DOI 10.18699/SBB-2022-350

Differential gene expression analysis in barley Nud gene mutants

Korotkova A.M.[1]*, Skripileva A.I., Vihorev A.V.[1], Kolosovskaya E.V.[1], Gerasimova S.V.[1, 2], Khlestkina E.K.[3]

[1] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*
[2] *Novosibirsk State University, Novosibirsk, Russia*
[3] *N.I. Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg, Russia*
** korotkova@bionet.nsc.ru*

Key words: CRISPR, transcriptome, Nud, barley

Motivation and Aim: Barley (*Hordeum vulgare* L.) is an important food crop in the world and in Russia in particular. Naked barley provides some benefits in terms of human nutrition and health. The *Nud* gene encodes a transcription factor conceivably involved in the formation of a lipidic layer at the grain surface of hulled barley. If the *Nud* gene function is lost, lemma and palea do no longer adhere to the grain's pericarp, which entails the non-adherent hull (i.e. naked) phenotype. In a recent study, we obtained *nud* loss-of-function lines by site-directed mutagenesis using customized RNA-guided Cas9 endonuclease in the spring barley cultivar 'Golden Promise'.
Methods and Algorithms: One of the obtained *Nud* mutant barley line showed the best results in analysis of agronomically relevant properties has been chosen for transcriptome analysis. The purpose of the experiment is to obtain a set of genes involved in the formation of the nakedness property in barley. Isogenic lines with contrasting phenotypes are a perfect experimental model system for the elucidation of gene function. For the experiment, two isogenic lines (differing only in one *Nud* gene) were taken: control Golden promise line and *nud* mutant line. Grain's pericarp were collected from the grain at two stages of spike development:
I). After the end of flowering (early milk development),
II). After filling the grain (dough development – the scales begin to adhere to the grain).
RNA was isolated from the samples and transferred for transcriptome analysis.
Results and Conclusion: After analysis we've got a large table with transcription activity levels of more than 20,000 genes. We selected a group of genes among them with upregulation (51) in the mutant and a group of genes with downregulation (185). 19 genes were chosen for further analysis. Primers were picked for each transcribed gene sequence, and upregulation or downregulation of the expression of these genes was confirmed using quantitative PCR analysis.
Acknowledgements: This work is carried out with the support of the Russian Science Foundation No. 21-66-00012.

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