

Mining differential gene expression in *Fagus crenata* seedlings in response to short-term soil drought stress

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Abstract

Despite concern about the stress drought imposes on tree species under a warming climate, their molecular responses to drought stress have not been well-documented. We analyzed the transcriptional response of seedling leaves after exposure to short-term drought stress in *Fagus crenata*. After well-watered and water-stressed treatments, we mapped the RNA-seq reads derived from sampled leaves and identified 127 differentially expressed genes (DEGs), of which 89 were up- and 38 down-regulated in water-stressed plants. Several dozen up-regulated DEGs were predicted to encode proteins that would facilitate mitigating processes or avoid the adverse effects caused by drought stress, including stomatal closure, reactive oxygen species (ROS) scavenging, abscisic acid (ABA) accumulation and response, and osmoprotectants. The evidence of down-regulation in several genes in response to drought stress was in accordance with the results of a literature survey. The functional category of sulfate assimilation was enriched in up-regulated DEGs, although there was also evidence of sulfur deficiency in the DEGs. These results suggest the existence of molecular mechanisms in beech that are common in other plant species, representing an acclimation response to drought stress as well as sulfur metabolism under drought stress conditions. This information provides the basis for further species-specific functional genomic research within the context of a warming climate.

Introduction

Fagus crenata, commonly known as Japanese beech, is a tall deciduous broad-leaved tree in the Fagaceae family; it is endemic to Japan and the dominant species in Japanese cool-temperate deciduous broad-leaved forests. However, it is predicted that global warming will reduce the habitat suitable for Japanese beech forests (Matsui et al. 2004). The main agent driving the decline of beech forests is increased evaporation of soil moisture, causing soil drought (Mouri & Shinoda 2005) and imposing physiological stress on the plants (i.e. drought stress). Climatic phenomena such as this are occurring more frequently, with time intervals shorter than those of tree turnovers (Hoffmann & Sgrò 2011). Given that seedlings are the most vulnerable to environmental stress compared with the other tree life history stages (Leck et al. 2008), investigating the short-term response of beech seedlings to drought stress is important for evaluating the ability of natural beech populations to persist in a future warming climate.

It is well-known that plants can respond rapidly to drought stress. Their physiological and morphological responses to short-term experimentally stimulated drought include cell elongation in leaves (Acevedo et al. 1971), retardation in cell wall and protein synthesis (Cleland 1967; Hsiao 1970), reductions in chlorophyll content as well as increased concentrations of reactive oxygen species, malondialdehyde (a marker for lipid peroxidation) (Chen et al. 2016), osmoprotectants such as free-proline, glycine betain and polyols (Ghosh et al. 2021), and reductions in tree size, specific leaf area, leaf relative water content and stomatal conductance (Amrutha et al. 2021). These changes in phenotypic traits over short timescales are driven by differential gene expression, which can be measured by assessing the expression levels of mRNA found in plant tissues (Shanker et al. 2014). Differential expression analyses comparing water-stressed plants with controls have identified candidate genes relating to acclimation under short-term drought stress in a wide range of species, including oak (Gugger et al. 2017), poplar (Yang et al. 2023) and pine (Li et al. 2024), as well as

annual plants such as maize (Le et al. 2012) and tomato (Liu et al. 2023). Recent advances in next-generation sequencing technology can yield large quantities of mRNA sequence data in a short time period (RNA-seq), and thus provide a cost-efficient and powerful tool for differential expression analyses (Deshpande et al. 2023). Thus, experimental research on stimulated drought stress in plants combined with RNA-seq technology can improve our understanding of the genomic background to the acclimation response of phenotypic traits.

Studies utilizing transcriptome profiles obtained from RNA-seq in the genus *Fagus* have focused on responses to drought stress in *F. sylvatica* (Müller et al. 2017) and flowering phenomena in *F. crenata* (Miyazaki et al. 2014; Satake et al. 2019): the molecular responses to drought stress remain unexplored in *F. crenata*. A previous study reported the species' phenotypical acclimation response to short-term drought stress in terms of biochemical concentrations in leaves (Ao et al. 2023). To better understand the species' potential for a rapid response to short-term stress, we performed a growth experiment under different watering regimes and obtained differential transcriptome profiles for the *F. crenata* seedlings using RNA-seq. A series of bioinformatic analyses were then conducted, including the mapping of RNA-seq reads to a reference genome, detection of differentially expressed genes (DEGs), annotations of gene ontology (GO) and metabolic pathways, and enrichment analyses. The specific objectives of the study were to (i) find putative genes associated with short-term drought stress and (ii) infer acclimation responses to short-term drought stress in *F. crenata* seedlings.

