ABSTRACT: 461

A MULTIFACETED APPROACH TO IMPROVE SOYBEAN PROTEIN QUALITY FOR FOOD AND FEED APPLICATIONS

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Soybean is an important protein source in human diets and animal feeds. This versatile legume is widely used throughout the world because of its high protein content and relatively balanced amino acid profile. The continual dominance of soybean in the world market, however, will necessitate alteration of soybean seed composition. Soybeans, which are extensively used as a high-protein feed ingredient in livestock production, do not contain sufficient sulfur-containing amino acids to meet the nutritional demands of rapidly growing swine or poultry. Additionally, some soy proteins can cause allergic responses in humans and monogastric animals. Our laboratory has taken a multifaceted approach to improve the sulfur amino acid content of soybean and eliminate the major allergens. We have developed a successful strategy to increase the cysteine content of soybean seed through the over-expression of O-acetylserine sulfhydrylase (OASS), a key sulfur assimilatory enzyme. OASS overexpressing transgenic soybean plants showed a 74% increase in protein-bound cysteine levels compared to non-transformed wild-type soybean seeds. The overall increase in soybean total cysteine content (both free and protein-bound) satisfies the recommended levels required for the optimal growth of monogastric animals. We have also demonstrated that soybean 7S globulins are major allergens for both humans and early weaned pigs. By employing RNAi technology we have successfully generated transgenic soybean plants that lack the 7S beta-conglycinin. These transgenic soybeans lacking all three subunits of beta-conglycinin will be a valuable source of hypoallergenic protein. The development of soybean cultivars lacking the major allergens along with improved sulfur amino acid content should further enhance the continued use of soybean in human diets and animal feeds.

A multifaceted approach to improve soybean protein quality for food and feed applications

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Composition of Soybean Seed



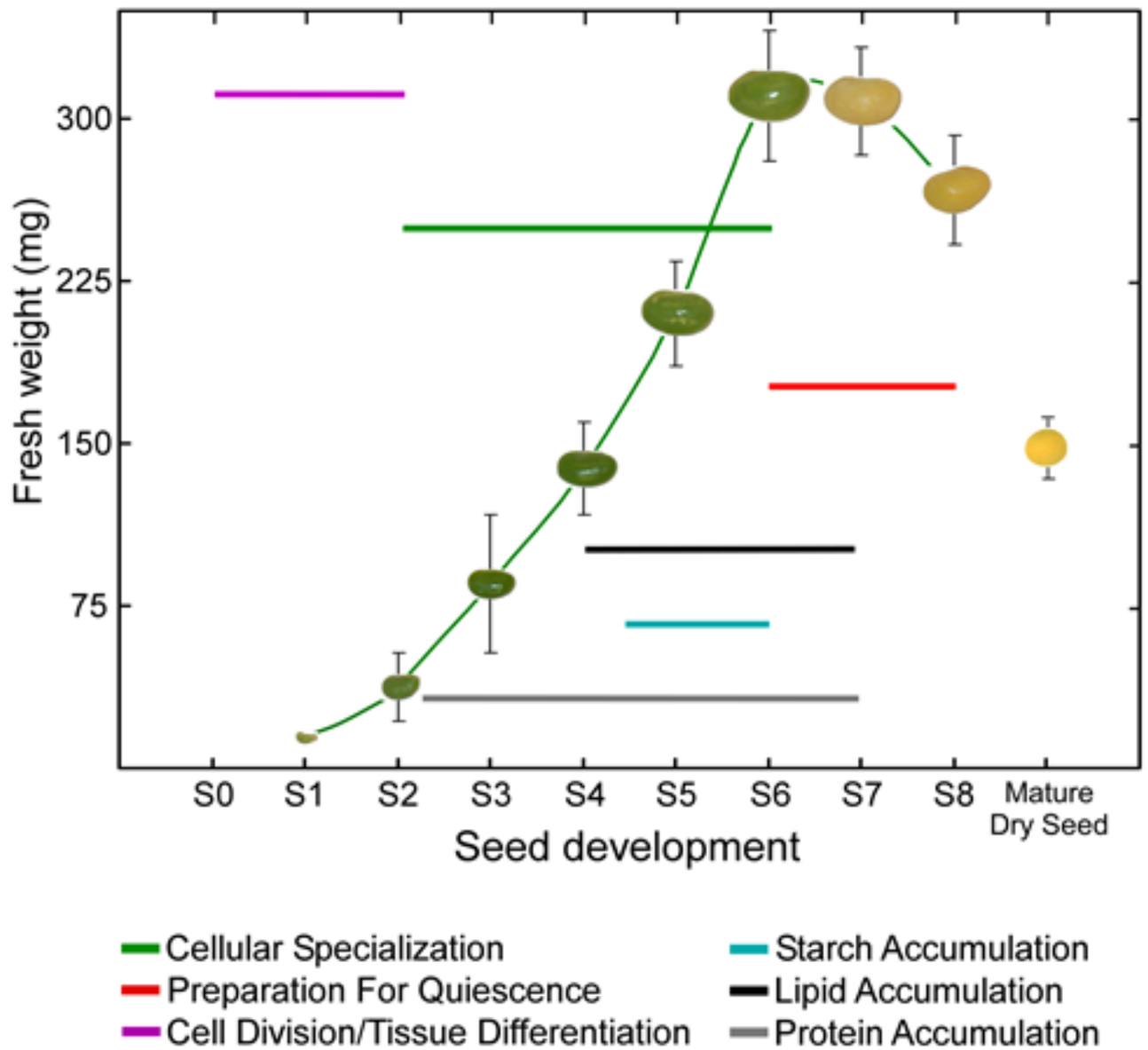
40% Protein

20% Oil

12 % Carbohydrate

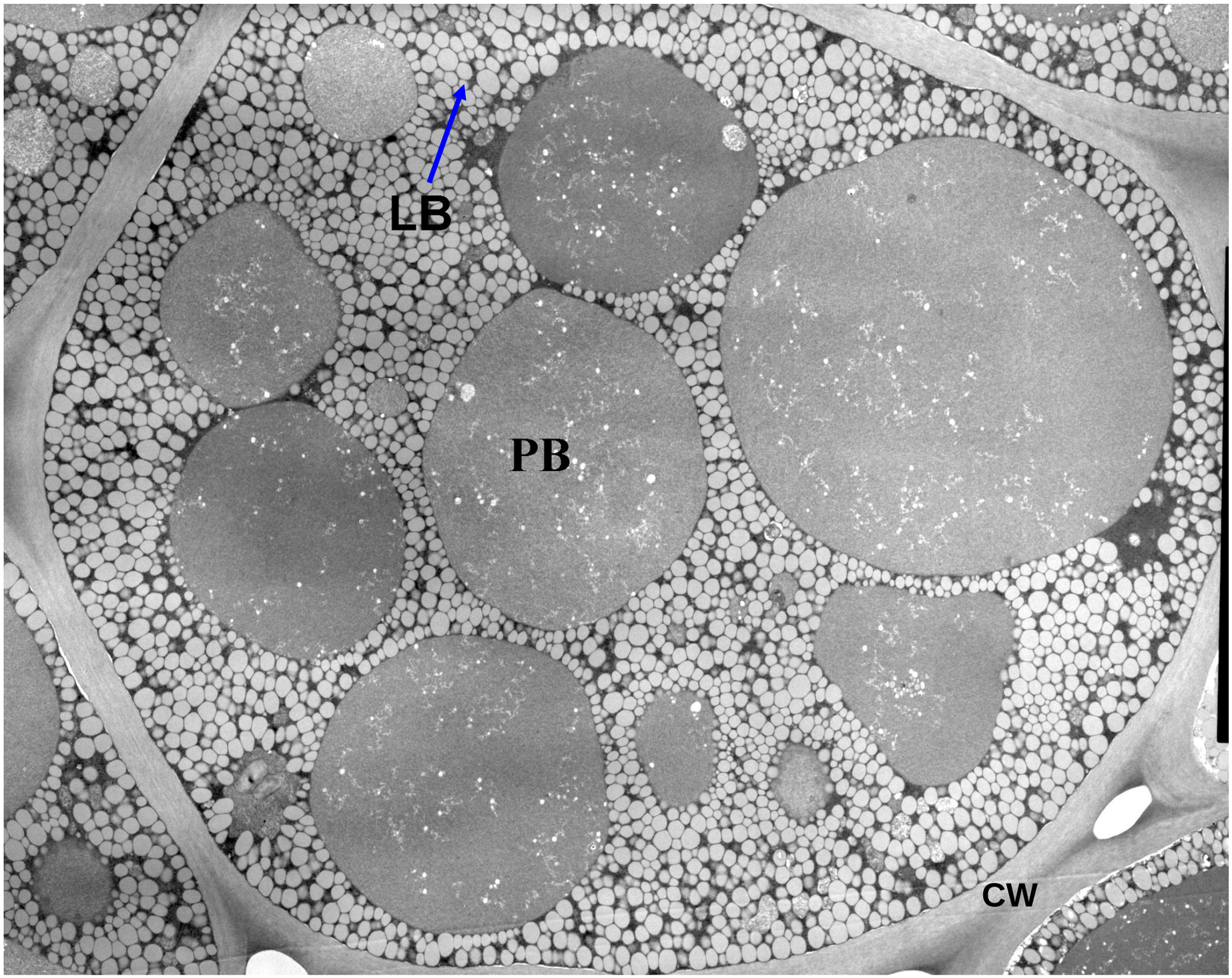
11 – 12 %	**Starch**
41 – 68 %	**Sucrose**
12 – 35 %	**Stachyose**
5 – 16 %	**Raffinose**

Soybean seed development



Miernyk JA. USDA-ARS

Electron Micrograph of Soybean Seed



Soybean Value: 0il and Meal

- **Soybean oil-primary source of edible vegetable oil in the US**
- **Meal-main consumers: swine and poultry**





Importance of soy meal

- **Soy meal is the single most important source of animal feed in the United States and Europe.**

- **More than one billion bushels of US soybeans are utilized for animal feed, which represents about 37% of total US soybean production.**

Amino acid composition of soy meal

- Soy meal - high protein content with superior amino acid profile.

- Rapidly growing swine and poultry have a higher dietary requirement for sulfur-containing amino acids.

- Lysine and methionine are the primary limiting amino acids in swine and poultry rations.

