SCIENCE CHINA
Life Sciences

The fungal endophyte Epichloë gansuensis increases NaCl-tolerance in Achnatherum inebrians through enhancing the activity of plasma membrane H+-ATPase and glucose-6-phosphate dehydrogenase

Wang Jianfeng, Hou Wenpeng, Christensen Michael, Xia Chao, Chen Tao, Zhang Zhixin and Nan Zhibiao

Citation: SCIENCE CHINA Life Sciences; doi: 10.1007/s11427-020-1674-y

View online: http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

Published by the Science China Press

Articles you may be interested in

Chemical composition and antifungal activity of the volatile oil from Epichloë gansuensis, endophyte-infected and non-infected Achnatherum inebrians
SCIENCE CHINA Life Sciences **58**, 512 (2015);

Interactive effects of water stress and powdery mildew (Blumeria graminis) on the alkaloid production of Achnatherum inebrians infected by Epichloë endophyte
SCIENCE CHINA Life Sciences **61**, 864 (2018);

Characterization of glucose-6-phosphate dehydrogenase deficiency and identification of a novel haplotype 487G>A/IVS5-612(G>C) in the Achang population of southwestern China
Science in China Series C-Life Sciences **50**, 479 (2007);

Effects of cutting frequency and height on alkaloid production in endophyte-infected drunken horse grass (Achnatherum inebrians)
SCIENCE CHINA Life Sciences **54**, 567 (2011);

Epichloë festucae var. lolii endophyte affects host response to fungal disease progression in perennial ryegrass (Lolium perenne)
SCIENCE CHINA Life Sciences **62**, 1264 (2019);

RESEARCH PAPER

The fungal endophyte *Epichlo ëgansuensis* increases NaCl-tolerance in *Achnatherum inebrians* through enhancing the activity of plasma membrane H^+-ATPase and glucose-6-phosphate dehydrogenase

Jianfeng Wang[1], Wenpeng Hou[1], Michael J. Christensen[2], Chao Xia[1], Tao Chen[1], Zhixin Zhang[3], Zhibiao Nan[1 *]

[1]State Key Laboratory of Grassland Agro-ecosystems; Center for Grassland Microbiome; Key Laboratory of Grassland Livestock Industry Innovation; Ministry of Agriculture and Rural Affairs; Engineering Research Center of Grassland Industry; Ministry of Education; College of Pastoral Agriculture Science and Technology; Lanzhou University, Lanzhou 730000, China

[2]Retired scientist of AgResearch, Grasslands Research Centre, Private Bag 11-008, Palmerston North 4442, New Zealand

[3]College of Grassland Agriculture, Northwest A&F University, Yangling, Shanxi 712100, P. R. China

[*]To whom correspondence should be addressed. E-mail: zhibiao@lzu.edu.cn

Received January 3, 2020; accepted March 8, 2020

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

Abstract

Salt stress negatively affects plant growth, and the fungal endophyte *Epichloë gansuensis* increases the tolerance of its host grass species, *Achnatherum inebrians*, to abiotic stresses. In this work, we first evaluated the effects of *E. gansuensis* on Glucose-6-phosphate dehydrogenase (G6PDH) and plasma membrane (PM) H^+-ATPase activity of *Achnatherum inebrians* plants under varying NaCl concentrations. Our results showed that the presence of *E. gansuensis* increased G6PDH, PM H^+-ATPase, SOD and CAT activity to decrease $O_2^{\cdot-}$, H_2O_2 and Na^+ contents in *A. inebrians* under NaCl stress, resulting in enhanced salt tolerance. In addition, the PM NADPH oxidase activity and NADPH/$NADP^+$ ratios were all lower in *A. inebrians* with *E. ganusensis* plants than *A. inebrians* plants without this endophyte under NaCl stress. In conclusion, *E. gansuensis* has a positive role on improving host grass yield under NaCl stress by enhancing the activity of G6PDH and PM H^+-ATPase to decrease ROS content. This provides a new way for the selection of stress-resistant and high quality forage varieties by the use of systemic fungal endophytes.

Keywords *Achnatherum inebrians*; *Epichloë* endophyte; NaCl tolerance; Glucose-6-phosphate dehydrogenase; Plasma membrane H^+-ATPase

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

Introduction

For world agriculture, a major question is how to grow enough food to feed about 2.3 billion additional people that will be present in 2050 (Fao 2009). It was reported that about 800 $\times 10^6$ million ha of soil is adversely affected by salt, and the area is increasing yearly (Munns and Tester 2008). Salt stress is a major environmental stress, and it limits the increase in the yield of crops. The understanding of plant responses to environment stresses is essential in achieving enhanced crop yields.

The pentose phosphate pathway (PPP) is the major pathway for the production of NADPH, which is used for biosynthesis and redox balance in plant cells (Kletzien et al. 1994; Valderrama et al. 2006; Cardi et al. 2011). Glucose-6-phosphate dehydrogenase (G6PDH) is the first and rate-limiting regulatory enzyme in the PPP. The majority of NADPH is produced by G6PDH (Kletzien et al. 1994; Huan et al. 2014). Several studies suggested that increased G6PDH activity would enhance the production of NADPH in order to scavenge excess reactive oxygen species (ROS) in plants (Wang et al. 2008; Li et al. 2011; Dal Santo et al. 2012). It was reported that G6PDH plays an important role in increasing plant resistance to NaCl stress (Wang et al. 2008; Li et al. 2011; Liu et al. 2012). The generation of H_2O_2 is mediated by the plasma membrane (PM) NADPH oxidase in plants. It had been demonstrated that ROS are scavenged through both non-enzymatic and enzymatic antioxidant pathways in plants (Thannickal and Fanburg 2000; Jiang et al. 2012; Ma et al. 2012; He et al. 2019). Plants can relieve the effects of salinity stress by removing excess ROS through increasing antioxidant enzyme activity (Yang et al. 2015). For example, salt stress increases SOD and CAT activity to scavenge excess ROS, and so play a key role in increasing salt-tolerance of plants (Sergio et al. 2012). In addition, ROS can activate the expression of stress responsive genes (Foyer and Noctor 2009; Boubakri et al. 2013). The reduction form of glutathione (GSH), which is a cysteine-containing tripeptide, is required to maintain the normal reduced state of cells in order to eliminate the harmful effects of excess ROS. As a protector and an antioxidant, GSH is oxidized to yield glutathione disulfide (GSSG). The reverse reaction is catalyzed by glutathione reductase (GR), and NADPH function as reducing potential in this process (Noctor and Foyer 1998). GSH is an important antioxidant molecule to eliminate excess ROS under abiotic stresses (Foyer and Halliwell 1976; Wang et al. 2008). Interestingly, in *Phragmites communis* dune reed, the G6PDH plays an important role in regulating GSH content under NaCl stress (Wang et al. 2008).

Under NaCl stress, tolerant plants generally maintain high potassium (K^+) content and low sodium

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

(Na^+) content (Song et al. 2015). The high Na^+ content inhibits K^+ absorption, and K^+ is an important element for plant growth (James et al. 2011). These processes are regulated by transport systems, including H^+-ATPase and ion channels associated with plasma membranes and tonoplasts (Rausch et al. 1996). Support for this is that callus of the dune reed ecotype of *P. communis*, an ecotype exposed to harsh stress conditions, maintained a higher K^+/Na^+ ratio, and that the activity of PM H^+-ATPase was higher than callus of the swamp reed, an ecotype exposed to low stress (Zhao et al. 2004). Another report also suggested that H_2O_2 up-regulates the level of Na^+/H^+ antiporter protein and PM H^+-ATPase activity, which subsequently results in plants tolerance to NaCl stress (Li et al. 2011). Furthermore, in *Chenopodium quinoa* and *Atriplex lentiformis* plants, rapid regulation of PM H^+-ATPase activity is important for NaCl resistance (Bose et al. 2015). Therefore, PM H^+-ATPase plays an important role in maintaining K^+/Na^+ balance in plants.

Some microorganisms, including those that colonize roots, can promote plant growth (Benizri et al. 2001; Compant et al. 2010; Baslam et al. 2011). An example of this is improved forage quality and growth linked to enhanced microorganism activity following magnesium fertilizer application (Chen et al. 2107). Endophytic microbes promoted rice (*Oryza sativa* L.) growth and Zn accumulation in rice grains (Wang et al. 2014). The fungal endophytes of Pooideae grasses belonging to the genus *Epichloë* are an excellent example of a beneficial association between plants and microorganisms (Müller and Krauss 2005). A key feature of grass/*Epichloë* associations is that the endophyte is present in all tissues except the roots, and in most associations, is also absent in vascular tissue (Christensen and Voisey, 2007). The nature of the association is mutualistic, with the endophyte confined to the intercellular spaces, where it obtains ongoing access to nutrients, a stable environment, freedom from microbial competition, and transmission to the next generation. In return, the plant receives protection against biotic and abiotic stresses. Most *Epichloë* endophytes are exclusively seed-transmitted with hyphae become incorporated into the embryo of developing seed (Zhang et al. 2017). Upon germination, the growth of hyphae is synchronized with the host grass (Christensen et al. 2008), being confined to when amongst dividing or elongating host cells, apart from when stromata are formed in some associations. Interestingly, the hyphae remain metabolically active until the senescence and death of leaves that they are in. There is strong communication between grass and endophyte, with some secondary metabolism genes being up-regulated in grass-endophyte symbiosis (Johnson et al. 2003). Some of the abiotic stresses that have been alleviated in plants by the presence of an *Epichloë* endophyte are the presence

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

in soil of cadmium (Zhang et al. 2010) and NaCl (Song et al. 2015; Wang et al. 2019), as well as cold tolerance (Chen et al. 2016), drought tolerance (Kannadan and Rudgers 2008; Clay and Schardl 2002; Xia et al. 2018) and low nitrogen tolerance (Wang et al. 2018a; Wang et al. 2018b). One study has demonstrated that *Epichloë* endophytes have an important role for the tolerance to NaCl stress in meadow fescue (*F. pratensis*) and tall fescue (*F. arundinacea*) (Reza Sabzalian and Mirlohi 2010). In addition, it was demonstrated that the presence of *Epichloë* endophyte can increase the host's resistance to pathogenic fungi (Xia et al. 2015; Xia et al. 2016; Tian and Nan 2019). The positive effects of *Epichloë* endophytes on grasses is now well established and cultivars of the important pasture grass perennial ryegrass (*Lolium perenne*) and tall fescue, the beneficial *Epichloë* strains can improve productively of the pasture (Johnson et al. 2013).

