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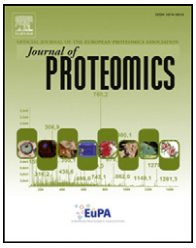

Comparative analysis of metabolic proteome variation in ascorbate-primed and unprimed wheat seeds during germination under salt stress

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ABSTRACT

Seed priming with ascorbic acid improves salt tolerance in durum wheat. For understanding the potential mechanisms underlying this priming effect a gel-free shotgun proteomic analysis was performed comparing unprimed to ascorbate-primed wheat seed during germination under saline and non-saline conditions. Since seed germination is the result of interplay or cross-talk between embryo and embryo-surrounding tissues, we studied the variation of metabolic proteome in both tissues separately. 167 of 697 identified and 69 of 471 identified proteins increase or decrease in abundance significantly in response to priming and/or salinity compared to untreated, unstressed control in embryo and embryo-surrounding tissues, respectively. In untreated wheat embryo salt stress was accompanied by change in 129 proteins, most of which are belonging to metabolism, energy, disease/defense, protein destination and storage categories. Ascorbate pretreatment prevents and counteracts the effects of salinity upon most of these proteins and changes specifically the abundance of 35 others proteins, most of which are involved in metabolism, protein destination and storage categories. Hierarchical clustering analysis revealed three and two major clusters of protein expression in embryo and embryo-surrounding tissues, respectively. This study opens promising new avenues to understand priming-induced salt tolerance in plants.

Biological significance
To clearly understand how ascorbate-priming enhance the salt tolerance of durum wheat during germination, we performed for the first time a comparative shotgun proteomic analysis between unprimed and ascorbate-primed wheat seeds during germination under saline and non-saline conditions. Furthermore, since seed germination is the result of

Abbreviations: UP-H_2O, UnPrimed control; UP-NaCl, UnPrimed salt stressed; AP-H_2O, Ascorbate-Primed non stressed; AP-NaCl, Ascorbate-Primed salt stressed.

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interplay or cross-talk between embryo and embryo-surrounding tissues we analyzed the variation of metabolic proteome in both tissues separately. 1168 proteins exhibiting greater molecular weight diversity (ranging from 5 to 258 kDa) were identified. Among them, 167 and 69 proteins were increased or decreased in abundance significantly by priming and/or salinity as compared to control, in embryo and embryo-surrounding tissues respectively. Ascorbate pretreatment alleviates the effects of salinity upon most of these proteins, particularly those involved in metabolism, energy, disease/defense, protein destination and storage functions. Hierarchical clustering analysis revealed three and two major clusters of protein accumulation in embryo and embryo-surrounding tissues, respectively. These results may provide new avenues for understanding and advancing priming-induced salt tolerance in crop plants.

1. Introduction

Salinity is a major abiotic stress that adversely influences plant growth and crop productivity. Seed germination and early seedling growth are the stages that most likely affect plant establishment and subsequent crop yield. Unfortunately, in many cereal crops such as wheat, seed germination and early seedling growth are the stages most sensitive to salt stress [1,2]. Wheat production is obviously limited by the availability of water resources and soil salinity, which are the primary cause of crop loss worldwide, reducing the average yields for most crop plants by more than 50% [3]. Therefore, understanding the molecular basis of abiotic stress responses is necessary for genetic improvement of stress tolerance in wheat crop [4,5]. A key to achieving this is the elucidation of the molecular mechanisms underlying seed germination and vigor in this species, especially under stress conditions.

It is well-documented that the wheat germination process is highly disturbed by salt and osmotic stress [6–8]. To date, several transcriptomic and proteomic studies have sought to identify both candidate gene and gene products in a range of crop and model plants under normal and stress conditions [9–14]. Cereal embryos have been largely used as a model system to study stress tolerance [15]. Seed protein content is of high value for defining the end-use quality of wheat grain [16], and it also determine the plant's performance during germination and affect the final crop productivity. Unlike storage proteins, albumins and globulins are proteins that control metabolic and cellular processes; therefore, they are of high interest when investigating germination under salinity stress [17].

Seed priming is a pre-sowing treatment that improves seed performance and alleviates the negative effects of environmental stresses on seed germination and seedling establishment [1]. To gain insights into the gap of knowledge on the molecular features of seed invigoration in wheat, we have previously used gel-free proteomic approach to investigate the metabolic proteome changes in quiescent dry seeds triggered by seed-priming treatments [18]. It has been reported that ascorbate-priming was accompanied by significant changes of 83 proteins, most of which are involved in protein metabolism, antioxidant protection, repair processes and in methionine-related metabolism.

In orthodox seeds (such as wheat seeds), neither ascorbate (AsA) nor ascorbate peroxidase (APX) activity exist at the quiescent stage, however, they appear within a few hours after the imbibition [9,19]. The rapid recovery of both AsA biosynthesis and APX activity is crucial for seed germination and early development of seedlings [20]. It is worth noting that the high dose of AsA suppress germination as reported for wheat [21] and rice seeds [22,23]. Despite its role as a substrate in gibberellins (GAs) biosynthesis, it has been reported that expression of GAs biosynthesis genes was suppressed by the low levels of AsA in abscisic acid (ABA)-treated rice seeds [22,23].

The ability of a seed to germinate has been described as a balance between the growth potential of the embryo under promoting conditions and the restrictions imposed by the embryo-surrounding tissues [14]. To date, the proteome analyses of endosperm, aleurone and embryo has been extensively studied in many monocot plants [9,15,24]. Nevertheless, limited information is available about salt-responsive proteins/genes in wheat seed during germination or post-priming germination, and the study of protein abundance and localization changes in response to salinity may therefore help identify the associated genes and provide a detailed network of the stress adaptation mechanisms in this important staple food crop [2,25]. Thus, to extend our understanding of the underlying mechanisms of seed vigor and seed invigoration, we carried out a comparative analysis between the metabolic proteome of unprimed and AsA-primed seeds during germination under control and salt stress conditions.

