Activated charcoal and promotion of growth regulation in wheat seedlings: transcriptome analysis

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Abstract

Background

Activated charcoal (AC) is highly adsorbent and is often used to promote seedling growth in plant tissue culture, although the molecular mechanism remains unclear. In this study, global transcriptome analysis by RNA sequencing was performed on 10 day old seedlings prepared from both underground and aboveground growing conditions to provide new insights into seedling growth in the presence of AC.

Results

A total of 18,555 differentially expressed genes (DEGs) were identified, in which 11,182 were found in roots and 7,373 were found in shoots. In seedlings grown with AC in immature embryo culture, 9,460 DEGs were up-regulated and 7,483 DEGs were down-regulated compared to the control. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that 254 KEGG pathways were enriched in DEGs, 226 of which were common in roots and shoots. Further analysis of major metabolic pathways showed that AC stimulated the expression of nine genes in the phenylpropanoid biosynthesis pathway, including PLA, CYP73A, COMT, CYP84A, and 4CL, which promoted cell differentiation and seedling growth. In the plant hormone signal transduction pathway, AC up-regulated genes related to stress resistance and disease resistance including EIN3, BZR1, JAR1, JAZ, and PR1, and down-regulated genes related to plant growth inhibition, including BKI1, ARR-B, DELLA, and ABF.

Conclusions

Growth medium containing AC not only promoted seedling growth, but also enhanced resistance to stress and disease.

Background

Activated charcoal (AC) is a porous carbonized substance with a large inner surface area on which many substances can be adsorbed. AC is often used in plant tissue culture to
improve cell growth and development [1]. It can adsorb harmful substances in culture media including impurities in agar, 5-hydroxymethylfurfural produced by sucrose during high-pressure sterilization, phenoquinones secreted by explants during culture, as well as beneficial substances in culture media such as growth regulators, vitamin B6, folic acid, and nicotinic acid [2]. There are many reports on its effects such as anti-browning, improvement of primary culture survival rates, promotion of bud proliferation and seedling growth in a dark environment, and promotion of rooting [3]. In recent years, high-throughput sequencing technology has been widely used in plant mechanism research and its efficiency has dramatically improved [4-7]. In this study, the difference in gene expression of 10 d old wheat seedlings cultured on two media types (with and without AC) was analyzed by transcriptome sequencing technology, which laid a foundation for further study of gene expression and the mechanism used by AC to promote the development of immature wheat embryos. Genes that promote wheat growth were analyzed and excavated to provide a theoretical basis for breeding high-yielding wheat varieties.

Methods

Plant material

The middle part of young spikes of wheat (Triticum aestivum liang xing 99 from Liang xing seed institute) at 15 d post-bloom was peeled, sterilized with 1.5 % NaClO for 15 min, and rinsed thoroughly with distilled water. Then, immature embryos were peeled off and the scutella were downward inoculated in base medium (N6 supplemented with 0.02 mg/L NAA, 0.05 mg/L 6-BA) and NAC. Two biological replicates were prepared for each group, with 10 immature embryos in each replicate. Ten of the 5 d and 10 of the 10 d seedlings were taken to determine leaf length, root length, leaf number, root number, fresh weight of stem and leaf, and fresh weight of roots of single plants. Trait differences were
analyzed by SPSS18.0 software (IBM, USA). Forty of the 10 d cultured seedlings were removed from N6 and NAC media, and roots and shoots of seedlings from each tank were collected separately. Each sample comprised 20 independent shoots or 20 independent roots; two biological replicates were paired for each sample, immediately frozen in liquid nitrogen, and stored at -80 °C.

