Bio-Rad, Irvine, CA, USA) using the NBT/BCIP for color development (Bio-Rad, Irvine, CA, USA).

2.7. Statistical Analysis

The experiment was designed with 3 three replicates to perform statistical analysis and presented as the mean with standard deviation. The significance of the data was calculated using an unpaired student t-test method with SPSS 22 software (IBM Corp, Armonk, NY, USA) and p-value <0.05 .

3. Results and Discussion

3.1. Confirmation of transgene integration by PCR

Tomato transformation was conducted using apical shoot explant, and the infected shoot was incubated in a selection medium under illumination. After five successive cycles in the selection media, the surviving shoot was acclimated in the growth chamber for two weeks and transferred to the greenhouse. To confirm insertion of the targeted gene, genomic DNA was isolated from the leaves of one-month-old transgenic and WT tomato grown in the greenhouse and subjected to PCR analysis using a pair of *nptII* gene primers. PCR analysis revealed the amplification of corresponding DNA with molecular size 550 bp in transgenic tomato lines but not in WT tomato. Among 16 acclimated plants, 12 transgenic lines were confirmed containing corresponding *nptII* DNA, one FL-SoSPS1 and 11 Δ N-SoSPS1 transgenic lines (Figure 2). Transgenic tomato lines expressed targeted gene were grown and collected their fruit for further analysis. The results clearly

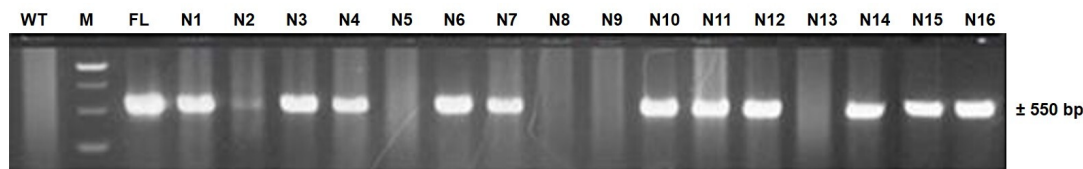


FIGURE 2 PCR amplification of *nptII* gene using genomic DNA isolated from WT and transgenic tomato leaves. The genomic DNA was extracted from tomato leaves and used for PCR analysis using specific primer pair to amplify 550 bp of the targeted *nptII* DNA fragment according to the method described in Materials and Methods. WT, M, FL, N1-N16 are wild-type, marker, transgenic full-length (FL-*SoSPS1*), and N-terminal truncated (Δ N-*SoSPS1*), respectively.

showed that both FL- and Δ N-*SoSPS1* successfully integrated into the genome of transgenic tomato lines.

3.2. Effect of FL- and Δ N-*SoSPS1* overexpression on growth and fruit number of tomato

To determine the effect of FL- and Δ N-*SoSPS1* introduction on growth and fruit number, the seed selected from FL, N1, N4, and N10 transgenic tomato lines were germinated and grown in the greenhouse. Growth of transgenic tomato lines FL and Δ N that were observed at 30 and 60 days after plantation (DAP) seemed higher than the WT plant (Figure 3a). To determine whether the higher vegetative growth increase productivity of transgenic tomato lines, the fruit number was measured at 90 DAP. The measurement of fruit number showed that overexpression of sugarcane SPS gene increased fruit number, both in FL and Δ N transgenic lines (Figure 3b). The increase was higher in Δ N transgenic lines of N1 and N10 compared to FL transgenic line. However, other agronomic traits such as flowering age and fruit size were comparatively unaffected in FL and Δ N transgenic lines. These results suggest that overexpression of SPS gene enhances sucrose synthesis and carbon partitioning toward the growth and productivity of transgenic tomatoes.