Material and Methods

Growth conditions and sampling for the drought stress experiment

On 13 July 2022, at Mokka-daira (35°54'N, 136°33'E, 957 m altitude), Mt Arajima (Fukui Prefecture, Japan), beech seedlings with two true leaves completely unfurled were dug up by their roots and transferred to polypots (12 cm in diameter × 10 cm in height) with local soil, and taken to the laboratory at Mie University (Mie, Japan). The seedlings were grown on in an environmental experimental room (LP1.9P-S, Nippon Medical & Chemical Instruments Co., Ltd, Japan) and were well-watered until the start of the experiment. From 25 July to 6 August 2022, the seedlings were grown for 12 days in an environmental experimental chamber (LH-241PFD-S, Nippon Medical & Chemical Instruments Co., Ltd). In the chamber, two watering regimes were applied as follows. Well-watered (WW) seedlings continued to be watered (100 ml per pot) once every 3 days, while water-stressed (WS) seedlings were not watered. There were five seedlings in each watering regime. There were no significant differences in seedling height between the treatments at the start of the experiment [WW: 9.3 ± 1.3 cm ($n = 5$), WS: 8.5 ± 1.4 cm ($n = 5$), t -test: $t = -0.852$, $P = 0.419$]. The positions of the pots were changed randomly every day to mitigate positional effects within the chamber. The environmental conditions in the chamber were: 25°C from 06:00 h to 18:00 h, 15°C from 18:00 h to 06:00 h; a photoperiod of 14 h light (06:00 h to 20:00 h)/10 h darkness (20:00 h to 06:00 h); and a photosynthetic photon flux density (PPFD) for the light period of 135–190 $\mu\text{mol}/\text{m}^2 \text{ s}$. The relative soil moisture content in the WW and WS treatment pots was measured at the beginning and end of the experiment, using a soil moisture meter (Lutron PMS-714, Taiwan) at a depth of 5 cm below the surface. On 27 July 2022, at the start

of the experiment, the average values for relative soil moisture content ($\pm$ SD) were 11.1% ($\pm$ 0.5) (n = 5) for WW, and 10.1% ($\pm$ 0.7) (n = 5) for WS. On 6 August 2022, at the end of the experiment, the values were 12.4% ($\pm$ 1.3) (n = 5) for WW, whereas for WS the soil in the pots had completely dried out (i.e. a value of 0.0%) (n = 5). These results confirmed that the WS beech seedlings had been subject to more drought stress than the WW seedlings.

At around 13:00–14:00 h on the last day of the experiment, two true leaves that had completed unfurled at the beginning of the experiment were collected per plant. These leaves were immediately frozen in liquid nitrogen and stored at -80°C until used for the RNA analyses. Total RNA was extracted from the leaves using a NucleoSpin[®] RNA Plant (TakaraBio Inc., Kusatsu, Japan) according to the manufacturer's instructions; 1 μl of the resulting RNA extract was used to measure the RNA concentration using a Qubit[®] 3.0 Fluorometer (ThermoFisher Scientific, Massachusetts, USA), and stored frozen at -80°C . The RNA samples were sent to Gene Bay Inc. (Tokyo, Japan), and the preparation of stranded RNA-seq libraries and next-generation sequencing by DNBSEQ-T7 (2x150 bp) (MGI Tech Co. Ltd) were performed at Novogene Co. Ltd (Beijing, China).

Transcriptome analyses

Adaptor sequences and low-quality regions were removed from the raw reads (DRR539888–DRR539897) for each sample using Trimmomatic (Bolger et al. 2014). Read quality was checked using FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The reads were then mapped to reference sequences for *F. crenata* (BKZX02000001–BKZX02000625, see also Supplementary Information Appendix 1, Table S1 and Table S2) using STAR (Dobin et al. 2013; Dobin & Gingeras 2015). The number of reads mapping to each gene was counted using RSEM (Li & Dewey 2011). In order to correct for any bias in the number of reads mapped to each gene as a result of variation in RNA quality [indicated by the RNA integrity number (RIN)] between samples, we calculated transcript integrity number (TIN) for each sample using RSeQC ver. 2.6.4 (Wang et al. 2016). Weighted local polynomial regression analyses were performed for the genes in each sample to which reads were mapped, with the ordinary logarithm of the number of reads as the response variable and the TIN as the explanatory variable (Wang et al. 2016). These corrected values were used in the following analyses to detect any differential gene expression between the treatments.

