Plant Physiology and Biochemistry 75 (2014) 36–44

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Differences in physiological features associated with aluminum tolerance in Tibetan wild and cultivated barleys

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ARTICLE INFO

Article history:
Received 8 March 2013
Accepted 25 November 2013
Available online 4 December 2013

Keywords:
Aluminum toxicity
ATPase
Citrate and malate secretion
Genotypic difference
Low pH
Tibetan wild barley

ABSTRACT

Aluminum (Al) toxicity is a major limiting factor for plant production in acid soils. Wild barley germplasm is a treasure trove of useful genes and offers rich sources of genetic variation for crop improvement. Al-stress-hydroponic-experiments were performed, and the physiochemical characteristic of two contrasting Tibetan wild barley genotypes (Al-resistant XZ16 and Al-sensitive XZ61) and Al-resistant *cv.* Dayton were compared. Ultrastructure of chloroplasts and root cells in XZ16 was less injured than that in Dayton and XZ61. Moreover, XZ16 secreted significantly more malate besides citrate and exhibited less Al uptake and distribution than both of XZ61 and Dayton in response to Al stress, simultaneously maintained higher H^+-, $Ca^{2+}Mg^{2+}$- and total-ATPase activities over XZ61. The protein synthesis inhibitor cycloheximide reduced citrate secretion from XZ16, but not from Dayton. In Tibetan wild barley, our findings highlight the significant correlations between Al tolerance, ATPase activity and citrate secretion, providing some insights into the physiological basis for Al-detoxification.

1. Introduction

Aluminum (Al) toxicity is the major factor limiting crop productivity on acid soils, affecting up to 50% of the world's potential arable land (Arroyave et al., 2013). Strategies to maintain production on acid soils include lime application to raise soil pH and the use of highly Al resistant plants. Considering the large-scale of acidic farmlands, approaches such as the development of Al resistant crop cultivars (Foy, 1996) with reduced Al uptake might offer a cost-effective and practically acceptable strategy to improve crop productivity on acid soil.

Barley (*Hordeum vulgare* L.) is one of the most Al-sensitive species among small grain cereals (Zhao et al., 2003). In order to breed barley cultivars resistant or resistant to Al toxicity, it is of importance to identify genetic resource with large potentials for Al tolerance. Wild barley offers a great source of useful genes and genetic variation for crop improvement (Pickering and Johnston, 2005). Annual wild barley from Qinghai-Tibet Plateau is regarded as one of the progenitors of cultivated barley and is rich in genetic diversity (Wang et al., 2009; Dai et al., 2011). However, the underlying physiological, biochemical and molecular mechanisms involved in Al tolerance remain unclear, thus it prevents the identification and exploiting of candidate genes into commercial barley cultivars.

One of the basic strategies proposed for Al tolerance is binding toxic Al to organic acids (OA) exported from root cells (Ma et al., 2001; Barceló and Poschenrieder, 2002). Physiological studies have shown that anion channels or transporters mediated secretion of OA regulates plant Al tolerance. Al-induced secretion of OA anions from roots has been reported in a wide range of plant species such as wheat, maize, sorghum, rye, and soybean (Magalhaes et al., 2007; Furukawa et al., 2007; Zhou et al., 2011). In barley, it was reported that Al-resistant barley cultivars detoxify Al by secreting citrate from the roots (Zhao et al., 2003; Fujii et al., 2012; Wang et al., 2007). Al-dependent secretion of malate from wheat roots occurred *via* plasma membrane (PM) anion channels of root cells (Zhang et al., 2001). Zhao et al. (2003) found that citrate secretion is inhibited by an anion-channel blocker niflumic acid (NIF) in barley. Furthermore, Al-induced secretion of malate and citrate from the roots of *Lespedeza bicolor* (Dong et al., 2008) and *Vigna umbellate* (Yang et al., 2006) were significantly reduced by a protein-synthesis

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http://dx.doi.org/10.1016/j.plaphy.2013.11.025

inhibitor cycloheximide (CHM) and an anion channel inhibitors 9-anthracenecarboxylic acid (A9C) and NIF.