RNA isolation and cDNA library construction

Total RNA was isolated using a TRIzol total RNA extraction kit (Invitrogen, USA), which yielded ~10 µg of total RNA from each sample. RNA quality was examined by 0.8 % agarose gel electrophoresis and spectrophotometry. High quality RNA with 28S:18S > 1.5 and 260/280 absorbance ratios between 1.8 and 2.2 was used for library construction and sequencing. Illumina HiSeq library construction was performed according to the manufacturer's instructions (Illumina, USA). Magnetic beads with poly T oligos attached were used to purify mRNA from total RNA. RNA was broken into 200-300 bp fragments using ion interruption. Using RNA as the template, 6-base random primers and reverse transcriptase were used to synthesize the cDNA chain, and the first chain of cDNA was used as a template for the synthesis of the second chain of cDNA, where the base T was replaced by the base U. After construction of the library, polymerase chain reaction (PCR) amplification was used to enrich the library fragments, and then the library was selected according to fragment size, which was 300-400 bp. The library was then accessed using an Agilent 2100 Bioanalyzer (Agilent, USA). After RNA extraction, purification, and library building, based on the Illumina HiSeq sequencing platform, the samples were subjected to double-end sequencing using next-generation sequencing (NGS) to obtain raw reads (Shanghai Personal Biotechnology Co., Ltd., China).
RNA sequencing and transcript analysis

Raw reads were filtered before data analysis; high quality clean reads with $Q > 20$ were obtained for subsequent analysis. Reference genome data were derived from the Ensembl database (http://www.ensembl.org/). The reference genome index was created by Bowtie2 software [19], and the clean reads were compared to the reference index using Tophat2 (http://tophat.cbcb.umd.edu/). The Read Count value on each gene was compared using HTSeq0.6.1p2 (https://github.com/genepattern/HTSeq.Count) as the original gene expression level. Expression levels were normalized using RPKM (Reads Per Kilo bases per Million reads) with RPKM values $> 1$ as the gene expression standard [20]. Differential analysis of gene expression was performed using DESeq with more than a two-fold change of expression level ($\log_2$ fold change value $> 1$) and the expression with significant difference (P-value $< 0.05$) as the differential gene screening condition [21]. KEGG pathway analysis was used to analyze the metabolic pathways and signaling pathways in which DEGs were primarily involved.

Quantitative real-time PCR analysis

To validate the expressions of DEGs, 15 candidate DEGs were randomly selected for a quantitative real-time PCR (qRT-PCR) analysis. Selected gene names and primer information are listed in Table 1. The housekeeping gene in wheat, TaRP15, was used as an internal control [22]. Using 1 µg RNA from each sample as a template, cDNA was synthesized using a PrimeScript™ RT reagent Kit with gDNA Eraser (TakaRa, JPN). RT-PCR was performed using a SYBR Premix Ex Taq kit (TakaRa, JPN) on an ABI ViiATM7 instrument.
The comparative 2-ΔΔCT method of quantification was used to quantify the relative expression of specific genes [23].

Results

Effect of AC on wheat seedling growth

Results from 5 d and 10 d old seedling measurement in both media treatments showed that seedling growth potential on base medium with 4 g/L AC (NAC) was significantly higher than on N6. Leaf and root length, root number, plant height, and fresh weight of 10 d old seedlings were significantly higher than those of 5 d old seedlings grown on NAC medium. On N6 medium, shoot length and fresh weight of the 10 d old seedlings were significantly higher than those of the 5 d old seedlings, but roots stopped growing and showed atrophy (t-test, P < 0.05) (Figure 1, 2).

Illumina sequence analysis and validation of selected differentially expressed genes (DEGs) using quantitative real-time polymerase chain reaction (qRT-PCR)

Two biological replicates were processed for each treatment, and a total of 10 roots and 10 stems from the 10 d old seedlings were analyzed for correlation with the cDNA libraries. Results showed that correlation between biological replicates was high, indicating that the sequencing data was reliable and repeatable (Figure 3). After removing joints and low quality sequences in the raw reads, high quality clean reads of Q > 20 were obtained, where 255,820,114 clean reads were found in the aboveground treatment and 283,192,836 clean reads were found in the underground treatment. A total of 461,062,200 filtered clean reads were compared to reference genomes using Tophat2. The percentage of clean reads (85.6 %) (452,832,933) was compared to gene regions, 97.7 %
(442,365,747) of which were compared with exon regions, indicating that clean reads were mostly effective.