Although the increased tolerance to abiotic stresses in endophyte-infected grasses is well known, the precise mechanisms involved with abiotic stresses remains largely unknown. This includes the possible role of G6PDH and PM H^+-ATPase activity in host grasses under NaCl stress. Therefore, under NaCl stress, we studied the relationship between *E. gansuensis* and the activity of G6PDH and PM H^+-ATPase in *A. inebrians* host grasses. In the present study, the response pattern of G6PDH and PM H^+-ATPase to NaCl stress was analyzed in *E. gansuensis*-infected (E+) and *E. gansuensis*-uninfected (E−) plants. Furthermore, under NaCl stress, the content of Na^+ and K^+ of E+ and E− *A. inebrians* plants was examined. Our aim was to confirm our hypothesis that the *E. gansuensis* regulates the activity of G6PDH and PM H^+-ATPase to decrease ROS and Na^+ content, and enhance K^+ content in E+ plants.

RESULTS

Effects of endophyte on dry weight of *A. inebrians* under NaCl stress

Our results demonstrated that NaCl stress negatively affected the dry weight of leaves and roots, which decreased with an increasing of NaCl concentrations (Fig. 1). Endophyte-infected and endophyte-uninfected *A. inebrians* plants showed no significant differences in the dry weights of leaves and roots under low NaCl stress (100 mM NaCl) conditions. However, compared with the 0 and 100 mM NaCl, 200, 300 and 400 mM NaCl had a significant impact on the dry weights of leaves and roots between E+ and E− *A. inebrians* (Fig. 1). For the dry weight of leaves or roots, there were no significant differences between E+ and E− *A. inebrians* under the 0 and low NaCl stress (100 mM

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

NaCl) treatments, however, there were significant differences between E+ and E− *A. inebrians* under high NaCl stress (200, 300 and 400 mM). For example, under 200 mM NaCl stress, the dry weight of E+ leaves was 0.52 ± 0.042 g, while the dry weight of E− leaves was only 0.36 ± 0.028 g (Fig. 1b). Similarly, the dry weight of E+ roots was higher than the dry weight of E− roots (Fig. 1c). These results showed that the presence of endophyte improved *A. inebrians* growth under NaCl stress.

Effects of NaCl on $O_2^{\cdot-}$ and H_2O_2 contents of E+ and E− plant

$O_2^{\cdot-}$ and H_2O_2 content of the leaves and roots in E+ and E− *A. inebrians* increased as the NaCl concentrations increased (Fig. 2). However, under the 0 mM NaCl concentration, the $O_2^{\cdot-}$ and H_2O_2 content of leaves and roots were not significantly different between E+ and E− *A. inebrians* plants (Fig. 2). Interestingly, the endophyte-infection alleviated this change, with leaves and roots of E+ plants having significantly lower content of $O_2^{\cdot-}$ and H_2O_2 than of E− plants under high NaCl stress (200, 300 and 400 mM) (Fig. 2).

Changes in G6PDH activity of E+ and E− plants under NaCl stress

The G6PDH activity of E+ and E− plants exposed to the elevated NaCl concentrations significantly increased compared with the control (Fig. 3). The results demonstrated that the G6PDH activity in E+ and E− *A. inebrians* plants reached a maximum at 200 mM NaCl stress. However, the prensence of endophyte enhanced the G6PDH activity in E+ *A. inebrians* plants, and E+ plants had significant higher activity compared with E− plants under high NaCl concentrations (Fig. 3). Further, the G6PDH activity of E+ leaves was enhanced by 25.3 %, 27.9 %, 20.0 % and 15.4 % compared with E− leaves under high NaCl stress (100, 200, 300 and 400 mM), respectively (Fig. 3a). Similarly, the G6PDH activity of roots of E+ plants was enhanced by 53.9 %, 29.6 %, 48.5 % and 20.9 % compared with roots E− plants under high NaCl stress (100, 200, 300 and 400 mM), respectively (Fig. 3b).

Effects of endophyte on the NADPH/$NADP^+$ ratio under NaCl stress

In E+ and E− plants, the NADPH content of leaves and roots was increased compared with the control plants under elevated NaCl concentrations, further, the leaves and roots of E+ plants had significantly lower NADPH level than of the E− plants under high NaCl stress (200, 300 and 400 mM) (Supplementary Fig. 1a and d). But the $NADP^+$ level in leaves and roots of E+ and E− plants was

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

decreased compared with the control plants at different NaCl concentrations, with the exception of the leaves of the 400 mM NaCl concentration (Supplementary Fig. 1b and e). It was found that the leaves and roots E+ plants had higher $NADP^+$ content than in the leaves and roots of E− plants under high NaCl stress (200, 300 and 400 mM). The leaves and roots of E− plants had higher NADPH/$NADP^+$ ratios than the leaves and roots of E+ plants under high NaCl stresses (Supplementary Fig. 1c and f).

Alterations in GSH content, and SOD and CAT activity of E+ and E− plants in response to NaCl stress

The GSH of E+ and E− plants decreased as the NaCl concentrations increased (Supplementary Fig. 2). Interestingly, the E+ plants had a higher GSH content than E− plants under high NaCl stress, however, the GSH content of leaves and roots were not significantly different in E+ and E− plants under low NaCl stress (0 and 100 mM) (Supplementary Fig. 2). Furthermore, in E+ and E− plants, our results showed that SOD and CAT activity of the leaves and roots increased as the NaCl concentration inccreased (Fig. 4). Under low NaCl concentration (0 and 100 mM), SOD and CAT activity of leaves and roots were not significantly different in E+ and E− plants; interestingly, E+ plants had a higher activity of SOD and CAT than E− plants under high NaCl stress (200, 300 and 400 mM) (Fig. 4). The SOD activity of leaves of E+ plants was enhanced by 9.3 %, 8.1 % and 6.4 % compared with E− plants under high NaCl stress (200, 300 and 400 mM), respectively (Fig. 4a). Similarly, the SOD activity of E+ roots was enhanced by 8.7 %, 14.4 % and 11.1 % compared with E− plants under high NaCl stress (200, 300 and 400 mM), respectively (Fig. 4b). The CAT activity of E+ leaves was enhanced by 3.9 %, 4.9 % and 10.9 % compared with E− plants under high NaCl stress (200, 300 and 400 mM), respectively (Fig. 4c). Similarly, the CAT activity of roots of E+ plants was enhanced by 10.7 %, 12.4 % and 10.4 % compared with E− plants under high NaCl stress (200, 300 and 400 mM), respectively (Fig. 4d).

Effects of endophyte on the PM NADPH oxidase and PM H^+-ATPase activity under NaCl stress

NaCl stress enhanced PM NADPH oxidase activity in the E+ and E− plants (Fig. 5). The results showed that the presence of endophyte decreased the activity of PM NADPH oxidase compared with E− counterparts under high NaCl stress. The PM NADPH oxidase activity of leaves of E+ plants was decreased by 13.4 %, 7.1 % and 4.8 % compared with E− plants under high NaCl stress (200, 300 and

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

400 mM), respectively (Fig. 5a). The PM NADPH oxidase activity of roots of E+ plantswas decreased by 8.8 %, 10.0 % and 11.9 % compared with E− plants under high NaCl stress (200, 300 and 400 mM), respectively (Fig. 5b). The data indicates that NaCl stress enhanced PM H^+-ATPase activity in the leaves and roots of E+ and E− plants (Fig. 6). The study demonstrated that the presence of the endophyte in *A. inebrians* plants enhanced PM H^+-ATPase activity compared with plants without the endophyte under high NaCl stress (100, 200, 300 and 400 mM). PM H^+-ATPase activity in leaves of E+ plants was increased by 8.5 %, 13.7 %, 9.9 % and 10.2 % compared with those of E− plants under the four NaCl stress (100, 200, 300 and 400 mM), respectively (Fig. 6a). The PM H^+-ATPase activity of roots of E+ plants was enhanced by 43.6 %, 15.2 %, 14.7 % and 17.0 % compare with the roots of E− plants under the four levels of NaCl stress (100, 200, 300 and 400 mM), respectively (Fig. 6b).

