
Research Article**Research Progress of Trehalose-6-phosphate synthase Gene (TPS) in Resurrection Plants****Zhengwei Yan¹, Yanhua Jia¹, Xiaohong Liu^{1, 2*}**¹ College of Life Science, China West Normal University, Nanchong, Sichuan province, China;² Key Laboratory of Southwest China Wildlife Resources Conservation, Nanchong, Sichuan province, China***Corresponding author**

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Abstract: Trehalose-6-phosphate synthase (TPS), as the key enzyme for the synthesis of plants trehalose, has effects of enhancing its anti-dehydration, as well as protecting biofilm, proteins, and others from harm. Additionally, reproductive isolation between species can be broken with transgenic technology, and beneficial gene of other species can be transformed and utilized to improve resistance of crops. Rice, maize and other crops once were transformed with SOD, DREB and other drought stress tolerance genes at home and abroad, trying to improve its drought tolerance. However, the drought tolerance of transgenic offspring cannot achieve the requirements of agricultural production due to weak drought resistant ability of these genes, incongruity between drought-resistance mechanism and metabolic mechanism of these crops, and other factors. The transformation of TPS genes will be a new direction for studying stress resistance of crops in recent years.**Keywords:** Resurrection Plants; Trehalose; Trehalose-6-Phosphate Synthase Gene; Gene Transformation.

INTRODUCTION

Trehalose, also known as yeast sugar, which was first discovered by Wiggers studying *claviceps purpurea* from rye in 1832. Afterwards, it was separated from mushrooms by Mitscherlich in 1858, and named as the “mushroom sugar” [1, 2]. As a non-reducing disaccharide, trehalose is connected by two glucose residues through α -1, 1 glycosidic bond, which is non-toxic, odorless, and slightly sweet. The irreducibility of trehalose determines its stability to acid, alkali, and high temperature, which can form glassy structure combined with two molecules of water, of which the water absorption of glassy structure is 3 times larger than sucrose, maltose, glucose and fructose. In drought, cold, high salinity and other adversity stress conditions, higher concentrations of trehalose is accumulated by partial organisms, in order to enhance its anti-dehydration, and protect biofilm, proteins, and others from harm [3-7]. Therefore, improving biological stress tolerance through metabolism regulation of trehalose is a hot topic in recent years.

Resurrection plants are the general term that describes a number of plants which can tolerate severe drought stress. These plants can maintain the integrity of cell membranes through enduring the injury during dehydration and repairing it during rehydration, which could maintain cell viability in a state similar to hibernation after losing 95% water. Hence, they are special mode plants used to explore and study the

mechanism of resistance to dehydration, and a type of plant with valuable drought-resistant genetic resources [8-11]. The study found that higher concentrations of trehalose are always accumulated in the resurrection plants. This paper summarizes the nature of trehalose, its biosynthetic pathway, and research progress of trehalose-6-phosphate synthase in the application of transgenesis, in order to provide reference for in-depth study of TPS gene in resurrection plants.

The Distribution and Nature of Trehalose

People have known trehalose for nearly a century, however, it was studied in depth after 1980s, especially, the rise of cloning technology in the 1990s created conditions for the study and research of in-depth study, and from then on the physiological effect, biosynthetic pathway and other aspects were studied thoroughly. Trehalose is widely distributed in resurrection plants such as *Selaginella leptophylla* Baker in desert, yeast, fungal spore and fruiting bodies and other organisms, while in higher plants, trehalose accumulation is not found except for *Myrothamnus flabellifolia*, *Selaginella lepidophylla*, and other resurrection plants [8-11], in addition, trehalose accumulation at very low level is detected in *Arabidopsis*, tobacco and potato and other plants under drought stress and in the experiments of validamycin A inhibiting the hydrolase activity of trehalose [12-14].

