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Strategies for improving the nutritional quality of *Phaseolus* beans through gene engineering

Geert Angenon [(1)], Janniek De Clercq [(1)], Willy Dillen [(1)], Alain Goossens [(1,2)], Jyoti Kapila [(1)], Mukund Zambre [(1)], Marc Van Montagu [(1)]

[(1)] Laboratorium voor Genetica, Department of Genetics. Flanders Interuniversity Institute for Biotechnology. Universiteit Gent. KL. Ledeganckstraat 35. B-9000 Gent (Belgium). E-mail: geang@gengenp.rug.ac.be
[(2)] Present address: Instituto de Biología Molecular y Celular de Plantas. Universidad Politécnica de Valencia-CSIC. Valencia (Spain).

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Although *Phaseolus* species are still difficult to transform, progress in this field now opens the way to engineering beans with a higher nutritional value. The opportunities for gene engineering in nutritional quality improvement, the strategies which can be adopted and the constraints we are still facing are briefly outlined, using the enhancement of the seed methionine content and the reduction in antinutritional factors as examples.
Keywords. *Phaseolus,* plant genetic transformation, seed nutritional quality.

Stratégies pour l'amélioration de la qualité nutritionnelle des haricots (*Phaseolus*) à l'aide de l'ingénierie génétique. Bien qu'il soit encore difficile de transformer génétiquement les espèces du genre *Phaseolus*, les progrès effectués dans ce domaine ouvrent des perspectives d'améliorer génétiquement la valeur nutritionnelle des haricots. Les possibilités de transformation génétique pour l'amélioration de la qualité nutritionnelle, les stratégies pouvant être adoptées et les contraintes actuelles sont brièvement décrites et illustrées par des exemples sur la teneur en méthionine et la réduction de facteurs antinutrionnels.
Mots-clés. *Phaseolus,* transformation génétique des plantes, qualité nutritionnelle des graines.

1. INTRODUCTION

Gene engineering can contribute significantly to *Phaseolus* improvement, especially where classical breeding cannot readily provide solutions. This is the case when germplasm with the desired characteristics is not available or when the trait is difficult to transfer to other genotypes, e.g. because of complex inheritance, close linkage to other undesirable traits, lack of knowledge about the underlying physiology, etc. Concerning nutritional quality, targets for a gene engineering approach include enhancement of the methionine content and reduction of phytate and raffinose-family-oligosaccharides (RFOs). The sulphur-containing amino acids methionine and cysteine are the first limiting amino acids in most grain legumes, including *Phaseolus* spp. (Nwokolo, Smartt, 1996), and so far classical breeding efforts to increase the concentration of these amino acids have not been successful. RFOs (α-1,6-galactosyl$_n$-sucrose) cannot be metabolised because of the absence of α-galactosidase in the human digestive system and are therefore considered as major determinants of flatulence, which may accompany bean consumption. Phytate (inositol hexaphosphate) forms complexes with minerals and thereby lowers the bioavailability of these minerals.

An important contribution of genetic engineering will be to elucidate the basis of nutritional deficiencies and as such facilitate improvement of nutritional quality by either conventional or biotechnological means. Indeed, the causes of many deficiencies are not fully established. For example, it is not clear to what extent RFOs on the one hand and factors such as fiber digestibility on the other hand, contribute to intestinal discomfort. Furthermore, compounds with antinutritional properties (phytate, RFOs, tannins, glucosinolates, etc.) may at the same time have beneficial effects for human health or constitute a defense of the plant towards biotic or abiotic stresses. RFOs are for instance implicated in cold and desiccation tolerance. Many of these issues can be addressed using transgene technology, which allows us to modify one single property of the plant. Moreover, the variation in transgene expression levels typically encountered

among different transgenic lines could be exploited to determine whether concentrations of antinutritional compounds exist that strike a balance between beneficial and adverse effects.

2. WHICH STRATEGIES CAN BE USED ?

In general the following strategies can be envisaged:
– overexpression of an endogenous (*Phaseolus*) gene,
– introduction of a modified endogenous gene,
– suppression of an endogenous gene by either antisense or co-suppression technology, and
– introduction of a foreign gene.

From the point of view of public acceptance, genes derived from *Phaseolus* or other food plants may be preferred. These strategies will be illustrated using the above mentioned traits as examples.

2.1. Enhancing methionine content

Several ways to enhance the seed methionine content can be followed, one of which is transformation with genes encoding methionine-rich seed proteins. Such proteins have not been detected in *Phaseolus* and it is therefore necessary to introduce foreign genes or to modify endogenous genes. Methionine-rich seed proteins and their corresponding genes have been identified in *Bertholletia excelsa*, *Helianthus annuus* and *Zea mays*. Transformation with these genes resulted in enhanced seed methionine levels in several grain legumes, including *Glycine max*, *Vicia narbonensis* and *Lupinus angustifolius* (reviewed by Tabe and Higgins, 1998). Alternatively, genes encoding highly abundant but methionine-poor seed storage proteins can be modified with extra methionine codons. To have a significant impact on the total methionine content, extensive modifications are required, while synthesis, intracellular transport and stability of the protein should not be affected. This can most easily be achieved if the three-dimensional structure of the protein is known. The crystal structure of two abundant seed proteins of *Phaseolus*, arcelin and phaseolin, are available (Lawrence *et al.*, 1990; Hamelryck *et al.*, 1996), making these proteins good candidates for this approach. One step further is to design completely artificial methionine-rich proteins (Keeler *et al.*, 1997). Whatever methionine-rich protein is chosen, high accumulation levels should be reached. This requires the use of appropriate regulatory sequences (see further) and can also be facilitated by reducing the accumulation of endogenous protein fractions. Antisense technology has for example been used to repress expression of napin and cruciferin in *Brassica napus* seeds (Kohno-Murase *et al.*, 1994; 1995) and of 2S albumin in *Arabidopsis thaliana* seeds (Goossens *et al.*, 1999) and to concomitantly increase seed-specific expression of other endogenous genes or transgenes.

