WOX family genes in somatic embryogenesis

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Somatic embryogenesis (SE) is one of the plant regeneration pathways. This process is very similar to zygotic embryogenesis, but embryos develop not from zygote, but from vegetative tissues. This process is widely used in biotechnology for plant transformation and propagation. Somatic embryos can derive directly from the vegetative tissues or through the formation of callus. The search for SE stimulators is very important for plant biotechnology.



We study SE in *Medicago truncatula* (*Fabaceae*). There are several Medicago lines, differing in capacity for somatic embryogenesis *in vitro*.



Several genes were reported to be the regulators of SE, among them the WOX (WUSCHEL-RELATED HOMEOBOX) family members are presented. These genes encode homeodomain-containing transcription factors, participating in different developmental processes.

WOX2 is known for its participation in zygotic embryogenesis. It is expressed in the zygote and later in the apical domain of the embryo. *WOX2* determines, together with the *WOX8* and *WOX9* genes, the formation of embryo domains. Several *WOX2* orthologs have been shown to be expressed during somatic embryogenesis in different species.

The aim of the project was to study the MtWOX2 role in the SE in M. truncatula



Due to qPCR results, expression of *MtWOX2* does not increase significantly during SE neither in non-embryogenic line, nor in embryogenic one.



But overexpression of *MtWOX2* in some cases leads to the development of embryogenic calli with increased size.



MtWOX2 overexpression



Wild type



We performed transcriptome analysis of *Medicago truncatula* calli with overexpression of this gene compared to the calli overexpressing *GUS*. It was shown that *MtWOX2* overexpression led to the changes in expression levels of genes, enriched with several GO pathways, including groups related to oxidative stress and ROS formation, response to toxic substance and auxinactivated signaling pathway. Among differentially expressed genes there are members of several TF families, e.g. MADS-box, BHLH, MYB, bZIP and others.







Previously, our laboratory has already performed transcriptome analysis of calli with overexpression of another member of the WOX family, *MtWOX9-1*. We compared the data obtained in the present study with the available data on overexpression of *MtWOX9-1*.



MtWOX9-1 is a participant in somatic embryogenesis; its overexpression leads to a significant increase in the embryogenicity of callus tissue and to changes in the expression levels of a number of genes involved in somatic embryogenesis.

GO enrichment analysis of genes, up-regulated by MtWOX9-1 and down-regulated by MtWOX2

During SE, MtWOX9-1 and MtWOX2 affect significantly overlapping gene sets and their influence is mostly opposite.



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OddsRatio

Pvalue

Count

2.55.0

• 7.5

10.0

0.008

0.006

0.004