Trehalose is white crystal, among which, its molecular formula is $C_{12}H_{22}O_{11} \cdot 2H_2O$, molecular weight is 378.33, melting point is 96.5~97.5°C, heat of fusion is 57.8kJ/mol, the specific rotation is +178.3° (20°C, 7% in water), and the density of crystalline trehalose is 1.512g/cm³. Theoretically, trehalose has three types of isomers, including α , β -1, 1-trehalose (neotrehalose), β , β -1, 1-trehalose (iso trehalose), and α -1, 1 trehalose (mycose), however, only α , α -1, 1 trehalose exists in Free State in nature [2]. It has weak sweetness, whose sweetness accounts for 45% of sucrose. Besides, trehalose can be dissolved in water, acetic acid and hot

ethanol, and insoluble in ether, acetone, it can also not restore Fehling's solution. In conclusion, the solubility of it in water is greatly affected by temperature, and trehalose will lose crystal water and change into anhydrous crystals when heated to 130°C.

Biosynthetic Pathway of Trehalose

In accordance with the different substrate during synthetic process of trehalose, the synthetic pathway can be divided into five types, consisting of TPS-TPP pathway, Tre-TreZ pathway, TreS pathway, TreT pathway, and TruP pathway. As shown in Figure 1:

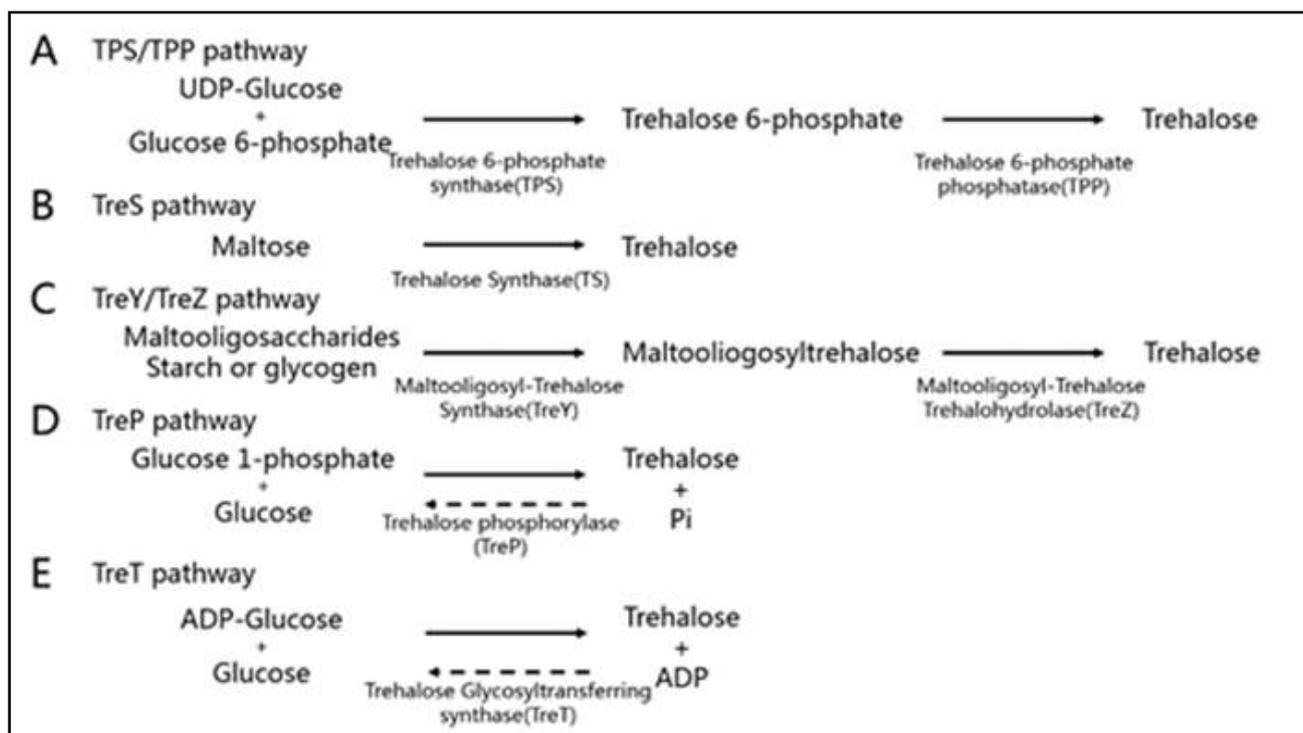


Fig-1: Five passways of trehalose synthesis

During biosynthetic pathway of trehalose, two key enzymes are involved, that is TPS and TPP, the metabolic pathways of trehalose in plants are shown in the figure 1 (A), the glucose in UDP-glucose and glucose 6-phosphate generates trehalose-6-phosphate (Tre6P) and UDP through catalytic reaction of TPS, moreover, trehalose-6-phosphate generates free trehalose through the reaction of TPP. Trehalose-6-phosphate synthase is rate-limiting enzyme of this reaction.

