Competency for shoot regeneration from Arabidopsis root explants is regulated by DNA methylation

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Abstract

Plants exhibit high capacity to regenerate in three alternative pathways: tissue repair, somatic embryo-genesis and de novo organogenesis. For most plants, de novo organ initiation can be easily achieved in tissue culture by exposing explants to auxin and/or cytokinin, yet the competence to regenerate varies among species and within tissues from the same plant. In Arabidopsis, root explants incubated directly on cytokinin-rich shoot inducing medium (SIM-direct), are incapable of regenerating shoots, and a pre-incubation step on auxin-rich callus inducing medium (CIM) is required to acquire competency to regenerate on the SIM. However the mechanism underlying competency acquisition still remains elusive. Here we show that the chromomethylase 3 (cmt3) mutant which exhibits significant reduction in CHG methylation, shows high capacity to regenerate on SIM-direct and that regeneration occurs via direct organogenesis. In WT, WUSCHEL (WUS) promoter, an essential gene for shoot formation, is highly methylated, and its expression on SIM requires pre-incubation on CIM. However, in cmt3 WUS expression induced by SIM-direct. We propose that pre-incubation on CIM is required for the re-activation of cell division. Following the transfer of roots to SIM, the intensive cell division activity continues, and in the presence of cytokinin leads to a dilution in DNA methylation that allows certain genes required for shoot regeneration to respond to SIM, thereby advancing shoot formation.