RESEARCH ARTICLE

Shotgun proteomic analysis of soybean embryonic axes during germination under salt stress

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Seed imbibition and radicle emergence are generally less affected by salinity in soybean than in other crop plants. In order to unveil the mechanisms underlying this remarkable salt tolerance of soybean at seed germination, a comparative label-free shotgun proteomic analysis of embryonic axes exposed to salinity during germination *sensu stricto* (GSS) was conducted. The results revealed that the application of 100 and 200 mmol/L NaCl stress was accompanied by significant changes (>2-fold, $P<0.05$) of 97 and 75 proteins, respectively. Most of these salt-responsive proteins (70%) were classified into three major functional categories: disease/defense response, protein destination and storage and primary metabolism. The involvement of these proteins in salt tolerance of soybean was discussed, and some of them were suggested to be potential salt-tolerant proteins. Furthermore, our results suggest that the cross-protection against aldehydes, oxidative as well as osmotic stress, is the major adaptive response to salinity in soybean.

Keywords:
Embryonic axes / Germination *sensu stricto* / *Glycine max* (L.) Merrill / Label-free quantification / Plant proteomics / Salt tolerance

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1 Introduction

Soybean (*Glycine max* (L.) Merrill), the world's most important food legume, provides an inexpensive source of protein (70%) and edible oil (35%) for human consumption (http://soystats.com/wp-content/uploads/SoyStats_2014.pdf). This important legume is adapted to grow in a wide range of environments; nevertheless, growth and yield of soybean are seriously affected by salinity [1, 2]. Thus, continuous improvements in yield, quality, and salt stress tolerance are the major targets in current soybean breeding programs [3], and therefore the identification of salt-responsive genes (SRGs)/proteins could facilitate the achievement of these objectives [3, 4].

Salt tolerance is a complex trait involving the function of many genes [5]. The germination of seeds in salt-affected soils is a primary factor in the agricultural production of soybean because it allows plant survival and reproduction [6]. Soybean is generally classified as a moderate salt-sensitive crop [4–6]. Salinity adversely affects plant growth and development through imposing osmotic and ionic stresses, and also by generating secondary effects such as oxidative stress [7]. However, these effects depend on the plant growth stage. While germination and seedling growth are often regarded as the most susceptible to salinity damage, seed imbibition and radicle emergence are generally less affected by salinity in soybean than in other crop plants [1, 8]. This makes embryonic axis an appropriate model system to investigating the potential mechanisms of salt tolerance in this species.

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Abbreviations: **ABA**, abscisic acid; **AKR**, aldo-keto reductase; **ALDH**, aldehyde dehydrogenase; **AQP**, Aquaporin; **CaEF**, calcium-binding EF-hand protein; **GSS**, germination sensu stricto; **LEA**, late embryogenesis abundant; **LFQ**, label-free quantification; **SRG**, salt-responsive gene; **SRP**, salt-responsive protein

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Significance of the study

This work describes for the first time the use of shotgun label-free proteomic approach in order to evaluate protein profiles of soybean embryonic axes during germination *sensu stricto* under salinity conditions. More than 1700 proteins have been identified. Among them 141 proteins were reproducibly (>2-fold increased or decreased) and statistically ($P<0.05$) identified and many of them were suggested to be salt-responsive proteins. Our results indicate the importance of proteins protection against salt-induced osmotic stress and most likely against salt-induced proteolytic damages due to ROS and aldehydes, in order to cope with high salinity. These finding might be extended towards the identification of reliable biomarkers useful for improving salt tolerance in soybean and related crops.

Recently several SRGs have been investigated in soybean [6, 9–12]. For instance, analyzing more than 190 accessions of soybean, Kan et al. [6] identified several candidate genes involved in salt tolerance at germination stage. However, SRGs may lead to increase or decrease of the salt-responsive proteins (SRPs), thus it is difficult to make the precise phenotyping of stress tolerance which is essential for breeding programs [13, 14]. By using the latest advancement in proteomic technology, however, proteomic approach seems to be the best choice for identifying master regulator proteins that play key roles in the abiotic stress response pathway, and providing opportunities for developing genetically engineered stress-tolerant crop plants [14].