3.3. Activity and level of sucrose-phosphate synthase

Measurement of SPS activity showed that the activity was increased up to 2.7-fold in transgenic tomato lines compared to WT tomato. Interestingly, the introduction of Δ N-*SoSPS1* resulted in more prominent increasing SPS activity than FL-*SoSPS1* (Figure 4a). The SPS activity clearly showed that the N-terminal truncation of SPS resulted in an active SPS and increased the activity approximately 1.8- and 2.7-fold compared to FL and WT tomato, respectively (Figure 4a). However, immunoblot with a specific antibody revealed that the antibody reacted with two protein bands with molecular size around 75 and 63 kDa (Figure 4b) both in FL and Δ N transgenic lines. These two protein bands should be produced from the degradation of SPS protein and did not distinguish the expected molecular size of the SPS protein between FL and Δ N transgenic tomato. Nevertheless, the intensity of degraded SPS protein bands were higher in transgenic lines compared to WT tomato. These results confirmed that introduction of SPS gene increase activity as well as the level of SPS and that N-truncation resulted in a higher activity.

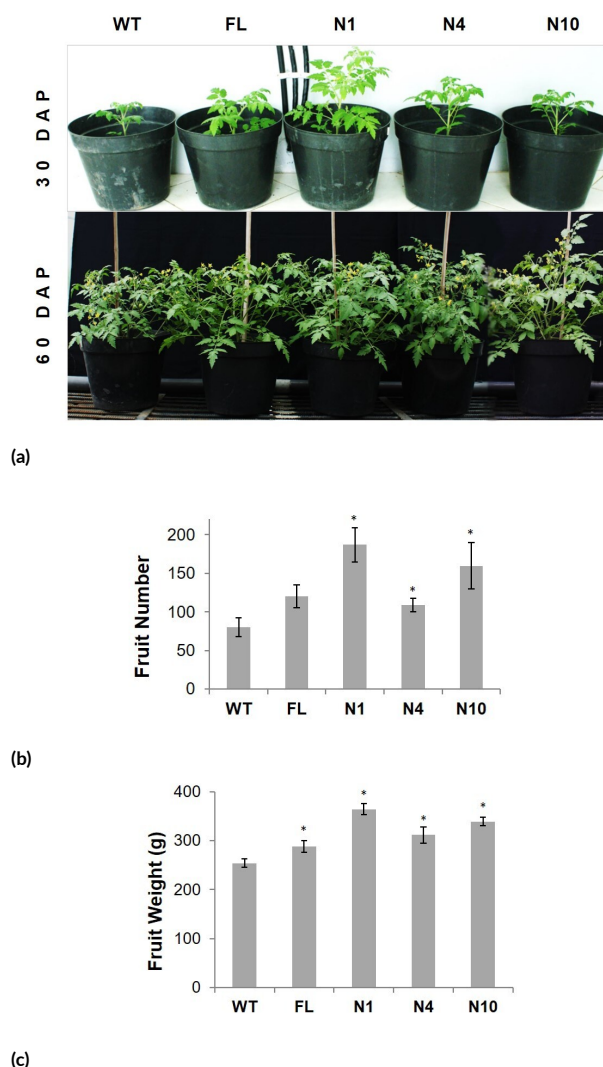


FIGURE 3 The morphological feature of WT (control) and selected tomato transgenic lines grown in a greenhouse. (a) Growth rate comparison between WT and transgenic lines observed at 30 and 60 DAP. (b) Total of tomato fruit number in WT and transgenic lines measured at 90 DAP. (c) Fruit Weight in WT and tomato transgenic lines measured for 50 tomato fruits. WT and FL were wild-type and FL-*SoSPS1* transgenic line of tomato, respectively. N1, N4, and N10 were tomato N-terminal truncated transgenic lines. Values are means \pm SD for three independent plants, (*) = denote significant different of tomato fruit number and fruit weight from WT ($p < 0.05$).