R package TCC (Sun et al. 2013) was used to detect differential gene expression between the treatments. The filterByExpr function in edgeR (Robinson et al. 2010) was used to filter out low-expression genes ("min.count" and "min.total.count" were set to 10 and 15, respectively). To normalize read counts and detect any differential expression, we applied DESeq2 (Love et al. 2014) in TCC, as DESeq2 has been reported to have superior detection capabilities when the number of samples in each treatment is small (< 12 samples) (Schurch et al. 2016). For the TCC analysis, the false discovery rate (FDR) set within the normalization procedure for the read count data was 0.05, the proportion of candidate differentially expressed genes removed during normalization (floorPDEG) was 0.05, the number of repeats for the above normalization procedure was 3, and the FDR for the detection of differentially expressed genes was set to 0.05. Genes with an FDR value of < 0.05 , as well as a $|\log_2\text{-fold change}| > 1$, were considered to be DEGs. In addition, based on normalized expression count data, a principal component analysis was used to visualize the results. These analyses were carried out using R 4.3.1 (R Development Core Team 2023).

A literature search was also carried out based on key terms within the names of the proteins encoded by the DEGs, as well as the phrases 'plant', 'drought stress', and 'water deficiency', which were input into search engines such as Web of Science, PubMed, and Google Scholar. The resulting literature was surveyed for whether the proteins discussed were involved in an acclimation response to drought stress showing the same trends as revealed in this study (i.e. up- or down-regulation).

To perform functional enrichment analyses, gene IDs from *Arabidopsis thaliana* were annotated with the reference genome as follows. A DIAMOND v2.0.15 blastp (Buchfink et al. 2021) search (with an E -value $< 10^{-5}$) was made against the protein database for *A. thaliana* (https://www.arabidopsis.org/download_files/Proteins/Araport11_protein_lists/Araport11_pep_20220914.gz). The results of the homology search were then input into Blast2GO Basic 6.0 to extract the *A. thaliana* gene IDs with the lowest E -values. Based on these annotated gene IDs, Metascape v3.5 (Zhou et al. 2019) was used to identify significantly enriched GO terms in the WS-specific up- and down-regulated DEGs, in which all the *Arabidopsis* gene IDs assigned to the beech reference sequence were used as a background. Similarly, enrichment of the Kyoto Encyclopedia of Genes and Genomes (KEGG) was conducted using the same Metascape. The FDR for the enrichment analyses was set at 0.05.

Results

Filtering low-quality reads for RNA sequences extracted from 10 samples of beech seedlings yielded a total of 51.6 Gb paired-end reads, with a total number of 347,371,569 reads (an average of 34,737,157 reads per individual) (Table S3). The reads were mapped to 25,883 of the 35,116 reference sequences, of which 17,533 were mapped to reads originating from all the samples. After correction based on TIN, the average number of reads mapped to the genes per sample was 13,543,365 (9,698,626–17,340,252).

There were 127 DEGs, of which 89 genes were up-regulated and 38 were down-regulated in WS plants (Fig. 1 and Fig. S1). This response was also apparent in a principal component analysis, in which samples collected from the WW plants clustered together and those from WS clustered together (Fig. 2). Based on the annotated database for the reference genome, the 82 up- and 35 down-regulated DEGs could be inferred to gene functions rather than "hypothetical protein" (Table S4 and Table S5). Furthermore, the literature survey identified 33 up-regulated DEGs that have been reported to encode proteins involved in acclimation responses to abiotic stresses, including drought stress (Table 1a). However, only two of the current down-regulated DEGs involving acclimation responses were supported by the literature survey (Table 1b).

Among the 8,868 GO terms found in our reference genome, up-regulated DEGs were annotated by 17 GO terms belonging to biological processes (14 terms) and molecular functions (three terms) (Table S6), whereas 14 GO terms comprised biological processes (eight terms) and molecular functions (six terms) in the down-regulated DEGs (Table S7). Based on the membership similarity between the GO terms, they were grouped into six and five clusters in up- and down-regulated DEGs, respectively (Table S6 and Table S7). In the up-regulated DEGs, two clusters contained significantly enriched GO terms, in which the representative terms with the lowest FDR were "cellular response to sulfur starvation" (GO:0010438) and "sulphate assimilation" (GO:0000103) in the first and second clusters, respectively (Table 2). No significantly enriched GO term was detected in the down-regulated DEGs.

Among the 141 KEGG pathways found in the reference genome, two pathways were detected in the up-regulated DEGs but were not significantly enriched (Table S8). No KEGG pathway was annotated in the down-regulated DEGs.

Discussion

DEGs of WS F. crenata seedlings

Of the 89 up-regulated DEGs in the WS plants, 33 genes could be annotated to encode proteins involved in acclimation responses to drought stress (Table 1a), whereas there were only two homologous genes associated with acclimation responses in the down-regulated DEGs (Table 1b). The additional literature survey identified several proteins encoded by genes down-regulated by drought stress, including a probable polygalacturonase (FCV25MIE_18960 in Table S5) in *A. thaliana* (Bray 2004), a putative pentatricopeptide repeat-containing protein (FCV25MIE_10987) (Pan et al. 2018) and LIM domain-containing protein WLIM2b (FCV25MIE_35051) (Yang et al. 2019) in foxtail millet (*Setaria italica*), and a DUF1666 domain-containing protein (FCV25MIE_10568) in sunflower (*Helianthus annuus*) (Wu et al. 2022). Although future studies should clarify the functions of such proteins in response to drought stress, our results suggest at least the existence of a molecular response to drought stress in beech, in common with other plant species.