- Supplementation with synthetic amino acids

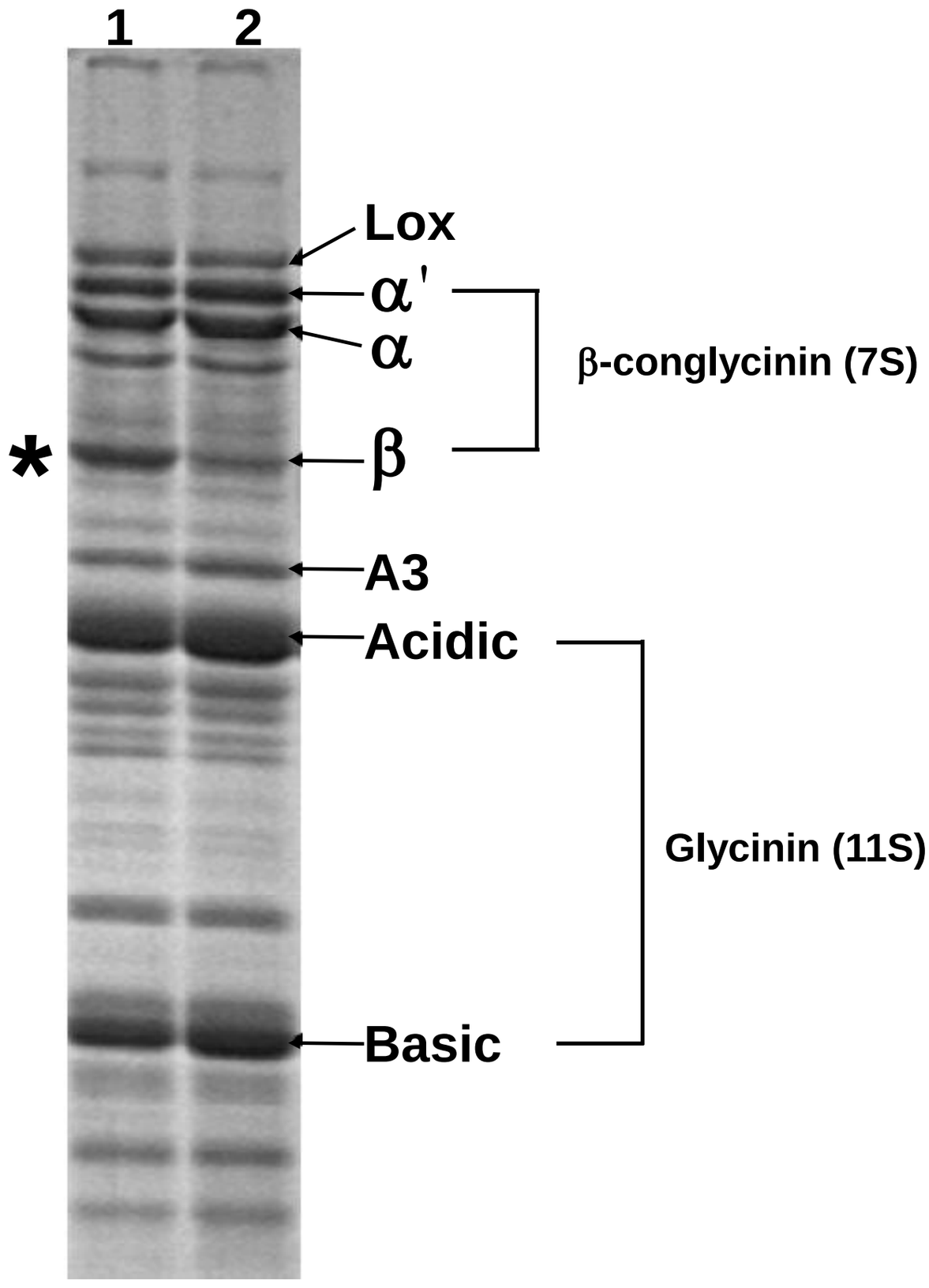
Limiting Amino Acids in Soybeans

- Sulfur-containing amino acids represent 2.2 to 3% of the total soybean seed protein.

- The minimum requirement for ruminant and non-ruminant nutrition is 3.5% and 5%, respectively.

- Mixtures with corn and supplements with synthetic amino acids are necessary to obtain a balanced diet.

SDS-PAGE Analysis of Soybean Seed Proteins



Sulfur Amino Acid Content of 7S and 11S Storage Proteins of Soybean

	β-conglycinin (7S)			Glycinin (11S)				
Amino Acid	α'	α	β	Gy1	Gy2	Gy3	Gy4	Gy5
Cysteine	0.8%	0.9%	0.0%	1.7%	1.7%	1.7%	1.1%	1.2%
Methionine	0.3%	0.2%	0.0%	1.3%	1.5%	1.1%	0.4%	0.6%

Our Goal

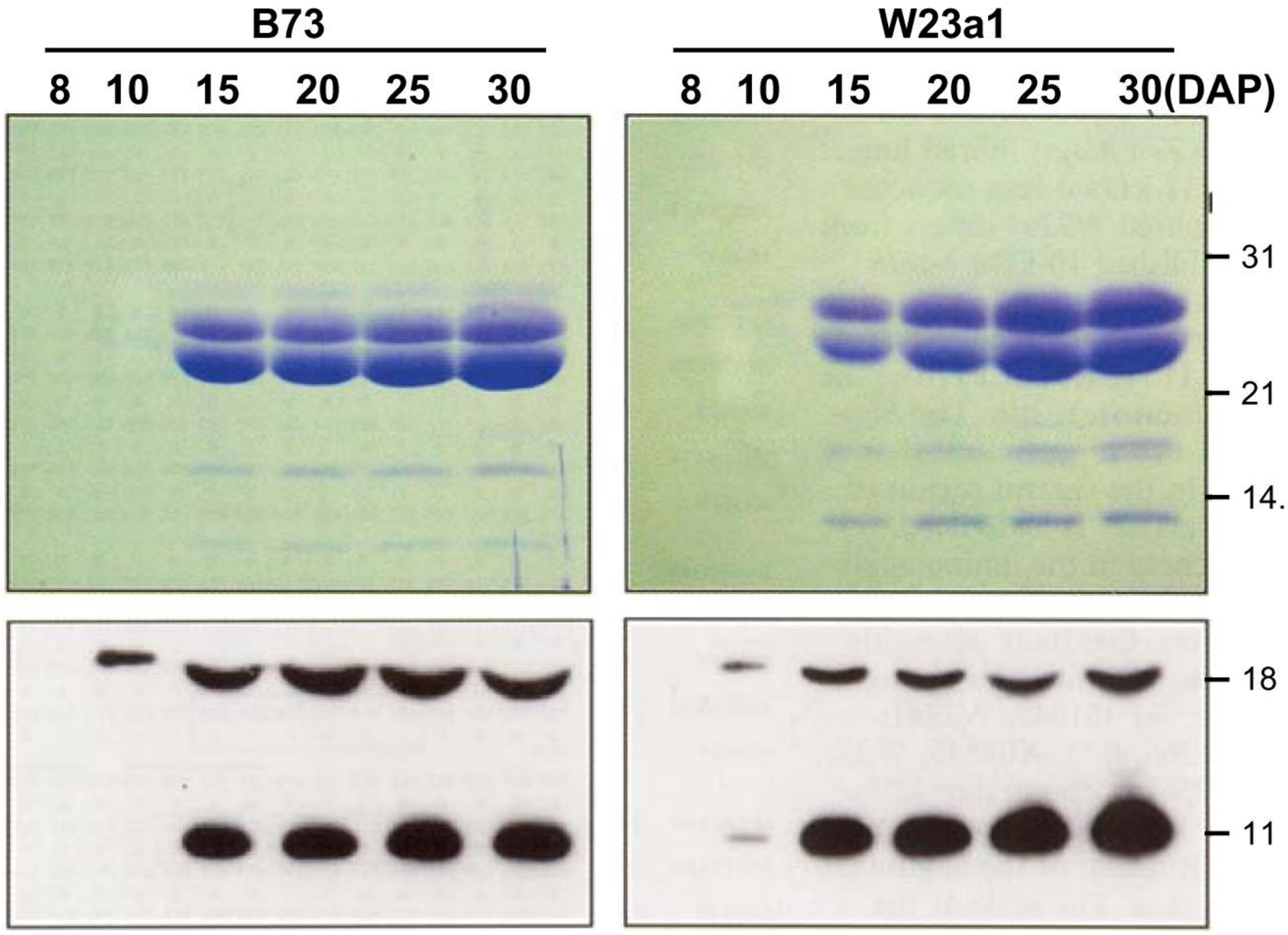
The overall goal of our lab is to enhance the nutritional quality of soybean seeds by increasing its total content of sulfur-containing amino acids, methionine and cysteine.

Approaches to Solve the Problem

1. By expressing heterologous seed proteins that are rich in cysteine and methionine

2. By manipulating the enzymes involved in sulfur metabolism

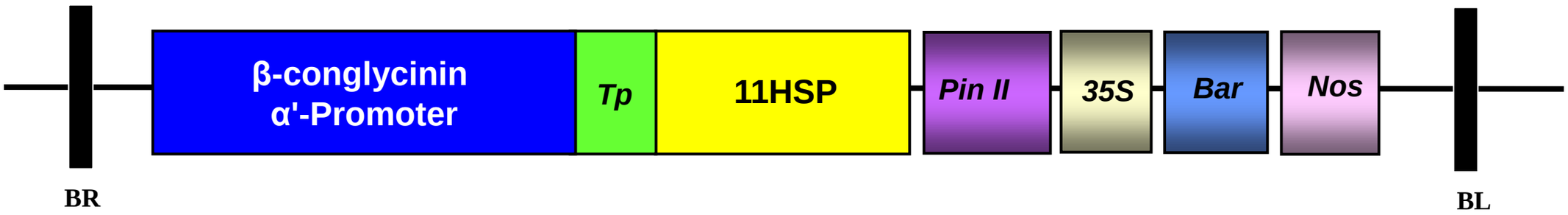
Maize Seed Storage Proteins

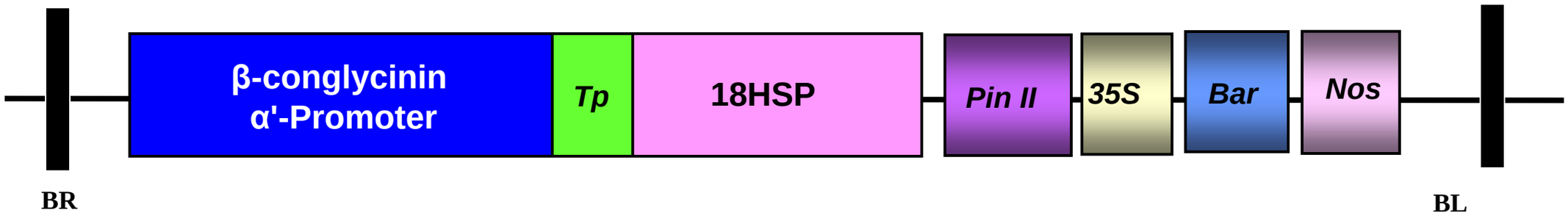


Amino Acid Composition of the 11 kD δ-zein

Amino Acid	No. of Residues	Percentage
Ala (A)	7	5.2%
Arg (R)	1	0.7%
Asn (N)	4	3.0%
Asp (D)	1	0.7%
Cys (C)	**6**	**4.4%**
Gln (Q)	14	10.4%
Glu (E)	0	0.0%
Gly (G)	4	3.0%
His (H)	3	2.2%
Ile (I)	3	2.2%
Leu (L)	17	12.6%
Lys (K)	0	0.0%
Met (M)	**30**	**22.2%**
Phe (F)	5	3.7%
Pro (P)	21	15.6%
Ser (S)	8	5.9%
Thr (T)	5	3.7%
Trp (W)	0	0.0%
Tyr (Y)	1	0.7%
Val (V)	5	3.7%

Soybean Transformation Constructs





Amino acid composition of isoproponal-extracted proteins from soybean.

