

Glucose-6-phosphate dehydrogenase plays a vital role in *Achnatherum inebrians* plants host to *Epichloë gansuensis* by improving growth under nitrogen deficiency

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Received: 4 February 2018 / Accepted: 8 June 2018 / Published online: 21 June 2018
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Abstract

Background and aims Low nitrogen negatively affects soil fertility and plant productivity. Glucose-6-phosphate dehydrogenase (G6PDH) and *Epichloë gansuensis* endophytes are two factors that are associated with tolerance of *Achnatherum inebrians* to abiotic stress. However, the possibility that *E. gansuensis* interacts with G6PDH in enhancing low nitrogen tolerance of host grasses has not been examined.

Methods *A. inebrians* plants with (E+) and without *E. gansuensis* (E-) were subjected to different nitrogen concentration treatments (0.1, 1, and 7.5 mM). After 90 days, physiological studies were carried out to investigate the participation of G6PDH in the adaption of host plants to low nitrogen availability.

Results Low nitrogen retarded the growth of *A. inebrians*. E+ plants had higher total dry weight, chlorophyll a and b contents, net photosynthesis rate, G6PDH activity, and GSH content, while having lower plasma membrane (PM) NADPH oxidase activity, NADPH/NADP⁺ ratios, and MDA and H₂O₂ than in E- *A. inebrians* plants under low nitrogen concentration. **Conclusions** The presence of *E. gansuensis* played a key role in maintaining the growth of the *A. inebrians* plants under low nitrogen concentration by regulating G6PDH activity and the NADPH/NADP⁺ ratio and improving net photosynthesis rate.

Responsible Editor: Katharina Pawlowski.

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Keywords *Achnatherum inebrians* · *Epichloë gansuensis* endophyte · Low nitrogen · Glucose-6-phosphate dehydrogenase

Introduction

Nitrogen (N) is an essential macronutrient for plants, and N availability is one of most important limiting factors for plant growth and crop productivity (Marschner 1995; McAllister et al. 2012; Xu et al. 2012). Due to the high demand for fertile soil for agriculture, crops are often established on marginal lands

where soil N is limiting (Bilodeau-Gauthier et al. 2011; Rennenberg et al. 2010). Substantial quantities of N fertilizers are applied to increase crop yields, and this results in serious environmental pollution (Ju et al. 2009). Extensive studies that are linked to growth in low-nitrogen field conditions have been conducted focusing on plant biomass, root architecture, N metabolism, nitrogen use efficiency, and cellular redox homeostasis (Abenavoli et al. 2016; Brouwer 1962; Drew and Saker 1975; Lea and Azevedo 2006; Luo et al. 2013; Wang et al. 2016a).

Glucose-6-phosphate dehydrogenase (G6PDH) is the first and the rate-limiting enzyme in the pentose phosphate pathway. It controls the carbon flow and produces reducing equivalents in the form of NADPH to meet cellular needs for reductive biosynthesis and maintenance of the cellular redox state (Kletzien et al. 1994). A large number of studies have shown that G6PDH was involved in tolerance to salt stress, aluminum stress, NaCl and UV-B cross tolerance, and pathogenesis (Li et al. 2011; Šindelář and Šindelářová 2002; Ślaski et al. 1996; Wang et al. 2016b; Wang et al. 2008; Zhang et al. 2013; Zhao et al. 2015). In addition, increased expression and activity of G6PDH can decrease oxidative stress (Leopold et al. 2003). In plants, G6PDH has been reported to be involved in nitrogen assimilation (Wang et al. 2003). Plasma membrane NADPH-dependent oxidase is thought to play a key role in ROS production (Jones et al. 2007). It was reported that glutathione (GSH), as an antioxidant and a protector, is oxidized to glutathione disulfide (GSSG), and the reverse reaction is catalyzed by glutathione reductase (GR), which uses NADPH as reducing potential (Noctor and Foyer 1998). Reduced glutathione (GSH) can metabolize H_2O_2 and is important as an antioxidant and redox buffer in plants exposed to environmental stresses (Foyer and Halliwell 1976; Liu et al. 2013; Wang et al. 2008). NADPH is one of the most important molecules of the cellular redox balance in the protection against oxidative damage (Zhang et al. 2013). To avoid stress damage, plants develop this adaptive strategy at the morphological, physiological, cellular, and metabolic levels (Chen et al. 2016; Desclaux et al. 2000; Hufstetler et al. 2007; Li et al. 2011). The photosynthetic capacity of leaves is related to the nitrogen content because the proteins of the Calvin cycle and thylakoids represent the majority of leaf nitrogen (Evans 1989). Thus, the role of N is connected with photosynthesis in agricultural production (Lawlor 2002). Under stress

environments, the excess reducing equivalents in chloroplasts cause over-reduction of the photosynthetic electron transport chain and accumulation of ROS, leading to photoinhibition (Müller et al. 2001; Zhang et al. 2012a; Zhang et al. 2011). Some studies have shown decreases in both the rate of net CO_2 assimilation and the quantum yield of photosynthesis in plants grown with low N supply (Evans 1989; Khamis et al. 1990).

