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Research article

Characterization of a wheat (*Triticum aestivum* L.) expansin gene, *TaEXPB23*, involved in the abiotic stress response and phytohormone regulation

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ABSTRACT

Expansins are proteins that are generally accepted to be key regulators of cell wall extension and plant growth. We examined the expression pattern of *TaEXPB23*, a wheat (*Triticum aestivum* L.) expansin gene, under exogenous phytohormone and abiotic stress treatments. In addition, we evaluated its function in the tolerance to salt stress and high temperature (HT) by overexpressing it in transgenic tobacco plants. In subcellular localization assays, *TaEXPB23* localized to the cell wall. Expression analysis demonstrated that the transcription pattern of *TaEXPB23* corresponded to wheat coleoptile growth. Real-time RT-PCR analysis revealed that *TaEXPB23* transcript expression was upregulated by exogenous methyl jasmonate (MeJA) and salt stress, but downregulated by exogenous gibberellins (GA_3), ethylene (ET), indole-3-acetic acid (IAA) and α-naphthlcetic acid (NAA). Overexpression of *TaEXPB23* in tobacco (*tabacum*) conferred tolerance to salt stress by enhancing water retention ability (WRA) and decreasing osmotic potential (OP). However, transgenic plants overexpressing *TaEXPB23* did not show any improvement in the tolerance to HT stress. These results suggested that *TaEXPB23* is regulated by phytohormones and is involved in the regulation of salt stress tolerance.

1. Introduction

Abiotic stresses, such as salt stress and high temperature (HT), frequently restrain crop growth and yield. Restriction of plant growth by retarding both cell division and extension is among the earliest visible effects of some stress conditions. The regulation of cell extension is critical for plant growth and stress resistance [1,2]. The plant cell wall determines the cell shape and is the main barrier against abiotic and biotic stresses. Cell extension in turgid plant organs is instigated by chemically loosening the structure of growth-limiting cell walls, resulting in the relaxation of wall tension and concomitant osmotic water uptake. Proteins in the cell wall are believed to play important roles in the regulation of cell wall extensibility, a key parameter in determining cell expansion [3].

Expansins are a class of cell wall proteins that mediate pH-dependent wall loosening, probably by disrupting hydrogen bonds between cellulose and matrix glycans [4]. They were identified because of their ability to restore long-term extension to cell walls and were first isolated from cucumber hypocotyls [5]. Since then, expansins have been found in many plant species and organs, including *Arabidopsis* [6], rice [7] and strawberry [8].

Expansins are encoded by a large gene superfamily, including *α-expansins* (*EXP A*), *β-expansins* (*EXP B*), *expansin-like A* (*EXL A*), and *expansin-like B* (*EXL B*) [4]. They participate in almost all aspects of plant development, including seed germination [9], stem elongation [10], floral development [3], and senescence [11]. Xu et al. [12] identified a heat-inducible expansin gene, *AsEXP1*, in *Agrostis scabra*, and the studies suggested that *AsEXP1* could be useful as a molecular marker to select for heat-tolerant grass germplasm. Water stress induced expansin gene expression was observed in maize [13], rice [14] and the resurrection plant, *Craterostigma plantagineum* [15]. Collectively, these studies point to an involvement of expansins in stress resistance of plants.

Plants have developed a series of complex response mechanisms to perceive and respond to diverse external signals. Phytohormones are important components in multiple signal pathways; they act synergistically or antagonistically, to regulate plant growth, development and defense response, generally by inducing gene expression. Most of the genes regulated by hormones are involved in the adjustment of plants to stress conditions, and they function in stress resistance. Previous studies suggested that expansin expression was regulated by phytohormones. For example, Nath and Trivedi [16] found that *MaExp1* from ripening

Abbreviations: ABA, abscisic acid; ET, ethylene; HT, high temperature; IAA, indole-3-acetic acid; GAs, gibberellins; MeJA, methyl jasmonate; MS, Murashige–Skoog; NAA, α-naphthlcetic acid; OP, osmotic potential; WT, wild type; WRA, water retention ability.

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bananas could be upregulated by treatment with ethylene (ET). Ding et al. [17] found that indole-3-acetic acid (IAA) induced the expression of expansin genes including *EXP-A* and *EXP-B* and Azeez et al. [18] identified that *GgEXPA1* is a gibberellins (GAs)-responsive expansin gene.

Wheat is one of the most important crops in the worldwide, and the wheat coleoptile is an ideal unit to investigate the mechanisms of cell elongation. The reason for this is that cell division rarely occurs in the growth of coleoptiles and its elongation is primarily attributed to cell expansion. To study the function of expansin genes in stress tolerance and its involvement in hormone regulation, we cloned *TaEXPB23* from wheat and transformed it to tobacco. Our previous research indicated that mRNA levels of *TaEXPB23* were induced by water stress, and transgenic tobacco overexpressing *TaEXPB23* had improved drought tolerance [19,20]. In the present study, we found that the transcripts of *TaEXPB23* were upregulated by NaCl and MeJA; but were downregulated by high temperature (HT), ET, α-naphthlcetic acid (NAA), and GA_3. Overexpression of *TaEXPB23* changed the root growth of transgenic tobacco seedlings and improved the tolerance to salt stress, but not to HT stress. These results are important in order to understand the function of *TaEXPB23* in plant development and stress defense.