Another way of enhancing methionine levels is through manipulating its biosynthesis. One of the key enzymes in the methionine biosynthetic pathway is aspartate kinase (AK). This enzyme is regulated by feedback inhibition. Transformation with an AK gene from *E. coli* that is insensitive to feedback inhibition resulted in enhanced free methionine levels in tobacco seeds (Karchi *et al.*, 1993). Other biosynthetic steps could similarly be manipulated. As free methionine is a minor fraction in comparison with protein-bound methionine, this approach is not expected to increase total seed methionine levels dramatically and it may be useful to introduce simultaneously a methionine-rich protein that can act as a strong sink for deposition of the extra methionine

2.2. Reduction of phytate and raffinose-family-oligosaccharides

RFO levels could be reduced through antisense or co-suppression constructs targeted at the genes encoding the α-galactosyl transferases that catalyze RFO synthesis. Alternatively, specific α-galactosidases could be expressed in a seed-specific manner. Similarly, reduction of phytate levels could be achieved by seed-specific expression of a phytase gene, for example the phytase cDNA recently cloned from maize seedlings (Maugenest *et al.*, 1997).

3. WHICH TOOLS ARE NEEDED ?

3.1. An efficient transformation system

So far, two systems for the production of transgenic plants in the genus *Phaseolus* have been described. One is a regeneration independent approach which resulted in transgenic *Phaseolus vulgaris* (common bean) plants and is based on particle bombardment of seedling apical meristems (Russell *et al.*, 1993; Aragão *et al.*, 1996). This procedure is comparatively inefficient and apparently not suitable for the production of a sufficiently large number of transgenic plants. In our group, transgenic *Phaseolus acutifolius* (tepary bean) plants have been generated with a procedure based on regeneration from callus and *Agrobacterium*-mediated gene transfer (Dillen *et al.*, 1997a). This procedure has been optimised (Dillen *et al.*, 1997b and unpublished results) and has become routine in our hands. So far, transgenic plants have been obtained in seven independent experiments. Within the grain legumes, *P. acutifolius* is now one of the few species for which the number of transformed plants that can be generated is large enough to permit

transgenic approaches for applied or fundamental research. Moreover, *P. acutifolius* can be hybridised with the economically more important species *P. vulgaris*. *P. acutifolius* is actually used in breeding programs as a source of useful traits for common bean improvement. Introducing transgenes in *P. vulgaris* through interspecific crosses with transgenic *P. acutifolius* is therefore feasible and would fit into existing breeding strategies. Because of progress made in regeneration of *P. vulgaris* genotypes (Zambre *et al.*, 1998), it may be possible to extend this transformation procedure to *P. vulgaris*.

3.2. Cloned coding sequences

For several traits, coding sequences are available at present (see some examples above). For other traits this is not yet the case. However, it can be expected that large scale sequencing projects for model plants and for the major crop species will provide most of the relevant genes in the near future. It will probably be necessary to clone the *Phaseolus* homologues of these genes when using antisense and co-suppression approaches, which rely on sequence homology.

3.3. Regulatory sequences

Preferentially, genes of interest should be expressed in a developmentally correct and tissue specific manner, i.e. in seeds and/or pods only. This is not a major constraint as most processes relevant to nutritional quality occur during the organ expansion and maturation phase of seed development. Several promoters which are active during this phase have been cloned, notably promoters of seed storage protein genes, and some of these direct very high seed specific expression. The available promoter sequences include those of the *Phaseolus* genes encoding phaseolin, phytohemagglutinin and arcelin (Slightom *et al.*, 1983; Voelker *et al.*, 1987; Anthony *et al.*, 1991; Goossens *et al.*, 1995). For some applications, however, promoters may be needed which are not readily available, e.g. seed coat specific promoters.

4. CONCLUSIONS

For several nutritional deficiencies of *Phaseolus*, the biochemical, physiological or molecular basis is not completely understood. Moreover, modifications meant to improve nutritional quality may create undesired side-effects. Transgenic approaches will undoubtedly be instrumental to resolve these uncertainties. *P. acutifolius*, for which an efficient transformation system is available, can serve as a very good model system in this regard. For some traits, e.g. methionine content, strategies for improvement are at hand and the available transformation procedures (for *P. vulgaris* and for *P. acutifolius*) can already be used to implement them. Nevertheless, more efficient transformation methodology for *P. vulgaris* would considerably reduce the efforts needed to introduce transgenes in commercial lines.

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