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Early activation of plasma membrane H^+-ATPase and its relation to drought adaptation in two contrasting oat (*Avena sativa* L.) genotypes

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A B S T R A C T

Major objective of this study is to elucidate the effect of early activation of root hair cell plasma membrane (PM) H^+-ATPase on drought adaptation in plants. Pot-culture experiments were carried out to determine oat (*Avena sativa* L.) genotypic differences in water maintenance, osmotic adjustment and PM H^+-ATPase activity at the seedling stage. Two oat genotypes with contrasting drought sensitivity, *Dingyou6* (*A. vernasativa*, drought-tolerant cultivar) and *Bende* (*A. venanuda*, drought-sensitive cultivar) were subjected to soil drought stress under environment-controlled growth chamber conditions. At 21 days after emergence, water supply was withheld to allow soils in pots to dry. Our results showed that drought-tolerant "*Dingyou6*" maintained significantly greater RWC and osmotic potential (OP) in roots and leaves, and also had larger root-to-leaf ratios of RWC and OP than drought-sensitive "*Bende*" along with 14-day drying process, suggesting that drought-tolerant cv. possesses superior root-to-leaf hydraulic conductivity, and stronger regulatory ability to drought stress. Analysis of the PM H^+-ATPase activity and the root and leaf osmolyte contents provided further chemical evidence for this result. Biosynthesis of leaf proline and glycine betaine (GB) followed a similar trend as the activities of root hair cell PM H^+-ATPase prior to intermediate stress (around 35% FWC). Significant increase in the activity of PM H^+-ATPase was observed at the SWC of about 45–50% FWC, without detectable changes in leaf and root RWC simultaneously. This demonstrated that there existed an early-warning response in roots before the onset of significant decrease in plant RWC. Moreover, the interspecific difference in the timing of triggering early response was obvious. Drought-tolerant "*Dingyou6*" initiated early response at about 50% FWC, but at about 45% FWC for drought-sensitive cv. This study implies that early activation of root hair cell PM H^+-ATPase triggers the increased biosynthesis of major osmolytes, which, in turn, leads to the up-regulation of water maintenance system.

1. Introduction

Plants are able to respond to drought stress by altering their cellular metabolism and invoking complex defense mechanisms (Bohnert and Jensen, 1996; Chaves et al., 2002). Their survival and adaptation under drying soil depend on their intrinsic regulatory ability to perceive stimulus, generate and transmit this "drying" signals, and initiate various biochemical changes (Blackman and Davis, 1985; Bohnert and Jensen, 1996; Ober and Sharp, 2003; Fan et al., 2008). During soil drying, early response of plants to stress is closely linked with immediate survival and gradual acclimation under drought stress (Augé and Duan, 1991; Bohnert and Sheveleva, 1998; Chaves et al., 2002; Xiong et al., 2006a; Fan et al., 2008). Due to the complexity of regulatory mechanism, this early response is extensively considered a coupled root-to-shoot process at the whole plant level. Previous efforts had been paid on elucidating how the early response operates, but little is known about the quantitative effect of this early-warning mechanism on crop growth and adaptation. Among recent progresses, non-hydraulic

Abbreviations: RWC, relative water content; SWC, soil water content; OP, osmotic potential; OA, osmotic adjustment; PM, plasma membrane; GB, glycine betaine.

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root-sourced signal is so far affirmed to be a unique "early-warning" response to soil drying in plants (Blackman and Davis, 1985; Chaves et al., 2002; Xiong et al., 2006b; Fan et al., 2008).

Since Blackman and Davis (1985) found root-sourced chemical signals formed when soil was drying, many subsequent experiments have elucidated how root–shoot communication might operate (Blackman and Davis, 1985; Ludlow et al., 1989; Croker et al., 1998; Mingo et al., 2003; Dodd et al., 2003; Norman et al., 2004; Xiong et al., 2006a,b). This mechanism enables plants to "sense" drought in the roots and is expressed as an alteration of physiological parameters in the leaves (Davis and Zhang, 1991; Gowing et al., 1990). This is a typical "early-warning" response of plants to drought (Blum and Johnson, 1993). Continuing drought initiates up a hydraulic gradient between the leaf and the drying soil, which speeds up the development of leaf water deficit by loss of leaf turgor pressure (Blum and Johnson, 1993; Comstock and Jonathan, 2002). On the basis of knowledge about plant water relation, the triggering mechanism of this "early-warning" response is likely summed up in the regulative role of root hair. Root hair as a major organ of water uptake can respond to reduced soil water availability, which may act as the signal resource from root-to-shoot. Growing root hair showed a variety of significant responses to osmotic stress including electrical signal, which is likely due to the presence of osmo-sensor (Lew, 1996). The plasma membrane (PM) H^+-ATPase of root hair cell is an essential protein that may be mainly responsible for the onset of root-to-shoot signal.

The PM H^+-ATPase (EC 3.6.1.35) has been called a 'master enzyme' responsible for a broad range of physiological processes (Samuels et al., 1992; Gévaudant et al., 2007; Liu et al., 2008), which include water maintenance, osmotic regulation and other adaptive mechanisms under drought stress (Ober and Sharp, 2003; Liu et al., 2005). It is an important proton pump that translocates proton out of cells when ATP is hydrolyzed (Liu et al., 2005). However, most of the information regarding the physiological effect of the PM H^+-ATPase has so far come from partial studies focusing on certain organ or tissue. In most cases, overall effect of the PM H^+-ATPase at the level of whole plant was largely lacked. The regulatory mechanism of root hairy cell PM H^+-ATPase to drought adaptation at whole plant level may be closely associated with root–shoot hydraulic conductance, osmotic adjustment and other unclear processes.