2. Results

2.1. TaEXPB23 encodes a cell wall protein

Choi et al. identified that expansins are a large family of cell wall proteins [21]. *TaEXPB23* is a β-expansin gene that was cloned from wheat plants in our lab by Xing et al. [19]. To determine the subcellular localization of *TaEXPB23* in plant cells, a *GFP* reporter gene was fused to *TaEXPB23* in a pBI121 vector. Onion epidermal cells were used to transiently express the fusion product using *Agrobacterium*-mediated transformation (Fig. 1). The cells transformed with the *35S::GFP* control construct showed GFP signals in the entire cytoplasm and nucleus (Fig. 1B). While individual cells transformed with *35S::TaEXPB23-GFP* (with or without plasmolysis) exhibited green fluorescence predominantly in the cell walls of onion epidermal cells (Fig. 1B), whereas indicating that *TaEXPB23* protein was localized at the cell walls.

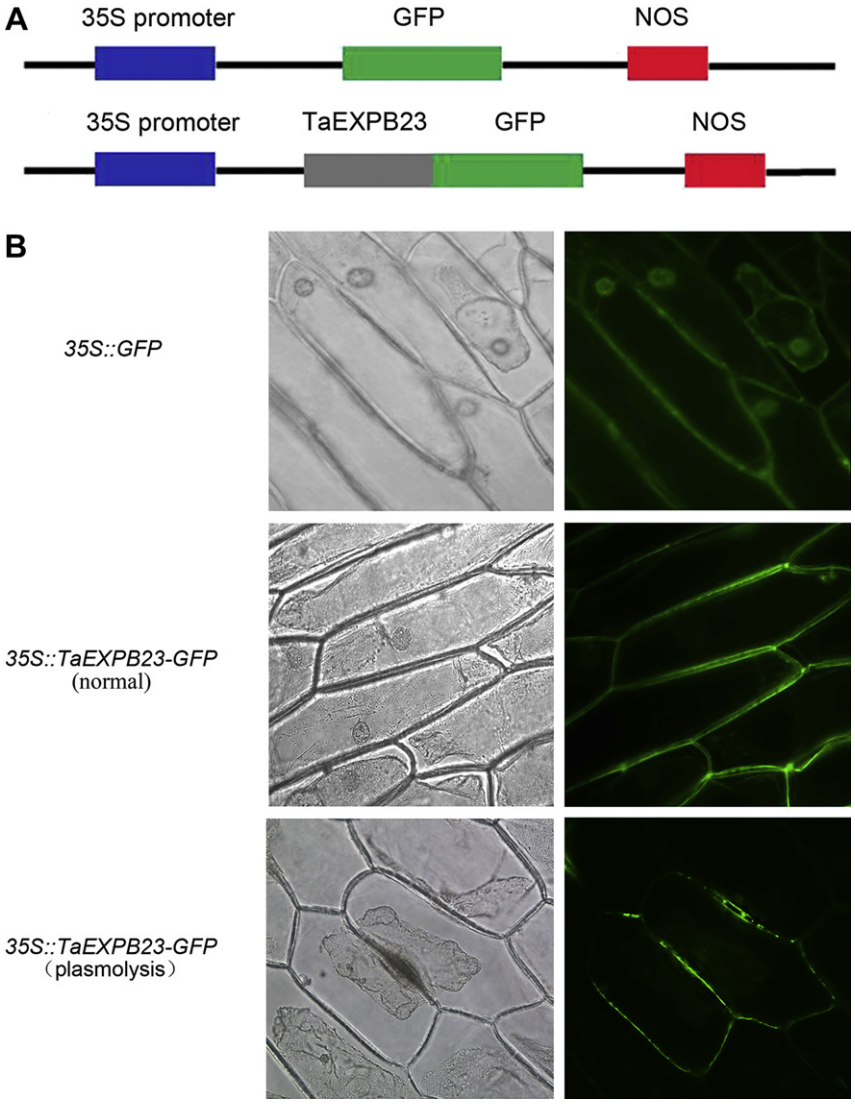

Fig. 1. Subcellular localization of a *TaEXPB23-GFP* fusion protein in onion epidermal cells. (A) Schematic representation of the *35S::TaEXPB23-GFP* fusion construct and the control *35S::GFP* construct. The *TaEXPB23* coding region was cloned upstream of the GFP coding region in the expression vector. (B) Transformed cells carrying the *35S::GFP* and *35S::TaEXPB23-GFP* constructs(normal and plasmolysis) were cultured on MS medium at 28 °C for two days and then observed under a microscope.