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Selection of methionine-enriched soybean seeds

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SELECTION OF METHIONINE-ENRICHED SOYBEAN SEEDS

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Protein accounts for approximately 40 % of the dry weight of a soybean seed; however, soy protein is deficient in both cysteine and methionine. To overcome this sulfur deficiency, animal feeders supplement soy protein rations with costly methionine, which is more stable than cysteine. To alleviate the need for methionine supplementation, we mutagenized seeds and selected genetic soybean lines, the protein of which is enriched approximately 20 % for both cysteine and methionine. Animal rations prepared from these genetic lines should not require methionine supplementation.

Soy protein is used world-wide as a major nutrient in the diets of humans and other animals. The main limitation of soy protein as an animal food, however, is its deficiency in the sulfur amino acids, especially methionine. A methionine deficiency is deleterious to animals for two reasons. First, animals can not synthesize cysteine *de novo* and second, dietary methionine is converted to cysteine more readily than is cysteine to methionine (Finkelstein *et al.* 1988). Also, because of the abundance of transmethylation reactions that rely upon *S*-adenosylmethionine, methionine utilization by animals exceeds that of cysteine. Hence, a primary concern of this research was to increase the mole percent of the sulfur amino acids in soybean seeds.

Two procedures were developed for the isolation of methionine over-producing soybean plants produced by chemically mutagenized seeds (Imsande 2001). Plants producing seeds with an elevated sulfur concentration were selected either by an ethionine resistance assay that relied upon hydroponic growth, for example mutants 20a2 and 86a1, or by a field phenotype of methionine over-producing plants, for example plants H52 and H82. Finally, genetic crosses were constructed using plants selected by the field-phenotype assay as recipients and the ethionine-resistant plants as pollen donors (Imsande 2001).

Pods containing the F1 seeds were collected and the seeds were planted in the greenhouse, producing F2 seeds. F2 seeds were field planted and F3 seeds from selected plants were collected. Seeds highest in percent seed sulfur (*i.e.*, S/N ratio) were selected and field grown. F3 seeds were field-planted yielding F4 seeds, which were analyzed for seed sulfur and S/N ratio (Imsande 2001). Plants selected by these procedures included H52 x 86a1 and H82 x 20a2. Seeds from these plants were

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assayed, and remnant seeds were planted and selected for elevated seed methionine, producing F5 seeds. Subsequently, protein from H82 x 20a2 seeds was analyzed for mole percent methionine and cysteine. These data show that the methionine content of line H82 x 20a2 is 31 % greater than that of typical soy protein whereas the cysteine content is 27 % greater (Imsande 2001).

Subsequently, M8 seeds were obtained from H52 #5 HF in 2000 and M9 seeds in 2001. Also, F5 seeds were obtained from crosses H52 x86a1 and H82 x20a2 in 2000 and F6 seeds from these two lines in 2001. The seeds produced by these lines were analyzed repeatedly for methionine concentration by growth support of an *E. coli* methionine auxotroph (Sambrook *et al.* 1989; Wright and Orman 1995). These analyses demonstrated a statistically significant increase in the methionine concentration of these lines (Table 1). A typical one-kg feed ration for animals contains approximately 150 g of soy protein. The mean mole percentage value for L-methionine of soybean protein is 1.40. Also, a typical one-kg ration is supplemented with 1 g of DL-methionine. Hence, the methionine concentration of soy protein must be increased approximately 21 % to provide as much L-methionine as is present in the supplemented ration, or 42 % to provide as much total methionine. The methionine mole percentage of our lines is approximately 1.84, which is approximately 31 % greater than the 1.40 mole percentage methionine found in a random sample of soybean meal. Because the mole percentage of cysteine in our lines also is increased approximately 27 %, rations prepared using these newly developed lines should not require methionine supplementation. Also, the increased cysteine content may enhance the firmness of tofu.

Table 1. Performance summary: 2000, without seed coat; 2001, with seed coat.

	Methionine content	CV	Increase in methionine (%)	Seed yield kg/10 foot row
2000 Samples				
H52 #5HF	0.308	5.6	9.2	ND
H52 x 86a1	0.317	6.7	12.4	ND
H82 x 20a2	0.312	5.5	10.6	ND
Kenwood 94	0.282	3.9	0 (control)	ND
2001 Samples				
H52 #5HF	0.340	29	25	0.760
H52 x 86a1	0.301	14	10	0.731
H82 x 20a2	0.312	15	14	0.675
Kenwood 94	0.273	14	0 (control)	0.753

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