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Proteome Profiling of Seed Storage Proteins Reveals the Nutritional Potential of *Salicornia brachiata* Roxb., an Extreme Halophyte

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Supporting Information

ABSTRACT: *Salicornia brachiata* is an extreme halophyte that grows in salty marshes and is considered to be a potential alternative crop for seawater agriculture. *Salicornia* seeds are rich in protein, and its tender shoots are eaten as salad greens. Seed storage proteins were fractionated by sequential extraction using different solvents, including distilled water for albumins, NaCl (1.0 M) for globulins, NaOH (0.1 N) for glutelins, and ethanol (70% v/v) for prolamins. Globulins accounted for 54.75% of the total seed storage proteins followed by albumins (34.30%) and glutelins (8.70%). The fractionated proteins were characterized using 2D-diagonal SDS-PAGE and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. The globulin fraction, composed of seven intermolecular disulfide-linked polypeptide pairs of molecular mass 63.5, 62.5, 54.7, 53.0, 43.2, 38.5, and 35.1 kDa, encompassed a basic and an acidic subunit. Two-dimensional gels revealed approximately 32 spots, with isoelectric points and molecular masses ranging from 4.93 to 11.6 and from ∼5.2 to ∼109.4 kDa, respectively. Protein spots were identified by MALDI-TOF MS peptide mass fingerprint analysis and further classified. Homology analysis demonstrated that 19% of the proteins were involved in metabolism, 16% were involved in signaling, and 15% were regulatory proteins. Peptide mass fingerprint analysis confirmed the presence of inter- and intramolecular disulfide linkages in the globulin fraction. Sulfur-rich proteins are of high nutritional value, and disulfides make *S. brachiata* a potential source of dietary supplementation.

KEYWORDS: *2D gel, globulins, MALDI-TOF, peptide fingerprinting, Salicornia, seed protein*

INTRODUCTION

Seed storage proteins (SSPs) are plant proteins that are abundantly consumed by humans. SSPs accumulate in seeds during the later stages of seed development and are degraded during germination. The resulting amino acids are utilized by developing seedlings as a nutritional source. Approximately 70% of human food is composed of plant proteins, whereas the remaining 30% comes from animals that feed on seed meals and meet the major dietary protein requirement of over half of the world's population.[1]

Salinity is emerging as one of the major constraints for profitable production of crops, as it is among the major limiting factors influencing plant growth and productivity. In coastal areas, extensive salt farming, scant rainfall, and uncontrolled use of groundwater for industrial purposes are important contributors to increasing salinity and drought. Cultivated areas are rapidly becoming depleted and therefore unsuitable for agricultural crops. During the past two decades, traditional crop production in coastal areas has become a major problem.[2] Halophytes that grow in elevated salinity conditions are plants with great potential importance to complement traditional agriculture in salt-affected areas and to support the agricultural economy as well to satisfy dietary requirements. Halophytes have also been regarded as a new source of crop used as vegetables, forage, and oilseed. Biomass production in halophytes varies from 10 to 20 ton/ha on seawater irrigation, which is equivalent to conventional crops. Halophyte forage and seed products can replace conventional ingredients in animal feeding systems, with some restrictions on their use due to high salt content and species-specific antinutritional compounds. These plants are also considered to be promising plant resources in arid coastal zones because of their high salt tolerance.[3]

Salicornia brachiata Roxb. (Dicotyledons, Caryophyllales, Amaranthaceae) is a leafless annual halophyte with green, jointed, succulent stems and terminal fruit-bearing spikes on which the seeds are borne. It is one of the most salt-tolerant halophytes, capable of growing under high salinity conditions.[4] The plants accumulate 30−40% NaCl in dry weight,[5] are considered to be a potential alternative seawater crop, and may contribute to reclamation of wasteland due to economic potential.[4−8] The application of its biomass as a vegetable salt (U.S. Patent 6929809) and shoots as salad greens and the plant's potential as a naturally adapted higher plant model for novel gene resources for salt adaptation make this extreme halophyte a promising candidate for industrial purposes and an alternative coastal area crop. Recent evidence of sulfur-rich proteins further contributes to its potential. Seeds of *Salicornia* spp. are rich in protein (35% of total seed content), similar to the soybean seed protein.[9] The leaves and shoots of *Salicornia bigelovii* and *S. brachiata* are eaten as greens,[10,11] and several species of the genus *Salicornia* cultivated in areas with elevated salinity are potential sources of commercial oil.[11]

A number of attempts have been made using leguminous seeds to determine the structural relationships among