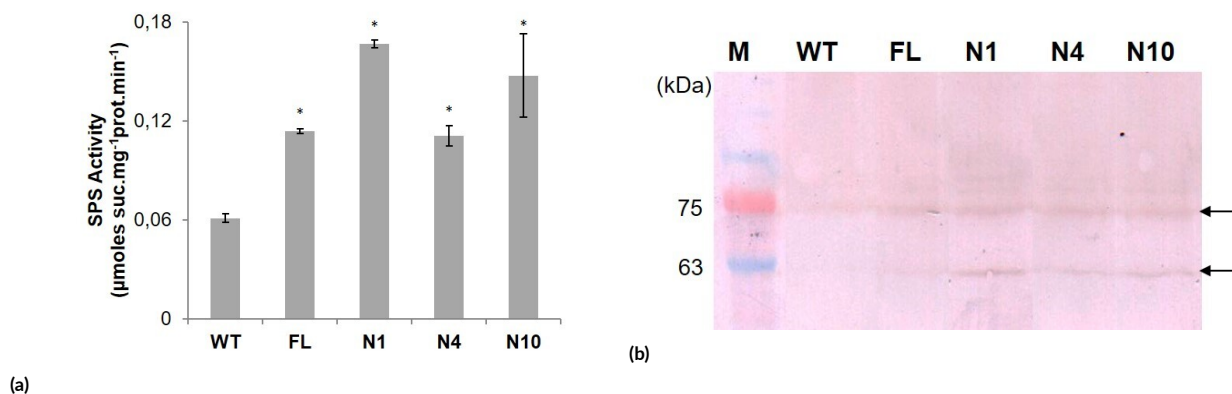


FIGURE 4 Activity and level of SPS in leaves of WT and transgenic tomato lines. (a) Activity of SPS was determined using saturated substrate by formation of sucrose (suc) as described in Materials and Methods. Values are means \pm SD for three independent plants, (*) = denote significant different of SPS activity from WT ($p < 0.05$). (b) Level of SPS protein determined by immunoblot using specific antibody against sugarcane SPS. Total soluble protein (30 μg) extracted from tomato leaves were used for the immunoblot analysis. WT, FL, N1-N10 are wild-type, full-length, and N-terminal truncated transgenic tomato respectively. M, molecular weight marker of protein.

3.4. Effect of glucose-6-phosphate on activity of sucrose-phosphate synthase

To examine the effect of N-truncation, SPS activity was measured in leaf of FL and ΔN transgenic tomato with various G6P levels. SPS was clearly activated by increasing addition of G6P concentration in saturating substrates (potential activity) both in FL transgenic and WT tomato, but not in ΔN transgenic tomato (Figure 5a). The SPS activity almost remains unchanged with increasing activator G6P in ΔN transgenic tomato. When the activity was measured in limiting fructose-6-phosphate (F6P) substrate (3 mM), addition of G6P still significantly increased the activity in both FL transgenic and WT tomato and did not in ΔN (Figure 5b). The activation of G6P on SPS activity at limiting F6P substrate has been reported in spinach⁵. However, lowered concentration of UDP-G substrate (1 mM) caused decreasing activation of SPS by G6P in FL and WT tomato but remain unchanged in ΔN (Figure 5c). These results indicated that SPS is activated by G6P in full length and no activation of N-terminal truncated SPS.

3.5. Discussion

Sucrose phosphate synthase is a key enzyme catalyzing the formation of sucrose-6-phosphate through the transfer of UDP-G as a donor to F6P as an acceptor (Barker 2000). As shown in Figure 4b, the SPS level in both transgenic tomatoes was higher than WT, but there is no difference in SPS protein band size between FL- and ΔN -SoSPS1. Immunoblot analysis with specific antibody as described by Sawitri et al. (2016) showed that the endogenous tomato SPS also reacts with polyclonal antibodies SPS. In this study, the immunoreactive protein was detected at 75 and 63 kDa (Figure 4b) with a predicted total molecular mass of about 138 kDa. The 75 and 63 kDa immunoreactive protein were presumably cleavage products arising from proteolysis of the plant SPS during the sample preparation of the SDS-PAGE procedure. Native plant SPS has a molecular mass of 120–138 kDa (Huber and Huber 1996).

