Physiological Response and Transcriptome Analysis of Wild Barley to Drought Stress Postprint

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Abstract

To investigate the physiological responses of wild barley under drought stress and to identify drought tolerance-related genes, physiological indices were determined and transcriptome sequencing analysis was performed on wild barley seedlings under different concentrations of polyethylene glycol (PEG) 6000 stress. The results showed that after 2 d of stress with different concentrations of PEG 6000, the proline content in wild barley leaves exhibited a continuous upward trend, while soluble sugar and superoxide dismutase (SOD) activity showed a trend of first increasing and then decreasing. Using the membership function method, leaves from the 30 mmol·L-1 PEG 6000 treatment group were selected for transcriptome sequencing analysis. Compared with the control group, 6 868 genes were up-regulated and 2 081 genes were down-regulated. GO (gene ontology) functional classification of the differentially expressed genes could be divided into 3 major categories containing 54 functional groups. Through KEGG pathway enrichment, 6 579 differentially expressed genes were enriched in 136 pathways, mainly including arginine and proline metabolism, glycolysis and gluconeogenesis, starch and sucrose metabolism, peroxisome and other pathways, and the relevant up-regulated genes in each metabolic pathway were identified.

Full Text

Physiological Response and Transcriptome Analysis of *Hordeum brevisubulatum* Under Drought Stress

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Abstract: This study investigated the physiological response of *Hordeum brevisubulatum* to drought stress to identify drought tolerance-related genes, physiological indices, and transcriptome changes in seedlings under different concentrations of polyethylene glycol (PEG) 6000 stress. The results showed that after 2 days of PEG 6000 treatment, proline content in *H. brevisubulatum* leaves increased continuously, while soluble sugar content and superoxide dismutase (SOD) activity initially increased and then decreased. The membership function method was used to select transcriptome sequencing and analysis of *H. brevisubulatum* leaves under 30 mmol · L ^{1} PEG 6000 treatment. Compared with the control group, 6,868 up-regulated genes and 2,081 down-regulated genes were identified. The differentially expressed genes were classified into three categories and 54 functional groups by Gene Ontology (GO) classification. A total of 6,579 differentially expressed genes were enriched in 136 pathways by KEGG pathway enrichment analysis. Key metabolic pathways including arginine and proline metabolism, glycolysis/gluconeogenesis, starch and sucrose metabolism, and peroxisome pathways were identified, with related up-regulated expression genes discovered in various metabolic pathways.

Keywords: *Hordeum brevisubulatum*; drought stress; physiological response; RNA-Seq; Zhalong Wetland; Songnen Plain

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1.2 Measurement of Physiological Indices

Physiological indices were measured according to standard methods. Proline content was determined using the sulfosalicylic acid method [11], soluble sugar content was measured by the anthrone colorimetric method [12], and SOD activity was assayed using the NBT photoreduction method [13].

1.4 Transcriptome Sequencing and Analysis

Total RNA was extracted from leaf samples using the TRIzol method. The RNA quality was assessed using an Agilent 2100 Bioanalyzer. Sequencing libraries were constructed and sequenced on an Illumina HiSeq platform. Raw reads were filtered to obtain clean reads, and the Trinity software (v2.0.6) was used for *de novo* assembly. The Tgicl software (v2.0.6) was then used to remove redundancy and obtain unigenes. Functional annotation was performed using Blast2GO for Gene Ontology (GO) analysis and Blast against the KEGG database for pathway annotation.

1.5 Statistical Analysis

All data were analyzed using SPSS 20.0 software with one-way ANOVA ($P < 0.05$). Values are presented as means ± standard deviation. GraphPad Prism

6.0 was used for graphical presentation. The membership function value was calculated as: R = (X - X)/(X - X), where X is the measured value, and X and X are the maximum and minimum values, respectively.

Results

[Figure 1: see original paper] shows the measured results of physiological indexes. Under PEG stress, proline content increased continuously with stress duration, while soluble sugar content and SOD activity showed initial increases followed by decreases.

presents the statistics of reading quality after filtration. The clean data yielded 52.25 Mb and 44.93 Mb of reads for the control and treatment groups, respectively, with Q20 values of 98.71% and 98.89%, and Q30 values of 85.99% and 86.81%. The GC content was 52.25% and 45.36%, respectively.

2.4 GO Functional Classification of Differentially Expressed Genes

Differentially expressed genes (DEGs) were classified into three main GO categories: biological process, cellular component, and molecular function, comprising 54 functional groups. The biological process category included metabolic process, cellular process, and single-organism process. The cellular component category included cell, cell part, and organelle. The molecular function category included catalytic activity and binding.

A total of 6,868 DEGs were up-regulated and 2,081 were down-regulated. These DEGs were enriched in 136 KEGG pathways, with significant enrichment in metabolic pathways, biosynthesis of secondary metabolites, and plant hormone signal transduction. The top 20 enriched pathways are listed in , including arginine and proline metabolism, glycolysis/gluconeogenesis, starch and sucrose metabolism, and peroxisome pathways.

Discussion

Drought stress induces the accumulation of reactive oxygen species (ROS) in plants, causing oxidative damage. SOD is a key antioxidant enzyme that scavenges ROS and protects cells from oxidative stress [18]. Under drought conditions, SOD activity initially increases as a protective response but may decrease under severe stress due to enzyme damage [19]. In this study, SOD activity in *H. brevisubulatum* leaves increased initially and then decreased under PEG treatment, consistent with previous studies on triticale [20].

Proline accumulation is a common adaptation mechanism in plants under drought stress, serving as an osmoprotectant and ROS scavenger [21]. The continuous increase in proline content observed in this study indicates its

important role in drought tolerance of *H. brevisubulatum*. Soluble sugars also function as osmolytes and energy sources during stress [22].

Transcriptome analysis revealed that DEGs were significantly enriched in pathways related to carbohydrate metabolism, amino acid metabolism, and ROS scavenging. The up-regulation of genes in arginine and proline metabolism, glycolysis, and peroxisome pathways suggests coordinated metabolic adjustments to maintain cellular homeostasis under drought stress. These findings provide valuable insights into the molecular mechanisms of drought tolerance in *H. brevisubulatum*.

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Note: Figure translations are in progress. See original paper for figures.