



# K<sup>+</sup> Uptake, H<sup>+</sup>-ATPase pumping activity and Ca<sup>2+</sup> efflux mechanism are involved in drought tolerance of barley



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## ABSTRACT

Chemical signals play a significant role in improving plant water use efficiency under drought stress. Hydroponic and pot experiments were conducted using three barley genotypes to study genotypic differences in K<sup>+</sup>, Ca<sup>2+</sup> and H<sup>+</sup> fluxes and physiological and biochemical traits of drought tolerant Tibetan wild barley XZ5 and cv Tadmor and drought sensitive cv ZJU9 in response to drought. Transient and steady-state ion fluxes were measured by noninvasive ion-selective microelectrode MIFE technique. We showed that exogenous PEG (polyethylene glycol 6000) and mannitol and soil drought stress all resulted in an immediate K<sup>+</sup> uptake from root epidermis and leaf mesophyll, with much more uptake in XZ5. Long-term drought stress are more detrimental to root K<sup>+</sup> homeostasis, and the degree of K<sup>+</sup> uptake differed due to severity of drought stress and was less presented in XZ5. Barley subjected to drought stress caused a large H<sup>+</sup> efflux in root epidermis and H<sup>+</sup> influx in leaf mesophyll, with significantly less alteration in XZ5 and Tadmor than in ZJU9. Meanwhile a dramatic Ca<sup>2+</sup> efflux was observed in root epidermis and leaf mesophyll under drought stress. PEG and mannitol treatments induced marked increases in H<sup>+</sup>-K<sup>+</sup>-ATPase in XZ5 and Tadmor. Our results demonstrate that K<sup>+</sup> uptake, Ca<sup>2+</sup> efflux and leaf H<sup>+</sup> influx/alkalization of apoplastic pH could be a chemical signal in barley in response to drought stress, and that stimulated H<sup>+</sup>-K<sup>+</sup>-ATPase and K<sup>+</sup> uptake, but less Ca<sup>2+</sup> efflux and H<sup>+</sup> alteration under drought, when concerning ionic mechanisms underlying drought tolerance, play an important role in drought tolerance in XZ5.

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## 1. Introduction

Drought is one of the most important abiotic stresses and is responsible for mechanical damage to roots, low soil nutrient availability and high soil salinity (Hameed et al., 2013). One of the cost-effective approaches for mitigating the effect of drought stress is to breed drought-resistant crops (Lakew et al., 2011). However, this progress is seriously hindered by the scarcity of germplasm resources and the complexity of physiological and genetic traits. Hence, understanding the mechanisms of plants in

response to drought stress and identifying genetic resources with high levels of drought tolerance is very crucial.

Barley (*Hordeum vulgare* L.) is a vital cereal crop and rank fourth in production following maize, rice and wheat (Janská et al., 2013). To breed drought-tolerant barley cultivars, it is crucial to identify genetic resources with significant potential for drought tolerance. Wild-barley germplasm is a precious treasure of available genes and provides rich sources of genetic variation for cereal improvement. In our previous work, we identified high-drought-tolerant Tibetan wild-barley genotypes (*H. vulgare* L. ssp. *spontaneum*) XZ5, with high K content in plants under drought (Zhao et al., 2010). It is essential to study the internal mechanisms concerning the differential drought tolerance between wild and cultivated barley for identification and exploiting of candidate genes for breeding drought-tolerant cultivars to deal with the global drought issue.

Drought stress caused restriction of K<sup>+</sup> diffusion in the soil towards the roots, thus limiting K<sup>+</sup> absorption (Wang et al., 2013). A direct causal relationship between the capacity to control fluxes of K<sup>+</sup> across the plasma membrane and PCD was documented for a range of abiotic stress conditions in plants. Hence, maintaining adequate cellular K<sup>+</sup> is vital for plant drought tolerance

**Abbreviation:** BSM, basal salts medium; BNS, basal nutrient solution; BSA, bovine serum albumin; PCD, programmed cell death; [Ca<sup>2+</sup>]<sub>cyt</sub>, cytosolic free Ca<sup>2+</sup>; Fv/Fm, maximal photochemical efficiency; HKTs, Na<sup>+</sup>/K<sup>+</sup> symporters; KOR, outward rectifying K<sup>+</sup>; H<sup>+</sup>/K<sup>+</sup>, symporters; MIFE, non-invasive microelectrode ion flux; OH, hydroxyl radicals; PAs, synergistic effects of polyamines; Pn, net photosynthetic rate; ROS, reactive oxygen species; SMC, soil moisture content; T-AOC, total antioxidant capacity.