Microorganisms, in particular Rhizobia in nodules of members of the Fabaceae, and arbuscular mycorrhizal fungi, can also greatly enhance the availability of usable nitrogen in soil (Wang et al. 2011). However, there are many gaps in the knowledge regarding how the efficiency of plants to grow in low-nitrogen conditions can be enhanced by the presence of microorganisms. One type of microorganism that is known to enhance the ability of host plants to better tolerate abiotic stress, as well as biotic stresses such as the feeding of insect, is the fungal endophytes belonging to the genus *Epichloë* that form associations with many grasses of the sub-family Pooideae (Schardl et al. 2004). These associations are entirely or largely mutualistic (Müller and Krauss 2005). The growth of these fungi, which are located in all parts of plants except for the roots, is synchronized with that of the plant (Christensen et al. 2008). The beneficial effects of these fungi against biotic stresses, especially grazing by livestock and invertebrate feeders, are largely associated with the presence of alkaloids produced when they are growing biotrophically (Fleetwood et al. 2007). The mechanisms by which *Epichloë* endophytes enhance growth and persistence when host plants are exposed to abiotic stresses (Sabzaljan and Mirlohi 2010; Song et al. 2015a; Chen et al. 2016) are not well understood.

Achnatherum inebrians usually grows in harsh conditions, such as in gullies, shady slopes, and roadsides, and it is found mainly in north and northwest China in the alpine and subalpine grasslands of Inner Gansu, Mongolia, Xinjiang, Qinghai, and Tibet (Shi 1997, Li et al. 2004). *A. inebrians* is commonly known as the drunken horse grass due to its narcotic and deterrent effects on grazing animals (Deng et al. 1998; Shi 1997). A survey of 20 populations of *A. inebrians* from low rainfall regions found that 19 populations were 100% infected with *E. gansuensis* while the other population was 80% infected (Nan and Li 2000). A feature of *A. inebrians* plants is that nearly all are host to the seed-transmitted *Epichloë* endophytes *E. gansuensis* or *E. inebrians* (Chen et al. 2015). Interestingly, it was showed that the presence of these endophytes increases

the tolerance of *A. inebrians* to waterlogging (Song et al. 2015b), pathogens (Xia et al. 2016; Xia et al. 2015), and cadmium (Zhang et al. 2010); salt tolerance (Song et al. 2015a); cold tolerance (Chen et al. 2016); and pests (Li et al. 2007; Zhang et al. 2012b). Studies involving associations between other grasses and their specific *Epichloë* species have provided further insights into the complex interactions between the fungal endophytes and the host grasses. The presence of *Epichloë* endophytes can affect mineral uptake, transport, and efficiency ratios in tall fescue (*Festuca arundinacea*) (Rahman and Saiga 2005). It has also been reported that the endophytes influence the relationship between the plant reproductive and vegetative above-ground biomass (Gundel et al. 2013). In addition, it was found that the presence of *E. festucae* affected antioxidants in *Festuca rubra* seeds (Gundel et al. 2012). The regions of grasslands where endophyte-infected *A. inebrians* plants are thriving provide harsh growing conditions in which the summers are hot and dry and the winters are cold, and the soils of the regions of grasslands are of low fertility. It is possible that one of the reasons why *Epichloë* endophyte-infected *A. inebrians* plants are thriving under these harsh conditions is that the presence of the endophyte assists in the uptake and utilization of nitrogen and other nutrients. As previously stated, one mechanism that has impacts of the uptake and utilization of nutrients is the activity of G6PDH and thus we studied the relationship between the presence of the *E. gansuensis* and the activity of G6PDH in *A. inebrians* host grasses under a low-nitrogen environment.

Materials and methods

Plant growth conditions and nitrogen treatments

A pot experiment was performed from 2 June to 15 October 2016 in the greenhouse of the College of Pastoral Agriculture Science and Pastoral Agriculture Science and Technology, the Yuzhong campus of Lanzhou University (104° 39' E, 35° 89' N, altitude 1653 m). Before planting, 20 seeds were selected randomly from the E+ and E− seeds and used to confirm the infection status of the seeds used to establish this part of the study. The result showed that all 20 E+ seeds were endophyte-infected, and the 20 E− seeds were all endophyte free. On June 2, healthy-looking and well-filled *A. inebrians* seeds of seedlines that differed only in being either infected or uninfected

with *E. gansuensis* were sown in 120 pots (10 cm in lower diameter × 18.5 cm in upper diameter × 19.5 cm in height), with each pot having 6 seeds. Sixty pots for E+ seeds and 60 pots for E− seeds had been filled with vermiculite (75 g) that had been sterilized in an oven at 150 °C for 3 h. Just three uniform seedlings were subsequently retained in each pot. Pots were placed in groups of six in individual trays, and all trays were irrigated with distilled water every 7 days. Following the appearance of the second fully expanded leaf, 1/2 strength Hoagland's solution was applied to the pots every 7 days. The trays were assigned at random to a position within a constant temperature greenhouse (temperature 26 ± 2 °C, moisture 42 ± 2%). After 45 days, E+ plants and E− plants were treated with modified 1/2 strength Hoagland nutrient solution (0.1, 1, and 7.5mM nitrogen) for a further 90 days. The abovementioned three different nitrogen concentrations were added to the pots every 7 days. The infection status of each seedling in the study was confirmed by microscopic examination of leaf sheath pieces stained with aniline blue. Each treatment was applied to both E+ and E− plants, and there were 12 independent replicates for each nitrogen concentration.