Changes in the content of Na^+ and K^+ and Na^+: K^+ of E+ and E− plants under NaCl stress

With the NaCl concentration increasing, Na^+ content exhibited a significant enhancement in the leaves and roots of E+ and E− plants (Fig. 7a and d). However, the presence of the endophyte alleviated this change, and the Na^+ content of the leaves of E+ plants and roots was lower than the leaves and roots of E− plants. With the increasing of the NaCl concentration, K^+ content decreased in roots of E+ and E− plants. But the leaves and roots of E+ plants had significantly higher K^+ content than of the E− counterpart plants (Fig. 7b and e). Further, the data showed that Na^+ content of the leaves of E+ plants was decreased by 38.1 %, 23.7 % and 37.7 % compared with the the leaves of E− plants under high NaCl stress (200, 300 and 400 mM), respectively (Fig. 7a). The Na^+ content of roots of E+ plants was decreased by 10.6 %, 13.3 %, 8.8 % and 30.8 % compared with the roots of E− plants under the all four NaCl concentrations (100, 200, 300 and 400 mM), respectively (Fig. 7d). These data implied that the endophyte had decreased the accumulation of Na^+. Moreover, an enhancing in the Na^+:K^+ ratios of leaves and roots were seen under NaCl stress compared with the control plants. The E+ plants had markedly lower ratios of Na:K compare with the E− plants when exposed to high NaCl stress (200, 300 and 400 mM).

Principal component analysis

The purpose of principal component analysis was to show the relationship among redox related molecules parameters, NaCl and endophyte. Some variables showed distinct separation in leaves, and

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

the factor 1 explained 62.75 % of the total variance, of which Na^+ contributed the most variation (14.95%); further, GSH, $O_2^{\cdot-}$ and PHA (PM H^+-ATPase) accounted for approximately 13.00%, 12.72% and 12.61%, respectively (Fig. 8a). G6PDH (66.12%) and $O_2^{\cdot-}$ (9.10%) were loaded on factor 2, and which could explained 13.06% of the variation. According to the average value of redox related molecules parameters in leaves between E+ and E− plants, the plot case factor coordinates of every NaCl concentration were analyzed. PCA plot showed a significant difference between the leaves of E+ and E− plants under 100, 200, 300 and 400 mM NaCl concentration (Fig. 8b). The factor 1 and factor 2 explained 68.44% and 13.53% of the total variance, respectively. The 0 mM NaCl concentration showed that the highest contribution (23.60%) to factor 1 in the leaves of E− plants, and 200 mM NaCl concentration was significantly loaded (26.14%) on factor 2 in the leaves of E+ plants (Fig. 8b). Furthermore, our data demonstrated that the E+ and E− leaves had no differences under 0 mM NaCl concentration (Fig. 8b).

Similarly, our results showed that, some variables showed obvious separation in roots. Factor 1 explained 64.96% of the total variance, of which Na^+ contributed the highest variation of about 13.95%, with plasma membrane (PM) NADPH oxidase (PNO), $O_2^{\cdot-}$ and GSH being about 13.44%, 11.65% and 11.18%, respectively (Fig. 9a). G6PDH (42.47%) and NADPA/$NADP^+$ (20.87%) were loaded on factor 2, and which explained 16.27% of the variation. Similarly, based on the mean values for the redox related molecules parameters between of roots of E+ and E− plants, the plot case factor coordinates of the NaCl concentration were analyzed. PCA plot showed a good distinction in 100, 200, 300 and 400 mM NaCl concentrations from roots of E+ and E− plants (Fig. 9b). The factor 1 and factor 2 explained 68.44% and 13.53% of the total variance, respectively. The 0 mM NaCl concentration showed the highest contribution (23.60%) to factor 1 in roots of E− plants, and 200 mM NaCl concentration was significantly loaded (26.14%) on factor 2 in roots of E+ plants (Fig. 9b). Furthermore, our results showed that roots of E+ and E− plants had no significant differences under the 0 mM NaCl concentration (Fig. 9b).

DISCUSSION

The study reported here is part of the research that is examining how the presence of *Epichloë* endophytes in *A. inebrians* enhances the ability of this grass to tolerate abiotic stresses that prevail in the arid and semi-arid grasslands of northwest China. Among the abiotic stress conditions where the

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

presence of an *Epichloë* endophyte in *A. inebrians* plants provides a real advantage is high salinity soil. Although previous reports have shown that G6PDH (Wang et al. 2008; Li et al. 2011; Liu et al. 2012), plasma membrane (PM) H^+-ATPase (Li et al. 2011; Bose et al. 2015; Liang et al. 2015; Vitart et al. 2001) and antioxidant enzymes (SOD and CAT) (Zhang et al. 2010; He et al. 2019) play an important role in enhancing plant tolerance to abiotic stresses, our study is the first report that the presence of a fungal endophyte up-regulates their activity, therefore, enhancing plant tolerance to NaCl stress. It is central to the understanding of the relationship between host grasses and *Epichloë* endophytes that the endophyte reprograms the physiology of the plant to adapt to abiotic stresses. A key feature of grass/*Epichloë* associations is that although the endophyte is present in all aboveground tissues, and it is absent in roots (Christensen and Voisey 2007). Importantly, there is strong communication between grass and endophyte, with some secondary metabolism genes being regulated when plant are host to *Epichloë* endophytes (Johnson et al. 2003; Dinkins et al. 2017; Schmid et al. 2017). In this study, the presence of the endophyte up-regulated the G6PDH and (PM) H^+-ATPase activity, including in the roots where hyphae are absent, and so increasing the persistence of grasses when faced with Na^+ toxicity.

In this study, the presence of the fungal endophyte resulted in higher dry weight of leaves and roots under high NaCl stress (200, 300 and 400 mM) compared with E− plants, which was consistent with previous research (Song et al. 2015a; Reza Sabzalian and Mirlohi 2010; Rodriguez et al. 2008). Generally, high Na^+ content is harmful for most plants by negatively affecting membrane stability, enzyme activity and by increasing ROS levels (He et al. 2019). Our results demonstrated that the contents of $O_2^{\cdot-}$ and H_2O_2 in *A. inebrians* leaves and roots increased with increasing NaCl concentrations. However, the presence of the endophyte significantly alleviated the NaCl-induced damage to *A. inebrians* plants as indicated by the reduced content of $O_2^{\cdot-}$ and H_2O_2. Previous research has reported that the endophyte significantly relieved cadmium-induced damage by reducing H_2O_2 content in *A. inebrians* plants (Zhang et al. 2016). Therefore, the reduction of ROS levels resulting from endophyte-infection might be a central physiological mechanism for the enhanced resistance of *A. inebrians* to NaCl stress.

G6PDH plays an important role in the pentose phosphate pathway, and it is the first rate-limiting enzyme, and which provides reducing power (NADPH) and controls the carbon flow, while NADPH maintains the redox state and reductive biosynthesis of cells (Kletzien et al. 1994). In this study, we

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

obtained evidence that the fungal endophyte regulated G6PDH activity in leaves and roots of *A. inebrians* plantsunder NaCl stress. What might be the biological role of *E. gansuensis* regulated G6PDH activity of *A. inebrians* plants under NaCl stress? It has been demonstrated that with *Phragmites communis* swamp reed and *Phragmites communis* dune reed that under long-term NaCl stress, the G6PDH activity was higher in *Phragmites communis* dune reed callus than that of *Phragmites communis* swamp reed callus under 600 mM NaCl stress (Wang et al. 2008). In this study, we got similar results regarding NaCl stress, with an increase in the activity levels of G6PDH with increased NaCl concentrations occurring in E+ plants as occurred with callus of the dune reed ecotype of *P. communis*. But unlike the improvement in G6PDH activity being linked to adaptation of the ecotype to exposure to stress, with *A. inebrians* these results from the presence of the endophyte.

NADPH plays a central function in the regulation of the cellular redox state. It had been reported that a high ratio of NADPH/$NADP^+$ could inhibit maize photosynthesis (Tsuchida et al. 2001). Our results indicated that the presence of the endophyte decreased the NADPH/$NADP^+$ ratio and NADPH content of leaves and roots, compared with *A. inebrians* plants without the endophyte. NADPH is oxidized by PM NADPH oxidase to transfer an electron to O_2 to form $O_2^{\cdot-}$, and further, SOD catalyzes $O_2^{\cdot-}$ and transform it to H_2O_2 (Van Gestelen et al. 1997). Under NaCl stress, our results demonstrated that the endophyte markedly decreased the PM NADPH oxidase activity of *A. inebrians* plants. In addition, a recent study suggested that NaCl stress enhanced the activity of SOD and CAT (He et al. 2019). Our results have shown that NaCl stress increased SOD and CAT activity, and importantly, the presence of *E. gansuensis* also enhanced SOD and CAT activity under NaCl stress. Together with the $O_2^{\cdot-}$ and H_2O_2 results, we propose that the PM NADPH oxidase plays a central function in this stress-reducing process. Under NaCl stress, studies suggested that NADPH oxidase regulated Na^+/ K^+ homeostasis of *Arabidopsis* in ROS-dependent (Ma et al. 2012). Another study demonstrated that reduced glutathione played a key role for increasing resistance and serves as the redox molecule for scavenging ROS under environmental stresses (Wang et al. 2008). Reduction of ROS may protect the stability and activity of other metabolic enzymes; for example; our previous study showed that the presence of the endophyte increased nitrate reductase (NR), nitrite reductase (NiR), and glutamine synthetase (GS) activity of *A. inebrians* under NaCl stress. In addition, the presence of the endophyte increases nitrogen use efficiency (NUE), providing sufficient nitrogen for plant growth under NaCl stress (Wang et al. 2019), which make E+ plants have higher biomass compared with E− plants.