To confirm the DEGs identified from sequencing and computational analysis, 15 DEGs were randomly selected for qRT-PCR. Correlation analysis between differential gene expression levels in RNA-seq and qRT-PCR was performed after log2 transformation. The Pearson correlation coefficient was 0.992, which was significantly correlated at the 0.01 level (Figure 4), indicating that expression of these genes was consistent across RNA-seq and qRT-PCR data.

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of DEGs

A total of 18,555 genes with unchanged expression were found by DESeq (Version 1.18.0) including 1,182 DEGs in roots and 7,373 DEGs in shoots, with a total of 1,612 DEGs common between roots and shoots. For genes affected by AC, there were 9,460 up-regulated DEGs and 7,483 down-regulated DEGs identified between the AC group and control (Figure 5). To further identify functional pathways, DEGs were found in 254 KEGG functional pathways by KEGG pathway analysis; there were 226 common KEGG pathways between roots and shoots. Among these pathways, the Metabolic category (105, 39.10 %) represented the largest group, followed by Organismal Systems (58, 25.66 %), Environmental Information Processing (24, 10.62 %), Genetic Information Processing (21, 9.29 %), and Cellular Processes (18, 7.96 %). A p value < 0.05 was considered as the screening condition. KEGG enrichment analysis showed that the AC treatment regulated genes were enriched in 37 KEGG pathways in roots and 30 KEGG pathways in shoots, which included phenylpropanoid biosynthesis, plant hormone signal transduction, starch and sucrose metabolism, biosynthesis of amino acids, and other metabolic pathways.
(Figure 6). We analyzed three major metabolic pathways: (1) phenylpropanoid biosynthesis, (2) plant hormone signal transduction, and (3) starch and sucrose metabolism. In these three major pathways, there were 29 genes with changed expression between the AC group and control; 21 genes were up-regulated, which promoted cell differentiation, seedling growth, or enhanced stress and disease resistance (e.g., PLA, HCT, ZIM, and JAC), and eight genes were down-regulated, which were related to the inhibition of plant growth (e.g., BKI1, ARR-B, DELLA, and ABF) (Table 2).

Discussion

Using KEGG enrichment analysis, 169 DEGs were mapped to the plant hormone signal transduction pathway, which represents the second largest group among the mapped functional pathways. Ninety-six DEGs mapped in this pathway were up-regulated and 73 DEGs were down-regulated in the AC group. In this study, the addition of AC to seedling culture medium increased the expression of EIN3, BZR1, JAR1, JAZ, and PR1 genes. These genes were previously found to be involved in the plant hormone signal transduction pathway, which directly or indirectly play an important role in regulating plant stress resistance or disease resistance [12-16]. For example, PR1 is a type of water-soluble protein that is produced by plants infected by pathogens or stimulated by a biotic factor. Its main functions include attacking pathogens, degrading cell wall macromolecules, degrading pathogen toxins, and binding viral coat protein to plant receptor molecules [17]. Alternatively, the expression of inhibited plant growth regulating genes (BKI1, ARR-B, DELLA, and ABF) was reduced (Table 3). For example, DELLA protein is a transcription factor that acts as an inhibitor of the GA (gibberellin) signal transduction pathway; it is a type of growth inhibitory protein located in the nucleus, which can directly interact with proteins of key transcription factors in plants and plays a central role in many plant signaling activities [18]. The addition of AC to the seedling culture medium causes
changes in the internal hormones to enhance both stress and disease resistance of seedlings.

Conclusions

AC can significantly promote wheat seedling growth while enhancing resistance to stress and disease. Our transcriptome data provide new insights into gene expression influenced by AC. Results show that AC stimulates gene expression in phenylpropanoid biosynthesis to promote cell differentiation and seedling growth; both stress and disease resistance genes increased expression, and the growth inhibiting genes decreased expression through the regulation of plant hormone signal transduction. Results of this study preliminarily show that AC can significantly promote the molecular mechanisms of wheat seedling growth, which is helpful for further growth studies.

