

Cloning and Characterisation of Promoter for N-Methyl Transferase Gene from Coffee

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SUMMARY

A PCR based method for walking in uncloned genomic DNA was adopted to successfully isolate and clone the 5' regulatory region of a coffee N-Methyl Transferase (NMT) gene. Sequence analysis performed with the aid of the Plant CARE, PLACE and PATCH programs, part of the TRANSFAC database, resulted in the identification of several core fragments of great importance for promoter function. The whole fragment was fused translationally in frame to the reporter gene β -glucuronidase (*uidA*) to study the ability of this genomic DNA fragment to direct gene expression. The resulting promoter + *uidA* translational fusion construct was used in *Agrobacterium tumefaciens* mediated transformation of *Nicotiana tabacum*. GUS assays showed that the isolated promoter directed the expression of the reporter gene in transgenic tobacco plants. A series of deletions in the NMT promoter region linked to the β -glucuronidase (*uidA*) coding region are being constructed and these will be analyzed for GUS activities by stable expression in transformants of *Nicotiana tabacum*.