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Research article

Effect of post-silking drought on nitrogen partitioning and gene expression patterns of glutamine synthetase and asparagine synthetase in two maize (*Zea mays* L.) varieties

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ABSTRACT

Glutamine synthetase (GS) and asparagine synthetase (AS) are proposed to have important function in plant nitrogen (N) remobilization, but their roles under drought stress are not well defined. In this study, the expression dynamics of GS and AS genes were analyzed in two maize varieties (ZD958 and NH101) in relation to post-silking drought stress induced nitrogen partitioning. ZD958 was a 'stay-green' variety with 5% nitrogen harvest index (NHI) lower than NH101. From silking to maturity, the amount of nitrogen remobilized from ear-leaves in ZD958 was evidently lower than NH101, and post-silking drought stress increased the nitrogen remobilization for both varieties. In ear-leaves, the expression of *ZmGln1-3* was enhanced under drought stress. Three AS genes (*ZmAS1, ZmAS2* and *ZmAS3*) were differentially regulated by post-silking drought treatment, of which the expression of *ZmAS3* was stimulated at late stage of leaf senescence. In NH101, the expression level of *ZmAS3* was markedly higher than that in ZD958. In developing grains, there were no significant differences in expression patterns of GS and AS genes between well water and drought treated plants. Drought stress altered maize N partitioning at the whole-plant level, and the up-regulation of GS and AS genes may contribute to the higher leaf nitrogen remobilization when exposed to drought treatments.

1. Introduction

Post-flowering senescence is the last stage of plant development and has very important function to the recycling and remobilization of plant assimilates, especially for nitrogen-containing molecules (Masclaux-Daubresse et al., 2010; McAllister et al., 2012). For maize, 40–70% of grain nitrogen comes from the translocation of the vegetative tissue during senescence (Lohaus et al., 1998; Masclaux-Daubresse et al., 2010). The nitrogen remobilization efficiency (NRE) from source to sink may differ genotypically or be affected by environmental factors, such as drought stress (Bauer et al., 1997; Lohaus et al., 1998).

The interaction between water and nitrogen (N) has been studied for a long time. On one hand, drought stress may inhibit N absorption and reduce the availability of N fertilizer, especially at higher fertility levels (Bennett et al., 1989; Fan and Li, 2001). On the other hand, drought stress at reproductive growth stage may hasten leaf senescence and cause premature of the whole-plant (Wang et al., 2005; Herrera-Rodríguez et al., 2007), for crops such as maize and sorghum, the delaying of leaf senescence confers better drought tolerance, and some tolerant varieties are found efficient in N assimilation (Kamara et al., 2014). However, the retention of leaf N may result in lower N remobilization (Chen et al., 2015). The optimizing of N balance is important for improving crop performance under stress conditions.

N remobilization requires the upload of free amino acids and ammonium which are released by leaf protein degradation (Bauer et al., 1997). Amide amino acids, glutamine and asparagine are considered as the major carriers of phloem N transport in many plants species, and the assimilation of ammonium into amide amino acids is an central route for N remobilization (Lea et al., 2007; Avila-Ospina et al., 2015). The increased amount of

Abbreviation: PD, post-silking drought; WW, well water; N, nitrogen; DAS, days after silking stage; GS, glutamine synthetase; AS, asparagine synthetase.

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glutamine and asparagine are observed in phloem sap during leaf senescence (Kamachi et al., 1991; Hwang et al., 2011).

Glutamine synthetase (GS) is responsible for ammonium assimilation by catalyze the conversion of glutamate and ammonium into glutamine (Valadier et al., 2008). There are two groups of GS in plants: the cytosolic GS (GS1) and plastidic GS (GS2). The role of the plastidic GS2 is mainly contribute to the assimilation of ammonium from the nitrate reduction in young leaves, and the cytosolic GS1 is responsible for the re-assimilation of ammonia released from the degradation of nitrogen-containing molecules in senescing leaves (Kamachi et al., 1991; Bauer et al., 1997; Masclaux-Daubresse et al., 2010; Avila-Ospina et al., 2015). For GS genes, the plastidic GS2 is encoded by one single gene in all the plant species studied up to now, while the cytosolic GS1 is encoded by a variable number of gene families in different plants. These GS1 isoforms have been found to participate in glutamine synthesis for various biological processes, including ammonium fixation, N storage and N transportation (Brugière et al., 2000; Avila-Ospina et al., 2015). In maize, five members of GS1 (*ZmGln1-1* to *ZmGln1-5*) genes have been identified, and the localization and expression of these GS1 genes are not in a similar manner. Among maize GS1 genes, *ZmGln1-3* and *ZmGln1-4* are highly accumulated in leaf tissues and have pivot role for N supply and partitioning (Martin et al., 2006; He et al., 2014).

Plant asparagine synthetase (AS) catalyzes the formation of asparagine in a glutamine dependent manner (Herrera-Rodríguez et al., 2007). A number of studies have documented that AS genes were stimulated by various environmental stresses, such as dark stress or carbon or N depletion, but the regulation of distinct AS genes are not similar (Wang et al., 2005; Lea et al., 2007; Gaufichon et al., 2013). In *Arabidopsis thaliana*, the expression of *ASN2* were down-regulated by aging, while *ASN1* was up-regulated at the same time (Gaufichon et al., 2013). In barley, the expression of *HvASN1, HvASN4*, and *HvASN5* were highly up-regulated by dark treatment while *HvASN3* was repressed by dark (Avila-Ospina et al., 2015). Several studies have reported that in wheat, soybean and sunflower, AS genes were also significantly induced by osmotic stress (Wang et al., 2005; Herrera-Rodríguez et al., 2007). In maize, four distinct AS genes: *ZmAS1* to *ZmAS4*, have been identified and were reported to have different kinetic properties (Todd et al., 2008; Duff et al., 2011).