There were several DEGs encoding for proteins related to detoxification of reactive oxygen species (ROS), including 5'-adenylylsulfate reductase (Park et al. 2012), homocysteine S-methyltransferase (Qiu et al. 2023), gamma-glutamylcyclotransferase (de María et al. 2020), Annexin (Konopka-Postupolska et al. 2009), S-adenosylmethionine synthase (Zhang et al. 2020b), ATP sulfurylase (Anjum et al. 2015), phenylcoumaran benzylic ether reductase (Niculaes et al. 2014), AAA-atpase asd (Xia et al. 2013) d box domain-containing protein (Song et al. 2017) in the up-regulated DEGs (Table 1a), and serine acetyltransferase (Ahmad et al. 2016) in the down-regulated DEGs (Table 1b). The accumulation of ROS through abiotic stresses, including water deficiency in the plant body, inflicts damage on cellular components through peroxidation of membrane lipids and the oxidation of proteins, DNA and RNA (Choudhury et al. 2017). Therefore, it is critical that plants protect themselves from harmful oxidants via detoxifying mechanisms, by using antioxidants and scavenging agents (Jubany-Marí et al. 2010). Our results suggest that detoxification of ROS is an available acclimation response to short-term drought stress in *F. crenata* seedlings.

Because abscisic acid (ABA) is a well-known trigger of the drought signaling cascade (Aslam et al. 2022), previous studies have reported genes potentially related to ABA metabolism (Long et al. 2019) and the ABA response (Umezawa et al. 2010). Our study identified an up-regulated DEG predicted to code for the B-box zinc finger protein (Table 1a), which regulates key genes related to ABA synthesis (*ABA1*, *ABA2*, *NCED3*) and those related to the ABA transduction pathway (*HY5* and *RD29A*) in the apple (*Malus domestica*) (Liu et al. 2019a). In addition, the homologous genes related to the ABA response under drought stress are found; serine hydroxymethyltransferase (Liu et al. 2019b), ninja-family protein AFP3-like (Rabara et al. 2015), serine/threonine-protein kinase SAPK3-like (Gao et al. 2018), MLO-like protein (Howlader et al. 2017) (Table 1a). In particular, serine hydroxymethyltransferase and MLO-like protein appear to relate to regulation of stomatal closure (Howlader et al. 2017; Liu et al. 2019b), which is in line with the increase seen in ABA concentration in leaves under drought stress conditions, to regulate stomatal closure and minimize water

loss from the leaves (Umezawa et al. 2010). These DEGs suggest that ABA-dependent responses lead to acclimation to short-term drought stress in the seedlings.

Avoidance of water loss from the plant body is often accompanied by changes in morphology and/or concentrations of biochemical molecules in the cells (Jaleel et al. 2009; Ghosh et al. 2021; Bawa et al. 2023). Our up-regulated DEGs encoded proteins involved with leaf morphology, including the transcription factor bHLH25-like (Table 1a), of which a homolog in rice is known to regulate positively the genes associated with cuticular wax biosynthesis, which mitigates water loss from the plant body (Gu et al. 2021), and xyloglucan endotransglucosylase/hydrolase in tomato, which is known to alter the cell-wall extensibility of guard cells mediated by cell-wall remodeling activity, positively regulating stomatal closure (Choi et al. 2011). There were also several up-regulated DEGs whose homologs are known to produce enzymes that facilitate the synthesis of osmotic adjustment substances (Table 1a), such as gamma-glutamylcyclotransferase for 5-oxo-proline (Xu et al. 2021), choline monooxygenase for glycine betaine (Russell et al. 1998), and NADP-dependent malic enzyme for malate (Sun et al. 2019). The accumulation of these substances in the cells increases cellular osmolality, which drives an influx of water or reduces its efflux, thus maintaining cell turgor pressure (Seleiman et al. 2021). A previous study has shown the accumulation of free-proline (a major osmoprotectant) in *F. crenata* seedlings exposed to drought stress for 12 days (Ao et al. 2023). This is in line with the present finding that the gene encoding proline-rich protein was down-regulated (Table 1b), as drought stress causes a drastic reduction in gene expression to avoid full use of the available proline molecules in plant cells, ensuring the availability of adequate proline molecules for osmolytes until their *de novo* synthesis begins (Gujjar et al. 2018).

