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Review

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Transgenic Soybeans and Soybean Protein Analysis: An Overview

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ABSTRACT: To meet the increasing global demand for soybeans for food and feed consumption, new high-yield varieties with improved quality traits are needed. To ensure the safety of the crop, it is important to determine the variation in seed proteins along with unintended changes that may occur in the crop as a result various stress stimuli, breeding, and genetic modification. Understanding the variation of seed proteins in the wild and cultivated soybean cultivars is useful for determining unintended protein expression in new varieties of soybeans. Proteomic technology is useful to analyze protein variation due to various stimuli. This short review discusses transgenic soybeans, different soybean proteins, and the approaches used for protein analysis. The characterization of soybean protein will be useful for researchers, nutrition professionals, and regulatory agencies dealing with soy-derived food products.

KEYWORDS: *soybean, 2D-PAGE, MS, β-conglycinin, glycinin, proteins, transgenic*

INTRODUCTION

Soybeans [*Glycine max* L. (Merr.)] are a major and inexpensive source of protein for animal feed and are also an increasingly important component of the diets of U.S. consumers.[1] The demand for soybean is mostly for oil and protein meal production.[2,3] Soybean proteins are used in human foods in a variety of forms including baby formulas, flours, protein isolates, concentrates, and textured fibers. The nutritional quality and quantity of proteins in the soybean seed are higher than in any other seed legumes. Soybean seed contains approximately 40% protein and 20% oil on a dry weight basis. Studies have shown a reduced risk of cancer, improved cardiovascular disease risk factors, and other reduced chronic illnesses in populations that consume soybeans and soy products on a regular basis.[4] Similarly, it is becoming the vital ingredient for many industrial products and pharmaceutical applications. However, soy foods also exhibit allergenic properties to sensitive consumers. Allergenic reactions are primarily due to antigenic proteins present in soybeans that perturb normal metabolism and can interfere with digestion and absorption of nutrients.[5,6]

To overcome future global food security challenges, it is critical to develop new improved soybean varieties (both quality and yield) and other crops using traditional breeding and new genetic engineering methods. Genetic engineering involves genetic modification (GM) by inserting a designed gene with a known gene sequence intended to develop a product with desired crop traits.[7] All methods of genetic modification change the quantity and/or quality of both primary and secondary metabolites, namely, proteins, lipids, carbohydrates, and isoflavones. Examples include reduction of allergens and antinutrients along with increase of value-added proteins, oil, and carbohydrates. However, like all new technologies, all new soybean varieties must be evaluated for safety and quality purposes. Several improved analytical approaches including genetic, proteomic, and metabolic profiling provide identification of protein and secondary metabolite profiles in GM crops.[8−10] These profiling approaches are used for evaluating the variability of protein profiles/expression due to genetic variables, environmental factors, nutrient stress, breeding methods, and interaction between genotype and environments.[11−16] These data analyses based on the natural variation of protein profiles are important for understanding potentially significant bilogical differences among GM and non-GM soybean varieties.[11] In this review, we discuss the benefits of analyzing new varieties in soybeans using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and mass spectrometry (MS). Analyzing the qualitative and quantitative variations of protein profiles in new varieties of soybeans is paramount to assessing unintended effects that might cause serious concern for soybean as food or feed. Although 2D-PAGE-based proteome analysis is still used widely, it has some limitations, including poor resolution of protein separation particularly high acidic and basic proteins.[17] These limitations have led to the development of gel-free MS-

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based proteomic techniques.[18] One of the technologies enables the identification of thousands of proteins in complex protein mixtures by directly coupling liquid chromatography to tandem mass spectrometry (LC-MS/MS), which is also referred to as multidimensional protein identification technology (MudPIT). Protein separation in MudPIT is also based on two dimensions using a biphasic column. The first phase consists of an SCX column followed by a reverse phase. MudPIT shows proteins outside the typical detection range of proteins in a 2D gel including hydrophobic proteins and proteins with higher molecular weight or isoelectric point.[19] Each technique offers various advantages and disadvantages. However, currently there is no single method that can provide quantitative and qualitative information of all proteins in a complex mixture. The choice of the methodologies to be used will depend on the objective of the study. Although MudPIT technology is able to identify many more proteins than the standard 2D gel-based technique for studying environmental effects including pathogens and other stress factors, proteins are better separated, visualized, and quantified in 2D gels than in the MudPIT approach.[20] It is also well-known that MudPIT cannot effectively separate abundant proteins from low-abundant proteins and tends to miss those proteins which are likely important enzymes regulating cell functions. In addition, unlike in 2D gel-based approaches (i.e., DIGE), MudPIT is unable to directly measure the relative abundance of proteins in different proteome samples. However, application of multiple techniques can provide complementary information.

BENEFITS OF GENETICALLY MODIFIED CROPS

Soybeans are used in the manufacture of a wide range of foods. However, there may be some health-associated issues due to the presence of allergens, including soybean hydrophobic proteins, hull protein, profilin, vascular protein, glycinin, and β-conglycinin, which affect some consumers.[21−23] In addition, soybean also contains some antinutrients, which limit its suitability as a food or feed. These include Kunitz trypsin inhibitors (KTI), lectins, protease inhibitors, phytin, and lipoxygenase.[24] Transgenic technology is a possible solution to reduce or eliminate the problems associated with allergens and antinutrients present in normal nongenetically engineered soybeans. Herman et al.[25] suppressed the expression of protein Gly m Bd 30K, which is a less abundant allergen protein in soybean. This protein is also referred to as P34, an immunodominant soybean allergen. The authors used gene silencing to prevent the accumulation of P34 and analyzed the results by 2D-PAGE coupled with MS but could not detect any differences in the protein profile other than the expected lack of the P34 protein. Transgenic approaches have also been used to benefit agriculture in producing quality traits by improving productivity, enhancing herbicide tolerance, and developing new value-added nutrients not present prior to modification.[25−28] Genetically engineered crops have been commercially available since 1996. Crops containing transgenes are carefully analyzed and regulated in the United States and other countries. Several companies have applied transgenic technologies to modify soybean and produced new value-added soybean varieties. Monsanto's Roundup Ready soybean, genetically modified to tolerate the herbicide glyphosate, is the most recognized transgenic soybean product on the market due to cost efficiencies for weed control. Other engineered traits of soybean can be categorized as agronomic, improvement in protein and oil quality, production of specialty oils, removal of allergenic proteins, and pharmaceutical products including γ-linolenic acid (GLA).[29−36] It is likely that soybean cultivars with a variety of transgenic modifications to enhance quality and productivity will be developed in the future. Therefore, it is important to determine if any unintended changes occur in the soybean seed as a result of conventional breeding/and or genetic modification. In 2010, the soybean genome sequence became available, allowing risk assessment at the whole genome level.[37]