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

G6PDH plays an important role to relieve the damage of oxidative stress by enhancing GSH content, and also GSH reduces ROS content (Wang et al. 2008). In addition, for the regeneration of GSH, NADPH is the main reducing potential. Compared to E− plants, E+ plants had significantly higher GSH content when at higher NaCl concentrations. Our results show that the role of *E. gansuensis* in the adaptation of *A. inebrians* to NaCl stress is through the regulation of G6PDH activity to maintain the intracellular content of GSH, and so decrease ROS content. G6PDH is the first and rate-limiting enzyme of the pentose phosphate pathway, which generates NADPH to maintain redox state equilibrium. The primary role in the regulation of G6PDH is played by the NADPH/$NADP^+$ ratio, and when the ratio is high the G6PDH activity decreases (Esposito et al. 2005). Our results showed that endophyte decreased the NADPH/$NADP^+$ ratio under NaCl stress; therefore, the presence of the endophyte increase G6PDH activity through regulating the cellular redox status and the NADPH/$NADP^+$ ratio under NaCl stress. In *Nicotiana tabacum*, G6PDH activity was induced by infection with *Phytophthora nicotianae* (Scharte et al. 2009). G6PDH activity is modulated by the protein kinase ASKa in *Arabidopsis* under salinity stress, and ASKa phosphorylates G6PD6 on a conserved Thr residue, therefore enhancing G6PDH activity and salt stress tolerance (Dal Santo et al., 2012). Our results also showed that the endophyte increases G6PDH activity of *A. inebrians* under high salinity conditions, increasing G6PDH activity probably through enhancing the G6PDH phosphorylation level. One study revealed that G6PDH activity in G6PDH loss-of-function mutants (*g6pd5*) was much lower than that in wild type (Col-0) seedlings, especially in *g6pd5* and *g6pd5/6* mutant seedlings under NaCl stress (Yang et al. 2009). Probabaly, the presence of the endophyte increased G6PDH activity in *A. inebrians* plants through enhancing the expression of *G6PDH* genes. In summary, G6PDH activity was increased by the presence of *E. gansuensis* through regulating the cellular redox status and the NADPH/NADP+ ratio, probably through regulating protein phosphorylation and the expression of *G6PDH* genes under NaCl stress.

The previous study demonstrated that PM H^+-ATPase plays a very central role in plant resistance to NaCl stress (Bose et al. 2015). Increasing activity of the PM H^+-ATPase had been showed to accelerate Na^+ efflux, therefore, alleviating Na^+ toxicity to plants (Li et al. 2011; Zhao et al. 2004). The presence of endophyte increased the activity of the PM H^+-ATPase compared with E− plants under NaCl stress. Meanwhile, E+ *A. inebrians* plants had lower Na^+ content and higher K^+ content than E− *A. inebrians* plants. These above results confirmed that *E. gansuensis* modulates the PM H^+-ATPase

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

activity of *A. inebrians* plants providing adaptation to NaCl stress. Therefore, the PM H^+-ATPase plays a very central function for maintaining the Na^+/K^+ balance in plants. As our results have shown that the endophyte significantly decreased Na^+ content and increased K^+ content in the leaves and roots of *A. inebrians* plants. With other plant growth-promoting fungi, including *Piriformospora indica*, arbuscular mycorrhizal fungi, and *Trichoderma* sp. activation of PM H^+-ATPase activity is an important mechanism of growth promotion (Felle et al. 2009; López-Coria et al. 2016; Krajinski et al. 2014; Wang et al. 2014). *Rhynchosporium secalis* activated PM H^+-ATPase of barley through secreting NIP1 and NIP3 protein to promote plant growth (Wevelsiep et al., 1993). Similarly, the fungal toxin fusicoccin, which is secreted by *Fusicoccum amygdali*, activates PM H^+-ATPase by stabilizing the association of the 14-3-3 protein (Falhof et al. 2016; Johansson et al. 1993; Korthout and de Boer 1994). A further other study showed that *Brevibacterium* RS16 improved salt tolerance to *Oryza sativa* by increasing H^+-ATPase activity (Chatterjee et al. 2018). The dark septate endophytic fungus A103 increased PM H^+-ATPase activity by enhancing the expression of the PM H^+-ATPase gene to promote plant growth (Vergara et al. 2019). The PM H^+-ATPase are the important pumps responsible for the establishment of cellular membrane potential in plants, which creates a proton electrochemical gradient across the membrane that is utilized by carrier proteins and channels to facilitate ion exchange through the plasma membrane (Sondergaard et al. 2004). Our results confirmed that *E. gansuensis* increases PM H^+-ATPase activity to provide energy for promoting Na^+/H^+ exchange, which provides adaptation to NaCl stress. In summary, PM H^+-ATPase activity was increased by the presence of endophyte, probably through secreting proteins, stabilizing the interaction of protein and the expression of PM H^+-ATPase genes. The restricting of Na^+ transport and ensuring a low Na^+/K^+ ratio are key mechanisms in which plants could positively respond to NaCl stress (Vitart et al. 2001). Moreover, increasing the K^+ content is related to mechanisms that enhance salt tolerance, which could decrease the level of toxic ions under NaCl stress. Another study has shown that some root-colonizing fungi can improve host growth under environment stress (Hacquard et al. 2016). Therefore, endophytic fungi have a powerful biological function to help plants adapt to various environments stresses. Based on our present research results and those in previous studies, we propose a model to indicate the key role of the endophyte is to mediate the activity of PM H^+-ATPase, G6PDH, SOD and CAT in host *A. inebrians* plants in order to relieve NaCl toxicity (Fig. 10).

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

CONCLUSIONS

In summary, our study provides a new thinking to understand how the presence of *E. gansuensis* regulated G6PDH activity and PM H^+-ATPase activity in leaves and roots of *A. inebrians* plants under NaCl stress. G6PDH oxidizes $NADP^+$ to produce NADPH and this is then oxidized to produce ROS by NADPH oxidase. NADPH is the main reducing power for GSH regeneration, which eliminates excess ROS levels. In addition, the presence of *E. gansuensis* increased SOD and CAT activity, which could scavenge excess ROS levels. During this process, the endophyte could increase the activity of PM H^+-ATPase in the host grass to decrease Na^+ content, and alleviate the toxicity. This understanding of the role of how this *Epichlo ë* endophyte enhances salt tolerance of its host grass provides a way for the breeding of new salt-tolerant forage germplasm for use in saline soils.

MATERIALS AND METHODS

Plant growth condition

Epichlo ë gansuensis-infected (E+) and *Epichlo ë gansuensis*-uninfected (E−) *Achnatherum inebrians* plants were established in an experimental field of Lanzhou University in 2013. *Epichlo ë gansuensis* was isolated and identified from *Achnatherum inebrians* by Chunjie Li, which is from our team (Li et al 2004; Chen et al. 2015). The seed used to establish these plants originated from a single E− plant and a single E+ to reduce variability within our plant material at the start of the study. In 2015, E− seeds were harvested from 36 E− *A. inebrians* plants, and E+ seeds were harvested from 36 E+ *A. inebrians* plants, bulked as E− or E+ seeds, and stored at a constant temperature of 4 ℃ for the present study. E+ and E− seeds were sown in 60 pots [lower diameter (10 cm) × upper diameter (18 cm) × height (19 cm)], respectively, and each pot had 6 seeds for each of the 60 E+ and E− pots, respectively. Eeach pot was filled with sterilized vermiculite (150 ^{o}C for 3 h). A pot experiment was performed in the greenhouse of the College of Pastoral Agriculture Science and Pastoral Agriculture Science and Technology, Yuzhong campus of Lanzhou University (35°89′ N, 104°39′ E, Altitude1653 m). One week after germination, three seedlings of the same growth state were kept in each pot, and six pots were in each of 20 trays. Every seven days, trays were watered with distilled water. Ten days after germination, 1/2 strength Hoagland solution was applied to the each pot every seven days. All trays were assigned at random to a position in the greenhouse (moisture: 42 ±2%, temperature: 26 ±2 ^{o}C). After 45 days, we used the aniline blue stain method to check the infection status of each seedling by microscopic

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

examination of leaf sheath pieces (Li 2005). E+ and E− plants were treated for 28 days with modified 1/2-strength Hoagland solution supplemented with NaCl to make up final concentrations of 0, 100, 200, 300 and 400 mM NaCl. Every seven days, 150 ml 1/2-strength Hoagland solution containing the five NaCl concentrations was applied to the corresponding pots of E+ and E− *A. inebrians* seedlings. In addition, 2.4 L distilled water was applied to each tray every 7 days. After 28 days with the various NaCl concentration treatment, physiological parameters, the dry weight of leaves and roots were measured.

The dry weight of leaves and roots

After the NaCl concentrations treatment for 4 weeks, the root and leaf laminas (no including pseudostems) were separated. The root and leaf laminas were dried at 80 ℃, to a constant weight to determine the dry weight. To determine the enzymatic activity, the leaves and roots of the remaining E+ and E− plants were frozen with liquid nitrogen and immediately stored at −80 °C

Determination of $O_2^{\cdot-}$, H_2O_2 and glutathione (GSH) content

The content of $O_2^{\cdot-}$ was determined according to the method described by Elstner and Heupel (1976). H_2O_2 content was determined according to the method described by Velikova et al (2000). The content of glutathione (GSH) was assayed according to a described method (Wang et al. 2018b).

Determination of superoxide dismutase **(SOD),** Catalase **(CAT) and** Glucose-6-phosphate dehydrogenase **(G6PDH) activity**

Antioxidant enzymes were extracted using the method described by He et al (2019). The activity of SOD was determined according to the method described by Prochazkova et al (2001). The CAT activity was determined according to the method described by Abdel Latef and Tran (2016). The extraction and measurement of G6PDH was assayed according to the method described by Hauschild and von Schaewen (2003).