Although several proteomic studies on soybean have been reported to date, little attention has been paid to the identification of SRPs in soybean seeds during germination [15–17]. Curiously, most of these proteomics studies use the term 'germination' when they are actually studying the post-germinative growth [18]. However, germination *sensu stricto* (GSS) encompasses the events beginning with hydration of the mature dry seed and terminates with the elongation of the embryonic axis and the emergence of the radicle [19]. For these reasons, to investigate the effect of salinity during GSS on the soybean embryonic axes, we carried out a comprehensive proteome profile analysis using high-resolution mass spectrometry-based proteomics with intensity-based label-free quantification.

2 Materials and methods

2.1 Seed treatment and Germination

Thirty dried soybean seeds (*Glycine max* (L.) Merrill. Cv. PR91M10) with uniform size were sterilized in 0.1% (w/v) aqueous solution of mercuric chloride for one minute and thoroughly washed three times with ddH_2O. Seeds were placed in Petri plates containing two layers of Whatman No. 1 filter paper initially moistened with ddH_2O (control) or with 100 mmol/L NaCl or 200 mmol/L NaCl solutions (NaCl-treated), and they were germinated in darkness at 24 ± 0.5°C. Soybean seeds that reached the end of stage II of germination or GSS stage (with the radicle protruded about 2–3 mm in length through the seed coat) in each treatment were hand dissected into embryonic axes and non-embryonic tissues (Fig. 1). Embryonic axes were stored at –80°C until use.

2.2 Extraction of total soluble protein from embryonic axes

The embryonic axes of early-germinating soybean seeds were ground into a fine powder and (100 mg) suspended in 500 μL of cold extraction buffer (175 mmol/L Tris-HCl, pH 8.8, 5% (w/v) SDS, 15% (v/v) glycerol, 5 mmol/L EDTA, 300 mmol/L

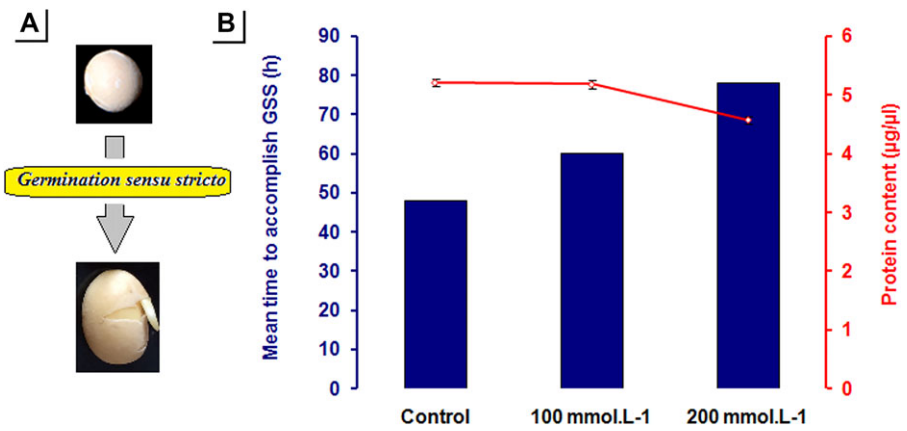

Figure 1. Germination of soybean seeds. (A) Germination *sensu stricto* (GSS). (B) Effect of salinity on the protein content (μg/μL) of the embryonic axes at different GSS times.

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DTT and 1% protease inhibitor cocktail). The suspension was incubated on ice for 1 h with intermittent mixing and then centrifuged at 500 x *g* for 15 min at 4°C to remove cell debris. The supernatant (400 μL) was transferred to a clean centrifuge tube, and four volumes of cold acetone with 0.07% (v/v) β-mercaptoethanol were added and incubated at −20°C overnight. The insoluble fraction was pelleted by centrifugation at 18 000 x *g* for 45 min at 4°C, and washed three times with ice-cold solution of 0.07% (v/v) β-mercaptoethanol in ddH_2O:acetone (20:80, v/v),. The protein pellet was slowly dried and solubilized in 8 mol/L urea, 50 mmol/L NH_4HCO_3 (pH 8.5) and 10 mmol/L DTT, and quantified by Bradford assay using BSA standard [20]. Three experimental replicates were performed for each germination conditions (control and NaCl-treated samples).

2.3 Enzymatic digestion and off-line desalting

Protein samples were reduced, alkylated and digested with trypsin as previously described [21]. After digestion and off-line desalting each sample was re-constituted with 0.1% (v/v) HCOOH aqueous solution and stored at −80°C until analysis.