IMPORTANCE OF PROTEOMIC ANALYSIS

Proteomic research and analysis are potentially useful for examining alterations in protein profiles including stress response proteins caused by mutations, introduction or silencing of genes, or responses to various stress stimuli including salt, drought, desiccation, cold, heat, mineral toxicity, mineral deficiency, and others.[38−41] In addition, proteomics has been applied to analyze the differences in food proteomes relevant to nutrition such as identification of markers for specific food-processing technologies and quality of processed food.[41] Houston et al.[42] quantified 10 soybean allergens such as glycinins G1, G2, G3, and G4, glycinin precursor, Gly m 5 β-conglycinin α subunit, Kunitz trypsin inhibitor 1 (KTI), and KTI 3, Gly m bd 28K, and Gly m bd 30K from 20 nongenetically modified commercial varieties by mass spectrometry approaches. The authors used two quantitative proteomic methods, namely, relative quantitation and absolute quantitation (AQUA), to quantify 10 soybean allergens. Relative quantitation was performed by spectral counting of trypsin-digested protein samples using linear ion trap tandem mass spectrometer (ProteomeXLTQ-ETD). The AQUA was performed by multiple reaction monitoring (MRM) with synthetic, isotope-labeled peptides as internal standards. Peptides were synthesized with a single C^{13}- and N^{15}-labeled lysine or arginine, depending upon the peptide sequence. For AQUA, the authors used an Agilent 6410 triple-quadrupole MS system coupled with an 1100 series LC system and an HPLC-Chip cube. The authors concluded that MRM analysis reduced technical variance of bovine serum albumin (BSA) internal standards to ∼7%. The authors further reported that of 10 allergens, 4 allergens showed differential expression profiles among 20 varieties of soybeans. Hajduch et al.[43] used a difference gel electrophoresis (2D DIGE) approach and compared achene protein in near-isogenic sunflower. This approach relies on the comparison of different protein samples labeled with different fluorescent dyes. Regnier et al.[44] reported perspective regarding comparative proteomics based on stable isotope labeling and affinity selection. In this process, they compared experimental samples derived from any protein modification state caused by diseases, external stimuli (drugs, toxins), and mutations in proteins from *Escherichia coli*, rat, cow, and human. The authors suggested that stable isotope coding strategies are efficient to provide protein concentration changes triggered by regulatory stimuli.

Proteomics encompasses many different subdivisions including structural proteomics, which is in-depth analysis of protein structure (high-throughput X-ray crystallography/modeling, high-throughput NMR spectroscopy/modeling); expression proteomics, which is an analysis of expression and differential expression of proteins (electrophoresis, protein chips, DNA chips, 2D-HPLC, mass spectrometry, microsequencing); and interaction proteomics, which is an analysis of interactions between proteins to characterize complexes and determine

functions (HT functional assays, ligand chips, yeast 2 hybrid, deletion analysis, motif analysis). Protein expression profiling involves the combined applications of separation and identification techniques such as 2D-PAGE, coupled with mass spectrometry methods including liquid chromatography−mass spectrometry (LC-MS) analysis or matrix-assisted laser desorption ionization (MALDI-TOF-MS), and the use of bioinformatics tools to characterize proteins in complex biological mixtures. Separation of proteins by 2D-PAGE has been practiced in laboratories for nearly 40 years, since its development in 1975.[45] 2D-PAGE systems are a combination of two different types of separation. In the first dimension, the proteins are separated on the basis of protein isoelectric point by isoelectric focusing (IEF). In the second dimension, the focused proteins are further separated by electrophoresis on the basis of protein molecular weight. 2D-PAGE is widely used to examine the compositional differences of protein profiles from various crop tissues including mature seeds, leaf, pulvinus, embryonic axis, and germinated seeds.[25,46−57] In addition, proteomic-based technologies have been successfully applied to the systematic study of the proteomic responses in many plant species to a wide range of abiotic stresses, including drought, nutrition deficiency, temperature, oxidative stress, herbicides, wounds, anoxia, salt, and heavy metals.[58−67] Furthermore, Hajduch et al.[68] employed a high-throughput proteomics approach to determine the expression of different classes of proteins during soybean seed filling. Although concerns have been raised regarding the reproducibility and sensitivity of 2D-PAGE results in characterizing all of the elements in a proteome, this technique is still considered to be most suitable for separating and visualizing proteins from a complex mixture.[20] The quality and discrete distribution of protein spots depends primarily on the choice of sample preparation method used for protein extraction.[69] Sample preparation greatly influences isoelectric focusing (IEF) in the first dimension.[70] Several publications are available for protein extraction methodologies suitable for 2D-PAGE separation.[25,71−76] The methods available for protein identification have progressed dramatically in the past several years, which resulted in several publications in plants, animals, and humans.[3,55,77,78] The traditional technique of Edman degradation to partially sequence an isolated protein has been replaced by mass spectrometry. In MS/MS analysis, proteins are enzymatically or chemically cleaved. The resulting peptides are fragmented and ionized by different ionization methods including MALDI or electrospray ionization (ESI). The resultant data consist of mass over charge (m/z) values, along with derived peptide sequence data in MS/MS mode, which is then subjected to a sequence similarity analysis over published databases. Recent advances in MS and the establishment of protein databases derived from genome and transcriptome sequence data have substantially increased the accuracy of protein identification in enriched and complex protein mixtures. These new techniques make it possible to now perform high-throughput analysis of uncharacterized protein isolates.[79,80] Major advances in MS instrumentation and the establishment of protein databases have substantially increased the accuracy of protein profile characterization from complex protein mixtures. Such analytical improvements also have led to a better ability to identify and quantify compositional changes associated with biological attributes and with variables regarding agricultural practices. In addition, the application of such techniques led to the recognition of the complexity of the biological material within plant cells and that the majority of plant chemical constituents have yet to be identified and structurally characterized. The potential of these modern and efficient methods in the detection of compositional changes in plant tissues has been discussed in previously published reviews by Kuiper et al.[81,82] Fiehn and Koo et al.[83,84] reported that plants are the most biochemically complex organism that can produce a wide array of different classes (lipids, carbohydrates, proteins, and other minor phyochemicals) of compounds. Therefore, several approaches and methodologies (lipidomics, proteomics, or general metabolomics) are often used, individually or in parallel, in attempt to resolve, quantify, and identify compounds in plant tissues.