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polypeptides present within the legumin protein complex, glycinin, one of the primary storage proteins in soybean seeds. These studies have demonstrated that glycinin consists of two major groups of polypeptides, designated the acidic and basic subunits, on the basis of their isoelectric points.[12] It is reported that at least some of the acidic and basic subunits are linked via disulfide bonds,[13,14] forming acid−base polypeptide complexes (AB complexes). However, the oligomeric structures of six subunits in the 12S globulins of legumes,[12] cucurbits,[15] ostrich fern,[16] and oat [17] are found to be composed of disulfide-linked large acidic and small basic polypeptides, which show similar mechanisms of synthesis in different genera. Disulfide bridges are also found in oilseeds from *Brassica* and other members of the Cruciferae family, in which the seed storage proteins are the 2S albumin (napin) and 12S globulin (cruciferin) types.[18]

Plant proteins lack essential amino acids and are considered of low nutrition compared to animal proteins. Humans require a diet with a balanced amino acid composition; however, seeds are often deficient in at least one of the essential amino acids. For years, plant breeders have tried to improve the balance of essential amino acids in important crops.[19] Molecular approaches for improving the nutritional quality of seed proteins provide alternatives to conventional approaches. In vitro mutagenesis of coding regions of certain seed proteins has been attempted in an effort to increase the levels of essential amino acids.[20] Current intensive research has focused on the isolation and characterization of seed proteins and introgression of gene(s) to produce transgenic plants that further enhance the nutritional value of seed proteins for food and fodder.

Amaranth's protein, AmAl, which is isolated from *Amaranthus hypochondriacus*, is a seed-specific protein that, unlike many seed storage proteins, contains high levels of the essential amino acids.[21] Because of its high nutritional value, the gene encoding this protein may compensate for the amino acid deficiencies of many seed proteins once it is genetically engineered into target plants.[21] In this way, sulfur-rich proteins may be ideal candidates for improving the nutritional content of the seed and the vegetative tissue of plants destined for ruminant feeding. Extensive research and deeper insight are needed before large-scale application of this approach for the improvement of the nutritional quality of seed proteins. Considering the dietary use of *S. brachiata*, an important plant of the Amaranthaceae family showing wide adaptation, the present work is focused on proteome profiling of seed proteins. To the best of our knowledge, this is the first study on the polypeptide subunit composition of the globulin fraction of seed proteins from *S. brachiata* showing the presence of inter- and intramolecular disulfide linkages in the major fraction of SSP.

MATERIALS AND METHODS

Plant Material. Mature seeds were extracted from dried *S. brachiata* Roxburgh (Amaranthaceae) plants grown at coastal marshy swamps on the Navabandar coast (N 21° 45′, E 72° 14′), Bhavnagar, Gujarat, India (Supporting Information, Supplementary S1).

Seed Protein Fractionation and Protein Estimation. Seeds were mechanically ground with an ice-cold pestle and mortar followed by vortexing in hexane at 4 °C for 1 h. The vortexing process was repeated three to four times to completely defat the seed meal. The defatted seed meal (100 mg) was dried under a vacuum, and seed storage proteins (e.g., albumins, globulins, glutelins, and prolamins) were fractionated by sequential extraction using different solvents, including distilled water for albumins, NaCl (1.0 M) for globulins, NaOH (0.1 N) for glutelins, and ethanol (70% v/v) for prolamins. Protein concentrations were estimated according to the Bradford method[22] using a standard curve of bovine serum albumin (BSA) and represented in relation to dry weight.

1D and 2D Nonreduced and Reduced SDS-PAGE. Samples were prepared by mixing the extracted fraction with sample buffer (0.2 mM Tris-HCl buffer (pH 6.8), 2% SDS, 10% glycerol, and 0.025% bromophenol blue) at a 1:1 ratio. Samples (15 μg of each protein fraction) were analyzed using 1D 12% SDS-PAGE (gel size 18 × 16 cm). For 2D nonreduced/reduced SDS-PAGE analysis, the proteins (15 μg) were first separated under nonreducing conditions in the first dimension followed by electrophoresis under reducing conditions in the second dimension. The gel strip from the first dimension was equilibrated in 0.2 mM Tris-HCl buffer (pH 6.8) containing 2% SDS and 2% 2-mercaptoethanol and was then loaded onto the second-dimension polyacrylamide gel. Both gel electrophoreses (1D and 2D) were repeated five times, and only reproducible spots (common spots) were taken further for the in-gel digestion followed by MALDI-TOF MS analysis.

