doi 10.18699/PlantGen-2025-291

Transcriptional activity of genes involved in the metabolism of abscisic acid in *Quercus robur* L. under drought conditions

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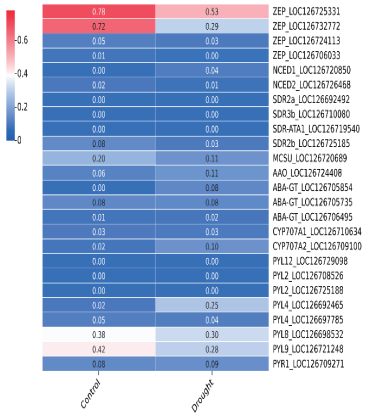
Motivation and aim: Evaluation of the transcriptional activity of genes involved in the abscisic acid metabolism in *Quercus robur* L. under soil drought conditions.
Materials and methods: *Q. robur* seedlings were divided into two groups of 10 plants each: control (75–80% moisture-holding capacity – MHC) and drought (8–12% MHC). Soil moisture was monitored daily using a Landtek MC-7828 SOIL moisture meter (AZ Instrument, China). Two weeks after the beginning of the experiment, leaf samples were collected, and total RNA was extracted using the R-Plants kit (Biolabmix, Russia) with DNase I treatment (Magen, China). RNA concentration was measured using a Qubit® 4 Fluorometer (Thermo Fisher Scientific, USA), while RNA quality was assessed based on A260/A280 and A260/A230 ratios using a SPECTROstar Nano spectrophotometer (BMG Labtech, Germany). Sequencing libraries were prepared following the Ligation Sequencing V14 protocol – Direct cDNA Sequencing (SQK-LSK114, version DCS_9187_v114_revI_31Jul2024). Sequencing was performed on a MinION device (Oxford Nanopore Technologies, UK) using MinKNOW 23.04.3 software. Base calling was conducted with the Dorado software (Oxford Nanopore, UK). Reads were mapped to the *Q. robur* reference genome (version 3.1, GCF_932294415.1) simultaneously with base calling. Gene read counts were quantified using samtools bedcov, and data normalization was performed using the RPG10K method (Reads Per Gene per 10,000 reads).
Results: The ZEP (zeaxanthin epoxidase) gene family exhibited high transcriptional activity in control group, particularly ZEP_LOC126725331 and ZEP_LOC126732772, with notable decrease under drought stress. This suggests that after two weeks of drought, the plants might have accumulated sufficient abscisic acid (ABA) levels to activate protective mechanisms. One of the key ABA biosynthesis regulators NCED1 and NCED2 (9-cis-epoxycarotenoid dioxygenase) showed low transcriptional activity in both groups. Similarly, the oxidation-stage enzymes SDR (short-chain dehydrogenase/reductase) and AAO (abscisic-aldehyde oxidase) demonstrated generally low expression levels, though AAO activity was nearly two-fold higher under drought conditions. ABA-GT (abscisate beta-glucosyltransferase) genes, responsible for ABA inactivation and long-distance transport of inactivated ABA, displayed enhanced activity during drought. In contrast, other ABA inactivation genes CYP707A1/2 (abscisic acid 8'-hydroxylase) remained weakly expressed in both conditions. Among ABA receptors, only PYL2_LOC126725188 showed increased transcriptional activity under drought, while other receptors maintained similar transcriptional activity between control and drought-stressed plants.



Transcriptional activity of genes involved in the metabolism of abscisic acid in *Q. robur* under drought conditions, which were identified using ONT long-read RNA-Seq technology

Conclusions: The study revealed drought-induced changes in ABA metabolism-related gene expression in *Q. robur*, including decrease of ZEP genes activity and increase of AAO and certain ABA inactivation genes activity. These results demonstrate complex regulation of ABA biosynthesis and catabolism under drought conditions, though further research is needed to fully understand drought adaptation mechanisms, particularly through investigating ABA dynamics at different stress stages.
Funding: The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation No. FNFE-2025-0013 "Regulation of gene expression involved in the adaptation to adverse environmental factors to improve economically valuable traits of woody and cultivated plants used in agroforestry".