SOYBEAN SEED PROTEINS

Molecular characterization of soybean seeds at the protein and DNA levels provides important data for assessing genetic diversity.[85] Results of the diversity patterns based on isozymes and RFLP markers were similar[86] and showed that wild genotype *Glycine soja* is much more diverse than cultivated *Glycine max*. The large amount of variation in seed composition between wild and cultivated genotypes may be due to a different complement of genes that control expression of β-conglycinin and glycinin protein composition compared to cultivated genotypes.[87,88] Sebolt et al.[89] reported that the wild soybean has increased protein content that was associated with a specific quantitative trait locus (QTL) allele. Soybean storage proteins are grouped into two types, namely, β-conglycinin and glycinin, based on the sedimentation coefficients. The β-conglycinin, a 7S globulin, consists of three types of nonidentical but homologous polypeptide subunits, namely, α, α', and β, with a molecular mass of 180 kDa.[50] The α' subunit consists of extension and core regions, whereas the β subunit is composed of only the core region. All three subunits' core regions exhibit high sequence identity with one another. The extension regions of α and α' subunits showed lower identities than the core regions.[50] In addition, β-conglycinin is a multigene family of about 20 genes encoding these subunits, therefore resulting in subunit variation among different cultivars.[90] Glycinin, a hexameric 11S globulin (360 kDa), consists of acidic (A) and basic (B) polypeptides. Glycinin is encoded by five nonallelic genes: Gy1, Gy2, Gy3, Gy4, and Gy5. These genes code for five precursor protein molecules, namely, G1, G2, G3, G4, and G5, respectively.[91] In addition, these five subunits are classified into two distinct major groups, groups I and II, based on their physical properties, including the identity of their amino acid sequences. The identities are ∼82−86% within the groups and 42−45% between the groups.[50] Group I subunits contain more methionine residues and consist of G1 (A1aBx), G2 (A2B1a), and G3 (A1aB1b) proteins. Group II contains G4 (A5A4B3) and G5 (A3B4) subunits. Beilinson et al.[92] identified and mapped an additional two genes in soybean Resnik, namely, gy6 and Gy7. Koshiyama[93] reported that the storage protein glycinin (acidic and basic polypeptides) showed genetic diversity among several soybean cultivars. Heterogeneity in glycinin subunits due to deletions of the G4 and G5 genes has been reported among Japanese soybean varieties.[94] Variation of glycinin and β-conglycinin subunits among 16 soybean genotypes including wild and cultivated genotypes was reported by Natarajan et al.[95] Saio et al.[96] reported that the proportion of β-conglycinin and glycinin is important and is responsible for the differences in the physical properties of tofu gel. Fukuda et al.[50] analyzed seed proteins

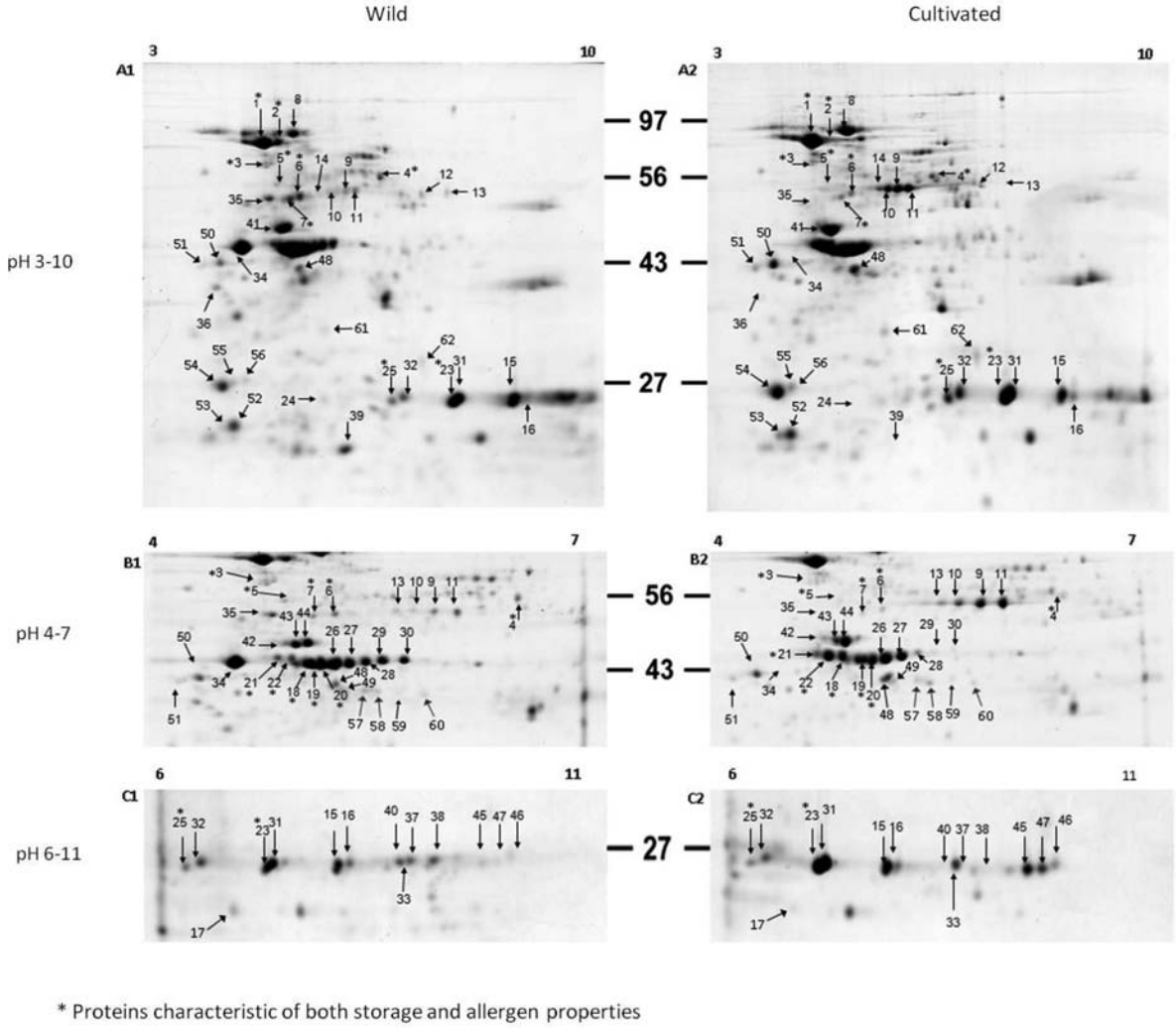

Figure 1. Two-dimensional gel proteome maps of soybean seed proteins. The first-dimension IEF was performed using pH 3.0−10.0, 4.0−7.0, and 6.0−11.0 linear IPG strips. Numbered arrows indicate the polypeptides identified by mass spectrometry.

Table 1. Soybean Seed Proteins Identified by Mass Spectrometry Application

spot ID	protein	accession no.	class
[1−7]	α-subunit of β-conglycinin	9967357	storage/allergen
[8]	α'-subunit of β-conglycinin	9967361	storage
[9−14]	β-conglycinin β-subunit	63852207	storage
[15−17]	glycinin Gl/AlaBx subunit	18635	storage
[18−25]	glycinin G2/A2B1 precursor	1212177	storage/allergen
[26−33]	glycinin subunit G3/AlablB	15988117	storage
[37−40]	glycinin G4/A5A4B3 precursor	81785	storage
[41−47]	glycinin G5/A3B4 subunit	33357661	storage
[50−51]	P34 probable thiol protease	129353	allergen
[48−49], [57−60]	soybean agglutinin	6729836/282898	antinutritional
[54−56]	Kunitz trypsin inhibitor	3318877	antinutritional
[52−53]	stress-induced protein SAM22 (allergen Gly m 4)	134194	allergen
[61−62]	allergen Gly m Bd 28K	12697782	allergen

from 390 lines of wild soybean to screen for genetic variants of storage proteins using electrophoresis and reported variants of glycinin and β-conglycinin subunits. In addition, the allergen/antinutritional factors also varied between genotypes.[97] In contrast, Yaklich et al.[98] reported that wild and cultivated genotypes showed little difference in P34 allergens. Koo et al.[84] screened several soybean accessions and found two soybean accessions (PI 603570A and PI 567476) with low P34 allergen by using immunoblot analysis. In addition, the authors analyzed these two accessions in detail by proteomic analysis using 2D-PAGE and MS and reported 19 differentially expressed proteins between these two soybean accessions. Piper and Boote[16] reported that maturity group, location, and environmental variation affect characteristics of soybean seeds, which

suggested temperature has a significant effect on protein expression.[99,100] There are many papers available on the natural variation of protein profiles by SDS-PAGE analysis of different soybean genotypes.[95,101,102] Natarajan et al.[73] reported that low-abundant soybean metabolic proteins varied among 16 different soybean genotypes including wild and cultivated using proteomics. We have compiled the variation of different classes of soybean seed proteins including storage, allergen, and antinutritional proteins in wild and cultivated soybeans (Figure 1 and Table 1). We found significant variation of protein between wild and cultivated soybean genotypes. The differential expression of proteins was reported in terms of number of proteins, intensity, and appearance of protein spots.[101,103,104]