2. Materials and methods

2.1. Seed treatment and germination

Seed priming with AsA solution (0.5 mmol L^{-1}) was performed essentially as previously described [18]. Thirty dried seeds of durum wheat (*Triticum durum* Desf. var. Waha), high yielding genotype moderately resistant to salinity at growth stage and widely cultivated in Algeria, both treated and untreated, were placed in each Petri-dish containing two layers of Whatman No. 1 filter paper initially moistened with 10 mL of saline solution (NaCl 250 mmol L^{-1}) or distilled water (control). Seeds were germinated in darkness in a temperature-controlled chamber held at 24 ± 0.5 °C until germination *sensu stricto* (about 42 hours).

2.2. Extraction and quantification of metabolic seed proteins

After 42 hours from the incubation, the embryo (embryonic axis and scutellum) was separated from the embryo-surrounding

tissues (embryo-ST, endosperm, aleurone layers and pericarp) and both of them were analyzed independently. Metabolic seed proteins were extracted using the method developed by Hurkman et al. [26] with some modifications. Briefly, the embryo and the embryo-ST from the wheat seeds were separately frozen in liquid N_2 and ground to a fine powder using a ceramic mortar and pestle. Seventy-five mg of the resulting powder was used to extract proteins as previously performed [18]. Metabolic proteins were quantified by Bradford assay using BSA standard [27]. Three experimental replicates were performed for each seed type.

2.3. In solution trypsin digestion and off-line desalting

For each sample, protein aliquots, were reduced, alkylated, and digested with trypsin. Reduction of disulphide bonds was performed with DTT (200 mmol L^{-1}) in incubation at 37 °C for 1 h, under slight agitation. Carbamidomethylation of thiol groups was performed by addition of IAA (200 mmol L^{-1}) and incubation for 1 h in the dark at RT. To consume any leftover alkylating agent and to avoid trypsin alkylation, DTT (200 mmol L^{-1}) was added and samples were incubated at 37 °C for 1 h, under slight agitation. The samples were then diluted with ammonium bicarbonate (50 mmol L^{-1}) to obtain a 1 mol L^{-1} final urea concentration. Sequencing grade-modified tryspin was added (1:20, w/w, enzyme to protein ratio) and the samples were incubated overnight at 37 °C. Enzymatic digestion was quenched with TFA. Digested samples were desalted using SPE C18 cartridges conditioned with ACN and rinsed with 0.1% TFA. Peptides were eluted from the SPE column with 500 μL ACN/ddH_2O (50/50, v/v) containing 0.05% TFA and were dried in a Speed-Vac SC 250 Express (Thermo S 164 avant, Holbrook, NY, USA). Each sample was re-constituted with 250 μL of 0.1% HCOOH aqueous solution and stored at -80 °C until LC-MS/MS analysis.

2.4. NanoHPLC-MS analysis

LC-MS/MS analysis was performed on Orbitrap Elite hybrid ion trap-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a nanoelectrospray ion source. Peptide mixtures were separated by RP chromatography using the Dionex Ultimate 3000 (Dionex Corporation Sunnyvale, CA, USA). The LC system was connected to an in-house manufactured 25 cm fused-silica nano-column, 75 μm i.d., packed with Acclaim-C18 2.2 μm silica microparticles, with outlet frit prepared using Kasil. Peptide mixtures were enriched on a 300 μm i.d. × 5 mm Acclaim PepMap 100 C18 (5 μm particle size, 100 Å pore size) μ-precolumn (Dionex), employing a premixed mobile phase ddH_2O/ACN 98/2 (v/v) (from loading pump) containing 0.1% (v/v) HCOOH at a flow-rate of 10 μL min^{-1}. LC gradient was optimized to detect the largest set of peptides, using ddH_2O/HCOOH (99.9/0.1, v/v) as phase A and ACN/HCOOH (99.9/0.1, v/v) as phase B. After an isocratic step at 5% B for 5 min, B was linearly increased to 15% within 2 min and then to 35% within 120 min; afterwards, phase B was maintained at 35% within 10 min, and increased to 80% within the following 10 min. Then, phase B was maintained at 80% for 10 min to rinse the column. Finally, B was lowered to 5% over 1 min and the column re-equilibrated for 19 min (177 min total run time). MS spectra were collected over an m/z range of 380–2000 Da using a resolution setting of 60000 (FWHM, *m/z* 400), operating in the data dependent mode to automatically switch between Orbitrap-MS and LTQ-MS/MS acquisition. MS/MS spectra were collected for the twenty most abundant ions in each MS scan. Rejection of +1, and unassigned charge states was enabled. All MS/MS spectra were collected using a normalized collision energy of 30%, and an isolation window of 2 *m/z*. Ion trap and Orbitrap maximum ion injection times were set to 100 and 200 ms, respectively. Automatic gain control (AGC) was used to prevent overfilling of the ion traps and was set to 1×10^6 for full FTMS scan, and 1×10^4 ions in MS^n mode for the linear ion trap. To minimize redundant spectral acquisitions, dynamic exclusion was enabled with a repeat count of 1 and a repeat duration of 30 s with exclusion duration of 70 s. In order to increase the number of identified proteins, we performed three technical replicates (LC-MS/MS runs) for each of the three experimental replicates.

2.5. Database searching and protein identification

Raw MS/MS data files from Xcalibur software (version 2.2 SP1.48, Thermo Fisher Scientific) were submitted to Proteome Discoverer software version 1.3 (Thermo Scientific) with the Mascot search engine for peptide/protein identification. The searches were performed against Swiss-Prot database (Release 2012_05, Number of sequences: 538585). Thermo Finnigan LCQ/DECA RAW file data import filter was used. The search was limited to proteins from species of the *Viridiplantae* (green plants) taxonomy entries and performed using the built-in decoy search option of Mascot. Enzymatic digestion with trypsin was selected, with maximum 2 missed cleavages, peptide charges +2 and +3, a precursor mass tolerance of 10 ppm and 0.8 Da fragment mass tolerance; acetylation (N-term), oxidation (M) and deamidation (N, Q) were used as dynamic modifications; carbamidomethylation (C) was used as static modification.

