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ORIGINAL PAPER

Transcriptomic analyses giving insights into molecular regulation mechanisms involved in cold tolerance by *Epichloë* endophyte in seed germination of *Achnatherum inebrians*

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Abstract Plants have developed mutualistic symbiosis with diverse fungal endophytes that increase their fitness by conferring abiotic and biotic stress tolerance. However, the molecular regulation mechanisms involved in stress tolerance remain largely unknown. Drunken horsegrass (*Achnatherum inebrians*), an important perennial bunchgrass in China, forms a naturally occurring symbiosis with an asexual symbiotic fungus *Epichloë gansuensis*. The effect of temperature on germination was determined for *E. gansuensis*-infected (E+) versus non-infected (E−) *A. inebrians*. Our results indicate that E+ seed have a higher germination rate under low temperature (10 °C) conditions compared with E− seed. To gain insight into the molecular mechanisms involved in the low temperature resistance of E+ drunken horsegrass, Solexa deep-sequencing was used to identify candidate genes showing differential expression. In total, 152 differentially expressed tags were identified, representing 112 up-regulated and 40 down-regulated genes, which were classified into eight functional categories. Many genes were found to be associated with low temperature response, such as genes participating in biosynthesis of alkaloids and unsaturated fatty acids. This study provides the first comprehensive examination of gene expression changes induced by fungal endophyte infection response to low temperature which is essential for understanding the molecular basis of this aspect of endophyte-enhanced plant improvement.

Keywords Cold tolerance · Gene expression · Molecular mechanisms · Mutualistic symbiosis · Seed germination

Introduction

In natural ecosystems, all plants appear to be symbiotic with fungal endophytes (Petrini 1986; Rodriguez et al. 2009). Such symbiotic associations can have profound impacts on plants through increasing fitness by conferring abiotic and biotic stress tolerance (Brundrett 2006). *Epichloë* (*Neotyphodium*) endophytes, found in many cool-season grasses (Schardl et al. 2004), have been shown to confer enhanced fitness by protecting the host plant against certain nematodes, fungal pathogens, insect herbivores and mammalian herbivores (Siegel et al. 1985; Latch 1993; Schardl and Phillips 1997), as well as enhanced tolerance to drought and high temperatures (Arechavaleta et al. 1989; Bacon 1993). In addition, there are some evidences that colony growth of plant-pathogenic fungi is inhibited by *Epichloë* endophytes (White and Cole 1985; Christensen et al. 1991; Christensen and Latch 1991; Holzmann-Wirth et al. 2000), and that disease tolerance can be imparted by some but not all *Epichloë* species (Gwinn and Gavin 1992, Welty et al. 1991). Enhanced tolerance obtained from these mutualistic symbioses have been hypothesized to be the result of a diverse range of fungal secondary metabolites including ergot alkaloids, the L-aminopyrrolizidine (loline) alkaloids, the

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pyrrolopyrazine alkaloid peramine and so on that are induced by endophyte infection (Bush et al. 1997; Siegel and Bush 1997).

Drunken horse grass (*Achnatherum inebrians*) is a perennial bunchgrass distributed on alpine and subalpine grasslands in China (Shi 1997), which can occur in natural symbiosis with *Epichloë gansuensis* (Li et al. 2004b) and has been detected mainly in seeds, leaf sheaths and peduncles (Bruehl et al. 1994). Due to the increased tolerance to stress conditions provided by endophyte infection (Li et al. 2007a, b; Zhang et al. 2010), the distribution of *A. inebrians* infected by *E. gansuensis* (E+) is more extensive than non-infected *A. inebrians* (E−), especially in harsh conditions such as arid or semi-arid grasslands (Li et al. 2004a). Interestingly, the high concentration of the ergot alkaloids ergonovine and lysergic acid amide were detected in E+ *A. inebrians* which could contribute to stress resistance similar to the other mutualistic symbiosis (Miles et al. 1996).

In contrast to our knowledge about some physiological responses of E+ *A. inebrians* to environmental stress, the information available on the molecular basis of stress tolerance is not strong. With the development of next generation high-throughput sequencing technology, which is also referred to as Solexa deep-sequencing, a number of researchers have uncovered molecular-based mechanisms involved in the environmental stress response in plants and animals (Xiao et al. 2010; Luan et al. 2011). Millions of short RNAs and differentially expressed genes can be identified in a sample using this technology (Bennett et al. 2005). Taking advantage of this technology, we present here the genome-wide analysis of the differential expression of genes between E+ and E− *A. inebrians* when germinated under low temperature. The difference of the germination rate at 10 °C between E+ and E− *A. inebrians* was investigated and the differential expression genes were also identified using Solexa deep-sequencing.

Materials and methods

Plant materials

Achnatherum inebrians plants with mature seeds were harvested in Sangke grassland, Gansu province, China in October 2007. After harvest, E+ and E− *A. inebrians* seeds were isolated according to the method described by Li et al. (2004b). The two specimens then were kept under constant 4 °C storage at the Official Forage Seed Testing Center of Ministry of Agriculture, Lanzhou, China. No specific permissions were required for the locations/activities in this study. The field studies did not involve endangered or protected species.

Seed germination at different temperatures

Both E+ and E− *A. inebrians* seeds which were washed with sterilized deionized distilled water twice were incubated at 5, 10, 15, 20 and 25 °C in Petri dishes (15-cm diameter) containing wet sterilized filter paper with 12 h light and 12 h dark cycle. After imbibition, germination rate of seeds was monitored every 48 h until 25 days. The seeds showing radical extension of 1 mm were scored and removed from the Petri dishes. After 25 days, when no additional seeds had germinated for 5 days, the germination experiment was terminated. Each treatment was repeated five times independently with 100 seeds in each replicate.

