EXPRESSION OF A METHIONINE-RICH STORAGE ALBUMIN FROM THE BRAZIL NUT (Bertholletia excelsa H.B.K., LECYTHIDACEAE) IN TRANSGENIC BEAN PLANTS (Phaseolus vulgaris L., FABACEAE)

F.J.L. Aragão1, L.M.G. Barros1, M.V. de Sousa2, M.F. Grossi de Sê, E.R.P. Almeida1, E.S. Gander1 and E.L. Rech1

ABSTRACT

Bean (Phaseolus vulgaris), an important component in the diet of people in developing countries, has low levels of the essential amino acid, methionine. We have attempted to correct this deficiency by introducing a transgene coding for a methionine-rich storage albumin from the Brazil nut via biolistic methods. The transgene’s coding sequence was driven by a doubled 35S CaMV promoter and AMV enhancer sequences. The transgene was stable and correctly expressed in homozygous R2 to R5 seeds. In two of the five transgenic lines the methionine content was significantly increased (14 and 23%) over the values found in untransformed plants.

INTRODUCTION

The advent of recombinant DNA technology combined with plant tissue culture methods has made it possible to introduce foreign genes into host plants. This has created hopes that nutritionally deficient crops could be corrected through the introduction and expression of suitable transgenes.

In Latin America, India and parts of Africa, the bean (Phaseolus vulgaris L., Fabaceae) and broad bean (Vicia faba L., Fabaceae) are among the most important grain legumes used for human consumption. However, although beans are rich in some essential amino acids, e.g., lysine, threonine, valine, isoleucine and leucine, their nutritional value is limited because of the small amounts of the essential amino acid methionine and cysteine (Ma and Bliss, 1978). One strategy to correct this deficiency is to introduce transgenes encoding a methionine-rich storage albumin from the Brazil nut (Bertholletia excelsa H.B.K., Lecythidaceae) into bean hosts.

We have isolated and characterized one of the Brazil nut’s 2S-albumin genes (be2s1 gene) (Gander et al., 1991). This gene codes for an 18-kDa precursor protein, which is processed by a complex series of steps to yield two subunits of 3 and 9 kDa, joined by disulfide bridges (Sun et al., 1987). This protein (2S-BN protein) contains 18.8% methionine and is targeted to the seed protein vacuoles (Altenbach et al., 1987).

Sequences coding for 2S albumins have been expressed in several species, such as tobacco (Nicotiana tabacum L., Solanaceae), canola (Brassica napus L., Cruciferae), field bean (Vicia naboensis L., Fabaceae), potato (Solanum tuberosum L., Solanaceae) and thale cress (Arabidopsis thaliana (L.) Heynh., Cruciferae). Increased levels of methionine in seeds of transgenic N. tabacum (30%), B. napus (11 to 33%) and A. thaliana (20%) have been reported (Altenbach et al., 1989, 1992; De Clercq et al., 1990; Guerche et al., 1990; Conceição et al., 1994; Saalbach et al., 1994; Tu et al., 1994; Pickardt et al., 1995).

A methionine-rich sunflower 2S-albumin gene has been used to increase the methionine content of grain and pasture legumes (Tabe et al., 1993).

We have reported the transient expression of the Brazil nut 2S-albumin gene in cells of the bean embryonic axis after transformation by particle bombardment (Aragão et al., 1992). However, a reproducible system for stable transformation and subsequent regeneration of bean plants was not established; thus, no transgenic seeds were obtained. Recently, we have overcome this handicap and transgenic bean plants (P. vulgaris) containing and expressing the 2S-albumin gene were obtained through biolistic processes (Aragão et al., 1996). Here we report the expression of the 2S-albumin gene from the Brazil nut in several lines of transgenic beans.

MATERIAL AND METHODS

Plant material

Transgenic bean plants (P. vulgaris) cv Olathe were obtained via particle bombardment of the apical meristematic region of embryos (Aragão et al., 1996). Plants were co-transformed with plasmids pEA23 and pBI426 (circular form). Plasmid pEA23 contains the β-glucuronidase (GUS) coding region (uidA gene) under control of the 35S CaMV promoter and the 2S-albumin gene from the Brazil nut.

1 Embrapa/Cenargen, S.A.I.N. Parque Rural, Caixa Postal 02372, 70849-970 Brasília, DF, Brasil. Send correspondence to F.J.L.A. Fax: +55-61-340-3624. E-mail: aragao@cenargen.embrapa.br
2 Centro Brasileiro de Seqüenciamento de Proteínas, Laboratório de Bioquímica e Química de Proteínas, Departamento de Biologia Celular, Universidade de Brasília, 70910-900 Brasília, DF, Brasil.
amino acid analysis

Extraction of seed proteins and SDS-PAGE-purified 2S-BN protein.

During biosynthesis, at least three stepwise cleavages are involved at the termini and internally. The final protein apparatus, into the protein bodies (Shewry et al., 1995). During biosynthesis, at least three stepwise cleavages are involved at the termini and internally. The final protein consists of two polypeptide chains of 9 and 3 kDa, linked by a disulfide bridge (Sun et al., 1987).