2.6. Scaffold analysis

Scaffold software (version Scaffold 3.1.2, Proteome Software Inc., Portland, OR.) [28] was used to validate MS/MS based peptide and protein identifications, and for label-free relative quantitation based on normalized spectral counting. The additional X! Tandem search engine (The GPM, Cyclone version 2010.12.01.1) was also used, keeping the same parameters previously used for Mascot. According to the Peptide and Protein Prophet algorithms [29,30] implemented into Scaffold, the peptide and protein identifications were accepted if they could be established at greater than 95% and 99% probability, respectively, and contained at least 2 unique identified peptides, resulting in a false discovery rate (FDR) for peptides and proteins of all samples ≤ 0.6% (including ≤ 5 decoys). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. ANOVA test was used to identify statistically significant differences between different treatments and control. Proteins, which had at least a two-fold difference for the mean ratio, as well as a P-value ≤ 0.05, a relative standard deviation (RSD) of experimental replicates ≤ 40%, and appear in more than one

experimental replicate, were considered present in the two samples in significant different quantities.

2.7. Functional classification

Gene Ontology (GO) data about the biological processes of identified proteins were obtained by means of Scaffold's built in option and according to Bevan et al. [31].

3. Results

3.1. Wheat embryo metabolic proteome

Of a total of 697 identified proteins, 167 were increased or decreased in abundance significantly in response to priming and/or salinity compared to untreated, unstressed control (see Fig. 1A). Among these proteins, 129 proteins (82 specifically affected) were found to be differentially accumulated in unprimed salt stressed wheat embryo (UP-NaCl vs. UP-H_2O), 45 among them were increased, and 84 were decreased in abundance (Fig. 1C and Table 1, Supplementary material Table S1). On the other hand, 51 proteins were differentially altered in AsA-primed non-stressed wheat embryo (AP-H_2O vs. UP-H_2O), among which 12 were increased and 39 were decreased in abundance (Fig. 1C and Table 1, Supplementary material Table S1). However, 77 proteins were found to be differentially changed in AsA-primed salt stressed wheat embryo (AP-NaCl vs. UP-H_2O), 34 of these were increased and 43 were decreased in abundance (Fig. 1C and Table 1, Supplementary material Table S1). Functional classification grouped the 167 identified proteins in 13 functional categories

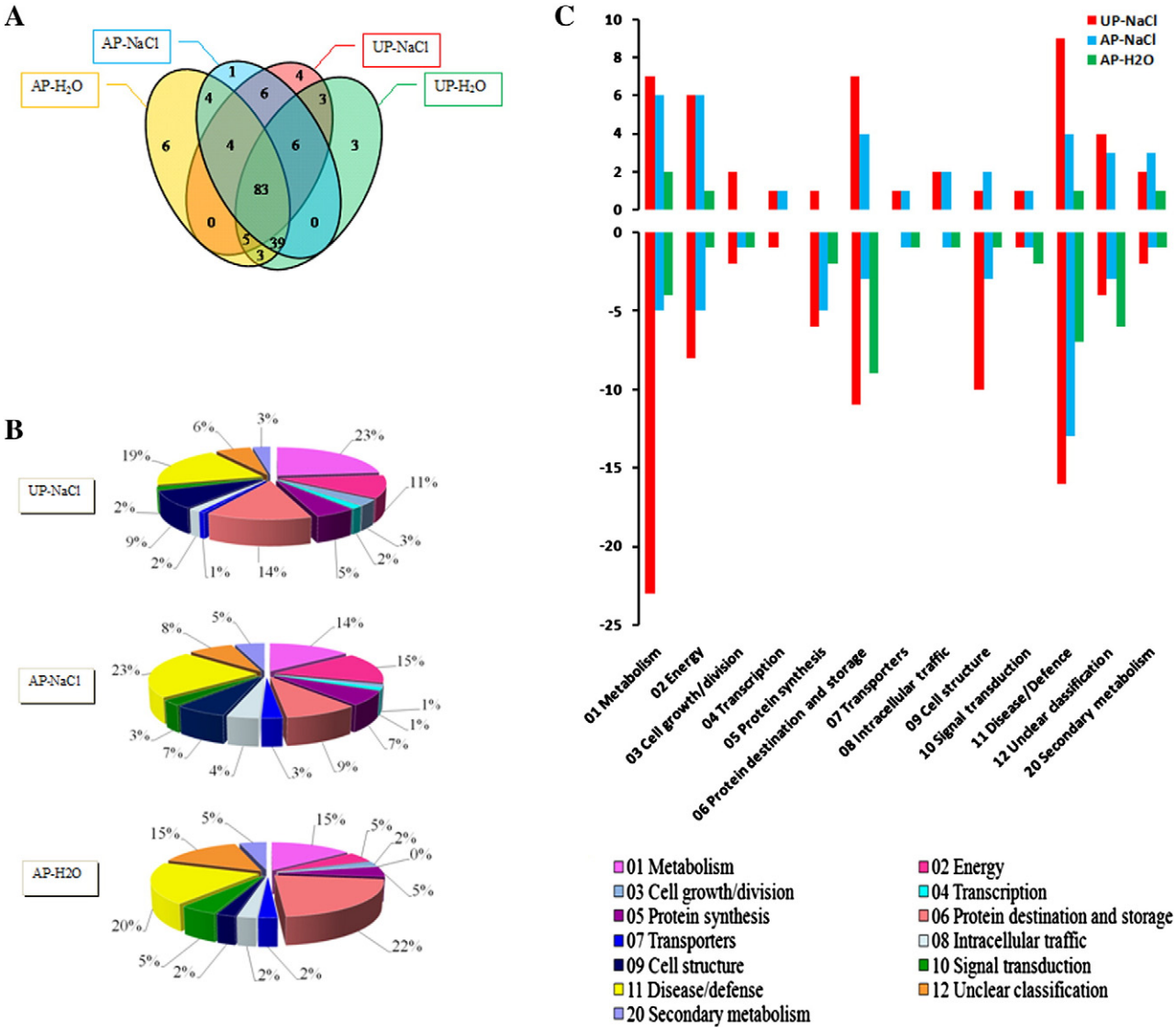

Fig. 1 – Metabolic proteome signature in Embryo of germinating primed and unprimed wheat seeds under salt stress as compared to control (UP-H_2O). A. Venn diagram based on the differentially accumulated proteins in primed and unprimed stressed seeds compared with controls; B. Functional classification of proteins according to Bevan et al. [31]; C. Functional distribution of proteins.