2.4 Nano HPLC-MS/MS analysis

Nano high performance liquid chromatography (nanoHPLC) coupled to tandem mass spectrometry (MS/MS) analysis was performed on a hybrid LTQ Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a nanospray ion source. The peptide mixtures were enriched on a 300 μm id × 5 mm Acclaim PepMap 100 C18 (5 μm particle size, 100 Å pore size) precolumn (Dionex) and separated by reversed phase chromatography using in-house made microcolumn (75 μm id packed with Acclaim-C18 2.2 μm silica microparticles) with a 2 h gradient from 11% to 31%, using ddH_2O:HCOOH (99.9:0.1, *v/v*) as mobile phase A and CAN:HCOOH (99.9:0.1, v/v) as mobile phase B, at a flow rate of 250 nL/min. The mass spectrometer was operated in the data dependent mode to simultaneously measure full scan MS spectra in the Orbitrap and the twenty most intense ions in the LTQ part by collisional induced dissociation, respectively. For each of the three experimental replicates, three technical replicates (nanoHPLC-MS/MS runs) were performed.

2.5 Database searching, protein identification and label-free quantification

All data files were analyzed using MaxQuant (version 1.5.1.2).The derived peak list was searched against the reference proteome of *Glycine max* (Uniprot, Nov. 2013) using the Andromeda search engine [22]. Strict trypsin specificity was required and a maximum of two missed cleavage sites was allowed. The minimum required peptide length was set to six amino acids. Carbamidomethylation of cysteine was set as a fixed modification and N-acetylation of proteins N-termini and oxidation of methionine were set as variable modifications. As no labeling was performed, multiplicity was set to 1. Peptide spectrum match and protein identifications were filtered using a target-decoy approach at an FDR of 1%. The second peptide feature was enabled. The match between runs option was applied with a match time window of 0.7 min and an alignment time window of 20 min. Protein identifications were accepted if they were established with >99.0% probability and contained at least two peptides. Relative label-free quantification (LFQ) of these proteins was carried out using the MaxLFQ algorithm [22] with fast LFQ, and with the following parameters: LFQ minimum ratio count set to 3, and the LFQ average number of neighbors to 6.

2.6 Data analysis

Further analysis of the data was carried out using the freely available Perseus software (version 1.5.0.31). The LFQ intensities were transformed at a base of 2 after elimination of contaminants and proteins only identified with modified peptides. For every experiment, data were filtered for proteins with at least six valid values in each specific experiment. After missing values were substituted a new distribution with a downshift of 1.8 standard deviations and a width of 0.3 standard deviations was produced. The total matrix was made using these values, enabling statistical analysis. Student's *t*-test ($P<0.05$) was performed on the new data to detect the significance of differences in protein intensities in samples (Ctrl vs. 100 mmol/L NaCl and Ctrl vs. 200 mmol/L NaCl). Hierarchical clustering analysis was also applied to this quantitative data using Perseus. The MS proteomics data have been deposited to the ProteomeXchange Consortium [23] via the PRIDE partner repository with the dataset identifier PXD001943.

2.7 Analysis of function and localization

Protein functions were categorized using GO annotation (Uniprot) and according to Bevan et al. [24]. Protein localization was analyzed using CELLO2GO, the web server for protein subCELlular LOcalization prediction with functional GO annotation (http://cello.life.nctu.edu.tw/cello2go/) [25].

3 Results

3.1 Experimental design and soybean seed proteome analysis

As expected, even at growth-inhibition levels of salinity (100 and 200 mmol/L NaCl), soybean embryonic axes maintain

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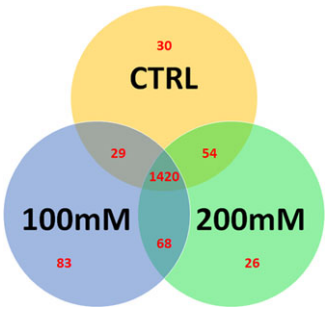

Figure 2. Venn diagram showing the proteins identified in the three sample typologies, i.e., control (Ctrl), subject to 100 mmol/L NaCl (100 mM) and to 200 mmol/L NaCl (200 mM).

their ability to germinate displaying only an increase in germination time in a concentration-dependant manner (Fig. 1A). As compared to the control, a significant decrease in the total protein content of embryonic axes was observed, but only at the highest salinity level (Fig. 1B).

A total of 15644 peptides were identified and quantified, with 9187 of them being unique peptides, corresponding to 1710 non-redundant proteins at an FDR of 1%. Proteins (with molecular mass ranging from 5.4 to 545 kDa) were identified as follows: 1533 proteins were detected in control samples, 1600 in 100 mmol/L NaCl treated samples and 1568 in 200 mmol/L treated samples (see Fig. 2). Peptide and protein identifications in all samples are listed in Supporting Information (Tables S1 and S2). Both the three technical replicates performed for each sample and the three biological replicates between samples were reproducible, with correlation coefficients ranging from 0.983 to 0.995 (see Supporting Information, correlation heatmaps).

