

Regeneration and transformation of plants from the Fabaceae family

V.E. Tvorogova^{1,2*}, E.A. Potsenkovskaia², V.Y. Simonova², K.V. Smirnov³, E.P. Efremova¹, Z.S. Konstantinov¹, A.V. Brynchikova², A.M. Artemiuk¹, D.B. Pavlova¹, D.V. Yakovleva¹, T.V. Dubenko⁴, A.A. Vanina¹, L.A. Lutova^{1,2}

¹ St Petersburg State University, Saint Petersburg, Russia

² Sirius University of Science and Technology, Sirius Federal Territory, Krasnodar region, Russia

³ All-Russia Research Institute for Agricultural Microbiology, Saint Petersburg, Russia

⁴ Federal Research Center N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), Saint Petersburg, Russia

* krubaza@mail.ru

Motivation and aim: Fabaceae family includes a lot of well-known agriculturally important species, which are currently gaining a special significance due to their high protein content and possibility of their usage as an alternative for meat. Nevertheless, the development of new cultivars with modern genetic engineering methods is hampered for many leguminous species, due to their recalcitrance to transformation and regeneration. Therefore, the investigation of factors, influencing on transformation effectiveness and regeneration of plants from transformed cells, is of great significance for Fabaceae family. Our research is focused on studying genetic control of regeneration and identifying genes capable of inducing somatic embryogenesis – a specific regeneration pathway – in leguminous species.

Materials and methods: We use *Medicago truncatula* as a model Fabaceae species. For *M. truncatula*, several lines contrasting in their capacity for somatic embryogenesis were obtained, which can be used to identify inducers and suppressors of this process. We also use *Pisum sativum* and *Lens culinaris*, species recalcitrant for transformation, to check the effects of ectopic expression of potential regeneration stimulators. The search of genes regulating somatic embryogenesis is performed using transcriptomic analysis, as well as functional analysis with evaluation of effects of CRISPR/Cas9 mediated knock-out and overexpression of potential regeneration regulators.

Results: We found several genes encoding for transcription factors from the WOX family and the NF-Y group, which can induce somatic embryogenesis or callus formation in *M. truncatula* when overexpressed. Such genes include *MtWOX9-1*, *MtWOX2*, and members of NF-YB and NF-YA families. CRISPR/Cas9-mediated knockout of these genes in *M. truncatula* demonstrated their importance for plant development *in vivo* and/or *in vitro*. The effects of ectopic expression of these identified regulators on regeneration in other leguminous species are currently under investigation.

Conclusions: Several transcription factors from the WOX family and the NF-Y group were shown to influence the development of embryogenic callus in *M. truncatula*. We are currently evaluating the possibility of their usage for transformation of other leguminous species.

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