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Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene

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ABSTRACT **With the aim of improving the nutritive value of an important grain legume crop, a chimeric gene specifying seed-specific expression of a sulfur-rich, sunflower seed albumin was stably transformed into narrow-leafed lupin (*Lupinus angustifolius* L.). Sunflower seed albumin accounted for 5% of extractable seed protein in a line containing a single tandem insertion of the transferred DNA. The transgenic seeds contained less sulfate and more total amino acid sulfur than the nontransgenic parent line. This was associated with a 94% increase in methionine content and a 12% reduction in cysteine content. There was no statistically significant change in other amino acids or in total nitrogen or total sulfur contents of the seeds. In feeding trials with rats, the transgenic seeds gave statistically significant increases in live weight gain, true protein digestibility, biological value, and net protein utilization, compared with wild-type seeds. These findings demonstrate the feasibility of using genetic engineering to improve the nutritive value of grain crops.**

Lupin (*Lupinus angustifolius* L.) is the major grain legume grown in Australia. Production exceeds 800,000 tons per annum, and the majority of this is used in the production of feed for the beef, pig, and poultry industries and for the supplementary feeding of sheep in summer when the feed quality of pastures is poor.

As a source of the 10 amino acids that are essential in the diet of nonruminant animals, lupin seed protein, in common with the protein of most other grain legumes, is deficient in the sulfur-containing amino acids methionine and cysteine (1). When diets containing lupin as the major protein source were supplemented with methionine, significant increases were found in growth of pigs (2) and in the efficiency of protein utilization in rats (3). Eggum *et al.* (4) reported significant increases in biological value and in net protein utilization when rats were given supplementary methionine while consuming diets in which the only protein source was lupin meal. In the pig and poultry industries, feed mixes are routinely supplemented with pure methionine to compensate for the low level of the sulfur-containing amino acids in grain legumes in general. Because nonruminant animals can convert methionine to cysteine (5), methionine can satisfy their total requirement for dietary sulfur-containing amino acids.

Ruminant animals also respond to dietary supplements of methionine. Increases in wool growth of 30–50% have been reported in response to supplementary methionine administered directly into the abomasum of grazing sheep (6). Because there is a loss of methionine and cysteine during conversion of ingested forage protein into rumen microbial protein, an ideal protein for ruminant nutrition, and especially for sheep, would be one that is rich in sulfur-containing amino acids and, at the same time, resists breakdown in the rumen, thereby delivering its amino acids directly to the hindgut of the animal.

It is common practice in southern Australia to feed lupin grain to sheep during the period of reduced pasture growth in summer and early autumn. It follows from the above that the introduction of a rumen-resistant, methionine/cysteine-rich protein into lupin grain should lead to increases in productivity in both nonruminant and ruminant animals. Genetic engineering offers one possible means by which this might be accomplished.

We have identified, in sunflower seeds, a suitable donor protein (sunflower seed albumin, SSA) which is rumen-stable and unusually rich in methionine and cysteine (7, 8). A high proportion of SSA therefore should escape conversion to microbial protein in the rumen, and thus be preserved for digestion and absorption in the lower gastrointestinal tract (9).

We have developed a transformation system for narrow-leafed lupin and introduced a gene for SSA into this species. We have used a stably transformed, high-expressing line to test the effects of this introduced gene on the amino acid composition of the transgenic seeds and carried out feeding trials with rats to determine nutritive value.

MATERIALS AND METHODS

Explant Material for Tissue Culture. Lupin (*Lupinus angustifolius* cv. Warrah) plants were grown in soil in the glasshouse. Pods were harvested when the embryonic axis was cream in color and the cotyledon was light green/cream. This corresponds to the stage of maximum seed dry weight. Pods were sterilized in 70% (vol/vol) ethanol for 1 min followed by 20 min in commercial bleach (10 g/liter of sodium hypochlorite, final concentration) and three to four rinses with sterile distilled water. Seeds were removed from the pods, and explants were prepared by slicing the embryonic axis longitudinally in two halves while it was still attached to the cotyledons. The root pole was removed 2 to 3 mm above the root tip and discarded, and thin longitudinal slices taken from the halves of the embryonic axis (three to five slices per axis) with the aid of a dissecting microscope. The cotyledons were used as a means of holding the axis for slicing.