Biomass production, determination of enzymatic activity, and chlorophyll a and b contents

After the different nitrogen concentration treatments for 90 days, all the plants from all the treatments were carefully removed from the growth medium and washed with distilled water before being manually separated into leaf blades and roots. To determine the total biomass, one plant of each pot of each treatment was oven-dried at 80 °C until a constant weight was reached. To determine enzymatic activity, leaves (excluding the pseudostems) and roots from the remainder of plants of each treatment were separately collected and were immediately frozen in liquid nitrogen and stored at −80 °C. Chlorophyll a and b contents were determined using a previously described method (Wellburn 1994).

Determination of net photosynthesis rate (Photo) and intercellular carbon dioxide concentration (Ci)

The measurement of net photosynthesis rate (Photo) and intercellular carbon dioxide concentration (Ci) and Photo and Ci data were determined using a previously described method (Xia et al. 2016). In brief, photosynthesis in the top leaf of six plants in each treatment was

measured using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) from 9:00 to 11:30 a.m. The chamber was equipped with a red/blue LED light source (LI6400-02B), with the PAR set at $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$, the detesting conditions at $T = 28 \pm 1 \text{ }^\circ\text{C}$, and air carbon dioxide concentration = $410 \pm 10 \mu\text{mol CO}_2 \text{ mol}^{-1}$.

Glucose-6-phosphate dehydrogenase activity assay

The extraction and assay of G6PDH was performed using a previously described method (Hauschild and von Schaewen 2003). Leaves (0.3 g) and roots (0.3 g) were ground in the extraction buffer containing 50 mM Hepes-Tris (pH 7.8), 3 mM MgCl_2 , 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at $12000g$ for 15 min at $4 \text{ }^\circ\text{C}$, and the supernatant was used to determine enzyme activity. A 200- μl aliquot of the extract was added to the assay buffer containing 50 mM Hepes-Tris (pH 7.8), 3.3 mM MgCl_2 , 0.5 mM glucose-6-phosphate disodium salt, and 0.5 mM NADPNa_2 . G6PDH activity was assayed at 340 nm for the initial 4 min.

Determination of NADP^+ and NADPH content

Leaves (0.4 g) and roots (0.4 g) were extracted in 4 ml of 0.2 M HCl and 0.2 M NaOH for the extraction of NADP^+ and NADPH, respectively. The homogenates were centrifuged at $12,000g$ for 10 min at $4 \text{ }^\circ\text{C}$. The supernatants were boiled for 1 min then cooled on ice, and 0.2 M NaH_2PO_4 was added, and then, these were neutralized with either NaOH or HCl for use for the quantification by the enzyme cycling method (Matsumura and Miyachi 1980).

Determination of glutathione content

Total glutathione content was measured according to a modified method (Anderson et al. 1992). With 7% sulfosalicylic acid in the extraction buffer, 0.3 g of leaves and roots were ground, homogenized with extraction buffer, and centrifuged for 10 min at $10,000g$ at $4 \text{ }^\circ\text{C}$. The supernatant was used to determine the glutathione content. Reagent 1 [110 mM Na_2HPO_4 , 40 mM NaH_2PO_4 , 15 mM EDTA, 0.3 mM 5,5-dithiobis (2-nitrobenzoic acid), and 0.04% BSA], reagent 2 [1 mM EDTA, 50 mM imidazole solution, and 0.02% BSA], 5% Na_2HPO_4 (pH 7.5), 1.5 U glutathione reductase (GR),

240 μl supernatant, and 9 mM NADPHNa_4 were mixed in a 2-ml centrifuge tube. The absorbance of the reaction mixture was measured at 412 nm at $25 \text{ }^\circ\text{C}$. Oxidized glutathione was measured as for total glutathione except that 1 ml of the supernatant extract was removed and then incubated with 40 μl 2-vinylpyridine for 1 h at $25 \text{ }^\circ\text{C}$ before mixing with the reaction buffer.

Assay of plasma membrane (PM) NADPH oxidase activity

PM was isolated using a previously described method (Qiu and Su 1999). The microsomal pellets were used for PM NADPH oxidase activity determination. PM NADPH oxidase activity was determined using a previously described method (Duan et al. 2009). Protein content was determined using a previously described method (Bradford 1976) with bovine serum albumin (BSA) as the standard. Determination of PM NADPH oxidase activity was carried out with three independent biological replicates.

