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Gene coexpression for stimulating regeneration in *Medicago truncatula*

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Motivation and aim: Somatic embryogenesis is used in tissue culture in the process of plant transformation and also in order to accelerate plant breeding. The WOX family proteins play a significant role in this process, as they are involved in the growth and development of plants. There are also NF-Y complex proteins which participate in the regulation of different aspects of plant development, including regeneration. NF-Y is a transcription factor composed of subunits A, B, and C, making it a heterotrimer. We previously demonstrated that the MtWOX9-1 protein stimulates somatic embryogenesis, whereas the MtNF-YB10 protein as well as other proteins from the NF-Y group also participate in this process in *Medicago truncatula*. Our goal is to identify combinations of genes from *WOX* and *NF-Y* groups that stimulate regeneration in *M. truncatula*.
Materials and methods: Genetic constructs for coexpression of *MtWOX9-1* and different *MtNF-Y* genes were created using Golden Gate method. Embryogenic (R108) and non-embryogenic (108-1) *Medicago truncatula* lines were transformed by the AGL1 strain of *Agrobacterium tumefaciens* using the modified AGROBEST protocol. *MtNF-YB3* gene was edited using the CRISPR/Cas9 system. Protein interactions were assessed using yeast two-hybrid system.
Results: At this stage of the research, genetic constructs for co-expression of *MtWOX9-1* and different *MtNF-Y* genes were introduced into *Agrobacterium tumefaciens,* and we plan to transform the non-embryogenic 108-1 line with these constructs to assess their ability to induce somatic embryogenesis. It was also found that according to the yeast two hybrid analysis MtWOX9-1 interacts with some proteins from the NF-YA, NF-YB and NF-YC families. Constructions encoding for chimeric proteins including MtWOX9-1 and MtNF-Y protein, are also checked for their ability to stimulate somatic embryogenesis.
Recently, we have shown that *MtNF-YB10* loss of function is not detrimental to plant development, although it significantly inhibits somatic embryogenesis. To further study and compare the functions of *MtNF-YB10* and its closest homolog, *MtNF-YB3*, we are obtaining a plant line with loss of *MtNF-YB3* function.
Conclusions: The MtWOX9-1 protein was found to interact with several different NF-Y proteins in yeast cells. At present, constructs for co-expression of *MtWOX9-1* and different *MtNF-Y* genes, as well as constructs encoding for chimeric proteins consisting of MtWOX9-1 and NF-Y proteins are obtained. Their capacity for stimulation of somatic embryogenesis is under investigation.
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