In-Gel Digestion and MALDI-TOF MS Analysis. Protein spots were excised manually from CBB stained gels and processed for in-gel digestion followed by MALDI-TOF MS analysis. Each protein-containing gel slice was completely destained in 50 mM NH_4HCO_3 and 50% v/v ACN for 1 h at 40 °C. Destained slices were then incubated with iodoacetamide (55 mM) for 30 min at room temperature followed by incubation with acetonitrile (50% v/v) for 15 min. Slices were then lyophilized, rehydrated, and incubated with sequencing grade modified trypsin (10 ng/μL) in 25 mM NH_4HCO_3 at 37 °C overnight. After digestion, peptides were collected, and the gels were washed (0.1% TFA in 50% ACN) three times to collect the remaining peptides. Peptides in the solution were desalted using ZipTip C18 pipet tips (Millipore, Bedford, MA, USA) and cocrystallized with 1 volume of saturated α-cyano-4-hydroxycinnamic acid in 50% v/v acetonitrile containing 1% TFA. The trypsin-digested peptides were characterized using a MALDI-TOF mass spectrometer (AXIMA CFR*plus*, Shimadzu Biotech, Kyoto, Japan) in reflector mode over the mass range of 700−3000 Da. Reproducibility of the spectrum was checked from five spot sets, and the spectra were analyzed after centroid and deisotoping. The monoisotopic peaks of peptide mass fingerprints were obtained by setting carbamidomethyl (C) as a fixed modification and Gln→pyro-Glu (N-term Q) and Glu→pyro-Glu (N-term E) as variable modifications, a peptide mass tolerance of ±0.5 Da, and one maximum mixed cleavage. The peptide mass fingerprint data were searched for comparative protein homology using the MASCOT database.

Protein Classification. The identified proteins were searched for their known functions using the UniProt database (http://www.ebi.uniprot.org/index). Proteins were classified into different groups according to biochemical functions using functional catalog software (http://mips.gsf.de/projects/funcat).

RESULTS AND DISCUSSION

On the basis of solubility criteria, four seed protein fractions, including albumins, globulins, glutelins, and prolamins, were fractionated and studied for their relative protein proportions in *S. brachiata* seeds. Analyses identified that of the four protein fractions, salt-soluble fraction globulins were the most abundant (54.75%), followed by water-soluble protein albumins (34.30%) and the alkali soluble glutelins (8.70%). The relative proportion of alcohol-soluble protein (prolamins) was the lowest (2.25%) of the total seed protein. The fractionation pattern was similar to that of the leguminous seed proteins.[23]

Seed protein fractions were further analyzed for their constituent polypeptides using SDS-PAGE under nonreducing and reducing conditions. Polypeptides were identified in the range of molecular mass of 10−124.4 kDa under both conditions (Figure 1). Salt-soluble globulins consisting of a large number of polypeptides (63.5, 62.5, 58.0, 54.7, 53.0, 44.5, 43.2, 38.5, 35.1, 34.2, 19.6, 13.3, and 11.4 kDa) were resolved as

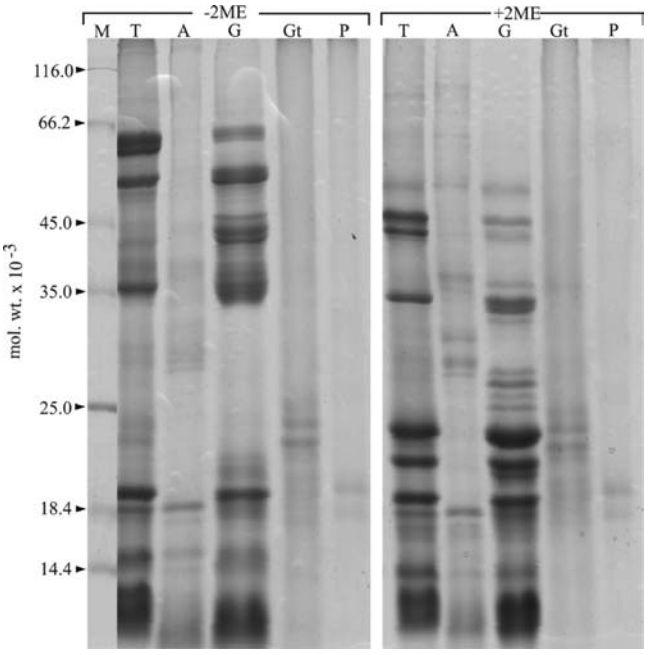

Figure 1. SDS-PAGE of seed protein fractions of *S. brachiata*. Lanes: M, standard protein molecular weight markers; T, total seed protein extract; A, albumins; G, globulins; Gt, glutelins; P, prolamins. Sections: −2ME, SDS-PAGE under nonreduced conditions; +2ME, SDS-PAGE under reduced conditions. About 15 μg of protein (of each fraction) was loaded in the gel of size 18 × 16 cm.