Abbreviations

AC, activated charcoal; DEGS, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; NAC, base medium with 4 g/L AC; PAL, phenylalanine ammonia-lyase; qRT-PCR, quantitative real-time polymerase chain reaction

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].
Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

SZ and FSD designed the experiments and wrote the manuscript. MYL, FSD, and JPW conducted the immature embryo culture and tissue sampling. XPS, YWL, and FY performed RNA extraction and qRT-PCR. FSD, HZ, and JFC analyzed the data. All authors read and approved the final manuscript.

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Appendix A.Supplementary data

Supplementary data to this article can be found online at

https://www.ncbi.nlm.nih.gov/sra/PRJN556084

References
1. Thomas TD. The role of activated charcoal in plant tissue culture. Biotechnol Adv. 2008;26(6):618-31.

2. Fridborg G, Eriksson T. Effects of activated charcoal on growth and morphogenesis in cell cultures. Physiol Plant. 1975;34(4):306-8.

3. Pan MJ, Van Staden J. The use of charcoal in in vitro culture—a review. Plant Growth Regul. 1998;26(3):155-63.

4. Liu C, Lu R, Guo G, He T, Li YB, Xu HW, et al. Transcriptome analysis reveals translational regulation in barley microspore-derived embryogenic callus under salt stress. Plant Cell Rep. 2016;35(8):1719-28.

5. Wang Y, Tao X, Tang XM, Xiao L, Sun JL, Yan XF, et al. Comparative transcriptome analysis of tomato (Solanum lycopersicum) in response to exogenous abscisic acid. BMC Genomics. 2013;14(1):841.

6. Han L, Li JL, Jin M, Su YH. Transcriptome analysis of Arabidopsis seedlings responses to high concentrations of glucose. Genet Mol Res. 2015;14(2):4784-801.

7. Zhao JL, Pan JS, Guan Y, Nie JT, Yang JJ, Qu ML, et al. Transcriptome analysis in Cucumis sativus identifies genes involved in multicellular trichome development. Genomics. 2015;105(5-6):296-303.

8. Hyun MW, Yun YH, Kim JY, Kim SH. Fungal and plant phenylalanine ammonia-lyase. Mycobiology. 2011;39(4):257-65.

9. Koukol J, Conn EE. The metabolism of aromatic compounds in higher plants IV. Purification and properties of the phenylalanine deaminase of Hordeum vulgare. J Biol Chem. 1961;236(10):2692-8.

10. Minami E, Ozeki Y, Matsuoka M, Koizuka N, Tanaka Y. Structure and some characterization of the gene for phenylalanine ammonia-lyase from rice plants. Eur J Biochem. 1989;185(1):19-25.
11. MacDonald MJ, D’Cunha GB. A modern view of phenylalanine ammonia-lyase. Biochem Cell Biol. 2007;85(3):273-82.

12. Konishi M, Yanagisawa S. Ethylene signaling in *Arabidopsis* involves feedback regulation via the elaborate control of EBF2 expression by EIN3. Plant J. 2008;55(5):821-31.

13. Oh E, Zhu JY, Wang ZY. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat Cell Biol. 2012;14(8):802.

14. Suza WP, Staswick PE. The role of JAR1 in jasmonoyl-L-isoleucine production during *Arabidopsis* wound response. Planta. 2008;227(6):1221-32.

15. Major IT, Yoshida Y, Campos ML, Kapali G, Xin XF, Sugimoto K, et al. Regulation of growth-defense balance by the JASMONATE ZIM-DOMAIN (JAZ)-MYC transcriptional module. New Phytol. 2017;215(4):1533-47.

16. Rushton PJ, Torres JT, Parniske M, Wernert P, Hahlbrock K, Somssich IE. Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes. EMBO J. 1996;15(20):5690-700.

17. Mitsuhara I, Iwai T, Seo S, Yanagawa Y, Kawahigasi H, Hirose S, et al. Characteristic expression of twelve rice PR1 family genes in response to pathogen infection, wounding, and defense-related signal compounds (121/180). Mol Genet Genomics. 2008;279(4):415-27.