Compared to the control samples, 141 proteins were significantly (*t*-test at $P<0.05$) changed in abundance under salinity conditions, and 31 of them showed similar differential abundance under both salt stress conditions (Supporting Information Table S3 and Fig. 3A). Ninety one proteins were successfully identified by querying the UniProt database using the MaxQuant search engine, whereas the others proteins were subsequently subjected to a homology search against the UniProt database (www.uniprot.org) using BLAST (Basic Local Alignment Search Tool) [26].

3.2 Functional classification of differential proteins

Of the 97 proteins displaying differential abundance in response to 100 mmol/L NaCl treatment, 58 were increased and 39 were decreased in abundance (Fig. 3A). These proteins were assigned into 14 functional categories. For the increased proteins, the most abundant categories were disease/defense (34%), metabolism (17%), and protein destination/storage (10%). The same classification for the decreased proteins

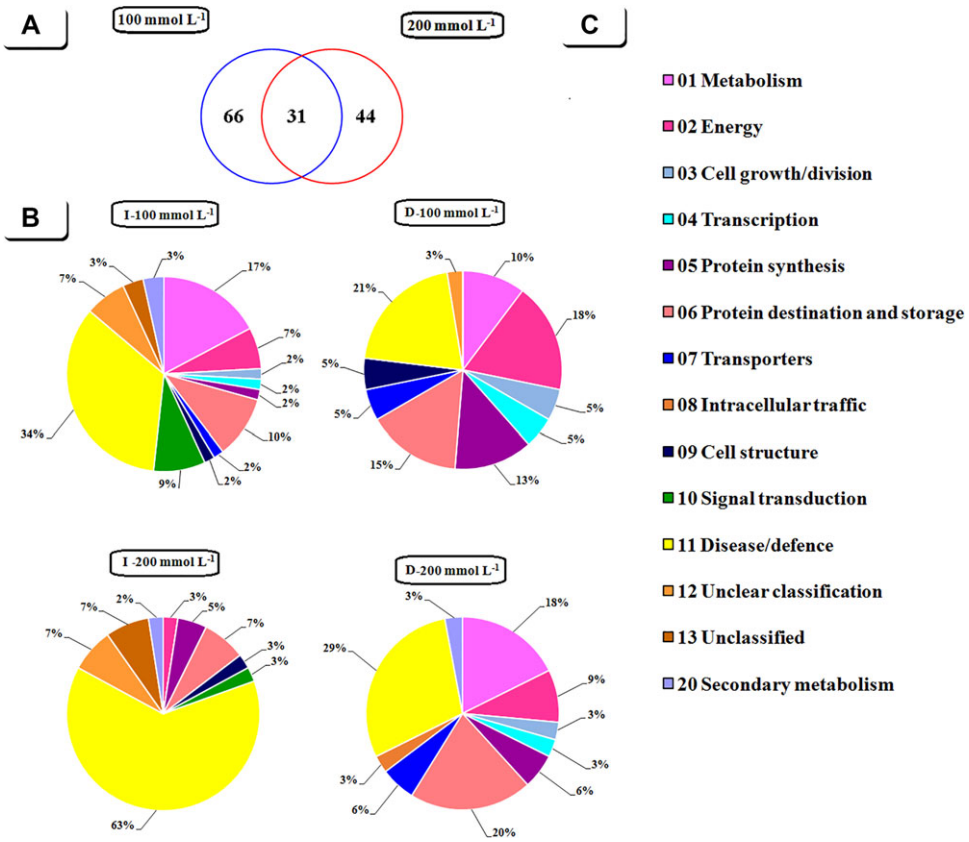

Figure 3. Proteome profile comparison of soybean embryonic axes germinating under salinity (100 and 200 mmol/L NaCl). (A) Venn diagram displays the number of differentially accumulated proteins under each level of salinity. (B) Functional classification of proteins shown in (A). (C) Functional classes according to GO and Bevan et al. [24]. The differentially accumulated proteins (>2-fold) were identified using *t*-test ($P<0.05$). I, increased; D, decreased.

