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BARD

FINAL REPORT

PROJECT NO. US-2805-96

Improving Methionine Content in Transgenic Forage Legumes

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Project Title: Improving methionine content in transgenic forage legumes.

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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Zein genes, aspartate kinase, Cystathionine gamma synthase, alfalfa.

Abbreviations commonly used in the report, in alphabetical order:

AK, CAB promoter, CaMV 35S promoter, CGS, 18KD zein, 15KD zein, 10KD zein

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)			1	1
Submitted, in review, in preparation	1	2		3
Invited review papers				
Book chapters				
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Master theses		1	3	
Ph.D. theses		1		
Abstracts		4		
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Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings				
Longer Visits (Sabbaticals)				

Description of Cooperation:

Exchange of ideas and gene constructs

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
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Licensed				

IMPROVING METHIONINE CONTENT IN TRANSGENIC FORAGE LEGUMES.
BARD Project No. US-2805-96
Final Scientific Progress Report

Abstract

Leguminous forage crops are high in proteins but deficient in S- amino acids. It has been shown that both wool quality and milk production can be limited by the post-ruminal supply of sulfur-containing amino acids. Efforts to use conventional plant breeding and cell selection techniques to increase the S-amino acid content of alfalfa have met with little success. With the objective to increase the S-amino acid content of forage legumes, the goal of this project was to co-express the methionine rich zein genes from corn along with a gene for a key enzyme in methionine biosynthesis, aspartate kinase (AK). The zeins are seed storage proteins from corn and are grouped into four distinct classes based on their amino acid sequence homologies.. The β -zein (15kd) and the δ -zein (10kd and 18kd) have proportionately high levels of methionine (10%, 22% and 28%, respectively). Initial studies from our lab had shown that while the 15kd zein accumulated to high levels in vegetative tissues of transgenic tobacco the 10kd zein did not. However, co-expression of the 10kd zein with the 15kd zein genes in tobacco showed stabilization of the 10kd zein and the co-localization of the 10kd and 15kd zein proteins in unique ER derived protein bodies. AK is the key enzyme for producing carbon skeletons for all amino acids of the aspartate family including methionine. It is, however, regulated by end-product feedback inhibition. The specific objectives of this proposal were: i. to co-express the 15kd zein with the 10/18kd zein genes in alfalfa in order to enhance the level of accumulation of the 10/18kd zein; ii. to increase methionine pools by expressing a feedback insensitive AK gene in transformants co-expressing the 15kd and 10/18kd zein genes. The Israeli partners were successful in expressing the AK gene in alfalfa which resulted in an increase in free and bound threonine but not in methionine (Galili et al., 2000). Since our target was to increase methionine pools, we changed our second objective to replace the AK gene with the gene for cystathionine gamma synthase (CGS) in the co-expression studies. The first methionine specific reaction is catalyzed by CGS. An additional objective was to develop a transformation system for Berseem clover, and to introduce the appropriate gene constructs into it with the goal of improving their methionine content.

Genes for the 15kd zein along with the genes for either the 10kd or 18kd zein have been introduced into the same alfalfa plant both by sexual crosses and by re-transformation. Analysis of these zein co-expressors have shown that both the 10kd and 18kd zein levels go up 5 to 10 fold when co-expressed with the 15kd zein (Bagga et al., MS in preparation). Incubation of the leaves of transgenic alfalfa co-expressing the 15kd and 10kd zein genes, in the rumen of cows have shown that the zein proteins are stable in the rumen. To increase the level of zein accumulation in transgenic alfalfa different promoters have been used to drive the zein genes in alfalfa and we have concluded that the CaMV 35S promoter is superior to the other strong leaf -specific promoters. By feeding callus tissue of alfalfa plants co-expressing the 15kd and 10kd zein genes with methionine and its precursors, we have shown that the zein levels could be significantly enhanced by increasing the methionine pools. We have now introduced the CGS gene (from Arabidopsis; kindly provided to us by Dr. Leustek), into the 15kd zein transformants and experiments are in progress to check if the expression of the CGS gene indeed increases the level of zein accumulation in alfalfa. We were not successful in developing a transformation protocol for Berseem clover.

Achievements

Significance: We have shown that the methionine content in alfalfa and other forage legumes could probably be enhanced significantly by increasing the free methionine pools and expressing the genes encoding for the stable methionine rich zein proteins, simultaneously. We have also shown that we can introduce multiple genes encoding for different proteins in the same alfalfa plant by sexual crosses and by re-transformation. We now have transgenic alfalfa plants co-expressing the methionine rich zein genes and the gene for cystathionine gamma synthase.

Agricultural and/or economic impacts: Alfalfa is a major forage crop and it has been bred for years for both forage quality and plant performance. However, all attempts to breed for improved amino acid balance has failed. This proposal is an attempt to improve the amino acid balance of alfalfa by genetic engineering. Improvement in the methionine content in forage legumes could have a great impact on wool growth in sheep and milk production by dairy animals.

Details of Cooperation: The US collaborators sent the 15kD zein and the 10kD zein gene constructs behind the CaMV 35S promoter to the lab in Israel. The Israeli collaborators shared the AK gene construct with the US lab. Even though the original intentions were to share plant material for sexual crosses or for re-transformation with the second gene but that was not feasible because of regulatory restrictions on transgenic plant material. There was exchange of ideas between the two labs. Alfalfa plants were transformed with the AK gene in Israel while the zein genes were introduced into alfalfa in the US. Both labs now have both sets of constructs in alfalfa and it should be feasible to introduce them in the same plant. However, in the second year we realized that co-expressing CGS gene might be more appropriate for increasing free methionine pools.

List of Publications:

- 1 Galili, S., Guenoune, D., Wininger, S., Hana, B., Schupper, A., Ben-Dor, B., and Kapulnik, S. 2000. Enhanced levels of free and protein-bound threonine in transgenic alfalfa (*Medicago sativa* L.) expressing a bacterial feedback-insensitive aspartate kinase gene. *Transgenic Research* 9: 137-144.
2. Bagga, S., Armendaris, A., Ray, I., Endres, M., Klypina, N., Sutton, D., Kemp, J.D., and Sengupta-Gopalan. Genetic engineering for ruminal stable high methionine protein in alfalfa. To be submitted to "Plant Science".
3. Bagga, S., Moguel, S., Potenza, C., Leustek, T., and Sengupta-Gopalan, C. Free methionine pools limit the expression of the methionine rich zein genes in transgenic alfalfa. MS in preparation.
4. Bagga, S., Leustek, T., Galili, S., and Sengupta-Gopalan, C. Effect of co-expression of the