Table 1 – Embryo proteome of unprimed and ascorbate-primed wheat seeds during germination under saline and non-saline conditions.

No	Identified Proteins	Entry name	MW (kDa)	P-Value	Functional Category	Functional Description	R1	R2	R3
1	Formate dehydrogenase, mitochondrial	FDH_SOLTU	42	<0.0001	20 Secondary metabolism	20 Secondary metabolism	I	N	N
2	Cationic peroxidase SPC4	PER1_SORBI	38	<0.0001	11 Disease/defense	11.06 Detoxification	N	N	I
3	Actin-2	ACT2_ORYSI	42	<0.0001	09 Cell structure	09.04 Cytoskeleton	D	D	D
5	Pyruvate decarboxylase isozyme 2	PDC2_ORYSI	65	<0.0001	12 Unclear classification	12 Unclear classification	I	N	I
6	Avenin-like b1	AVLB1_WHEAT	33	<0.0001	06 Protein destination/storage	06.20 Storage proteins	N	D	D
7	Serine carboxypeptidase 2	CBP2_WHEAT	50	<0.0001	06 Protein destination/storage	06.13 Proteolysis	I	D	D
8	Peroxidase	PER1_WHEAT	32	<0.0001	11 Disease/defense	11.06 Detoxification	D	N	D
9	Oxalate oxidase 2	OXO2_HORVU	23	<0.0001	11 Disease/defense	11.05 Stress responses	D	N	N
10	Basic endochitinase A	CHIA_SECCE	34	<0.0001	11 Disease/defense	11.02 Defense-related	I	N	N
11	Chaperone protein ClpB1	CLPB1_ORYSJ	101	<0.0001	06 Protein destination/storage	06.01 Folding and stability	I	N	I
12	DEAD-box ATP-dependent RNA helicase 21	RH21_ORYSJ	85	<0.0001	11 Disease/defense	11.05 Stress responses	I	D	N
15	Fructose-1,6-bisphosphatase, cytosolic	F16P2_ORYCO	38	<0.0001	02 Energy	02.02 Gluconeogenesis	I	N	I
17	Probable xyloglucan endotransglucosylase/hydrolase	XTH_WHEAT	33	<0.0001	09 Cell structure	09.01 Cell wall	D	I	I
20	S-adenosylmethionine synthase 3	METK3_HORVU	43	<0.0001	01 Metabolism	01.01 Amino acid	D	N	N
21	Cysteine proteinase EP-B 1	CYSP1_HORVU	40	<0.0001	06 Protein destination/storage	06.13 Proteolysis	N	D	D
22	Serine/threonine-protein phosphatase 5	PPP5_ARATH	60	<0.0001	10 Signal transduction	10.0407 Phosphatases	D	D	D
26	RuBisCO large subunit-binding protein subunit alpha, chloroplastic (Fragment)	RUBA_WHEAT	58	<0.0001	06 Protein destination/storage	06.10 Complex assembly	D	N	N
27	Calreticulin	CALR_ORYSJ	48	<0.0001	06 Protein destination/storage	06.01 Folding and stability	D	N	N
28	Acetolactate synthase 1, chloroplastic	ILVB1_ORYSJ	69	<0.0001	01 Metabolism	01.01 Amino acid	D	N	N
29	Protein SGT1 homolog	SGT1_ORYSJ	41	<0.0001	11 Disease/defense	11.05 Stress responses	D	N	D
31	Alpha-amylase inhibitor 0.28	IAA2_WHEAT	17	<0.0001	12 Unclear classification	12 Unclear classification	N	D	N
32	Wheatwin-2	WHW2_WHEAT	16	<0.0001	11 Disease/defense	11.02 Defense-related	N	D	D
34	S-adenosylmethionine synthase 1	METK1_TRIMO	43	<0.0001	01 Metabolism	01.01 Amino acid	D	N	N
36	Guanine nucleotide-binding protein subunit beta-like protein A	GBLPA_ORYSJ	36	<0.0001	11 Disease/defense	11.02 Defense-related	D	N	N
37	Alcohol dehydrogenase 1	ADH1_PENAM	41	<0.0001	11 Disease/defense	11.03 Cell death	I	N	I
39	Dehydrin COR410	CO410_WHEAT	28	<0.0001	11 Disease/defense	11.05 Stress responses	D	N	D
40	Aldehyde dehydrogenase family 2 member B4, mitochondrial	AL2B4_ARATH	59	<0.0001	11 Disease/defense	11.05 Stress responses	D	D	D
41	L-ascorbate peroxidase 1, cytosolic	APX1_ORYSI	27	<0.0001	11 Disease/defense	11.06 Detoxification	D	N	N
43	Probable phospholipid hydroperoxide glutathione peroxidase	GPX4_CITSI	19	<0.0001	11 Disease/defense	11.06 Detoxification	N	I	N
45	Glutamate decarboxylase 2	DCE2_ARATH	56	<0.0001	01 Metabolism	01.01 Amino acid	I	N	N
46	T-complex protein 1 subunit alpha	TCPA_ARATH	59	<0.0001	06 Protein destination/storage	06.01 Folding and stability	N	D	N
47	Wheatwin-1	WHW1_WHEAT	16	<0.0001	11 Disease/defense	11.02 Defense-related	N	D	N
48	Fructan 6-exohydrolase	6FEH_WHEAT	66	<0.0001	01 Metabolism	01.05 Sugars/ Polysaccharides	I	N	N
49	Small ubiquitin-related modifier 1	SUMO1_ARATH	11	<0.0001	06 Protein destination/storage	06.07 Modification	D	N	N
51	Dynamin-related protein 1B	DRP1B_ARATH	68	<0.0001	01 Metabolism	01.03 Nucleotides	N	I	I
53	Avenin-like a2	AVLA2_WHEAT	20	0.0001	06 Protein destination/storage	06.20 Storage proteins	N	D	N
54	Mitogen-activated protein kinase 3	MPK3_ORYSJ	42	0.00011	10 Signal transduction	10.0404 Kinases	I	N	I
56	Oxalate oxidase GF-3.8	GER3_WHEAT	24	0.00012	11 Disease/defense	11.05 Stress responses	D	D	N
60	Linoleate 9S-lipoxygenase 1	LOX1_HORVU	96	0.00013	01 Metabolism	01.06 Lipid and sterol	I	N	N
61	Serine–tRNA ligase	SYS_ARATH	52	0.00013	05 Protein synthesis	05.10 tRNA synthases	N	D	N
62	Phenylalanine ammonia-lyase	PAL1_ORYSJ	75	0.00013	20 Secondary metabolism	20.1 Phenylpropanoids/ phenolics	D	N	N
63	Serpin-Z1A	SPZ1A_WHEAT	43	0.00013			I	N	N