Sugarcane SPS expressed in *E. coli* has a molecular mass of 120 kDa (Sawitri et al. 2016). In addition, this difference is thought to be due to post-translational modifications of SPS in plant cells. Likewise, maize SPS expressed in *E. coli* has a molecular mass of 3 kDa shorter than maize SPS expressed in tomato (Worrell et al. 1991). The difference in molecular mass of maize SPS in *E. coli* and tomato is due to protein phosphorylation. Phosphorylation is one of several post-translational modifications that occur in protein synthesis. Phosphorylation affects the structure and function of a protein (Huber and Huber 1991).

Overexpression of the SPS gene has been reported to increase activity and level of SPS protein in transgenic tomato (Nguyen-Quoc et al. 1999), potato (Ishimaru et al. 2008), and sugarcane (Anur et al. 2020). Similarly, the results obtained in this study showed that the two transgenic tomatoes (FL and ΔN) had higher activity compared to the non-transgenic tomato (WT). The increase of SPS activity in transgenic tomato plants reached 2.7-fold in transgenic ΔN tomato compared to WT (Figure 4a). Previous research explained that expression of ΔN -SoSPS1 in *E. coli* had 10-fold higher activity than full-length SoSPS1. This is due to the removal of the N-terminal domain from SPS. Previous studies reported that the N-terminal truncated SoSPS1 showed higher specific activity with longer truncation (Sawitri et al. 2016). Deletion of the N-terminal domain is considered an intrinsic disorder in the structure of the SPS protein. A polypeptide region with intrinsic disorder plays a role in modulating its activity, such as optimizing high or low binding affinity for the substrate or controlling its ability to interact with other proteins (Hilser and Thompson 2007; Ferreon et al. 2013). Furthermore, the roles of SPS in sucrose metabolism and plant growth were reported. The overexpression of SPS increased sucrose content and improved growth of transgenic sugarcane (Anur et al. 2020), improved biomass production of *B. distachyon* (Falter and Voigt 2016), increased yield of transgenic potato (Ishimaru et al. 2008),