Sample preparation and RNA extraction

Both E+ and E− *A. inebrians* seeds were washed with sterilized deionized distilled water twice and sown on sterilized filter paper and grown at 10 °C in constant temperature incubators for 2 days. Total RNAs were isolated from about 100 seeds of either E+ or E− drunken horse grass using the TRIzol reagent (Invitrogen) and incubated with DNase I (Takara) to remove residual genomic DNA according to manufacturer's instruction. The concentration and quantification of all RNA were determined spectrophotometrically. Three independent replicates were performed to each sample.

Tag library construction and Solexa sequencing

For Solexa deep sequencing, the 3′-tag digital gene expression libraries were prepared as described in the Illumina DGE protocol. In brief, about 6 μg of total RNA was used to fractionate mRNA with oligo(dT) magenic beads. First- and second-strand cDNA was synthesized and bead-bound cDNA was subsequently digested with NlaIII. And then, the 3′-cDNA fragments attached to the oligo(dT) beads were ligated to the Illumina GEX NlaIII Adapter 1 containing a recognition site for the MmeI which cuts 17–18 bp downstream from the NlaIII site (CATG site) to produce tags with adapter 1. Subsequently, the restriction enzyme MmeI was applied to produce 21–22 bp tags starting with the NlaIII recognition sequence. After removing 3′ fragments with magnetic beads precipitation, an Illumina GEX adapter 2 was ligated at the site of MmeI cleavage. The resulting adapter ligated cDNA tags were enriched by 15 cycles of PCR performed with two primers that anneal to the ends of the two adapters. The resulting 85 bp fragments were purified using a 6 % Novex TBE PAGE gel and subjected to the Illumina/Solexa sequencing system (Illumina Inc., San Diego, CA, USA). Image recognition and base calling were performed using the Illumina Pipeline.

Data processing and statistical analysis

To obtain high quality data in the two libraries, raw sequence reads were filtered by the Illumina pipeline, removing 3′ adaptor tags, low quality tags and one copy tags. After filtering, we classified the clean tags according their copy number in the library and showed their percentage as total clean tags. Subsequently, due to the limited listing of *A. inebrians* mRNAs and ESTs in GenBank, all tags were mapped to rice reference sequences GCA_000005425.2 by SOAP (Li et al. 2008) as rice is relatively close to *A. inebrians* in genetic relationship. Only one mismatch was allowed and tags mapped to more than one transcript were excluded from our analysis. The ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) accession number is E-MTAB-4199.

Cloning of selected transcripts 3′ end and semi-quantitative RT-PCR analysis

3′ end fragment sequence information of selected transcripts was obtained via 3′ RACE according to the GeneRcerTM Kit (Invitrogen). Briefly, reverse transcription was performed with about 1 μg of the total RNA of *A. inebrians* seeds using the GeneRacerTM oligo-dT primer [5′-GCTGTCAACGA-TACGCTACGTAACGGCATG ACAGTG (T)18-3′], to create RACE-ready first-strand cDNA with known priming sites at the 3′ ends. To obtain the 3′ ends, the PCR reaction was performed using the first strand of cDNA with forward gene-specific primer as shown in Table S1 and the GeneRacerTM 3′ reverse primer (5′-GCTGTCAACGA-TACGCTACGTAACG-3′), and then nested PCR was carried out with the same forward primers and 3′ nested primer (5′-CGCTACGTAACGGCATGACAGTG-3′). Individual PCR products were cloned into PMD18-T vector (TaKaRa) and sequenced with the universal T7 primer on an ABI 3730 DNA Sequence Analyzer. For RT-PCR analysis, primers were designed based on 3′ end sequence of selected genes using primer 5.0 version software, and a housekeeping gene, ACTIN, used as the internal control. The PCR primers are shown in Table S1.

Results

Seed germination at different temperatures

The germination rate of *Achnatherum inebrians* seeds was influenced by temperature (Fig. 1). With decreasing temperature, the germination rate of *A. inebrians* seeds decreased gradually. Interestingly, the decreased germination rate of E+ *A. inebrians* seeds was significantly slow compared with E− *A. inebrians* seeds at 10 °C (Fig. 1).

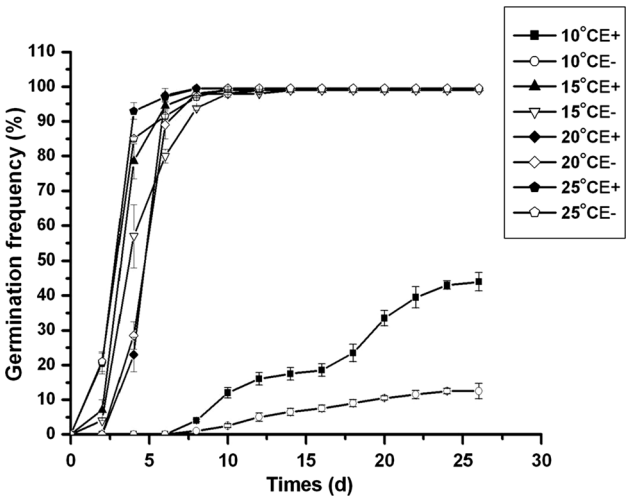

Fig. 1 Germination of E+ (*filled shape*) and E− (*unfilled shape*) *A. inebrians* seeds in relation to incubation temperature. Each point is the average of n = 5 (100 seeds each) ± SE

However, a similar germination rate was observed in E+ and E− *A. inebrians* seeds at other temperature (15, 20, 25 and 30 °C) conditions. Thus, endophyte can improve the germination rate of *A. inebrians* seeds within a certain range of low temperature.

