

	すにーる くまーる たろーる
氏名（本籍地）	SUNIL KUMAR THALOR
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論文題目	Generation of sucrose-accumulating and drought tolerant plants by engineering of a single transcription factor （単一の転写因子の改変によるショ糖高蓄積そして乾燥耐性植物の創出）
博士論文審査委員	（主査） 教授 草野 友延 教授 十川 和博 教授 東谷 篤志

論文内容の要旨

Available agronomical important crops have been developed through extensively yield oriented selection and plant breeding approaches. But growing need for plant biomasses drives us to fix the maximum solar energy by photosynthesis. Sucrose is an end product of photosynthesis and a sort of easily extractable form of energy. Therefore, my concern is to find the agronomic strategy to enhance sucrose content *in planta*.

As noted sucrose is an end product of photosynthesis, also functions as a signaling molecule. One of such activities was reported in the regulation of translation of main ORF in a sub-group of Arabidopsis group S *bZIP* transcription factor-encoding genes. The phenomenon is called as sucrose-induced repression of translation (SIRT). Enhanced sucrose acts as repressor of main ORF translation mediated by one of the upstream open reading frames (uORFs) located in such group S *bZIP* genes' transcripts. Thus the uORF was termed as sucrose controlled upstream open reading frame (SC-uORF). SIRT has been well characterized in five members out of group S *bZIP* transcription factor family in Arabidopsis. Searching the redundant and non-redundant databases, I found 3-4 copies of the genes carrying conserved SC-uORFs in almost all plant organisms, suggesting the presence of SIRT beyond Arabidopsis. *lip19* members, which have been investigated in my laboratory, are phylogenetically closely related with Arabidopsis group S *bZIP* members. With this background, I used *Nicotiana tabacum tbz17*, which belongs to *lip19* members, to prove SIRT experimentally and found that its main ORF translation is repressed in presence of high level of sucrose.

The expression of *lip19* subfamily members are known to be upregulated in various abiotic stresses. Those abiotic stresses, such as drought, high salt, cold and so on, lead to low energy state because most of the biochemical processes including photosynthesis are compromised and still available energy is consumed in cellular activities for survival. As I described, the translation of *tbz17* main ORF was tightly controlled by sucrose via SC-uORF and its expression was associated with abiotic stresses and aging, while its physiological relevance in terms of sucrose metabolism and abiotic stress response has not been addressed. Keeping in mind these two objectives, I generated the tobacco transgenic plants overexpressing SIRT-insensitive *tbz17*. The resulting plants, termed *tbz17-ox*, had thicker

leaves consisting of enlarged cells and contained 3- to 4- times more sucrose compared to that of wild type plants. The gene expression analysis revealed that some of sucrose synthetic pathway genes (especially *fbpase*, *spsC*, *spp2*) have enhanced transcript levels along with asparagine synthase (*asn1*). The expressional correlation between *tbz17* and sucrose synthetic pathway genes and *asn1* was further confirmed by virus-induced gene silencing (VIGS) method using *tbz17* homologue in *N. benthamiana*. Transactivation assay using *bZIP53*, the closest *tbz17* homolog in Arabidopsis, showed that bZIP53 transactivates *asn1* but not all kinds of *sps* genes. The results suggested that *tbz17* bZIP protein transactivates *asn1* but the induction of *sps* and *spp* genes occurs in an indirect manner. I assumed that *asn1*, one of the target genes of *tbz17*, synthesizes asparagine hence changes nitrogen metabolism, which brings metabolic reprogramming and results in enhanced sucrose anabolism with unknown mechanism.

Along with sucrose accumulation, *tbz17-ox* plants were tolerant to dehydration stress compared to wild types plants. Drought tolerance is supposed to be achieved *via* enhanced *in vivo* content of biocompatible sucrose molecules along with enhanced transcript level of some seed specific protein genes like *cruciferin* and *late embryogenesis abundant-76* (*lea-76*).

To conclude, in my Ph.D. study, I demonstrated that *tbz17* exhibits SIRT mediated by its SC-uORF in a manner similar to genes belonging to the group S of *bZIP* family in Arabidopsis. Furthermore, constitutive transgenic expression of SIRT-insensitive *tbz17* leads to production of tobacco plants with thicker leaves composed of enlarged cells with 3-4 times higher sucrose content compared to wild type plants. Moreover, the transgenic plants became drought tolerant, in which some seed-specific genes such as *cruciferin* and *lea-76* were upregulated in whole plants. My finding provides a novel strategy to generate high-content sucrose plants with concomitant drought tolerance. Genes coding for the type of hypothetical sucrose-sensitive bZIP transcription factors investigated in this work are present in at least 3-4 copies per plant. Theoretically, my proposed strategy might be applicable to the genes belonging to the group of S *bZIP* genes.

Publication

Sunil Kumar Thalor, Thomas Berberich, Sung Shin Lee, Seung Hwan Yang, Ryozo Imai, Yoshihiro Takahashi, Tomonobu Kusano (2011) Generation of sucrose-accumulating and drought tolerant plants by engineering of a single transcription factor. *Paper submitted*

論文審査結果の要旨

植物においてショ糖は、光合成の最終産物のひとつであると同時にシグナル分子としても機能する。Smeekens らは、シロイヌナズナ *bZIP11* mRNA の翻訳がショ糖により抑制されること、このショ糖誘導性の翻訳抑制 (Sucrose-induced repression of translation, SIRT と略) には *bZIP11* mRNA の 5'-leader 配列にある配列 (upstream open reading frame, uORF と略) が関与することを明らかにした。当分野で低温誘導性、老化関連遺伝子として研究してきたタバコの *tbz17* は、分子系統解析から *bZIP11* と極めて近縁関係にあることが明らかとなっていた。Sunil Kumar Thalor 君は、*tbz17* mRNA にも *bZIP11* mRNA に見出された uORF と類似の uORF 配列が存在したことから、一過的な発現解析系、形質転換植物を用いて、*tbz17* mRNA からの翻訳が、uORF を介してショ糖によって抑制されるかを検討し、*tbz17* での SIRT の存在を実証した。次に、構成的プロモーターの下流にショ糖反応性配列である uORF を削除した *tbz17* 配列を置いたコンストラクトをタバコ植物に導入した。得られた植物 (*tbz17-ox* 植物) は、野生型タバコに比べ、葉のサイズが小さく、葉厚が 1.5 倍ほどになっていた。また走査電子顕微鏡により、柵状組織そして柔組織のいずれの細胞も野生株タバコ植物より大きくなっていることを明らかにした。さらにこれらの植物はショ糖を特異的に蓄積していること、それに呼応するようにショ糖合成系の酵素遺伝子の発現が亢進していること、*tbz17* の発現を抑制した植物ではショ糖合成系の酵素遺伝子の発現抑制が起こっていること、等を明らかにした。

彼は、*tbz17-ox* 植物が乾燥ストレスに耐性であることも見出した。種々の解析から、*tbz17-ox* 植物では種子の成熟過程で発現するクルシフェリン遺伝子と *LEA76* 遺伝子が高発現していることを明らかにし、ショ糖高蓄積と共に、こうした種子特異的遺伝子の高発現が、乾燥ストレス耐性形質を与えていると考察した。

上述の研究成果を導き出したことは、Sunil Kumar Thalor 君が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、Sunil Kumar Thalor 君提出の論文は、博士 (生命科学) の博士論文として合格と認める。