high-intensity bands. Polypeptides at 124.4, 65.7, 64.5, 56.8, 49.8, 48.6, 38.6, 37.7, 21.7, 21.0, 20.5, 18.7, 18.2, 15.6, and 14.9 kDa were resolved as low-intensity bands under nonreducing condition. Under reducing conditions, the polypeptides at 63.5, 62.5, 54.7, 53.0, 43.2, 38.5, and 35.1 kDa disappeared and new bands at 46.2, 43.8, 36.5, 34.5, 23.4, 23.2, 21.4, 19.0, and 11.0 kDa emerged with high intensities.

SDS−polyacrylamide gels of the albumin fraction showed polypeptides of molecular mass 117.9, 89.8, 79.2, 64.6, 44.4, 43.4, 38.6, 34.6, 30.1, 28.7, 28.0, 18.7, 15.6, 14.7, 11.4, and 10.0 kDa. These polypeptides showed no differences in their molecular masses and therefore exhibited similar patterns under both nonreducing and reducing conditions. Glutelin and prolamin fractions contained fewer polypeptides and exhibited low-intensity resolution. The glutelin fraction consisted of polypeptides at 25.2, 23.9, 22.9, 21.7, 20.5, 18.7, and 18.2 kDa. Only two polypeptides, 19.7 and 18.2 kDa, were observed with very low intensities in the prolamin fraction under both reducing and nonreducing conditions. Polypeptide profiling under reducing and nonreducing conditions showed the presence of disulfide bonds in the globulin fraction.

Because the globulin fraction exhibited significant disulfide bonds, individual bands observed under nonreducing conditions were compared with those obtained under reducing conditions using two-dimensional polyacrylamide gel electrophoresis. The 2D gel with nonreducing conditions in the first dimension followed by reducing conditions in the second dimension demonstrated that polypeptides possessing disulfide

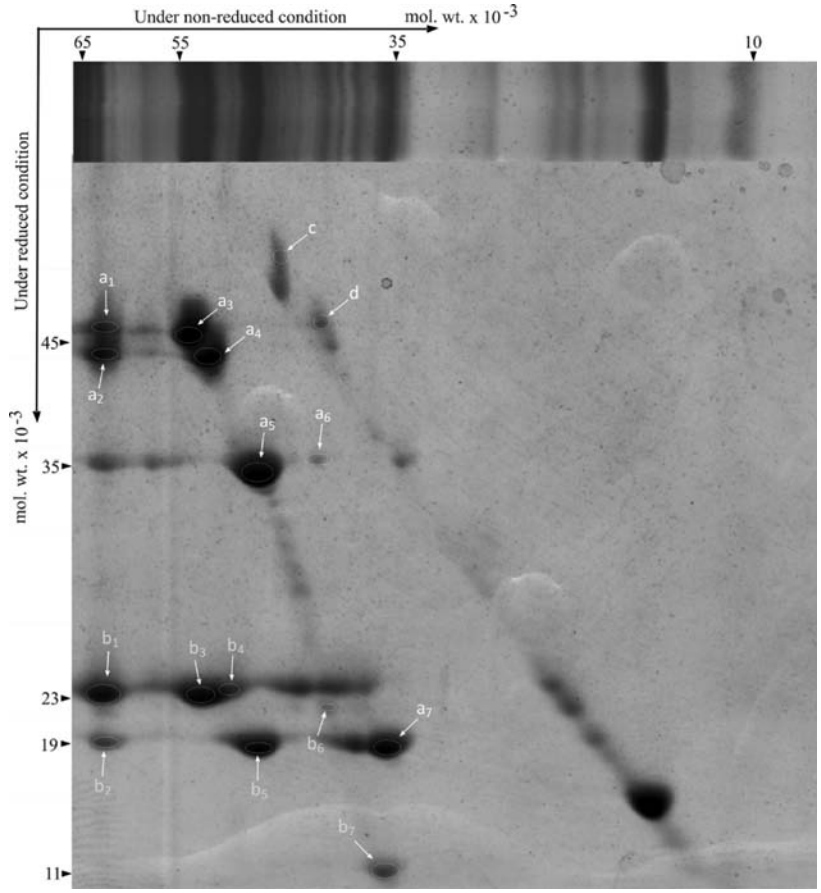

Figure 2. Two-dimensional polyacrylamide gel electrophoresis showing disulfide-linked polypeptide subunit composition in globulin fraction of seed protein of *S. brachiata* under nonreducing/reducing conditions. About 15 μg of globulin protein fraction was loaded on the strip.