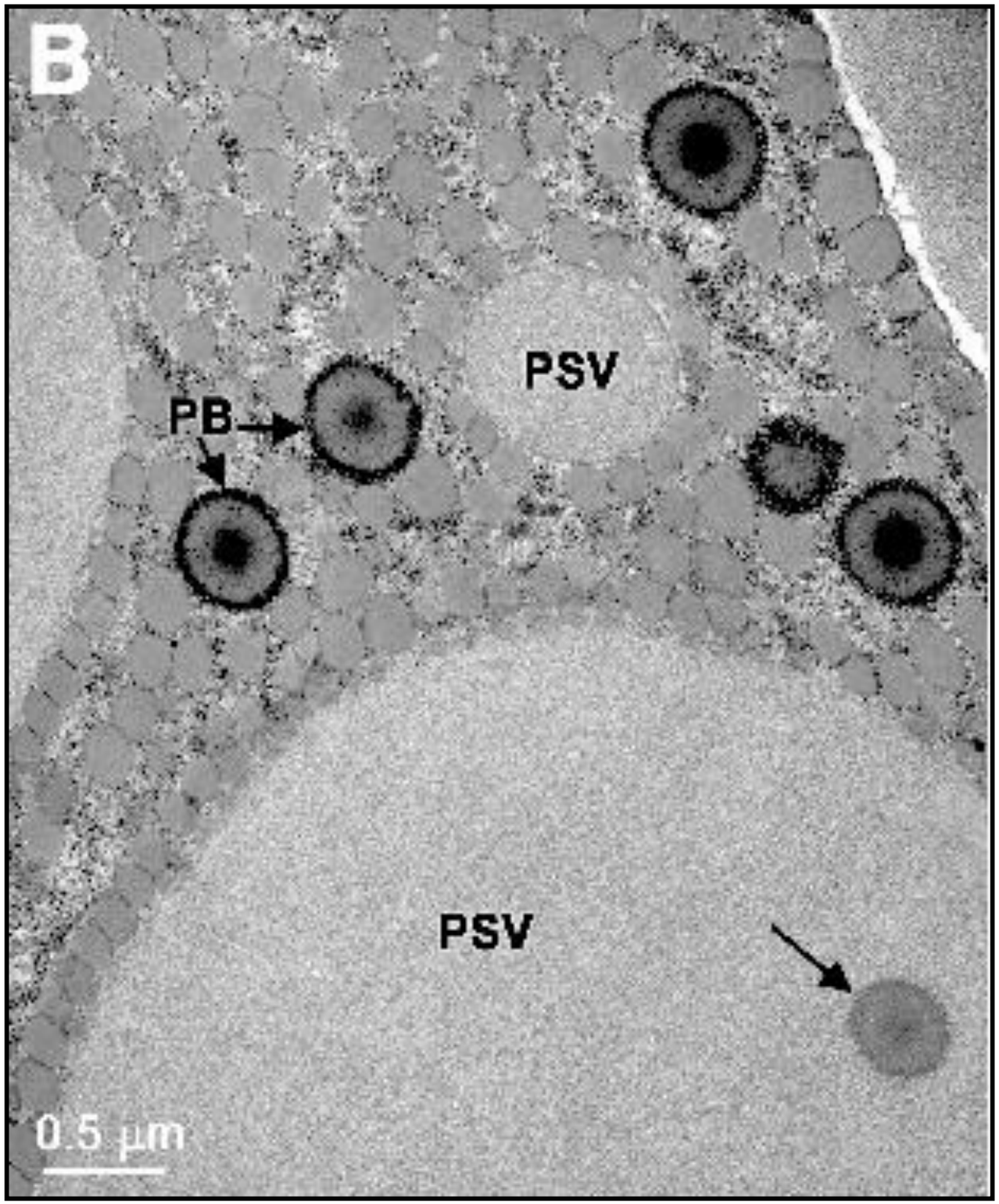
Amino acid	Control	A11*	A13*
Aspartic acid	14.40 (0.03)	13.56 (0.07)	13.64 (0.06)
Threonine	8.00 (0.22)	7.75 (0.02)	7.85 (0.27)
Glutamic acid	19.17 (0.27)	18.48 (0.42)	20.25 (0.28)
Proline	6.62 (0.05)	7.04 (0.08)	6.86 (0.14)
Glycine	6.29 (0.02)	5.96 (0.05)	6.31 (0.04)
Alanine	7.40 (0.07)	7.28 (0.06)	7.56 (0.10)
Cysteine	5.41 (0.14)	5.22 (0.08)	4.30 (0.15)
Valine	5.26 (0.02)	5.46 (0.02)	5.92 (0.11)
Methionine	2.52 (0.02)	4.36 (0.06)	3.74 (0.10)
Isoleucine	4.36 (0.14)	4.48 (0.10)	4.69 (0.14)
Leucine	8.99 (0.12)	9.77 (0.07)	9.55 (0.16)

*Transgenic soybean lines expressing 11 kD δ-zein
Data are the average of three different preparations of prolamin fractions.
Standard deviation is shown in parenthesis.

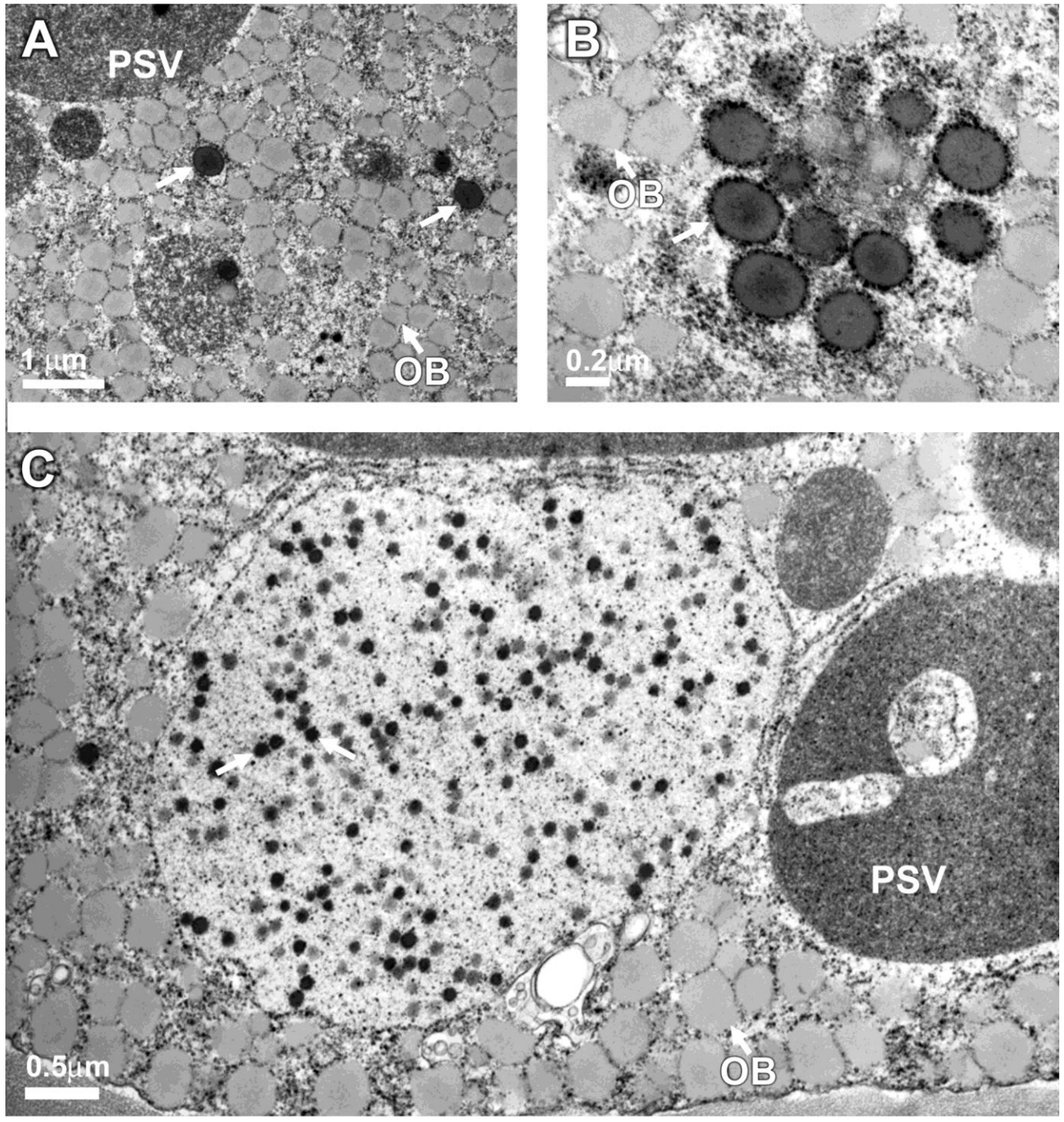
11 kD δ-zein accumulation is restricted to transitional cells



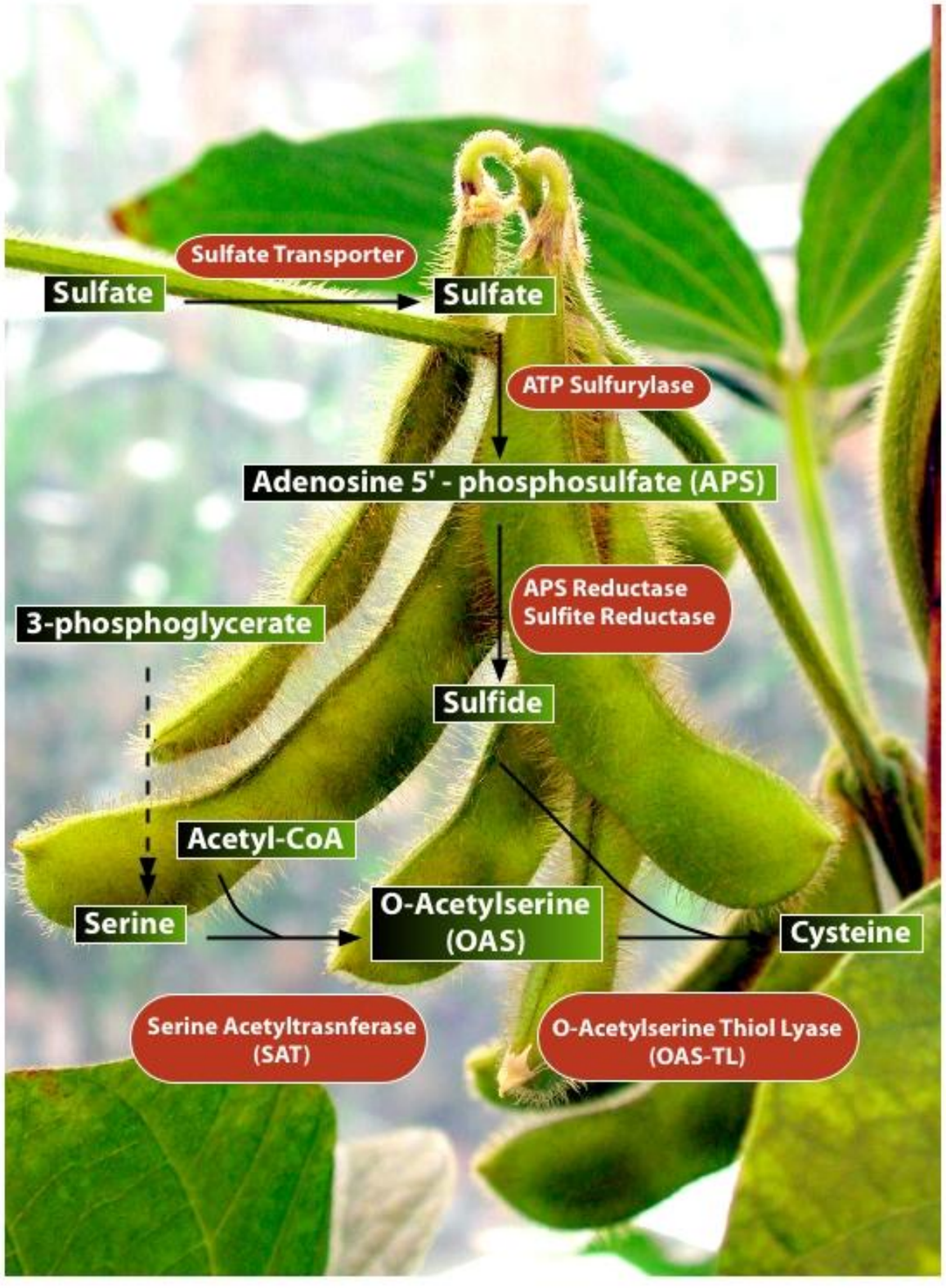
ER-derived Protein Bodies in Transgenic Soybeans



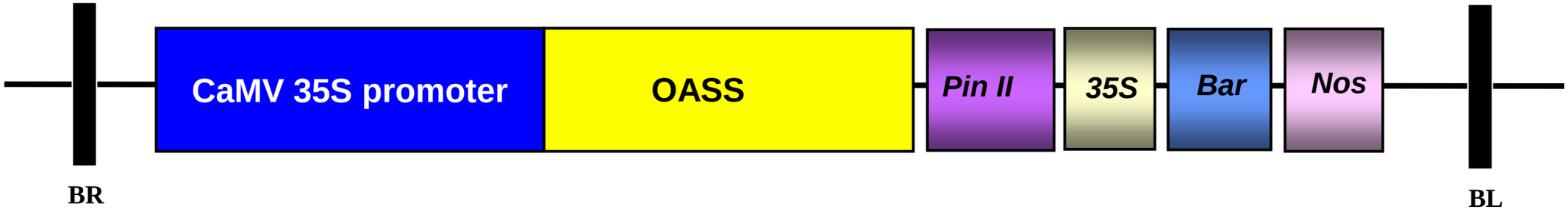
Sulfur Supplementation Promotes ER-derived Protein Body Formation in Transgenic Soybean



