Genetic Modification of Trehalose Metabolism in Cereal Crops to Improve Grain Yield

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Abstract

Rice and maize are the most important cereal crops in the world and are the primary source of food energy for more than half of humanity. Drought and salinity are the major environmental stresses reducing agricultural productivity worldwide. Efforts to improve crop performance under abiotic stress conditions have met with limited success because the fundamental mechanisms of stress tolerance in plants are still not well understood. Fortunately, it is now possible to use transgenic approaches to improve abiotic stress tolerance and grain yield in agriculturally important crops with far fewer target traits than had been previously anticipated. Trehalose is a non-reducing disaccharide of glucose that functions as a compatible solute and in the stabilization of biological structures under abiotic stress in bacteria, fungi and invertebrates. With the notable exception of the desiccation-tolerant "resurrection plants", trehalose does not accumulate to significant levels in the vast majority of plants, in spite of the proliferation of plant trehalose pathway genes. Phylogenetic analyses of protein sequences derived from the corresponding DNA sequences from the completely annotated genomes of a monocot (rice) and a dicot (Arabidopsis) plant species showed the presence of gene families for both trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP), indicating the genomic complexity of trehalose biosynthetic genes in plants. Also, very recent studies have shown that trehalose metabolism is of immense importance in numerous plant processes and its manipulation has great potential in crop improvement. Here, we report our results on the regulated overexpression of a trehalose biosynthetic fusion gene in cereal crops (rice and maize) for the purposes of improving abiotic stress tolerance and other agronomic traits. The expression of the transgene was under the control of light-inducible or stress-dependent promoters. Compared with non-transgenic plants, several independent transgenic lines showed higher levels of trehalose accumulation and total soluble carbohydrates, increased photosynthetic capacity, exhibited sustained plant growth and improved grain yield under non-optimal conditions. These results demonstrate the potential of manipulating trehalose metabolism in cereal crops to improve abiotic stress tolerance and yield traits.

Keywords: Rice, maize, abiotic stress, trehalose-6-phosphate synthase, phosphatase

Introduction

The first green revolution-led in the 1960s by several eminent scientists resulted in a dramatic increase in wheat, rice and maize grain yields. During the 20th century, agricultural researchers were reasonably successful in developing new elite cultivars that met with the demand for staple foods for the growing world population. However, abiotic stresses such as extreme temperatures, drought, salinity and mineral deficiency and toxicity as well as climate change are now reducing cereal crops yields initiating a major concern about global food security. Traditional approaches to breeding monocot plants with improved stress tolerance have thus far met with limited success. This lack of desirable progress is attributable to the fact that abiotic stress tolerance is a complex trait that is influenced by coordinated and differential expression of a network of genes. Fortunately, recent developments in transgenic research offer new opportunities for elucidating the function of many useful candidate genes from numerous organisms that will lead to the improvement of stress tolerance of agriculturally important crop plants.

The discovery of trehalose metabolism has been one of the most exciting developments in plant metabolism and plant science in recent years. Trehalose [α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside], a dimer of glucose, is present in diverse organisms such as bacteria, fungi, insects, and some invertebrates, and known to have various functions that distinguish it from another non-reducing sugar sucrose [α-D-glucopyranosyl-(1→4)-β-D-fructofuranoside]. There is considerable evidence for a role for trehalose in protection from desiccation, salinity, osmotic stress as well as extreme tolerance to temperatures by stabilizing dehydrated enzymes, proteins, and lipid membranes, regulation of carbohydrate metabolism, and protection of biological structures from damage against a variety of environmental stresses (Crowe et al 1992; Wingler 2002; Crowe 2007).

In higher plants, trehalose-6-phosphate (T6P) or trehalose has been shown to control plant development and inflorescence branching by acting as a signaling molecule (Satoh-Nagasawa et al 2006; Ramon and Rolland 2007). In bacteria there are five different trehalose biosynthetic routes, whereas in fungi and plants there is only one (Avonce et al 2006). The single pathway for trehalose biosynthesis that is common to both prokaryotes and eukaryotes consists of two reactions. First, trehalose-6-phosphate is generated from UDP-glucose (UDP-Glu) and glucose-6-phosphate (G6P) in a reaction catalyzed by trehalose-6-phosphate synthase (TPS, EC 2.4.1.15). T6P is then dephosphorylated to form trehalose via trehalose-6-phosphate phosphatase (TPP, EC 3.1.3.12). TPS and TPP genes were functionally identified in *Arabidopsis thaliana* by complementation of yeast mutants (Blazquez et al 1998; Vogel et al 1998). Homologous TPS and TPP genes have now been identified in many other plant species. These results suggest that trehalose synthesis may in fact be ubiquitous among angiosperms, although the levels to which it accumulates are generally low (Goddijn and Van Dun 1999).