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(Demidchik et al., 2010). Moreover, plasma-membrane ion channels are probably associated with ROS sensing and ROS-mediated regulatory reactions in plants. Hydroxyl radical-producing property can activate  $K^+$ -efflux GORK channels (Demidchik et al., 2010) and  $H_2O_2$  can trigger opening SKOR channels. There are numerous genes encoding proteins involved in  $K^+$  transporter in plants. For example, there are KT, HKT,  $K^+/H^+$  antiporter and Shaker-type  $K^+$  channels in *Arabidopsis* (Shabala, 2003). Although many studies have been reported on salinity and water logging-induced  $K^+$  loss in barley (Zeng et al., 2014), there are still great gaps in our understanding on whether  $K^+$  plays any roles as a chemical signal in plant tissues to communicate between roots and leaves under drought, and whether higher K content is involved in better K retention from the leaf mesophyll.

Drought stress usually cause alteration in xylem sap pH, which is a significant component of chemical signaling in plant and may conduct synergistically with ABA (Abscisic acid) (Schachtman and Goodger, 2008). Many authors have shown that pH of xylem sap extracted from plants grown in drought conditions becomes more alkaline, and ABA concentration was increased (Wilkinson and Davies, 1997). Bacon et al. (1998) reported that barley xylem sap pH is also extremely sensitive to soil water content. The change of apoplast pH may be a chemical signal and has direct effects on ion fluxes across the plasma membrane (Ehlert et al., 2011). Hence, it is of importance to measure the  $H^+$  fluxes from roots and leaves in drought treatment in order to assess the dynamic pH changes.

Calcium ions, an essential plant nutrient and a key second messenger (Dodd et al., 2010), play a crucial structural role in cell walls and maintain membrane integrity (Bose et al., 2011). However, calcium can be a toxic ion in the cytoplasm at higher concentrations as it triggers aggregation of proteins and nucleic acids, precipitation of phosphates (Case et al., 2007). Stomatal closure caused by drought stress was produced by ABA-regulated repetitive rise of  $[Ca^{2+}]_{cyt}$  waves (Blatt, 2000), which are involved in the inhibition of  $H^+$ -ATPase and KIRs, and activation of anion channels (Chen et al., 2012). Meanwhile, plants also have evolved  $Ca^{2+}$  efflux mechanisms via  $Ca^{2+}/H^+$  exchangers and  $Ca^{2+}$ -ATPase pumps that can keep  $[Ca^{2+}]_{cyt}$  at a steady and very low level by releasing  $Ca^{2+}$  out of the cell or into the intracellular organelles (Bose et al., 2011).

Meanwhile, another significant component of plant drought tolerance is the appropriate osmotic adjustment, which can be achieved by the accumulation of compatible solutes in cytoplasm (Reddy et al., 2004). One of the consequences of drought-induced inhibition of photosynthesis is the exposure of plants to excess energy, which may be detrimental to PSII due to increased production of ROS in the chloroplasts and over-reduction of reaction centers. A very efficient antioxidative defense system in plant cells utilizes both nonenzymic and enzymic constituents to reduce the toxicity of ROS. It is reported that carotenoids are associated with defending the photosynthetic apparatus from photoinhibitory damage by singlet oxygen, which is achieved by the excited triplet state of chlorophyll (Loggini et al., 1999). Hence, it is important to assess the impact of drought on photosynthetic, osmolality, ROS and T-AOC.

In this study, we hypothesized that maintaining cytosolic  $K^+$  homeostasis with integration of  $H^+$ -ATPase activity, and  $Ca^{2+}$  efflux mechanism may confer drought tolerance in Tibetan wild barley XZ5. This hypothesis was investigated using MIFE measuring technique. We compared the kinetics of drought-induced  $K^+$ ,  $Ca^{2+}$  and  $H^+$  fluxes from the mesophyll tissue of three contrasting barley genotypes drought tolerant Tibetan wild barley XZ5 and cv Tadmor, and drought sensitive cv ZJU9 (Zhao et al., 2010) and physiological traits to illuminate the ionic mechanisms underlying drought tolerance in XZ5. Our measurements revealed that XZ5 showed more  $K^+$  uptake, less  $Ca^{2+}$  efflux, and the least  $H^+$  alteration under

drought stress, compared with Tadmor and ZJU9. Our results demonstrate that drought has substantial effect on root/leaf ion flux patterns, and that this effect is different in drought-tolerant wild barley XZ5 and cv Tadmor and drought-sensitive ZJU9.