Transformation and Regeneration Procedures. Slices of the embryonic axis were immediately incubated for 30 to 90 min in a suspension of *Agrobacterium tumefaciens* containing the plasmid pBSF16 (see below). Explants were removed from the *Agrobacterium* suspension and transferred to solid cocultiva-

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Abbreviations: SSA, sunflower seed albumin; GUS, β-glucuronidase; CaMV, cauliflower mosaic virus.

tion medium that consisted of B5 medium (10) together with 3 mg/liter naphthaleneacetic acid and 0.5 mg/liter benzylaminopurine. Coniferyl alcohol (100 μM) was added as a filter-sterilized solution to medium that previously had been autoclaved and cooled. The explants were cocultivated in a horizontal position for 3 days at 24 ± 1°C with a 16-hr photoperiod. After cocultivation, explants were washed three to four times in sterile distilled water and blotted dry on sterile filter paper before plating on regeneration medium.

Shoot formation was initiated on Murashige and Skoog (MS) medium (11) as modified (12). Nitrogen was supplied as 23.7 mM ammonium nitrate and 4 mM asparagine. Naphthaleneacetic acid and benzylaminopurine each were added at 1 mg/liter, while sucrose was added at 0.3% wt/vol. Bacto agar (Difco) (0.8%, wt/vol) was used for solidification and the pH adjusted to pH 5.8 before autoclaving. Timentin (Beecham Research Laboratories, Dandenong, Victoria, Australia) was filter-sterilized and added to cooled media (150 mg/liter) to control *Agrobacterium* growth. Phosphinothricin (5 mg/liter) was used for selection.

After 14 days culture at 24°C, shoots were transferred to MS medium containing 0.1 mg/liter naphthaleneacetic acid and 1 mg/liter benzylaminopurine. Phosphinothricin and timentin levels were kept constant throughout the regeneration and selection procedures. Once shoots had reached 10 mm in height, the clumps of shoots were divided and cultured every 2 weeks on either MS medium containing 0.5 mg/liter benzylaminopurine for further shoot growth, or individual shoots were transferred to B5 medium (10) without phosphinothricin but containing 1 mg/liter indolebutyric acid to induce root growth. Regenerated plantlets were transferred to soil in the glasshouse with an inverted polycarbonate jar over the plant for the first few weeks until they became acclimated to glasshouse conditions.

Gene Construction. A three-gene construct containing the *bar*, *ssa*, and *uidA* genes was prepared (Fig. 1). These genes coded for phosphinithricin acetyltransferase, SSA and β-glucuronidase (GUS), respectively. The *bar* and *uidA* genes each were under the control of 5′ flanking sequences from the gene for the 35S RNA of cauliflower mosaic virus (CaMV), and the *ssa* gene had a seed-specific promoter from a pea vicilin gene (13).

The *Escherichia coli uidA* gene was reconstructed in two steps from pKiwi 101 (14). In the first step, the ORF was excised as a *Sal*I-*Eco*RI fragment, end-filled with the Klenow fragment of DNA polymerase I (Pol1K), and ligated into *Sma*I-cut pDH51 (15). This resulted in a chimeric *uidA* gene (pDHGUS) controlled by the CaMV *35S* promoter. To prevent GUS expression in bacteria, the 5′ untranslated region and the translational initiation codon of pDHGUS were replaced with the corresponding region of pKiwi 101 by substituting a 0.7-kb *Eco*RV fragment containing the 3′ proximal region (96 bp) of the CaMV *35S* promoter and approximately 570 bp of the 5′ end of the GUS coding region, to yield pGUS2. Plasmid pGUS2 was digested with *Eco*RI, and the fragment containing the chimeric *uidA* gene was inserted into *Eco*RI-digested pTAB10 (16), a binary vector that contained a chimeric CaMV *35S-bar* gene. The CaMV *35S-bar* gene, which conferred resistance to the herbicide phosphinothricin, was used as the selectable marker in plant transformation. The resulting plasmid was partially digested with *Eco*RI, end-filled with Pol1K, and recircularized to create pTAB16, a binary plasmid containing the CaMV *35S-bar* and CaMV *35S-uidA* genes separated by a unique *Eco*RI site.