Research Progress of Trehalose-6-Phosphate Synthase

The synthesis of E. coli trehalose consists of two steps: first of all, TPS with *otsA* gene coding catalyze UDP-glucose and 6-glucose phosphate, so as to synthesize trehalose-6-phosphate connected by α -1,1glucosidic bond, and then TPS with *otsA* gene coding transforms it into trehalose. *otsA* and *otsB* gene expression are regulated by the same operon [15-17]. In yeast, TPS (56kDa) and TPP (102kDa) enzyme are

encoded by TPS1 and TPS2 gene, so as to form trehalose synthase complex combined with TSL1 and TPS3 regulatory subunits, and catalyze the synthesis of trehalose. In the study conducted by Garg *et al.*, one expression vector formed by *otg* and *usB* genes is transformed into rice genome, in order to improve the accumulation level of trehalose in transgenic rice, and its resistance to adversity. Whereas, many studies show that TPS enzyme monomer can also catalyze the synthesis of trehalose in cells [18-20].

At present, there are few reports on enzymatic characteristics of TPS derived from plants at home and abroad. Valenzuela-Soto and others [10] found that molecular mass of TPS in resurgent selaginella tamariscina was 115kDa, isoelectric point was 4.69, optimum pH was 7.0, after separation and purification of immobilized metal affinity chromatography (IMAC), and its activity was activated by Ca²⁺, Mg²⁺, K⁺, Na⁺ and other ions with low concentration, fructose 6-phosphate, fructose and glucose, and restrained by

proline. There was no depressant effect of substrate and the end product on the activity of TPS enzyme. Márquez-Escalantea [46] comprehensively utilized molecular-exclusion chromatography, ion-exchange chromatography, electrophoresis and other technologies and proved that the size of TPS enzyme monomer of resurgent selaginella tamariscina was 115kDa, which existed in polymer form in cells, namely dimer, tetramer and hexamer with 224, 434, 624kDa of molecular size respectively. These three polymers can be maintained in a stable structure in the range of from 5 to 11 of pH value, but the enzymatic activity of different polymers has quite different optimum temperature.

The Application of TPS Gene in Plant Genetic Transformation

The most important role of TPS is to catalyze substrate, so as to generate trehalose through transformation. Trehalose is accumulated in plants, playing a protective function, so that some mal-character of plants can be improved, and the effect of optimization of breeding can be achieved. During the study of plant stress resistance, metabolism regulation of trehalose and gene transformation of plant draws people's great attention. The application of TPS gene in plant genetic transformation has achieved some progress (as shown in table 1).