The question then arises as to whether the trehalose pathway is omnipresent and important in plants. The answer is that although the presence of trehalose biosynthesis genes in higher plants has been demonstrated, details of both the physiological functions and the regulation of this pathway remain largely unknown. In general, only one or two copies of TPS and TPP genes, with highly conserved substrate binding and catalytically relevant amino acid residues, exist in most bacteria, fungi and insects (Gibson et al 2002). On the other hand, similar genes in higher plants constitute a large gene family, but lack several of the catalytically relevant residues. For example, genome sequencing of *Arabidopsis* and rice has revealed complex genomic organization of plant trehalose biosynthesis genes (Leyman et al 2001; Paul et al 2001; Lunn 2007). Eleven putative TPS genes were identified within the genome of *Arabidopsis* and rice, whereas 10 putative TPP genes were found within the *Arabidopsis* and eleven in rice. However, only one putative trehalase (TRE) gene is known to exist in both model species. Interestingly, trehalose does not accumulate to any appreciable level in either species. Notably, there are more putative genes for the synthesis of trehalose than for sucrose. It now appears that even though the chemistry of trehalose and sucrose is similar, the biological functions performed by these sugars are quite different in crop plants.

Recently, several research groups have been trying to genetically modify trehalose biosynthetic pathways in plants to enable a study of its effect on plant growth, development, biotic and abiotic stress tolerance and yield traits (Table 1). In most cases, however, constitutive overexpression of TPS and/or TPP encoding genes from yeast or *Escherichia coli* in model plants resulted in enhanced trehalose levels, but also caused stunted plant growth, lancelet leaves, altered roots and changes in carbohydrate metabolism under normal growth conditions (Goddijn et al 1997; Romero et al 1997; Pilon-Smits et al 1998; Yeo et al 2000; Dai et al 2001).

As an alternative strategy for engineering enhanced trehalose accumulation in rice or maize, we used an ABA stress-inducible ABRC promoter (Su et al 1998) to drive the overexpression of *E. coli* trehalose biosynthetic genes (*otsA* and *otsB)*. The resulting fusion gene (TPSP) has the dual advantages of requiring only a single transformation event to introduce both genes simultaneously into the plant genome, while at the same time imitating naturally occurring putative bipartite TPS/TPP-like genes in plants (Seo et al 2000). We introduced the TPSP gene into rice or maize via *Agrobacterium*-mediated gene transfer and created a large number of transgenic rice and maize plants that grow well under normal growth conditions and are completely fertile (Garg et al 2002; Garg et al 2012). Furthermore, the homozygous transgenic rice plants exhibited sustained plant growth, less photo-oxidative damage and more favorable mineral balance under both salt and low-temperature stress conditions, as compared to non-transgenic plants (Garg et al 2002). Under non-stress conditions, transgenic rice or maize lines accumulate trehalose in shoots at concentrations from 50 to 200 ug/g fresh weight (FW) depending on growing conditions.