## 2. Materials and methods

### 2.1. Plant materials, growth conditions and treatments

Three barley genotypes were used for all experiments: one drought tolerant Tibetan wild barley XZ5, two cultivars (*Hordeum vulgare* L.) of drought tolerant Tadmor and drought sensitive ZJU9 (Zhao et al., 2010). For ion flux measurements, seeds were thoroughly rinsed in distilled water for 30 min after sterilized with 10% NaClO for 15 min (Zeng et al., 2014). The plants were then cultivated in a dark growth cabinet at room temperature ( $24 \pm 1^\circ C$ ) with aerated BSM solution containing 0.1 mM  $CaCl_2$  and 0.5 mM KCl for 3 d (Chen et al., 2005). Healthy and uniform seedlings with a root length of  $70 \pm 10$  mm were sampled for treatment. Four treatments (5% and 10% PEG 6000; 3% and 6% mannitol) were chosen to measure transient ion fluxes of 3-d old seedlings. For steady-state ion fluxes measurements, drought stress was conducted by exposing their roots to 10% PEG or 3% mannitol for 0, 1, 4, and 24 h.

The hydroponic experiment was carried out to study the response of seedlings to PEG and mannitol treatments. Viable seeds were sown in petri dishes with wet filter paper bed in a growth room at  $22^\circ C/18^\circ C$  (day/night). Uniform three-leaf stage seedlings were transplanted to 5-L containers filled with half-strength Hoagland nutrient solution. The solution was aerated continuously and changed every 5 d. Plants were subjected to Control, BNS, PEG (BNS with 20% PEG 6000) and Mannitol (BNS with 6% Mannitol) treatments for 5 d after exposure, respectively. Roots and shoots were separated and sampled at 1, 3, and 5 d.

A greenhouse pot experiment was conducted using the 3 genotypes on Zijingang Campus at Zhejiang University, Hangzhou, China, with natural light and a temperature of  $20 \pm 2^\circ C$ /day and  $15 \pm 2^\circ C$ /night. Air-dried soil was sieved and filled in 2-L plastic pots. Ten seeds were sown and filtered to five healthy and uniform seedlings. The soil was watered with half-strength Hoagland's solution and maintained at  $\sim 35\%$  SMC. When seedlings grew to three functional leaves, drought stress was imposed by stopping irrigation until SMC was down to 10% (mild drought) and 4% (severe drought), respectively. In this experiment, the third fully expanded leaves under normal conditions were sampled for steady-state and transient ion flux measurements in response to PEG, mannitol treatment, and the third fully expanded leaves under control and drought stress were sampled for steady-state ion flux measurements in response to real drought stress (Mak et al., 2014). Also, the same leaves were sampled to measure leaf sap  $Ca^{2+}$  and  $K^+$  concentration,  $H_2O_2$ , MDA,  $\cdot OH$ , T-AOC, total protein contents with six replications respectively.

### 2.2. Ion flux measurements

Net  $K^+$ ,  $Ca^{2+}$  and  $H^+$  fluxes were measured from mature zone (10–12 mm from the root tip) by MIFE technique (University of Tasmania, Hobart, Australia; Shabala et al., 1997). Three micro-electrode tips were positioned in one plant abounded with ion-selective cocktails ( $K^+$  60031;  $H^+$  95297;  $Ca^{2+}$  21048, Switzerland Sigma, Buchs, Switzerland), respectively, 40  $\mu m$  above the tissue. At the time of measurement, electrodes were shifted in a slow 6-s cycle square wave with 40- $\mu m$  amplitude by a computer-driven micromanipulator (Patchman NP2, Eppendorf, Hamburg, Germany). Net ion fluxes were calculated using cylindrical diffusion geometry by the MIFEFLUX program (Newman, 2001).

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