18. Djakovic-Petrovic T, Wit M, Voesenek LACJ, Pierik R. DELLA protein function in growth responses to canopy signals. Plant J. 2007;51(1):117-26.

19. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9(4):357-9.

20. Wagner GP, Kin K, Lynch VJ. Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. Theory Biosci. 2012;131(4):281-85.
21. Anders S, Huber W. Differential expression of RNA-Seq data at the gene level—the DESeq package. Heidelberg: European Molecular Biology Laboratory (EMBL); 2012.

22. Xue GP, Sadat S, Drenth J, McIntyre CL. The heat shock factor family from *Triticum aestivum* in response to heat and other major abiotic stresses and their role in regulation of heat shock protein genes. J Exp Bot. 2013;65(2):539-57.

23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta DeltaC(T)) method. Methods. 2001;25:402-8.

### Tables

#### Table 1. Primer information for qRT-PCR

| Gene ID                  | Forward (5→3)                        | Reverse (5→3)                        |
|--------------------------|--------------------------------------|--------------------------------------|
| TaRP15                   | GCCACACGTGCTTTGCAGATAAG              | GCCCTCAAGCTCAACCATATA                |
| TRIAE_CS42_2DL_TGACv1    | CCCTGGGAGACTTTAGTGGGA                | CCCGTGCTTTCCCTTTGTTGGA               |
| TRIAE_CS42_7DS_TGACv1    | GCCAACCGCCTGGACGAGTA                 | CCATCCCTGCGATGACTCT                 |
| TRIAE_CS42_1DL_TGACv1    | TTTCACATCGGTGAATTTCC                 | CCCGTGATGGGTTGTC                    |
| TRIAE_CS42_4AS_TGACv1    | ATACGGGTTATATCTCC                  | CCCAGCCACGCTTCA                     |
| TRIAE_CS42_2AL_TGACv1    | AGGTGAAACCGCAAGGTA                   | AGGATGAAGTCCGTGTTGGA                 |
| TRIAE_CS42_2AS_TGACv1    | GGATGCCCCGTCGGCGAAGA                 | AGGATGAAGTCCGTGTTGGA                 |
| TRIAE_CS42_U_TGACv1      | GGACGCGGACTTCATCGC                  | TGGTGGGAGTTAGTCCGTAC                  |
| TRIAE_CS42_4BL_TGACv1    | CCTCCTCGCTCCGAAATA                   | CCAATACGTATCGCCACC                   |
| TRIAE_CS42_1DL_TGACv1    | AAGATGCTGGTATAGAGTGGT              | GGGTGGTGGTGCGTCCGGTTGGA              |
| TRIAE_CS42_5AL_TGACv1    | TGAACCTCGCATCTCGACAG                 | GGGTGGTGGTGCGTCCGGTTGGA              |
| TRIAE_CS42_4DL_TGACv1    | CACTGCTCTCCCTCT                  | TTCAATCCGCATCACCT                    |
| TRIAE_CS42_1BL_TGACv1    | TTAACAGCTCGGATGCGG                  | GGGTGGTGGTGCGTCCGGTTGGA              |
| TRIAE_CS42_5DL_TGACv1    | GTGACGGTGACGAGGTG                 | GGGTGGTGGTGCGTCCGGTTGGA              |
| TRIAE_CS42_U_TGACv1      | TGGACCGGAGCAGCGC                   | GGGTGGTGGTGCGTCCGGTTGGA              |
| TRIAE_CS42_2AS_TGACv1    | ATGAGGCAAGTATGGAGA                  | GGGTGGTGGTGCGTCCGGTTGGA              |

#### Table 2. Part of three major pathways and related genes identified by KEGG enrichment analysis

| Pathway | Gene ID | Fold change (NAC/N6) | Expression in NAC | Description |
|---------|---------|----------------------|-------------------|-------------|
|         |         |                      |                   |             |

