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Metabolic analysis of two contrasting wild barley genotypes grown hydroponically reveals adaptive strategies in response to low nitrogen stress

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ABSTRACT

Nitrogen (N) is an essential macronutrient for plants. The increasingly severe environmental problems caused by N fertilizer application urge alleviation of N fertilizer dependence in crop production. In previous studies, we identified the Tibetan wild barley accessions with high tolerance to low nitrogen (LN). In this study, metabolic analysis was done on two wild genotypes (XZ149, tolerant and XZ56, sensitive) to understand the mechanism of LN tolerance, using a hydroponic experiment. Leaf and root samples were taken at seven time points within 18 d after LN treatment, respectively. XZ149 was much less affected by low N stress than XZ56 in plant biomass. A total of 51 differentially accumulated metabolites were identified between LN and normal N treated plants. LN stress induced tissue-specific changes in carbon and nitrogen partitioning, and XZ149 had a pattern of energy-saving amino acids accumulation and carbon distribution in favor of root growth that contribute to its higher LN tolerance. Moreover, XZ149 is highly capable of producing energy and maintaining the redox homeostasis under LN stress. The current results revealed the mechanisms underlying the wild barley in high LN tolerance and provided the valuable references for developing barley cultivars with LN tolerance.

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1. Introduction

N is an essential mineral nutrient required for plant growth and development. N fertilization is a key factor affecting crop yield, with much increase achieved over the last half century because of extensive use of N fertilizer (Miller and Cramer, 2005). However, only less than half of the applied N is used by crops (Socolow, 1999), thus bringing the severe environmental problems while adding productive cost for farmers. Therefore, it is quite important to alleviate the dependence of crop production on N fertilizer (tolerance to LN stress) or to improve N use efficiency (NUE) of crops in order to ensure the agricultural sustainability. Development of the crop cultivars with high LN tolerance or NUE is a most fundamental and efficient approach for coping with low N availability in soils.

N availability in soils varies drastically with space and time, and correspondingly plants have evolved versatile mechanisms/strategies to cope with N limitation. Moreover, it was found that such N limitation adaptability in crops is closely associated with their yield performance (McCullough et al., 1994; Ding et al., 2005). Obviously genetic improvement of LN tolerance in crops is possible and practical. In addition, it has been well documented that NUE is a genetically controlled trait, differing dramatically among genotypes, in the crops including wheat, rice, maize and barley (Le Gouis et al., 2000; Anbessa et al., 2009; Namai et al., 2009; Presterl et al., 2003). However, genetic diversity in the cultivated barley becomes narrower, forming a bottleneck for genetic improvement (Ellis et al., 2000). On the other hand, the Tibetan annual wild barley, proved as one of the ancestors of the cultivated barley (Dai et al., 2012), is rich in genetic variation and shows much better adaptation to poor soil fertility, such as K deficiency (Zeng et al., 2015) and N deficiency (Quan et al., 2016). In a previous study, we identified some wild barley genotypes with high LN tolerance (Yang et al., 2014), suggesting that the wild barley may provide the elite genetic materials or genes for improving LN tolerance of barley as well as other cereal crops. It is therefore increasingly important to understand the mechanisms underlying the wild barley in high NUE or LN tolerance.

Abbreviations: N, nitrogen; LN, low nitrogen; G-6-P, glucose-6-phosphate; 2-KG, 2-ketoglutaric acid; PPP, pentose phosphate; Glu, glutamic acid; Gln, glutamine; Asp, aspartic acid; Asn, asparagine; Ser, serine; Gly, glycine; Ala, alanine; Leu, leucine; Thr, threonine; Lys, lysine; Val, valine; Phe, phenylalanine; Tyr, tyrosine; GABA, 4-aminobutyric acid.

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The development of gas chromatography-mass spectrometry (GC–MS) technology has facilitated the comprehensive analysis of metabolite profile of a specific genotype or sample, allowing us to make insight into the multiple physiological processes in responses to various conditions. In recent years, metabolomics analysis has been widely used to investigate the tolerance of plants to various abiotic stresses, including temperature (Kaplan et al., 2004), drought (Guo et al., 2009), salt stress (Kim et al., 2007; Patterson et al., 2009), potassium nutrition (Armengaud et al., 2009) and phosphate deficiency (Huang et al., 2008). It has also been performed to dissect the metabolic changes of some crops under LN stress, such as tomato (Urbanczyk-Wochniak and Fernie, 2005), maize (Schlüter et al., 2012), cultivated barley (Comadira et al., 2015). However, no relevant study of metabolics has been done on the wild barley under LN stress, although it shows higher LN tolerance than the cultivated barley.

In this study, a GC–MS-based method was used to investigate the impact of LN stress on metabolite profiles in different tissues of the two Tibetan wild barley accessions, so as to reveal the possible difference of metabolic profiles in response to LN stress between the two wild barleys differing in LN tolerance.