Table 1 (*continued*)

No	Identified Proteins	Entry name	MW (kDa)	P-Value	Functional Category	Functional Description	R1	R2	R3
					06 Protein destination/storage				
67	Tryptophan synthase beta chain 1 (Fragment)	TRPB1_MAIZE	43	0.00015	01 Metabolism	01.01 Amino acid	D	N	N
68	Probable lactoylglutathione lyase, chloroplast	LGUC_ARATH	39	0.0002	20 Secondary metabolism	20 Secondary metabolism	I	N	I
69	Alcohol dehydrogenase 1	ADH1_HORVU	41	0.00021	11 Disease/defense	11.03 Cell death	I	N	N
70	Glutamine synthetase leaf isozyme, chloroplastic	GLNA2_HORVU	47	0.00022	01 Metabolism	01.01 Amino acid	D	N	D
74	Alpha-1,4-glucan-protein synthase [UDP-forming]	UPTG_MAIZE	41	0.00029	09 Cell structure	09.01 Cell wall	D	N	D
78	Pyruvate, phosphate dikinase 1, chloroplastic	PPDK1_MAIZE	103	0.00035	02 Energy	02.30 Photosynthesis	D	N	D
79	Transketolase, chloroplastic	TKTC_SOLTU	80	0.00035	02 Energy	02.07 Pentose phosphate	I	N	N
80	Defensin-like protein 2	DEF2_WHEAT	5	0.00035	11 Disease/defense	11.05 Stress responses	I	D	I
82	Glucose-6-phosphate isomerase, cytosolic	G6PI_MAIZE	62	0.00038	02 Energy	02.01 Glycolysis	I	N	N
83	Histone H2B.4	H2B4_MAIZE	15	0.00040	09 Cell structure	09.13 Chromosomes	D	N	D
84	V-type proton ATPase subunit C	VATC_HORVU	40	0.00040	07 Transporters	07.22 Transport ATPases	I	I	I
86	Trypsin/alpha-amylase inhibitor CMX1/CMX3	IACX1_WHEAT	14	0.00041	12 Unclear classification	12 Unclear classification	N	D	N
88	Monothiol glutaredoxin-S10	GRS10_ORYSJ	18	0.00041	11 Disease/defense	11.06 Detoxification	D	D	N
89	Catalase isozyme 2	CATA2_HORVU	57	0.00042	11 Disease/defense	11.06 Detoxification	D	N	N
91	Proliferating cell nuclear antigen	PCNA_ORYSJ	29	0.00045	03 Cell growth/ division	03.16 DNA synth/ replication	D	N	N
92	L-ascorbate peroxidase 2, cytosolic	APX2_ORYSJ	27	0.00046	11 Disease/defense	11.06 Detoxification	D	N	D
94	DnaJ protein homolog	DNJH_CUCSA	46	0.00055	06 Protein destination/storage	06.01 Folding and stability	N	D	N
99	U1 small nuclear ribonucleoprotein C	RU1C_ARATH	22	0.00066	12 Unclear classification	12 Unclear classification	D	D	D
101	Late embryogenesis abundant protein B19.3	LE193_HORVU	15	0.00067	03 Cell growth/ division	03.30 Seed maturation	N	D	D
102	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1, mitochondrial	DHSA1_ARATH	70	0.00067	02 Energy	02.20 E-transport	D	N	I
104	Chaperone protein ClpB2, chloroplastic	CLPB2_ORYSJ	109	0.00075	06 Protein destination/storage	06.01 Folding and stability	N	D	N
105	Flavone O-methyltransferase 1	FOMT1_WHEAT	39	0.00076	11 Disease/defense	11.06 Detoxification	D	N	N
109	Fructan 1-exohydrolase w1	1FEH1_WHEAT	67	0.00089	01 Metabolism	01.05 Sugars/ Polysaccharides	D	N	D
111	Alpha-1,4-glucan-protein synthase [UDP-forming] 2	UPTG2_SOLTU	42	0.00097	09 Cell structure	09.01 Cell wall	D	N	N
113	Phenylalanine ammonia-lyase	PAL2_ORYSI	77	0.00098	20 Secondary metabolism	20.1 Phenylpropanoids/ phenolics	D	I	I
114	Sucrose synthase 2	SUS2_ORYSJ	92	0.001	11 Disease/defense	11.05 Stress responses	D	D	D
115	ATP-dependent (S)-NAD(P)H-hydrate dehydratase	NNRD_SORBI	41	0.001	12 Unclear classification	12 Unclear classification	I	N	N
118	Linoleate 9S-lipoxygenase 2	LOX2_ORYSJ	97	0.0012	01 Metabolism	01.06 Lipid and sterol	D	N	N
121	Acyl-[acyl-carrier-protein] desaturase 2, chloroplastic	STAD2_ORYSI	45	0.0012	01 Metabolism	01.06 Lipid and sterol	N	I	N
122	Sulfite reductase [ferredoxin], chloroplastic	SIR_MAIZE	70	0.0014	12 Unclear classification	12 Unclear classification	D	D	D
127	60S ribosomal protein L13a	RL13A_PICMA	24	0.0017	05 Protein synthesis	05.01 Ribosomal proteins	D	N	D
128	5-methyltetrahydropteroyltriglutamate–homocysteine methyltransferase	METE_CATRO	85	0.0017	01 Metabolism	01.01 Amino acid	D	N	N
129	Eukaryotic translation initiation factor 5A-1/2	IF5A1_SOLTU	17	0.0018	05 Protein synthesis	05.04 Translation factors	D	N	N
132	Serpin-Z2A	SPZ2A_WHEAT	43	0.0019	06 Protein destination/storage		I	D	N
133	Coatomer subunit beta′-1	COB21_ORYSJ	103	0.002	08 Intracellular traffic	08.07 Vesicular	N	N	D