Determination of MDA content and H_2O_2 content

Malondialdehyde (MDA) content was determined using a previously described method (Liu et al. 2007). H_2O_2 content was performed using a previously described method (Velikova et al. 2000).

Statistical analysis

Data analyses were performed with SPSS version 17.0 (SPSS, Inc., Chicago, IL). Significance of differences between E+ and E- plants in all of the parameters was carried out by independent *T* tests. Statistical significance was defined at the 95% confidence level. Means are reported with their standard errors. Each experiment was carried out with three independent biological replicates.

Results

Total dry weight and chlorophyll a and b contents under the different nitrogen concentrations

Our results showed that *A. inebrians* plants with and without *E. gansuensis* showed significant differences in total dry weight and chlorophyll a and b contents under the 0.1 mM N concentration. Further, the presence of the

endophyte enhanced the total dry weight and chlorophyll a and b contents compared with the E⁻ plants under the 0.1 mM N concentration (Fig. 1), and the total dry weight of E⁺ plants was higher than that of E⁻ plants under the 1 mM N concentration (Fig. 1a). However, our data indicated that E⁺ and E⁻ plants had no significant differences in chlorophyll a and b under the 1 and 7.5 mM N concentrations (Fig. 1b, c). In summary, the total dry weight and chlorophyll a and b contents of E⁺ plants were increased by 49.9% and 13.5 and 13.6 % compared with those of E⁻ plants under the 0.1 mM N concentration, respectively (Fig. 1).

Photo and C_i of E⁺ and E⁻ plants under the different nitrogen concentrations

Our results showed that N concentration positively affected net photosynthetic rate (Photo); however, N concentration negatively affected intercellular carbon dioxide concentration (C_i) (Fig. 2). Further, we found that there were no significant differences of the net photosynthetic rate and intercellular carbon dioxide concentration between E⁺ and E⁻ plants under the 7.5 mM N concentration. Interestingly, the Photo of E⁺ plants was higher than in E⁻ plants under the 0.1 mM N concentration, and the Photo of E⁺ plants was increased by 26.4% compared with that of E⁻ plants (Fig. 2a). E⁺ plants had higher C_i than E⁻ plants under the 1 mM N concentration, being 18.7% higher than E⁻ plants (Fig. 2b).

G6PDH activity of E⁺ and E⁻ plants under the different N concentrations

Our results showed that the N concentrations affected G6PDH activity of *A. inebrians*, with G6PDH activity decreasing as the N concentrations increased (Fig. 3). Further, our results showed that there was significant difference in G6PDH of leaves and roots between E⁺ and E⁻ plants under the 0.1 mM N concentration (Fig. 3), and there was also significant difference in G6PDH of leaves between E⁺ and E⁻ plants under the 1 mM N concentration. Interestingly, endophyte infection resulted in higher activity of G6PDH in leaves than in those of endophyte-free plants under the 0.1 and 1 mM N concentrations, being 10.3 and 41.9% higher, respectively, than in leaves of E⁻ plants (Fig. 3a). Our results showed that the endophyte-infected plants had 27.6% higher activity of G6PDH in roots than of those of endophyte-free plants under the 0.1 mM N concentration (Fig. 3b).

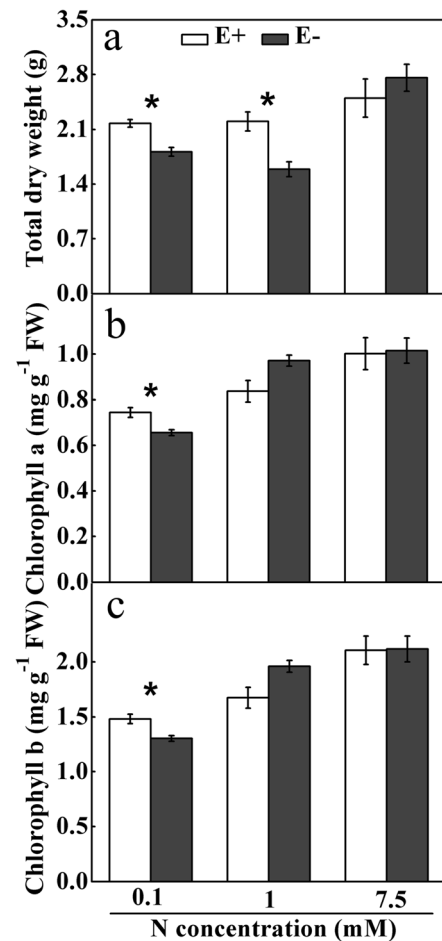


Fig. 1 Total dry weight (a), chlorophyll a (b), and chlorophyll b (c) of *Achnatherum inebrians* with and without endophyte under different N concentrations. The data presented are the means \pm SE of four independent biological replicates. Asterisk (*) means significant difference ($P < 0.05$) between E⁺ and E⁻ plants

Effect of the different N concentrations on the NADPH and NADP⁺ content of E⁺ and E⁻ plants

