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The involvement of expansins in response to water stress during leaf development in wheat

Shan Zhou [a], Yang-yang Han [b], Yanhui Chen [a], Xiangzhu Kong [a], Wei Wang [a,*]

[a] State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an 271018, Shandong, PR China
[b] Plastic Surgery Institute, Weifang Medical University, Weifang 261053, Shandong, PR China

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ABSTRACT

Expansins are cell wall proteins that are generally considered to be the key regulator of cell wall extension during plant growth. In this study, we used two different wheat (*Triticum aestivum* L.) cultivars to demonstrate that expansins are involved in wheat leaf growth and response to water stress, by regulating the expansin activity and cell wall susceptibility to expansins. Expansin activity was associated with the relative elongation rate of leaves during leaf development, suggesting their involvement in leaf elongation. Moreover, cell wall extension characteristics and expansin gene transcription were closely involved in the leaf cell elongation region. Water stress restrains leaf growth, but the growth rate of leaves was changed after rehydration, which is consistent with the response of expansin activity to water stress. Meanwhile, increased cell wall susceptibility to expansin by water deficit played an important role in maintaining cell wall extension. Furthermore, the expansin activity in drought-resistant cultivar HF9703 was always higher than that in drought-sensitive cultivar 921842 under water stress condition, which may be correlated with the higher expansin gene expression in HF9703 *versus* 921842. These data provide evidence for a role of expansins in the growth and response of wheat leaves to water stress.

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Introduction

Drought is one of the adversities that crops frequently encounter during growth and development. In recent years, the droughts caused by climate change are becoming more frequent. Wheat is a worldwide crop, whose growth and yield are directly related to the human food supply. However, water stress can seriously inhibit the normal growth and development of wheat and affect the quality and yield (Chaves and Oliveira, 2004). The organic matter needed for wheat growth and grain filling depends mainly on the leaf supply, as this is the primary organ for manufacturing carbohydrates. Understanding the determinants of wheat leaf growth and their response to drought has great significance in production.

Leaf growth mainly relates to cell division and cell elongation, and the elongation growth of the cells is more sensitive to drought stress than cell division. Cells are continuously produced in the meristematic region near the leaf base and expand in distal regions of the growing zone, while pushed forward by younger cells. Cell elongation depends on loosening of cell walls, uptake of water into expanding cells and synthesis of cell wall polysaccharides (Cosgrove, 1999). Because cell turgor pressure changes little along the growing region (Tomos and Pritchard, 1994; Bouchabké et al., 2006), the primary factors that regulate plant growth are most often attributed to cell wall loosening and cell wall extensibility (Wu et al., 1996). Water deficit affects the elongation rate of the leaf and the spatial distribution of the relative elongation rate (Tardieu et al., 2000), most likely linked to local changes in cell wall properties (Wu et al., 1996).

Expansins are a family of cell wall proteins proposed to play a key role in the regulation of tissue elongation and cell wall differentiation (McQueen-Mason and Rochange, 1999; Cosgrove, 2000). Expansin is hypothesized to induce an increase in cell wall extensibility in vitro and cell expansion *in vivo*, by breaking hydrogen bonds between hemicelluloses and cellulose micro fibrils (McQueen-Mason and Cosgrove, 1994). During growth, plant cells secrete expansin, which unlocks the network of wall polysaccharides, permitting turgor-driven cell enlargement (Cosgrove, 2000). Various analyses have revealed that expansins are expressed in all expanding parts of plants or organs that undergo cell wall modifications, including roots (Wu et al., 2001; Guo et al., 2011), leaf elongation regions (Reidy et al., 2001; Muller et al., 2007), internodes (Sasayama et al., 2009; Lee and Kende, 2001), ripening fruits

* Corresponding author. Tel.:+86 538 8246166; fax: +86 538 8242288.
E-mail address: wangw@sdau.edu.cn (W. Wang).

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(Rose et al., 1997), flowers (Gookin et al., 2003; Dai et al., 2012), and pollen (Cosgrove et al., 1997). Sloan et al. (2009) identifies that induction of expansin gene expression leads to elevated expansin activity and leaf area, only during the mid phase of leaf growth. The mid phase of leaf growth corresponds to the inflection point of relative growth rate and thus, to the period of maximum leaf growth rate. Cookson et al. (2005) reported on potential correlations between maximum value of absolute leaf growth rate and final leaf size. Thus, expansin is correlated to maximum leaf growth rate and further controls the final leaf size.

Meanwhile, there is increased evidence showing that expansins are vitally involved in the responsive mechanisms of plant species to water stress. For instance, overexpression of the expansin gene *RhEXPA4* in *Arabidopsis* confers strong drought tolerance to transgenic plants (Dai et al., 2012). Barcia et al. (2014) analyzed the consequences of water deficit during the first 4 days of wheat development, focusing on root growth. They found that two expansin genes (*TaEXPB8* and *TaEXPA5*) were up-regulated in roots under water deficit. Yan et al. (2014) reported that *AtEXP2* is involved in seed germination and abiotic stress response in *Arabidopsis*. The growth of coleoptiles in the drought-resistant wheat HF9703 is obviously faster than that in the drought-sensitive cultivar 921842 under water stress conditions, which correlated with higher expansin activity and expansin protein expression (Zhao et al., 2011).