Table-1: The application of TPS gene in plant genetic transformation

Gene	Source	Promoter	Plants	Main function	References
otsA, otsB	Escherichia coli	CaMV35 S	Potato	Transgenic potato has dwarf plant and enhanced drought tolerance.	Goddijn O <i>et al.</i> [12]
otsA, otsB	Escherichia coli	Rsu, Abai	Meal of rice	Transgenic rice includes trehalose accumulation. Which can grow normally and has light oxidative damage under stress conditions; While under abiotic stress conditions, the balance of its mineral nutrition and stress resistance will be enhanced.	Garg AK <i>et al.</i> [17]
otsA, otsB	Escherichia coli	Ubi1	Rice	Transgenic rice includes trehalose accumulation; its performance has deficiency changes, which is resistant to drought, salt damage and chilling injury.	Jang IC <i>et al.</i> [21]
otsA, otsB	Escherichia coli	CaMV35 S	Tobacco	Photosynthesis of transgenic tobacco changes, which will grow fast under drought stress.	Pellny TK <i>et al.</i> ; [22]
TPSP	Escherichia coli	pABA	Wheat	Transgenic wheat shows strong drought resistance in the greenhouse	Wang Xiao <i>et al.</i> [23]
TPSP	Escherichia coli	pABA	Wheat	The germination rate of transgenic lines is significantly higher than the control plants, showing certain drought resistance ability.	Li Jinhua. [24]
otsA	Escherichia coli	CaMV35 S	Tobacco	Transgenic tobacco will be hypo genetic, having less water loss from detached leaf, which can help to improve salt tolerance, drought resisting and other adverse-resistant characteristics.	Wang Yi qin <i>et al.</i> ; [25]
otsA	Escherichia coli	CaMV35 S	Barbado s aloe	Transgenic aloe contains trehalose accumulation.	Chen Jie <i>et al.</i> ;[26]
otsA	Escherichia coli	Prd29A	Wheat	Transgenic wheat contains trehalose accumulation, whose drought resisting and salt tolerance may be enhanced, however, it needs to be further identified.	Kang Xusheng <i>et al.</i> ; [27]
otsA	Escherichia coli	CaMV35 S	Nicotian a benthamiana	The seeding of transgenic nicotiana benthamiana has significantly enhanced salt resistance.	Lu Yujian <i>et al.</i> ; [28]
otsB	Escherichia coli	CaMV35 S	Nicotian a benthamiana	The adverse resistance of transgenic nicotiana benthamiana will be improved obviously.	Lu Yujian <i>et al.</i> [47]
TPS1	Yeast	CaMV35 S	Tobacco	Transgenic tobacco contains trehalose accumulation, with the characteristics of slow growth, phenotypic changes, and enhanced drought resistance. The drought resistanceof plants extent of morphological changes are positively correlated with each other.	Romero C <i>et al.</i> ; [29]
TPS1	Yeast	CaMV35 S	Potato	Transgenic potato has dwarf plant and enhanced drought resistance.	Yeo ET <i>et al.</i> ; [30]

TPS1	Yeast	CaMV35 S	Tomato	Transgenic tomato has dwarf plant and little trehalose accumulation, with the characteristics of drought resistance, salt damage tolerance, and oxidative stress resistance.	Cortina C <i>et al.</i> ; [31]
TPS1	Yeast	Rsu	Tobacco	Transgenic tobacco has dwarf plant, whose osmotic stress and tolerance are enhanced.	Holmstrom KO <i>et al.</i> ; [32]
TPS1	Arabidopsis	CaMV35 S	Tobacco	Transgenic tobacco can grow normally in medium with glucose.	Leyman B <i>et al.</i> ; [33]
TPS1	Saccharom vces cerevisiae	p-wcs120	Maize	The drought tolerance of transgenic Maize plants may be improved, however, it needs to be further identified.	Zhang Zhiyong <i>et al.</i> ; [34]
TPS1	TPS genes	rd29A	Maize	The drought resistance of transgenic Maize plants may be enhanced.	An Lihun <i>et al.</i> ; [35]
TPS	Yeast	Rsu	Tobacco	Transgenic tobacco contains trehalose accumulation, having less water loss from detached leaf,	Holmstrom KO <i>et al.</i> [32]
TPS	Saccharom vces cerevisiae	Prd29A	Tobacco	Transgenic tobacco has morphological changes, and the drought resistance will be enhanced.	Zhao Huiwu <i>et al.</i> ; [36]
TPS	Yeast	Ubi	Ryegrass	Under water stress, the water retaining capacity and drought resistance of transgenic ryegrass are enhanced.	Jia Weilong <i>et al.</i> ; [37]
TPS	Arabidopsis	CaMV35 S	Tobacco	Transgenic tobacco contains trehalose accumulation, whose stress resistance is improved.	Guo Bei <i>et al.</i> [38]
TPS	TPS genes	Ubiquitin	Maize inbred line	The drought resistance of transgenic Maize plants (strain) is higher than control plants.	Dong Chunlin <i>et al.</i> ; [39]
AtTPS1	Arabidopsis	CaMV35 S	Arabidopsis	Transgenic arabidopsis plants contain trehalose accumulation, whose dehydration and tolerance are enhanced.	Avonce N <i>et al.</i> ; [40]
AtTPS2	Saccharom vces cerevisiae	mwcs120	Maize	The drought tolerance of transgenic Maize plants may be improved; however, it needs to be further identified.	Mou Yu <i>et al.</i> ; [41]
SpTPS1	Selaginella pulvinata	PMA1	Deletion mutant yeast strains	Trehalose of deletion mutant yeast strains is recoverd.	Lin Jing <i>et al.</i> [42]
SpTPS1	Selaginella pulvinata	Ubiquitin	Hemarthria compressa	The stress resistance of transgenic regeneration plant is enhanced.	Xu Yaohua [43]
PyTPS	Pyropia yezoensis	Prd29A	Peanut	The salt tolerance of transgenic peanut is enhanced, having influence on the quality of some seed progeny.	Wang Ya <i>et al.</i> ; [25]
GaTPS	Asiatic cotton	CaMV35 S	Tobacco	Control tobacco plants wilting evident is obvious wilted, which has worse affected degree than transgenic tobacco.	Chen Yajuan [44]