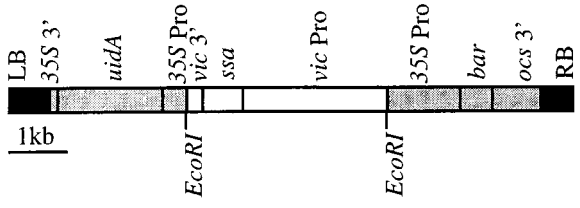

FIG. 1. The multigene construct transferred to lupins. The construct contained three chimeric genes: *35S-uidA*, encoding the reporter enzyme β-glucuronidase (GUS); *vic-ssa*, encoding the sunflower seed albumin; and *35S-bar* encoding the selectable marker phosphinothricin acetyltransferase. LB and RB, left and right T-DNA borders from *A. tumefaciens*; *35S* 3′, 3′ flanking region from the *35S* gene of CaMV; *uidA*, protein-coding region from the *uidA* gene of *E. coli*; *35S* Pro, promoter from the *35S* gene of CaMV; *vic* 3′, 3′ flanking region from the *vicilin* gene from pea; *ssa*, protein-coding region from the *ssa* gene from sunflower (*Helianthus annuus*); *vic* Pro, promoter from the pea *vicilin* gene; *bar*, protein-coding region from the *bar* gene of *Streptomyces hygroscopicus*; *ocs* 3′, 3′ flanking region from the octopine synthase gene of *A. tumefaciens*.

Enzyme Assays and Western Blot Analyses. Activity of the GUS enzyme was determined histochemically in leaf tissue (17, 18). Expression of the *bar* gene was monitored by measuring the phosphinithricin acetyltransferase activity (19). SSA level was measured in flour filed from the cotyledons of lupin seeds at a position distal to the embryonic axis. Protein was extracted with a solution containing 0.5 M NaCl, 0.1 M *N*-tris(hydroxymethyl)methylaminoethanesulfonic acid at pH 7.8, and 1 mM EDTA, and the concentration was determined by Bradford assay (20). The protein (1 μg) was fractionated by SDS/PAGE and electroblotted onto nitrocellulose membrane (21). SSA was detected with SSA antiserum from goat in combination with rabbit anti-goat IgG conjugated to alkaline phosphatase. Coomassie blue-stained gels were loaded with 40 μg of total seed protein.

Chemical Analyses. The amino acid composition of lupin seed meal was determined (22) on samples that were oxidized with performic acid before hydrolysis and analysis by ion exchange chromatography. Total N was determined in an autoanalyzer after Kjeldahl digestion of finely ground meal (23). The data are based on duplicate determinations on duplicate samples. Total sulfur, oxidized S (corresponding to sulfate) and carbon-bonded S (corresponding to amino acid sulfur), were determined by x-ray fluorescence spectrometry (24) of four replicate subsamples of seed meal.

Electron Microscope Immunolocalization of SSA. Tissue preparation and immunogold labeling were carried out as described earlier (25).

Rat Feeding Trials. Feeding trials with meal from both transgenic and nontransgenic lupins were as described (4). Each day two groups of five male Wistar rats, each weighing approximately 70 g, were offered 10.47 g of a diet consisting of finely ground (<0.05 mm) lupin-seed meal, a nitrogen-free mixture and a vitamin-mineral mixture. The diet contained, in each 100 g of dry matter, 1.5 g of N derived from either transgenic or nontransgenic lupin seeds. Values for true protein digestibility, biological value, net protein utilization, and digestible energy were determined (4). True protein digestibility is the apparent digestibility corrected for metabolic N in the fecal material, and biological value is the percentage of absorbed N that is retained by the animal (after correction for urinary N and endogenous urinary N). Net protein utilization is derived from true protein digestibility × biological value per 100.

