OVEREXPRESSION OF *SoSUT1* GENE ON TRANSGENIC SUGARCANE (*Saccharum* spp. hybrids)

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Abstract

In most plants, sucrose is the major export organic form of photoassimilate from the photosynthetic tissue to sink tissue, where it is stored or metabolized. The translocation of sucrose is facilitated by sucrose transporter protein (SUT). To study the role of sucrose transporter in sugarcane, overexpression of *SoSUT1* gene on transgenic sugarcane was evaluated. Based on the cDNA bands intensity, it can be illustrated that the expression of *SoSUT1* gene on transgenic leaves is higher than non transgenic. The increasing of *SoSUT1* gene expression is followed by SUT1 content improvement detected by Western blot method using specific SUT1 polyclonal antibody. The increased *SoSUT1* gene expression improved sucrose translocation from transgenic sugarcane leaves to its stems.

Keywords: overexpression, *SoSUT1*, sugarcane.

Introduction

Sucrose as the major transported form of fixed carbon must be translocated from source tissue to the sites of consumption and storage or sink tissues. The translocation of sucrose is facilitated by some distinct sucrose transporters proteins (SUT) located in the plasma membrane [1]. ShSUT1 sucrose transporter may have a role in partitioning of sucrose between the vascular tissue and sites of storage in the parenchyma cells of sugarcane stems internodes [2]. To study sucrose transporters in sugarcane, we had conducted characterization of transgenic sugarcane plant expressing *SoSUT1*. Characterization includes the analysis of gene expression by RT-PCR, protein by Western Blot, and sucrose content by HPLC.

Materials and Methods

Plant Material
Transgenic sugarcane plant inserting *SoSUT1*[3]