TRANSGENIC SOYBEAN CROPS

Soybeans can be transformed by microprojectile bombardment or *Agrobacterium*-mediated transformation methods.[105−107] In 2011, about 94% of the soybeans planted in the United States were genetically modified for herbicide resistance, Roundup Ready soybean.[108] The Roundup Ready soybean was demonstrated to have no significant differences in protein profiles in comparison to nontransgenic soybean. However, the levels of phytoestrogens were lower in genetically modified, herbicide-tolerant soybeans compared to their isogenic conventional soybeans grown under similar conditions using high-pressure liquid chromatographic analysis.[109] Other research investigations showed that there are no effects of the CP4 5-enolpyruvylshikimatae-3-phosphate synthase (CP4 EPSPS) gene, which confers glyphosate resistance (GR) to contents of isoflavone, antinutrients, and several different secondary metabolites of soybeans.[110−113] In addition, others have produced transgenic soybean with reduced allergenicity or added nutritional qualities. Similarly, a methionine-rich protein of corn has been expressed in soybean with no significant variation in protein profile.[27] Kinney and Knowlton[114] and McCabe et al.[115] studied seed protein profiles of a high-oleic acid transgenic soybean line versus its normal counterpart and reported no differences. Kim et al.[116] developed a transgenic soybean with overexpression of a cytosolic isoform of *O*-acetylserine sulfhydrylase (OASS). The authors reported 4−10-fold increases of OASS and also more accumulation of Bowman−Birk protease inhibitor, a cystine-rich protein. In another investigation, Qi et al.[117] successfully produced transgenic soybean seeds with enhanced threonine levels, which is an essential amino acid not able to be synthesized by humans and monogastric animals. In addition to high production of threonine, these transgenic soybean seeds also showed substantial increase of other major free amino acid levels exhibiting normal seed morphology and germination under greenhouse conditions. In another study, Kim et al.[118] developed a line of transgenic soybean seeds through seed-specific overexpression of two carotenoid biosynthetic genes, namely, capsicum phytoene synthase and pantoea carotene desaturase. The authors reported that the seeds showed a ∼62-fold accumulation of β-carotene when compared to non-transgenic soybeans. The authors concluded that the increase of β-carotene was of the highest levels when compared with previously reported results in Golden Rice 2, maize endosperm, and potato tuber.

New and improved varieties of crops and agriculture products are rapidly being introduced in the global market place. Therefore, it is important to determine the variation in protein content along with unintended changes that may occur in the crop as a result of modifications or several different stress stimuli. This mini-review provides an overview of benefits of transgenic soybeans and the analysis of different soybean proteins using 2D-PAGE and MS. The identification, characterization, and subsequent quantification of different classes of soybean proteins will be useful for researchers, nutrition professionals, and regulatory agencies to investigate changes in the protein profiles and their impact on health.

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REFERENCES

(1) Boulter, D. Ontogeny and development of biochemical and nutritional attributes in legume seeds. In *Advanced Legume Science*; Summerfield, R. J., Bunting, A. H., Eds.; Royal Botany Gardent Publishing: Kew, UK, 1980; pp 127−134.

(2) Boerma, H. R. *Soybeans: Improvement, Production, and Uses*, 3rd ed.; American Society of Agronomy: Madison, WI, 2004.

(3) Stevenson, S. E.; Woods, C. A.; Hong, B.; Kong, X.; Thelen, J. J.; Ladics, G. S. Environmental effects on allergen levels in commercially grown non-genetically modified soybeans: assessing variation across north america. *Front. Plant Sci.* **2012**, *3*, 196.

(4) Cassidy, A.; Faughnan, M. Phyto-oestrogens through the life cycle. *Proc. Nutr. Soc.* **2000**, *59*, 489−496.

(5) Berneder, M.; Bublin, M.; Hoffmann-Sommergruber, K.; Hawranek, T.; Lang, R. Allergen chip diagnosis for soy-allergic patients: Gly m 4 as a marker for severe food-allergic reactions to soy. *Int. Arch. Allergy Immun.*. **2013**, *161*, 229−233.

(6) Hei, W.; Li, Z.; Ma, X.; He, P. Determination of β-conglycinin in soybean and soybean products using a sandwich enzyme-linked immunosorbent assay. *Anal. Chim. Acta* **2012**, *734*, 62−68.

(7) *Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects*; National Academies Press: Washington, DC, 2004.

(8) Gayen, D.; Sarkar, S. N.; Datta, S. K.; Datta, K. Comparative analysis of nutritional compositions of transgenic high iron rice with its non-transgenic counterpart. *Food Chem.* **2013**, *138*, 835−840.

(9) Ricroch, A. E.; Berge, J. B.; Kuntz, M. Evaluation of genetically engineered crops using transcriptomic, proteomic, and metabolomic profiling techniques. *Plant Physiol.* **2011**, *155*, 1752−1761.

(10) Wang, Y.; Xu, W. T.; Zhao, W. W.; Hao, J. R.; Luo, Y. B.; Tang, X. G.; Zhang, Y.; Huang, K. L. Comparative analysis of the proteomic and nutritional composition of transgenic rice seeds with Cry1ab/ac genes and their non-transgenic counterparts. *J. Cereal Sci.* **2012**, *55*, 226−233.

(11) Doerrer, N.; Ladics, G.; McClain, S.; Herouet-Guicheney, C.; Poulsen, L. K.; Privalle, L.; Stagg, N. Evaluating biological variation in non-transgenic crops: executive summary from the ILSI Health and Environmental Sciences Institute workshop, November 16−17, 2009, Paris, France. *Regul. Toxicol. Pharmacol.* **2010**, *58*, S2−S7.

(12) Krishnan, H. B.; Natarajan, S. S.; Mahmoud, A. A.; Nelson, R. L. Identification of glycinin and β-conglycinin subunits that contribute to the increased protein content of high-protein soybean lines. *J. Agric. Food Chem.* **2007**, *55*, 1839−1845.