We therefore proposed a hypothesis that the initiation of PM H^+-ATPase in root hair cells might play a critical role in regulating the root-to-shoot communication. In higher plants, there is now substantial evidence that glycine betaine (GB) and proline are two major organic osmolytes that accumulate in a variety of plant species in response to drought stress (Hanson and Burnet, 1994; Ma et al., 2004; Ashraf and Foolad, 2007). These compounds normally accumulate in large quantities in response to dehydration stress (Mohanty et al., 2002; Yang et al., 2003; Kavi Kishore et al., 2005). This process is thought to play adaptive roles in mediating osmotic adjustment (OA) and protecting subcellular structures in stressed plants. Thus, the changes of PM H^+-ATPase activity have been contemplated to increase the concentrations of these compounds in plants grown under stress conditions to increase their stress tolerance. Increased proline and GB may also function as protein compatible hydrotropes (Srinivas and Balasubramanian, 1995) that help the generation of ATP for repairing of stress-induced damages and accordingly improve the ability of water maintenance in leaves. Osmotic adjustment is generally considered an important component of drought resistance (Blum et al., 1999; Cattivelli et al., 2008). Those cultivars with better osmotic adjustment ability had better performance of stress adaptation under drought stress, enabling plants to maintain water absorption and turgor pressure (Morgan, 1983, 1995; Moinuddin et al., 2005; Fan et al., 2008).

Existing researches showed that the difference among genotypes or species in drought adaptation can be traced to different capacities for water acquisition and transportation (Chaves et al., 2002; Ma et al., 2004; Xiong et al., 2006b). The ability of plants to water maintenance and hydraulic conductance may be critical to drought acclimation development. This type of drought acclimation in plants is evolutionarily innate defense ability and can mechanistically act as immune responses as in animals (Nürnberger and Kemmerling, 2006). Plant species (or plant non-cultivar-specific) and plant cultivar-specific resistance are two distinct but evolutionarily interrelated types of resistance that constitute plant innate immunity. Drought-tolerant type and drought-sensitive one in crops had differentiated stress response to drought (Schwanz and Polle, 2001; Zhu et al., 2005; Fan et al., 2008; Li et al., 2009). Hydraulic properties of roots are regulated by root-sourced signal such as ABA (Mahdieh and Mostajeran, 2009).

Our previous work showed that the soil moisture at which non-hydraulic root-sourced signal (nHRS) was triggered was positively correlated with that of hydraulic root signal (HRS) in eight old or modern wheat varieties. Earlier onset of nHRS significantly affected drought tolerance and yield performance (Xiong et al., 2006b). However, its regulatory mechanism and the difference between cultivars are so far unclear. In this study, we chose two oat genotypes with contrasting drought resistant, *Dingyou6* (drought-tolerant cultivar) and *Bende* (drought-sensitive cultivar), as experimental materials to reveal whether the early activation of PM H^+-ATPase in root hair cells is related to osmotic adjustment and water maintenance. The results will provide a better understanding on the root-to-shoot regulatory mechanism on whole plant level for the crops grown in drying soil.

2. Materials and methods

2.1. Description of experiments

Seeds of two oat (*Avena sativa* L.) genotypes with contrasting drought sensitivity, *Dingyou6* (*A. vernasativa*, drought-tolerant type) and *Bende* (*A. venanuda*, drought-sensitive type) that were supplied by Dryland Agricultural Research Center of Dingxi, Gansu, PR China, were grown in pot-culture condition as follows. Plant material preparation and water supply control was made according to our previous methods (Xiong et al., 2006a).

Oat seeds were surface-sterilized in 0.5% NaOCl for 15 min, rinsed in distilled water for 15 min, and grown in plastic pots of diameter 36 cm and height 30 cm in a growth cabinet (Conviron PGV36, Asheville, North Carolina, USA) under controlled environmental conditions (light/dark regime of 16/8 h at 20–25 °C, relative humidity of 60–70%, photosynthetic photon flux density of 300 $\mu mol\ m^{-2}\ s^{-1}$). Twenty seedlings were placed into each pot. After 21 days of growth in cabinet, drought stress treatment was started by withholding water to well-watered seedlings. The soil water contents (SWCs) were determined gravimetrically everyday by weighing pots throughout the whole drying period (Xiong et al., 2006a,b).

According to soil water characteristic curve calculated by the relationship between soil suction and soil moisture, water availability gradients were categorized into sufficient water supply (CK, 65% field water capacity (FWC)), mild stress (MS, 45% FWC), intermediate stress (IS, 35% FWC) and serious stress (SS, 20% FWC), respectively (Table 1). To facilitate development of the relationship between soil moisture and plant physiological parameters (RWC, OP and enzyme activities), a variety of SWCs measured in a continuous drying episode were classified into a series of soil water gradients, in which the soil water content was at the levels of 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60% and 65% FWC (with a fluctua-