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Short Communication

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED ABIOTIC STRESS-INDUCED GENES IN TEFF GRASS (*ERAGROSTIS TEF*) LEAVES

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ABSTRACT

The responses of plants to salinity and heavy metals have been extensively studied; however, the relevant stress-responsive genes in teff grass have not previously been investigated. Here, we identified differentially expressed genes (DEGs) in teff grass (*Eragrostis tef* cv. Tiffany) leaves under conditions of salt and arsenic stress, by using an annealing control primer (ACP)-based differential display reverse transcriptase-PCR method with 120 arbitrary ACPs. We observed the up-regulation of 6 DEGs, including 2 newly induced DEGs. Under conditions of arsenic stress, the 2 newly induced DEGs were identified as Bax inhibitor 1. We propose that the newly induced and up-regulated genes play a central role in plant detoxification metabolisms against salt and arsenic. In addition, these genes may enhance the phytotoxic response of teff grass.

Keywords: Arsenic, DEGs, Salt stress, Teff grass.

INTRODUCTION

Forage crops are exposed to various environmental stresses throughout their life cycle. For example, high salinity leads to a decline in plant growth and a decrease in production yield (Yan *et al*., 2005). Arsenic is considered to be an important toxic metalloid that reduces crop productivity (Ahsan *et al*., 2008). Increasing evidence suggests that high salinity or arsenic induces the generation of excess reactive oxygen species, which are highly toxic and can affect many important plant cellular components such as DNA, RNA, lipids, and proteins (Jiang *et al*., 2007; Requejo and Tena *et al*., 2006;). The resulting cellular damage impedes plant growth and development, thereby resulting in reduced crop quality.

The biochemical and physiological responses of plants to salinity and heavy metals have been extensively studied by different researchers such as (Gul *et al.,* 2017; Jan *et al.,* 2017; Jan *et al.,* 2016[a, b]; Rahman *et al.,* 2016, 2015; Hamayun *et al.,* 2010; Narusaka *et al.,* 2003; Shinwari *et al.,* 1998); however, to the best our knowledge, there is no published literature involved in salt and arsenic stress of teff grass. Thus, identification of novel salt and arsenic stress-responsive genes in teff grass is essential.

The objective of the present study was to identify differentially expressed genes (DEGs) in teff grass leaves under conditions of salt and arsenic stress, by using an annealing control primer (ACP)-based differential display reverse transcriptase-PCR method. We discuss the potential roles of the identified genes. We propose that these genes represent a valuable tool for the development of crop plants with enhanced tolerance to abiotic stresses such as salinity or arsenic.

MATERIALS AND METHODS

Plant materials and stress treatments: Teff grass (*Eragrostis tef* cv. Tiffany) seeds were planted in plastic pots containing horticulture nursery medium (Biomedia, Seoul, Republic of Korea). The pots were placed in a growth chamber and were maintained at 25 ± 1°C under a light intensity of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a 12-h photoperiod (day/night). After growing for 2 weeks, seedlings were subjected to salt (NaCl, 300 mM) or arsenic ($Na_2HAsO_4 \cdot 7H_2O$, 500 µM) treatments for a period of 6 h. After each treatment, the leaves were individually excised. Plants grown in the absence of salt and arsenic treatments were used as controls. Leaf samples were collected and stored at −80°C until being used in analyses.

RNA extraction and cDNA synthesis: Total RNA was extracted from control leaf tissues and from leaf tissues of plants subjected to salt and arsenic treatments, by using the Plant RNeasy Mini Kit (Qiagen, Valensia, CA, USA). Integrity assessment was conducted by using electrophoresis on a 1.2% formaldehyde gel. Total RNA was used for reverse-transcriptase-catalyzed first-strand cDNA synthesis. The reverse transcription reaction was performed as described previously (Lee *et al*., 2011, 2009. The synthesized first-strand cDNAs were used as templates in Gene Fishing™ PCR.

ACP-based GeneFishing™ reverse transcription PCR and sequencing: DEGs were identified by using an ACP-based PCR method with the GeneFishing™ Kit

(Seegene Inc., Seoul, Republic of Korea) (Lee *et al*., 2011, 2009). The amplified PCR products were separated on 2% agarose gels stained with ethidium bromide and the DEGS were visually selected by comparison with the control. These genes were purified from the gel by using the GENCLEAN® II Kit (Q-BIO gene, Carlsbad, CA, USA) and were cloned into a TOPO TA cloning vector (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cloned plasmids were sequenced by using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with M13 forward primer (5′-CGC CAG GGT TTT CCC AGT CAC GA-3′) or M13 reverse primer (5′-AGC GGA TAA CAA TTT CAC ACA GGA-3′). The homologies of the sequences were determined through comparison with the GenBank database by using the NCBI BlastX program (http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS AND DISCUSSION