All the above analyses were carried out on seeds from field-grown lupin plants.

RESULTS

Transgene Expression. Expression of the three-gene construct (*bar-ssa-uidA*) in lupin was monitored by assaying the enzymes encoded by the *bar* and *uidA* genes in young leaves of developing plants from the T_0 (primary transgenic) generation. Expression of the *ssa* gene, which was regulated by a seed-specific promoter from the pea vicilin gene, was assayed by immunoblots of protein from T_1 seeds. SSA-positive, T_1 seeds of a line expressing the transgenes at high levels were germinated, and 10 T_2 seeds from each of the resultant 18 T_1 plants were screened for SSA. In 14 of these 18 plants, all 10 seeds were positive. All the T_2 seeds from four of these lines were germinated to produce T_2 plants, and in one of these lines all 54 T_2 plants were positive with respect to GUS and phosphinithricin acetyltransferase activities. This result indicated that the line was homozygous with respect to the transgenes. Southern blotting analysis showed that the selected line contained a tandem insertion of the transferred DNA at a single locus (data not shown).

Analysis of 20 T_2 seeds from this line by SDS/PAGE and by immmunoblotting confirmed the homozygosity of the *ssa* gene in the selected transgenic line. The SSA protein was detected by both Coomassie blue staining and by immunoblotting (Fig. 2). Apart from the additional band due to SSA, no major alterations were obvious in the total protein profile of the transgenic seeds.

The gene for SSA encodes a precursor protein of 141 amino acids. In sunflower, SSA is posttranslationally modified at the amino terminus in two steps: first by the removal of a signal peptide sequence of 25 amino acids, and second by the elimination of a further 13-residue hydrophobic pro-sequence to yield a mature protein of 103 amino acids with a molecular mass of 12,133 Da (7). After SDS/PAGE, the SSA protein from transgenic lupins comigrated with SSA purified from sunflower seeds (Fig. 2), indicating that the pre-pro-protein was processed in transgenic lupin in the same way as in sunflower. This was further supported by electron microscopic immunolocalization studies on developing cotyledons in transgenic lupin seeds, which showed that SSA protein accumulated in small, electron-dense protein bodies (Fig. 3) similar to those containing the endogenous lupin seed storage proteins (S.C. and K. R. Gayler, unpublished observations).

Composition of Transgenic Seeds. Seven thousand T_3 seeds of the homozygous transgenic lupin line and 7,000 seeds of the nontransgenic parent lupin cultivar were sown in a field trial conducted in insect-proof enclosures. Seed yields were compared for transgenic and nontransgenic plants. No statistically significant difference was found between the two populations (Table 1). A sample of seed of the transgenic line and of the nontransgenic parent line was analyzed for nitrogen and sulfur contents (Table 1). Lupin seeds expressing the SSA gene were not significantly different from the parent with respect to total nitrogen and total sulfur contents. In spite of this lack of difference in total sulfur contents, there were marked differences in the distribution of sulfur between the oxidized sulfur fraction (sulfate) and the carbon-bonded sulfur fraction (sulfur amino acids). In the transgenic seeds, the level of sulfate was reduced by 528 ppm and the level of carbon-bonded sulfur increased by 446 ppm. Consistent with these changes, amino acid analyses indicated that the transgenic seeds contained 94% more methionine than the wild type. This was accompa-