As already shown for gamma-glutamylcyclotransferase, putative up-regulated DEGs encoding for proteins were inferred to regulate multiple acclimation processes (e.g. ROS detoxification, ABA metabolism and responses, and the production of osmoprotectants), including NAC domain-containing protein (Nakashima et al. 2012), ankyrin repeat-containing protein (Zhao et al. 2020), and glucosyltransferase families (crocin glucosyltransferase and 7-deoxyloganetin glucosyltransferase-like) (Liu et al. 2021) (Table 1a). In particular, NAC is a well-known transcription factor (TF) located upstream of the stress-tolerance pathways that regulate downstream genes (Nakashima et al. 2012). In plants, there are 11 major TFs (NAC, ERF, WRKY, bZIP, MYB, HD-ZIP, ZnF, bHLH, ASR, NF-Y, and HSF) that form a regulatory network in response to drought stress (Hu et al. 2022). Our present study found DEGs encoding for bHLH (basic/helix-loop-helix) as well as NAC TFs, whereas Matsuda et al. (2011) identified beech homologs of *MYB* genes with transcriptomes that varied with drought stress treatments over several hours. Given that the number of DEGs varies depending on the period and/or strength of drought stress [e.g. Müller et al. (2017) in *F. sylvatica*], more experiments are needed to clarify the regulatory network of TFs that target the downstream gene expression in response to drought stress in *F. crenata*.

GO enrichment and inference of metabolic processes in WS F. crenata seedlings

The GO term “cellular response to sulfur deficiency” was significantly enriched in the up-regulated DEGs in our beech seedlings, in accordance with other reports suggesting drought stress inhibits plants’ absorption of sulfur from soils (Lee et al. 2016). Sulfur starvation in the plant body has been reported to enhance the

activities of several enzymes relating to sulfur assimilation, including ATP sulfurylase (Takahashi et al. 2001) and 5'-adenylylsulfate reductase (an enzyme associated with the step following ATP sulfurylase in the sulfur assimilation process) (Takahashi et al. 1997). These enzymes are putatively encoded by the genes for FCV25MIE_22545 and FCV25MIE_32112 (Table 1a), which are annotated by the GO term “sulfate assimilation” (Table 2). ATP sulfurylase, an enzyme in the first committed step of sulfur assimilation, is considered to be sensitive to redox regulation in plants (Prioretti et al. 2014), and is negatively regulated by the concentration of cysteine, which is the final product of the sulfur assimilation process (Vauclare et al. 2002), and that of reduced glutathione (GSH), which is a major redox regulator synthesized from cysteine (Lappartient & Touraine 1996). In our study, because the gene encoding serine acetyltransferase, an enzyme that is essential for synthesizing cysteine (i.e. the endpoint of sulfur assimilation), was found to be down-regulated (Table 1b), cysteine concentrations would be low in the leaves, allowing the up-regulation of genes encoding ATP sulfurylase and 5'-adenylylsulfate reductase. In maize exposed to a restricted water supply for 12 days, leaves showed significant reductions in the activity of serine acetyltransferase, as well as cysteine concentrations, compared with those receiving a sufficient water supply, whereas there were substantial increases in cysteine concentrations, without the decrease in serine acetyltransferase activity, in the roots of the same plants (Ahmad et al. 2016). Ahmad et al. (2016) point out that re-translocation of sulfur occurs from the shoots to the roots in the plant body, while sulfur compounds accumulate in the roots and enable development of the root system and ROS detoxification under drought conditions. Further research should consider simultaneous examination of the transcriptome in leaves and roots, as well as their comparison, to facilitate our understanding of the overall mechanism of acclimation response to drought stress in *F. crenata* seedlings.

In conclusion, we have identified putative genes encoding proteins associated with short-term drought stress in beech seedlings, from which positive regulators against the adverse effects driven by drought stress can be inferred, including ABA metabolism and response, ROS scavenging, osmotic adjustment, and stomatal closure. Functional categories of genes related to sulfur and its metabolic processes were also enriched in the leaf transcriptome of seedlings exposed to drought stress, which might have been involved in the shoot-to-root sulfur translocation as a result of drought stress acclimation. These genes, as well as their annotated functions, could be used to search for range-wide as well as local genetic variation in relation to functional traits, including acclimation potential to drought stress. This would provide crucial information for establishing a species-specific conservation policy within the context of a future warming climate.

Declarations

Competing interests

The authors declare no competing interests.

Data Archiving Statement

The raw data generated in this study were archived in the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (DRA) under accession number PRJDB15599.