In addition, ATPase activity is a crucial factor for plant survival under various environmental stresses, such as Al (Shen et al., 2005), salt (Alvarez-Pizarro et al., 2009), and low pH (Yan et al., 1998). Hamilton et al. (2001a)) found that Al stress triggers vacuolar H^+-ATPase (V-ATPase) and mitochondrial ATP synthase (F_1F_0-ATPase) in an Al-resistant wheat cultivar and only the V-ATPase is specifically required for its Al tolerance. Shen et al. (Shen et al., 2005) observed that regulation of H^+-ATPase activity (mainly ATPase) was critical for soybean plant survival under Al stress. Al inhibited H^+-ATPase by permanently altering the plasma membrane potentials (Ahn et al., 2001), and Al tolerance has been proposed to correlate with plasma membrane H^+-ATPase-catalyzed proton efflux in roots of white lupin (Tomasi et al., 2009) and in rice bean (Yang et al., 2007). Therefore, the question arises whether the mechanism of Al-tolerance in wild barley is different from that in cultivated barley or other plant species, and whether ATPase activity and secretion of OAs are crucial for Al exclusion in Tibetan wild barley. Most of the previous reports have focused on the role of H^+-ATPase or V-ATPase in Al tolerance, whereas the ATPase activities including H^+-, $Ca^{2+}Mg^{2+}$- and total-ATPase responding to Al stress in Tibetan wild barley genotypes have never been compared with those in elite Al-resistant barley cultivars.

Recently, Dai et al. (2011) described the extent of genotypic variation of both low pH and Al tolerance among some Tibetan wild annual barley genotypes. In this study, two of these genotypes XZ16 (high acid and Al resistant) and XZ61 (acid and Al sensitive) were examined and compared with the Al-resistant barley cultivar Dayton (Wang et al., 2007), which was used as the control. We demonstrated that, compared to Dayton and XZ61, XZ16 was less affected by Al toxicity by secreting significantly more malate besides citrate and accumulated less Al. Furthermore, Al-induced OA secretion was significantly correlated to the ATPase activities in barley, which may be responsible for the genotypic differences in Al tolerance.

2. Results

2.1. Tibetan wild barley XZ16 is highly resistant to Al toxicity

The severity of Al-induced symptoms differed significantly between the three genotypes, where root growth inhibition were severe and appeared rapidly in XZ61 (Figure S1). Root dry weight of XZ16 (resistant genotype) was less affected by 15 d of exposure to 100 μM Al than that of Dayton. However, in Al-sensitive XZ61, the dry weight of root and individual plant was inhibited by 46.4% and 23.7% under Al stress, respectively.

The dose- and time-responses for Al accumulation in roots and shoots are summarized in Fig. 1. Root Al accumulation increased with an increase in external Al levels. On average, XZ16 accumulated 19.2% less Al than that of Dayton when exposed to 50–200 μM Al. However, both Al-resistant genotypes exhibited significantly ($P < 0.05$) less accumulation than that of Al-sensitive XZ61 in response to 25–200 μM Al (Fig. 1A) and over 3–24 h of exposure to 25 μM Al (Fig. 1B).

Interestingly, shoot Al accumulation was significantly lower ($P < 0.05$) in XZ16 than in Dayton and XZ61 in response to all the Al treatments (Fig. 1C) and after 6–24 h of exposure to 25 μM Al (Fig. 1D). After exposing to 200 μM Al, XZ16 showed 22.8% and 17.1% lower ($P < 0.05$) shoot Al content than Dayton and XZ61, respectively. However, there is no obvious difference in shoot Al content between the tolerant *cv.* Dayton and sensitive wild genotype XZ61. We worked out that an Al transfer rate from root to shoot was 15.6% lower in XZ16 than that of Dayton when exposed to 100 μM $AlCl_3$ for 24 h. Our finding indicates that Tibetan wild barley XZ16 is highly resistant to the combined low-pH/Al treatment by allowing much less root-to-shoot Al transport.

