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Asparagine synthetase gene *TaASN1* from wheat is up-regulated by salt stress, osmotic stress and ABA

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Summary

Differences in gene expression between salinity stressed and normally grown wheat seedlings were compared by the differential display (DD) technique. One DD-derived cDNA clone was characterized as a partial sequence of the wheat asparagine synthetase (AS) gene by sequence analysis and homology search of GenBank databases. Two AS genes of wheat, *TaASN1* and *TaASN2*, were further isolated by the RT-PCR approach. Comparison of the deduced polypeptide of *TaASN1* and *TaASN2* with AS proteins from other organisms revealed several homologous regions, in particular, the conserved glutamine binding sites and Class-II Glutamine amidotransferases domain. The functionality of *TaASN1* was demonstrated by complementing an *Escherichia coli* asparagine auxotroph. *TaASN1* transcripts were detected in roots, shoots, anthers and young spikes by RT-PCR analysis. Abundance of *TaASN1* mRNA in young spikes and anthers was higher than that in shoots and roots under normal growth conditions. *TaASN1* was dramatically induced by salinity, osmotic stress and exogenous abscisic acid (ABA) in wheat seedlings. *TaASN2* transcripts were very low in all detected tissues and conditions and were only slightly induced by ABA in roots.

Introduction

Soil salinity limits agricultural production throughout the world. The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed (Zhu, 2001). As one of the most important crops, the biomass of wheat (*Triticum*

Abbreviations: ABA, abscisic acid; Asn, asparagine; AS, asparagine synthetase; DD, differential display; IPTG, isopropyl β-thiogalactoside

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aestivum) is reduced by over 90% in 50% seawater (250 mM NaCl) (Kingsbury and Epstein, 1984), and grain yield is reduced by 50–90% in only 50 mM NaCl (Marschner, 1995). Clearly, there is considerable scope for improvement in yield of wheat grown in a salt-stressed environment. Salt stress affects plant growth in many ways, such as the Na ionic toxicity and hyperosmotic stress, which cause plant nutritional imbalance and oxidation stress. In order to adapt to a high-salt environment, plants change gene expression patterns, metabolic activity, and ion and water transport to minimize stress damage and to reestablish ion and water homeostasis.

Glutamine-dependent asparagine synthetase (AS: EC 6.3.5.4) catalyses the transfer of an amide group from glutamine to aspartate-forming asparagine (Asn) in an ATP-dependent reaction. In most plant species, AS seems to be encoded by a small gene family. Three AS genes have been identified in Arabidopsis (Lam et al., 1998) and sunflower (Herrera-Rodriguez et al., 2002), but in most species only one or two genes are known. Asn plays a prominent role in nitrogen transport and storage in plants. High concentrations of Asn can also be found in various plant tissues under stress conditions, such as mineral deficiencies, salt stress or drought (Moller et al., 2003). In barley, soybean and *Coleus blumei*, the Asn pool increased markedly when the plants were subjected to severe water stress or salt stress (Gilbert et al., 1998). The accumulation of Asn may be caused by stimulated synthesis, inhibited degradation of amino acids, impaired protein synthesis, and/or enhanced protein degradation (Ranieri et al., 1989), but much information about these processes is still unknown.

In this paper, using a differential display (DD) technique, we isolated and characterized one salt-inducible gene, *TaASN1*, which encodes the glutamine-dependent AS. The functional activity of *TaASN1* was tested by a complementation assay. The expression patterns of *TaASN1* in different tissues of wheat (shoots, roots, spikes, anthers) and different stress conditions (ABA, osmotic, salt) were also investigated.

Materials and methods

Plant growth condition and NaCl stress treatments

Seeds of *Triticum aestivum L.* cv. Keyi26 were germinated in a Petri dish, then were cultivated hydroponically in full-strength Hoagland solution (Elberse et al., 2003) in a greenhouse (16/8 h daily light period, 25 °C temperature (night/day) and 60–70% relative humidity). Two-week-old seedlings were transferred to fresh Hoagland medium supplemented with 250 mM NaCl, 5.0% (w/v) mannitol or 20 μM abscisic acid (ABA). After exposure to the stress treatments for 3, 7 or 24 h, shoots and roots were collected in liquid nitrogen and stored at −80 °C until further processing.

Differential display

The roots of NaCl-treated and untreated control seedlings were used for DD RT-PCR. Total RNA was extracted with Trizol reagent (GIBCO-BRL, USA) and was digested with DNAase I (Promega, Madison, WI). DD was performed according to the procedures described previously (von der Kammer et al., 1999) with modification. Three reverse transcription reactions per sample are set for the first strand cDNA synthesis. 2 μg RNA from roots of control and stressed seedlings was used for reverse transcription with either one-base anchor oligonucleotides HT1 (5′-TGC CGA AGC TTT TTT TTT TTA-3′), HT2 (5′-TGC CGA AGC TTT TTT TTT TTG-3′) or HT3 (5′-TGC CGA AGC TTT TTT TTT TTC-3′) and 200 units M-MLV reverse transcriptase (Promega) in a volume of 20 μL according to the manufacturer's instructions. The reverse-transcription reactions were diluted fivefold, and 2 μL was initially subjected to PCR employing the corresponding one-base anchor oligonucleotide (1 μM) along with either one of the DD random primers (von der Kammer et al., 1999), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 × buffer, and 0.2 μL Tag DNA polymerase (TaKaRa, Dalian, China). Reactions were performed in a thermal cycler (PCR system 9700, Applied Biosystems, USA) with the following cycling conditions: one round at 94 °C for 4 min for denaturing; cooling 40 °C for 4 min for low-stringency annealing of primer; and heating 72 °C for 1 min for extension. This round was followed by 35 high-stringency cycles: 94 °C for 45 s; 60 °C for 2 min; and 72 °C for 1 min. One final step at 72 °C for 10 min was added to the last cycle. The denature loading buffer was added to PCR products and heated to 94 °C for 5 min and stored on ice prior to loading on 6% denaturing polyacrylamide DNA sequencing gel (BIO-RAD, USA). Electrophoresis was performed at **a** constant 85 W for 3 h and then the gel was subjected to silver staining (Bassam et al., 1991). The differential cDNA fragments were recovered from the sequencing gel and eluted. The elusions were used as templates for reamplification using the same sets of primer pairs. The reamplified fragments were