(13) Maestri, D. M.; Labuckas, D. O.; Meriles, J. M.; Lamarque, A. L.; Zygadlo, J. A.; Guzman, C. A. Seed composition of soybean cultivars

evaluated in different environmental regions. *J. Sci. Food Agric.* **1998**, *77*, 494−498.
(14) Natarajan, S. S.; Xu, C. P.; Garrett, W. M.; Lakshman, D.; Bae, H. Assessment of the natural variation of low abundant metabolic proteins in soybean seeds using proteomics. *J. Plant Biochem. Biotechnol.* **2012**, *21*, 30−37.
(15) Paek, N. C.; Imsande, J.; Shoemaker, R. C.; Shibles, R. Nutritional control of soybean seed storage protein. *Crop Sci.* **1997**, *37*, 498−503.
(16) Piper, E. L.; Boote, K. J. Temperature and cultivar effects on soybean seed oil and protein concentrations. *J. Am. Oil Chem. Soc.* **1999**, *76*, 1233−1241.
(17) Santoni, V.; Molloy, M.; Rabilloud, T. Membrane proteins and proteomics: un amour impossible? *Electrophoresis* **2000**, *21*, 1054−1070.
(18) Abdallah, C.; Dumas-Gaudot, E.; Renaut, J.; Sergeant, K. Gel-based and gel-free quantitative proteomics approaches at a glance. *Int. J. Plant Genomics* **2012**, DOI: 10.1155/2012/494572.
(19) Gorg, A.; Weiss, W.; Dunn, M. J. Current two-dimensional electrophoresis technology for proteomics. *Proteomics* **2004**, *4*, 3665−3685.
(20) McDonald, H.; Friedman, D. Leveraging technologies: DIGE and MudPIT. *J. Biomol. Tech.* **2010**, *21*.
(21) Ballmer-Weber, B. K.; Vieths, S. Soy allergy in perspective. *Curr. Opin. Allergy Clin Immunol.* **2008**, *8*, 270−275.
(22) Gonzalez, R.; Polo, F.; Zapatero, L.; Caravaca, F.; Carreira, J. Purification and characterization of major inhalant allergens from soybean hulls. *Clin. Exp. Allergy* **1992**, *22*, 748−755.
(23) Helm, R. M.; Cochrell, G.; Herman, E. M.; Burks, A. W., Jr.; Simpson, H. A.; Bannon, G. A. Cellular and molecular characterization of a major soybean allergen. *Int. Arch. Allergy Immunol.* **1998**, *117*, 29−37.
(24) Burks, A. W., Jr.; Brooks, J. R.; Sampson, H. A. Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. *J. Allergy Clin. Immunol.* **1988**, *81*, 1135−1142.
(25) Herman, E. M.; Helm, R. M.; Jung, R.; Kinney, A. J. Genetic modification removes an immunodominant allergen from soybean. *Plant Physiol.* **2003**, *132*, 36−43.
(26) El-Shemy, H. A.; Khalafalla, M. M.; Fujita, K.; Ishimoto, M. Improvement of protein quality in transgenic soybean plants. *Biol. Plant.* **2007**, *51*, 277−284.
(27) Kim, W. S.; Krishnan, H. B. Expression of an 11 kDa methionine-rich delta-zein in transgenic soybean results in the formation of two types of novel protein bodies in transitional cells situated between the vascular tissue and storage parenchyma cells. *Plant Biotechnol. J.* **2004**, *2*, 199−210.
(28) Tseng, S. T.; Johnson, C. W.; Mckenzie, K. S.; Oster, J. J.; Hill, J. E.; Brandon, D. M. Registration of 'L-205' rice. *Crop Sci.* **2001**, *41*, 2004−2004.
(29) Buhr, T.; Sato, S.; Ebrahim, F.; Xing, A.; Zhou, Y.; Mathiesen, M.; Schweiger, B.; Kinney, A.; Staswick, P. Ribozyme termination of RNA transcripts down-regulate seed fatty acid genes in transgenic soybean. *Plant J.* **2002**, *30*, 155−163.
(30) Cahoon, E. B.; Marillia, E. F.; Stecca, K. L.; Hall, S. E.; Taylor, D. C.; Kinney, A. J. Production of fatty acid components of meadowfoam oil in somatic soybean embryos. *Plant Physiol.* **2000**, *124*, 243−251.
(31) Dinkins, R. D.; Reddy, M. S. S.; Meurer, C. A.; Yan, B.; Trick, H.; Thibaud-Nissen, F.; Finer, J. J.; Parrott, W. A.; Collins, G. B. Increased sulfur amino acids in soybean plants overexpressing the maize 15 kDa zein protein. *In Vitro Cell. Dev. Biol.* **2001**, *37*, 742−747.
(32) Falco, S. C.; Guida, T.; Locke, M.; Mauvais, J.; Sanders, C.; Ward, R. T.; Webber, P. Transgenic canola and soybean seeds with increased lysine. *Biotechnol. N. Y.* **1995**, *13*, 577−582.
(33) Qin, F.; Kang, L.; Guo, L.; Lin, J.; Song, J.; Zhao, Y. Composition of transgenic soybean seeds with higher γ-linolenic acid content is equivalent to that of conventional control. *J. Agric. Food Chem.* **2012**, *60*, 2200−2204.
