Plant Physiology and Biochemistry 111 (2017) 257–265

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Ionomic and physiological responses to low nitrogen stress in Tibetan wild and cultivated barley

Xiaoyan Quan, Jianbin Zeng, Zhigang Han, Guoping Zhang*

Agronomy Department, Institute of Crop Science, Zhejiang University, Hangzhou, 310058, China

ARTICLE INFO

Article history:
Received 9 October 2016
Received in revised form
26 November 2016
Accepted 4 December 2016
Available online 6 December 2016

Keywords:
Glutamine synthetase
Ionome
Nitrogen
Nitrate reductase
Tibetan wild barley

ABSTRACT

In a previous study, we identified the low-nitrogen (LN) tolerant accessions from the Tibetan wild barley (*Hordeum vulgare* subsp. *spontaneum*). In this study, two wild barley genotypes (XZ149, LN-tolerant and XZ56, LN-sensitive) and a barley cultivar ZD9 (*H. vulgare*) were used to determine the LN tolerant mechanism underlying the wild barley in the ionomic and physiological aspects. XZ149 exhibited higher LN tolerance with highest relative dry weight and N accumulation among three barley genotypes under LN stress. When exposed to LN stress, XZ149 had more N transportation from roots to leaves, and remained relatively higher activities of nitrate reductase (NR, EC.1.7.1.1) and glutamine synthetase (GS, EC.6.3.1.2) in leaves than other two genotypes, ensuring its higher capacity of N assimilation and utilization. The ionome analysis showed that LN stress had a significant effect on tissue ionome and the effect was genotypic and tissue-specific difference. On the whole, XZ149 maintained more stable Mn and Cu contents in roots, and less reduction of root P, K and Ca contents than XZ56 and ZD9 when exposed to LN stress. It may be assumed that more N movement into shoots, greater N assimilating capacity and specific rearrangement of nutrient element levels in tissues under LN stress are attributed to LN tolerance in XZ149.

1. Introduction

Nitrogen (N) is an essential mineral nutrient required in quantity and is frequently a key factor limiting crop yield and quality (Marschner, 2012). However, the environmental problems brought by the excessive N fertilizer application in crop production become increasingly severe (Socolow, 1999). Thus, well understanding of the mechanisms of low nitrogen tolerance is imperative for improving N use efficiency. The Tibetan annual wild barley has been proved as one of the ancestors of cultivated barley (Dai et al., 2012), and shows generally better adaption to poor soil fertility, including N deficiency (Quan et al., 2016) and K deficiency (Zeng et al., 2015). Some wild barley genotypes with high LN tolerance have been identified in our previous study (Yang et al., 2014), providing elite materials for understanding the mechanisms of LN stress tolerance.

Extensive studies have been performed on plant biomass, nitrate uptake and root architecture to link the traits with LN stress tolerance (Brouwer, 1962; Drew and Saker, 1975; Lea and Azevedo, 2006). Nevertheless, it is less documented about the fine-tuning of plant metabolism under LN stress. Meanwhile, adaptation to steady-state low N in plants is also poorly studied (Forde and Lea, 2007). It would be of significance to obtain an overview of the modifications in plant N metabolism when subjected to N stress. In fact, some N-containing compounds and enzyme activity have been used as diagnostic indicators to reveal the N level response of the different plant genotypes.

Meanwhile, plants require at least 13 mineral elements for adequate development, including so-called macronutrients - phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) in addition to N, as well as iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), boron (B), chloride (Cl), nickel and molybdenum, which are referred to as micronutrients (Marschner, 2012). All of these elements are associated with plant growth and crop yield, and in many instances, deficiency of one element interconnects with the metabolism of other nutrients under cross-talking regulation (Schachtman and Shin, 2007; Liu et al., 2009). Their interactions have been highlighted in some recent reviews (Amtmann and Armengaud, 2009; Williams and Salt, 2009). For example, N metabolism and allocation could be altered by B and Fe deficiency in tobacco and cucumber separately (Camacho-Cristóbal

* Corresponding author.
E-mail address: zhanggp@zju.edu.cn (G. Zhang).

http://dx.doi.org/10.1016/j.plaphy.2016.12.008
0981-9428/

and González-Fontes, 1999; Borlotti et al., 2012). Similarly, improve N status enhanced root uptake, remobilization and root-to-shoot translocation of Zn in wheat and ammonium toxicity decreased levels of Ca and Mg in cucumber (Erenoglu et al., 2011; Roosta and Schjoerring, 2007). These results demonstrate that mineral homeostasis in plants is highly monitored, indicating that changes in availability of a single element could exert an effect on the uptake and accumulation of other elements in plants. Thus, LN stress may change mineral balances in plants, directly or indirectly affecting mineral accumulation (Kutman et al., 2011; Xue et al., 2012; Schreiner et al., 2013). However, the genotypic responses of elements and their interactions to LN stress have not been fully elucidated up to date.

The ionome has been defined as the mineral nutrient composition of an organism or tissues (Salt et al., 2008). Ionomics is the study of elemental accumulation in living organisms using high-throughput elemental profiling. There are four major techniques in the studies of ionomics, i.e. neutron activation analysis (NAA), inductively coupled plasma-atom/optical emission spectrometry (ICP-AES/OES), inductively coupled plasma-mass spectrometry (ICP-MS) and X-ray fluorescence (XRF) (Wu et al., 2013). High-throughput elemental profiling has been applied to study the ionome response to the environment or the genetics underlying the changes of ionome over the environments (Baxter, 2009). For instance, multivariable ionomic signatures in Arabidopsis were established to investigate physiological responses using ICP-MS, such as P and Fe homeostasis (Baxter et al., 2008). In addition, ionome has been employed on the studies of element contents under different N supply (Watanabe et al., 2015; Lecourt et al., 2015). However, almost no experiment has been reported on studying the effect of LN stress on plant ionome profiles comprehensively.