2S-albumin expression

Expression of the 2S-BN protein in the cotyledon tissues of transgenic bean plants was determined by Western blot (Figure 1) and ELISA (Figure 2). The Western
Expression of a methionine-rich albumin in transgenic beans

Western blot analysis revealed that the 2S-BN protein was correctly expressed in some of the transgenic bean lines (B34-5, E36-6, F40-4 and G41-14), as judged from the presence of the mature 12-kDa protein (Figure 1). No signals were observed in the control D35-11, containing only the uidA-neo fusion gene, and in the untransformed control. It was not possible to detect differences in the amount of 2S protein in the transgenic mature seeds. ELISAs were performed on immature and mature seeds (Figure 2). Lines B34-5, D35-11 and F40-4 revealed no significant signal increase when compared to the control plants. Both lines, E36-6 and G41-14, showed 2S-BN protein accumulation in immature and mature seeds, with greater accumulation in immature seeds. During maturation, the 2S-BN protein content in these lines decreased (Figure 2). This suggests that the 2S proteins are either not stored correctly and degraded prematurely or that the 2S-specific mRNA is less stable in the storage cells of beans than in those of the Brazil nut. The Western blot analyses performed in this study were over-incubated and stained, so it is possible that saturation had an influence, making it impracticable to read these results quantitatively.

Amino acid composition of seeds

The amino acid composition of seeds of five transgenic lines and one control was analyzed (Table I). The methionine content significantly increased in all 2S-transgenic lines (B34-5, E36-6, F40-4 and G41-14) from 10 to 23% over the values of the control. As expected, line D35-11, which contains only the uidA-neo fusion genes, showed no significant increase.

Transgenic canola and tobacco seeds expressing the be2s1 sequence driven by the β-phaseolin promoter had an increase of 11 to 33% in methionine content (Altenbach et al., 1989, 1992). In V. nabornensis transformed with 2S-albumin-coding sequences under the control of the legumin B4 promoter, a 3% increase in total seed proteins was reported (Saalbach et al., 1995). Brazil nut 2S sequences driven by the analogous 2S promoter from A. thaliana caused a 20% increase in methionine content in transgenic A. thaliana (Conceição et al., 1994). However, whenever 2S-BN sequences were expressed under the control of the 35S CaMV promoter, the expression levels observed were insufficient to alter the methionine content in the target plants, e.g., S. tuberosum (Tu et al., 1994), V. narbonensis (Saalbach et al., 1995) and N. tabacum (Marcellino et al., 1996).

Since the pioneering work of Murai et al. (1983),
expressing the bean phaseolin gene in sunflower (*Helianthus annuus* L., Compositae), numerous attempts to express chimeric genes encoding storage proteins such as the 2S-BN protein have been made. With the exceptions cited above, very low levels of trans-protein accumulation have been achieved (De Clercq *et al.*, 1990; Guerche *et al.*, 1990; Saalbach *et al.*, 1994) and in almost no case could observable changes in seed amino acid composition be observed.

In our study, we transformed bean plants with a chimeric construct containing the doubled 35S CaMV promoter plus the AMV enhancer sequence, assuming that its performance would be superior to the native 35S promoter. Indeed, in two of the transgenic lines a 14 and 23% increase of methionine was achieved.

In view of these results, we are now assessing the possibility of achieving higher levels of methionine in transgenic beans by using homologous and seed-specific promoters such as the β-phaseolin promoter. These results will form the foundation for the production of genetically engineered commercial varieties of beans containing high levels of proteins rich in essential amino acids.

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**REFERENCES**


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**Table I** - Amino acid compositions (%) of salt-soluble proteins from transgenic and non-transgenic (control) mature bean seeds and from the Brazil nut. The transgenic lines B34-5, E36-6, F40-4 and G41-14 contain the *be2s1* and *gus* gene. The transgenic line D35-11 contains only the *gus* gene.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>B34-5</th>
<th>D35-11</th>
<th>E36-6</th>
<th>F40-4</th>
<th>G41-14</th>
<th>Control</th>
<th>Brazil nut</th>
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<tbody>
<tr>
<td>Ala</td>
<td>6.76a</td>
<td>6.78a</td>
<td>6.72a</td>
<td>6.76a</td>
<td>6.79a</td>
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<td>Arg</td>
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<td>8.57a</td>
<td>8.55a</td>
<td>8.26a</td>
<td>8.43a</td>
<td>8.53a</td>
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<td>Asp</td>
<td>11.90a</td>
<td>11.70a</td>
<td>12.00a</td>
<td>11.69a</td>
<td>11.63a</td>
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<td>CySO.H</td>
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<td>1.27a</td>
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<td>1.41a</td>
<td>1.06a</td>
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<td>5.07a</td>
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<td>4.97a</td>
<td>3.42a</td>
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</table>

a, b, c, d: Mean values in the same columns with different superscripts are significantly different (P < 0.05) according to Tukey’s test.

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