(34) Suszkiw, J. Researchers develop first hypoallergenic soybeans. *Agric Res.* **2002**, *50*, 16−17.
(35) Abdeen, A.; Schnell, J.; Miki, B. Transcriptome analysis reveals absence of unintended effects in drought-tolerant transgenic plants overexpressing the transcription factor ABF3. *BMC Genomics* **2010**, *11*, 69.
(36) Barros, E.; Lezar, S.; Anttonen, M. J.; van Dijk, J. P.; Rohlig, R. M.; Kok, E. J.; Engel, K. H. Comparison of two GM maize varieties with a near-isogenic non-GM variety using transcriptomics, proteomics and metabolomics. *Plant Biotechnol. J.* **2010**, *8*, 436−451.
(37) Schmutz, J.; Cannon, S. B.; Schlueter, J.; Ma, J.; Mitros, T.; Nelson, W.; Hyten, D. L.; Song, Q.; Thelen, J. J.; Cheng, J.; Xu, D.; Hellsten, U.; May, G. D.; Yu, Y.; Sakurai, T.; Umezawa, T.; Bhattacharyya, M. K.; Sandhu, D.; Valliyodan, B.; Lindquist, E.; Peto, M.; Grant, D.; Shu, S.; Goodstein, D.; Barry, K.; Futrell-Griggs, M.; Abernathy, B.; Du, J.; Tian, Z.; Zhu, L.; Gill, N.; Joshi, T.; Libault, M.; Sethuraman, A.; Zhang, X. C.; Shinozaki, K.; Nguyen, H. T.; Wing, R. A.; Cregan, P.; Specht, J.; Grimwood, J.; Rokhsar, D.; Stacey, G.; Shoemaker, R. C.; Jackson, S. A. Genome sequence of the palaeopolyploid soybean. *Nature* **2010**, *463*, 178−183.
(38) Dubey, H.; Grover, A. Current initiatives in proteomics research: the plant perspective. *Curr. Sci. India* **2001**, *80*, 262−269.
(39) Iwahashi, Y.; Hosoda, H. Effect of heat stress on tomato fruit protein expression. *Electrophoresis* **2000**, *21*, 1766−1771.
(40) Luo, J.; Ning, T.; Sun, Y.; Zhu, J.; Zhu, Y.; Lin, Q.; Yang, D. Proteomic analysis of rice endosperm cells in response to expression of hGM-CSF. *J. Proteome Res.* **2009**, *8*, 829−837.
(41) Salekdeh, G. H.; Komatsu, S. Crop proteomics: aim at sustainable agriculture of tomorrow. *Proteomics* **2007**, *7*, 2976−2996.
(42) Houston, N. L.; Lee, D. G.; Stevenson, S. E.; Ladics, G. S.; Bannon, G. A.; McClain, S.; Privalle, L.; Stagg, N.; Herouet-Guicheney, C.; MacIntosh, S. C.; Thelen, J. J. Quantitation of soybean allergens using tandem mass spectrometry. *J. Proteome Res.* **2011**, *10*, 763−773.
(43) Hajduch, M.; Casteel, J. E.; Tang, S.; Hearne, L. B.; Knapp, S.; Thelen, J. J. Proteomic analysis of near-isogenic sunflower varieties differing in seed oil traits. *J. Proteome Res.* **2007**, *6*, 3232−3241.
(44) Regnier, F. E.; Riggs, L.; Zhang, R. J.; Xiong, L.; Liu, P. R.; Chakraborty, A.; Seeley, E.; Sioma, C.; Thompson, R. A. Comparative proteomics based on stable isotope labeling and affinity selection. *J. Mass Spectrom.* **2002**, *37*, 133−145.
(45) O'Farrell, P. H. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **1975**, *250*, 4007−4021.
(46) Agrawal, G. K.; Hajduch, M.; Graham, K.; Thelen, J. J. In-depth investigation of the soybean seed-filling proteome and comparison with a parallel study of rapeseed. *Plant Physiol.* **2008**, *148*, 504−518.
(47) Boonmee, A.; Srisomsap, C.; Chokchaichamnankit, D.; Karnchanatat, A.; Sangvanich, P. A proteomic analysis of *Curcuma comosa* Roxb. rhizomes. *Proteome Sci.* **2011**, *9*, 43.
(48) Danchenko, M.; Skultety, L.; Rashydov, N. M.; Berezhna, V. V.; Matel, L.; Salaj, T.; Pret'ova, A.; Hajduch, M. Proteomic analysis of mature soybean seeds from the Chernobyl area suggests plant adaptation to the contaminated environment. *J. Proteome Res.* **2009**, *8*, 2915−2922.
(49) Flengsrud, R.; Kobro, G. A method for two-dimensional electrophoresis of proteins from green plant tissues. *Anal. Biochem.* **1989**, *177*, 33−36.
(50) Fukuda, T.; Maruyama, N.; Kanazawa, A.; Abe, J.; Shimamoto, Y.; Hiemori, M.; Tsuji, H.; Tanisaka, T.; Utsumi, S. Molecular analysis and physicochemical properties of electrophoretic variants of wild soybean *Glycine soja* storage proteins. *J. Agric. Food Chem.* **2005**, *53*, 3658−3665.
(51) Gorg, A.; Postel, W.; Weiss, W. Detection of polypeptides and amylase isoenzyme modifications related to malting quality during malting process of barley by two-dimensional electrophoresis and isoelectric focusing with immobilized pH gradients. *Electrophoresis* **1992**, *13*, 759−770.

