

Survey of Endogenous β -glucuronidase (GUS) Activity in Coffee Tissues and Development of an Assay for Specific Elimination of this Activity in Transgenic Coffee Tissues

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SUMMARY

The β -glucuronidase (GUS) gene (*uidA/gusA*) of *E.coli* is often used as a reporter gene to study the expression of foreign genes and genetic engineering of crop plants because of advantages over other reporter genes. However, the presence of endogenous GUS activity could cause difficulties in the assay and result in false positives. To avoid these difficulties we conducted a histochemical survey of endogenous GUS activity in various untransformed tissues of coffee (*Coffea arabica* and *C. canephora*) using X-gluc as substrate and compared with that of transformed embryogenic calli and somatic embryos carrying *gusA*-intron gene. We tested the effect of pH between 5 and 9 and methanol (20%) on GUS activity. Different untransformed tissues of coffee revealed various levels of endogenous GUS activity. Endosperm, integument, zygotic embryos, somatic embryos and stigmas exhibited a high level of endogenous GUS activity. Hypocotyl, ovary and pericarp showed medium level endogenous GUS activity, while the leaf, root, embryogenic and non-embryogenic calli, corolla tube and anthers showed low activity. Style did not exhibit any GUS activity. In all untransformed tissues, maximum GUS activity was noticed at pH 5 which was reduced with increasing pH. Methanol also reduced endogenous GUS activity in untransformed tissues. Contrary to the results obtained with untransformed tissues, the transformed tissues showed positive GUS reaction at all the pH tested and the intensity of GUS reaction was more in presence of methanol. Histochemical GUS assay reaction carried out at pH 9 in presence of 20% methanol did not detect endogenous GUS activity in untransformed coffee tissues but detected strong expression of transgenic bacterial GUS activity.