The salinity and heavy metal stress responses of forage crops have previously been investigated (Ahsan *et al.,* 2008; Lee *et al.,* 2009; Moons, 2003; Requejo and Tena, 2006) however, there are no published literature reports regarding the effects of salt and arsenic stress on teff grass leaves. Hence, screening of the stress-responsive genes in teff grass under conditions of high salinity and heavy metals is essential. In the present study, we showed that 6 DEGs were differentially expressed under conditions of salt and/or arsenic stress (Figure 1). Among these DEGs, DEG2 and DEG3 were newly induced during arsenic treatment; DEG1, DEG4, DEG5, and DEG6 were up-regulated during arsenic treatment; and DEG1 and DEG5 were up-regulated during salt treatment (Figure 1 and Table 1).

The sequence similarities of the differentially expressed DEGs are presented in Table 1. The nucleotide sequences of the identified genes were 503–708 bp in length. The 6 DEGs were identified as highly homologous with known genes and they contained partial sequence information that included probable protein phosphatase 2C BIPP2C1-like (PP2C1, DEG1), Bax inhibitor 1 (DEG2 and DEG3), putative T-complex protein 11 (DEG4), transaldolase 2 (DEG5), and glutathione *S*-transferase 1 (DEG6). In addition, the 2 genes that were newly induced during arsenic treatment were identified as Bax inhibitor 1, suggesting that Bax inhibitor plays an important role in preventing apoptosis damage under conditions of heavy metal stress.

Similar to PP2C1, DEG1 was up-regulated under conditions of salt and arsenic stress. This finding implies that DEG1 belongs to a group of reversible modification enzymes that are involved in the protein dephosphorylation of serine, threonine, or tyrosine residues; these genes play crucial roles in plant signal transduction networks, as well as in animal systems (Vlad *et al.,* 2009). Yu *et al.* (2003) reported that *Arabidopsis* PP2C-like phosphatase gene regulated stem cell identity through CLAVATA receptor kinase signaling. Moreover, Hu *et al.* (2006) showed that overexpression of *OsBIP2CI* in tobacco conferred enhanced biotic and abiotic tolerances. The up-regulation of PP2C1 further suggests that this gene plays a critical role in the activation of defense response to conditions of biotic and abiotic stress in teff grass.

DEG2 and DEG3 were newly induced under conditions of heavy metal stress; these genes were identified as encoding a Bax inhibitor-1 (BI-1), which was first documented as a suppressor of Bax-induced cell death in yeast (Xu and Reed, 1998). Programmed cell death in higher plants is essential for the maintenance of cellular homeostasis and environmental stress responses. Watanabe and Lam (2006) reported that the *Arabidopsis* mutant BI-1 exhibited accelerated progression of cell death under conditions of biotic and abiotic stress. Complementary analysis of *AtBI-1* transgenic plants in this mutant rescued the accelerated cell death phenotypes. Watanabe and Lam (2008) further showed that *AtBI-1* regulates endoplasmic reticulum stress-induced cell death following exposure to tunicamycin. Moreover, the *Arabidopsis* BI-1 constitutive overexpression line shows delayed methyl jasmonate-induced leaf senescence through suppression of the cytosolic calcium-dependent activation of MAP kinase 6 (Yue *et al.,* 2012). Taken together, these findings suggest that BI-1 plays a critical role in plant survival under various conditions of environmental stimuli. Future functional studies to elucidate the mechanism whereby teff grass BI-1 functions as a survival factor in enhanced stress tolerance are required. In the present study, the nucleotide sequences of the 2 newly induced DEGs were dissimilar (data not shown). Thus, it is possible that teff grass BI-1 has 2 isoforms; however, further whole-genome sequencing analysis of these genes is required.

Putative T-complex protein 11 (DEG4), transaldolase 2 (DEG5), and glutathione *S*-transferase (GST; DEG6) were up-regulated under conditions of salt and/or arsenic stress. Ahnert *et al.* (1996) proposed that T-complex protein (TCP) functions as cytosolic folding machinery in mammalian cells and yeast; however, the role of TCP in plants remains unclear. Transaldolase is an important enzyme for metabolite balance in the non-oxidative segment of the pentose phosphate pathway. Moehs *et al.* (1996) observed the accumulation of the transaldolase mRNA of potato in tubers in response to wounding stress. GST is a key enzyme in the reactive oxygen species-scavenging systems of plants and animals. Moons (2003) used transcriptional analysis to show that tau class GST is up-regulated in rice roots under conditions of salt and heavy metal stress. In addition, Ahsan *et al.* (2008) demonstrated that two isoforms of GST (omega and tau classes) were up-regulated in rice

Table 1. Differentially expressed genes (DEGs) identified in tall fescue leaves subjected to salt and/or arsenic treatments. The genes were identified by using annealing control primer-based differential display reverse transcriptase-PCR analysis.

