Generation of transgenic tall fescue plants with enhanced abiotic stress tolerance

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Introduction

Environmental stresses, such as drought, salinity and temperature extremes significantly decrease the yield of forage crops. Therefore, traits associated with abiotic stress-tolerance are of prime importance for their improvement. Biotechnological approaches have the potential to accelerate and complement conventional breeding by extending the range of gene sources for valuable traits, thus offering new opportunities for forage improvement. Transgenic technology appears as an efficient biotechnological tool of molecular breeding for improving forage quality and yield as well as tolerance to various environmental stresses. Occurrence of high level of reactive oxygen species (ROS) is a common phenomenon in abiotic stress-challenged plants. Plants have been evolved with a number of protective mechanisms to counteract such oxidative stress. Molecular chaperones play crucial roles against oxidative stress. Heat shock proteins (HSPs) are molecular chaperones that provide thermotolerance in plants (Heckathorn et al. 1998). It has been reported that chloroplast-localized small HSPs protect thermolabile photosystem II (PSII) in isolated chloroplasts in vitro and are important for heat acclimation (Heckathorn et al. 1998). A number of previous reports have demonstrated that ABA is essential for the adaptive response to drought stress (Xiong et al. 2002). ABA-responsive elements (ABRE)-binding bZIP proteins (ABFs) have been isolated (Choi et al. 2000) and mediate ABA-dependent stress signaling in Arabidopsis (Uno et al. 2000). Overexpression of ABF increased tolerance to drought or heat stress (Vanjildorj et al. 2006). In this project, we generated transgenic tall fescue plants over-expressing chloroplastic small HSP or ABF, and investigated their performance under abiotic stresses.

Methods

Agrobacterium strains EHA105 harboring expression vector containing chloroplast-localized small HSP (OsHSP26) or ABF were used for infection of mature seed-derived embryogenic calli of tall fescue. OsHSP26 or ABF were driven by the constitutive CaMV35S and oxidative stress inducible SWPA2 promoter, respectively. Integration of the T-DNA into the genomes of putative transgenic plants was confirmed by PCR and DNA gel blot analyses. Transgene expression was verified by RT-PCR or RNA gel blot analysis. To examine abiotic stress tolerance, leaf segments of transgenic plants were subjected to stress treatments such as methyl viologen (MV), H$_2$O$_2$, NaCl or heat and measured several physiological parameters such as ion leakage, 

Results and Discussion

The OsHSP26 transgenic tall fescue plants showed significantly lower electrolyte leakage and reduced accumulation of thiobarbituric acidreactive substances (TBARS) when exposed to heat or MV stress treatment. The photochemical efficiency of PSII (Fv/Fm) in the OsHSP26 tall fescue plants was higher than that in the control plants during heat stress (42°C, Fig. 1). These results suggest that the
OsHSP26 protein plays an important function in the protection of PSII during heat and oxidative stress (Kim et al. 2012). The induction of ABF expression in transgenic tall fescue under drought stress (limited irrigation) was confirmed by RT-PCR and RNA gel blot analyses. ABF plants showed decreased electrolyte leakage when exposed to MV or H₂O₂ stress treatment. Evans blue staining after drought stress treatment indicated that cell death occurred in greater extent in non-transgenic control plants compared to the ABF transgenics (Figure 2A). In addition, ABF transgenic plants showed enhanced tolerance against drought stress at the whole plant level (Figure 2B). Tolerance to other abiotic stresses will be shown in the poster.

Conclusion

We have generated transgenic tall fescue plants using Agrobacterium-mediated genetic transformation and evaluated their performance to abiotic stresses. Transgenic plants showed enhanced tolerance to multiple abiotic stresses. These results suggest that transgenic plants may be useful for cultivation in unfavorable marginal soil conditions.

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References


