

Grapevine Protoplasts as a Transient Expression System for Comparison of Stilbene Synthase Genes Containing cGMP-Responsive Promoter Elements

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A method for preparing elicitor-responsive protoplasts from grapevine cells kept in suspension culture was established. The protoplasts were employed in order to perform transient gene expression experiments produced by externally added plasmids. Using the gene coding for bacterial β -glucuronidase as the reporter gene, the transient expression under the control of various promoters of stilbene synthase genes were analyzed. The elicitor-responsiveness of promoters from grapevine genes and heterologous promoters were assayed: the grapevine stilbene synthase gene VST-1 and pine stilbene synthase genes PST-1, PST-2 and PST-3. Compared to the expression effected by the cauliflower mosaic virus 35S RNA-promoter, the stilbene synthase promoters caused a 2–5-fold increase in GUS-activity. Incubation of transformed protoplasts with fungal cell wall further stimulated the stilbene synthase promoters but not the 35S RNA-promoter. An even more pronounced differentiation between the promoters was observed when cGMP was included in the transient expression assays. Instead of treating transformed protoplasts with fungal cell wall we administered simultaneously cGMP and the plasmid to be tested. The cGMP-responsive increase was (a) specific concerning the nucleotide applied, (b) characteristic of grapevine protoplasts, and (c) not seen with shortened promoter-GUS constructs or GUS under the control of the 35S RNA-promoter. The highest cGMP-dependent response to stress was shown by the promoter of the grapevine stilbene synthase gene VST-1.

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