Plant Molecular Biology **20**: 357–359, 1992.

Update section

Short communication

Particle bombardment-mediated transient expression of a Brazil nut methionine-rich albumin in bean (*Phaseolus vulgaris* L.)

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Received 13 February 1992; accepted 7 May 1992

Key words: Brazil nut, 2S albumin gene, gene transfer, *Phaseolus vulgaris*, particle bombardment

Abstract

Bean (*Phaseolus vulgaris* L.) mature embryos were transformed using biolistic methods with a plasmid containing 2S albumin and β-glucuronidase structural sequences, both under the control of the 35S CaMV promoter. We have shown that chimaeric tissues could be obtained and that both structural sequences were expressed to similar levels.

Legumes are economically important crops and there is a worldwide interest that they become amenable through recombinant DNA technology. We have isolated and characterized a gene from Brazil nut encoding a methionine-rich protein (2S gene) [1]. However, the lack of systems to introduce foreign genes into beans and soybeans has seriously hampered studies on the expression of this gene in transgenic plants. Based on the biolistic concept [4], we have developed a simple and quick protocol to introduce the 2S gene in *Phaseolus vulgaris* cv. Carioca tissues. The method involves the use of an electrical particle acceleration gun [6]. Embryonic axes excised from mature seeds were used in the bombardment experiments. Per bombardment, 250 μg of gold particles (1–3 μm) and 1.8 μg DNA were used. Coating procedures and bombardment conditions are specified elsewhere [6]. The vector constructed, p35SBN, is a pTZ18 derivative that includes the complete sequence encoding the 2S [1] and the β-glucuronidase (GUS) [3] coding region, each under the control of a 35S CaMV promoter (short version, not enhanced). After bombardment, the embryonic axes were incubated on basal medium [5] without growth regulators at 28 °C for 24 h. Subsequently, the axes were frozen in liquid nitrogen and ground to a fine powder. Proteins were extracted in 10 mM sodium phosphate buffer pH 7.2, containing 0.5 M NaCl, 1 mM PMSF and 10 mM 2-mercaptoethanol. Samples of the extracted proteins were fractionated using SDS-PAGE and analysed through immunodetection methods. The antibodies utilized were risen in BalbC mice against $HPLC^-$ and subsequently SDS-PAGE-purified 2S albumins and against SDS-PAGE-purified β-glucuronidase (Sigma). Details on antibody preparation

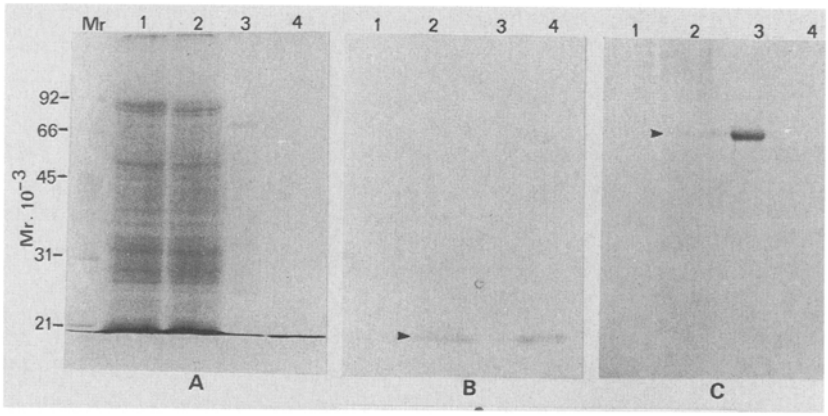

Fig. 1. A. SDS-PAGE of total protein from: (1) unbombarded and (2) bombarded bean embryonic axes; (3) β-glucuronidase protein (SIGMA); (4) methionine-rich 2S protein purified from Brazil nut. B and C. Western blots of the same gel as in A, with 2S and GUS antibodies respectively. The arrows in lanes 2 refer to the expression of 2S and GUS proteins in bombarded embryonic axes. Each slot was loaded with a 500 μg of total protein.

as well as western blot and ELISA techniques are given in Grossi de Sa *et al.* [2]. Western blots showed that the polypeptides which react with the polyclonal antibodies specific for the 2S and GUS proteins respectively, are present in bombarded but not in control tissues (Fig. 1). A single bombarded embryonic axis expressed enough proteins to be detected by the assay. Figure 2 shows the activity of the 2S and GUS genes in bean embryonic axes. From this figure it is clear that both, 2S and GUS structural sequences are expressed in bean cells and neither 2S nor GUS sequences do influence the rate of transcription, since the 35S promoter drives both to similar levels of expression. This is the first demonstration that a nutritionally important storage protein gene from the tropical family of Lecithidacea can be expressed in a legume species, which opens the possibility for the correction of methionine deficiency of legumes through genetic engineering.

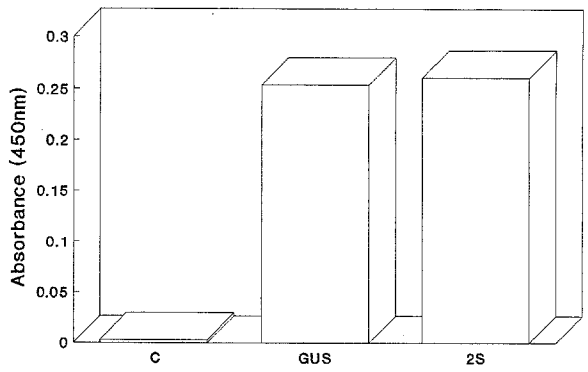

Fig. 2. Activity of the GUS and 2S genes in bombarded embryonic axes, analysed by enzyme-linked immunosorbent assay. C, control; GUS, β-glucuronidase; 2S, methionine-rich protein. Each well was loaded with a 250 μg of total protein.

Acknowledgements

This work was supported, in part, by a World Bank loan to the Brazilian government.

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