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Auxin-related functions of LEAFY COTYLEDON2 gene in the induction of somatic embryogenesis in Arabidopsis

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Somatic embryogenesis (SE) induced *in vitro* exemplifies the developmental plasticity of the plant somatic cells while genes that trigger embryogenic transition in differentiated explant cells are intensively identified. A significant progress in SE studies has been made recently owing to the application of expressive genomic tools, transgenic lines and mutants of Arabidopsis, a model plant in genomics. Several transcription factor (TF) genes were indicated to be essential for SE induction and among them LEAFY COTYLEDON2 (LEC2), a master regulator of zygotic embryogenesis (ZE) in Arabidopsis. To reveal the genetic components of LEC2-dependent pathway of SE induction, explants of Arabidopsis undergoing embryogenic transition on 2,4-dichlorophenoxyacetic acid (2,4-D) -supplemented medium were studied. Gene expression profiling and mutant/transgenic line analysis indicated auxin related functions of LEC2 during SE induction. Accordingly, LEC2 was found to promote the embryogenic pathway in somatic cells through stimulation of auxin biosynthesis. Three of YUCCA (YUC) genes, YUC1, YUC4 and YUC10 which encode flavin monooxygenases involved in tryptophan depended pathway of auxin biosynthesis, were proven to be up-regulated by LEC2 in explants undergoing SE induction. In addition, GFP-monitored expression of YUC genes was detected in SE-involved regions of the explants i.e. cotyledons, shoot apical meristem and developed somatic embryos. Further support on the essential role of YUC genes in SE induction allowed to observe embryogenic capacity of yuc mutants. Two of them, yuc2 and yuc4, were found to display an impaired embryogenic potential. Relevantly to the hypothesis on LEC2-stimulated activity of auxin biosynthesis genes in SE induction, an elevated level of indole-3-acetic acid (IAA) was observed in tissues overexpressing LEC2 gene. The results also suggest that LEC2 may impact SE induction through the regulation of the components of auxin signaling pathway. Accordingly, two of AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) genes, the key regulators of auxin-response pathway, IAA30 and IAA31, were observed to be highly stimulated by LEC2 during SE. However, the relation between LEC2 and AUX/IAA genes needs further analysis.

Auxin-stimulated expression of LEC2 and a presence of Auxin Response Element (AuxRE) in LEC2 regulatory region suggest that the gene may be controlled by AUXIN RESPONSE FACTORS (ARFs), the key regulators of auxin-responsive genes. Thus, to identify ARFs engaged in SE induction, especially those involved in regulation of LEC2 activity, an expression profiling of all 22 ARF genes of Arabidopsis was conducted in an embryogenic culture. The qRT-PCR and GFP-reporter lines analysis indicated that the majority (14) of ARFs were active in tissues subjected to SE induction. Moreover, the arf mutants and overexpressor lines were evaluated in the embryogenic culture. ARFs with a significantly modulated expression in SE coupled with an impaired embryogenic response of the relevant mutant and/or overexpressor line were identified, including ARF1, ARF2, ARF3, ARF5, ARF6, ARF8 and ARF11. Among the candidate ARFs involved in SE induction, ARF5 encoding MONOPTEROS (MP) protein of a key role in ZE, was indicated. ARF5 was found to be highly up-regulated in SE and arf5 mutant displayed a distinctly reduced SE response. However, ARF5-regulated targets that control SE induction remain to be revealed.