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Molecular mechanism of drought stress tolerance in barley (*Hordeum vulgare* L.) via a combined analysis of the transcriptome data

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Electronic Supplementary Material (ESM)

The authors are fully responsible for both the content and the formal aspects of the electronic supplementary material. No editorial adjustments were made.

SUPPLEMENTARY FILE 1

Commonly, each GSE was analysed separately following:

– The data and AnnotGPL were download by getGEO.
– The data were extracted by the exprs method and the log2 fold change was investigated.
– The data were normalised by the normalizeQuantiles method through the Limma package
– Data quality was controlled using boxplot plot.
– RNA quality was checked by the AgiMicroRna package.
  – boxplotMicroRna and RleMicroRna for normalised data were drawn.
  – hierclusMicroRna was drawn.
– The groups were determined by factor (groups, levels = unique(groups)) and model. matrix.
– The means were calculated for every group through lmFit.
– Log2 fold change was determined using makeContrasts and contrasts.fit methods.
– Statistical analysis was performed by eBayes.
– Groups were analysed by topTable method and djust.method = "fdr" was used.
– Some factors including: PROBEID, Gene.ID, Gene. title, Gene. symbol, log FC, AveExpr and adj. P. Val were selected for future analyse.
– Statically table, Probe ID and AnnotGPL table were combined.

For merging *P*-values which are unadjusted, the sum logs of the Fisher method were performed. For setting the combined *P*-values for the datasets, the p.adjust function in metaRNASeq was applied employing the 'fdr' method. In case the *P*-values were ≤ 0.01 and median log2-fold change values were >1 and < –1, the genes were considered as differentially expressed.

Overall, to analyse the data, the following packages were used:

```
BiocManager::install("Biobase")
BiocManager::install("GEOquery")
BiocManager::install("limma")
BiocManager::install("AgiMicroRna")
BiocManager::install("plier")
install.packages("metaRNASeq")
```