doi 10.18699/bgrs2024-06-35

Development of genetic models for validation of candidate genes associated with cold stress response in tea plant

Egorova A.[1, 2]*, Fizikova A.[1, 3], Fomin I.[2], Kostina N.[2], Gerasimova S.[1, 2], Samarina L.[1, 3], Malukova L.[1]

[1] *Federal Research Centre the Subtropical Scientific Centre, RAS, Sochi, Russia*
[2] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*
[3] *Sirius University of Science and Technology, Sirius, Russia*
* *egorova@bionet.nsc.ru*

Key words: *Camellia sinensis*; Cas9/gRNA; protoplasts; genetic transformation

Motivation and Aim: Tea (*Camellia sinensis* (L.) Kuntze) is a perennial woody plant, one of the most important agricultural crops for human consumption, medicinal and functional purposes, grown in more than 60 countries worldwide. Cold is the main factor limiting the expansion of tea plantations to more northern latitudes and limiting their productivity. However, knowledge of cold response in woody plants, including tea, is fragmented and the genetic basis of cold tolerance requires further investigation. The genetic diversity of tea in the Northwest Caucasus can serve as a valuable source of cold tolerant germplasm for the development of new tolerant cultivars. Kolkhida is the best local large leaf tea cultivar, known for its high-quality leaves used in black and green tea production. Transcriptional analysis of cv. Kolkhida identified candidate genes involved in long-term cold stress responses [1]. The aim of this study is to functionally validate these candidate genes using knockout and overexpression methods.
Methods and Algorithms: Cas9/gRNA-mediated site-directed mutagenesis was used to knock out candidate genes in cv. Kolkhida. Overexpression of candidate genes was performed in *Nicotiana tabacum* SR1 plants.
Results: Two candidate genes (*COR413PM1-like* and *ELIP1*) were selected for functional validation. Two target sites were selected in the coding sequences of each gene. Cas9/gRNA vectors were constructed for the target sites in these genes. The extraction and transformation of protoplasts from young tea leaves was established and the mutagenic activity of the vectors was demonstrated in protoplasts. The cas9/gRNA-carrying cassettes were then introduced into the binary vectors which are planned to be used for stable transformation of tea and subsequent regeneration of plants carrying mutations in the two target genes.
The candidate genes were cloned from the cv. Kolkhida and overexpression vectors were constructed. *Agrobacterium*-mediated genetic transformation of *N. tabacum* was performed, resulting in ten transgenic lines for each gene. Based on qPCR analysis, five lines with the highest expression for each gene were selected and T1 generations were obtained.
Conclusion: During this work, the development of genetic models was initiated, which will be used to study the functions of candidate genes in response to cold stress.
Funding: The study is supported by a grant from the Russian Science Foundation (No. 23-46-00002).

References

1. Samarina L., Wang S., Malyukova L. et al. Long-term cold, freezing and drought: Overlapping and specific regulatory mechanisms and signal transduction in tea plant (*Camellia sinensis* (L.) Kuntze). *Front Plant Sci.* 2023;14:1145793. doi 10.3389/fpls.2023.1145793