Analysis of differential gene expression libraries

To obtain a global view of differences in the transcriptional profile of E+ and E− *A. inebrians* seeds germinating under low temperature, Solexa deep-sequencing was employed to construct two gene expression profiles from E+ and E− *A. inebrians* seeds germinated for 48 h at 10 °C. Major characteristics of the two libraries were summarized (Table 1). In total, 3,649,469 (E+) and 3823829 (E−) tags were sequenced and filtered by the Illumina pipeline removing 3′ adaptor tags, low quality tags and one copy tags, and a total of 3,480,742 (E+) and 3,643,237 (E−) clean tags were obtained. The number of corresponding distinct tags were 112,754 (E+) and 113,028 (E−). All clean tags of the two libraries were subsequently mapped to the rice genome by the tolerances of one mismatch in each alignment. With this criteria set only 12.32 % (E+) and 12.40 % (E−) total clean tags were aligned to the reference genome, 10.38 % (E+) and 10.14 % (E−) of the distinct clean tags mapped unambiguously to the rice genes, and more than 80 % of the distinct clean tags in both libraries did not map to the reference database (Table 1).

Since the copy number of a tag reflects the quantitative level of gene expression, the distribution of tag expression was used to evaluate the normality of the differential gene expression data (’t Hoen et al. 2008). As shown in Fig. 2, similar patterns of the distribution of total tags and distinct

Table 1 Summary of Solexa deep-sequencing profiles and their mapping to the reference genes

Summary	E+	E−
Raw data		
Total	3,649,469	3,823,829
Distinct tag	280,493	292,592
Clean tag		
Total number	3,480,742	3,643,237
Distinct tag number	112,754	113,028
All tag mapping to gene		
Total number	428,664	451,857
Total % of clean tag	12.32	12.40
Distinct tag number	21,039	14,811
Distinct tag % of clean tag	18.66	13.10
Unambiguous tag mapping to gene		
Total number	360,834	369,690
Total % of clean tag	10.38	10.14
Distinct tag number	18,237	12,873
Distinct tag % of clean tag	5.24	3.53
All tag-mapped genes		
Number	11,208	10,121
% Of reference genes	19.74	17.81
Unambiguous tag-mapped genes		
Number	8764	7798
% Of reference genes	15.44	13.73
Unknown tag		
Total number	3,052,098	3,191,380
Total % of clean tag	87.68	87.60
Distinct tag number	91,715	98,217
Distinct tag % of clean tag	81.34	86.9

tags over different tag abundance categories were found for the two libraries, suggesting there was no bias in the construction of the libraries from the two specimens. In addition, high-expression tags with copy numbers larger than 100 were overwhelmingly dominant (Fig. 2a, c) whereas low-expression tags with copy numbers less than 5 occupied the majority of distinct tag distributions (Fig. 2b, d). These results suggest that while the majority of mRNA is expressed at low levels, a small proportion of mRNA is highly expressed.

To identify genes showing significant change in expression level, we mapped all clean tag sequences of these two gene expression libraries to the rice genome using the method described by Audic and Claverie (1997). Our results indicated that a total 254 genes showing differential expression with a log2 ratio >1 or <−1 ($P < 0.001$) were found between these two specimens (Fig. 3). Of those genes, 152 genes were considered significant with log2 ratio >2 or <−2, with 112 genes up-regulated and 40 genes down-regulated in the E+ *A. inebrians.*

Validation of representative genes by semi-quantitative RT-PCR

In order to experimentally validate differentially expressed genes derived from Solexa sequencing, the expression level of 6 modulated genes was analyzed further by semi-quantitative RT-PCR with gene-specific primers (Table S1). Due to the limited information available of *A. inebrians* genes, 3′ RACE was performed to obtain 3′ end sequence of corresponding gene (Table S2). The gene-specific primers were designed based on 3′ end sequence using Primer 5 software. The results normalized against *ACTIN* expression levels, which are among the more stably-expressed genes, show that expression of seven genes behaved similarly between RT–PCR and Solexa sequencing results (Fig. 4). Only one gene did not show consistent expression in the two results. Overall, the RT-PCR results mainly supported the Solexa sequencing results and confirmed differentially expressed genes in the E+ *A. inebrians*.

Functional categorization of differentially expressed genes

To characterize the functional consequences of differentially expressed genes in E+ *A. inebrians,* all 152 differentially expressed genes were functionally annotated based on careful analysis of the Gene Ontology database and the scientific literature (Ashburner et al. 2000). All of the genes were manually assigned to eight categories of observed differences (Fig. 5). It was found that approximately 9 % of these genes are involved in the biosynthesis of alkaloids, 6 % are involved in biosynthesis of fatty acids, 10 % belong to stress response genes, about 24 % are involved in protein turnover, 11 % belong to transcription and signal transduction and further 13 % genes are proteins of unknown function.