bonds were easily reduced and moved as spots off of the diagonal (Figure 2). Disulfide-linked polypeptides were not observed in the second major fraction of albumins (Supporting Information, Supplementary S2). Electrophoretic mobility of proteins was altered because of cleavage of inter- or intramolecular disulfide bonds by β-mercaptoethanol. Breakage of intermolecular disulfide bonds between subunits yielded different subunit polypeptides in the second dimension. Cleavage of intramolecular disulfide bonds within a single polypeptide caused a conformational change that either increases or decreases the mobility during SDS-PAGE. The diagonal SDS-PAGE used in the present study restricted unreactive proteins to a diagonal, whereas proteins outside the diagonal contained thiols in close proximity to form intramolecular disulfide bonds.[24] Seven intramolecular disulfide-linked polypeptide pairs (63.5, 62.5, 54.7, 53.0, 43.2, 38.5, and 35.1 kDa) were observed possessing acidic (large) and basic (small) subunits in the ranges of 19.0−46.2 and 11.0−23.2 kDa, respectively (Table 1). Polypeptides of 47 and 44 kDa represented intramolecular disulfide-bonded pairs that resolved (spots c and d) at higher molecular mass positions of 53 and 46.2 kDa, respectively (Figure 2). Polypeptides lacking disulfide bonds were resolved along the diagonal with a relatively low intensity.

Table 1. Molecular Masses of Polypeptide Pairs and Their Constituent Subunits of Albumin Fraction[a]

	molecular mass (kDa)	
subunit	nonreducing condition[b]	reducing condition[b,c]
1	63.5	46.2 and 23.2
2	62.5	43.8 and 19.0
3	54.7	45.5 and 23.2
4	53.0	43.8 and 23.4
5	43.2	34.5 and 19.0
6	38.5	36.5 and 21.4
7	35.1	19.0 and 11.0

[a]Proteins was altered by the cleavage of inter- and intramolecular disulfide bonds of the subunits of a multisubunit complex, yielding different subunit polypeptides in the second dimension. [b]Separated as subunits a_1−a_7 linked to b_1−b_7, respectively, as shown in Figure 2. [c]Large and small subunits are acidic and basic in nature, respectively.

Globulins of leguminous seeds are classified as 11S (legumin) and 7S (vicilin). Globulin of *Vicia faba* is a hexameric protein comprising six monomers (60 kDa), with each monomer containing two subunits of molecular mass 40 and 20 kDa joined by disulfide bonds.[25] Approximately 10 disulfide-linked polypeptide pairs in the range of molecular mass 37−79 kDa have been observed in *V. faba*.[26] Legumin of *Glycine max*, known as glycinin, is a hexameric protein (350 kDa) that consists of two subunits, an acidic (37−40 kDa) and a basic (18−20 kDa) subunit.[14] Disulfide-bonded polypeptide pairs were found in the range of 39−85 kDa in globulins of *Lathyrus sativus* seeds,[27] whereas six disulfide-linked polypeptide pairs (53, 52, 50, 42, 39, and 23 kDa) have been reported in *Cucumis melo*.[28] The globulin fraction of seed protein from *S. brachiata* is composed of seven disulfide-linked polypeptide subunit pairs (35.1−63.5 kDa), similar to globulin (legumin) of leguminous seed, the latter of which further consists of an acidic (19.0−46.2 kDa) and a basic (11.0−23.2 kDa) subunit.

Protein Identification through MALDI-TOF MS. The purified globulins of seed protein of *S. brachiata* from 2D SDS-PAGE were further analyzed using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS). A total of 32 protein spots detected on the 2D-diagonal gel showed disulfide linkages (Supporting Information, Supplementary S3). Spots were excised and processed for in-gel trypsin digestion. Trypsin-digested polypeptides were analyzed by MALDI-TOF MS, and the peptide mass fingerprint data (Figure 3) obtained were searched for functional identification using the UniProt database. Proteins were further categorized into seven groups on the basis of their biochemical functions (Figure 4 and Supporting Information, Supplementary S4). Approximately 19% of proteins were associated with metabolism, whereas 16% of proteins were involved in cellular processes and signaling. Transcription and translation regulatory proteins represented 15% of the total identified proteins; however, 41% of the proteins showed no homology with existing databases and thus were categorized as novel or uncharacterized proteins. Approximately 14 different major protein spots (Figure 2) showed homology to proteins containing isoelectric points (p*I*) and molecular masses ranging from 4.93 to 11.6 and from ∼5.2 to ∼109.4 kDa, respectively (Table 2). The identified proteins were related to 13 different plant species: *Arabidopsis thaliana*, *Oryza sativa*, *Triticum aestivum*, *Zea mays*, *Brassica compestris*, *Medicago truncatula*, *Allium cepa*, *Lycopersicon esculentum*, *Cyathea alata*, *Timmia megapolitana*, *Fragaria ananassa*, *Antirrhinum majus* and *Gossypium*.