Assays of $NADP^+$, NADPH and NADPH/$NADP^+$

The content of $NADP^+$ and NADPH was determined using the method described by Matsumura and Miyachi (1980). The ratio of NADPH/$NADP^+$ is based on the content of NADPH and $NADP^+$.

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

Assays of the activity of PM NADPH oxidase and PM H^+-ATPase

The plasma membrane was isolated according to the method described by Qiu and Su (1999). Further, the microsomal pellets were used to determine the activity of PM NADPH oxidase and PM H^+-ATPase. The activity of PM NADPH oxidase was determined using the method described by Duan et al (2009), and PM H^+-ATPase activity was measured using the method described by Liang et al (2015). The contents of protein was measured using the method described by Bradford (1976).

Determination of Na^+ and K^+ content

For measuring the content of Na^+ and K^+ in leaves and roots, the method was used as described by Bao et al (2016).

Statistical analysis

Data analyses were carried out with SPSS version 17.0. The significance of differences of all the parameters between E+ and E− *A. inebrians* plants was performed by independent T-tests. The significance difference was at $P<0.05$. Means are represented with standard errors. Additionally, principal component analysis (PCA) was carried out with Statistic 6.0 (USA) to assess relationships among the parameters of stresses, as well as between NaCl/endophyte and these parameters. Each parameters was performed using independent T-tests with three independent biological replicates.

Acknowledgments

This research was financially supported by National Basic Research Program of China (2014CB138702), the Joint Fund of the Natural Science Foundation of China and the Karst Science Research Center of Guizhou Province (Grant No. U1812401), Changjiang Scholars and innovative Research Team in University (IRT_17R50), Lanzhou University “Double First-Class” guiding special project-team construction fund-scientific research start-up fee standard (561119206), the Natural Science Foundation of China (31901378), Guizhou education department program (Qianjiaohe-KY-2018-130), Major science and technology sub-project of Guizhou science and technology program (Qiankehe-2019-3001-2).

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

Competing interests The author(s) declare that they have no conflict of interest.

References

Abdel Latef AA, Tran L-SP (2016) Impacts of priming with silicon on the growth and tolerance of maize plants to alkaline stress. Front Plant Sci 7: 243.

Bao AK, Du BQ, Touil L, Kang P, Wang QL, Wang SM. (2016) Co-expression of tonoplast Cation/H^+ antiporter and H^+-pyrophosphatase from xerophyte *Zygophyllum xanthoxylum* improves alfalfa plant growth under salinity, drought and field conditions. Plant Biotechnol J 14(3): 964-975.

Baslam M, Garmendia I, Goicoechea N (2011) Arbuscular mycorrhizal fungi (AMF) improved growth and nutritional quality of greenhouse-grown lettuce. J Agr Food Chem 59: 5504-5515.

Benizri E, Baudoin E, Guckert A (2001) Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Science Techn 11: 557-574.

Bose J, Rodrigo-Moreno A, Lai D, Xie Y, Shen W, Shabala S. (2015) Rapid regulation of the plasma membrane H+-ATPase activity is essential to salinity tolerance in two halophyte species, *Atriplex lentiformis* and *Chenopodium quinoa*. Ann Bot 115(3): 481-494.

Boubakri H, Wahab MA, Chong J, Gertz C, Gandoura S, Mliki A, Bertsch C, Soustre-Gacougnolle I. (2013) Methionine elicits H_2O_2 generation and defense gene expression in grapevine and reduces Plasmopara viticola infection. J Plant Physiol 170(18): 1561-1568.

Bradford MM. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1-2): 248-254.

Cardi M, Chibani K, Cafasso D, Rouhier N, Jacquot J-P, Esposito S (2011) Abscisic acid effects on activity and expression of barley (*Hordeum vulgare*) plastidial glucose-6-phosphate dehydrogenase. J Exp Bot 62: 4013-4023.

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am Nat 160: S99-S127.

Chatterjee P, Samaddar S, Niinemets Ü, Sa T-M (2018) *Brevibacterium* linens RS16 confers salt tolerance to *Oryza sativa* genotypes by regulating antioxidant defense and H^+-ATPase activity. Microbiol Res 215: 89-101.

Chen L, Li X, Li C, Swoboda GA, Young CA, Sugawara K, Leuchtmann A, Schardl CL (2015) Two distinct *Epichloë* species symbiotic with *Achnatherum inebrians*, drunken horse grass. Mycologia 107: 863-873

Chen J, Li Y, Wen S, Rosanoff, A, Yang, G, Sun X. (2017) Magnesium fertilizer-induced increase of symbiotic microorganisms improves forage growth and quality. J Agr Food Chem, 65: 3253-3258.

Chen N, He R, Chai Q, Li C, Nan Z (2016) Transcriptomic analyses giving insights into molecular regulation mechanisms involved in cold tolerance by *Epichloë* endophyte in seed germination of *Achnatherum inebrians*. Plant Growth Regul 80: 367-375.

Christensen MJ, Bennett RJ, Ansari HA, Koga H, Johnson RD, Bryan GT, Simpson WR, Koolaard JP, Nickless EM, Voisey CR. (2008) *Epichloë* endophytes grow by intercalary hyphal extension in elongating grass leaves. Fungal Genet Biol 45(2): 84-93.

Christensen M, Voisey C (2007) The biology of the endophyte/grass partnership. Proceedings of the 6th international symposium on fungal endophytes of Grasses. New Zealand Grassland

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

Association Christchurch.

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42: 669-678.

Dal Santo S, Stampfl H, Krasensky J, Kempa S, Gibon Y, Petutschnig E, Rozhon W, Heuck A, Clausen T, Jonak C (2012) Stress-induced GSK3 regulates the redox stress response by phosphorylating glucose-6-phosphate dehydrogenase in *Arabidopsis*. Plant Cell 24: 3380-3392.

Dinkins RD, Nagabhyru P, Graham MA, Boykin D, Schardl CL (2017) Transcriptome response of *Lolium arundinaceum* to its fungal endophyte *Epichloë coenophiala*. New Phytol 213: 324-337.

Duan ZQ, Bai L, Zhao ZG, Zhang GP, Cheng FM, Jiang LX, Chen KM. (2009) Drought-stimulated activity of plasma membrane nicotinamide adenine dinucleotide phosphate oxidase and its catalytic properties in rice. Jf Int Plant Biol 51(12): 1104-1115.

Elstner EF, Heupel A (1976) Inhibition of nitrite formation from hydroxylammoniumchloride: a simple assay for superoxide dismutase. Anal Biochem 70: 616-620.

Esposito S, Guerriero G, Vona V, Di Martino Rigano V, Carfagna S, Rigano C (2005) Glutamate synthase activities and protein changes in relation to nitrogen nutrition in barley: the dependence on different plastidic glucose-6P dehydrogenase isoforms. J Exp Bot 56: 55-64.

Fao U. (2009) How to feed the world in 2050. Rome: High-Level Expert Forum.

Falhof J, Pedersen JT, Fuglsang AT, Palmgren M (2016) Plasma membrane H^+-ATPase regulation in the center of plant physiology. Mol Plant 9: 323-337.

Felle HH, Waller F, Molitor A, Kogel K-H (2009) The mycorrhiza fungus *Piriformospora indica* induces fast root-surface pH signaling and primes systemic alkalinization of the leaf apoplast upon powdery mildew infection. Mol Plant Microbe In 22: 1179-1185.

Foyer CH, Halliwell B. (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133(1): 21-25.

Foyer CH, Noctor G. (2009) Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid Redox Sign 11(4): 861-905.

Hacquard S, Kracher B, Hiruma K, Münch PC, Garrido-Oter R, Thon MR, Weimann A, Damm U, Dallery J-F, Hainaut M. (2016) Survival trade-offs in plant roots during colonization by closely related beneficial and pathogenic fungi. Nat Commun 7.

Hauschild R, von Schaewen A. (2003) Differential regulation of glucose-6-phosphate dehydrogenase isoenzyme activities in potato. Plant Physiol 133(1): 47-62.

Huan L, Xie X, Zheng Z, Sun F, Wu S, Li M, Gao S, Gu W, Wang G (2014) Positive correlation between PSI response and oxidative pentose phosphate pathway activity during salt stress in an intertidal macroalga. Plant Cell Physiol 55: 1395-1403.

He Q, Wang X, He L, Yang L, Wang S, Bi Y (2019) Alternative respiration pathway is involved in the response of highland barley to salt stress. Plant Cell Reports 38: 295-309.

James RA, Blake C, Byrt CS, Munns R. (2011) Major genes for Na^+ exclusion, *Nax1* and *Nax2* (wheat *HKT1*; 4 and *HKT1; 5*), decrease Na^+ accumulation in bread wheat leaves under saline and waterlogged conditions. J Exp Bot 62(8): 2939-2947.

Jiang YP, Cheng F, Zhou YH, Xia XJ, Mao WH, Shi K, Chen Z, Yu JQ. (2012) Cellular glutathione redox homeostasis plays an important role in the brassinosteroid-induced increase in CO_2

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

assimilation in *Cucumis sativus*. New Phytol 194(4): 932-943.

Johnson LJ, de Bonth AC, Briggs LR, Caradus JR, Finch SC, Fleetwood DJ, Fletcher LR, Hume DE, Johnson RD, Popay AJ (2013) The exploitation of epichloae endophytes for agricultural benefit. Fungal Divers 60: 171-188.

Johansson F, Sommarin M, Larsson C (1993) Fusicoccin activates the plasma membrane H^+-ATPase by a mechanism involving the C-terminal inhibitory domain. Plant Cell 5: 321-327.