(*continued on next page*)

Table 1 (*continued*)

No	Identified Proteins	Entry name	MW (kDa)	P-Value	Functional Category	Functional Description	R1	R2	R3
134	Histone H3.2	H32_MEDSA	15	0.002	09 Cell structure	09.13 Chromosomes	D	N	N
135	Regulator of nonsense transcripts 1 homolog	RENT1_ARATH	137	0.002	04 Transcription	04.22 mRNA processing	I	I	I
137	Methionine S-methyltransferase	MMT1_HORVU	120	0.0022	01 Metabolism	01.01 Amino acid	I	I	I
138	Serpin-Z1C	SPZ1C_WHEAT	43	0.0022	06 Protein destination/storage		I	D	D
140	Ubiquitin-conjugating enzyme E2 7	UBC7_WHEAT	19	0.0022	06 Protein destination/storage	06.07 Modification	N	I	N
141	Putative diaminopimelate epimerase, chloroplastic	DAPF_ORYSI	38	0.0022	01 Metabolism	01.01 Amino acid	D	N	N
142	Uridine 5′-monophosphate synthase (Fragment)	UMPS_TOBAC	50	0.0022	01 Metabolism	01.03 Nucleotides	I	D	N
144	Histone H2A.1	H2A1_WHEAT	16	0.0026	09 Cell structure	09.13 Chromosomes	D	N	N
145	Chaperone protein ClpB3, mitochondrial	CLPB3_ORYSJ	109	0.0026	06 Protein destination/storage	06.01 Folding and stability	D	D	N
148	DNA replication licensing factor MCM3 homolog 2	MCM32_MAIZE	85	0.0028	03 Cell growth/ division	03.16 DNA synth/ replication	D	N	N
152	Bowman-Birk type proteinase inhibitor II-4 (Fragment)	IBB2_WHEAT	6	0.0032	12 Unclear classification	12 Unclear classification	I	D	I
153	Chaperonin CPN60-1, mitochondrial	CH61_CUCMA	61	0.0033	06 Protein destination/storage	06.01 Folding and stability	D	N	N
154	Fumarate hydratase 1, mitochondrial	FUM1_ARATH	53	0.0033	02 Energy	02.10 TCA pathway	D	I	D
157	Probable eukaryotic translation initiation factor 5-1	IF5Y_ARATH	49	0.0034	05 Protein synthesis	05.04 Translation factors	D	D	D
158	Non-specific lipid-transfer protein (Fragment)	NLTP1_WHEAT	12	0.0034	07 Transporters	07.13 Lipids	N	D	D
160	Serine carboxypeptidase 3	CBP3_HORVU	56	0.0034	06 Protein destination/storage	06.13 Proteolysis	D	D	D
164	40S ribosomal protein S3a	RS3A_ORYSJ	30	0.0038	05 Protein synthesis	05.01 Ribosomal proteins	D	N	N
167	Glutamate-1-semialdehyde 2,1-aminomutase, chloroplastic	GSA_HORVU	49	0.0041	01 Metabolism	01.01 Amino acid	D	N	N
168	Gamma-gliadin (Fragment)	GDB3_WHEAT	27	0.0041	06 Protein destination/storage	06.20 Storage proteins	N	D	N
169	12-oxophytodienoate reductase 1	OPR1_ORYSJ	42	0.0041	11 Disease/defense	11.05 Stress responses	D	N	N
171	Aldehyde dehydrogenase family 7 member A1	AL7A1_BRANA	53	0.0043	11 Disease/defense	11.06 Detoxification	I	N	D
172	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	PMGI_MAIZE	61	0.0044	02 Energy	02.01 Glycolysis	D	N	N
175	S-adenosylmethionine synthase 4	METK4_HORVU	43	0.0051	01 Metabolism	01.01 Amino acid	D	N	N
181	Protochlorophyllide reductase A, chloroplastic	PORA_WHEAT	41	0.0054	20 Secondary metabolism	20 Secondary metabolism	N	I	N
184	Acyl-[acyl-carrier-protein] desaturase, chloroplastic	STAD_HELAN	45	0.0056	01 Metabolism	01.06 Lipid and sterol	D	I	I
187	GTP-binding protein SAR1	SAR1_TOBAC	23	0.006	08 Intracellular traffic	08.07 Vesicular	I	I	I
188	Nucleoside diphosphate kinase IV, chloroplastic/mitochondrial	NDK4_ARATH	26	0.0061	01 Metabolism	01.03 Nucleotides	D	I	I
189	Probable acetyl-CoA acetyltransferase, cytosolic 2	THIC2_ARATH	43	0.0061	12 Unclear classification	12 Unclear classification	I	D	N
191	Probable UDP-glucose 6-dehydrogenase 2	UGDH2_ARATH	53	0.0064	01 Metabolism	01.03 Nucleotides	D	I	N
192	NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, mitochondrial	NDUS1_ARATH	82	0.0064	02 Energy	02.20 E-transport	D	N	N
196	Aconitate hydratase 2, mitochondrial	ACO2M_ARATH	108	0.0072	02 Energy	02.10 TCA pathway	I	D	D
197	17.4 kDa class I heat shock protein	HSP17_ARATH	17	0.0075	06 Protein destination/storage	06.01 Folding and stability	I	N	N
198	Probable methionine–tRNA ligase	SYM_ORYSJ	89	0.0076	05 Protein synthesis	05.10 tRNA synthases	D	N	D
199	4-alpha-glucanotransferase DPE2	DPE2_ORYSJ	108	0.0082	01 Metabolism	01.05 Sugars/ Polysaccharides	N	I	N
202	Arginase	ARGI1_ARATH	37	0.0091	01 Metabolism	01.01 Amino acid	D	D	D
203	Endoplasmin homolog	ENPL_ARATH	94	0.0098	06 Protein destination/storage	06.01 Folding and stability	D	N	N
204	Glutathione S-transferase 1	GSTF1_WHEAT	26	0.010	11 Disease/defense	11.06 Detoxification	I	N	I