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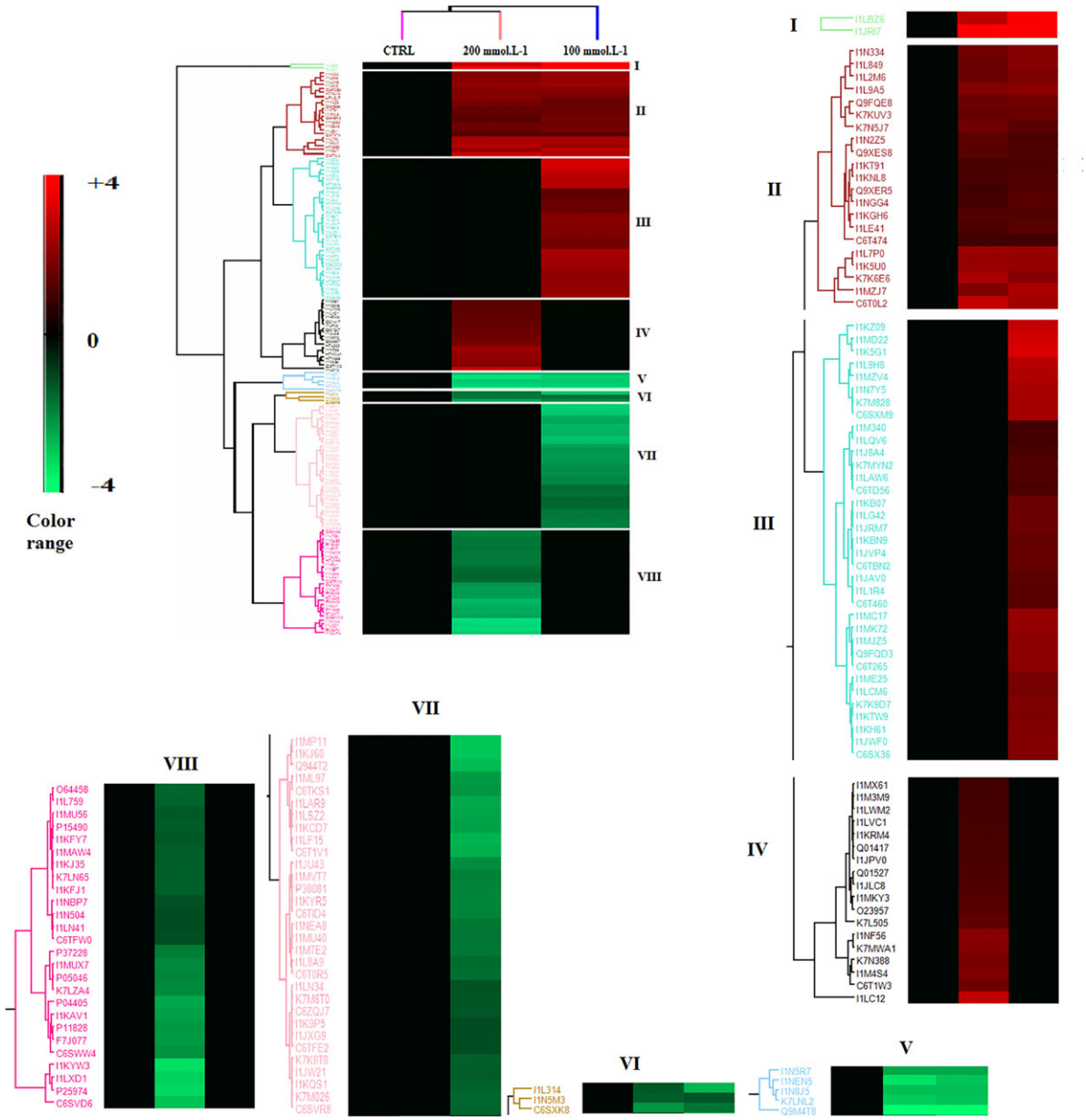

Figure 4. Comparative protein accumulation patterns. Accumulation patterns of the 141 significantly changed proteins. After hierarchical clustering analysis, the proteins were divided into eight groups, as shown by different colors. Two major branches of dendrogram consisting either of mostly increased (clusters: I, II, III, IV) or decreased (clusters: V, VI, VII, VIII) proteins grouped in clusters according to the relative abundance. The color scale ranges from saturated red (log2(R)≤ +4) for increased proteins to saturated green (log2(R)≥–4) for decreased proteins.

provided disease/defense (21%), protein destination/storage (15%), metabolism (10%) and energy (18%) as the most represented categories (Fig. 3B).