Johnson LJ, Johnson RD, Schardl CL, Panaccione DG. (2003) Identification of differentially expressed genes in the mutualistic association of tall fescue with *Neotyphodium coenophialum*. Physiol Mol Plant P 63(6): 305-317.

Kannadan S, Rudgers J (2008) Endophyte symbiosis benefits a rare grass under low water availability. Funct Ecol 22: 706-713.

Kletzien R, Harris P, Foellmi L. (1994) Glucose-6-phosphate dehydrogenase: a" housekeeping" enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. The FASEB J 8(2): 174-181.

Korthout H, de Boer AH (1994) A fusicoccin binding protein belongs to the family of 14-3-3 brain protein homologs. Plant Cell 6: 1681-1692.

Li CJ. (2005) Biological and ecological characteristics of *Achnatherum Inbrians*/*Neotyphodiun* endophyte symbiont. Lanzhou University, China. PhD dissertation.

Li C, Nan Z, Paul VH, Dapprich PD, Liu Y (2004) A new *Neotyphodium* species symbiotic with drunken horse grass (*Achnatherum inebrians*) in China. Mycotaxon 90: 141-147.

Li J, Chen G, Wang X, Zhang Y, Jia H, Bi Y. (2011) Glucose-6-phosphate dehydrogenase-dependent hydrogen peroxide production is involved in the regulation of plasma membrane H^+-ATPase and Na^+/H^+ antiporter protein in salt-stressed callus from *Carex moorcroftii*. Physiol Plantarum 141(3): 239-250.

Liang C, Ge Y, Su L, Bu J. (2015) Response of plasma membrane H^+-ATPase in rice (*Oryza sativa*) seedlings to simulated acid rain. Environ Sci Pollut R 22(1): 535-545.

Liu Y, Wan Q, Wu R, Wang X, Wang H, Wang Z, Shi C, Bi Y. (2012) Role of hydrogen peroxide in regulating glucose-6-phosphate dehydrogenase activity under salt stress. Biol Plantarum 56(2): 313-320.

López-Coria M, Hernández-Mendoza J, Sánchez-Nieto S (2016) Trichoderma asperellum induces maize seedling growth by activating the plasma membrane H^+-ATPase. Mol Plant Microbe In 29: 797-806.

Ma L, Zhang H, Sun L, Jiao Y, Zhang G, Miao C, Hao F. (2012) NADPH oxidase *AtrbohD* and *AtrbohF* function in ROS-dependent regulation of Na^+/K^+ homeostasis in *Arabidopsis* under salt stress. J Exp Bot 63(1): 305-317.

Matsumura H, Miyachi S. (1980) [43] Cycling assay for nicotinamide adenine dinucleotides. Method Enzymol 69: 465-470.

Munns R, Tester M. (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol. 59: 651-681.

Müller C B, Krauss J. (2005) Symbiosis between grasses and asexual fungal endophytes.Curr Opin Plant Biol 8: 450-456.

Noctor G, Foyer CH. (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Biol 49(1): 249-279.

Prochazkova D, Sairam R, Srivastava G, Singh D (2001) Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Sci 161: 765-771.

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

Qiu Q-S, Su X-F. (1999) The influence of extracellular-side Ca^{2+} on the activity of the plasma membrane H^+-ATPase from wheat roots. Funct Plant Biol 25(8): 923-928.

Rausch T, Kirsch M, Löw R, Lehr A, Viereck R, Zhigang A. (1996) Salt stress responses of higher plants: the role of proton pumps and Na^+/H^+-antiporters. J Plant Physiol 148(3-4): 425-433.

Reza Sabzalian M, Mirlohi A. (2010) *Neotyphodium* endophytes trigger salt resistance in tall and meadow fescues. J Plant Nutr Soil Sci 173(6): 952-957.

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y-O, Redman RS. (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2(4): 404-416.

Scharte J, Schön H, Tjaden Z, Weis E, von Schaewen A (2009) Isoenzyme replacement of glucose-6-phosphate dehydrogenase in the cytosol improves stress tolerance in plants. P Natl Acad Sci USA 106: 8061-8066.

Schmid J, Day R, Zhang N, Dupont P-Y, Cox MP, Schardl CL, Minards N, Truglio M, Moore N, Harris DR (2017) Host tissue environment directs activities of an *Epichloë* endophyte, while it induces systemic hormone and defense responses in its native perennial ryegrass host. Mol Plant Microbe In 30: 138-149.

Sergio L, De Paola A, Cantore V, Pieralice M, Cascarano NA, Bianco VV, Di Venere D (2012) Effect of salt stress on growth parameters, enzymatic antioxidant system, and lipid peroxidation in wild chicory (*Cichorium intybus* L.). Acta physiologiae plantarum 34: 2349-2358.

Sondergaard TE, Schulz A, Palmgren MG (2004) Energization of transport processes in plants. Roles of the plasma membrane H^+-ATPase. Plant Physiol 136: 2475-2482.

Song M, Chai Q, Li X, Yao X, Li C, Christensen MJ, Nan Z. (2015a) An asexual *Epichloë* endophyte modifies the nutrient stoichiometry of wild barley (*Hordeum brevisubulatum*) under salt stress. Plant Soil 387(1-2): 153-165.

Tian P, Nan Z (2019) *Epichloë* festucae var. lolii endophyte affects host response to fungal disease progression in perennial ryegrass (*Lolium perenne*). Sci China Life Sci 62(9): 1264-1265.

Thannickal VJ, Fanburg BL. (2000) Reactive oxygen species in cell signaling. Am J Physiol-Lung C 279(6): L1005-L1028.

Tsuchida H, Tamai T, Fukayama H, Agarie S, Nomura M, Onodera H, Ono K, Nishizawa Y, Lee B-H, Hirose S. (2001) High level expression of C4-specific NADP-malic enzyme in leaves and impairment of photoautotrophic growth in a C3 plant, rice. Plant Cell Physiol 42(2): 138-145.

Valderrama R, Corpas FJ, Carreras A, GÓMEZ-RODRÍGUEZ MV, Chaki M, Pedrajas JR, FERNÁNDEZ-OCAÑA A, DEL RÍO LA, Barroso JB. (2006) The dehydrogenase-mediated recycling of NADPH is a key antioxidant system against salt-induced oxidative stress in olive plants. Plant Cell Environ 29(7): 1449-1459.

Van Gestelen P, Asard H, Caubergs RJ. (1997) Solubilization and separation of a plant plasma membrane NADPH-O_2-Synthase from Other NAD(P)H oxidoreductases. Plant Physiol 115(2): 543-550.

Velikova V, Yordanov I, Edreva A. (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151(1): 59-66.

Vergara C, Araujo KEC, Sperandio MVL, Santos LA, Urquiaga S, Zilli JÉ (2019) Dark septate endophytic fungi increase the activity of proton pumps, efficiency of ^{15}N recovery from ammonium sulphate, N content, and micronutrient levels in rice plants. Braz J Microbiol 50: 825-838.

Vitart V, Baxter I, Doerner P, Harper JF. (2001) Evidence for a role in growth and salt resistance of a

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

plasma membrane H^+-ATPase in the root endodermis. Plant J 27(3): 191-201.

Wang E, Yu N, Bano SA, Liu C, Miller AJ, Cousins D, Zhang X, Ratet P, Tadege M, Mysore KS (2014) A H^+-ATPase that energizes nutrient uptake during mycorrhizal symbioses in rice and Medicago truncatula. Plant Cell 26: 1818-1830.

Wang J, Nan Z, Christensen MJ, Zhang X, Tian P, Zhang Z, Niu X, Gao P, Chen T, Ma L (2018a) Effect of *Epichlo ë gansuensis* endophyte on the nitrogen metabolism, nitrogen use efficiency, and stoichiometry of *Achnatherum inebrians* under nitrogen limitation. J Agr Food Chem 66: 4022-4031.

Wang J, Nan Z, Christensen MJ, Li C (2018b) Glucose-6-phosphate dehydrogenase plays a vital role in *Achnatherum inebrians* plants host to *Epichlo ë gansuensis* by improving growth under nitrogen deficiency. Plant and Soil 430: 37-48.

Wang J, Tian P, Christensen MJ, Zhang X, Li C, Nan Z (2019) Effect of *Epichlo ë gansuensis* endophyte on the activity of enzymes of nitrogen metabolism, nitrogen use efficiency and photosynthetic ability of *Achnatherum inebrians* under various NaCl concentrations. Plant Soil 435: 57-68.

Wang X, Ma Y, Huang C, Wan Q, Li N, Bi Y. (2008) Glucose-6-phosphate dehydrogenase plays a central role in modulating reduced glutathione levels in reed callus under salt stress. Planta 227(3): 611-623.

Wang Y, Yang X, Zhang X, Dong L, Zhang J, Wei Y, Feng Y, Lu L. (2014) Improved plant growth and Zn accumulation in grains of Rice (*Oryza sativa* L.) by inoculation of endophytic microbes isolated from a Zn hyperaccumulator, *Sedum alfredii* H. J Agr Food Chem, 62: 1783-1791.

Xia C, Christensen MJ, Zhang X, Nan Z (2018) Effect of *Epichlo ë gansuensis* endophyte and transgenerational effects on the water use efficiency, nutrient and biomass accumulation of *Achnatherum inebrians* under soil water deficit. Plant Soil 424: 555-571.

Xia C, Li N, Zhang X, Feng Y, Christensen MJ, Nan Z (2016) An *Epichlo ë* endophyte improves photosynthetic ability and dry matter production of its host *Achnatherum inebrians* infected by *Blumeria graminis* under various soil water conditions. Fungal Ecol 22: 26-34.

