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Removal of antinutritional factors from bean (*Phaseolus vulgaris* L.) seeds

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Phytohemagglutinin and the lectin-related proteins present in bean seeds are toxic to monogastric animals and lower the nutritional value of beans. Since these antimetabolites are present in substantial amounts, a breeding program aimed to the removal of phytohemagglutinin was developed. The character "absence of phytohemagglutinin" was transferred into a bean cultivar by backcrossing. The lines obtained maintained the agronomic performance of the recurrent parent. Preliminary results show that removal of phytohemagglutinin results in a higher true protein digestibility. Further modification in the composition of the lectin-related protein family is now under way.
Keywords. *Phaseolus,* antinutrients, genetic improvement.

Élimination de facteurs antinutritionnels des graines du haricot commun (*Phaseolusvulgaris* L.). La phytohémagglutinine et les protéines apparentées à la lectine présentes dans les graines du haricot sont toxiques pour les monogastriques et diminuent leur valeur nutritionnelle. Vu la présence de ces antimétabolites en quantité substantielle, un programme d'amélioration visant à éliminer la phytohémagglutinine a été initié. Le caractère "absence de la phytohémagglutinine" a été transféré chez un cultivar du haricot commun par rétrocroisements. Les lignées obtenues conservent les qualités agronomiques du parent récurrent. Les résultats préliminaires montrent que l'élimination des phytohémagglutinines aboutit à une plus grande digestibilité des protéines. D'autres modifications portant sur la composition des protéines apparentées à la lectine sont actuellement en cours.
Mots-clés. *Phaseolus,* antinutrients, amélioration génétique.

1. INTRODUCTION

Improvement of yield, protein content, and quality are, together with the introduction of resistance to diseases, the main aims of common bean breeding. Protein content of bean (*Phaseolus vulgaris*) seeds is lower than that of other grain legumes. A large fraction of seed protein is represented by the storage protein phaseolin (up to about 50% of the total) and by the components of the lectin-related protein family (Vitale, Bollini, 1995). Belonging to this family are arcelin and α-amylase inhibitor (α-AI), and the true lectin, phytohemagglutinin (PHA). Both PHA and α-AI are responsible for the lowering of the nutritional value of bean seeds. Furthermore, all three proteins have been related to defence mechanisms against predators (Chrispeels, Raikhel, 1991).

In bean genome, genes coding for lectin-related proteins are linked, behave as a single Mendelian character, and share a high degree of sequence homology (Mirkov *et al.*, 1994). Relative amount of members of this family may vary in different genotypes. In particular, arcelin has been detected only in wild material collected in Mexico, whereas the two other proteins are widely distributed (Osborn, 1988). The molecular bases for the absence of PHA have been described (Staswick, Chrispeels, 1984). Apparently, arcelin-containing seeds (with exception of arcelin 3 and arcelin 4) are devoid of α-AI (Santino *et al.*, 1991) but this point needs further investigation.

Feeding trials carried out on rats with pure PHA indicated this protein as a major antimetabolite of raw beans (Pusztai, Palmer, 1977). To investigate on the role of these proteins in the seeds, a breeding project aimed at the production of nearly isogenic bean lines differing in the composition of the lectin-related bean family is in progress.

We present the results related to the influence of PHA on field performance and nutritional value of seed proteins of two groups of breeding lines, one lacking and the second with the presence of this lectin. Part of these results have been published previously (Confalonieri *et al.*, 1992).

2. MATERIALS AND METHODS

Cultivars Pinto UI 111 (P) and Heidi (H) were used as PHA-null parents (lec/lec). The cv. Taylor's Horticultural (Asgrow Seed Company) was used as the PHA-containing parent (Lec/Lec). Since the lec character segregates as a single Mendelian unit it was easily introduced by backrossing in the recurrent Lec cultivar. Determining, through erythroagglutination assay, the presence of PHA in single seed extracts performed screening for lec/lec recombinant lines. Two field trials were also performed to estimate the influence of PHA on the main agronomic traits (see below) and on the nutritional value of seed proteins, using for the first one 13 BC2F3 and the second one 14 BC6F5 lines.

The agronomic traits analysed were: grain yield/plant, number of seeds/pod, number of pods/plant, 1000 seed weight, protein % on dry matter, ash % on dry matter. An analysis of variance was applied on the data.

Measurement of digestibility of raw Taylor's Horticultural seeds was made possible only by modifying the protocol, using adult rats, instead of weaning ones (FAO, 1991).