Biosynthesis of alkaloids

One of the important differences in the transcriptional profiling between E+ and E− specimens was genes involved in the biosynthesis of tryptophan which is the precursor of ergot alkaloids (Robbers et al. 1972). Previous research has shown that exogenous tryptophan feeding or over-expression of genes involved in the biosynthesis of tryptophan can result in a significant increase of indole alkaloid accumulation (Robbers et al. 1972; Whitmer et al. 1998, 2002). In our present studies, almost all of the genes in this category were up-regulated dramatically an

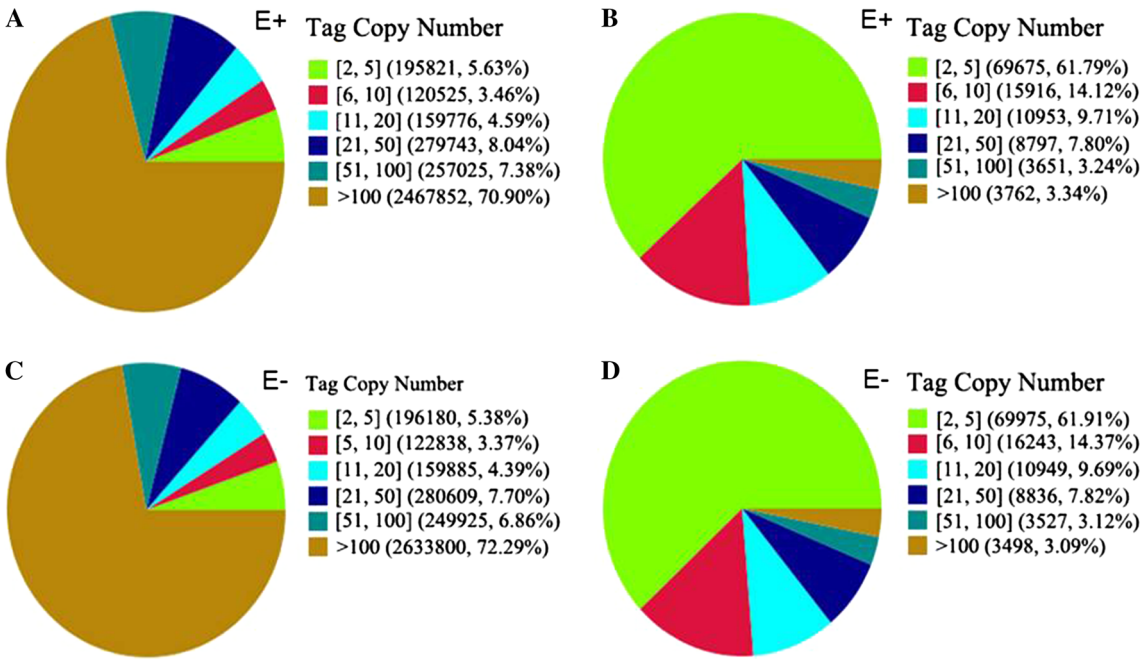

Fig. 2 Distribution of total and distinct clean tags from imbibed E+ and E− *A. inebrians* seeds. **a** and **b**, distribution of E+ total and distinct clean tags respectively; **c** and **d**, distribution of E− total and distinct clean tags respectively

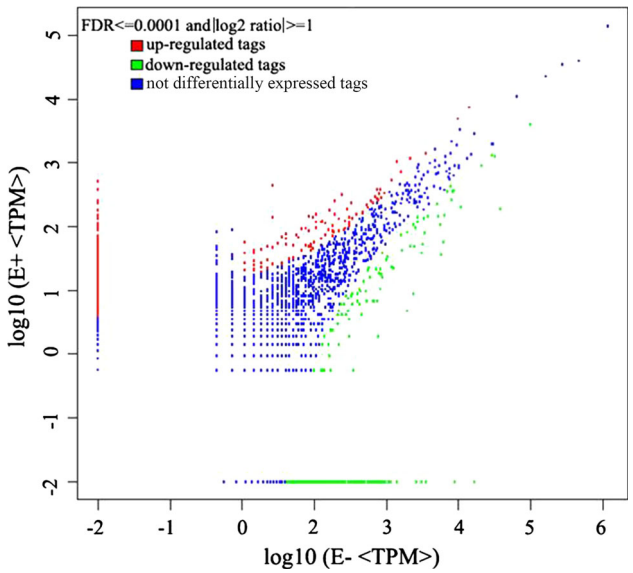

Fig. 3 Comparison of gene expression levels between imbibed E+ and E− *A. inebrians* seeds. TPM (transcript per million clean tags): the number of mapped tags per transcript per million clean tags (Li and Dewey 2011). With an estimated false-discovery rate (FDR) of <0.001 and |log2Ratio| ≥ 1 (ratio: E+/E−), the *red* represents up-regulated genes, the *green* represents down-regulated genes and the *blue* represents genes that are not differentially expressed. (Color figure online)

exception being a UDP-glucosyl transferase in the E+ specimen. Most of these genes regulate different key steps in the tryptophan biosynthesis pathway (Table S3), including glycolysis (two genes identical to 2, 3-bisphosphoglycerate-independent phosphoglycerate mutase and UDP-glucoronosyl/UDP-glucosyl transferase), shikimate synthesis (phospho-2-dehydro-3-deoxyheptonate aldolase, 3-phosphoshikimate 1-carboxyvinyltransferase and indole-3-glycerol phosphate synthase) and tryptophan conversion (transketolase and aminotransferase). In summary, up-regulation of tryptophan synthesis related genes may result in increased ergot alkaloids biosynthesis.