In this article, using a rainproof shelter to simulate post-silking drought stress, by means of RT-qPCR, we characterized the influence of post-silking drought on expression patterns of genes coding for GS and AS (*ZmGln1-3, ZmGln1-4, ZmGS2, ZmAS1, ZmAS2 and ZmAS3*) in different tissues (ear-leaves, developing grains) of two maize varieties with comparing nitrogen harvest index (NHI), the objective of this study is to investigate the expression patterns of these genes with respect to drought stress induced N remobilization.

2. Materials and methods

2.1. Plant materials and growth conditions

This work was conducted at Northwest A&F University, Shaanxi province, China. Two maize hybrid varieties were used in this study. 'Zhengdan958' (ZD958) was a 'stay-green' hybrid (Ning et al., 2013), which has a larger green leaf area at physiological maturity. 'Nonghua101' (NH101) was selected as a rapidly leaf senescence hybrid under our experimental conditions. The experiment was carried on in a large-scale rainproof sheds (32 m length × 15 m width × 3 m height), the roof of sheds were transparently plastic-covered, with all four sides open. Maize were cultivated in 2 m length × 1.5 m width pools with concrete waterseal walls around. The soil in the depth of 1.2 m was Eum-Orthrosols (Chinese soil Taxonomy). The property of the 0–20 cm topsoil was as follows: soil organic matter: 15.80 $g \cdot kg^{-1}$, Alkaline-N: 72.5 $mg \cdot kg^{-1}$, $NaHCO_3$-P: 29.87 $mg \cdot kg^{-1}$ and NH_4OAc-K: 127.35 $mg \cdot kg^{-1}$, respectively. The two maize varieties were grown from 22 June to 18 October 2014.

2.2. Experimental design and sampling

Two water treatments were: well-water (WW) and post-silking drought (PD). Before silking, the total irrigation amount was 220 mm (amount of irrigation was controlled by a water meter and calculated by the area of the pool) to keep enough water supply for both of the treatments. After that stage, the irrigation amount for WW and PD treatments were 80 and 30 mm, respectively, keeping soil relative water content at about 80–85% (WW) and 45–55% (PD). Each treatment had five pools for replications.

Fresh samples of ear-leaves (refers to the 3 leaves closest to the ear) and developing grains were collected on 20, 35, and 50 days after silking (DAS), respectively. For dry samples, the whole up-ground plant was harvested (husk and tassel excluded) at maturity, and then divided into: stem, cob, ear-leaves, other leaves and grains. Ear-leaves at silking stage were also collected for calculating the amount of remobilized N in ear-leaves from silking to maturity (Ning et al., 2013). Fresh samples were immediately frozen in liquid nitrogen and stored at −80 °C for gene expression analysis. The dry samples were dried to constant weight at 80 °C, then milled for the measurement of dry matter, total nitrogen, total amino nitrogen and sucrose concentration.

2.3. Measurements

Net photosynthetic rate was measured by Li-6400 photosynthesis system (USA) and chlorophyll was measured by SPAD-502 (Japan).

N concentration was determined by the standard macro-Kjeldahl procedure (Horneck and Miller, 1998). To evaluate N partitioning in different upper-ground parts of maize, we calculated as follows: N content in stem, cob, ear-leaves, other-leaves and grain was calculated by dry weight × N concentration of different parts, respectively; N partitioning rate (%) was calculated by (N content in different parts/total N of upper-ground plant) × 100%; N harvest index = (N content in grains/total N of upper-ground plant) × 100% (Ciampitti and Vyn, 2012). The amount of N remobilized in ear-leaves was calculated by subtracting the N content in ear-leaves at maturity from the N content in ear-leaves at silking stage (Ning et al., 2013). Total green leaf area = ∑leaf length × leaf width × 0.75 (Aliu et al., 2008). The concentration of nitrogen in the form of free amino acids (amino acid nitrogen) was determined by ninhydrin colorimetric method (Xiong et al., 2006), sucrose concentration was determined by colorimetric method as described by (Verma et al., 2011).

Total RNA was isolated from fresh leaves and grains by the Plant total RNA extraction Kit (Rakara, Japan) according to the manufacturer's protocols. The quality and concentration of RNA were checked by NanoDrop 2000 UV–Vis Spectrophotometer (Thermo Scientific, USA). Reverse transcription of RNA into cDNA was carried out with the ABM Reverse Transcription Kit (ABM, Canada) following the manufacturer's protocol. The newly synthesized first-stand cDNA was used for gene expression analysis.

The real-time quantitative (RT-qPCR) was performed on CFX96 real-time PCR system (Bio-rad, USA) with SYBR Green reagent (ABM, Canada), relative level of gene expression was calculated by $2^{-\Delta\Delta Ct}$ method, using maize *actin* gene as reference gene to normalize all samples. Primers for *ZmGln1-3*, *ZmGln1-4*, *ZmGS2*,