Sulfur Assimilatory Pathway in Plants

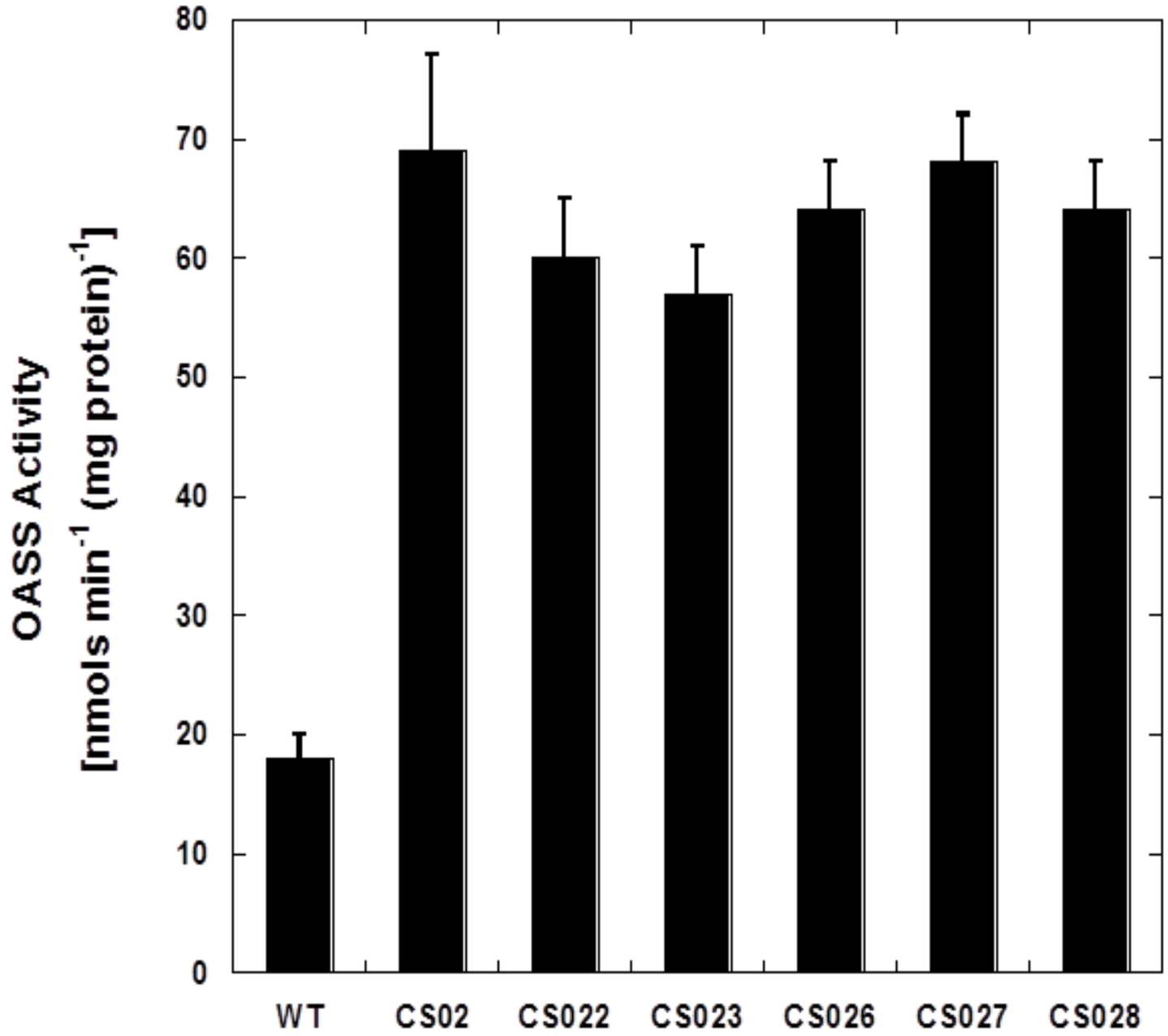


Soybean Transformation Construct

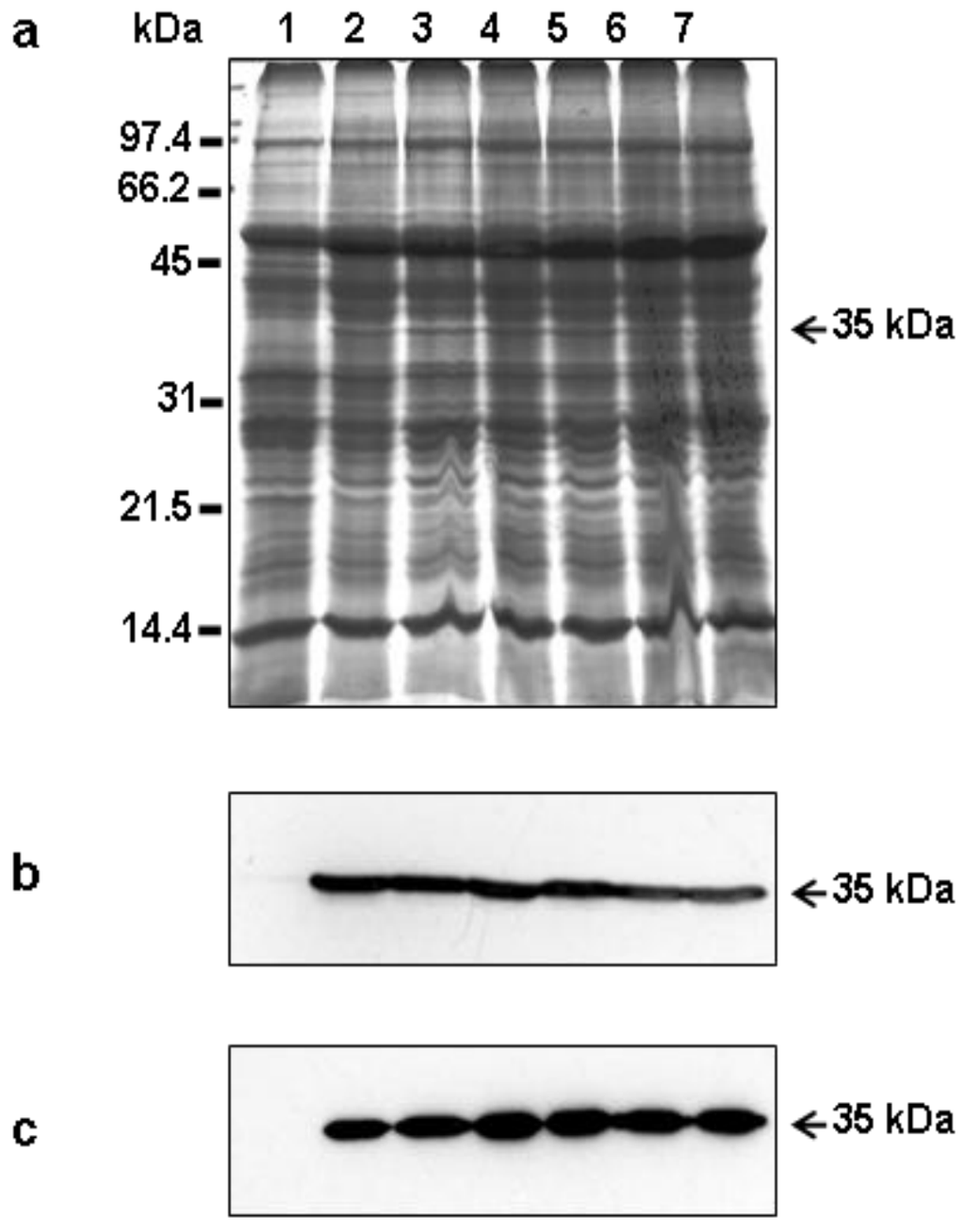


OASS: *O*-acetylserine sulfhydrylase

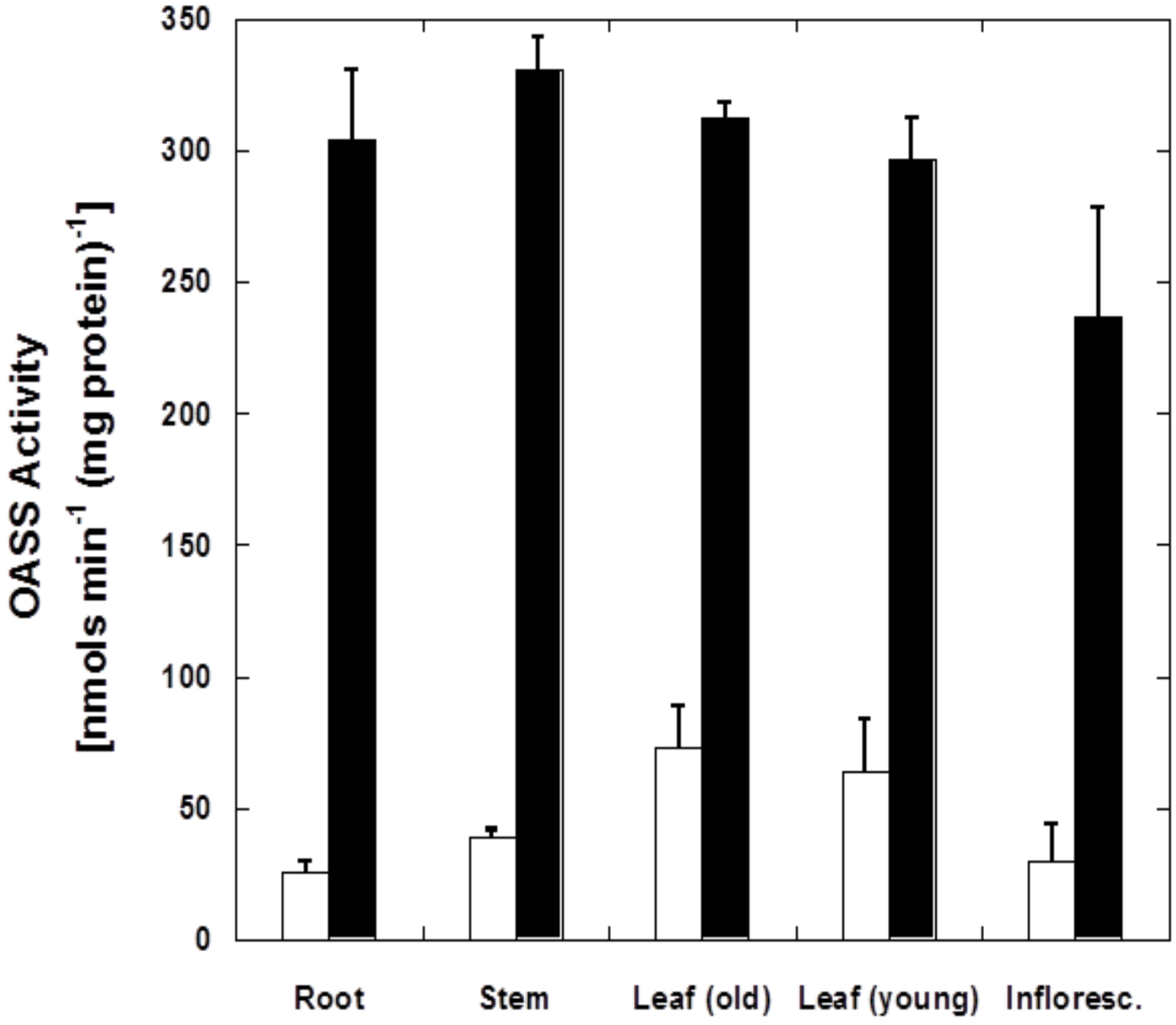
Transgenic soybeans reveal high OASS activity