Fatty acid metabolism

The regulation of biosynthesis of fatty acids is important for the acclimation of higher plants to low temperature. In the present work we identified a series of genes involved in fatty acid metabolism (Table S3). Among these genes, two genes identical to acyl-desaturase which are involved in unsaturated fatty acids synthesis (Keil et al. 2010) were dramatically up-regulated. Furthermore, some genes related to fatty acid synthesis were also up-regulated in the E+ specimen, including one gene similar to acetyl-CoA carboxylase which catalyzes the formation of malonyl-CoA. In addition, an acyl-coenzyme A oxidase related gene was significantly down-regulated in the E+ specimen. Goto et al. (2011) have demonstrated that down-regulation of acyl-coenzyme A oxidase gene could enhance the fatty acid oxidation which results in the decrease of the levels of circulating and accumulating lipids in mammals. Different expression of these genes in E+ seeds of *A. inebrians* could

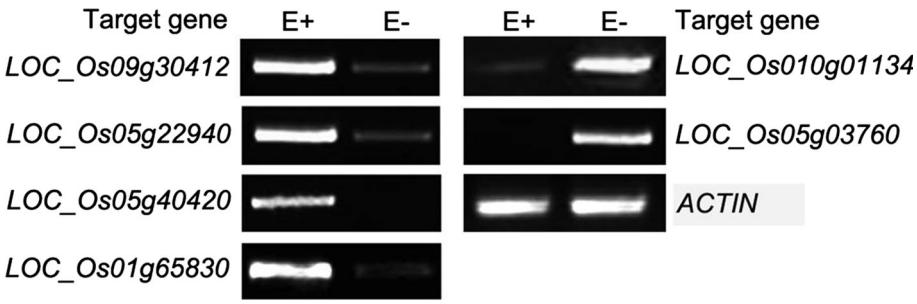

Fig. 4 RT-PCR analysis of gene expression level for six selected genes. All data were normalized to the *ACTIN* expression level. The RT-PCR products were separated on agarose gels and were visualized with ethidium bromide under UV light

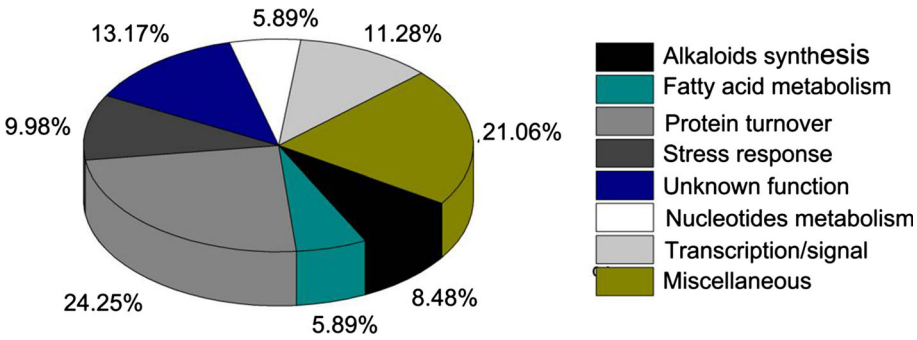

Fig. 5 Pie chart of functional categories of differential expressed genes from E+ and E− *A. inebrians* seeds in the germination process at low temperature

change the composition and accumulation of fatty acids consequently improving its fitness to low temperatures.

Stress response

In our investigation, a set of modulated genes related to stress response were detected (Table S3). Among them, several genes associated with ROS scavenging function were upregulated in the E+ specimen, including homologs of peroxiredoxin, peroxidase precursor and copper/zinc superoxide dismutase. It is well known that stress conditions including cold, drought and salt can induce the accumulation of ROS such as super oxide, hydrogen peroxide, and hydroxyl radicals (Mittler 2002; Deng et al. 2014; Garg and Chandel 2015). Therefore, those ROS, which are known as signals, induced up-regulation of ROS scavenger related genes (Prasad et al. 1994). Moreover, two genes similar to heat shock proteins (HSPs) were up-regulated in E+ samples. HSPs, often called the stress proteins, are now recognized as important for stress resistance (Grover 2002). In addition, several stress respond proteins also dramatically increased, including a universal stress protein which is accumulated in plants infected with fungi under various environmental stress condition (Kvint et al. 2003). However, two genes similar to *DnaJ* domain containing protein were down-regulated. Blumberg and Silver (1991) have proved that the increased expression of *SCJ1*, a homologue of the bacterial heat-shock gene *DnaJ*, results in mis-sorting of a nuclear-targeted test protein.

Protein turnover

Genes related to protein turnover were also identified in our experiment. Most of them were prevalently up-regulated in E+ specimen (Table S3), including the genes identical to protein synthesis (e.g. ribosomal proteins) and protein degradation genes (e.g. protease and ubiquitin-conjugating enzymes). On the one hand, the levels of several proteins associated with protein translation were increased including ribosomal proteins (e.g. subunit of 26, 40 and 60 s ribosome and eukaryotic translation initiation factor) and protein translation initiation factor. Regulation of the translational machinery is considered as an integral component in the cellular stress response (Wood and Oliver 1999; Dhanaraj et al. 2007). On the other hand, there are several modulated genes related protein degradation were detected in our analysis, including ubiquitin-conjugating enzymes and some proteases (e.g. FtsH protease, Serine Carboxypeptidase and T1 family peptidase). Protein degradation during stress progress is a highly conserved and regulated phenomenon in all the organisms reported so far (Vierstra and Callis 1999).

Discussion

Numerous studies have shown that endophytes provide their grass partners with a series of fitness improvements (Siegel et al. 1985; Latch 1993; Schardl and Phillips 1997; Pedro et al. 2006). Germination is a complex process that

could be affected by endophytes. It has been found that endophyte infection can increase the germination ability under environmental stress in *Festuca arundinacea* (Soleimani et al. 2010), *Lolium perenne* (Clay 1987) and *Bromus setifolius* (Novas et al. 2003). However, little is known regarding the molecular mechanisms involved in germination capability under stress conditions in endophyte-infected plants. In the present study, we analyzed the germination superiority of *A. inebrians* seeds infected with *Epichloë* endophyte at low temperature stress using Solexa deep-sequencing to identify candidate genes showing differential expression compared to endophyte-free *A. inebrians*. Germination experiments between two populations showed that E+ *A. inebrians* seeds have significantly higher germination ability at low temperatures. Especially at 10 °C, germination rate of E+ *A. inebrians* seeds is almost up to 50 %, whereas little is germinated in E− *A. inebrians*. Previous work has shown that *Epichloë* endophyte infection was of benefit to the germination of *A. inebrians* under high $CdCl_2$ concentrations stress (Zhang et al. 2010).