3. RESULTS

3.1. Field trials

Detailed results for the field trials are shown in Confalonieri *et al.*, 1992. The major conclusions are as follow:

– There are significant differences of agronomic traits between single lines.

– There are no difference in seed yield between PHA-null and PHA-containing lines in both field trials, one using BC2F3 and the other using BC6F5.

– A significant negative coefficient of correlation (-0.46) was found between grain yield/plant and seed protein content in the BC2F3 lines, in accordance with the results described by other authors (Osborn, 1988) but no significant correlation was found between these two characteristics in the BC6F5 lines (in fact this material is more homogeneous from a genetic point of view).

The effects produced by the absence of PHA in the seeds on the expression of other traits like seed germination and resistance to diseases and pests should still be evaluated before claiming any real advantage of the lectin-null genotypes.

3.2. Seed protein analysis

PHA null-lines derived from both crosses showed electrophoretic profiles identical to that of cv. Taylor's Horticultural, with exception in the area of the gel containing PHA, which was absent. Immunochemical analysis of the proteins revealed that the PHA-null lines contained the α-AI variant characteristic of Pinto and Heidi cultivars. This character therefore cosegregates with the PHA-null one, further confirming their linkage.

Evaluation of nitrogen utilization and protein quality of PHA-null beans in comparison with the recurrent parent is under way. Preliminary results are shown in **table 1**. PHA-null lines were pooled before analysis and protein quality determined on both raw and cooked material.

The variations in lectin-related protein profile was also studied by screening bean collections with polyclonal antibodies. The crossreaction between the polyclonal antibodies and all the components of the lectin-related protein family allows the assessment of the variations in this family and could lead to the identification of accessions with particularly interesting profiles. Some extent of this variation is shown in **figure 1**.

Table 1. Protein quality of bean samples — *Qualité protéinique des échantillons de haricot*.

Protein quality	**Raw bean**		**Cooked bean**	
	Taylor's Horticultural	Lectin-null	Taylor's Horticultural	Lectin-null
Chemical score (**1**)	0.92	0.92	0.91	0.91
Limiting amino acids	Sulphur a.a.	Sulphur a.a.	Sulphur a.a.	Sulphur a.a.
True protein digestibility (**2**)	17.55 ± 2.41 (**3**)	40.13 ± 2.29 (**4**)	66.77 ± 1.49 (**4**)	72.23 ± 1.51* (**4**)
Protein digestibility-corrected a.a. score (**1**)	0.16	0.37	0.61	0.66

(**1**) According to FAO/WHO (1991). (**2**) Mean ± SE (**3**) Adult rat weight: 100 g. (**4**) Weaning rat weight: 60 g. * Lectin-null bean *versus* recurrent parent: $p < .02$.

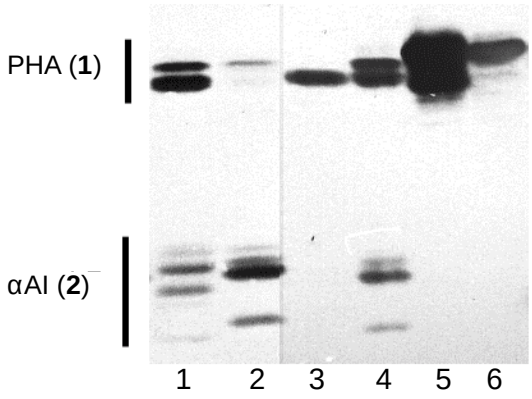

Figure 1. Immunoblot profiles showing variations in lectin-related protein of beans. Total seed proteins were analysed using antibodies against recombinant α-AI — *Profils immunologiques montrant des variations au niveau des proteines assimilées à la lectine du haricot.*

(**1**) PHA: Phytohemagglutinin. (**2**) αAI: α-amylase inhibitor.
Lane 1: Tailor's Horticultural-cultivated;
lane 2: Pinto 111-cultivated;
lane 3: G08386-wild;
lane 4: G12896-wild;
lane 5: G11051-wild;
lane 6: G02771-wild.

Lectin-related profiles corresponding to Taylor's Horticultural and Pinto 111 are shown in lanes 1 and 2 respectively. Bean in lane 5 contains arcelin type 6 and apparently lacks α-AI (Sparvoli, Bollini, 1998). Accessions shown in lanes 3 and 6 also are devoid of a-AI and are now under study. Finding of beans lacking one or more components of this protein family should allow to develop near isogenic lines to investigate on the relevance of each single protein.

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