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Agrobacterium-mediated transformation and detection of GUS reporter gene activity in IAC sugarcane genotypes

Boer, APM[1,2]; Festucci, CDS[2]; Oliveira, IC[2]; Landell MGA[2]; Molinari, HBC[3]; Goldman, MHS[1]; Creste, S[2];

[1] Laboratory of Plant Molecular Biology - Department of Genetics, Faculty of Medicine of Ribeirao Preto, USP.
[2] Centro de Cana - Instituto Agronômico de Campinas - IAC
[3] Embrapa Agroenergia

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Sugarcane (*Saccharum spp*) is a major crop in Brazil, being the main source of raw material for producing sugar and ethanol. Despite the importance of the culture for the country, the average national productivity is low, with an average of 85 t/ha. The main strategy for increasing productivity is the development of improved cultivars, coupled with an optimized management system. Thus, new *in vitro* techniques such as somatic embryogenesis and *Agrobacterium*-mediated transformation show great potential for application in breeding programs to increase productivity. The present study evaluated the potential of genetic transformation of three sugarcane genotypes (IACSP96-2042, IACSP96-3060 and IACSP95-5000) belonging to the IAC sugarcane breeding program, considering their regeneration and *in vitro* morphogenesis capacities. Embryogenic calli induced with 2.4 D were used as starting material since they exhibited *in vitro* regeneration rates suitable for transformation protocols. We have tested the use of two *Agrobacterium tumefaciens* strains (C58C1 and EHA-105) and cultures at optical densities (OD) of 0.4, 0.6, and 0.8 The binary vectors used were pSoup and pBract304, which have the BAR and GUS coding sequences under the control of the *Ubi-1* promoter. The plates containing the explants in selective culture media were transferred to the germination chamber and incubated under appropriate conditions of light and temperature for plant regeneration. The highest regeneration rate (~ 58%) was achieved with the genotype IACSP96-3060, inoculated with the strain C58C1 at a bacterial OD of 0.4 and an initial concentration of 1 mg/L of PPT. The lowest rate (~ 4%) was obtained with the genotype combination IACSP96-3060, strain EHA-105 and a bacterial OD of 0.8. We have been able to obtain PPT-resistant (5 mg/L) and GUS-positive shoots only for the IACSP95-5000 genotype, with a variable frequency between 3.3% and 6.6%. Many factors seem to affect the efficiency of *Agrobacterium*-mediated transformation of sugarcane. Undoubtedly, the explant source and the plant genotype have been suggested by several authors as key factors in the success of genetic transformation. Moreover, the correct choice of bacterial strains and OD's also appear to be relevant to achieve a good efficiency in the transformation process. Thus, the establishment of more efficient protocols for sugarcane transformation will contribute for developing commercial interesting transgenic sugarcane varieties as well as for studies focusing on sugarcane functional genomics.

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