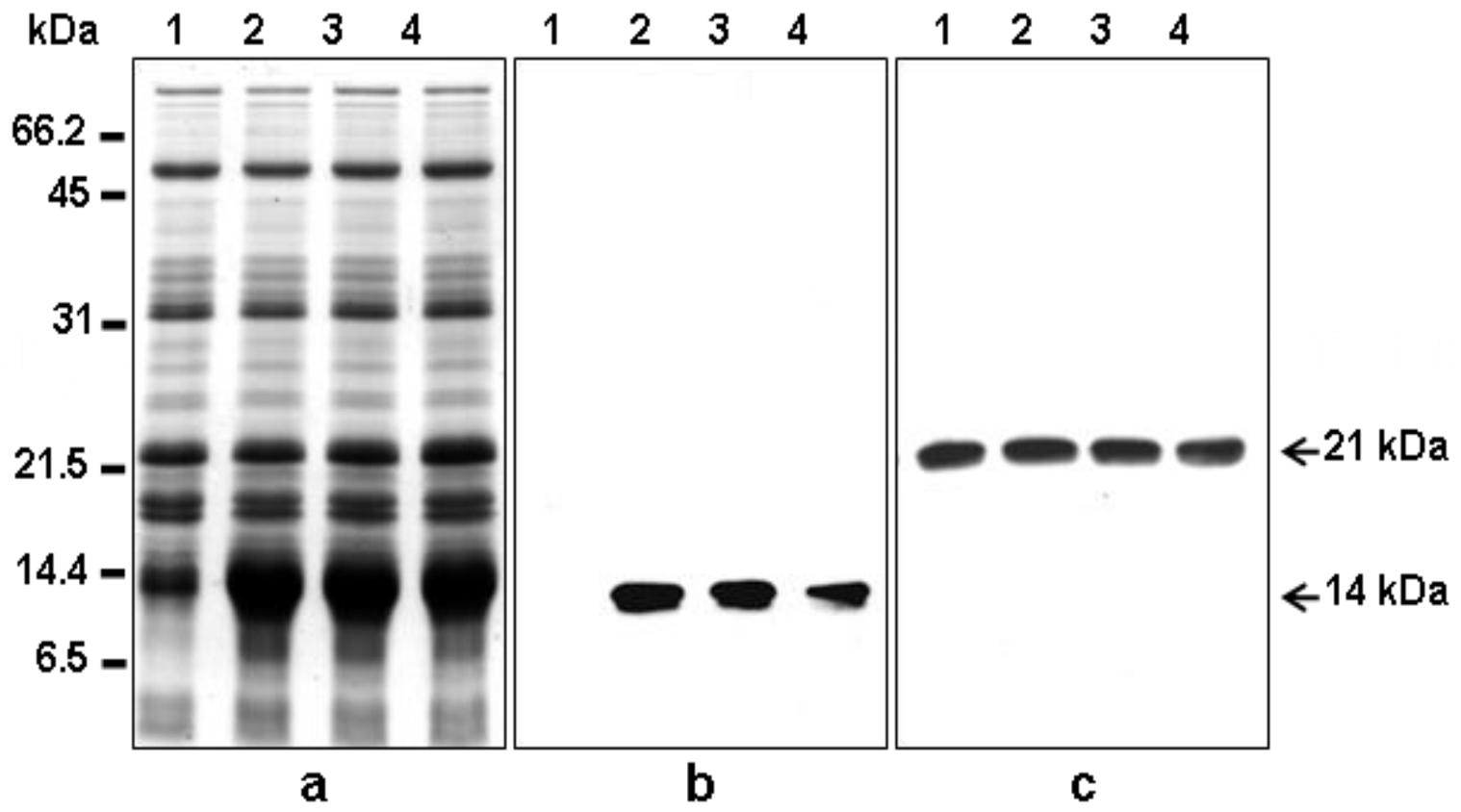
Accumulation of OASS in transgenic soybeans



Transgenic soybeans reveal high OASS activity in all organs



Bowman-Birk protease inhibitor accumulation is enhanced in transgenic soybean seeds overexpressing OASS

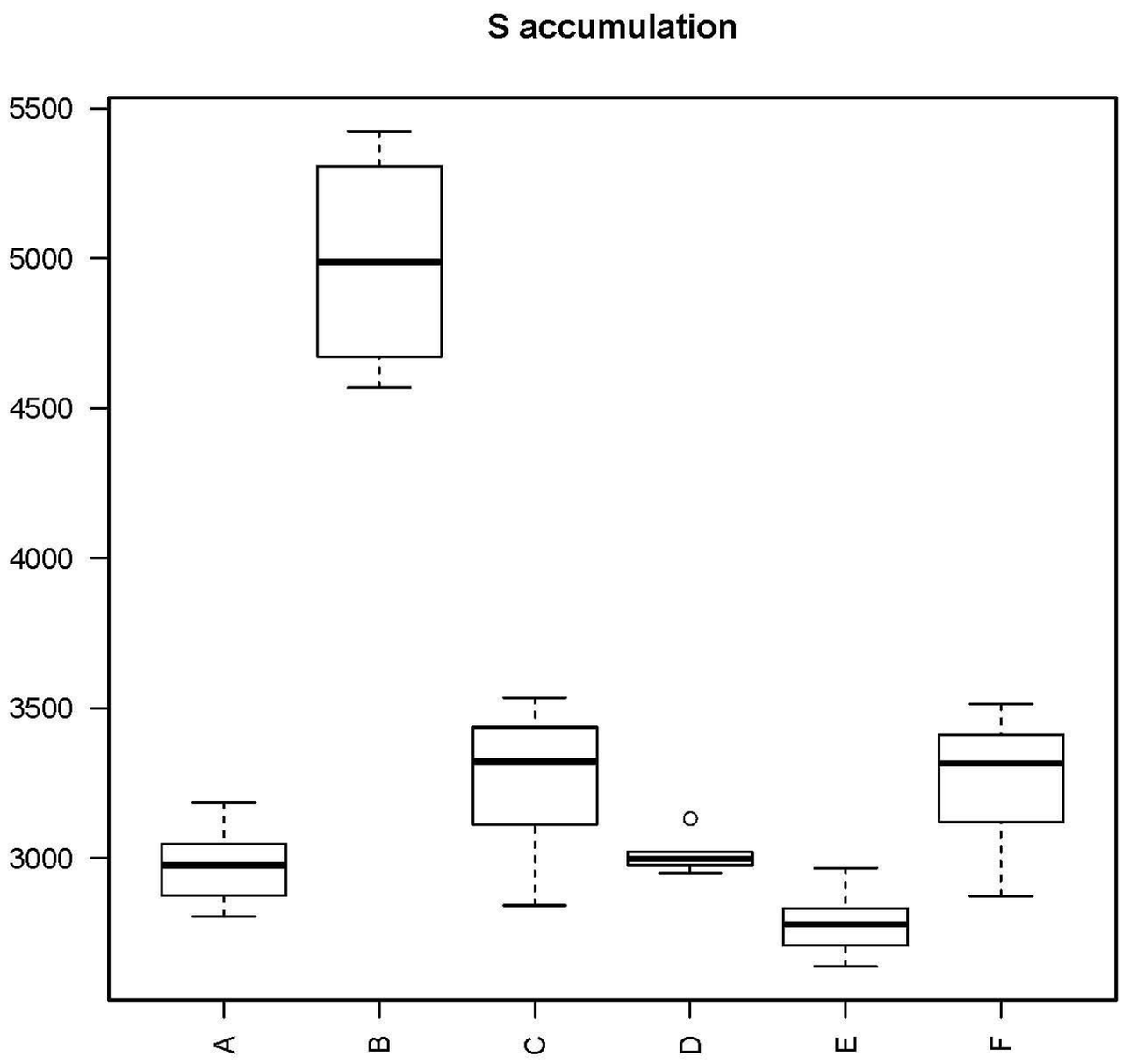


Amino acid composition of soybean seed (% per 100g protein)

Amino acid	Control	CS02*
Aspartic Acid	11.89 (0.17)	12.07 (0.11)
Threonine	4.20 (0.07)	4.28 (0.04)
Serine	4.69 (0.11)	5.28 (0.02)
Glutamic Acid	17.14 (0.30)	17.14 (0.14)
Proline	5.09 (0.05)	5.05 (0.05)
Glycine	4.55 (0.07)	4.42 (0.03)
Alanine	4.45 (0.08)	4.50 (0.04)
Cysteine	**1.59 (0.03)**	**2.77 (0.01)**
Valine	5.09 (0.08)	4.67 (0.06)
Methionine	**1.57 (0.03)**	**1.84 (0.02)**
Isoleucine	4.82 (0.07)	4.55 (0.06)
Leucine	8.06 (0.12)	7.60 (0.08)
Tyrosine	3.63 (0.04)	3.15 (0.05)
Phenylalanine	5.07 (0.07)	4.67 (0.07)
Lysine	6.84 (0.11)	7.04 (0.06)
Histidine	2.92 (0.04)	2.92 (0.03)
Arginine	7.25 (0.09)	6.71 (0.07)
Tryptophan	1.14 (0.02)	1.35 (0.01)

*Transgenic soybean line overexpressing OASS. Dry seed powder from three independent plants were combined and subjected to amino acid analysis.

Transgenic soybeans overexpressing OASS contain elevated elemental sulfur



Soybean as a food allergen

- **Soybean has been recognized as one of the “big 8” food allergens.**

- **Soybean allergy is most common among children nourished with soybean-based infant formula.**

- **0.5% of the U.S. population is allergic to soybean.**

- **Allergic symptoms to soybean include skin, gastrointestinal and respiratory reactions and in some cases anaphylaxis.**

- **Several soybean proteins have been identified as allergens.**

Pig's immunological response to soybean

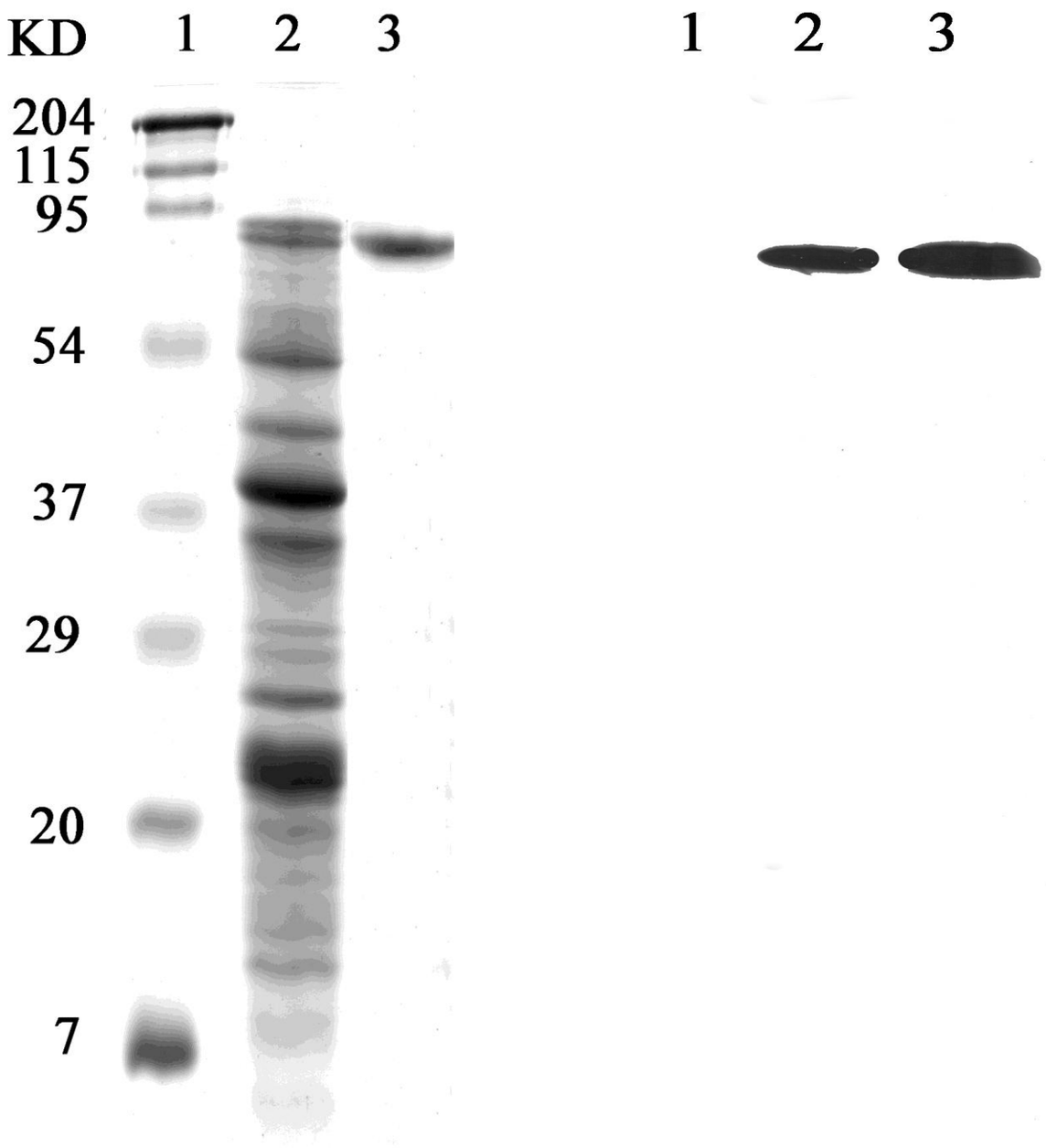
- **Can cause an inflammatory response in early-weaned pigs.**