Xia C, Zhang X, Christensen MJ, Nan Z, Li C (2015) *Epichlo ë* endophyte affects the ability of powdery mildew (*Blumeria graminis*) to colonise drunken horse grass (*Achnatherum inebrians*). Fungal Ecol 16: 26-33.

Yang L, Wang X, Chang N, Nan W, Wang S, Ruan M, Sun L, Li S, Bi Y (2019) Cytosolic glucose-6-phosphate dehydrogenase is involved in seed germination and root growth under salinity in *Arabidopsis*. Front Plant Sci 10: 182.

Yang T, Zhang L, Hao H, Zhang P, Zhu H, Cheng W, Wang Y, Wang X, Wang C (2015) Nuclear-localized At HSPR links abscisic acid-dependent salt tolerance and antioxidant defense in *Arabidopsis*. Plant J 84: 1274-1294.

Zhang W, Card SD, Mace WJ, Christensen MJ, McGill CR, Matthew C. (2017) Defining the pathways of symbiotic *Epichlo ë* colonization in grass embryos with confocal microscopy. Mycologia 109(1): 153-161.

Zhang X, Li C, Nan Z. (2010) Effects of cadmium stress on growth and anti-oxidative systems in *Achnatherum inebrians* symbiotic with *Neotyphodium* gansuense. J Hazard Mater 175(1): 703-709.

Zhao L, Zhang F, Guo J, Yang Y, Li B, Zhang L. (2004) Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. Plant Physiol 134(2): 849-857.

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

FIGURES AND LEGENDS

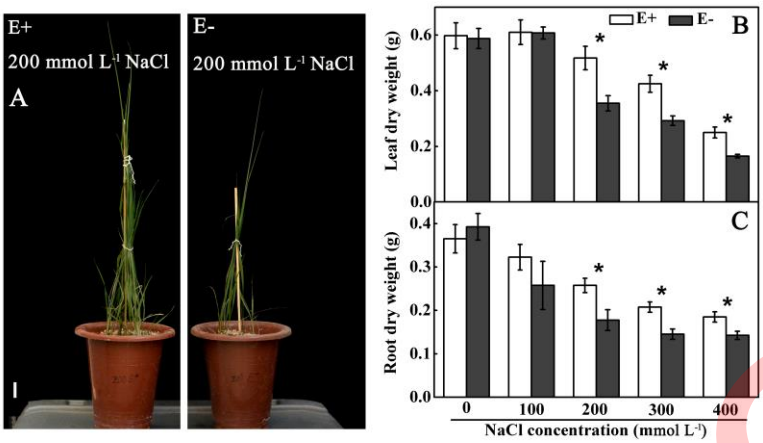

Fig. 1. Effects of endophyte on the dry weight of *A. inebrians* under NaCl stress. (a) E+ and E− *Achnatherum inebrians* plants grown in exposure to 200 mM NaCl. (b) Leaf dry weight. (c) Root dry weight. Data are the means ± standard error (SE). Asterisk indicate significant difference at $P<0.05$ between E+ and E− *A. inebrians*.

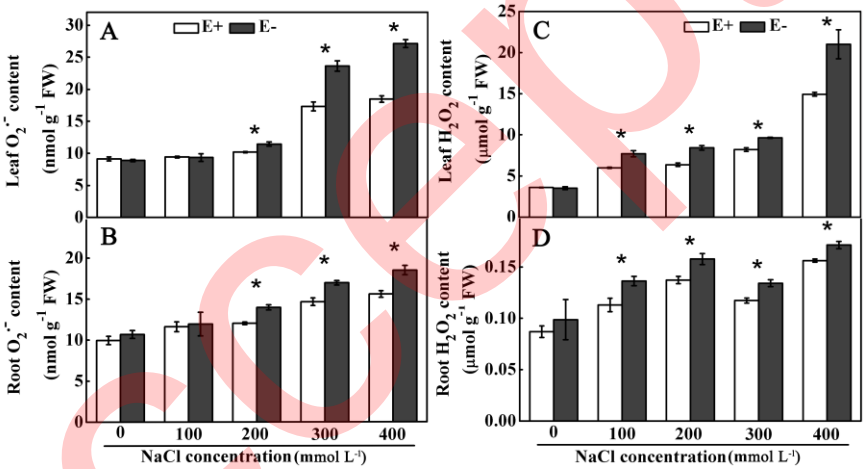

Fig. 2. Effects of endophyte on $O_2^{\cdot-}$ and H_2O_2 contents of *A. inebrians* plants under the various NaCl concentrations. (a) $O_2^{\cdot-}$ content in leaves. (b) $O_2^{\cdot-}$ content in roots. (c) H_2O_2 content in leaves. (d) H_2O_2 content in roots. Data are the means ± standard error (SE). Asterisk indicate significant difference at $P<0.05$ (independent T-tests) between E+ and E− *A. inebrians* plants under the same stress conditions.

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

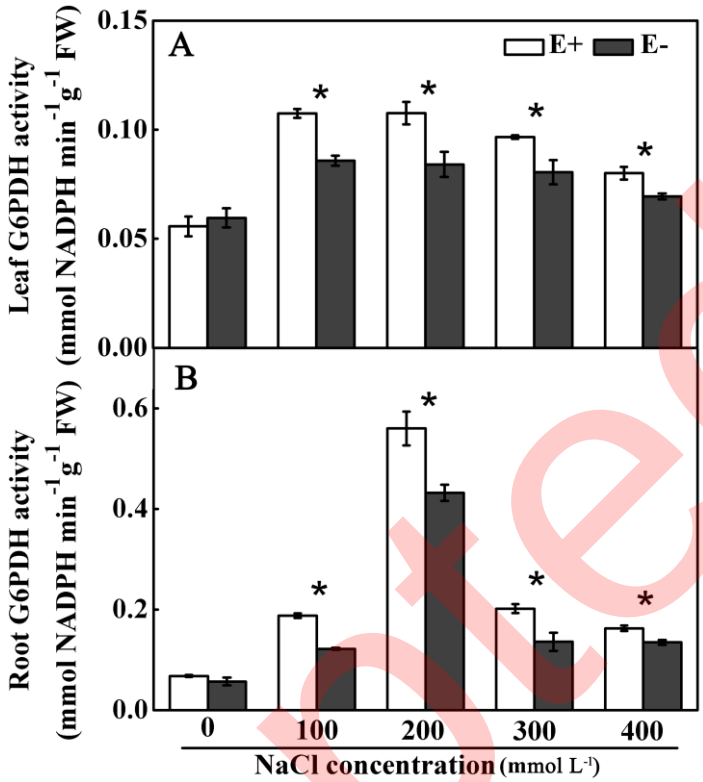

Fig. 3. Effects of endophyte on *A. inebrians* G6PDH activity under NaCl stress. (a) The activity of G6PDH in leaves. (b) The activity of G6PDH in roots. Data are the means ± stardard error (SE). Asterisk indicate significant difference at $P<0.05$ (independent T-tests) between E+ and E− *A. inebrians* plants under the same stress conditions.

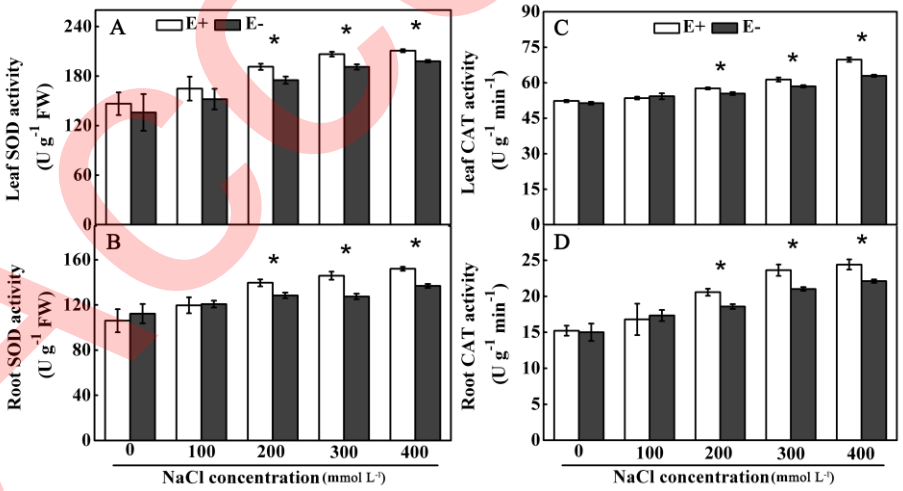

Fig. 4. Effects of endophyte on the activity of SOD and CAT in *A. inebrians* plants under NaCl stress. (a) The activity of SOD in leaves. (b) The activity of SOD in roots. (c) The activity of CAT in leaves. (d) The activity of CAT in roots. Data are the means ± standard error (SE). Asterisk indicate significant difference at $P<0.05$ (independent T-tests) between E+ and E− *A. inebrians* plants under the same stress conditions.