Analysis of Solexa deep-sequencing identified candidate genes that may contribute to low temperature tolerance due to endophyte infection. In general, approximately eight thousands genes were investigated (Table 1), 152 of which (1.9 %) were differentially expressed (up or down-regulated). Moreover, semi-quantitative RT-PCR analysis of selected genes confirmed the reliability of the Solexa deep-sequencing data (Fig. 4). Further functional annotation indicated that a number of genes could be assigned to categories concordant with low temperature response. Induced changes of alkaloid biosynthesis genes were detected in the host plant, including induction of genes related to biosynthesis of tryptophan (Table S3) which is the precursor of ergot alkaloids (Robbers et al. 1972). In previous studies, the over-expression of tryptophan decarboxylase in *Trichoderma harzianum* resulted in enhancing alkaloids accumulation in a transgenic cell line (Canel et al. 1998; Whitmer et al. 1998, 2002). Our earlier work has demonstrated that an endophytic fungus and ergot alkaloids existed in *A. inebrians* (Li et al. 2007b). The presence of such fungi and associated high levels of ergot alkaloids (Miles et al. 1996), have become central in mechanisms to explain beneficial effects of endophytes on host plants (Schardl et al. 2004, 2007). Therefore, the increasing ergot alkaloid accumulation result from over production of tryptophan should contribute to the low temperature resistance in seed germination progress.

Several changes which are commonly associated with responses to various stress conditions were found in our study. Firstly, differential expression of genes related to fatty acid biosynthesis was observed (Table S3). Interestingly, some genes involved in unsaturated fatty acids synthesis were up-regulated in E+ *A. inebrians*. In higher plants, the most thoroughly accepted mechanism as a response to low temperature is increasing the level of unsaturated fatty acids of membrane lipids. For example, in the progress of maize germination at low temperature, Santis et al. (1999) found that the content of unsaturated fatty acid was dramatically increased with the decrease of the temperature. In addition, Prasad (1996) also suggested a possible involvement of the content of the unsaturated fatty acids in chilling tolerance of maize seedlings. Therefore, differential expression of those fatty acids related genes maybe result in a changed plasma membrane lipid composition of E+ *A. inebrians* seeds which could contribute to the enhancement of low temperature resistance. Moreover, most of the universal stress response related genes were up-regulated in E+ *A. inebrians* seeds (Table S3), including some genes homologous to antioxidase and heat shock proteins which are involved in scavenging ROS and maintaining proteins in their functional conformations respectively under various stress conditions. In addition, the expression of many genes related to protein turnover were modulated in E+ specimen, which may have resulted in accelerated protein translation and degradation (Table S3). It has been reported that the levels of protein synthesis is increased by stress conditions, in which many defensive proteins are produced resulting in improved stress resistance of plants (Ndimba et al. 2005). A number of previous studies have shown that protein degradation of numerous short-lived and regulatory proteins that control these cellular functions are involved in the regulation of stress response (Bochtler et al. 1999; Liu 2004; Reinstein 2004).

In summary, based on sequence similarities to genes in the rice genome database, a number of genes modulated in their response to low temperature in E+ *A. inebrians* were identified by Solexa deep-sequencing. The Solexa deep-sequencing technology is a powerful tool for high-throughput genome research, which has been also adapted for differentially expressed genes (Velculescu and Kinzler 2007; Licatalosi and Darnell 2010; Metzker 2010; Wang et al. 2016). Based on the knowledge of these differentially expressed genes, we have speculated their possible functional role in the germination process and stress tolerance of *A. inebrians-Epichloë* symbioses under low temperature. However, due to the limited genome information of *A. inebrians*, more than 60 % of candidate genes that may have novel functional roles in endophyte-grass associations could not be analyzed. Once more EST and genome projects of *A. inebrians* are completed, some of these differentially expressed genes could be further identified and functionally characterized in future. Briefly, this study provides the first global catalogue of modulated genes of *A. inebrians* infected with endophytes in germination under

low temperature, together with their functional annotations and will help to elucidate the molecular basis of the stress resistance enhancement that endophytes provide their host plants.

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Compliance with ethical standards

Conflict of interests The authors have declared that no conflicts of interests exist.

Ethical statement Every parts of the research did not involve human participants and other animals. Our manuscript complies to the Ethical Rules applicable for Plant Growth Regulation.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Arechavaleta M, Bacon CW, Hoveland CS, Radcliffe DE (1989) Effect of the tall fescue endophyte on plant response to environmental stress. Agron J 81:83–90

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H et al (2000) Gene Ontology: tool for the unification of biology. Nat Genet 25:25–29

Audic S, Claverie JM (1997) The significance of digital gene expression profiles. Genome Res 7:986–995

Bacon CW (1993) Abiotic stress tolerances (moisture, nutrients) and photosynthesis in endophyte-infected tall fescue. Agric Ecosyst Environ 44:123–141

Bennett ST, Barnes C, Cox A, Davies L, Brown C (2005) Toward the 1,000 dollars human genome. Pharmacogenomics 6:373–382

Blumberg H, Silver PA (1991) A homologue of the bacterial heat-shock gene DnaJ that alters protein sorting in yeast. Nature 349:627–630

Bochtler M, Ditzel L, Groll M, Hartmann C, Huber R (1999) The proteasome. Annu Rev Biophys Biomol Struct 28:295–317