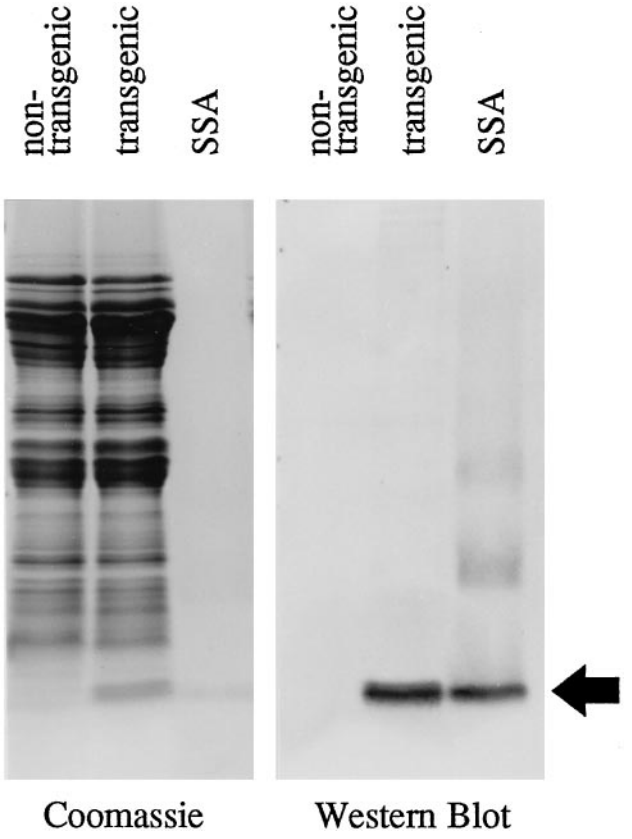

FIG. 2. Total extractable protein from nontransgenic and transgenic lupin seeds fractionated in duplicate gels by SDS/PAGE and protein was detected either with Coomassie blue or with antiserum to SSA (Western blot). A lane with purified SSA (indicated by an arrow) was included as a reference in both cases.

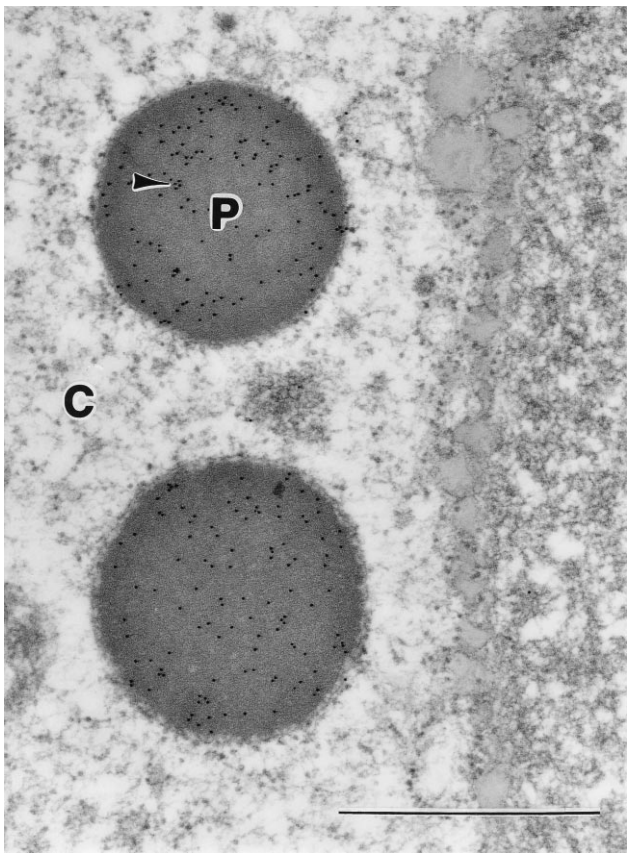

FIG. 3. Electron microscope immunolocalization of SSA in a cotyledon cell of maturing transgenic lupin seed. Electron microscope sections were treated with antiserum to SSA, and the bound antibody was detected with protein A coated on gold particles. The gold particles (arrow) are confined to the dense protein bodies (P). They are distinguishable in size and appearance from ribosomes in the surrounding cell cytoplasm (C). (Bar = 1 μm.)

