

TsMIP6 Enhances the Tolerance of Transgenic Rice to Salt Stress and Interacts with Target Proteins

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Abstract Aquaporins (AQPs), a large family of channel proteins in plants, play an important role in regulating the balance of osmotic potential in cells. We isolated an AQP gene, *TsMIP6*, from the halophyte *Thellungiella salsuginea* and functionally characterized it in transgenic rice (*Oryza sativa*). This gene belongs to a subfamily of tonoplast intrinsic proteins and is localized at the plasma membrane. Real-time PCR showed that expression of *TsMIP6* in shoots or roots of *T. salsuginea* was markedly induced by salinity, whereas its ectopic expression in ‘Kitaake’ lines of rice significantly increased plant tolerance to salt stress. Physiological data suggested that *TsMIP6* is involved in regulating ion homeostasis and water channel activity in salt-stressed transgenic rice. Heterologous expression analysis indicated that *TsMIP6* specifically interacts with a member of the glycoside hydrolase family 64 protein #617 in yeast cells. This suggests that the relationship between *TsMIP6* and #617 has a crucial role in mediating osmotic balance in plant cells. Moreover, *TsMIP6* might help to modulate the transport of some neutral molecules and may function through a pathway regulating solute equilibrium to maintain osmotic potential.

Keywords: Aquaporin, Interaction, Real-time PCR, Salt stress, Transgenic Kitaake

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Introduction

As major intrinsic proteins, aquaporins (AQPs) play an important role in facilitating the passive transport of water across membranes (Johanson et al. 2001). Based on their structural characteristics, plant AQPs can be divided into four subfamilies: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs), and small basic intrinsic proteins (SIPs) (Wang et al. 2014). Aquaporins support the movement of small neutral molecules, e.g., urea, boric acid, silicic acid, glycerol, ammonia, carbon dioxide, and H₂O₂ (Biela et al. 1999; Maurel et al. 2008; Wang et al. 2014). Peng et al. (2007) have found that NtAQP1 from tobacco (*Nicotiana tabacum*) promotes CO₂ membrane transport in a homologous plant system (*Arabidopsis thaliana*) at the cellular level, and functions in photosynthesis and stomatal opening. In a yeast system, AQPs within the TIP, NIP, and SIP subfamilies enhance the capacity to transport H₂O₂ (Bienert et al. 2008, 2011; Maurel et al. 2008).

The involved response by AQPs to abiotic stress provides important insight into how plants tolerate high salt and drought, which is accomplished through a pathway of osmotic regulation (Wang et al. 2014). However, due to diverse efficiency, they can exhibit different expression profiles under such stresses (Alexandersson et al. 2005; Guo et al. 2006). Their physiological responses are also multifaceted (Guo et al. 2006) and overexpression by AQP genes can lead to increased stress tolerance in transgenic plants. For example, overexpression of *TaNIP* in transgenic *Arabidopsis* improves tolerance to excess salt (Gao et al. 2010) and can