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Author contributions

Conceptualization: TT and HA; Methodology: TT and HA; Formal analysis and investigation: TT and HA; Writing - original draft preparation: TT and HA; Writing - review and editing: YA, SA, YO, YM, HK, and NT; Funding acquisition: TT and NT; Resources: YA, SA, YO, YM, and HK. All authors read and approved the final manuscript.

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Tables

Table 1 Based on a literature survey, the (a) up-regulated and (b) down-regulated differentially expressed genes of *Fagus crenata* seedlings whose functions in response to abiotic stress can be inferred

(a)

Locus_tag in the beech reference genome*	Putative protein	Involvement in functions related to abiotic stress	References
FCV25MIE_12444	Serine hydroxymethyltransferase 7	ROS scavenging and stomatal closure	Liu et al. (2019b)
FCV25MIE_07246	Transcription factor bHLH25-like	Cuticular wax biosynthesis	Gu et al. (2021)
FCV25MIE_33838	Transcription factor bHLH113		
FCV25MIE_16266	DNA damage-repair/toleration protein DRT102	DNA repair	Li et al. (2016)
FCV25MIE_05115	5'-adenylylsulfate reductase 1 chloroplastic-like	ROS-related defense pathway	Park et al. (2012)
FCV25MIE_32112	5'-adenylylsulfate reductase 3 chloroplastic-like		
FCV25MIE_08137	Ninja-family protein AFP3-like isoform X1	ABA response	Rabara et al. (2015)
FCV25MIE_22587	Homocysteine S-methyltransferase 3	ROS scavenging	Qiu et al. (2023)
FCV25MIE_25115	Serine/threonine-protein kinase SAPK3-like	ABA metabolism	Gao et al. (2018)
FCV25MIE_12920	Gamma-glutamylcyclotransferase 2-1-like	ROS scavenging	de María et al. (2020)
FCV25MIE_30298	Ankyrin repeat-containing protein At5g02620-like isoform X1	Proline accumulation, antioxidant, and activation of stress-related TFs	Zhao et al. (2020)
FCV25MIE_15802	Ankyrin repeat-containing protein		
FCV25MIE_30221	Ankyrin repeat and protein kinase domain-containing protein 1		

Table 1 *Continued*

Locus_tag in the beech reference genome*	Putative protein	Involvement in functions related to abiotic stress	References
FCV25MIE_28347	NAC domain-containing protein 86-like	Regulation of osmoprotectant and antioxidants, and regulation of stress-tolerant genes	Nakashima et al. (2012)
FCV25MIE_05204	Xyloglucan endotransglucosylase/hydrolase protein 9	Stomatal closure via cell-wall remodeling	Choi et al. (2011)
FCV25MIE_23256	S-adenosylmethionine synthase	ROS scavenging and ABA metabolism	Zhang et al. (2020b)
FCV25MIE_00576	Auxin-responsive protein SAUR68-like	Regulation of plant growth	Bouzroud et al. (2018)
FCV25MIE_17904	Annexin D3	ROS scavenging	Konopka-Postupolska et al. (2009)
FCV25MIE_17905	Annexin D4		
FCV25MIE_14061	Wall-associated receptor kinase-like 10 isoform X1	Regulation of plant growth	Zhang et al. (2020a)
FCV25MIE_14564	Wall-associated receptor kinase-like 8		
FCV25MIE_22545	ATP sulfurylase 1 chloroplastic-like isoform X1	ROS scavenging	Anjum et al. (2015)
FCV25MIE_19833	UDP-glycosyltransferase 74B1-like	ABA response	Rabara et al. (2015)
FCV25MIE_27360	Crocetin glucosyltransferase chloroplastic-like	ABA accumulation, stomatal closure, ROS scavenging, accumulation of osmoprotectant, and up-regulation of stress-related gene expressions	Liu et al. (2021)
FCV25MIE_15921	7-deoxyloganetin glucosyltransferase-like		

Table 1 *Continued*

Locus_tag in the beech reference genome*	Putative protein	Involvement in functions related to abiotic stress	References
FCV25MIE_32676	MLO-like protein 12	ABA response	Howlader et al. (2017)
FCV25MIE_23637	Phenylcoumaran benzylic ether reductase Betv6	ROS scavenging	Niculaes et al. (2014)
FCV25MIE_08783	AAA-atpase asd mitochondrial	ROS scavenging	Xia et al. (2013)
FCV25MIE_13390	U-box domain-containing protein 21-like	ROS scavenging	Song et al. (2017)
FCV25MIE_20137	U-box domain-containing protein 19-like		
FCV25MIE_30527	Choline monooxygenase chloroplastic	Glycine betaine production	Russell et al. (1998)
FCV25MIE_12792	NADP-dependent malic enzyme isoform X2	Malate production	Sun et al. (2019)
FCV25MIE_33628	B-box zinc finger protein 32-like	ABA response and ROS scavenging	Liu et al. (2019a)

(b)

Locus_tag in the beech reference genome*	Putative protein	Involvement in functions related to abiotic stress	References
FCV25MIE_26236	Serine acetyltransferase 3 mitochondrial	ROS scavenging	Ahmad et al. (2016)
FCV25MIE_24515	14 kDa proline-rich protein DC2.15-like	Regulation of proline production level	Gujjar et al. (2018)

* Obtained from our *de novo* assembly, which has been registered in DDBJ (accession no. BKZX02000001-BKZX02000625).