- **SBM can affect pig performance in the first few weeks following weaning.**

- **Elicit specific antibodies in weaned piglets.**

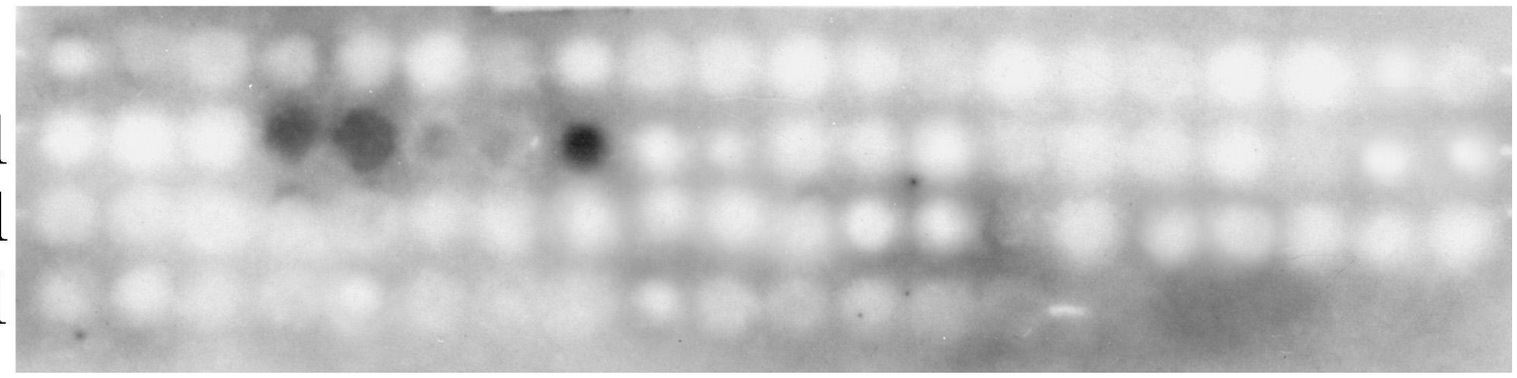
- **Soy allergens have not been identified and characterized.**

SDS-PAGE/immunoblot analysis of the soybean seed proteins



Multiple IgG-binding regions identified on the α-subunit allergen

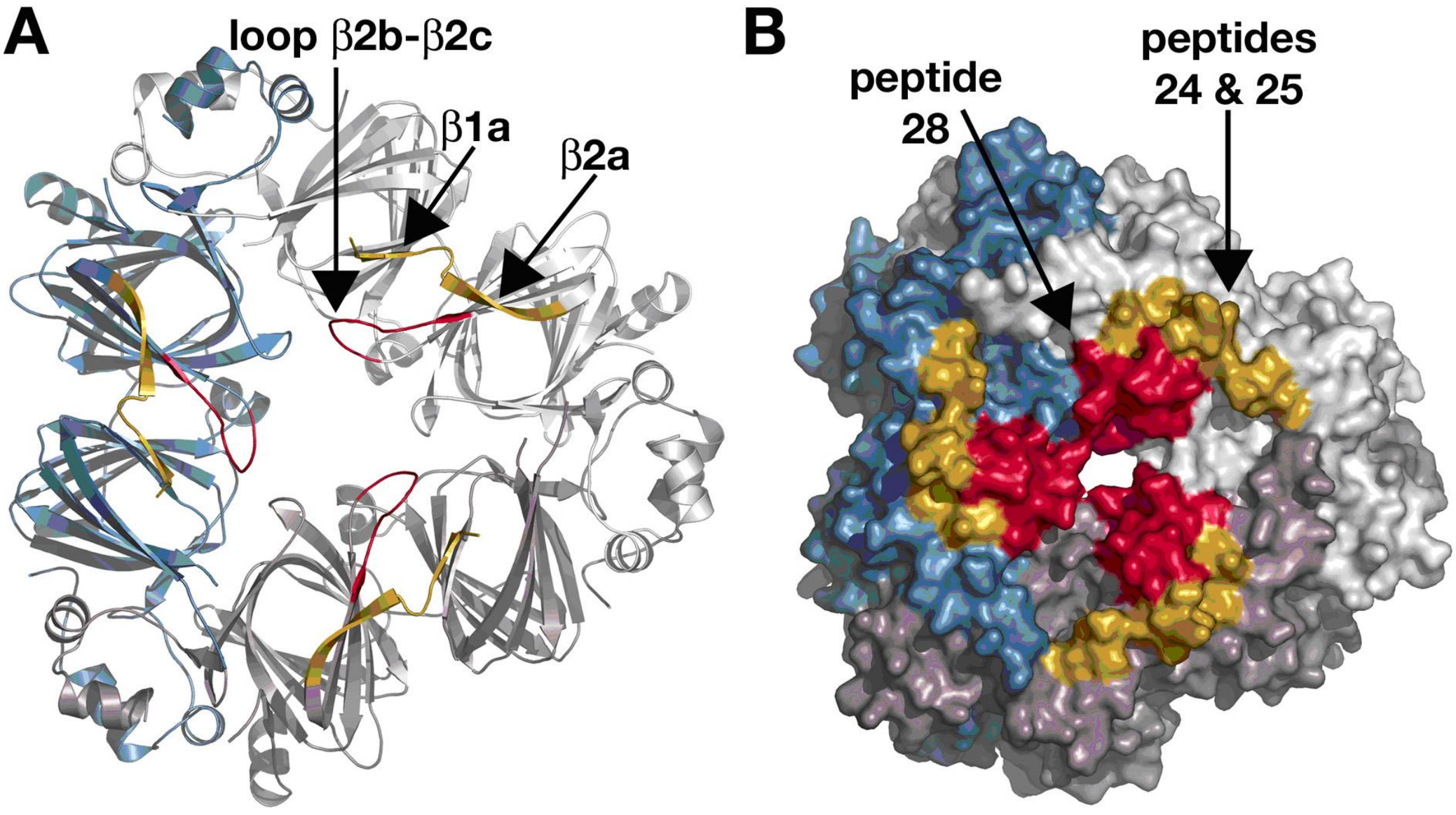
A



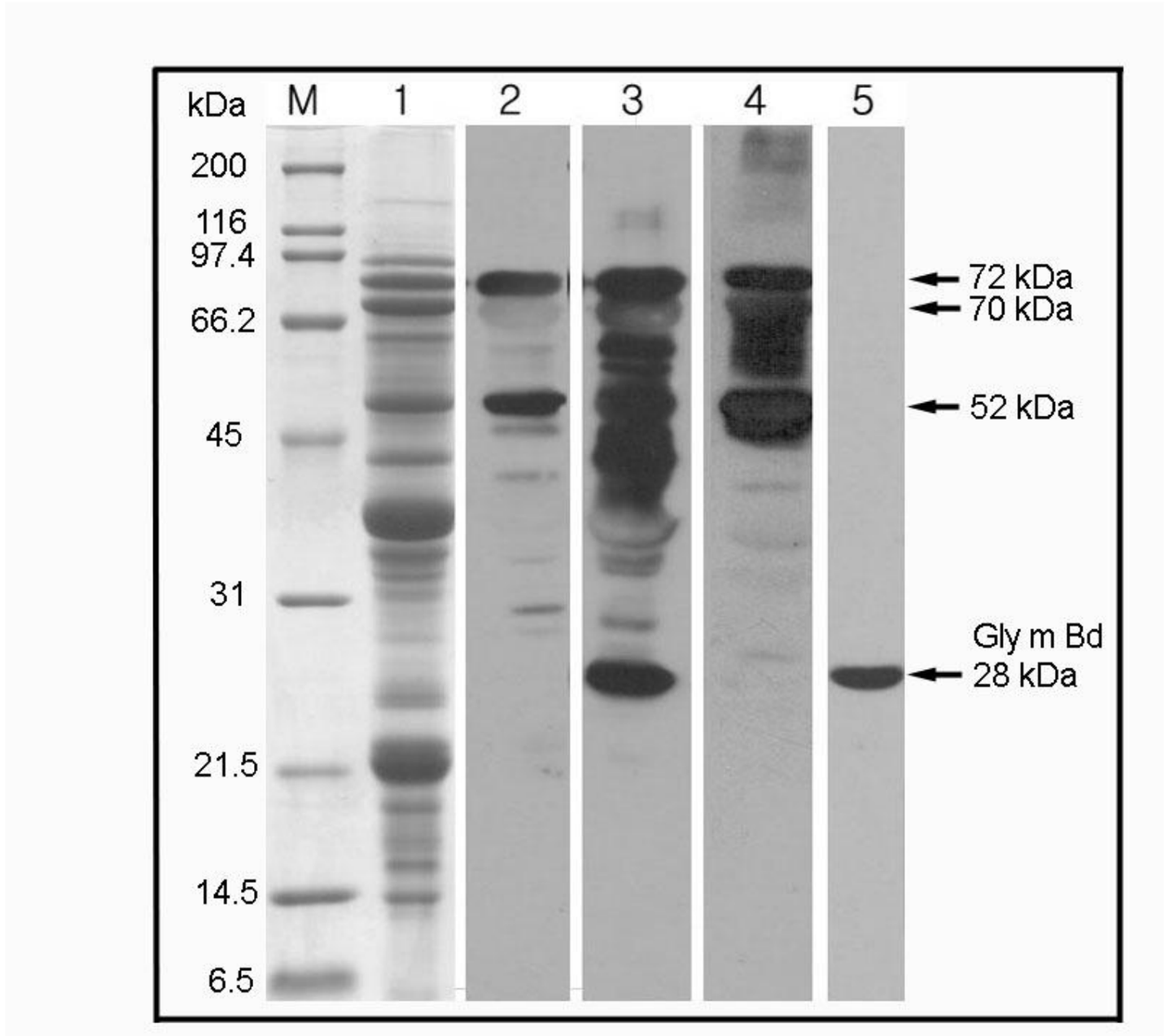
B

1 MMRARFPLLLLGLVFLASVSVSFGIAYWEKENPKHNKCLQSCNSERDSYR
51 NQACHARCNLLKVEKEECEEGEIPRPRPRPQHPEREPQQPGEKEEDEDEQ
101 PRPIPFPRPQPRQEEEHEQREEQEWPRKEEKRGEKGSEEEDEDEDEEQDE
151 RQFPFPRPPHQKEERNEEEDEDEEQQRESEESED**SELRRHKNKNPFLFGS**
201 **NRFETLFKNQYGRIRVLQRFNQRSPQLQNLR**DYRILEFNSKPNTLLLPNH
251 ADADYLIVILNGTAILSLVNNDDRDSYRLQSGDALRVPSGTTYYVVNPDN
301 NENLRLITLAIPVNKPGRFESFFLSSTEAQQSYLQGFSRNILEASYDTKF
351 EEINKVLFSREEGQQQGEQRLQESVIVEISKEQIRALSKRAKSSSRKTIS
401 SEDKPFNLRSRDPIYSNKLGKFFEITPEKNPQLRDLDIFLSIVDMNEGAL
451 LLPHFNSKAIVILVINEGDANIELVGLKEQQQEQQQEEQPLEVRKYRAEL
501 SEQDIFVIPAGYPVVVNATSNLNFFAIGINAENNQRNFLAGSQDNVISQI
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601 LRAFY