Table 1. Characteristics of field-grown lupin seeds from the nontransgenic parent line and from a homozygous transgenic line expressing SSA

Characteristic	Nontransgenic	Transgenic
Seed yield,* kg	4.64 (0.15)†	4.48 (0.12)†
N, %	5.79 (0.07)†	5.81 (0.08)†
Total S, ppm	3,534 (85)†	3,451 (99)†
Oxidized S, ppm	1,076 (63)‡	548 (15)‡
Carbon-bonded S, ppm	2,457 (53)§	2,903 (73)§

Standard errors are shown in parentheses.
*Mean seed yield from six replicate field trial plots, each 27 m^2.
†Difference between transgenic and nontransgenic not significant.
‡Difference significant at $P = 0.001$.
§Difference significant at $P = 0.003$.

Table 3. Nutritional evaluation of transgenic and nontransgenic lupins in a rat feeding trial

Lupin seeds	True protein digestibility, %	Biological value, %	Net protein utilization %	Digestible energy, %
Nontransgenic	89.4 (2.0)*	63.2 (3.16)*	56.4 (2.6)†	77.7 (4.3)‡
Transgenic	95.7 (1.4)*	73.0 (2.0)*	69.8 (1.3)†	78.4 (3.1)‡

Standard errors are shown in parentheses.
*Difference between transgenic and nontransgenic significant at $P = 0.03$.
†Difference between transgenic and nontransgenic significant at $P = 0.002$.
‡Difference between transgenic and nontransgenic not significant.

nied by a 12% reduction in cysteine, while the levels of the other amino acids were not changed (Table 2).

Feeding Trials. The nutritive values of wild-type and transgenic lupin seeds were compared in feeding trials with rats (Table 3). Large, statistically significant increases were observed in true protein digestibility, biological value, and net protein utilization of transgenic seeds, but there was no change in digestible energy. The results with transgenic seeds expressing the SSA gene are comparable to results seen earlier (4) when a lupin-based diet was supplemented with pure methionine. In the trial described here, rats were offered 10.47 g per day of a ration that contained 22.6% of either the transgenic or the nontransgenic lupin meal. A notable difference was observed between the two diets in the amount of residue left at the end of each day. In the case of the control ration, an average of 4.67 g was left uneaten, whereas only 2.18 g remained in the case of the transgenic diet. Consistent with this, rats receiving transgenic lupins as their sole nitrogen source made significantly higher live weight gains than the control group (Table 4).

DISCUSSION

Grain legumes are an important, protein-rich food and feed source, and their deficiency in sulfur amino acids for animal diets makes them an obvious target for modification by genetic engineering. However, development of the necessary procedures for transformation and regeneration of grain legumes has proved more difficult than for some other dicotyledonous species. Although the necessary procedures have been developed for engineering a limited number of grain legumes, to date there have been no reports of genetically engineered improvement in the nutritive value of any grain legume crop. To introduce foreign genes into lupins, we first developed a robust regeneration protocol that could be used in combination with a gene delivery system such as *A. tumefaciens*. Although the regeneration of lupin explants from tissue culture has been reported (26–28), attempts to reproduce these results in our laboratory using Australian commercial cultivars were unsuccessful. After extensive testing of a range of tissue explants and media, we developed a reproducible regeneration system, based on organogenesis, using thinly sliced embryonic axes of maturing seeds. Exposure of these explants to *A. tumefaciens* containing the plasmid BSF16 resulted in the recovery of transformed lupin plants at a frequency of 0.01%. This paper reports the characteristics of a single transformed line in which the SSA gene was expressed at a high level in the seed. In this transgenic line, SSA protein accounted for approximately 5% of the total, extractable seed protein.

Although it has been recognized for many years that grain legumes in general are deficient in the sulfur-containing amino acids, methionine and cysteine (1), traditional plant breeding methods have not been successful in increasing the level of these amino acids. Indeed, from a survey of 45 naturally occurring lines of peas from a wide range of origins, there is evidence that an increase in one sulfur-rich protein fraction (legumin) is invariably accompanied by a decrease in the other major sulfur-rich fraction (the albumins) (29). The consequence of this negative correlation is that the sulfur amino acid content relative to protein content remains fairly constant.