DEG no.	GP no.[1]	Salt	Arsenic	Accession no.[2]	Annotation (Species)	Length (bp)[3]	Score	E Value
1	GP17	↑[4]	↑	XP_003558609.1	Probable protein phosphatase 2C BIPP2C1-like (*Brachypodium distachyon*)	607	97.8	3.00e-20
2	GP25	—[5]	New[6]	ABR25531.1	Bax inhibitor-1, partial (*Oryza sativa indica* group)	543	69.3	2.00e-12
3	GP27	—	New	ABR25531.1	Bax inhibitor-1, partial (*Oryza sativa indica* group)	503	67.8	5.00e-12
4	GP71	—	↑	BAD15465.1	Putative T-complex protein 11 (*Oryza sativa japonica* group)	630	130	2.00e-31
5	GP84	↑	↑	NP_001150674.1	Transaldolase 2 (*Zea mays*)	563	150	9.00e-40
6	GP114	—	↑	NP_001105412.1	Glutathione *S*-transferase 1 (*Zea mays*)	708	182	3.00e-53

[1]GeneFishing primer identity number
[2]Accession number in NCBI
[3]Nucleotide sequence length
[4]↑, Up-regulated
[5]—, Non-differentially expressed
[6]New, newly induced

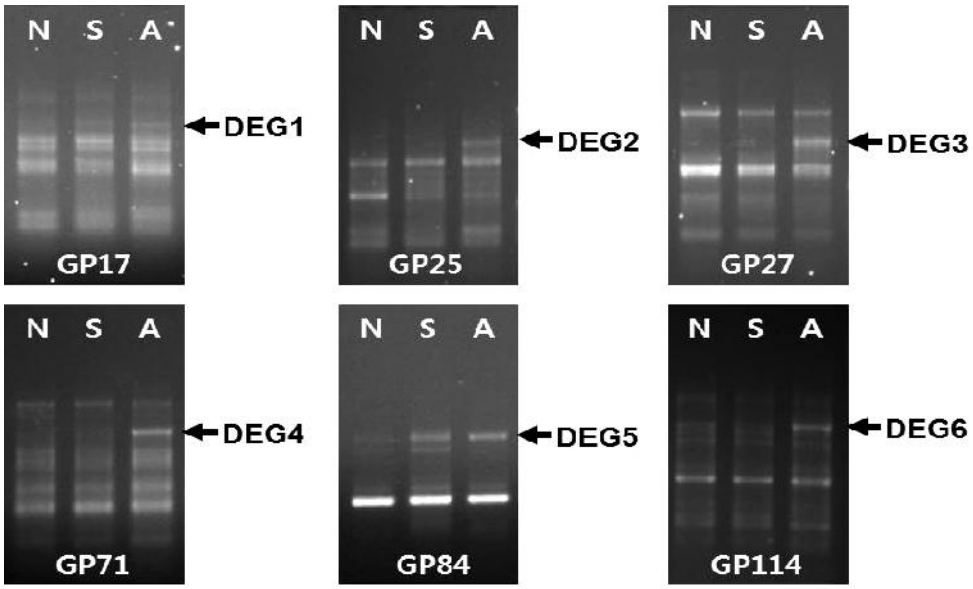

Fig. 1. Agarose gel electrophoresis. The gels show the results of annealing control primer (ACP)-based differential display reverse transcriptase-PCR analysis for the identification of differentially expressed genes (DEGs) in response to salt and arsenic treatments. Arrows indicate DEGs. N, no-treatment control; S, salt treatment 300 mM; A, arsenic treatment 500 μM ; GP, GeneFishing™ PCR primer.

roots under conditions of arsenic stress. Taken together, these findings suggest that TCP, transaldolase, and GST function in the detoxification of ROS-induced lipid peroxidation or that these genes are involved in protection against oxidative stresses such as salt and/or heavy metals.

We believe that our present findings provide a valuable insight into the stress adaptation response of crop plants. In addition, the identification of novel genes may facilitate the development of effective strategies for improving heavy metal tolerance, especially of teff grass. Further comparative analysis, such as overexpression studies to clarify the detoxification mechanism involved in oxidative damage to teff grass, are required.

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