Bruehl GW, Kaiser WJ, Klenin RE (1994) An endophyte of *Achnatherum inebrians*, an intoxicating grass of northwest China. Mycologia 86:73–776

Brundrett MC (2006) Understanding the roles of multifunctional mycorrhizal and endophytic fungi. In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial root endophytes. Springer, Berlin, pp 281–293

Bush LP, Wilkinson HH, Schardl CL (1997) Bioprotective alkaloids of grass–fungal endophyte symbioses. Plant Physiol 114:1–7

Canel C, Lopes-Cardoso MI, Whitmer S, van der Fits L, Pasquali G et al (1998) Effects of over-expression of strictosidine synthase and tryptophan decarboxylase on alkaloid production by cell cultures of *Catharanthus roseus*. Planta 205:414–419

Christensen MJ, Latch GCM (1991) Variation among isolates of *Acremonium* endophytes (*A. coenophialum* and possibly *A. typhinum*) from tall fescue (*Festuca arundinacea*). Mycol Res 95:1123–1126

Christensen MJ, Latch GCM, Tapper BA (1991) Variation within isolates of *Acremonium* endophytes from perennial ryegrasses. Mycol Res 95:918–923

Clay K (1987) Effects of fungal endophpjytes on the seed and seedling biology of *Lolium perenne* and *Festuca arundinacea*. Oecologia 73:358–362

Deng B, Jin X, Yang Y, Lin Z, Zhang Y (2014) The regulatory role of riboflavin in the drought tolerance of tobacco plants depends on ROS production. Plant Growth Regul 72:269–277

Dhanaraj AL, Alkharouf NW, Beard HS, Chouikha IB, Matthews BF et al (2007) Major differences observed in transcript profiles of blueberry during cold acclimation under field and cold room conditions. Planta 225:735–751

Garg N, Chandel S (2015) Role of arbuscular mycorrhiza in arresting reactive oxygen species (ROS) and strengthening antioxidant defense in *Cajanus cajan* (L.) Millsp. nodules under salinity (NaCl) and cadmium (Cd) stress. Plant Growth Regul 75:521–534

Goto T, Lee JY, Teraminami A, Kim YI, Hirai S et al (2011) Activation of PPAR-alpha stimulates both differentiation and fatty acid oxidation in adipocytes. J Lipid Res 52:873–884

Grover A (2002) Molecular biology of stress responses. Cell Stress Chaperones 7:1–15

Gwinn KD, Gavin AM (1992) Relationship between endophyte infestation level of tall fescue seed lots and *Rhizoctonia zeae* seedling disease. Plant Dis 76:911–914

Holzmann-Wirth A, Dapprich P, Eierdanz S, Heerz D, Paul VH (2000) Anti-fungal substances extracted from *Neotyphodium* endophytes. In: Proceedings of the 3rd international conference on harmful and beneficial microorganisms in Grassland, Pasture and Turf, pp 65–69

Keil S, Müller M, Zoller G, Haschke G, Schroeter K et al (2010) Identification and synthesis of novel inhibitors of acetyl-CoA carboxylase with in vitro and in vivo efficacy on fat oxidation. J Med Chem 53:8679–8687

Kvint K, Nachin L, Diez A, Nyström T (2003) The bacterial universal stress protein: function and regulation. Curr Opin Microbiol 6:1401–1445

Latch GCM (1993) Physiological interactions of endophytic fungi and their hosts. Biotic stress tolerance imparted to grasses by endophytes. Agric Ecosyt Environ 44:143–156

Li B, Dewey CN (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinform 12:323

Li CJ, Nan ZB, Gao JH, Tian P (2004a) Detection and distribution of *Neotyphodium-Achnatherum inebrians* association in China. In: Kallenbach R, Rosenkrans C, Lock R (eds) Proceedings of 5th international *neotyphodium*/grass—interactions symposium, Arkansas, USA, vol 210, pp 24–27

Li CJ, Nan ZB, Paul V, Dapprich P, Liu Y (2004b) A new *Neotyphodium* species symbiotic with drunken horse grass (*Achnatherum inebrians*) in China. Mycotaxon 90:141–147

Li CJ, Gao JH, Nan ZB (2007a) Interactions of *Neotyphodium gansuense*, *Achnatherum inebrians* and plant-pathogenic fungi. Mycol Res 111:1220–1227

Li CJ, Zhang XX, Li F, Nan ZB, Schardl CL (2007a) Disease and pests resistance of endophyte infected and non-infected drunken horse grass. In: Popay A, Thom ER (eds) Proceedings of the 6th international symposium on fungal endophytes of grasses. New Zealand Grassland Association, Dunedin, New Zealand, pp 111–114

Li R, Li Y, Kristiansen K, Wang J (2008) SOAP: short oligonucleotide alignment program. Bioinformatics 24:713–714

Licatalosi DD, Darnell RB (2010) RNA processing and its regulation: global insights into biological networks. Nat Rev Genet 11:75–87

Liu CY (2004) Ubiquitin ligases and the immune response. Annu Rev Immunol 22:81–127

Luan JB, Li JM, Varela N, Wang YL, Li FF et al (2011) Global analysis of the transcriptional response of whitefly to tomato yellow leaf curl China virus reveals the relationship of coevolved adaptations. J Virol 85:3330–3340

Metzker ML (2010) Sequencing technologies—the next generation. Nat Rev Genet 11:31–46

Miles CO, Lane GA, Di Menna ME, Garthwaite I, Piper EL et al (1996) High levels of ergonovine and lysergic acid amide in toxic *Achnatherum inebrians* accompany infection by an *Acremonium*-like endophytic fungus. Agric Food Chem 44:1285–1290