Table 1. Genetic modification of trehalose metabolism in plants

Plant species	Impact of genetic modification on traits	Reference
Tobacco	Increased trehalose levels; transgenic plants showed less water loss upon leaf detachment	Holmström et al (1996)
Tobacco potato	Low levels of trehalose in leaves of tobacco; no detectable level of trehalose in potato; inhibition of trehalase activity improves trehalose accumulation	Goddijn et al (1997)
Tobacco	Higher levels of trehalose; phenotypic alterations (stunted growth; lancet-shaped leaves); improved drought tolerance	Romero et al (1997)
Tobacco	Phenotypic alterations (larger leaves and shorter stems under drought stress)	Pilon-Smits et al (1998)
Potato	Phenotypic alterations (dwarfism); drought tolerance	Yeo et al (2000)
Tobacco	Altered phenotype (stunted growth); transgenic plants showed less water loss upon leaf detaching	Dai et al (2001)
Rice	Higher trehalose levels; sustained plant growth under drought, salt and cold stress; less photo-oxidative damage; favorable mineral balance under abiotic stress; increased photosynthetic capacity	Garg et al (2002)
Rice	Increased trehalose levels; absence of phenotypic alterations; salinity and drought stress tolerance	Jang et al (2003)
Arabidopsis	T6P is indispensable for carbohydrate utilization and growth	Schluepmann et al (2003)
Tobacco	Increased photosynthetic capacity per leaf unit area; increased growth rate and whole-plant biomass	Pellny et al (2004)
Arabidopsis	Normal vegetative growth and transition to flowering	Van Dijken et al (2004)
Tomato	Higher trehalose content; altered phenotypes (dwarfism and lancet shaped leaves); tolerance to drought, salt and oxidative stress	Cortina and Culiáñez-Macià (2005)
Tobacco	Higher trehalose content; no morphological alteration; tolerance to water deficit	Han et al (2005)
Tobacco	Tolerance to osmotic stress; transgenic plants smaller than wild type	Almeida et al (2005)
Arabidopsis	Increased starch content in leaves	Kolbe et al (2005)
Arabidopsis	Activation of ADP glucose pyrophosphorylase and higher rates of starch synthesis	Lunn et al (2006)
Tobacco	Plants able to grow in media containing glucose (glucose-insensitive phenotypes)	Leyman et al (2006)
Sugarcane	Very high levels of trehalose accumulation in transgenic plants (9-13 mg/g FW); improved drought tolerance	Zhang et al (2006)
Tobacco *Arabidopsis*	Higher trehalose content; alteration of root development in *Arabidopsis*; improved drought tolerance	Karim et al (2007)
Arabidopsis	Tolerance to multiple and extreme abiotic-stress conditions; trehalose accumulation in transgenic plants	Miranda et al (2007)
Common bean	Improved drought tolerance, increase grain yield, trehalose acts as a key metabolite in plant-rhizobacteria interactions	Suarez et al (2008)
Potato	Marker-free transgenic plants showed higher stomatal conductance and altered drought response as compared to control plants	Stiller et al (2008)
Arabidopsis	Regulates cell shape and plant architecture	Chary et al (2009)
Potato	Altering T6P content affects tuber growth and alters responsiveness to hormones during sprouting	Debast et al (2011)
Maize Rice	Higher levels of trehalose and other soluble carbohydrates; increased capacity for photosynthesis; differences in plant growth, development and improved grain yield under non-optimal conditions; multiple stress tolerance	Garg et al (2012)
Arabidopsis	T6P is required for the onset of leaf senescence associated with high carbon availability	Wingler et al (2012)
Rice	Transgenic rice produced high amounts of soluble sugars and showed enhanced tolerance to both drought and salt stress.	Redillas et al (2012)

Under non-stress conditions, however, the range of trehalose accumulation is significantly higher at concentrations up to 850 ug/g FW when salt and Non-transgenic plant displayed a considerable decline in soluble carbohydrates in shoots under salt stress. In contrast, transgenic plants showed a significant increase in the accumulation of trehalose, glucose, sucrose and soluble starch in shoot tissue in response to abiotic stresses. The level of stress-induced trehalose accumulation in transgenic rice or maize plants was far below that observed in resurrection plants (Garg et al. 2002; Redillas et al. 2012). These data indicate that the enhanced stress tolerance we observed in transgenic plants was not a direct effect of trehalose acting as an osmoprotectant. Rather, the correlation between enhanced stress tolerance and total soluble carbohydrate levels suggests that trehalose may be acting as a general regulator of carbon metabolism, as previously reported for some microorganisms (Thevelein and Hohmann 1995).

The discovery of a plethora of trehalose metabolism enzymes in higher plants, and its role in modulating photosynthesis, carbon metabolism and stress protection, has led to a new series of scientific surprises and offers new challenges for researchers in this field. Furthermore, it has been demonstrated that trehalose has a fundamental role in embryo development (Eastmond et al 2002; Gómez et al 2005), and in abscisic acid and sugar signaling (Avonce et al 2004) in *Arabidopsis*. Therefore, analysis of the tissue-specific expression of trehalose biosynthesis and degradation should shed light on the role of trehalose in abiotic stress tolerance, plant metabolism, growth and development, plant-pathogen interactions and seed development. In view of the latest findings, plant trehalose research should be seen as an opportunity to use multidisciplinary approaches for the dissection of plant metabolic networks, including the interface between sugar sensing-signaling and carbohydrate metabolism. Clearly, further studies are needed to resolve the functions of several plant TPS and TPP proteins to understand the intricacies of trehalose metabolism in plants.

Conclusions

Engineering of trehalose overproduction in rice and maize plants can be achieved by stress-inducible expression of a bifunctional TPSP fusion gene without any detrimental effect on plant growth or grain yield. During abiotic stress, transgenic plants accumulated increased amounts of trehalose and exhibited high levels of tolerance to abiotic stresses, as compared to non-transgenic plants. These results demonstrate the potential for utilizing our transgenic approach to develop new improved rice and maize cultivars with increased abiotic stress tolerance and enhanced drought stress is imposed. It is clear that many factors, in addition to endogenous trehalose levels, function to regulate plant stress responses. productivity. In principle, this same technique can be used to confer stress tolerance to other economically important cereal crops such as wheat, sorghum and millets.

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