In the present study, we employed two wild barley genotypes (XZ149, LN-tolerant and XZ56, LN-sensitive) and one barley cultivar (ZD9) to study the effect of LN on N metabolism and ionomic changes using ICP-OES with aims at understanding the mechanisms of LN stress tolerance underlying Tibetan wild barley.

2. Materials and methods

2.1. Plant materials and treatments

Healthy seeds of the two Tibetan wild barley accessions XZ149 (LN tolerant) and XZ56 (LN sensitive), a cultivar ZD9 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. At three-leaf-stage, seedlings were exposed to 2 mM N (control), 0.2 mM N (LN) and 0 mM (N starvation).

2.2. Sampling and measurement

The plant samples were taken and separated into shoots (or leaves and stems) and roots at 14 d after N treatment. Six biological replications were prepared for dry weight, and oven-dried for use in total N content measurement. Meanwhile fresh plant tissues were taken for physiological analysis, with three biological replications.

Soluble protein content was determined according to Andrews et al. (1999), and nitrate-N content was determined according to Mosier et al. (1998). NR activity was measured according to Kaiser et al. (1999). GS activity was measured as described by Masclaux-Daubresse et al. (2006).

For ionome analysis, only the plants exposed to 2 and 0.2 mM N were used. The plant samples were taken at 18 d after N treatment. Dry roots and shoots were finely ground, and approximately 0.2 g tissue samples were predigested with a mixture of 6 ml HNO_3 and 1 ml of H_2O_2 for about 20 min at 130 °C, and then digested in a microwave (Multiwave 3000, Anton Paar GmbH, Australia) after adding 1 ml of H_2O_2. The digested solution was boiled to eliminate acid for 1.5 h at 160 °C. The contents of K, P, Mg, Ca, Zn, Fe, Cu and Mn were determined using an ICP-OES spectrometer (Optima 6000 series, PerkinElmer Inc, USA).

2.3. Data statistics

Significant difference for physiological traits and element

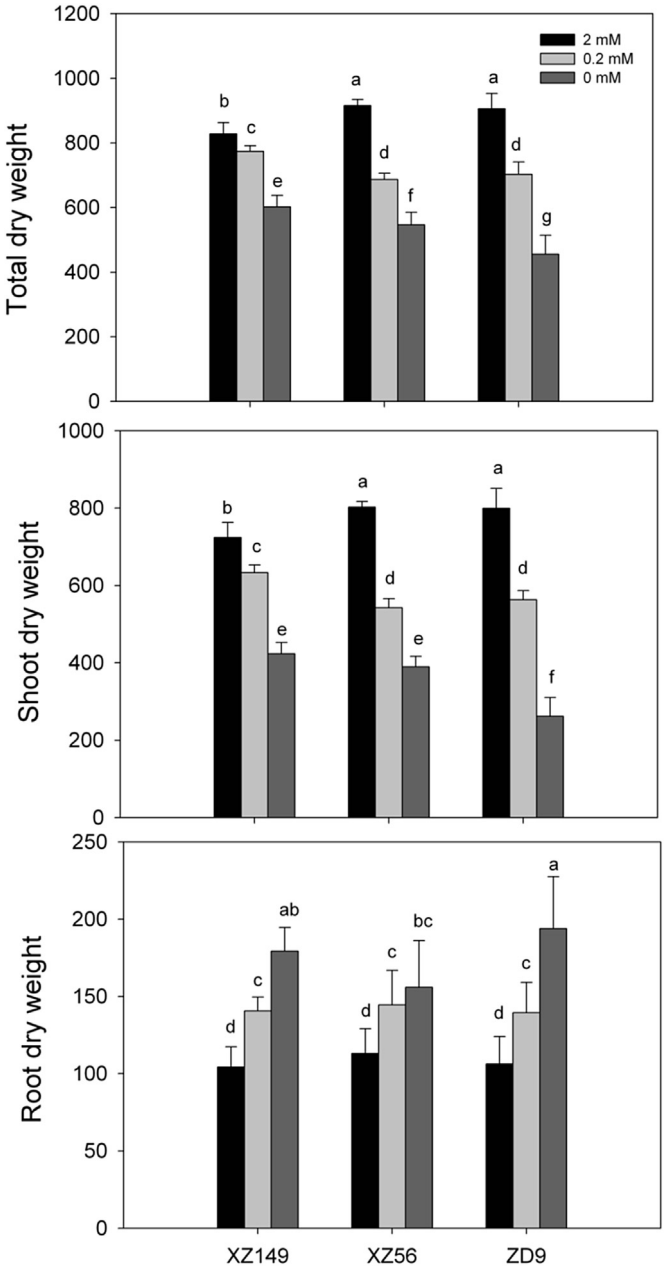

Fig. 1. Effects of low nitrogen stress on dry weight (mg.plant^{-1}) of three barley genotypes. The different letters mean significant difference among treatments and genotypes according to the Duncan's multiple range, $P < 0.05$.