Our results indicated that the NADPH content in leaves of E⁺ and E⁻ plants of the 0.1 and 1 mM N treatments was increased compared with the 7.5 mM N treatment plants, and the leaves of E⁺ plants had significantly lower content of NADPH than in the leaves of E⁻ plants under the 0.1 and 1 mM N concentrations (Fig. 4a). But the endophyte-infected plants had a higher content of NADP⁺ in leaves than endophyte-free plants under the 0.1 mM N concentration (Fig. 4b). The NADPH content in root samples of E⁺ plants was lower than in roots of E⁻ plants under the 0.1 and 1 mM N concentrations (Fig. 4d). In contrast, the NADP⁺ content of E⁺ plants

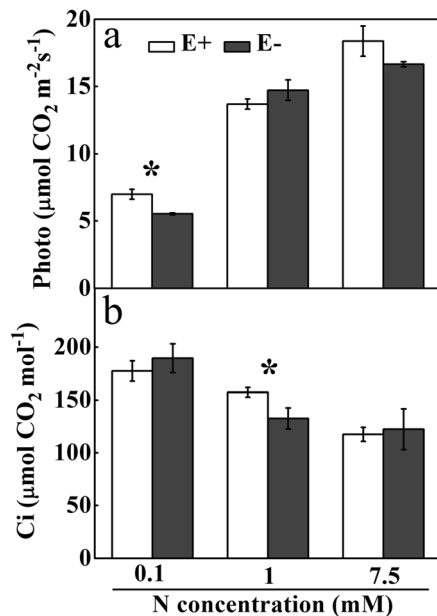


Fig. 2 The net photosynthetic rate (a) and intercellular carbon dioxide concentration (b) of *Achnatherum inebrians* with and without endophyte under different N concentrations. The data presented are the means \pm SE of three independent biological replicates. Asterisk (*) means significant difference ($P < 0.05$) between E+ and E- plants by independent *T* tests

was higher than in E- plants under the 0.1 and 1 mM N concentrations (Fig. 4e). Further, E- plants had higher NADPH/NADP⁺ ratios in leaves and roots than in E+ plants under the 0.1 mM N concentration (Fig. 4c, f).

Changes in GSH content of E+ and E- plants under the different N concentrations

Higher N concentration positively affected the GSH content of the leaves and roots in E+ and E- plants (Fig. 5). Further, our data showed that the GSH content of E+ plants was higher than in E- plants under the 0.1 mM N concentration, with the GSH content of leaves of E+ plants being increased by 20.0% compared with E- plants (Fig. 5a). Similarly, the GSH content of roots of E+ plants was increased by 52.4% compared with E- plants under the 0.1 mM N concentration (Fig. 5b).

Effects of the different N concentrations on the activity of PM NADPH oxidase of E+ and E- plants

In the leaves and roots of E+ and E- plants, the 0.1 mM N treatment activated PM NADPH oxidase activity (Fig. 6). Further, our data demonstrated that endophyte infection

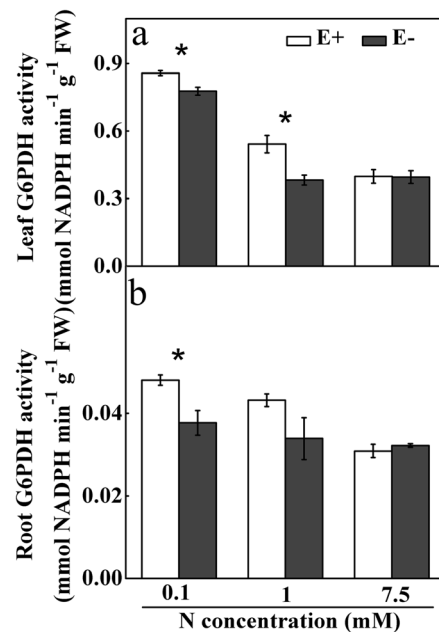


Fig. 3 G6PDH activity in leaves (a) and roots (b) of *Achnatherum inebrians* with and without endophyte under different N concentrations. The data presented are the means \pm SE of three independent biological replicates. Asterisk (*) means significant difference ($P < 0.05$) between E+ and E- plants by independent *T* tests

decreased PM NADPH oxidase activity compared with E- plants under the 0.1 mM N concentration. Further, the results showed that PM NADPH oxidase activity in leaves and roots of E+ plants was decreased by 10.1 and 13.7% compared with E- plants under the 0.1 mM N treatment, respectively (Fig. 6a, b).

Changes in MDA and H₂O₂ contents of E+ and E- plants under the different N concentrations

The 0.1 mM N treatment increased the MDA content in leaves and roots of E+ and E- plants (Fig. 7a, b). MDA content had significant differences in leaves and roots between E+ and E- plants, being lower in E+ than E- plants under the 0.1 mM N concentration (Fig. 7a, b). Further, our results showed that N concentrations affected H₂O₂ content of leaves (Fig. 7c). Interestingly, the presence of the endophyte decreased H₂O₂ content in leaves of E+ plants compared with the E- plants under the 0.1 and 1 mM N concentrations (Fig. 7c). However, the 0.1 mM N concentration significantly increased the H₂O₂ content of roots of E+ and E- plants, and the H₂O₂ content of roots of E+ plants was lower than that of E- plants under the 0.1 mM N concentration (Fig. 7d).