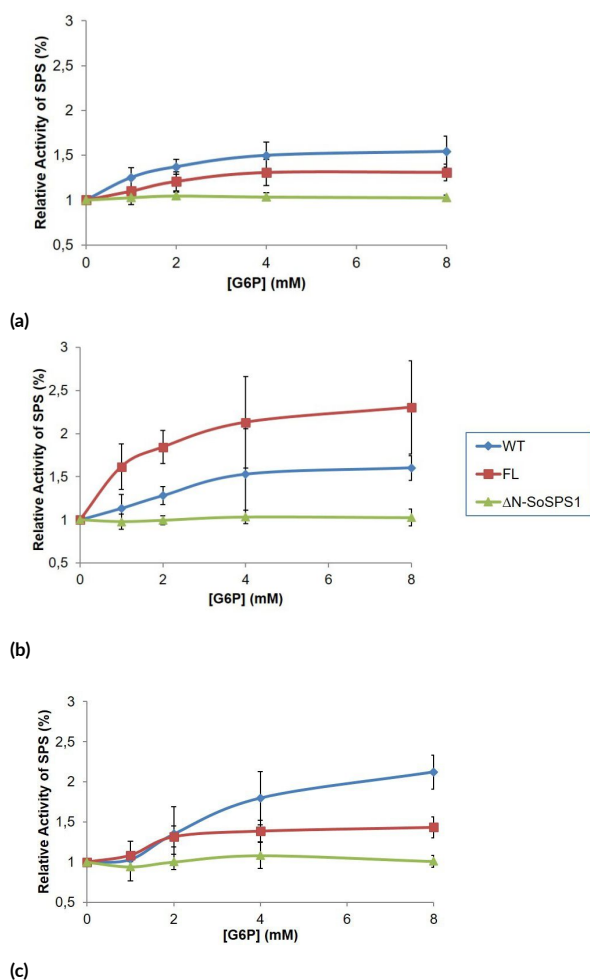


FIGURE 5 Effect of activator G6P concentration on the activity of sucrose phosphate synthase in tomato plants. (a) Activity was measured in the assay mixture containing saturated substrate (20 mM UDP-G and 20 mM F6P), (b) Activity was measured in the assay mixture containing 20 mM UDP-G and limiting substrate F6P at 3 mM, (c) Activity was measured in the assay mixture containing 20 mM F6P and limiting substrate UDP-G at 1 mM. The results are expressed as relative activity of SPS when the activity at 0 mM G6P was calculated as 1. Protein samples extracted from WT (blue), FL-SoSPS1 (red) and ΔN-SoSPS1 line N1 (green) were used in this experiment. Values are means \pm SD for three independent plants.

and also altered growth and development of transgenic tobacco (Park et al. 2008; Seger et al. 2015). In line with the previous finding, this study demonstrated that overexpression of *SoSPS1* also improves growth and fruit number in transgenic tomato lines compared to WT (Figure 3). Similar result was reported that overexpression of maize SPS increases the sink strength of transgenic tomato fruit (Nguyen-Quoc et al. 1999). Furthermore, overexpression of ΔN-*SoSPS1* resulted in higher sucrose synthesis and tomatoes fruit production compared to FL-*SoSPS1* (Figure 3). These results imply that the ΔN-*SoSPS1* is an important target gene to improve carbon partitioning for plant growth and production.

SPS activity was activated by G6P in FL and WT tomato at the concentration-dependent manner to 8 mM,

but the allosteric effect was not observed in the ΔN SPS (Figure 5). The absence of G6P influence on the ΔN activity is due to the absence of an N-terminal domain part. It was reported that the N-truncation of SPS was not affected by the addition of activator G6P in the *E. coli* expression system (Sawitri et al. 2016). The N-terminal domain is a domain involved in the allosteric regulation of SPS. SPS cyanobacteria and SPS *H. orenii* (HoSPS) do not have an N-terminal domain and their activities are not allosterically regulated (Lunn et al. 1999; Teck et al. 2008). The ΔN-*SoSPS1* expressed in *E. coli* was also shown to be unaffected by the allosteric effect of G6P compared to FL-*SoSPS1*. The N-terminal domain of sugarcane SPS contains serine residue at position 162, which is considered to be the phosphorylation site and involved in the modulation of SPS activity. The truncation of S162 residue in the N-terminal domain can eliminate the allosteric effect of G6P on SPS activity (Sawitri et al. 2016). Similar to the S158 residue from SPS spinach which also plays a role in modulating SPS activity (Toroser et al. 1999). Taken together, the results of our study support the notion that the N-terminal domain of *SoSPS1* is involved in allosteric regulation. The SPS kinetic activity of ΔN is apparently unaffected by the increase of G6P, whereas the FL or WT raises its kinetic activity in response to the gradual increase of G6P.

4. Conclusions

The results obtained in this study showed that the two transgenic tomato lines expressing *SoSPS1* (FL and ΔN) had higher activity compared to the non-transgenic tomatoes (WT). The highest increase of SPS activity was in transgenic ΔN tomato reached 2.7-fold. The SPS kinetic activity of ΔN-*SoSPS1* is apparently unaffected by the increase of G6P, whereas the FL-*SoSPS1* or WT raises its kinetic activity in response to the gradual increase of G6P.

Acknowledgments

This work was supported by the Indonesian Ministry of Education and Culture: grant number (SPPK) 116/E4.1/AK.04.PT/2021-World Class Research for BS.

Authors' contributions

BS, SNA (conception and designed the study, validation, data curation, writing review, and editing manuscript). SNA, IDA, PD (carried out the laboratory work and investigation). All authors read and approved the final version of the manuscript.

Competing interests

The authors have declared that no competing interest exists.

