

The screening vector system of morphogenic regulators in *Fabaceae*

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Legumes are important agricultural and food crops, however, some legume species have a low regenerative capacity *in vitro*, which complicates obtaining the genetically modified plants with improved properties and analysing gene function.

To search for genes that stimulate somatic embryogenesis and to increase the regeneration frequency in legumes *in vitro*, we designed a screening vector system that will allow faster cloning of genes encoding potential regulators of morphogenesis by preserving restriction sites in the final vector. The construction of vectors is based on the Golden Gate [1] modular cloning method. Using type II restriction endonucleases, DNA fragments form sticky ends and are combined in a given order to form multigene constructs intended for *Agrobacterium*-mediated transformation. In order to identify efficient variants for gene expression, we used a number of promoters: CaMV 35S(long), CaMV 35S(double), nopaline synthase (nos), actin 2 (act2), and a number of terminators: 35S CaMV, nos, act2 for *MtWOX9-1* overexpression. *Medicago truncatula* WOX9-1 (*MtWOX9-1*) is a WUSCHEL-related homeobox transcription factor for which a positive effect on the formation of somatic embryos in callus culture was previously shown [2].

Based on the analysis of embryogenic tissue, the optimal combination of promoters and terminators will be selected to assemble the vector for screening of morphogenetic regulators.

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REFERENCES

1. Weber E, Engler C, Gruetzner R, et al. A modular cloning system for standardized assembly of multigene constructs. *PLoS One*. 2011;6(2):16765. DOI: 10.1371/journal.pone.0016765
2. Tvorogova VE, Fedorova YuA, Potsenkovich EA, et al. The WUSCHEL-related homeobox transcription factor MtWOX9-1 stimulates somatic embryogenesis in *Medicago truncatula*. *Plant Cell Tissue and Organ Culture*. 2019;138(3):517–527. DOI: 10.1007/s11240-019-01648-w

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