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

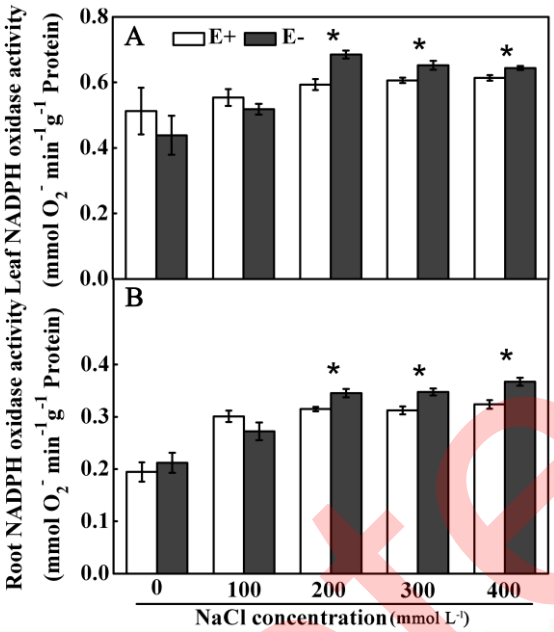

Fig. 5. Effects of endophyte on the PM NADPH oxidase activities of *A. inebrians* plants under NaCl stress. (a) The PM NADPH oxidase activity in leaves. (b) The PM NADPH oxidase activity in roots. Data are the means ± standard error (SE). Asterisk indicate significant difference at $P<0.05$ (independent T-tests) between E+ and E− *A. inebrians* plants under the same stress conditions.

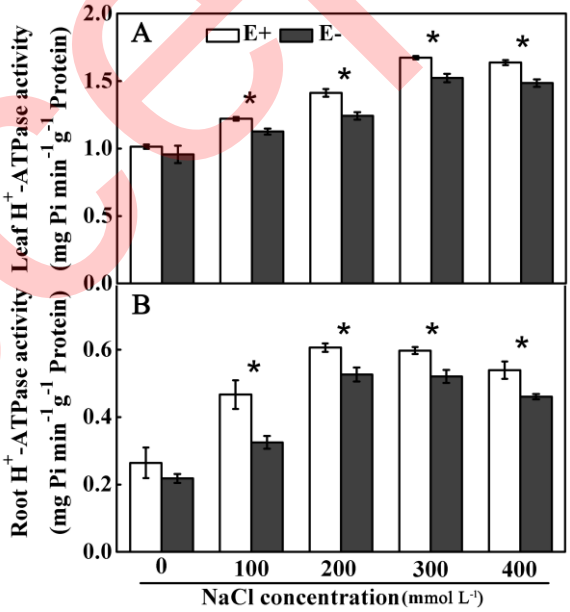

Fig. 6. Effects of endophyte on the PM H^+-ATPase activity of *A. inebrians* plants under NaCl stress. (a) The PM H^+-ATPase activity in leaves. (b) The PM H^+-ATPase activities in roots. Data are the means ± standard error (SE). Asterisk indicate significant difference at $P<0.05$ (independent T-tests) between E+ and E− *A. inebrians* plants under the same stress conditions.

Accepted

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

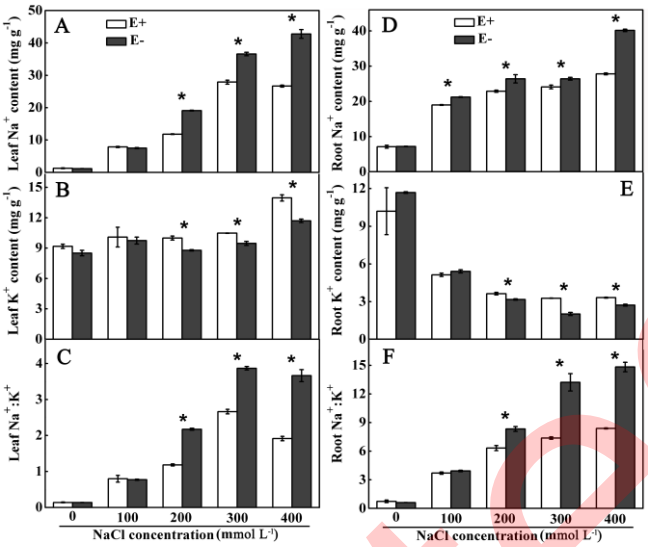

Fig. 7. Effects of endophyte on Na^+ and K^+ content, and Na^+:K^+ ratios of *A. inebrians* plants under NaCl stress. (a) Na^+ content in leaves. (b) K^+ content in leaves. (c) Na^+:K^+ ratio in leaves. (d) Na^+ content in roots. (e) K^+ content in roots. (f) Na^+:K^+ ratio in roots. Data are the means ± standard error (SE). Asterisk indicate significant difference at P<0.05 (independent T-tests) between E+ and E− *A. inebrians* plants under the same stress conditions.

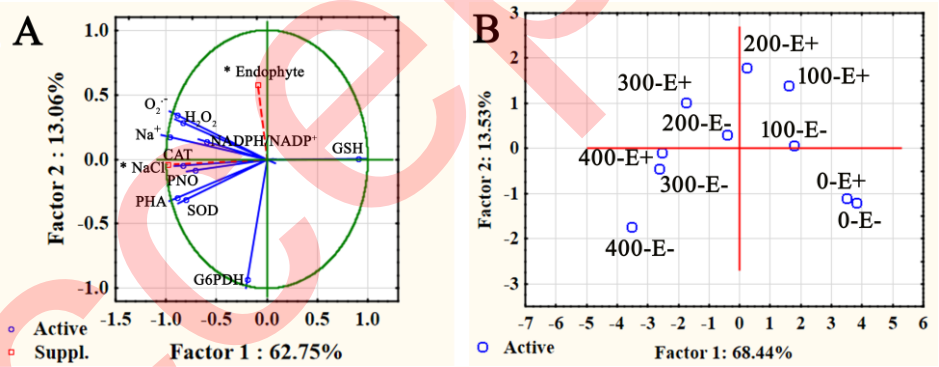

Fig. 8. Projection of the variables on the factor-plane (1×2), active and supplementary variables, *supplementary variable (a) and projection of the cases on the factor-plane (1×2), case with sum of cosine square>=0.00 (b) in leaves of E+ and E− plants. (a) Projection of redox related molecules parameters. The redox related molecules parameters were used as active variables, and NaCl concentration and E+/E− plants as auxiliary variables. Based on the correlation principal component analysis, factor 1 and factor 2 were found by referring to the relative contributions of every redox related molecules. (b) Projection of each NaCl concentration and E+/E− plants on the factor plane. The results of PCA of NaCl content in E+ and E− leaves were summarized. Plasma membrane (PM) NADPH oxidase: (PNO), plasma membrane (PM) H^+-ATPase: (PHA).

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y

Accepted

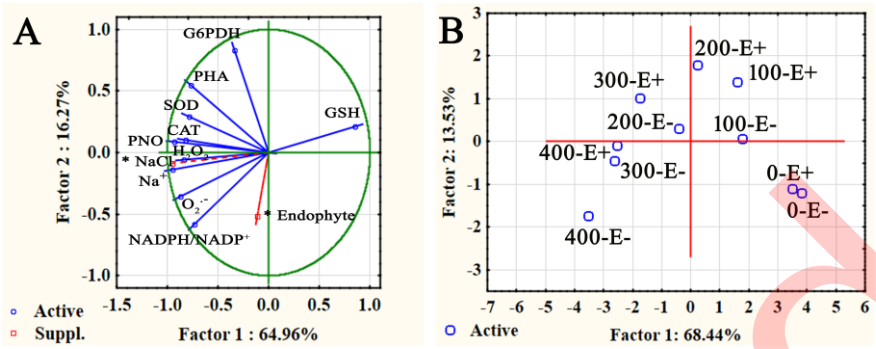

Fig. 9. Projection of the variables on the factor-plane (1×2), active and supplementary variables, *supplementary variable (a) and projection of the cases on the factor-plane (1×2), case with sum of cosine square>=0.00 (b) in roots of E+ and E− plants. (a) Projection of redox related molecules parameters. The redox related molecules parameters were used as active variables, and NaCl concentration and E+/E− plants as auxiliary variables. Based on the correlation principal component analysis, factor 1 and factor 2 were found by referring to the relative contributions of every redox related molecules. (b) Projection of each NaCl concentration and E+/E− plants on the factor plane. The results of PCA of NaCl content in E+ and E− roots were summarized. Plasma membrane (PM) NADPH oxidase: (PNO), plasma membrane (PM) H^+-ATPase: (PHA).

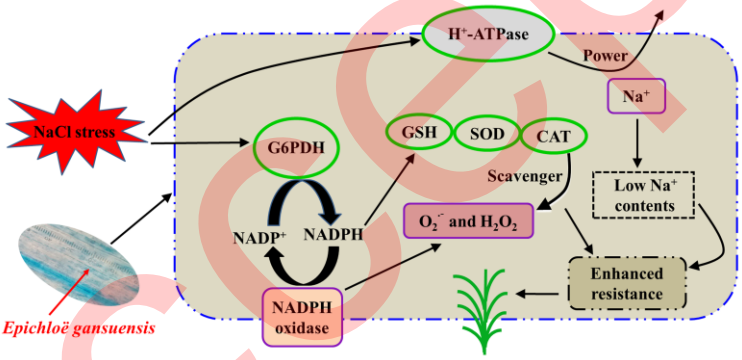

Fig. 10. A proposed model of an *Epichlo ë* gansuensis endophyte enhanced host NaCl-tolerance by modulating PM H^+-ATPase, G6PDH and antioxidant enzymes activity in *A. inebrians* plants. Green ellipse: activity or content was increased by *Epichlo ë gansuensis*; Purple square: activity or content was decreased by *Epichlo ë gansuensis*.

SUPPORTING INFORMATION

Figure S1 Effects of endophyte on NADPH content, $NADP^+$ content and the NADPH:$NADP^+$ ratios in *A. inebrians* plants under NaCl stress.

Figure S2 Effects of endophyte on the GSH content of *A. inebrians* plants under NaCl stress.

Downloaded to IP: 192.168.0.213 On: 2020-05-08 09:30:17 http://engine.scichina.com/doi/10.1007/s11427-020-1674-y