(52) Klubicova, K.; Danchenko, M.; Skultety, L.; Berezhna, V. V.; Uvackova, L.; Rashydov, N. M.; Hajduch, M. Soybeans grown in the Chernobyl area produce fertile seeds that have increased heavy metal resistance and modified carbon metabolism. *PLoS One* **2012**, *7*, e48169.
(53) Lee, H.; Garrett, W. M.; Sullivan, J. H.; Forseth, I.; Natarajan, S. Differentially expressed proteins of soybean (*Glycine max*) pulvinus in light and dark conditions. *J. Basic Appl. Sci.* **2013**, *9*, 157−171.
(54) Mooney, B. P.; Krishnan, H. B.; Thelen, J. J. High-throughput peptide mass fingerprinting of soybean seed proteins: automated workflow and utility of UniGene expressed sequence tag databases for protein identification. *Phytochemistry* **2004**, *65*, 1733−1744.
(55) Natarajan, S. S.; Krishnan, H. B.; Khan, F. H.; Chen, X.; Garrett, W. M.; Lakshman, D. Analysis of soybean embryonic axis proteins by two-dimensional gel electrophoresis and mass spectrometry. *J. Basic Appl. Sci.* **2013**, *9*, 309−332.
(56) Neilson, K. A.; Gammulla, C. G.; Mirzaei, M.; Imin, N.; Haynes, P. A. Proteomic analysis of temperature stress in plants. *Proteomics* **2010**, *10*, 828−845.
(57) Ren, N.; Xie, T.; Xing, D. Composition of extracellular polymeric substances influences the autoaggregation capability of hydrogen-producing bacterium *Ethanoligenens harbinense*. *Bioresour. Technol.* **2009**, *100*, 5109−5113.
(58) Alves, M.; Francisco, R.; Martins, I.; Ricardo, C. P. P. Analysis of *Lupinus albus* leaf apoplastic proteins in response to boron deficiency. *Plant Soil* **2006**, *279*, 1−11.
(59) Castro, A. J.; Carapito, C.; Zorn, N.; Magne, C.; Leize, E.; Van Dorsselaer, A.; Clement, C. Proteomic analysis of grapevine (*Vitis vinifera* L.) tissues subjected to herbicide stress. *J. Exp. Bot.* **2005**, *56*, 2783−2795.
(60) Chang, W. W.; Huang, L.; Shen, M.; Webster, C.; Burlingame, A. L.; Roberts, J. K. Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings acclimated to a low-oxygen environment, and identification of proteins by mass spectrometry. *Plant Physiol.* **2000**, *122*, 295−318.
(61) Labra, M.; Gianazza, E.; Waitt, R.; Eberini, I.; Sozzi, A.; Regondi, S.; Grassi, F.; Agradi, E. *Zea mays* L. protein changes in response to potassium dichromate treatments. *Chemosphere* **2006**, *62*, 1234−1244.
(62) Pinheiro, C.; Kehr, J.; Ricardo, C. P. Effect of water stress on lupin stem protein analysed by two-dimensional gel electrophoresis. *Planta* **2005**, *221*, 716−728.
(63) Shen, S.; Jing, Y.; Kuang, T. Proteomics approach to identify wound-response related proteins from rice leaf sheath. *Proteomics* **2003**, *3*, 527−535.
(64) Sule, A.; Vanrobaeys, F.; Hajos, G.; Van Beeumen, J.; Devreese, B. Proteomic analysis of small heat shock protein isoforms in barley shoots. *Phytochemistry* **2004**, *65*, 1853−1863.
(65) Wang, S. B.; Chen, F.; Sommerfeld, M.; Hu, Q. Proteomic analysis of molecular response to oxidative stress by the green alga *Haematococcus pluvialis* (Chlorophyceae). *Planta* **2004**, *220*, 17−29.
(66) Yan, S.; Tang, Z.; Su, W.; Sun, W. Proteomic analysis of salt stress-responsive proteins in rice root. *Proteomics* **2005**, *5*, 235−244.
(67) Yan, S. P.; Zhang, Q. Y.; Tang, Z. C.; Su, W. A.; Sun, W. N. Comparative proteomic analysis provides new insights into chilling stress responses in rice. *Mol. Cell. Proteomics* **2006**, *5*, 484−496.
(68) Hajduch, M.; Ganapathy, A.; Stein, J. W.; Thelen, J. J. A systematic proteomic study of seed filling in soybean. Establishment of high-resolution two-dimensional reference maps, expression profiles, and an interactive proteome database. *Plant Physiol.* **2005**, *137*, 1397−1419.
(69) Rabilloud, T.; Blisnick, T.; Heller, M.; Luche, S.; Aebersold, R.; Lunardi, J.; Braun-Breton, C. Analysis of membrane proteins by two-dimensional electrophoresis: comparison of the proteins extracted from normal or *Plasmodium falciparum*-infected erythrocyte ghosts. *Electrophoresis* **1999**, *20*, 3603−3610.
(70) Shaw, M. M.; Riederer, B. M. Sample preparation for two-dimensional gel electrophoresis. *Proteomics* **2003**, *3*, 1408−1417.
(71) Alam, I.; Sharmin, S.; Kim, K. H.; Kim, Y. G.; Lee, J.; Lee, B. H. An improved plant leaf protein extraction method for high resolution two-dimensional polyacrylamide gel electrophoresis and comparative proteomics. *Biotechnol. Histochem.* **2013**, *88*, 61−75.
(72) Chatterjee, M.; Gupta, S.; Bhar, A.; Das, S. Optimization of an efficient protein extraction protocol compatible with two-dimensional electrophoresis and mass spectrometry from recalcitrant phenolic rich roots of chickpea (*Cicer arietinum* L.). *Int. J. Proteomics* **2012**, DOI: 10.1155/2012/536963.
(73) Mesquita, R. O.; de Almeida Soares, E.; de Barros, E. G.; Loureiro, M. E. Method optimization for proteomic analysis of soybean leaf: Improvements in identification of new and low-abundance proteins. *Genet. Mol. Biol.* **2012**, *35*, 353−361.
(74) Rodrigues, E. P.; Torres, A. R.; da Silva Batista, J. S.; Huergo, L.; Hungria, M. A simple, economical and reproducible protein extraction protocol for proteomics studies of soybean roots. *Genet. Mol. Biol.* **2012**, *35*, 348−352.
(75) Rodrigues, S. P.; Ventura, J. A.; Zingali, R. B.; Fernandes, P. M. Evaluation of sample preparation methods for the analysis of papaya leaf proteins through two-dimensional gel electrophoresis. *Phytochem. Anal.* **2009**, *20*, 456−464.
(76) Zhang, L. L.; Feng, R. J.; Zhang, Y. D. Evaluation of different methods of protein extraction and identification of differentially expressed proteins upon ethylene-induced early-ripening in banana peels. *J. Sci. Food Agric.* **2012**, *92*, 2106−2115.
(77) Atherton, M. J.; Braceland, M.; Fontaine, S.; Waterston, M. M.; Burchmore, R. J.; Eadie, S.; Eckersall, P. D.; Morris, J. S. Changes in the serum proteome of canine lymphoma identified by electrophoresis and mass spectrometry. *Vet. J.* **2013**, *196*, 320−324.
(78) Ossipova, E.; Oliynyk, G.; Cerqueira, C.; Becker, S.; Ytterberg, J.; Auer, G.; Klareskog, L.; Jakobsson, P. J. Identification of novel ACPA targets in rheumatoid arthritis synovial tissues using 2D gel electrophoresis and mass spectrometry. *Ann. Rheum. Dis.* **2013**, *72*, A77−A77.
(79) Hunt, D. F.; Yates, J. R.; Shabanowitz, J.; Winston, S.; Hauer, C. R. Protein sequencing by tandem mass-spectrometry. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6233−6237.
(80) Karas, M.; Hillenkamp, F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 Da. *Anal. Chem.* **1988**, *60*, 2299−2301.
(81) Kuiper, H. A.; Kleter, G. A.; Noteborn, H. P.; Kok, E. J. Assessment of the food safety issues related to genetically modified foods. *Plant J.* **2001**, *27*, 503−528.
(82) Kuiper, H. A.; Kok, E. J.; Engel, K. H. Exploitation of molecular profiling techniques for GM food safety assessment. *Curr. Opin. Biotechnol.* **2003**, *14*, 238−243.
(83) Fiehn, O. Metabolomics − the link between genotypes and phenotypes. *Plant Mol. Biol.* **2002**, *48*, 155−171.
(84) Koo, S. C.; Bae, D. W.; Seo, J. S.; Kyoung, M. P.; Choi, M. S.; Kim, S. H.; Shim, S. I.; Kim, K. M.; Chung, J.; Kim, M. C. Proteomic analysis of seed storage proteins in low allergenic soybean accession. *J. Korean Soc. Appl. Biol. Chem.* **2011**, *54*, 332−339.
(85) Carter, T.; Nelson, R. L.; Sneller, C.; Cui, Z. Genetic diversity in soybean. In *Soybeans: Improvement, Production, and Uses*, 3rd ed.; Boerma, H. R., Specht, J., Eds.; American Society of Agronomy: Madison, WI, 2004.
(86) Clikeman, A. D.; Palmer, R. G.; Shoemaker, R. C. The effect of pre-selection on diversity detection in exotic germplasm. *Soybean Genet. Newsl.* **1998**, *25*, 149.
(87) Apuya, N. R.; Frazier, B. L.; Keim, P.; Roth, F. J.; Lark, K. G. Restriction fragment length polymorphisms as genetic-markers in soybean, *Glycine max* (L) Merrill. *Theor. Appl. Genet.* **1988**, *75*, 889−901.
(88) Griffin, J. D.; Palmer, R. G. Variability of 13 isozyme loci in the USDA Soybean Germplasm collections. *Crop Sci.* **1995**, *35*, 897−904.
(89) Sebolt, A. M.; Shoemaker, R. C.; Diers, B. W. Analysis of a quantitative trait locus allele from wild soybean that increases seed protein concentration in soybean. *Crop Sci.* **2000**, *40*, 1438−1444.
(90) Maruyama, N.; Adachi, M.; Takahashi, K.; Yagasaki, K.; Kohno, M.; Takenaka, Y.; Okuda, E.; Nakagawa, S.; Mikami, B.; Utsumi, S.