Spot a_1 (46.2 kDa, p*I* 11.6) was identified as a putative splicing factor-like protein and showed an intermolecular disulfide linkage with spot b_1 (23.2 kDa, p*I* 5.87), which showed resemblance to a hypothetical protein of *Medicago truncatula*. The majority of splicing factors are widely distributed throughout the nucleus (excluding the nucleoli) and are involved in premRNA splicing and the regulation of gene expression. The polypeptide resolved as spot a_3 (45.5 kDa, p*I* 5.79) showed homology with H^+-transport ATP synthase proteins and showed an intermolecular disulfide linkage with spot b_3 (23.2 kDa, p*I* 5.03), identified as a hypothetical protein. Protein H^+-ATPases play a vital role not only in respiration and photosynthesis but also in organelle energy production. There are four types of H^+-ATPases, mitochondrial, chloroplast, plasma membrane, and vacuolar ATPases, which have been reported in cells from higher plants. The plasma membrane H1-ATPase is involved in nutrient uptake, phloem loading, elongation growth, and establishment of turgor.[29] The plasma membrane H1-ATPase is considered to be essential for plant growth, and little is known about its regulation in response to a variety of physiological stimuli including light, salt stress, pathogenic elicitors, hormones (such as auxin and abscisic acid), and fungal toxins (such as fusicoccin).

Polypeptide spot a_4 (43.8 kDa, p*I* 8.92) showed homology with AY156708 NID-like protein of *Brassica oleracea* var. *botrytis*. The protein was identified as xyloglucan endotransglycosylase (XET) C-terminus-like protein of a family of cell wall enzymes, which play an important role in the assembly,[30] modification, synthesis, development, and senescence of the plant cell walls.[31,32] Spot a_5 (34.5 kDa, p*I* 5.07) was found to be homologous to a GTP-binding protein and showed intermolecular disulfide bonding with spot b_5, which was homologous to a ribosomal protein (19 kDa, p*I* 10.44). GTPase superfamily proteins play key roles in various structural and molecular mechanisms and are considered to be molecular switches in diverse biological processes, such as cell