Among the 75 significantly altered proteins in response to 200 mmol/L NaCl treatment, 41 of them were increased and 34 were decreased (Fig. 3A). Likewise, these proteins were assigned into 14 functional categories. The most abundant categories are disease/defense (63%) and protein destination/storage (7%) for the increased proteins and disease/defense (29%), protein destination/storage (20%) and metabolism (18%) for the decreased proteins (Fig. 3B).

3.3 Cluster hierarchical analysis of differential proteins

All identified proteins were classified into eight accumulation patterns (Fig. 4). Clusters I and II grouped proteins with

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an increased abundance at both levels of salinity (100 and 200 mmol/L); cluster III included proteins with an increased abundance at lower salinity level. Cluster IV consisted of proteins showing an increased abundance at higher salinity level. Clusters V and VI included proteins with a decreased abundance at both levels of salt stress. Cluster VII grouped proteins with a decreased abandance at low salinity level, and finally, cluster VIII which include proteins with a decreased abundance at high salinity level.

4 Discussion

Seed germination is a critical phase in the life history of plants; therefore, salt tolerance during the germination phase may be especially critical for successful establishment and productivity of soybean in saline soils [6]. High salinity negatively affect germination, growth, nodulation, seed quality and quantity, thus reducing the yield of soybean production [1, 2, 6, 9, 27–30].Therefore, it is of great interest to understand the genetic basis of salinity tolerance traits in soybean, mainly at seed germination stage [6].

It is evident that the seedling stage of soybean is much more sensitive to salinity than the germination [1, 9, 28]. For instance, soybean seedling growth is suppressed when the concentration of Na^+ in tissues exceeded 6% of the fresh weight, whereas germination remains possible even when the concentration of Na^+ exceeded 9% of fresh weight [29]. This finding was supported by our results (Fig. 1) and those of several studies [2, 28] highlighting the importance of embryonic axis as an appropriate model system for investigating salt tolerance of soybean. Up to now, very few studies have devoted to investigating the proteomic alteration of soybean seeds/seedlings under salinity [16–18, 31–33]. In all of these studies the identification of SRPs was performed using gel-based proteomic techniques. However, shotgun proteomic methods allow for higher resolution analysis compared with gel-based methods, facilitating deep proteome analysis and the identification of lower-abundance proteins [34]. Thus, given the essential role for embryonic proteins in regulation of seed germination, in this study we attempt to analyze for the first time the proteome alteration of soybean embryonic axes during early-germination under moderate (100 mmol/L NaCl) and high (200 mmol/L NaCl) salinity stress, by using a shotgun proteomic approach based on nanoHPLC-MS/MS.

4.1 Proteome profiles of soybean embryonic axes similarly influenced by both salt treatments

In proteomic studies, it has been generally presumed that proteins associated with salt tolerance (SRPs) are those whose abundance increases in salt-tolerant model systems (plants, organs, etc.) or decreases in salt-sensitive ones [14–16, 32, 33]. However, plants can face unpredictable and varying salt stress throughout their life cycle; therefore, SRPs involved in plant adaptation to different soil salinity levels may represent interesting targets for engineering plants with improved salt tolerance. Thus, considering the salt tolerance of germinating soybean seeds (Fig. 1), it is presumed that proteins showing increased abundance under both stress conditions are potential SRPs.

Of all the identified proteins, 31 showed similar differential abundance under both salt treatments (Supporting Information Table S3). Of these, 23 proteins showed increased abundance in response to salinity and most of which are stress-related proteins (Fig. 3). Eight proteins among them are late embryogenesis abundant (LEA) proteins. Comparable results were obtained in previous works [15, 16, 32, 33]. LEA proteins accumulate in immature embryos in response to abscisic acid (ABA) treatment [35] as well as to several abiotic stresses such as cold and salinity [36]. The accumulation of LEA proteins has been associated with dehydration tolerance in soybean, which may be induced by salinity, suggesting that osmotic stress and ABA-mediated regulation of LEA proteins play an important role in soybean salt tolerance [18, 36, 37]. In support to this latter assumption, one of the suggestive SRPs was found to be a putative aquaporin TIP-type alpha which is probably a tonoplast-localized aquaporin. Aquaporins (AQPs) are water selective channels, which belong to the major intrinsic protein family that has representatives in all kingdoms and presents multiple isoforms. The soybean genome has 66 major intrinsic protein genes [38]. AQPs are known to transport water, CO_2, H_2O_2 and a wide range of small uncharged solutes. Also, AQPs appear to modulate abiotic stress-induced signaling. Consequently, they contribute to several plant growth and developmental processes, such as seed germination or emergence of lateral roots [39]. Thus, AQPs seem to be good candidates for the engineering of abiotic stress tolerance in soybean as well as in other plants [40]. This assumption was supported by recent findings showing that the constitutive overexpression of GmPIP1;6 is able to confer salt tolerance in soybean [10].