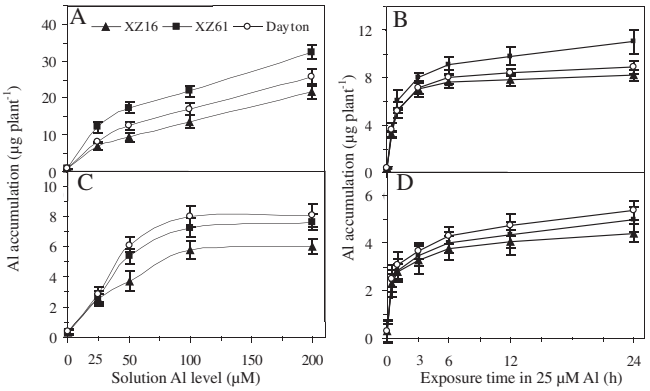

Fig. 1. Dosage response (A, C) and time-course experiments (B, D) of Al accumulation in roots (up panel) and shoots (down panel) of barley seedlings of XZ16 (▲), XZ61 (■) and Dayton (○).

Fig. 2 shows Al distribution (morin staining) at different time points in longitudinal and cross sections of barley roots exposed to

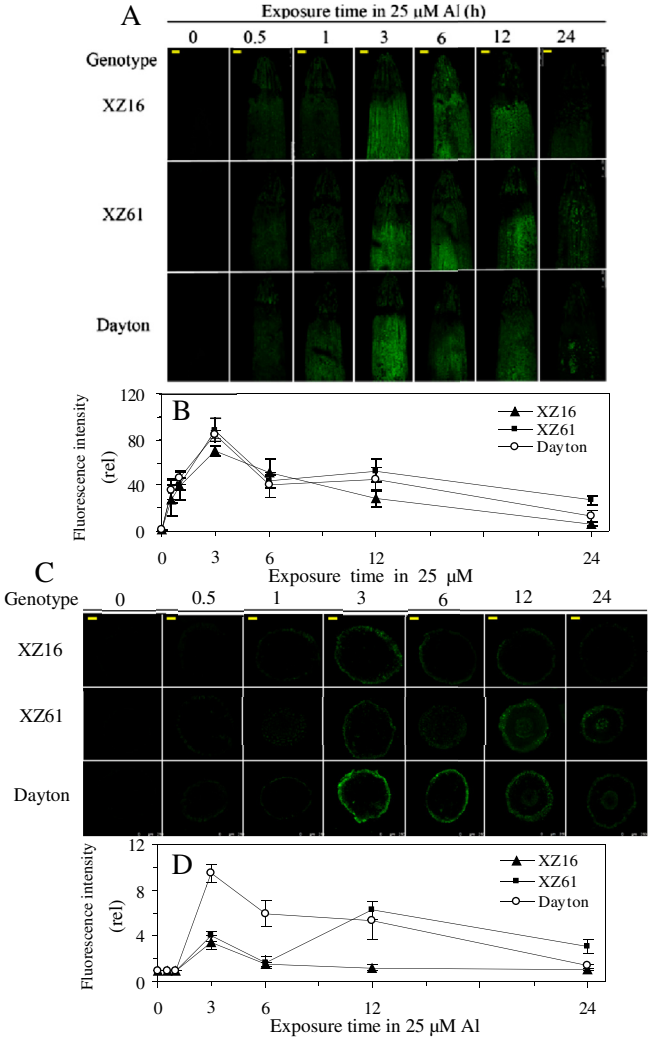

Fig. 2. Al localization in longitudinal (A) and cross (B) sections of XZ16, XZ66 and Dayton roots exposed to 25 μM Al for different time intervals as detected by the morin fluorescence. Relative fluorescence intensities on longitudinal (C) and cross (D) section were calculated using Image J software.