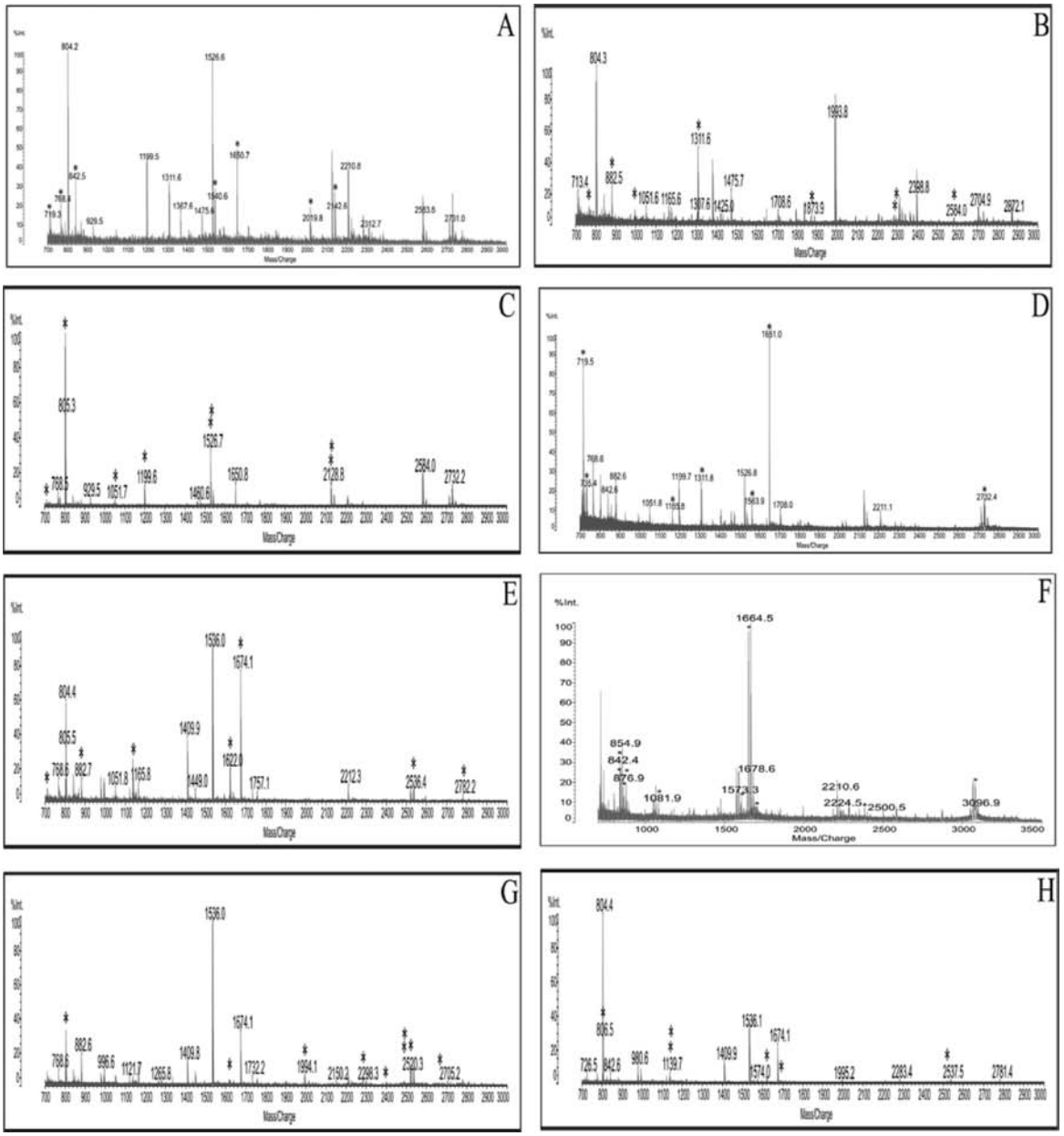

Figure 3. MALDI-TOF MS spectra of protein spots (A–F, spots a_1–a_7, excluding a_5; G, spot b_2; H, spot b_7). Proteins spots were subjected to in-gel digestion by trypsin and thereafter analyzed by MALDI-TOF MS in reflector mode over the mass range of 700–3000 Da. Reproducibility of the spectrum was checked from five spot sets, and the spectra were analyzed after centroid and deisotoping. Peaks marked with asterisks (∗) correspond to tryptic peptides that were matched with a protein from a database search.

proliferation, development, protein synthesis, signal transduction, membrane trafficking, intracellular transport of proteins, and cytoskeletal organization.[33−35] The ribosomal protein (spot b_5) was reported to have an extraribosomal function in addition to protein synthesis,[36] and the genes encoding ribosomal proteins are highly expressed in actively dividing tissues.[37−39]

Protein spot b_2 (19.0 kDa, p*I* 5.09), identified as a chlorophyll *a*/*b*-binding protein (CAB protein), exhibited an intermolecular disulfide bond with spot a_2 (43.8 kDa, p*I* 5.97), which showed homology to sequence 85 from *Fragaria ananassa*. The chlorophyll pigments are anchored in thylakoid membranes of chloroplasts through their interaction with a set of proteins known as the CAB proteins.[40] Plants have an extensive membrane-integrated light-harvesting system consisting of the LHCA1−4 and LHCB1-6 chlorophyll *a*/*b*-binding proteins as the main subunits, which associate in single or multiple copies to PSI and PSII and form large supercomplexes.[41] LHCI containing chlorophyll *b* consisted of a group of polypeptides with apparent molecular masses of 19−24 kDa.[42] Spot a_7 (19 kDa, p*I* 5.76, putative α subunit of ATP synthase) showed an intermolecular disulfide linkage with hypothetical protein P0640E12.107 of *Oryza sativa* (spot b_7). Seed protein spots a_6, b_1, b_3, b_4, b_6, and b_7 showed homology to hypothetical proteins from *Oryza sativa*, *Medicago truncatula*, and *Arabidopsis thaliana* (Table 2).