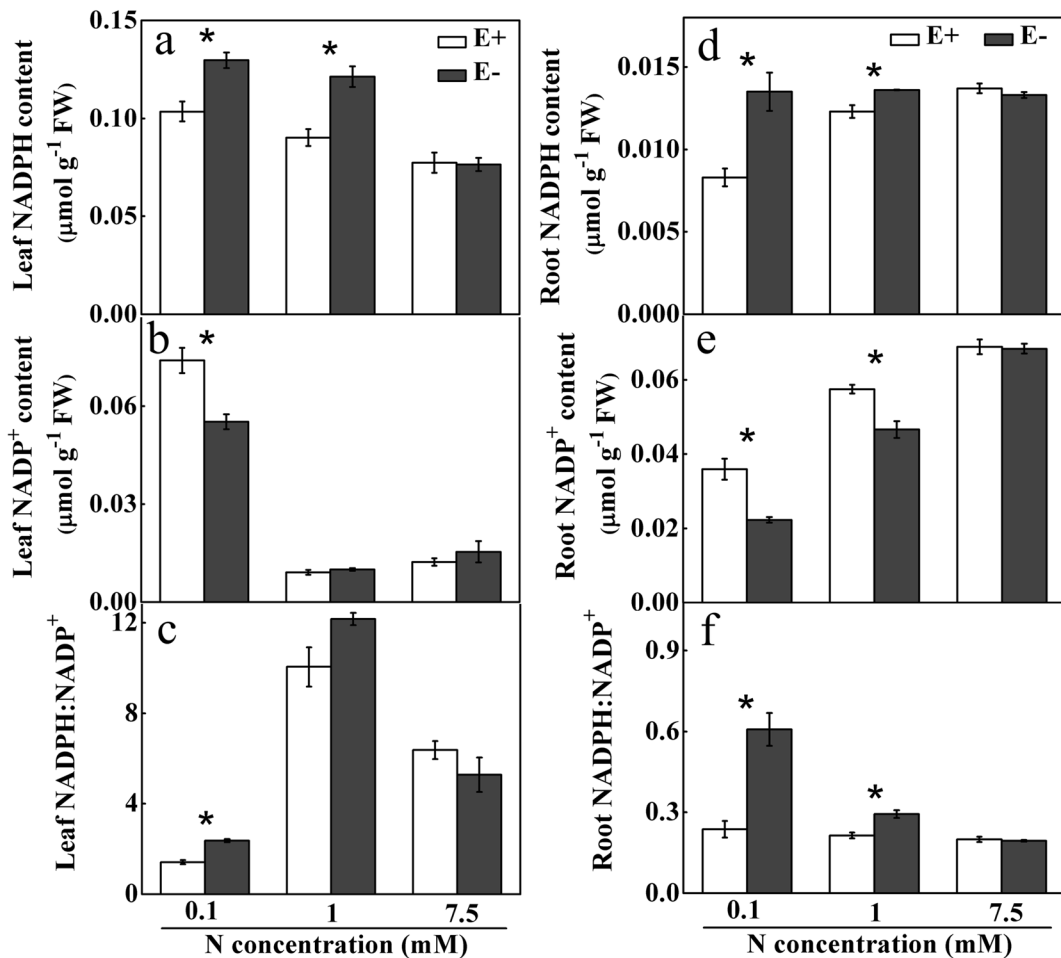


Fig. 4 NADPH and NADP⁺ content and NADPH:NADP⁺ ratios in leaves (a–c) and roots (d–f) of *Achnatherum inebrians* with and without endophyte under different N concentrations. The data

presented are the means \pm SE of three independent biological replicates. Asterisk (*) means significant difference ($P < 0.05$) between E+ and E- plants by independent *T* tests

Discussion

The present work is one of the few studies on the impact of the *E. gansuensis* on *A. inebrians* plants growing under low nitrogen concentration. The important finding of this study was that although low nitrogen significantly reduced the total dry weight, the chlorophyll a and b contents, and the net photosynthesis rate, the presence of the endophyte alleviated the adverse effects on the plants. This finding is in agreement with two previous studies that had shown that the presence of an *Epichloë* endophyte in host plants under stress alleviated the extent of the reduction in dry weight, the chlorophyll a and chlorophyll b contents, and the net photosynthesis rate (Song et al. 2015a; Xia et al. 2018; Xia et al. 2016).

It is important to consider the nature of the relationship between *E. gansuensis* and the host grass in order to understand the process of how the presence of this fungus activates plant physiological responses to abiotic stresses. In nature, *Epichloë* endophytes are only found in association with grasses belonging to the sub-family Pooideae. The synchronized systemic colonization of host plants and the mutual benefit to both partners of the association is a feature of the unique plant/fungus association (Christensen et al. 2008). Clearly, there is strong communication between the host grass and the fungal endophyte, with some genes involved with secondary metabolism being upregulated when in association with host grasses.