The recent development of tissue culture procedures for the stable transformation of a limited range of grain legumes such as soybean (*Glycine max*; ref. 30), common bean (*Phaseolus vulgaris*; ref. 31), pea (*Pisum sativum*; refs. 19 and 32), narbon bean (*Vicia narbonensis*; ref. 33), and lupin (this report) has made possible a new phase in the efforts to improve the sulfur amino acid content of these important seeds. Two approaches have been used for genetic engineering of grain legumes for increased sulfur amino acid content. One approach has been to use *in vitro* mutagenesis of a gene coding for a legume seed storage protein to increase its methionine content—for example, β-phaseolin from *P. vulgaris* (34), glycinin from soybean (35), or vicilin from *Vicia faba* (36). In the first two cases, the modified protein was extremely unstable when expressed in tobacco seeds, and in the latter case, although the modified protein was as stable as unmodified vicilin in transgenic tobacco seeds, the level of expression was not high enough to result in a significant increase in the total sulfur amino acid

Table 2. Amino acid composition (g/16 g N) of lupin seed meal from nontransgenic and transgenic plants

Amino acid	Nontransgenic	Transgenic	Amino acid	Nontransgenic	Transgenic
Asx	9.61	9.49	Phe	3.71	3.52
Thr	3.11	3.01	His	2.73	2.65
Ser	4.99	4.81	Lys	4.36	4.57
Gly	3.92	3.75	Arg	11.31	11.38
Ala	3.08	3.01	Cys	1.36	1.20
Val	3.85	3.99	Met	0.55	1.07
Ile	4.30	4.34	Glx	20.90	19.54
Leu	6.46	6.18	Pro	4.00	3.98
Tyr	3.54	3.50			

Table 4. Live weight of rats fed a diet containing meal from either transgenic or nontransgenic lupins as the sole protein source

Time	Mean live weight, g	
	Nontransgenic	Transgenic
Start of adaptation period	66.8 (3.4)	67.1 (2.7)
Start of balance period	66.7 (2.4)	73.6 (2.3)
End of balance period	69.0 (2.8)	79.1 (2.2)

Each group of rats was fed on the appropriate diet for an adaptation period of 3 days before a period of 5 days during which nitrogen balance studies were carried out. Standard errors are shown in parentheses.

content. A second approach involves the identification of seed proteins with unusually high methionine/cysteine contents from nonlegumes, and the expression of genes coding for these proteins at a high level in the seeds of the target grain legume species. Two such proteins have emerged as promising candidates for this role, one from Brazil nut (*Bertholletia excelsa* H.B.K.; ref. 37) and the other from sunflower (*Helianthus annuus*; ref. 7). These are both proteins from the 2S albumin seed protein fraction, and they contain 18% methionine/8% cysteine and 16% methionine/8% cysteine, respectively. Saalbach *et al.* (38) introduced the gene for the Brazil nut protein into narbon bean and found the protein accumulated at up to 4% of extractable protein. However, work with transgenic soybeans has cast doubt on the usefulness of this protein as a means of improving nutritional quality. The 2S Brazil nut protein is highly allergenic in some human subjects both in the purified form and in extracts of transgenic soybean seeds (39).

The 2S albumin from sunflower seeds (SSA) is a more promising donor of sulfur-amino acids from two points of view. First, a search of the literature has not revealed any reports of allergenicity associated with sunflower seeds, therefore SSA would provide a good source of supplementary sulfur amino acids for both animals and humans. Second, SSA has been shown to be resistant to microbial degradation in the rumen of sheep (8), raising the possibility of obtaining increases in wool growth in sheep given supplementary feeding with lupins containing additional methionine during periods of poor pasture quality.