Crystal structures of recombinant and native soybean β-conglycinin β homotrimers. *Eur. J. Biochem.* **2001**, *268*, 3595−3604.
(91) Nielsen, N. C.; Dickinson, C. D.; Cho, T. J.; Thanh, V. H.; Scallon, B. J.; Fischer, R. L.; Sims, T. L.; Drews, G. N.; Goldberg, R. B. Characterization of the glycinin gene family in soybean. *Plant Cell* **1989**, *1*, 313−328.
(92) Beilinson, V.; Moskalenko, O. V.; Livingstone, D. S.; Reverdatto, S. V.; Jung, R.; Nielsen, N. C. Two subtilisin-like proteases from soybean. *Physiol. Plant.* **2002**, *115*, 585−597.
(93) Koshiyama, I. Storage proteins of soybean. In *Seed Proteins: Biochemistry, Genetics, and Nutritive Value*; Gottschalk, W., Muller, H. P., Eds.; Nijhoff/Junk: The Hague, The Netherlands, 1983; pp 427−450.
(94) Yagasaki, K.; Takagi, T.; Sakai, M.; Kitamura, K. Biochemical characterization of soybean protein consisting of different subunits of glycinin. *J. Agric. Food Chem.* **1997**, *45*, 656−660.
(95) Natarajan, S.; Xu, C.; Bae, H.; Bailey, B. A.; Cregan, P.; Caperna, T. J.; Garrett, W. M.; Luthria, D. Proteomic and genetic analysis of glycinin subunits of sixteen soybean genotypes. *Plant Physiol. Biochem.* **2007**, *45*, 436−444.
(96) Saio, K.; Kamiya, M.; Watanabe, T. Food processing characteristics of soybean 11S and soybean 7S proteins. Part I. Effect of difference of protein components among soybean carieties on formation of tofu-gel. *Agric. Biol. Chem. Tokyo* **1969**, *33*, 1301−1308.
(97) Stahlhut, R. W.; Hymowitz, T. Variation in the low-molecular weight proteinase-inhibitors of soybeans. *Crop Sci.* **1983**, *23*, 766−769.
(98) Yaklich, R. W.; Helm, R. M.; Cockrell, G.; Herman, E. M. Analysis of the distribution of the major soybean seed allergens in a core collection of *Glycine max* accessions. *Crop Sci.* **1999**, *39*, 1444−1447.
(99) Gibson, L. R.; Mullen, R. E. Soybean seed composition under high day and night growth temperatures. *J. Am. Oil Chem. Soc.* **1996**, *73*, 733−737.
(100) Hurburgh, C. R.; Brumm, T. J.; Guinn, J. M.; Hartwig, R. A. Protein and oil patterns in United-States and world soybean markets. *J. Am. Oil Chem. Soc.* **1990**, *67*, 966−973.
(101) Natarajan, S.; Xu, C. P.; Bae, H. H.; Bailey, B. A. Proteomic and genomic characterization of Kunitz trypsin inhibitors in wild and cultivated soybean genotypes. *J. Plant Physiol.* **2007**, *164*, 756−763.
(102) Yaklich, R. W. β-Conglycinin and glycinin in high-protein soybean seeds. *J. Agric. Food Chem.* **2001**, *49*, 729−735.
(103) Natarajan, S.; Xu, C. P.; Bae, H.; Caperna, T. J.; Garrett, W. M. Proteomic analysis of allergen and antinutritional proteins in wild and cultivated soybean seeds. *J. Plant Biochem. Biotechnol.* **2006**, *15*, 103−108.
(104) Natarajan, S. S.; Xu, C.; Bae, H.; Caperna, T. J.; Garrett, W. M. Characterization of storage proteins in wild (*Glycine soja*) and cultivated (*Glycine max*) soybean seeds using proteomic analysis. *J. Agric. Food Chem.* **2006**, *54*, 3114−3120.
(105) Parrott, W. A.; Hoffman, L. M.; Hildebrand, D. F.; Williams, E. G.; Collins, G. B. Recovery of primary transformants of soybean. *Plant Cell Rep.* **1989**, *7*, 615−617.
(106) Reddy, M. S.; Ghabrial, S. A.; Redmond, C. T.; Dinkins, R. D.; Collins, G. B. Resistance to bean pod mottle virus in transgenic soybean lines expressing the capsid polyprotein. *Phytopathology* **2001**, *91*, 831−838.
(107) Sanford, J. C.; Decit, M. J.; Bruner, R. F.; Johnston, S. A. Method and apparatus for introducing biological substances into living cells. *U.S. Patent* **1993**, *5*, 204−253.
(108) USDA-NASS, Agricultural statistics, 2011.
(109) Lappe, M. A.; Bailey, E. B.; Childress, C.; Setchell, K. D. R. Alterations in clinically important phytoestrogens in genetically modified, herbicide-tolerant soybeans. *J. Med. Foods* **1999**, *1*, 241−245.
(110) Duke, S. O.; Rimando, A. M.; Pace, P. F.; Reddy, K. N.; Smeda, R. J. Isoflavone, glyphosate, and aminomethylphosphonic acid levels in seeds of glyphosate-treated, glyphosate-resistant soybean. *J. Agric. Food Chem.* **2003**, *51*, 340−344.
(111) Padgette, S. R.; Taylor, N. B.; Nida, D. L.; Bailey, M. R.; MacDonald, J.; Holden, L. R.; Fuchs, R. L. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *J. Nutr.* **1996**, *126*, 702−716.
(112) Taylor, N. B.; Fuchs, R. L.; MacDonald, J.; Shariff, A. R.; Padgette, S. R. Compositional analysis of glyphosate-tolerant soybeans treated with glyphosate. *J. Agric. Food Chem.* **1999**, *47*, 4469−4473.
(113) Zhou, J.; Berman, K. H.; Breeze, M. L.; Nemeth, M. A.; Oliveira, W. S.; Braga, D. P.; Berger, G. U.; Harrigan, G. G. Compositional variability in conventional and glyphosate-tolerant soybean (*Glycine max* L.) varieties grown in different regions in Brazil. *J. Agric. Food Chem.* **2011**, *59*, 11652−11656.
(114) Kinney, A. J.; Knowlton, S. Designer oils: the high oleic soybean. In *Genetic Modification in the Food Industry*; Roller, S., Harlander, S., Eds.; Blackie: London, UK, 1998.
(115) McCabe, D. E.; Swain, W. F.; Martinell, B. J.; Christou, P. Stable transformation of soybean by particle acceleration. *Bio/Technol.* **1998**, *6*, 923−926.
(116) Kim, W. S.; Chronis, D.; Juergens, M.; Schroeder, A. C.; Hyun, S. W.; Jez, J. M.; Krishnan, H. B. Transgenic soybean plants overexpressing *O*-acetylserine sulfhydrylase accumulate enhanced levels of cysteine and Bowman-Birk protease inhibitor in seeds. *Planta* **2012**, *235*, 13−23.
(117) Qi, Q.; Huang, J.; Crowley, J.; Ruschke, L.; Goldman, B. S.; Wen, L.; Rapp, W. D. Metabolically engineered soybean seed with enhanced threonine levels: biochemical characterization and seed-specific expression of lysine-insensitive variants of aspartate kinases from the enteric bacterium *Xenorhabdus bovienii*. *Plant Biotechnol. J.* **2011**, *9*, 193−204.
(118) Kim, M. J.; Kim, J. K.; Kim, H. J.; Pak, J. H.; Lee, J. H.; Kim, D. H.; Choi, H. K.; Jung, H. W.; Lee, J. D.; Chung, Y. S.; Ha, S. H. Genetic modification of the soybean to enhance the β-carotene content through seed-specific expression. *PLoS One* **2012**, *7*, e48287.