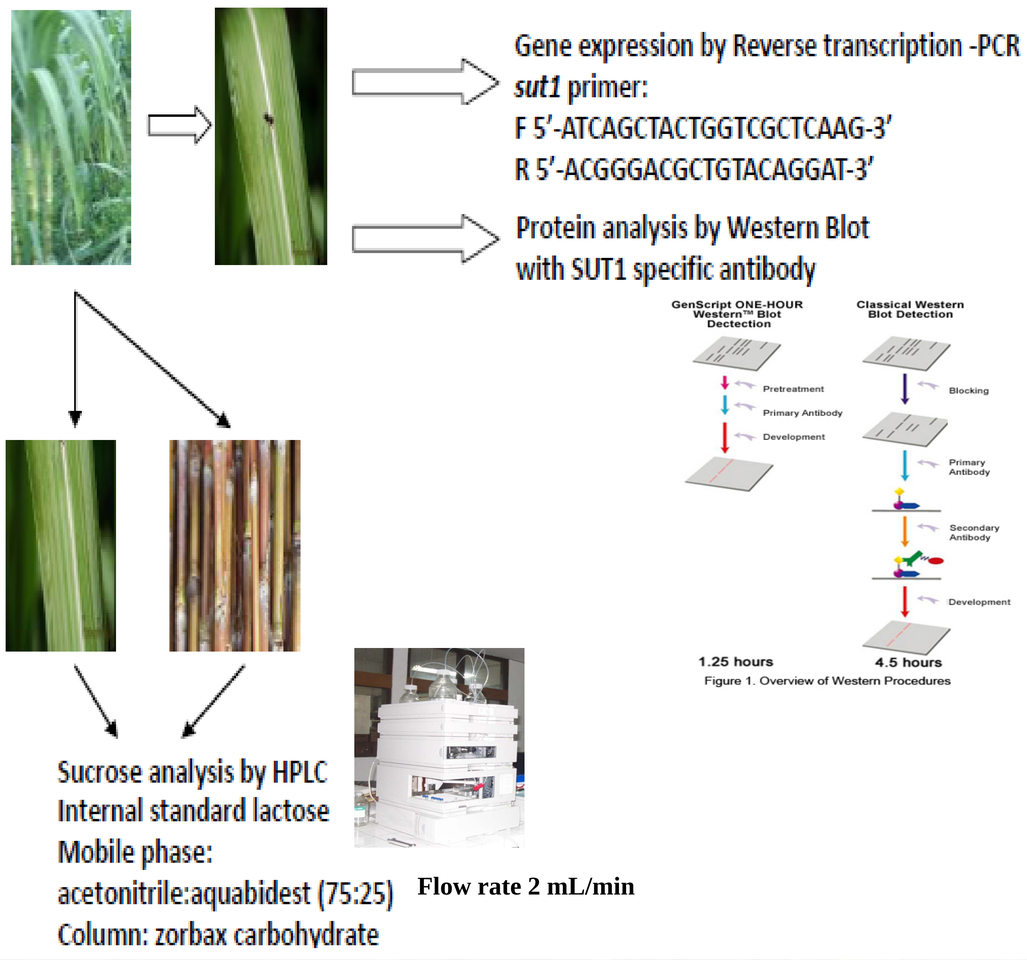

Figure 1. Overview of Western Procedures

Flow rate 2 mL/min

Result and Discussion

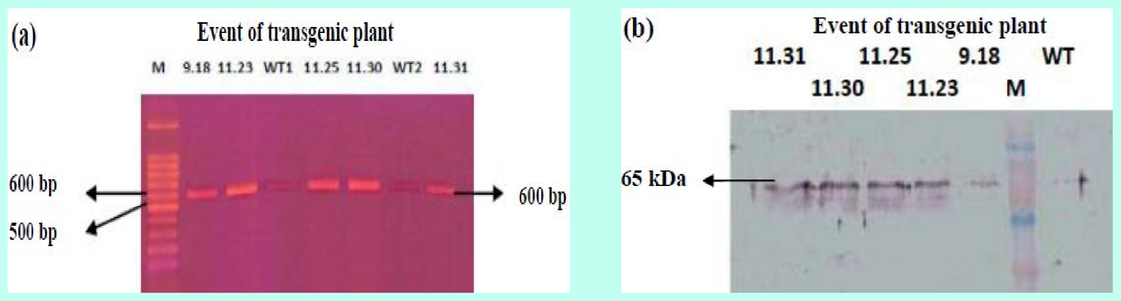

Figure 1. Overexpression of *SoSUT1* gene ; bp : base pair, M : marker, WT : wild type, non transgenic plant
(a) RT-PCR analysis of transgene expression. RNA was extracted from leaf of non transgenic and transgenic sugarcane plant.
(b) Western blot analysis of a crude protein extract prepared from leaf of non transgenic and transgenic sugarcane plant.

Detection of *SoSUT1* gene expression level using RT-PCR method showed that the *SoSUT1* gene expression in transgenic sugarcane leaf was higher compare to the wild type, the non transgenic plants (Fig. 1a). The increasing of *SoSUT1* gene expression in the transgenic sugarcane leaf were followed by increased of protein SUT1 (Fig. 1b). This finding indicates overexpression of *SoSUT1* can increase transription and translation on transgenic sugarcane plant. A similar phenomenon was observed in spinach sucrose transporter (*SoSUT1*) overexpressing from potato [4].

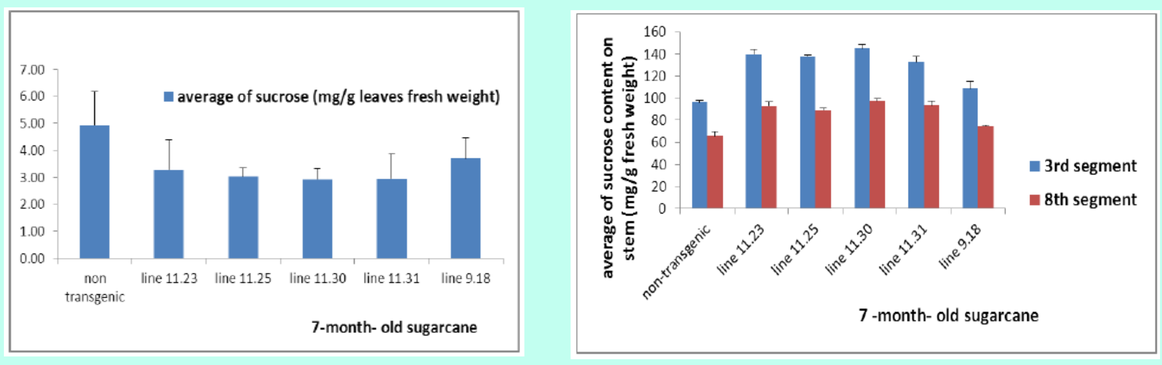

Figure 2. Sucrose content in leaves and stem of transgenic sugarcane plant
(a) Sucrose content in leaves ; (b) Sucrose content in stem on the 3rd segment (mature internodes) or 8th segment (immature internodes).

Sucrose content in transgenic leaves was lower compared to the non-transgenic leaves ((Fig. 2a). The increased of *SoSUT1* gene expression improved the sucrose translocation from leaves to the stem of transgenic sugarcane as illustrated in Figure 2b.