ROS which are responsible for peroxidative cell damage are continuously generated within living systems especially during germination under stress conditions [41]. ROS detoxification is an important part of salt-stress tolerance, thus, it is not surprising that the abundance of many proteins involved in ROS detoxification, particularly Cu/Zn-SOD, glutathione S-transferase and aldose reductase-like protein, was substantially increased in response to salinity. ROS detoxification pathway may also involve proteins of the superfamily of aldehyde dehydrogenases (ALDHs) [42]. In line with this, a cytosolic ALDH C4 (RF2C) was showed an increased abundance (>9-fold). ALDHs are members of $NAD(P)^+$-dependent protein superfamily that catalyze the oxidation of a wide range of endogenous and exogenous highly reactive aliphatic and aromatic aldehyde molecules. The soybean genome contains 18 unique ALDH sequences encoding members of five ALDH families [43]. The overexpression of ALDH genes is considered to be an option for improving tolerance against a wide range of stress [44]. For example, salt-stressed transgenic

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Arabidopsis plants overexpressing grapevine ALDH2B8 showed sustained growth, and increased oxidative stress tolerance, which was correlated with decreased accumulation of ROS and malondialdehyde derived from membrane lipid peroxidation [45]. In addition, the aldo-keto reductase (AKR) superfamily include a large number of primarily monomeric proteins, which reduce a broad spectrum of substrates ranging from simple sugars to potentially toxic aldehydes [46]. Based on this, the detoxification of aldehydes in soybean embryonic axes may also be critical for germination under salinity stress, suggesting that ALDHs and AKRs represent promising classes of proteins/genes to improve salt tolerance in soybean.

Interestingly, one of the signaling proteins whose abundance increases in response to salinity was 28 kDa heat- and acid-stable phosphoprotein-like protein. Curiously, this protein which is believed to be involved in regulation of cell growth and proliferation (Uniprot) had never been reported before to be induced in response to any abiotic stress. However, it has been previously identified in a study investigating the possible causal genes for salt tolerance in wild soybean [47]. Thus, we suggest that this protein may be involved in signal transduction during salt stress response.

4.2 Proteome profiles of soybean embryonic axes differentially influenced by both salt treatments

According to the biphasic model of growth reaction to salt stress, the ionic stress impact on plant growth occurs much later, and with a minor effect than the osmotic stress, especially at low to moderate salinity levels [5]. Consequently, the major difference in the observed protein expression profiles could generally be attributed to either the degree or the nature of stress encountered by soybean embryonic axes and/or to the adaptation strategy adopted by these organs at each salinity level. In order to well understand the salt tolerance strategies adopted by embryos to cope with different levels of salinity, we also compared the proteome alterations specific to each levels of salinity. Out of the 141 proteins, 66 and 44 proteins showed responses specific to moderate and high salinity stress, respectively (Supporting Information Tables S4 and S5).

4.2.1 Proteins specific to moderate salinity

In addition to the proteins discussed above (Section 4.1), about 30 other proteins showed increased abundance only under moderate salinity. Interestingly, most of these proteins (13) are involved in primary metabolism and energy production namely amino acids biosynthesis, lipid degradation/peroxidation and ATP synthesis (Supporting Information Table S4). Previously it has been reported that the abundance of several enzymes involved in amino acid biosynthesis increases when storage protein mobilization is inhibited [48]. Consistent with this, at moderate salinity, where no apparent change in abundance of storage proteins was observed, several proteins involved in amino acids biosynthesis showed an increased abundance. In addition, abundance of three enzymes involved in lipids mobilization was increased. One of these enzymes is a lipoxygenase. Lipoxygenases are lipid-oxidizing enzymes involved in various physiological processes, such as growth, development, and response to biotic and abiotic stresses [49]. They catalyze the oxidation of polyunsaturated fatty acids and could also function as ROS scavenger when seed reserves are being mobilized most rapidly during germination [48].

