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CAMV 35S PROMOTER-REGULATED EXPRESSION OF GREEN FLUORESCENT PROTEIN IN COTTON: FROM TRANSIENT ACTIVITY TO TRANSGENIC PLANTS

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Abstract

CaMV 35S promoter is the most commonly used promoter for investigating transgene activity in a transient and a stable manner in plants. It is also the promoter that drives the *Bt* gene in Bollgard cotton. Though it is presumed to be a constitutive promoter, some reports suggest that it is not expressed in all cell-types. In addition, the information available on its expression profile in all possible cell- and tissue-types and during early stages of development is incomplete. We have examined activity of this promoter using the green fluorescent protein (GFP) gene as a reporter system following T-DNA transfer to cotton cells, from transient expression to regenerated, stably transformed plants. The promoter/GFP system proved to be very effective in elucidating the cell-types that were competent for *Agrobacterium*-mediated transformation. Majority of the wounded cells in the cotyledon expressed GFP transiently while only a limited number of cells in the stele area of the hypocotyl segments showed transient activity. Stable transformation events from both explants showed good activity from callus to different stages of regeneration. Following regeneration, the promoter expression profile was studied during development of zygotic embryo and in all the vegetative and floral cell- and tissue-types. GFP expression was not detected during the early stages of embryogenesis. The first perceptible GFP expression was observed in a small area at the junction of hypocotyl and cotyledons in embryos at around 13 days post anthesis. The GFP fluorescence progressively became stronger and expanded throughout the cotyledon and hypocotyl as embryo development advanced. Following germination, varying levels of promoter activity were observed in all cell- and tissue-types in the hypocotyl, cotyledon, stem, leaf, petiole, and root. The promoter also expressed in all floral parts. Although cotton-pollen exhibited a low-level of greenish autofluorescence, it was possible to discern GFP-dependent fluorescence in some of the pollen from all the T0 plants examined. Developing cotton fibers also exhibited GFP fluorescence suggesting that the 35S promoter was active in these specialized epidermal cells. Thus, we show that the expression of the 35S promoter was developmentally regulated during embryogenesis and that beyond a certain stage during embryogenesis, the promoter was expressed in most cell- and tissue-types in cotton albeit at different levels.