Table 1 (*continued*)

No	Identified Proteins	Entry name	MW (kDa)	P-Value	Functional Category	Functional Description	R1	R2	R3
205	Aspartate carbamoyltransferase 2, chloroplastic	PYRB2_PEA	44	0.011	01 Metabolism	01.03 Nucleotides	N	D	N
206	40S ribosomal protein S30	RS30_ARATH	7	0.011	05 Protein synthesis	05.01 Ribosomal proteins	N	D	N
209	Actin-8	ACT8_ARATH	42	0.012	09 Cell structure	09.04 Cytoskeleton	D	N	N
214	ATP-citrate synthase alpha chain protein 3	ACLA3_ORYSJ	47	0.013	01 Metabolism	01.06 Lipid and sterol	D	I	N
215	NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial	NDUV1_ARATH	53	0.013	02 Energy	02.20 E-transport	D	N	I
219	Agglutinin isolectin 3 (Fragment)	AGI3_WHEAT	19	0.013	12 Unclear classification	12 Unclear classification	N	D	I
221	Histone-binding protein MSI1 homolog	MSI1_ORYSJ	48	0.014	09 Cell structure	09.13 Chromosomes	D	N	N
224	U-box domain-containing protein 72	PUB72_ORYSJ	58	0.014	06 Protein destination/storage	06.07 Modification	D	I	I
227	Phosphoethanolamine N-methyltransferase 1	PEAM1_ARATH	56	0.014	01 Metabolism	01.06 Lipid and sterol	D	N	N
228	17.0 kDa class II heat shock protein	HSP23_MAIZE	17	0.015	06 Protein destination/storage	06.01 Folding and stability	N	D	N
230	Protein-L-isoaspartate O-methyltransferase	PIMT_WHEAT	25	0.015	06 Protein destination/storage	06.01 Folding and stability	I	N	I
232	Em protein CS41	EM2_WHEAT	10	0.016	11 Disease/defense	11.05 Stress responses	I	D	D
233	Phospholipase D alpha 1	PLDA1_MAIZE	92	0.016	01 Metabolism	01.06 Lipid and sterol	I	I	I
234	Acetyl-CoA acetyltransferase, cytosolic 1	THIC1_ARATH	41	0.017	20 Secondary metabolism	20.2 Terpenoids	N	D	D
235	Phosphomannomutase	PMM_WHEAT	28	0.018	01 Metabolism	01.03 Nucleotides	D	N	D
238	Heat shock protein 82	HSP82_MAIZE	82	0.018	06 Protein destination/storage	06.01 Folding and stability	D	D	N
240	Histone H1	H1_WHEAT	24	0.018	09 Cell structure	09.13 Chromosomes	D	N	N
241	60S ribosomal protein L34-2	RL342_ARATH	14	0.018	05 Protein synthesis	05.01 Ribosomal proteins	I	I	D
243	Lactoylglutathione lyase	LGUL_SOLLC	21	0.019	20 Secondary metabolism	20 Secondary metabolism	N	I	I
244	Ferredoxin-dependent glutamate synthase, chloroplastic	GLTB_ORYSJ	175	0.020	01 Metabolism	01.01 Amino acid	N	I	I
245	Probable L-ascorbate peroxidase 6, chloroplastic	APX6_ORYSJ	34	0.020	11 Disease/defense	11.06 Detoxification	D	D	D
246	Splicing factor U2af large subunit B	U2A2B_WHEAT	61	0.021	04 Transcription	04.22 mRNA processing	D	I	N
248	Elongation factor Tu, chloroplastic	EFTU_PEA	53	0.022	05 Protein synthesis	05.04 Translation factors	D	N	D
250	GTP-binding protein yptV4	YPTV4_VOLCA	24	0.023	08 Intracellular traffic	08.07 Vesicular	N	D	N
251	Probable phosphoglucomutase, cytoplasmic 2	PGMC2_ARATH	63	0.023	01 Metabolism	01.05 Sugars/ Polysaccharides	I	N	N
252	Glucose-6-phosphate isomerase 1, chloroplastic	G6PIP_ARATH	67	0.023	02 Energy	02.02 Gluconeogenesis	N	I	N
253	Heat shock 70 kDa protein, mitochondrial	HSP7M_PHAVU	73	0.024	06 Protein destination/storage	06.01 Folding and stability	N	D	N
254	Lon protease homolog, mitochondrial	LONM_MAIZE	106	0.024	06 Protein destination/storage	06.13 Proteolysis	D	D	N
257	Malate dehydrogenase, cytoplasmic	MDHC_MAIZE	36	0.024	02 Energy	02.10 TCA pathway	N	I	I
258	GDP-mannose 3,5-epimerase 2	GME2_ORYSJ	42	0.025	01 Metabolism	01.07 Cofactors	D	N	D
260	Anthranilate synthase component I-1, chloroplastic	TRPE_ARATH	66	0.026	01 Metabolism	01.01 Amino acid	D	N	N
262	Phosphoenolpyruvate carboxykinase [ATP]	PCKA_CUCSA	74	0.027	02 Energy	02.02 Gluconeogenesis	I	I	I
263	Pyrophosphate–fructose 6-phosphate 1-phosphotransferase subunit alpha	PFPA_SOLTU	67	0.027	02 Energy	02.01 Glycolysis	D	D	D
265	Thioredoxin reductase NTRA	NTRA_ORYSJ	38	0.028	11 Disease/defense	11.06 Detoxification	D	N	N
271	Actin-related protein 4	ARP4_ORYSI	48	0.032	09 Cell structure	09.04 Cytoskeleton	I	N	I
272	6-phosphofructokinase 4, chloroplastic	K6PF4_ARATH	58	0.032	02 Energy	02.01 Glycolysis	I	N	D
275	Adenine phosphoribosyltransferase 1	APT1_WHEAT	20	0.033	01 Metabolism	01.03 Nucleotides	D	N	D
278	Dihydrolipoyl dehydrogenase 1, mitochondrial	DLDH1_ARATH	54	0.036	11 Disease/defense	11.05 Stress responses	N	N	D