The present study is the first report that the presence of an *Epichloë* endophyte in a plant upregulates activity of G6PDH and enhances tolerance to low nitrogen stress.

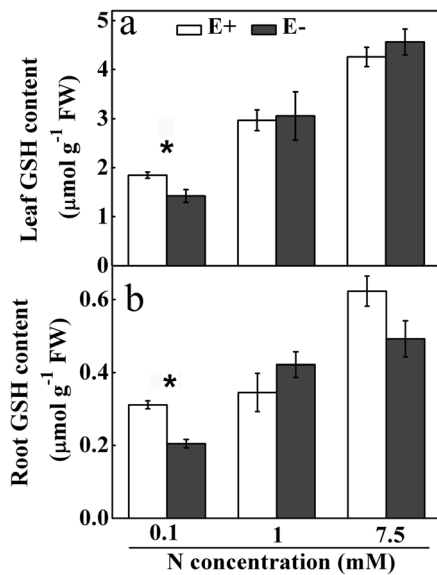


Fig. 5 GSH contents of leaves (a) and roots (b) of *Achnatherum inebrians* with and without endophyte under different N concentrations. The data presented are the means \pm SE of three independent biological replicates. Asterisk (*) means significant difference ($P < 0.05$) between E+ and E- plants by independent *T* tests

Related to this finding is that the induction of G6PDH activity in an aluminum-resistant wheat cultivar may be associated with aluminum resistance (Ślaski et al. 1996), and this is consistent with our results presented here. Further, our data showed that the NADPH content and the NADPH/NADP⁺ ratio of leaves and roots of E+ plant were lower than those of E- *A. inebrians* plants growing under the 0.1 mM N concentration. A previous study had shown that reducing equivalents in the form of NADPH is the main product of G6PDH, and NADPH plays an important role in the cellular redox state regulation, but even so it had harmful effects on cells (Zhang et al. 2013). It had been shown that an increased NADPH/NADP⁺ ratio made photosynthesis susceptible to photoinhibition in maize (Tsuchida et al. 2001). PM NADPH oxidase oxidizes cytoplasmic NADPH to transfer an electron to molecular O₂ to form O₂⁻, which is then converted to H₂O₂ by SOD, to keep the NADPH balance (Van Gestelen et al. 1997; Zhang et al. 2013). Thus, PM NADPH oxidase is very important for resistance of plants to stress (Ma et al. 2012), which is consistent with our results presented here. Our study showed that the PM NADPH oxidase activity markedly increased under the 0.1 mM N concentration, and the presence of the *E. gansuensis* decreased the activity of the PM NADPH oxidase under the 0.1 mM N concentration. Further, it

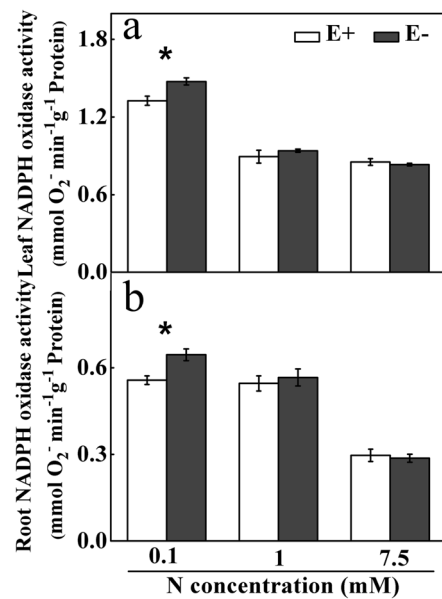


Fig. 6 PM NADPH oxidase activities in leaves (a) and roots (b) of *Achnatherum inebrians* with and without endophyte under different N concentrations. The data presented are the means \pm SE of three independent biological replicates. Asterisk (*) means significant difference ($P < 0.05$) between E+ and E- plants by independent *T* tests

was reported that GSH was also an important antioxidant molecule produced in plants in response to environment stress (Foyer and Noctor 2005; Liu et al. 2013; Wang et al. 2008; Zhao et al. 2015). In addition, a previous study demonstrated that NADPH produced by G6PDH is the main reducing potential for the regeneration of GSH (Noctor et al. 1998). It has also been shown that G6PDH plays a central role in modulating reduced glutathione levels in reed callus under salt stress (Wang et al. 2008). Our data showed that the 0.1 mM N condition decreased GSH content; however, the presence of *E. gansuensis* in *A. inebrians* plants increased GSH content compared with endophyte-free plants. Taken together, our results showed that *E. gansuensis* upregulates the activity of G6PDH and GSH content and downregulates NADPH content and PM NADPH oxidase activity that enhance the adaptation of *A. inebrians* to low N stress. In general, increased MDA and H₂O₂ content indicates lipid peroxidation in response to abiotic stress (Song et al. 2015b; Zhao et al. 2015), and MDA has been used to estimate the peroxidation of lipids in membranes and loss of membrane integrity (DeLong and Steffen 1997). Further, our results showed that the presence of *E. gansuensis* decreased MDA and H₂O₂ content and increased host growth under the low N concentration. To our knowledge,