References

- Aluko OO, Li C, Wang Q, Liu H. 2021. Sucrose Utilization for Improved Crop Yields : A Review Article. *Int. J. Mol. Sci.* 22:1–29. doi:10.3390/ijms22094704.
- Anur RM, Mufithah N, Sawitri WD, Sakakibara H. 2020. Overexpression of Sucrose Phosphate Synthase Enhanced Sucrose Content and Biomass Production in Transgenic Sugarcane. *Plants* 9(200):1–11. URL doi:10.3390/plants9020200.
- Apriasti R, Widyaningrum S, Hidayati WN, Sawitri WD, Darsono N. 2018. Full sequence of the coat protein gene is required for the induction of pathogen-derived resistance against sugarcane mosaic virus in transgenic sugarcane. *Mol. Biol. Rep.* 45(6):2749–2758. doi:10.1007/s11033-018-4326-1.
- Barker L. 2000. SUT2, a putative sucrose sensor in sieve elements. *the Plant Cell* 12(7):1153–1164. doi:10.1105/tpc.12.7.1153.
- Castleden CK, Aoki N, Gillespie VJ, MacRae EA, Quick WP, Buchner P, Foyer CH, Furbank RT, Lunn JE. 2004. Evolution and function of the sucrose-phosphate synthase gene families in wheat and other grasses. *Plant Physiol.* 135(3):1753–1764. doi:10.1104/pp.104.042457.
- Cumino A, Curatti L, Giarrocco L, Salerno GL. 2002. Sucrose metabolism: Anabaena sucrose-phosphate synthase and sucrose-phosphate phosphatase define minimal functional domains shuffled during evolution. *FEBS Letters* 517(1-3):19–23. doi:10.1016/S0014-5793(02)02516-4.
- Doehlert DC, Huber SC. 1983. Regulation of Spinach Leaf Sucrose Phosphate Synthase by Glucose-6-Phosphate, Inorganic Phosphate, and pH. *Plant Physiol.* 73:989–994. doi:10.1104/pp.73.4.989.
- Falter C, Voigt CA. 2016. Improving biomass production and saccharification in *Brachypodium distachyon* through overexpression of a sucrose-phosphate synthase from sugarcane. *J. Plant Biochem. Biotechnol.* 25(3):311–318. doi:10.1007/s13562-015-0343-5.
- Ferreon ACM, Ferreon JC, Wright PE, Deniz AA. 2013. Modulation of allostery by protein intrinsic disorder. *Nature* 498(7454):390–394. doi:10.1038/nature12294.
- Hilser VJ, Thompson EB. 2007. Intrinsic disorder as a mechanism to optimize allosteric coupling in proteins. *Proc. Natl. Acad. Sci. U. S. A.* 104(20):8311–8315. doi:10.1073/pnas.0700329104.
- Huber SC, Huber JL. 1991. Regulation of maize leaf sucrose-phosphate synthase by protein phosphorylation. *Plant Cell Physiol.* 32(3):319–326. doi:10.1093/oxfordjournals.pcp.a078083.
- Huber SC, Huber JL. 1996. Role and Regulation of Sucrose-Phosphate Synthase in Higher Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47(1):431–444. doi:10.1146/annurev.arplant.47.1.431.
- Huber SC, Huber JL, McMichael RW. 1994. Control of Plant Enzyme Activity by Reversible Protein Phosphorylation. *Int. Rev. Cytol.* 149:47–97. doi:10.1016/S0074-7696(08)62086-0.
- Ishimaru K, Hirotsu N, Kashiwagi T, Madoka Y, Nagasuga K, Ono K, Ohsugi R. 2008. Overexpression of a maize SPS gene improves yield characters of potato under field conditions. *Plant Prod. Sci.* 11(1):104–107. doi:10.1626/pp.11.104.
- Kurniah NI, Sawitri WD, Rohman MS, Nugraha Y, Hase T, Sugiharto B. 2021. Mutation of UDP-glucose binding motif residues lead to increased affinity for ADP-glucose in sugarcane sucrose phosphate synthase. *Mol. Biol. Rep.* 48(2):1697–1706. doi:10.1007/s11033-021-06181-8.
- Lunn JE, Gillespie VJ, Furbank RT. 2003. Expression of a cyanobacterial sucrose-phosphate synthase from *Synechocystis* sp. PCC 6803 in transgenic plants. *J. Exp. Bot.* 54(381):223–237. doi:10.1093/jxb/erg023.
- Lunn JE, Price GD, Furbank RT. 1999. Cloning and expression of a prokaryotic sucrose-phosphate synthase gene from the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Mol. Biol.* 40(2):297–305. doi:10.1023/A:1006130802706.
- Nguyen-Quoc B, N'Tchobo H, Foyer CH, Yelle S. 1999. Overexpression of sucrose phosphate synthase increases sucrose unloading in transformed tomato fruit. *J. Exp. Bot.* 50(335):785–791. doi:10.1093/jxb/50.335.785.
- Osorio S, Ruan YL, Fernie AR. 2014. An update on source-to-sink carbon partitioning in tomato. *Front. Plant Sci.* 5:1–11. doi:10.3389/fpls.2014.00516.
- Park JY, Canam T, Kang KY, Ellis DD, Mansfield SD. 2008. Over-expression of an arabidopsis family A sucrose phosphate synthase (SPS) gene alters plant growth and fibre development. *Transgenic Res.* 17(2):181–192. doi:10.1007/s11248-007-9090-2.
- Salerno GL, Curatti L. 2003. Origin of sucrose metabolism in higher plants: When, how and why? *Trends Plant Sci.* 8(2):63–69. doi:10.1016/S1360-1385(02)00029-8.
- Sawitri WD, Afidah SN, Nakagawa A, Hase T, Sugiharto B. 2017. Identification of UDP-glucose binding site in glycosyltransferase domain of sucrose phosphate synthase from sugarcane (*Saccharum officinarum*) by structure-based site-directed mutagenesis. *Biophys. Rev.* 10(2):293–298. doi:10.1007/s12551-017-0360-9.
- Sawitri WD, Narita H, Ishizaka-Ikeda E, Sugiharto B, Hase T, Nakagawa A. 2016. Purification and characterization of recombinant sugarcane sucrose phosphate synthase expressed in *E. Coli* and insect Sf9 cells: An importance of the N-Terminal domain for an allosteric regulatory property. *J. Biochem.* 159(6):599–607. doi:10.1093/jb/mvw004.
- Seger M, Gebril S, Tabilona J, Peel A, Sengupta-Gopalan C. 2015. Impact of concurrent overexpression of cytosolic glutamine synthetase (GS1) and sucrose phosphate synthase (SPS) on growth and development in transgenic tobacco. *Planta* 241(1):69–81.