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**Phenylpropanoid biosynthesis**

| Accession       | Gene ID       | Log2 Fold Change | Expression   |
|-----------------|---------------|------------------|-------------|
| TRIA_ECS42_1BS_TGACv1_049914_A01641 | 47.15         | up (PAL) phenylalanine ammm | |
| TRIA_ECS42_1DS_TGACv1_080107_A02393 | 12.22         | up (PAL) phenylalanine ammm | |
| TRIA_ECS42_1BS_TGACv1_049965_A01648 | 10.79         | up (PAL) phenylalanine ammm | |
| TRIA_ECS42_2AL_TGACv1_096113_A03172 | 10.21         | up (PAL) phenylalanine ammm | |
| TRIA_ECS42_6DL_TGACv1_527273_AA17016 | 8.24          | up (PAL) phenylalanine ammm | |
| TRIA_ECS42_1AS_TGACv1_019041_AA00587 | 7.14          | up (PAL) phenylalanine ammm | |
| TRIA_ECS42_3AL_TGACv1_194598_AA06365 | 9.78          | up (CYP73A) trans-cinnamati | |
| TRIA_ECS42_3B_TGACv1_220699_AA071585 | 6.61          | up (CYP73A) trans-cinnamati | |
| TRIA_ECS42_2BS_TGACv1_148390_AA04925 | 6.15          | up (CYP73A) trans-cinnamati | |
| TRIA_ECS42_5AL_TGACv1_137838_AA12530 | 5.77          | up (CYP73A) trans-cinnamati | |
| TRIA_ECS42_6DL_TGACv1_543204_AA17370 | 4.28          | up (COMT) caffeic acid 3-O-n | |
| TRIA_ECS42_6BS_TGACv1_514476_AA16603 | 2.19          | up (COMT) caffeic acid 3-O-n | |
| TRIA_ECS42_2BL_TGACv1_132718_AA04393 | 7.96          | up (CYP84A, F5H) ferulate-5- | |
| TRIA_ECS42_2AS_TGACv1_113803_AA03608 | 6.15          | up (4CL) 4-coumarate--CoA l | |
| TRIA_ECS42_6BL_TGACv1_502904_AA16266 | 5.14          | up (4CL) 4-coumarate--CoA l | |
| TRIA_ECS42_7BS_TGACv1_591841_AA19232 | 8.26          | up (HCT) shikimate O-hydroxycinnamoyltransfe | |
| TRIA_ECS42_2DS_TGACv1_178855_AA06018 | 5.80          | up (HCT) shikimate O-hydroxycinnamoyltransfe | |
| TRIA_ECS42_7AS_TGACv1_569782_AA18240 | 5.73          | up (HCT) shikimate O-hydroxycinnamoyltransfe | |
| TRIA_ECS42_3AL_TGACv1_194329_AA06311 | 2.42          | up (CYP98A, C3'H) S-O-(4-co-monoxygenase | |
| TRIA_ECS42_7BS_TGACv1_592306_AA19353 | 3.59          | up caffeoyl-CoA O-methyltra | |
| TRIA_ECS42_1DL_TGACv1_621454_AA20162 | 4.05          | up caffeoyl-CoA O-methyltra | |
| TRIA_ECS42_5DL_TGACv1_436308_AA14599 | 6.25          | up (CCR) cinnamoyl-CoA red | |
| TRIA_ECS42_5BL_TGACv1_406204_AA13421 | 5.56          | up (CCR) cinnamoyl-CoA red | |
| TRIA_ECS42_5AL_TGACv1_375041_AA12145 | 5.15          | up (CCR) cinnamoyl-CoA red | |
| TRIA_ECS42_3AS_TGACv1_321177_AA10566 | 2.09          | up (ARR-A) two-component r ARR-A family | |