In this study, we first analyzed the relationship between the growth of wheat leaves and expansin activity. Then, we investigated the response of expansin to different drought stresses, using two wheat cultivars with different drought tolerance. Our results suggest that expansin is involved in the leaf growth and mainly expressed in the leaf elongation region. In addition, water stress inhibited the growth of leaf, but the change in expansin activity and cell wall susceptibility to expansin during water stress could increase the growth rate after rehydration. These results indicated that expansin is a contributing factor to leaf growth maintenance and involved in the response of leaf growth to water stress.

Materials and methods

Plant materials, growth conditions, and treatments

Two wheat (*Triticum aestivum* L.) lines, HF9703 and 921842, were used in this study. HF9703 is a drought-resistant cultivar, 921842 is a drought-sensitive cultivar. Wheat seeds were soaked overnight in aerated water and germinated on moist filter paper at 26 °C for 1 day. Subsequently, the germinated wheat seeds were placed in a well-ordered fashion on a nylon gauze sheet at the appropriate density and cultured in trays (25 cm × 18 cm × 5 cm) containing half-strength Hoagland solution. Growth conditions for wheat seedlings were 26 °C day/22 °C night under a 16 h photoperiod, light intensity of 300 μmol m^{-2} s^{-1} and a relative humidity of 70%.

For detecting the dynamic growth of the wheat leaves, the length of leaf 3 was measured daily from when it emerged until its length did not change for three consecutive days. Relative elongation rate at time j (RER_j) over two time points was determined using the following equation: $RER_j = LN(m_j\text{-}m_{j-1})/(t_j - t_{j-1})$, where m is the measurement in length, t is time point, and m_j and m_{j-1} are measurements at times t_j and t_{j-1}, the previous time point. On day 2, 4, 6, 8 and 10 after leaf 3 initial, the leaf 3 was cut from the leaf base, frozen with liquid nitrogen and stored at −80 °C until use.

When leaf 3 reached the length of 11–12 cm (about day 5), the consistent growth of seedlings was treated with different levels of water stress. Water stress was induced using polyethylene glycol 6000 (PEG-6000) solution, at three different concentrations, *i.e.* 10% PEG-6000 (−0.15 MPa) to induce moderate water stress, 20% PEG-6000 (−0.73 MPa) to induce intermediate water stress and 30% PEG-6000 (−1.55 MPa) to induce severe water stress. The leaf length, the characteristic of cell wall (including cell wall extension, expansin activity and the susceptibility of cell wall to expansin proteins) and expansin gene expression were detected. After 24 h of water stress treatment, leaf 3 was cut into three segments (named 0–40 mm, 40–80 mm, 80–120 mm, respectively), then these segments were frozen with liquid nitrogen and stored at −80 °C until use.

We also carried out a drought–rehydration experiment. After water stress treatment, the treated seedlings moved to normal water condition and then we measured the leaf length after 24 h of re-watering.

Microscopic analysis

The fingernail oil blot was used to measure cell length. We first chose different lengths of wheat leaves, respectively divided the blade into 1 cm long pieces and spread the colorless fingernail oil. Coating should be uniform, thickness in the 0.3–0.5 mm range, and then placed in a clean and ventilation place. When the fingernail oil was fully dried, we cut a 5 mm × 5 mm square on the fingernail oil blot with a blade, then used tweezers to tear the fingernail oil blot along the incision direction. The fingernail oil blot was directly observed using optical microscope. Cell lengths were measured using the software analysis package Nano Measurer.

Measurement of expansin activity

The extraction of the cell wall proteins were performed according to Gao et al. (2008) based on McQueen-Mason et al. (1992). Quantification of cell wall proteins was performed following the method of Bradford (1976), with bovine serum albumin (BSA) as a standard. All samples were adjusted to 2 mg ml^{-1}. An extensometer was installed as described by Cosgrove (1989) with some modifications (Gao et al., 2007).

Wheat coleoptiles were inserted between two clamps spaced 5 mm apart and subjected to a constant tension of 12 g. Wheat coleoptiles were abraded with carborundum, boiled in water (20 s) to eliminate endogenous expansin activity, pressed, and clamped onto the extensometer. After pretreatment with sodium acetate buffer (50 mM, pH 4.5) for 40 min, the solution was exchanged with the same buffer containing cell wall proteins of wheat leaves and the extension was recorded for a further 60 min. Expansin activity was assayed by measuring the wall extension after addition of protein extracts.

Measurement of acid-induced cell wall extension

Acid-induced cell wall extension was carried out according to the method of Cosgrove (1989). Segments (3-cm long, 2-mm wide) prepared from the leaf sections were abraded with carborundum and then squeezed between two glass slides to remove cell sap. The segments were fixed on an extensometer between two clamps 5 mm apart, under a constant load of 12 g. The wall sections were first incubated in HEPES buffer (50 mM, pH 6.8) for 40 min and then in sodium acetate buffer (50 mM, pH 4.5) for 60 min to measure cell wall extension.

Measurement of cell wall susceptibility to expansin proteins

Cell wall susceptibility to expansins was carried out according to the method of Cosgrove (1989).For the measurement of wheat leaves cell wall susceptibility to expansin action, the wheat cell wall

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