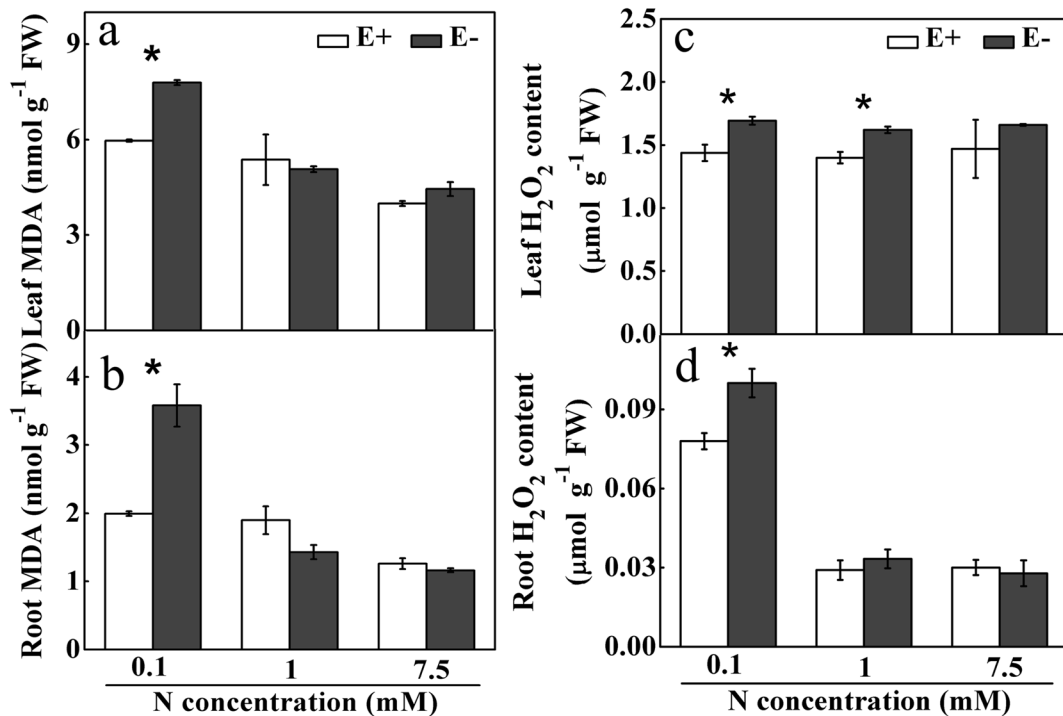


Fig. 7 MDA and H₂O₂ content in leaves (a, c) and roots (b, d) of *Achnatherum inebrians* with and without endophyte under different N concentrations. The data presented are the means \pm SE of

three independent biological replicates. Asterisk (*) means significant difference ($P < 0.05$) between E+ and E- plants by independent *T* tests

this is the first study demonstrating that an *Epichloë* species can increase host grass growth under low N concentration.

In this study, the presence of *E. gansuensis* upregulated G6PDH activity and GSH content and downregulated NADPH content, the NADPH/NADP⁺ ratio, and the PM NADPH oxidase activity so enhancing the persistence of host grasses when exposed to the low N concentration. Our results were similar with previous research in that the presence of *E. gansuensis* significantly increased the antioxidant contents (GSH), and the reduced levels of MDA and H₂O₂ are consistent with higher antioxidant activity (Zhang et al. 2010). As a consequence, its reduced oxidative damage to photosynthetic proteins could also extend the life of chloroplasts and result in an overall greater number of functional chloroplasts, and higher rates of fixed carbon accumulation, and this would be a factor for why E+ plants had higher total dry weight than E- plants. Similarly, the presence of *E. gansuensis* reduced oxidative damage to other metabolism enzymes of host growth, for example, the enzymes of nitrogen metabolism. Further, the presence of the endophyte may increase

nitrogen use efficiency and so improve host growth under low nitrogen concentration, and this will be the focus of our research in the future.

In summary, our results have both theoretical and applied importance. First, the presence of *E. gansuensis* increased tolerance of *A. inebrians* to low N stress. Second, we propose that the presence of an *Epichloë* endophyte will provide a selection advantage in natural grasslands.

Acknowledgements We wish to thank Xingxu Zhang, Chao Xia, Shan Xu, and Yane Guo for their help and advice during the preliminary stages of this project.

Author contributions Designed the research: Jianfeng Wang and Zhibiao Nan. Performed the research: Jianfeng Wang and Zhibiao Nan. Analyzed the data: Jianfeng Wang and Zhibiao Nan. Wrote the paper: Jianfeng Wang, Zhibiao Nan, Michael Christensen, and Chunjie Li.

Funding information This research was financially supported by National Basic Research Program of China (2014CB138702) and the National Nature Science Foundation of China (31772665), and the Open Foundation of Research Institute of Qilian Mountains, Lanzhou University.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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