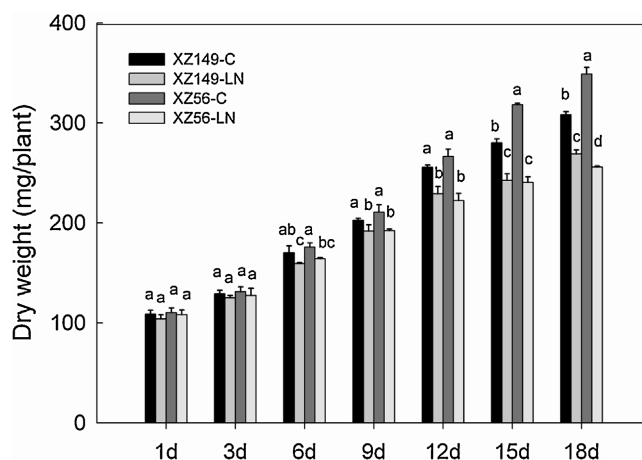

Fig 1. The temporal changes of plant biomass in the two barley genotypes under normal N (C) and low N (LN) conditions.
The lowcase letters represent significant difference (P, 0.05) among treatments and genotypes at each time point (n = 4, bars show the SD).

2. Materials and methods

2.1. Plant materials and treatments

Healthy seeds of two Tibetan wild barley accessions, *i.e.* XZ149 (LN tolerant) and XZ56 (LN sensitive) were germinated in a plant growth chamber (22/18 °C, day/night). Ten-day-old seedlings with uniform size were transplanted into black plastic containers (5 l) with aerated hydroponic solution in a greenhouse with natural light. The hydroponic solution was prepared according to Quan et al. (2016), and renewed every five days. Three-leaf-stage seedlings were exposed to 0.2 mM N (LN treatment) and 2 mM N (control).

2.2. Sampling and metabolite extraction

The plant samples were taken at seven time points, namely 1, 3, 6, 9, 12, 15, and 18 d after LN treatment, respectively. Shoots and roots were separated, dried for constant weight at 80 °C, and then the plant biomass was recorded. For metabolite profiling analysis, the topmost fully expanded leaves in plants were used as leaf sample. All leaf and root samples with four biological replicates were frozen in liquid nitrogen.

The metabolites were extracted according to Lisec et al. (2006) with small modification. The fresh samples were quickly milled in a mortar (pre-cooled) with liquid nitrogen. Then 100 mg accurately weighed fine powder was transferred to a 2-ml, screw cap, round bottom tube, and extracted in 1400 μl of 100% methanol (pre-cooled at −20 °C), and finally 60 μl of ribitol (0.2 mg/ml stock in dH_2O) was subsequently added as internal quantitative standard. The mixture was shaken for 10 min at 70 °C in a thermo-mixer at 950 rpm, followed by centrifugation for 10 min at 11,000*g*. In the obtained supernatant, 750 μl chloroform (−20 °C) and 1500 μl dH_2O (4 °C) were added, then centrifuged for 15 min at 2, 200*g*. Totally 150 μl supernatant was dried in a vacuum freeze dryer and the derivatization of the dried samples was initiated at 37 °C for 2 h by adding 40 μl of 20 mg/ml methoxyamine hydrochloride in pyridine (Sigma-Aldrich) and then treated with 70 μl MSTFA (Sigma-Aldrich) for 30 min.

2.3. GC–MS analysis

Metabolites contents of the extracted samples were determined using 7890A/5975C GC–MS system (Agilent, USA). The prepared sample of 1 μl was injected into the HP-5 capillary column and the injection temperature was set at 230 °C. The analysis was performed as following temperature-rising program: initial temperature of 80 °C for 2 min, then at 15 °C/min rate up to 300 °C, kept 300 °C for 10 min. Mass spectrometry was identified by fully-scanning method with range from 70 to 600 (*m*/*z*). The mass spectra data were analyzed for resolution of co-eluting peaks using AMDIS_32 software (Nashalian and Yaylayan, 2015).

2.4. Data analysis and statistics

Significant difference of each metabolite between treatments was tested using a data processing system (DPS) software, and the difference at $P < 0.05$ and $P < 0.01$ was considered as significant and highly significant, respectively. Principle component and heatmap analysis (PCA) of the identified metabolites was performed using Metaboanalyst 3.0 (Xia et al., 2015).

3. Results

3.1. The temporal changes of plant biomass in response to LN stress

The two Tibetan wild barley genotypes, namely XZ149 and XZ56, were identified as LN tolerant and sensitive, respectively (Yang et al., 2014). In this study, LN stress reduced shoot dry weight of the two wild barley accessions, with the reduction being significant from 6 d after stress treatment (Fig. 1). The genotypic difference caused by LN stress become larger with the treated time, with no difference at 1 d and the reduction being 12.8% and 26.6% at 18 d for XZ149 and XZ56, respectively (Fig. 1).

3.2. The changes of metabolite profiles of the two genotypes in response to LN stress

Totally 51 metabolites content changed significantly under LN stress relative to the control (C) in both leaves and roots of XZ149 and XZ56. Hierarchical clustering analysis clearly grouped 224 samples into two classes, namely leaves and roots (Fig. 2, Tables A1 and A2). Moreover, 112 samples were clearly divided into two groups, i.e. the samples taken at early stage (before 9 d after stress treatment, the left group) and those taken at late stage (12–18 d after treatment, the right group) (Figs. A1 and A2, Tables A1 and A2). Furthermore, four subclasses could be divided, mainly represent-

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