doi:10.1007/s00425-014-2165-4.

- Sonnenwald U, Quick WP, MacRae E, Krause KP, Stitt M. 1993. Purification, cloning and expression of spinach leaf sucrose-phosphate synthase in *Escherichia coli*. *Planta* 189(2):174–181. doi:10.1007/BF00195074.
- Teck KC, Bujnicki JM, Tan TC, Huynh F, Patel BK, Sivaraman J. 2008. The structure of sucrose phosphate synthase from *Halothermothrix orenii* reveals its mechanism of action and binding mode. *Plant Cell* 20(4):1059–1072. doi:10.1105/tpc.107.051193.
- Toroser D, McMichael R, Krause KP, Kurreck J, Sonnenwald U, Stitt M, Huber SC. 1999. Site-directed mutagenesis of serine 158 demonstrates its role in spinach leaf sucrose-phosphate synthase modulation. *Plant Journal* 17(4):407–413. doi:10.1046/j.1365-313X.1999.00389.x.
- Worrell AC, Bruneau Jm, Summerfelt K, Boersig M, Toni A. 1991. Expression of a Maize Sucrose Phosphate Synthase in Tomato Alters Leaf Carbohydrate Partitioning. *The Plant Cell* 3(10):1121–1130. doi:10.1007/s11033-009-9510-x.