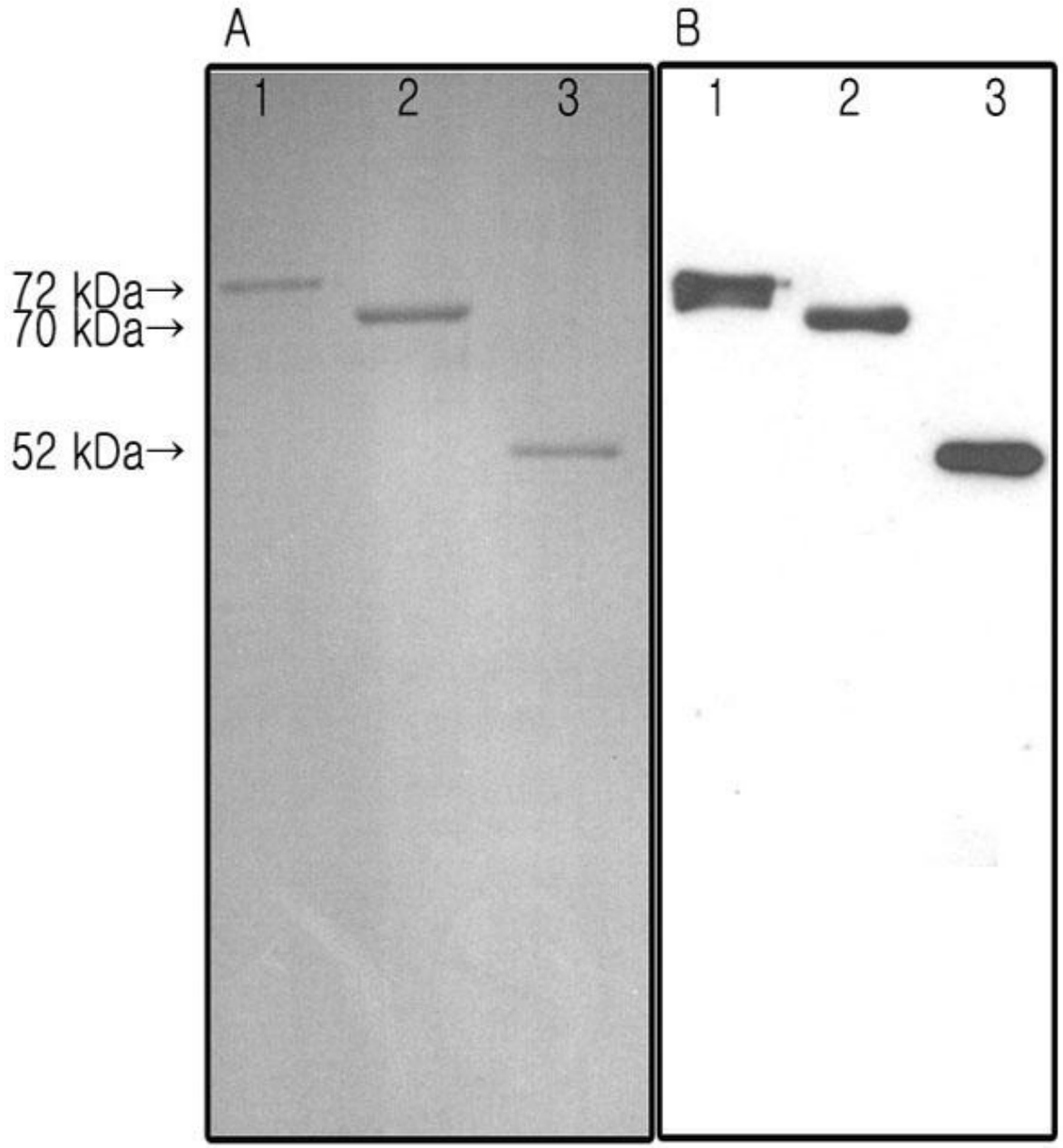
Mapping of immunogenic epitopes on the homology-modeled structure of the soybean β-conglycinin



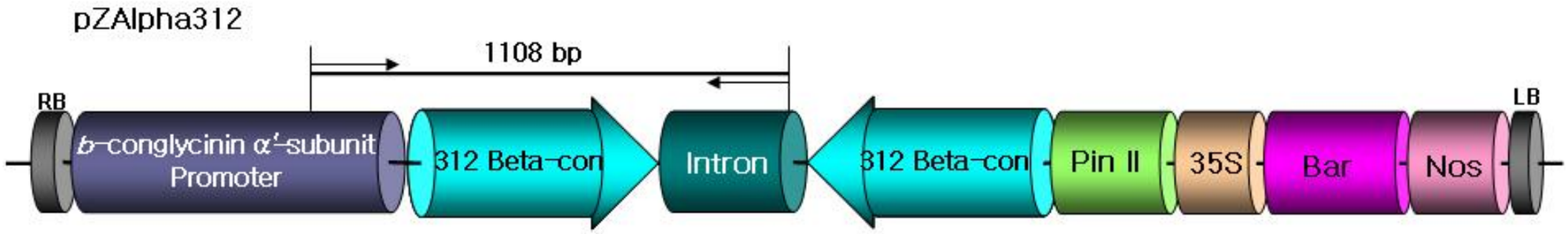
SDS-PAGE and reactivity analysis of soybean seed proteins probed with sera from soybean sensitive patients

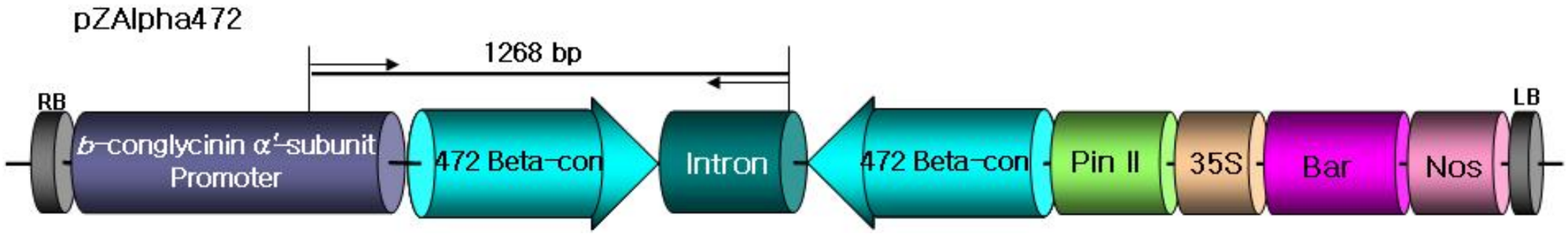


IgE binding to purified α', α and β-subunits of β-conglycinin

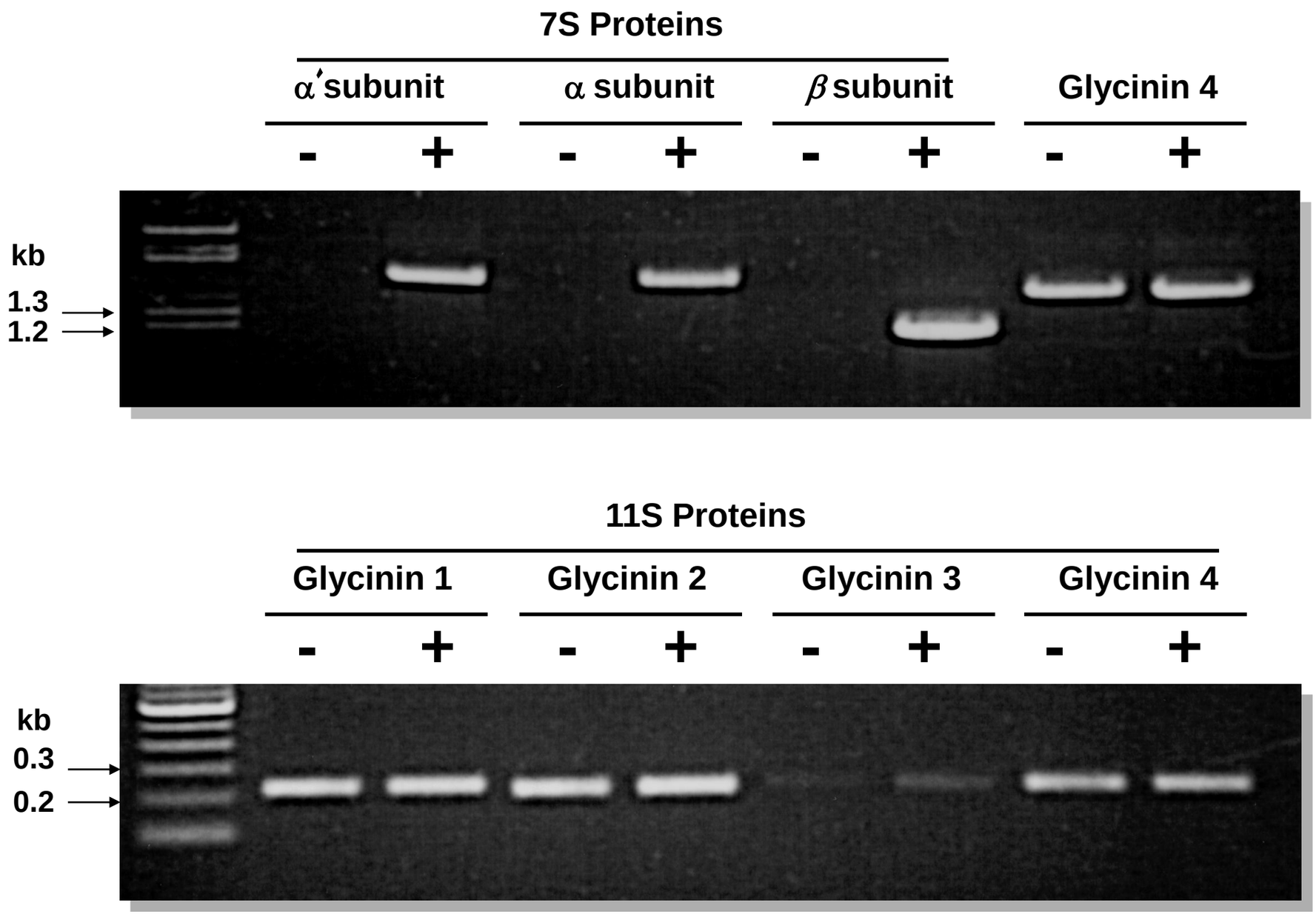


Design of RNAi construct for seed-specific silencing of β-conglycinin in transgenic soybean