The only substantial changes in the amino acid composition of lupin seeds as a result of the expression of SSA were in the methionine and cysteine levels (Table 2). Based on a protein content of 36.1% and measured cysteine and methionine contents per unit dry weight (data not shown), methionine level was increased by 94% (from 0.199 to 0.386 g/100 g dry weight) and, unexpectedly, cysteine was reduced by 12% (from 0.491 to 0.433 g/100 g dry weight). The net effect was an increase of 19% in the total sulfur amino acid content on a dry weight basis. Although no change was observed in seed nitrogen level and total seed sulfur content, there was a marked redistribution of sulfur between the organic (carbon-bonded sulfur) and inorganic (sulfate) fractions. Pinkerton *et al*. (24) showed that carbon-bonded sulfur, measured by x-ray fluorescence spectrometry, was an accurate measurement of the combined methionine and cysteine contents, and the 18% increase in carbon-bonded sulfur is in close agreement with the 19% increase in cysteine plus methionine content calculated from the amino acid analyses. However, based on a level of 5% SSA in the lupin seed protein, we predicted that there would be a 24% increase in cysteine and a 140% increase in methionine levels.

The unexpected finding that the cysteine and methionine levels in the transgenic grain were not increased in direct proportion to the level of SSA indicates that associated changes must have occurred in the other components of the protein or nonprotein fraction of the seed. Phenotypic plasticity of the seed protein fraction has been previously observed; for example, under conditions of limiting sulfur nutrient supply, there is a major reduction in the content of the more sulfur-rich protein components and a compensating increase in the level of the more sulfur-poor proteins in legume seeds such as lupins (40). At moderate degrees of sulfur stress, there is no net change in the total protein content per seed. It is possible that in transgenic lupins expressing SSA there are similar changes in the composition of the seed protein fraction, in spite of the fact that no major changes were detectable in total seed protein profiles on Coomassie blue-stained polyacrylamide gels (Fig. 2). For example, conglutin δ (M_r 14,000) is a quantitatively minor globulin component of lupin seeds with a relatively high level of cysteine (8 residues percent). It accounts for about 70% of the total seed sulfur (41). A reduction in the level of this protein, which is not readily detected by staining with Coomassie blue, could, in part, account for the reduction in cysteine level observed in the seed of the transgenic lupins. From the point of view of animal nutrition, an increase in methionine is more important than an increase in cysteine, because, while animals synthesize cysteine from methionine, they do not carry out the reverse reaction (5).

A marked difference between the two seed samples was further indicated by the apparent increase in palatability of the transgenic seed, as indicated by increased feed intake by rats. The reason for this unknown. It is possible that competition between SSA and other methionine- or cysteine-rich proteins for limiting sulfur amino acids may have resulted in altered synthesis of key catalytic or regulatory proteins that are involved in other metabolic pathways, leading to other changes in seed components. At this stage, it is not possible to discriminate between possible effects of the transgenes, including *uidA* and *bar*, and possible effects due to somaclonal variation arising in tissue culture. Importantly, we found that the expression of SSA in lupin seed under field conditions did not result in any yield penalty for the transgenic plants (Table 1).

The results of the rat feeding trial provide the first report of an improvement in the nutritive value of a feed grain as a result of a modification using genetic engineering (Table 3). Increases were found in the two directly measured parameters, biological value, and true protein digestibility, and in the derived value for net protein utilization. Because it takes into account excreted urinary N corrected for endogenous urinary N, biological value is the most sensitive of these parameters to amino acid imbalance in the diet. No change was seen in digestible energy. These results are similar to those obtained earlier by Eggum *et al*. (4), where rats were fed diets containing meal from 11 different cultivars of *L. angustifolius* supplemented with synthetic methionine. Methionine supplementation had no effect on true protein digestibility, but increased biological value and net protein utilization for all cultivars by between 4.6% and 19.7%. We are currently conducting further field trials to assess the agronomic performance of the transgenic lupins and also will assess further their nutritive value in feeding trials with chickens, pigs, and sheep.

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