ROS, reactive oxygen species.

ABA, abscisic acid.

Table 2 Significantly enriched gene ontology (GO) terms in the differentially expressed genes (DEGs) up-regulated by drought stress in *Fagus crenata* seedlings

ID ^a	Category	Description	Log ₁₀ (<i>p</i> -value ^b)	Log ₁₀ (FDR ^c)	Test group ^d	Reference group ^e
GO:0010438	Biological processes	Cellular response to sulfur starvation	-6.607	-2.659	3/70	3/10996
GO:0000103	Biological processes	Sulfate assimilation	-5.071	-1.424	3/70	7/10996

^a The GO terms detected were clustered based on their membership similarity (see also Supplementary Information Table S4), and those exhibiting the lowest significant log₁₀(false discovery rate; FDR) are shown here.

^b Based on the cumulative hypergeometric distribution

^c Based on the Benjamini-Hochberg procedure to account for multiple testing when a given *Q* number of GO terms was typically identified in the reference genome, as follows. First, all *p*-values were sorted from small to large. Second, given a *p*-value of *p* at rank *i*, it would be expected that *pQ* GO terms could be found with the same or a better *p*-value by chance under a Bonferroni correction. As we only observed *i* such GO terms, the portion of our observations that were false (i.e. FDR) was min(*pQ*/*i*, 1). See Table S6 for full list of FDR.

^d The number of DEGs annotated by the corresponding GO term/the number of all DEGs annotated by GO terms.

^e The number of genes annotated by the corresponding GO term in the reference genome/the number of all genes annotated by GO terms in the reference genome.