Spots c and d, which are homologous to hypothetical protein OSJNBa0036C12.20 (p*I* 9.22) and cyclin-like F-box protein (p*I* 4.93), respectively, showed intramolecular disulfide bonds. In addition to disulfide-linked proteins, other proteins similar to B

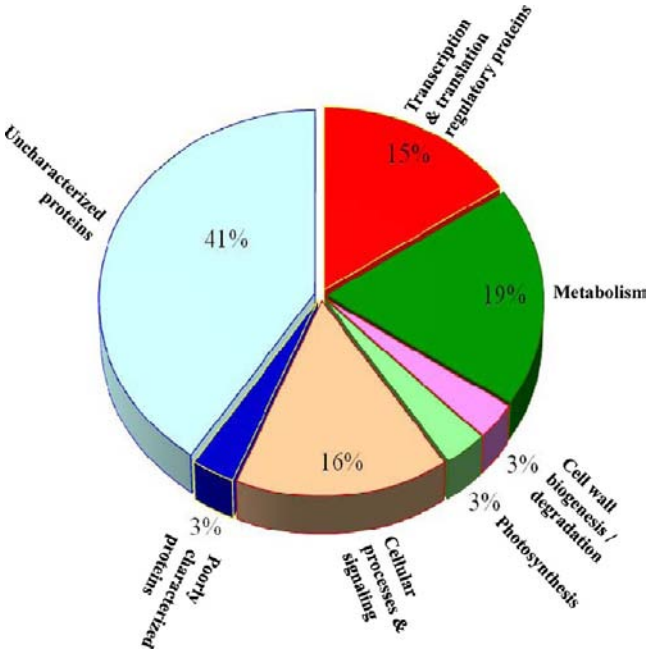

Figure 4. Functional classification of the proteins identified in salt-soluble fraction globulin based on comparative homology as listed in the Supporting Information, Supplementary S4.

transcriptional activator, including cysteine-sulfoxide lyase, M25 protein, myb-related protein, proline-rich protein precursor, protein kinases, putative inositol polyphosphate 5-phosphatase, small heat shock protein, and sulfotransferase, were also identified (Supporting Information, Supplementary S4).

In conclusion, peptide mass fingerprint analysis confirms the presence of inter- and intramolecular disulfide bonds in the globulins, the major fraction of seed storage proteins. Plant proteins are deficient in essential amino acids and are therefore inferior to animal protein sources. An extreme halophyte, *S. brachiata*, exhibits robust growth in saline conditions and is considered to be a potential alternative crop for seawater agriculture. The sulfur-rich proteins are considered to be highly nutritious, and the presence of inter- and intramolecular disulfide bonds makes this plant a potential source for dietary supplementation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Table 2. Major Polypeptides Containing Intermolecular Disulfide Linkages Resolved in the Second Dimension below the Diagonal and Identified by MALDI-TOF MS[a]

spot	protein homologue [accession no.][b]	p*I*[c]	M_r[d] (kDa)	score	query coverage[e]
a_1	splicing coactivator subunit like, *Oryza sativa* [Q6ZB62]	11.6	46.2	70	24
a_2	sequence 85 (fragment) from *Fragaria ananassa* (strawberry) [CAD57104]	5.97	43.8	76	19
a_3	H^+ transporting ATP synthase chain like protein, *Oryza sativa* [Q84PA4]	5.79	45.5	69	31
a_4	AY156708 NID, *Brassica oleracea* var. *Botrytis* [AAO00727]	8.92	43.8	61	24
a_5	GTP-binding protein (At5g18570), *Arabidopsis thaliana* [Q8L7L0]	5.07	34.5	72	16
a_6	hypothetical protein P0680C01.8, *Oryza sativa* [Q6YSV1]	5.8	36.5	72	52
a_7	putative α subunit of ATP synthase (fragment), *Cyathea alata* [Q1H9R4]	5.76	19.0	108	27
b_1	hypothetical protein, *Medicago truncatula* [Q1SQW5]	5.87	23.2	62	68
b_2	chlorophyll *a*/*b* binding protein type III precursor (cab-13), tomato [CDTO33]	5.09	19.0	81	35
b_3	hypothetical protein T20N10.170, *Arabidopsis thaliana* [T49165]	5.03	23.2	68	17
b_4	hypothetical protein T20N10.170, *Arabidopsis thaliana* [T49165]	5.03	23.4	68	17
b_5	ribosomal protein 4 (fragment), *Timmia megapolitana* [Q8MG47]	10.44	19.0	60	37
b_6	hypothetical protein OSJNBb0011H15.27, *Oryza sativa* [Q6Z3I6]	7.52	21.4	52	34
b_7	hypothetical protein P0640E12.107, *Oryza sativa* [Q84NN7]	10.82	11.0	77	53

[a]Subunits a_1−a_7 are linked to b_1−b_7, respectively. [b]With which spots show homology. [c]p*I* of the protein with which spots show close proximity. [d]Molecular mass of spots. [e]Query coverage in percentage sequence coverage.

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