Identification of salt and drought inducible glutathione S-transferase genes of hybrid poplar

Soon-Ho Kwon · Hye-Kyoung Kwon · Wook Kim · Eun Woon Noh · Mi Kwon* · Young Im Choi*

Research Article

Received: 25 November 2013 / Revised: 28 November 2013 / Accepted: 29 January 2014
© Korean Society for Plant Biotechnology

Abstract Recent genome annotation revealed that Populus trichocarpa contains 81 glutathione S-transferase (GST) genes. GST genes play important and varying roles in plants, including conferring tolerance to various abiotic stresses. Little information is available on the relationship – if any – between drought/salt stresses and GSTs in woody plants. In this study, we screened the PatgGST genes in hybrid poplar (Populus alba × Populus tremula var. glandulosa) that were predicted to confer drought tolerance based on our expression analysis of all members of the poplar GST superfamily following exposure to salt (NaCl) and drought (PEG) stresses, respectively. Exposure to the salt stress resulted in the induction of eight PatgGST genes and down-regulation of one PatgGST gene, and the level of induction/repression was different in leaf and stem tissues. In contrast, 16 PatgGST genes were induced following exposure to the drought (PEG) stress, and two were down-regulated. Taken together, we identified seven PatgGSTs (PatgGSTU15, PatgGSTU18, PatgGSTU22, PatgGSTU27, PatgGSTU46, PatgGSTU51 and PatgGSTU52) as putative drought tolerance genes based on their induction by both salt and drought stresses.

Keywords Glutathione S-transferases, salt stress, drought stress, Populus alba × Populus tremula var. glandulosa

Introduction

Gene/genomic duplication events have been very common occurrences during the evolution of eukaryotes. Some of these duplicated genes/genomic sequences would have been prone to mutation and may have acquired novel functions in addition to their original function(s), or they may have changed their original function(s) completely. In extreme cases, they might have become pseudogenes (Hughes 1994; Force et al. 1999; Moore and Purugganan 2005). Thus, it is not at all uncommon to identify duplicated genes in eukaryotes, especially in plants (Tuskan et al. 2006). These duplicated copies of the genes form a unique gene family and it is a commonly accepted assumption that all of those genes have evolved and diversified after repeated duplication events, as exemplified by the glutathione S-transferase (GST) gene family in plants. GSTs (EC 2.5.1.18) are multi-functional proteins that can be found in all organisms (Smith et al. 2004). They protect cells from both biotic and abiotic stresses, including xenobiotics, heavy metals, pathogens, and oxidative bursts (Kampranis et al. 2000; Mueller et al. 2000; Agrawal et al. 2002).

Plant GSTs are subdivided into eight distinct classes. These are Phi, Tau, Theta, Zeta, Lambda, glutathione-dependent dehydroascorbate reductases (DHARs), tetrachlorohydroquinone dehalogenase (TCHQD) (Basantani and Srivastava 2007) and γ-subunit of the eukaryotic translation elongation factor 1B (EF1Bγ) with last subfamily only being included as a member of the GST family based on structural similarities to GSTs (Jeppesen et al. 2003; Oakley 2005; Lan et al. 2009). Among these, Phi and Tau are plant specific whereas the Theta and Zeta classes are found in mammals, fungi, and insects (Sheehan et al. 2001; Smith et al. 2004). In plants, each GST family has a large number of members. For example, the GST gene family in Arabidopsis thaliana has 53 members (Dixon et al. 2002; Wagner et al. 2002), 79 members in Oryza sativa (Soranzo et al. 2004) and 81
members in *Populus trichocarpa* (Lan et al. 2009). In *Populus trichocarpa*, the Tau and Phi GSTs were the most numerous, being represented by 58 and 9 members, respectively (Lan et al. 2009). The Lambda, DHAR, and EF1B gamma GST classes were each represented by three members, both the Zeta and Theta classes by two members, and the TCHQD class by just one member (Lan et al. 2009).