Figures

Fig. 1

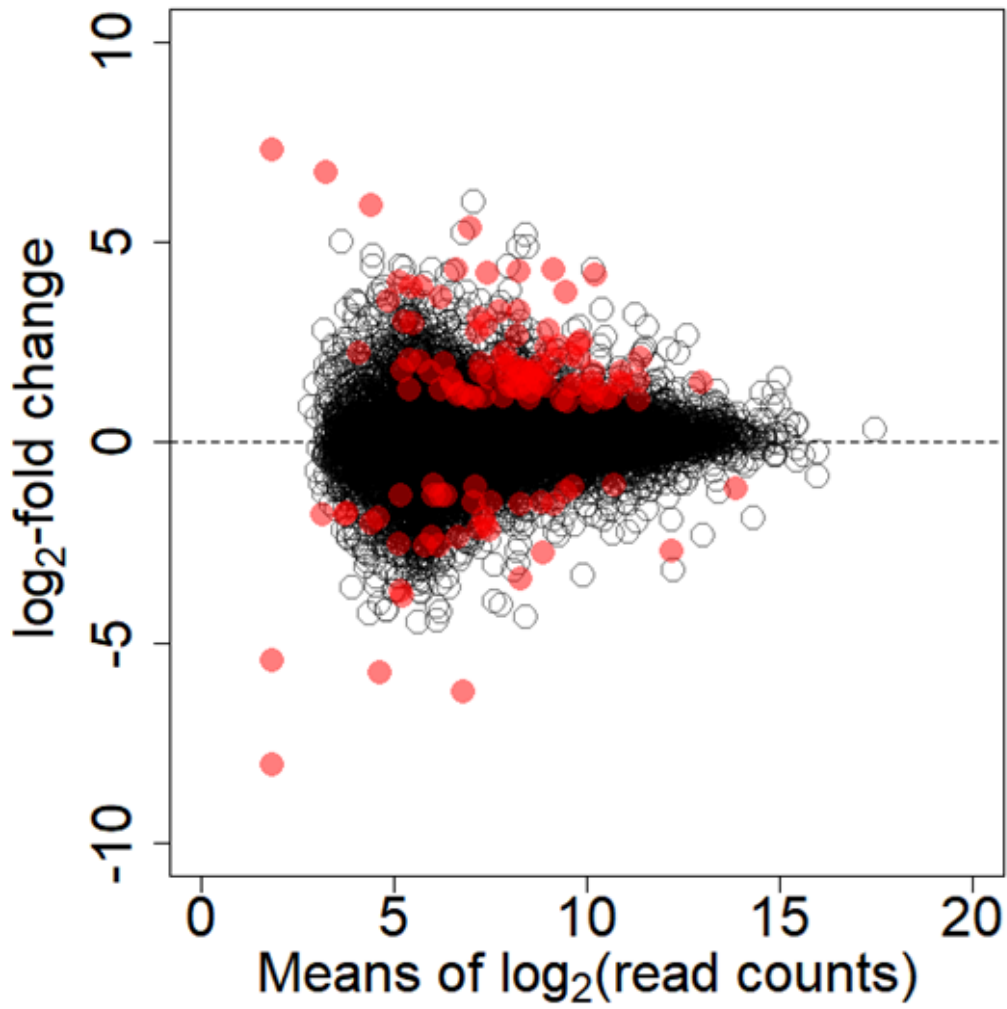


Figure 1

Log₂-fold gene expression change versus mean expression level for drought stress-treated *Fagus crenata* seedlings. Red points are statistically significant after false discovery rate (FDR) adjustment.

Fig. 2

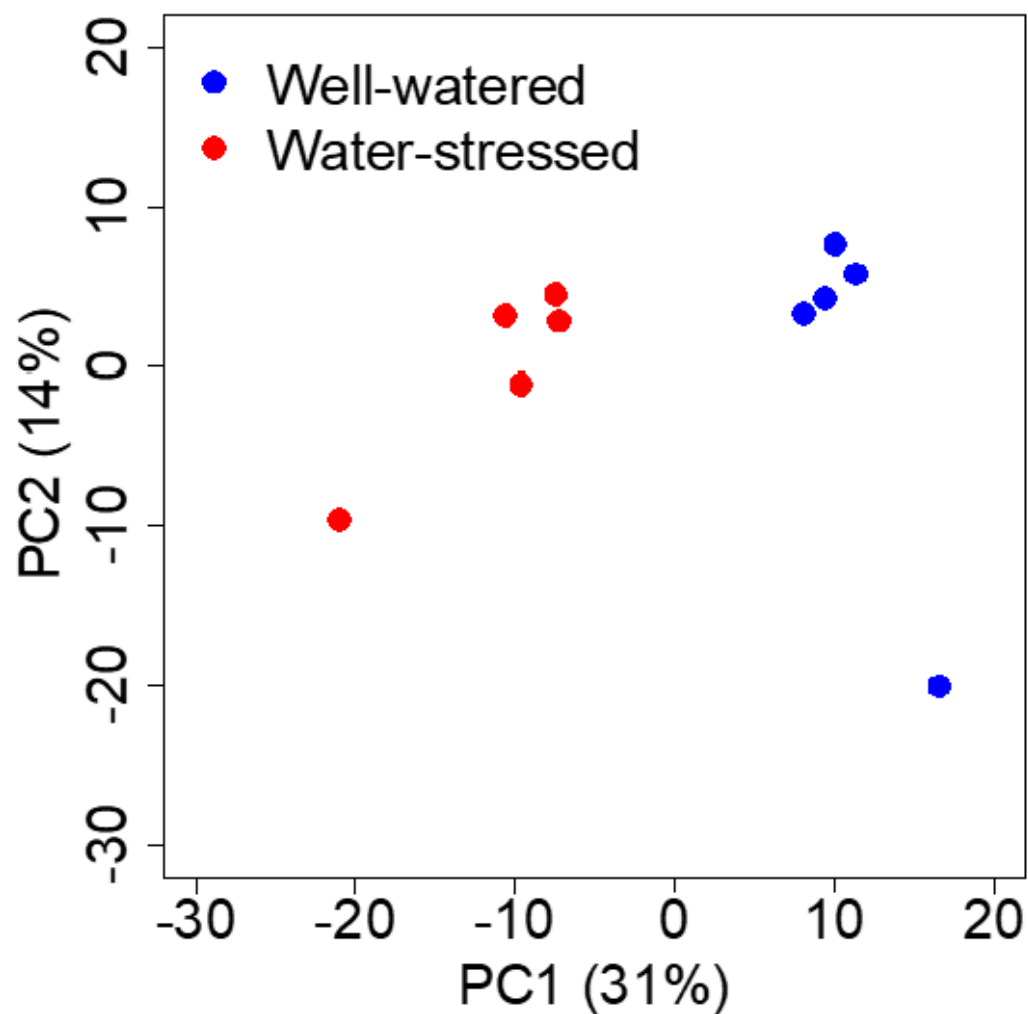


Figure 2

Principal component (PC) analysis of gene expression data for the 500 most variable genes in *Fagus crenata* seedlings. All samples from the well-watered treatment clustered on the right and all those from the water-stressed treatment clustered on the left.

Supplementary Files

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- [BeechshorttermtranscriptomeSlver.3.pdf](#)