There is a general acceptance that under salt stress, many stress-responsive signals (e.g., Ca^{2+}, ROS and ethylene) are perceived by their receptors/sensors and transduced through kinase-mediated protein phosphorylation and/or G-proteins to regulate the corresponding signaling and metabolic pathways [50]. In agreement, in this study a G-protein, a phosphatase methylesterase 1 and two calcium-binding EF-hand proteins (CaEF) were identified (Supporting Information Table S4). Almost similar result was obtained before when the roots of a salt tolerant soybean cultivar were exposed to moderate salinity (100 mmol/L) [50]. When transferred into tobacco, the CaEF gene considerably increases its salt tolerance [51]. Also, the overexpression of GmCaM4 in soybean results in an enhanced salt tolerance [11]. Comparable results are reported in recent study [52]. Furthermore, Yin et al. [16] reported that calcium treatment could increases the salt stress tolerance of germinating soybean seeds via enriching signal transduction.

4.2.2 Proteins specific to high salinity

In addition to the aforementioned proteins (Section 4.1), several proteins involved in protein synthesis/destination/storage, primary metabolism and energy showed decreased abundance (Supporting Information Table S5) indicating the importance of protein turnover and protection in response of *Glycine max* to high salinity [53]. Stress acclimation reveals an enhanced demand on protein metabolism, including both protein biosynthesis and degradation [54]. The selective degradation of regulatory proteins by the ubiquitin-proteasome pathway controls key aspects of plant growth, development and stress defense [55]. Ubiquitin-proteasome system regulates plant development and cell division by regulating different cellular signals [56]. Also, accumulation of these proteins may be involved in degradation of proteins used during cell division and cell growth. Therefore, the proteasome mediated protein degradation could be an essential pathway for seed germination and development under normal and stress conditions. Nevertheless, overexpression of the ubiquitin-conjugating enzyme gene GmUBC2 in Arabidopsis confers improved salt tolerance by regulating the expression of a set of important stress-responsive genes [57]. Consistent with this finding, our results indicates the contrast

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regulation of Ubiquitin-conjugating enzymes at both level of salinity. Otherwise, given the remarkable decrease in abundance of the 20S proteasome induced by both levels of salinity (>35-fold and >45-fold at 100 and 200 mmol/L, respectively), and based on recent findings [58], we thought that 20S proteasome might participate in the salt stress tolerance of soybean at the germination stage as a negative factor.

Among the proteins decreased with increasing salinity, six proteins are identified as storage proteins (Supporting Information Table S5). Similar results was reported recently [15], suggesting that storage proteins might not only function as seed storage reserves but also have additional roles in plant defense. Almost 90% of the proteins in soybean are storage proteins, which mostly consist of β-conglycinin (7S) and glycinin (11S) [9]. Recent proteomics studies have revealed the presence of several isoforms of glycinin and conclycinin in the embryonic axis of soybean [59]. The breakdown of such proteins might help to nourish the germinating soybean embryos and young seedlings [18, 48]. The mobilization of seed storage proteins was partially or completely inhibited under stress conditions [60]. This process might be mediated by specific stored proteases [61]. Although protease inhibitors are present in soybean seeds to protect them against predators [62], however, during germination or when seeds are subjected to salt stress, they are degraded to a lower level by stored proteases [17]. The two major types of inhibitors in soybean are Bowman–Birk inhibitors (BBI) and the Kunitz trypsin inhibitor [63]. In the present study, several protease inhibitors, such as Kunitz trypsin inhibitor and lectin were found to be decreased only under high salinity. These findings are in good agreement with the observed decrease in abundance of storage protein under high salinity and also with the low level of protein content in the germinating embryonic axes exposed to high salinity (Fig. 1).

4.3 Concluding remarks

In order to identify potential SRPs in soybean, a comparative label-free shotgun proteomic analysis of the proteome profiles of soybean embryonic axes during early germination under salt stress conditions (100 and 200 mmol/L NaCl) was performed. The relative abundance of 141 proteins was found to be significantly influenced by salinity. The importance of most of these proteins in salt tolerance of soybean was discussed, and some of them were suggested to be potential SRPs. Furthermore, findings indicate that, at moderate salinity, the embryo seems to successfully protect its proteins against the action of proteases, ROS and aldehydes generated during germination under stress. In contrast, at high salinity, the soybean embryo does not appear to fully protect its proteins, especially against the action of ROS and aldehydes the abundance of which substantially increases under high salinity. These findings should be relevant to the identification of reliable markers useful for improving salt tolerance in soybean and related crops.

The authors declare no conflict of interest.

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