GST genes play very important and varying roles in plants. Some of the roles reported to date include conferring tolerance to oxidative stress and UV-radiation, protecting cells from biotic and abiotic stress, and providing defense against cadmium toxicity (Kampranis et al. 2000; Loyall et al. 2000; Agrawal et al. 2002; Bianchia et al. 2002). Tau- and Phi class GSTs, the two largest classes and the most abundant GSTs in plants, participate in endogenous cellular metabolism by functioning as glutathione peroxidases that counteract oxidative stress, as flavonoid-binding proteins and as stress signaling proteins (Dixon et al. 2002; Mueller et al. 2000; Loyall et al. 2000; Basantani and Srivastava 2007). Although detailed knowledge of the physiological and molecular mechanisms of stress tolerance in plants is not yet available, considerable evidence has been accumulated to indicate that GSTs play a protective role in mitigating the effects of drought and salinity stresses in plants (George et al. 2010; Ji et al. 2010; Wei et al. 2010; Jha et al. 2011; Chen et al. 2012). For example, Ji et al. (2010) observed that the overexpression of *Glycine soja* GST gene in tobacco enhanced the drought and salt stress tolerance of the transgenic tobacco plants. In another study, transgenic tobacco plants overexpressing the *Salicornia brachiata* GST gene were more tolerant of the salt stress condition than wild-type tobacco plants (Jha et al. 2011).

Poplar species are fast-growing temperate woody plants with high potential for biomass production. The recent sequencing of the entire genome of *P. trichocarpa* has led to an abundance of gene expression data becoming available. Based on poplar genome annotation, Lan et al. (2009) showed that *Populus* spp. have the largest GST family known to date. Thus, this plant would appear to be an ideal model species to study the function of GSTs in plants. GSTs are a particularly interesting research topic since the functions of some GST genes might be fully or partially overlapping in terms of salt and drought stress tolerance.

In this study, our aim was to screen the members of the poplar GST family that respond to salt and drought stresses by semi-quantitative reverse transcriptase (RT)-PCR analysis. Seven members of the Tau class of the GST family were screened based on transcript levels in the hybrid poplar (*Populus alba × P. tremula var. glandulosa*) under conditions of salt (NaCl) and drought [polyethylene glycol (PEG)] stresses.

**Materials and methods**

Plant culture and stress treatments

A hybrid aspen clone BH1 (*Populus alba × P. tremula var. glandulosa*) was vegetatively propagated via shoot-tip culture on solid rooting medium [half-strength MS medium (Murashige and Skoog 1962) supplemented with 3% sucrose, 0.2 mg/L indole-3-butyric acid, and 0.8% agar; pH 5.8]. The cultures were maintained at 22°C and 40% humidity under white-fluorescent lighting provided at an intensity of 200 µmol/m²s for 16 h per day. The plants were subcultured at 4-week intervals until being transferred into liquid medium prior to the stress treatments. For the stress treatments, plants were removed from the solid medium, washed with sterile distilled water, and placed in test tubes containing liquid medium with the same composition as the rooting medium (except for a higher concentration of agar) supplemented with 150 mM NaCl or 10% PEG 6000. Plants were harvested after 0 (Control), 30 m, or 2 h exposure to the stress (Fig. 1).

RNA extraction and cDNA library construction

Fresh tissues (leaves and stems) were ground into a fine powder in liquid nitrogen using a mortar and pestle. After homogenization, total RNA was purified using the RNeasy Plant Mini kit (Qiagen, Venlo, The Netherlands). The quality and quantity of purified total RNA were confirmed by spectrophotometric analysis and gel electrophoresis. Total RNA (5 µg) was used to synthesize first-strand cDNAs by the PrimeScript™ RT Reagent kit (TaKaRa, Otsu, Japan).

![Control](image)

**Fig. 1** Shoot-tip cultures of the hybrid poplar (*Populus alba × P. tremula var. glandulosa*) for stress treatments.