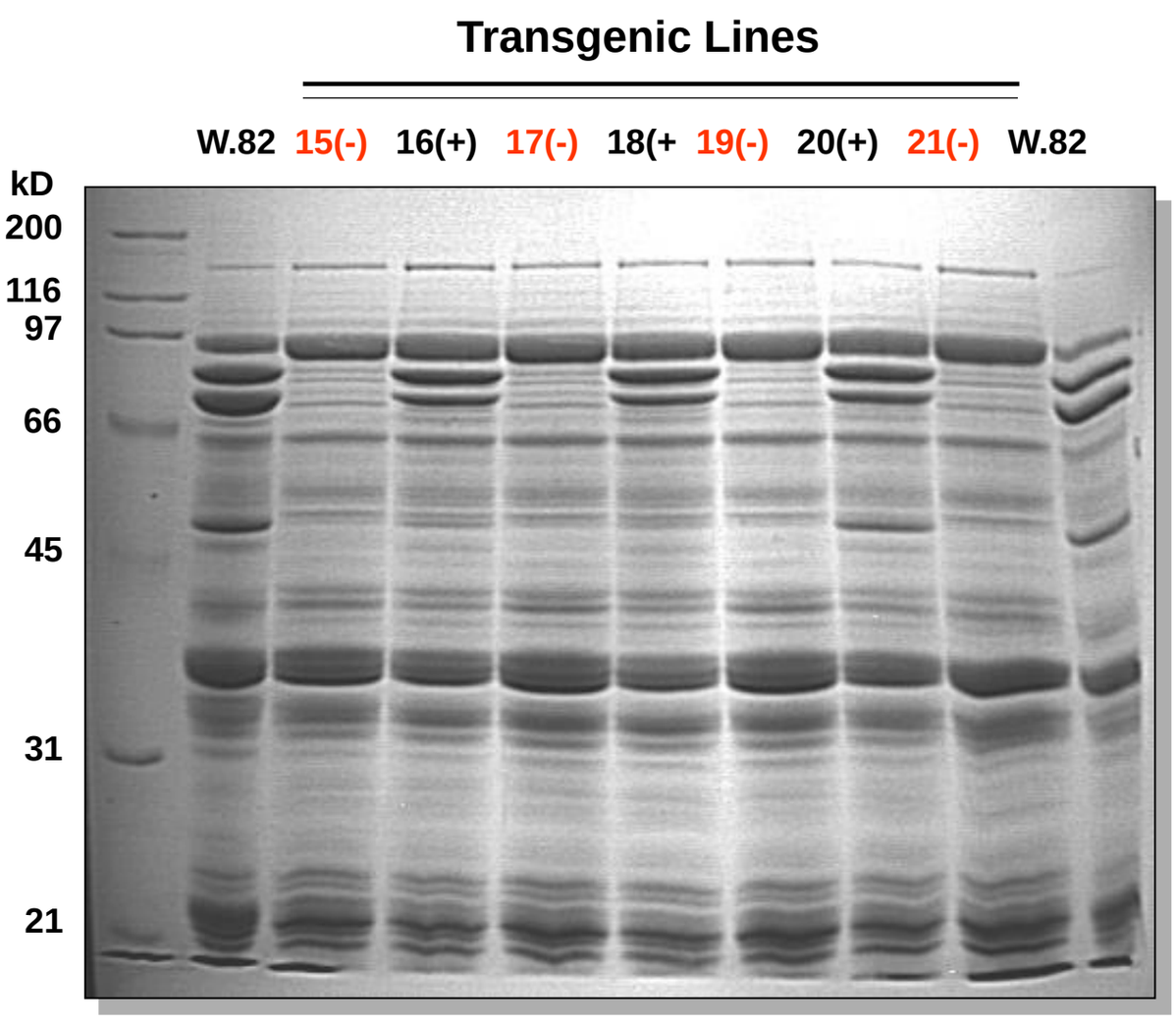


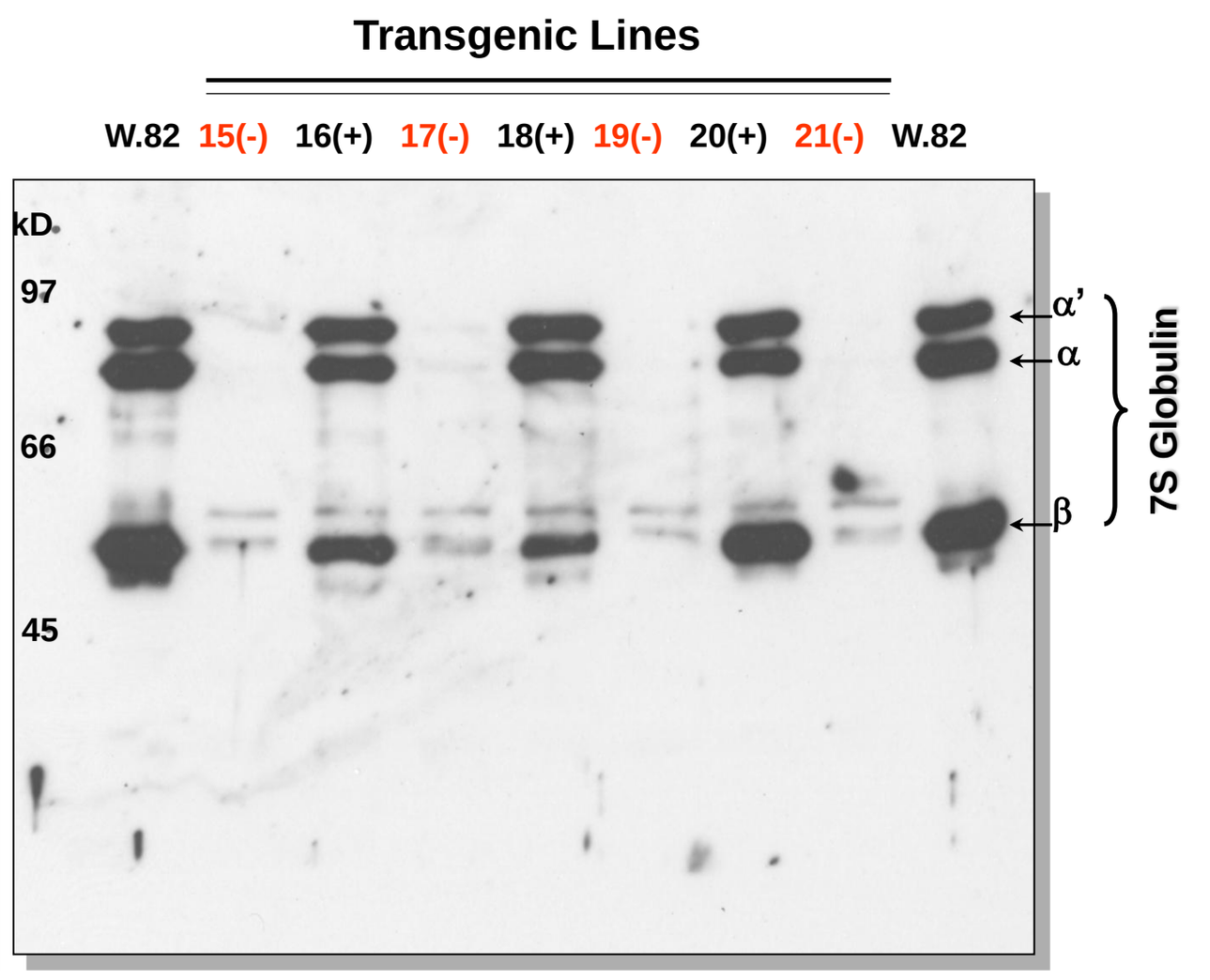


7S and 11S Globulin Transcripts in Transgenic Soybean

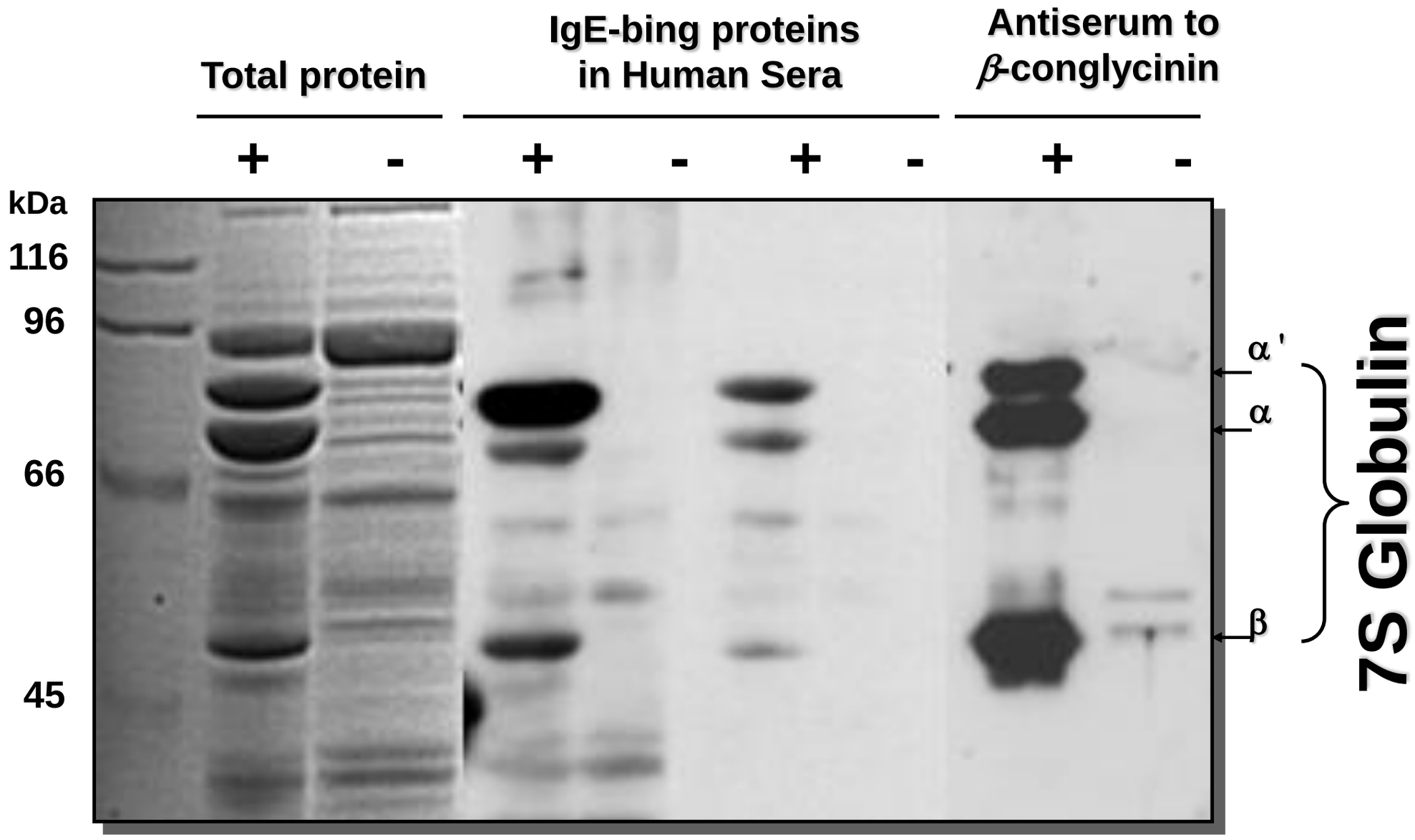


Seed-specific silencing of β-conglycinin in transgenic soybean

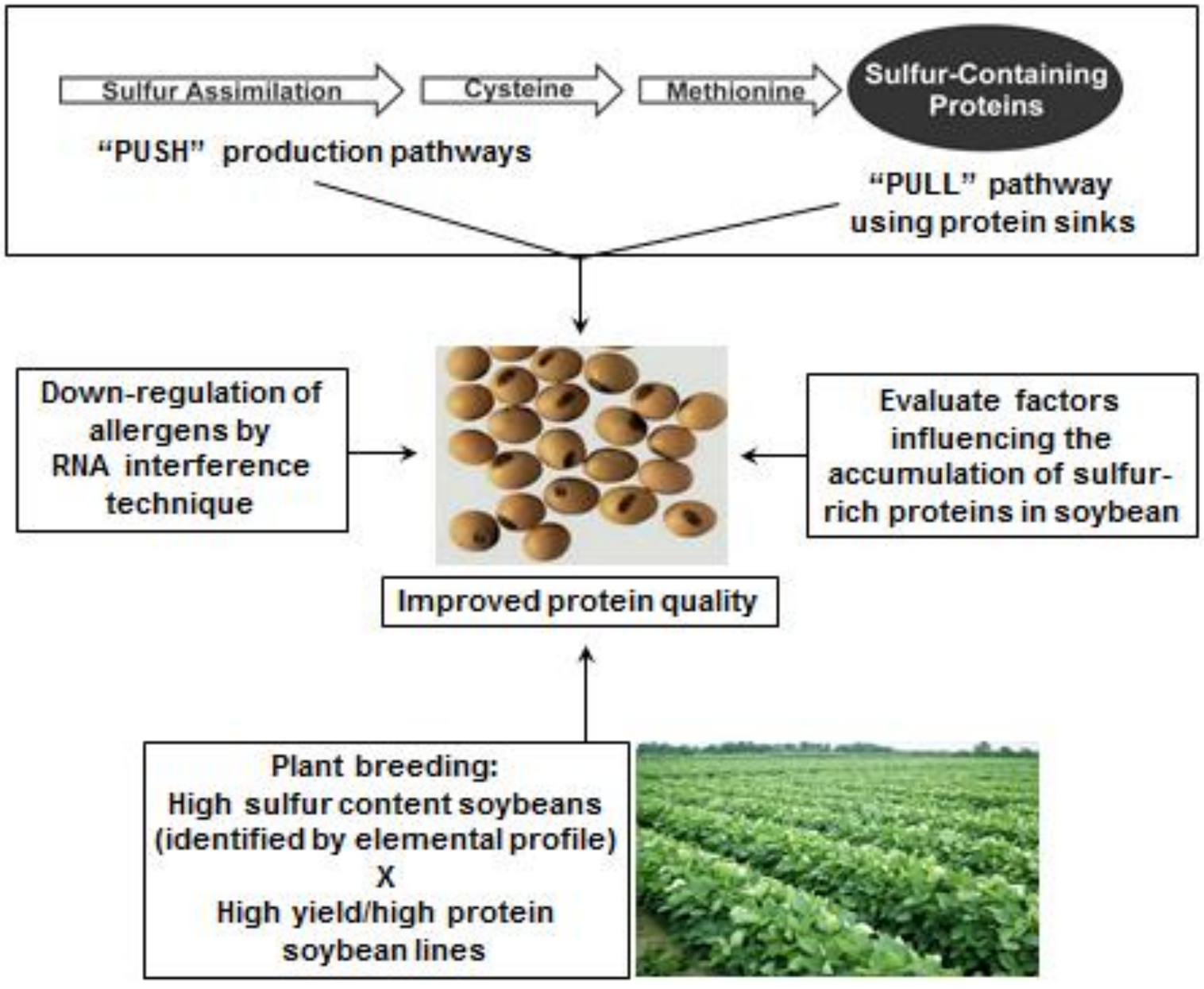




Immunoreactive proteins are reduced in transgenic soybean seeds



Strategies to produce increased S-containing amino acids and decreased allergens in soybean seeds



Acknowledgments

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