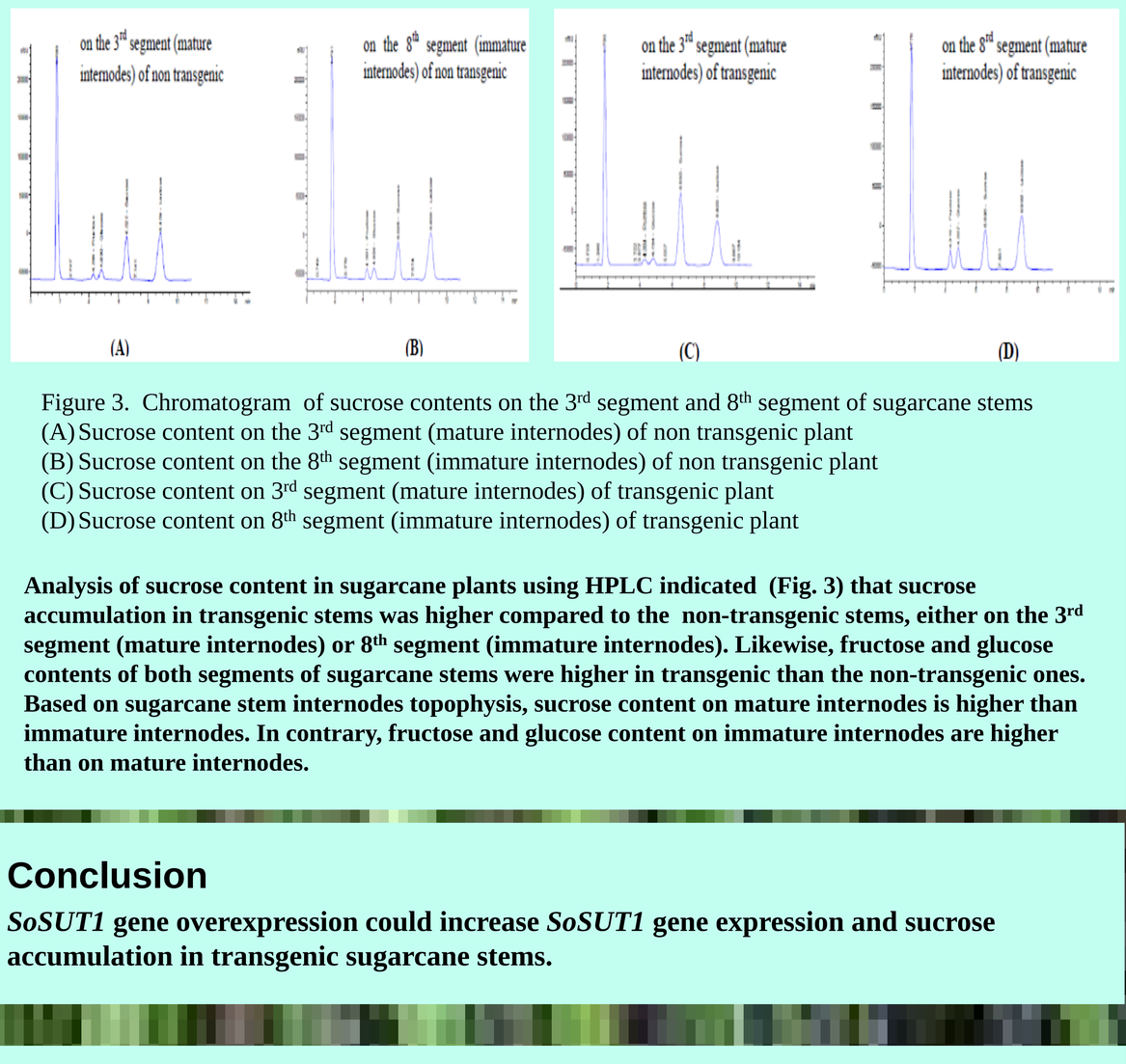

Figure 3. Chromatogram of sucrose contents on the 3rd segment and 8th segment of sugarcane stems
(A) Sucrose content on the 3rd segment (mature internodes) of non transgenic plant
(B) Sucrose content on the 8th segment (immature internodes) of non transgenic plant
(C) Sucrose content on 3rd segment (mature internodes) of transgenic plant
(D) Sucrose content on 8th segment (immature internodes) of transgenic plant

Analysis of sucrose content in sugarcane plants using HPLC indicated (Fig. 3) that sucrose accumulation in transgenic stems was higher compared to the non-transgenic stems, either on the 3rd segment (mature internodes) or 8th segment (immature internodes). Likewise, fructose and glucose contents of both segments of sugarcane stems were higher in transgenic than the non-transgenic ones. Based on sugarcane stem internodes topophysis, sucrose content on mature internodes is higher than immature internodes. In contrary, fructose and glucose content on immature internodes are higher than on mature internodes.

Conclusion

***SoSUT1* gene overexpression could increase *SoSUT1* gene expression and sucrose accumulation in transgenic sugarcane stems.**

Acknowledgements

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Captions to be set in Times or Times New Roman or equivalent, italic, 18 to 24 points, to the length of the column in case a figure takes more than 2/3 of column width.