Validation of Coffee seed promoter in *Nicotiana tabacum*

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Promoters that restrict transgenic expressions in a specific organ or tissue and, at a certain stage of plant development or in response to stress are extremely desirable for the improvement of agronomic species by transgenesis. Coffee is among the five most valuable crops to developing nations, and its production is of great economic interest to Brazil. Commercial production is based mainly on the specie *Coffea arabica*, which is tetraploid, perennial and has low genetic variability, impairing the genetic improvement by traditional methods. Thus, genetic modification appears as a good way to obtain coffee plants more suitable for different purposes. The aim of this work was to isolate an active promoter from endosperm of coffee seeds. Initially, a contig preferentially expressed in coffee fruits was identified by Electronic Northern, using the database of the Brazilian Coffee Genome Project. The organ specificity of the transcript was confirmed in fruit, particularly in seed endosperm, by RT-PCR, RT-qPCR and Northern blot. Then, the promoter was isolated by the 5' RACE method using the Universal Genome Walker Kit (Clontech). A fragment of 1.2 kb and three truncated versions (407 bp, 785 bp and 1.0 kb) were isolated, starting at the first ATG codon of the protein, in order to evaluate the expression profile of different modules. The sequences were in silico analyzed and all fragments exhibited promoter-specific *cis* elements. In the present work we constructed expression cassettes in which the four versions of the promoter were cloned in the binary vector pBI121 by replacing the 35S promoter that regulates the β-glucuronidase (*uidA*/*gus*) gene. The constructed binary vectors were used to transform tobacco (*Nicotiana tabacum*) through *Agrobacterium tumefaciens*. Histochemical analysis of GUS activity show that the 1.2 kb, 1.0 kb e 785 pb fragments promotes expression in leaves, flowers and fruits, with differences in the activity level, and all three fragments have low activity in roots. Finally, the 407 bp fragment promotes the expression of Gus only in tobacco seeds. Future work will be performed to determine in which seed tissue this promoter is active and which domains are involved in the tissue-specificity.