CONSTITUTIVE COSUPPRESSION OF THE GA 20-OXIDASE 1 GENE IN TOMATO LEADS TO SEVERE DEFECTS IN VEGETATIVE AND REPRODUCTIVE DEVELOPMENT

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Knowledge on the role of hormones in plant growth and development has resulted from the studies of exogenous application and endogenous measurements of hormone levels in various wild-type and mutant genotypes. The recent identification of several genes encoding hormone biosynthesis and response-related enzymes has greatly facilitated our understanding of the function and regulation of plant growth regulators. For instances, along the gibberellin (GA) biosynthesis pathway, the transcription of genes involved in the final part of the metabolic chain has been reported as being strictly controlled by both endogenous and exogenous cues. In particular, the transcription of the GA 20-oxidase (GA20ox) gene family is recognized as a key regulation step for the control of active GAs. In tomato, GA20ox genes form a small multigenic family composed of three members, which are differentially transcribed during development. Previous results obtained in our laboratory have shown that LeGA20ox1 is a tightly regulated gene, which responds to the stimulus of pollination and fertilization to drive the growth of the ovary into a fruit.

To study in more depth the role of LeGA20ox1 in the vegetative and reproductive development of tomato plants, a cosuppression approach was undertaken in order to silence the expression of the gene. A 638-bp truncated 5’ sequence of the gene was cloned in sense orientation downstream to the CaMV 35S promoter in the pBI121 vector. Cotyledon explants from cv Chico III tomato plants were transformed with the described construct via Agrobacterium-based standard protocols. Seven primary transformants that grew extremely slowly were rescued, controlled with PCR amplification and grown in tunnel; all of them showed a more or less severe brachitic habit. Since such plants were not fertile when open pollinated, one plant showing a severe dwarf phenotype was selected and in vitro micropropagated in order to have a clone of 25 individuals. Untransformed control plants were obtained by in vitro propagation of a wild-type near-isogenic plant. Transgenic plants were dramatically affected in the vegetative development; internodes were much shorter than in the control determining the severely dwarf phenotype. Moreover, transformed plants showed leaflets that were smaller and narrower than the control.

Although no difference was noticed in the inflorescence phenotype, flowers of the cosuppressed plants were somehow defective in comparison to the control: the insertion of floral organs was not regularly alternated, the corolla was paler and the anther cone was more elongated and slightly distorted. Male sporogenesis was severely affected in transformed plants and the number of viable pollen grains was very low. In the pistil, ovule development and female meiosis appeared unaffected and ovaries were...
regularly stimulated to grow when pollinated with normal pollen. On going pollination experiments will reveal if and at which extent the inhibition of ga biosynthesis is important in the completion of seed and fruit development. If these plants will prove to be female fertile, they could be easily crossed with mutants involved in hormone synthesis or response or with other genetic stocks suitable to study the interaction of gas with other classes of growth regulators.