Notes: CaMV35S: Cauliflower mosaic virus 35's promoter; Rsu: Rubisco small subunit; Abai: ABA-inducible; Ubi1: Maize Ubi-1 promoter; Prd29A: Osmotic stress inducible promoter; mwcs120: Monocots stress inducible promoter; p-wcs120: Plant stress inducible promoter; PMA1: PMA promoter; rd29A: Drought responsive promoter; PABA: Chimeric drought responsive promoter; Ubiquitin: Maize Ubiquitin promoter; TPSP: Fusion gene of ostA and ostB of E. coli.

Environmental stress is a major factor affecting plant growth and development, among which, drought and salt stress have the most serious impact on the plant. The stress resistance of plants is improved through establishing genetic transformation system, offering us the possibility that TPS gene could be modified. Trehalose, as a storage carbohydrate, is an

important product of stress metabolism, which is greatly influenced by the environment, moreover, after influencing of abiotic stress, the expression of TPS gene is strengthened, so as to improve the vitality of enzyme, and enhance cell enrichment ability of trehalose. The ability of plants to resist various abiotic stresses will be improved through genetic engineering technology,

which has very important significance for promoting grain yield increase and cultivating superior crops.

Outlook

Drought is the main limiting factor for many crops production. Improving drought tolerance of crop varieties through breed improvement is the most cost-effective method to overcome the drought hazard. However, different drought tolerance among most crops is just relative, even the germplasm with the drought tolerance cannot meet the production requirement for drought tolerance. Generally, drought tolerance is a type of quantitative characters controlled by micro-effect polygene; its genetic improvement has more difficult, which has little progress over the years. Transgenic technology can break reproductive isolation between species, transforming and utilizing beneficial gene of other species. Rice, maize and other crops once were transformed with SOD, DREB and other drought stress tolerance genes at home and abroad, trying to improve its drought tolerance. However, the drought tolerance of transgenic offspring cannot achieve the requirements of agricultural production due to weak drought resistant ability of these genes, incongruity between drought-resistance mechanism and metabolic mechanism of these crops, and other factors. In order to develop the genes with higher drought resistant ability, which are suitable for physiological metabolism of crops and agricultural production requirements, exogenous trehalose-6-phosphate synthase gene is transformed by many researchers at home and abroad into the plant in recent years, improving the stress resistance of transgenic plants, especially drought resistant ability, which provides a new research direction for improving stress resistance of crops.

In recent years, the studies on stress resistance of crops at home and abroad shows that the selection of stress resistance gene is partial to adverse-resistant plants rather than bacteria and fungi gradually, for instance, in resurrection plants of ferns, the trehalose content of some Selaginellaceae xeric species in dry conditions can reach up to 20% of the total. Lin Jing [45] compared the amino acid sequence of typical TPS1 in multiple species with Clustalx1.8 program, and established phylogenetic tree in neighbor-joining method and maximum parsimony provided by MEGA software, indicating that the traditional classification of phylogenetic tree established with neighbor-joining method and that established by maximum parsimony is consistent. Resurrection plants have closer affinity than bacterium and fungus, which provides us another important reference for selecting stress resistance genes. I believe that there will be more research findings about transgenosis of resurrection plants in the future.

At present, the gene transformation of TPS gene has obtained a wide range of applications and markets, and the application of trehalose has entered into biological field from agriculture, food and medical

domain. Furthermore, throughout the development process, TPS gene has obtained rapid development, and the trehalose called “the sugar of life” has been attracts universal attention of the society and people.

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