Mittler R (2002) Oxidative stress, antioxidants, and stress tolerance. Plant Sci 7:405–410

Ndimba BK, Chivasa S, Simon WJ, Slabas AR (2005) Identification of *Arabidopsis* salt and osmotic stress responsive proteins using two-dimensional difference gel electrophoresis and mass spectrometry. Proteomics 5:4185–4196

Novas MV, Gentile A, Cabral D (2003) Comparative study of growth parameters on diaspores and seedlings between populations of *Bromus setifolius* from Patagonia, differing in *Neotyphodium* endophyte infection. Flora 198:421–426

Pedro EG, Pablo HM, Claudio MG, Roberto LB (2006) Effects of the *Neotyphodium* endophyte fungus on dormancy and germination-rate of *Lolium multiflorum* seeds. Austral Ecol 31:767–775

Petrini O (1986) Taxonomy of endophytic fungi of aerial plant tissues. In: Fokkema NJ, van den Huevel J (eds) Microbiology of the phyllosphere. Cambridge University Press, Cambridge, pp 175–187

Prasad TK (1996) Mechanism of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities. Plant J 10:1017–1026

Prasad T, Anderson M, Martin B, Stewart C (1994) Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell 6:65–74

Reinstein E (2004) Immunologic aspects of protein degradation by the ubiquitin-proteasome system. Isr Med Assoc J 6:420–424

Robbers JE, Robertson LW, Hornemann KM, Jindra A, Floss HG (1972) Physiological studies on ergot: further studies on the induction of alkaloid synthesis by tryptophan and its inhibition by phosphate. J Bacteriol 112:791–796

Rodriguez RJ, JrJF White, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330

Santis AD, Landi P, Genchi G (1999) Changes of mitochondrial properties in maize seedlings associated with selection for germination at low temperature. Fatty acid composition, cytochrome c oxidase, and adenine nucleotide translocase activities. Plant Physiol 119:743–754

Schardl CL, Phillips TD (1997) Protective grass endophytes: where are they from and where are they going? Plant Dis 81:430–437

Schardl CL, Leuchtmann A, Spiering M (2004) Symbiosis of grasses with seed borne fungal endophytes. Annu Rev Plant Biol 55:315–340

Schardl CL, Grossman RB, Nagabhyru P, Faulkner JR, Mallik UP (2007) Loline alkaloids: currencies of mutualism. Phytochemistry 68:980–996

Shi ZC (1997) Important poisonous plants of China grassland (in Chinese). China Agricultural Press, Beijing, pp 166–176

Siegel MR, Bush LP (1997) Toxin production in grass/endophyte associations. In: Carroll GC, Tudzynski P (eds) The Mycota. Part A, plant relationships, vol 5. Springer, Berlin, pp 221–238

Siegel MR, Latch GCM, Johnson MC (1985) *Acremonium* fungal endophytes in tall fescue and perennial ryegrass: significance and control. Plant Dis 69:179–183

Soleimani M, Hajabbasi MA, Afyuni M, Mirlohi A, Borggaard OK et al (2010) Effect of endophytic fungi on cadmium tolerance and bioaccumulation by *Festuca arundinacea* and *Festuca pratensis*. Int J Phytoremediation 12:535–549

't Hoen PAC, Ariyurek Y, Thygesen HH, Vreugdenhil E, Vossen RHAM et al (2008) Deep sequencing-based expression analysis shows major advances in robustness, resolution and inter-lab portability over five microarray platforms. Nucleic Acids Res 36:389–399

Velculescu VE, Kinzler KW (2007) Gene expression analysis goes digital. Nat Biotechnol 25:878–880

Vierstra R, Callis J (1999) Polypeptide tags, ubiquitous modifiers for plant protein regulation. Plant Mol Bio 41:435–442

Wang B, Xue Y, Zhang Z, Ding D, Fu Z, Tang J (2016) Transcriptomic analysis of maize kernel row number-associated miRNAs between a single segment substitution line and its receptor parent. Plant Growth Regul 78:145–154

Welty RE, Barker RE, Azevedo MD (1991) Reaction of tall fescue infected and noninfected by *Acremonium coenophialum* to *Puccinia graminis* subsp. *graminicola*. Plant Dis 75:883–886

White JF, Cole GT (1985) Endophyte-host association in forage grasses. III. In vitro inhibition of fungi by *Acremonium coenophialum*. Mycologia 77:487–489

Whitmer S, Canel C, Hallard D, Gonçalves C, Verpoorte R (1998) Influence of precursor availability on alkaloid accumulation by transgenic cell line of *Catharanthus roseus*. Plant Physiol 116:853–857

Whitmer S, van der Heijden R, Verpoorte R (2002) Effect of precursor feeding on alkaloid accumulation by a tryptophan decarboxylase over-expressing transgenic cell line T22 of *Catharanthus roseus*. J Biotechnol 96:193–203

Wood A, Oliver M (1999) Translational control in plant stress: formation of messenger ribonucleoprotein complexes (mRNPs) in *Tortula ruralis* in response to desiccation. Plant J 18:359–370

Xiao SQ, Jia JY, Mo DL, Wang QW, Qin LM et al (2010) Understanding PRRSV infection in porcine lung based on genome-wide transcriptome response identified by deep sequencing. PLoS One 5:e11377

Zhang XX, Fan XM, Li CJ, Nan ZB (2010) Effects of cadmium stress on seed germination, seedling growth and antioxidative enzymes in *Achnatherum inebrians* plants infected with a *Neotyphodium* endophyte. Plant Growth Regul 60:91–97