**Plant hormone signal transduction**

| Accession       | Gene ID       | Log2 Fold Change | Expression   |
|-----------------|---------------|------------------|-------------|
| TRIA_ECS42_3B_TGACv1_221378_AA073875 | 20.04         | up (PYL) abscisic acid recept | |
| TRIA_ECS42_7DL_TGACv1_602538_AA19597 | 2.44          | up (EBF1_2) EIN6-binding F-t | |
| TRIA_ECS42_6BS_TGACv1_514535_AA16611 | 2.40          | up (EBF1_2) EIN3-binding F-t | |
| TRIA_ECS42_3DL_TGACv1_251912_AA08858 | 3.87          | up (EIN3) ethylene-insensitiv | |
| TRIA_ECS42_2DS_TGACv1_178626_AA05984 | 2.61          | up (BZR1_2) brassinosteroid | |
| TRIA_ECS42_1BL_TGACv1_030488_AA00922 | 5.34          | up (JAR1_4_6) jasmonic acid- | |
| TRIA_ECS42_4BL_TGACv1_320580_AA10437 | 6.24          | up (JAZ) jasmonate ZIM dom | |
| TRIA_ECS42_5BL_TGACv1_405157_AA13213 | 24.45         | up (PR1) pathogenesis-relate | |
| Gene Symbol | Description | Log2 Fold Change | Log2 Fold Ratio | Annotation |
|-------------|-------------|-----------------|----------------|------------|
| TRIAE_CS42_7DS_TGACv1_625472_AA20652 | (PR1) pathogenesis-related protein | 8.73 | up | |
| TRIAE_CS42_3B_TGACv1_221831_AA075087 | (AHP) histidine-containing protein | 0.32 | down | |
| TRIAE_CS42_7AS_TGACv1_569714_AA18224 | (ARR-B) two-component response regulator family | 0.31 | down | |
| TRIAE_CS42_7AS_TGACv1_569714_AA18224 | (DELLA) DELLLA protein | 0.31 | down | |
| TRIAE_CS42_3AL_TGACv1_197036_AA06644 | (ABF) ABA responsive element binding factor | 0.19 | down | |
| TRIAE_CS42_5BL_TGACv1_404247_AA12921 | (BKI1) BRI1 kinase inhibitor | 0.22 | down | |
| TRIAE_CS42_3DL_TGACv1_250531_AA08698 | (NPR1) regulatory protein | 0.48 | down | |
| TRIAE_CS42_2AS_TGACv1_114089_AA03639 | (otsB) trehalose 7-phosphatase | 10.52 | up | |
| TRIAE_CS42_2DS_TGACv1_178535_AA05972 | (otsB) trehalose 8-phosphatase | 4.84 | up | |
| TRIAE_CS42_1AL_TGACv1_003899_AA00518 | (TREH, treA, treF) alpha, alpha-trehalase | 2.29 | up | |
| TRIAE_CS42_1DL_TGACv1_061138_AA01866 | (TREH, treA, treF) alpha, alpha-trehalase | 2.03 | up | |
| TRIAE_CS42_3DL_TGACv1_249164_AA08400 | (scrK) fructokinase | 2.79 | up | |
| TRIAE_CS42_7DS_TGACv1_624145_AA20592 | (glgA) starch synthase | Inf | up | |
| TRIAE_CS42_4DS_TGACv1_361541_AA11698 | sucrose-phosphate synthase | 0.31 | down | |
| TRIAE_CS42_6DL_TGACv1_526359_AA16803 | (AMY, amyA, malS) alpha-amylase | 0.08 | down | |
| TRIAE_CS42_2DL_TGACv1_158310_AA05153 | (GBE1, glgB) 1,5-alpha-glucan branching enzyme | 0.43 | down | |

Figures
Figure 1

The growth potential of seedlings cultured for 5 and 10 days compared to the control.
Figure 2

Differences in 5 and 10 d seeding growth potential between NAC and N6 medium with regard to leaf length, root length, leaf number, root number, fresh weight of stem and leaf, and fresh weight of roots (t test, *: p < 0.05, **: p < 0.01, respectively).
Figure 3

Correlation of sample detection.
Correlation analysis of DEGs between RNA-seq and qRT-PCR. Scatter plot indicates the log2 transformed gene expression values in RNA-seq and qRT-PCR.
Figure 5

DEGs in wheat roots and shoots under added AC conditions compared to the control condition.

Figure 6

The major pathways under AC by KEGG enrichment analysis.