(*continued on next page*)

Table 1 (*continued*)

No	Identified Proteins	Entry name	MW (kDa)	P-Value	Functional Category	Functional Description	R1	R2	R3
279	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	ODPB_PEA	39	0.037	02 Energy	02.01 Glycolysis	D	I	I
280	Asparagine synthetase [glutamine-hydrolyzing]	ASNS_ASPOF	66	0.038	01 Metabolism	01.01 Amino acid	N	I	N
281	Coatomer subunit gamma-1	COPG1_ORYSJ	99	0.038	08 Intracellular traffic	08.07 Vesicular	I	N	I
283	Methylenetetrahydrofolate reductase 1	MTHR1_MAIZE	66	0.039	01 Metabolism	01.01 Amino acid	D	I	N
284	Probable 26S proteasome non-ATPase regulatory subunit 3	PSMD3_TOBAC	56	0.040	06 Protein destination/storage	06.13 Proteolysis	I	I	I
285	COP9 signalosome complex subunit 4	CSN4_ARATH	45	0.040	10 Signal transduction	10.04 Mediators	I	N	N
286	Late embryogenesis abundant protein 1	LEA1_ORYSI	36	0.040	03 Cell growth/ division	03.30 Seed maturation	I	N	N
288	Villin-like protein ABP41 (Fragments)	ABP41_LILDA	16	0.042	12 Unclear classification	12 Unclear classification	D	N	D
292	1-Cys peroxiredoxin	REHY_MEDTR	24	0.045	11 Disease/defense	11.06 Detoxification	I	N	N
293	Probable protein phosphatase 2C 69	P2C69_ARATH	38	0.047	10 Signal transduction	10.047 Phosphatases	N	D	N
295	Protein disulfide-isomerase like 2-1	PDI21_ARATH	39	0.048	06 Protein destination/storage	06.01 Folding and stability	N	D	N
296	3-isopropylmalate dehydrogenase, chloroplastic	LEU3_SOLTU	40	0.048	01 Metabolism	01.01 Amino acid	D	D	N
297	Phospho-2-dehydro-3-deoxyheptonate aldolase 2, chloroplastic	AROG_ORYSJ	59	0.049	01 Metabolism	01.01 Amino acid	D	N	N
298	Dynamin-related protein 1E	DRP1E_ARATH	70	0.049	01 Metabolism	01.03 Nucleotides	N	I	I

Embryo proteins found to be differentially expressed in unprimed and ascorbate-primed wheat seeds during germination under saline and non-saline conditions, as described in Materials and methods section and identified by MS, with relative expression ratios. Ratio salinity/ control (R1), ratio ascorbate-priming/control (R2), ratio salinity × ascorbate-priming/control (R3) are reported, where the proteins that were decreased in abundance are indicated with D, not changed proteins with N, and the proteins that were increased in abundance with I.

(Fig. 1B). The most over-represented ones are those of metabolism (14–23%), energy (5–15%), protein destination and storage (9–22%) and disease/defense (19–23%). Comparative functional distribution however revealed 5 classes that represented the most variation observed between the three treatments versus the control (Fig. 1B).

3.2. Wheat embryo-ST metabolic proteome

As expected (since endosperm is a dead tissue), of a total of 471 identified proteins only 69 proteins were significantly accumulated (Fig. 2A,Table 2, Supplementary material Table S2). Of these proteins, 53 (17 specifically affected) were found to be differentially altered in unprimed salt stressed wheat embryo-ST (UP-NaCl vs. UP-H_2O), 26 of these were increased and 27 were decreased in abundance (Fig. 2C and Table 2, Supplementary material Table S2). On the other hand, 49 proteins (13 specifically affected) were differentially accumulated in AsA-primed non stressed wheat embryo-ST (AP-H_2O vs. UP-H_2O), among which 26 were increased and 23 were decreased in abundance (Fig. 2C and Table 2, Supplementary material Table S2). However, 48 proteins were found to be differentially changed in AsA-primed salt stressed wheat embryo-ST (AP-NaCl vs. UP-H_2O), 23 of these were increased and 25 were decreased in abundance (Fig. 2C and Table 2, Supplementary material Table S2). Functional classification grouped the 69 identified proteins into 11 functional categories (Fig. 2B). The most over-represented categories are those of metabolism (15–21%), energy (9–12%), protein synthesis (15–21%), protein destination and storage (16–24%) and disease/ defense (13–15%). Comparative functional distribution however revealed 2 classes that represented the most variation observed between the three treatments versus the control (Fig. 2B).

3.3. Hierarchical clustering of AsA- and salt stress-responsive proteins

In order to identify proteins with similar accumulation patterns, a two-way hierarchical clustering methodology known as Ward's method [32] was applied using PermutMatrix software (http://www.atgc-montpellier.fr/permutmatrix/; [33, 34]). In embryo, at least, three major clusters of protein accumulation were found, with the first containing proteins that were less abundant in embryo of salt stressed wheat seeds with or without AsA pretreatment (60 proteins), the second consisting of proteins that were more abundant in embryo of UP-NaCl wheat seeds but less abundant in embryo of AP-H_2O and AP-NaCl wheat seeds (50 proteins), and finely the third cluster include proteins that were less abundant in embryo of UP-NaCl seeds but more abundant in AsA pretreated seeds (57 proteins), compared to control group (Fig. 4). On the other hand, the protein accumulation patterns of UP-H_2O, AP-H_2O and AP-NaCl treatments were clustered together reflecting the close similarity between these treatments; however, the protein accumulation pattern of UP-NaCl treatment was put in a separate cluster. This result was confirmed by principal

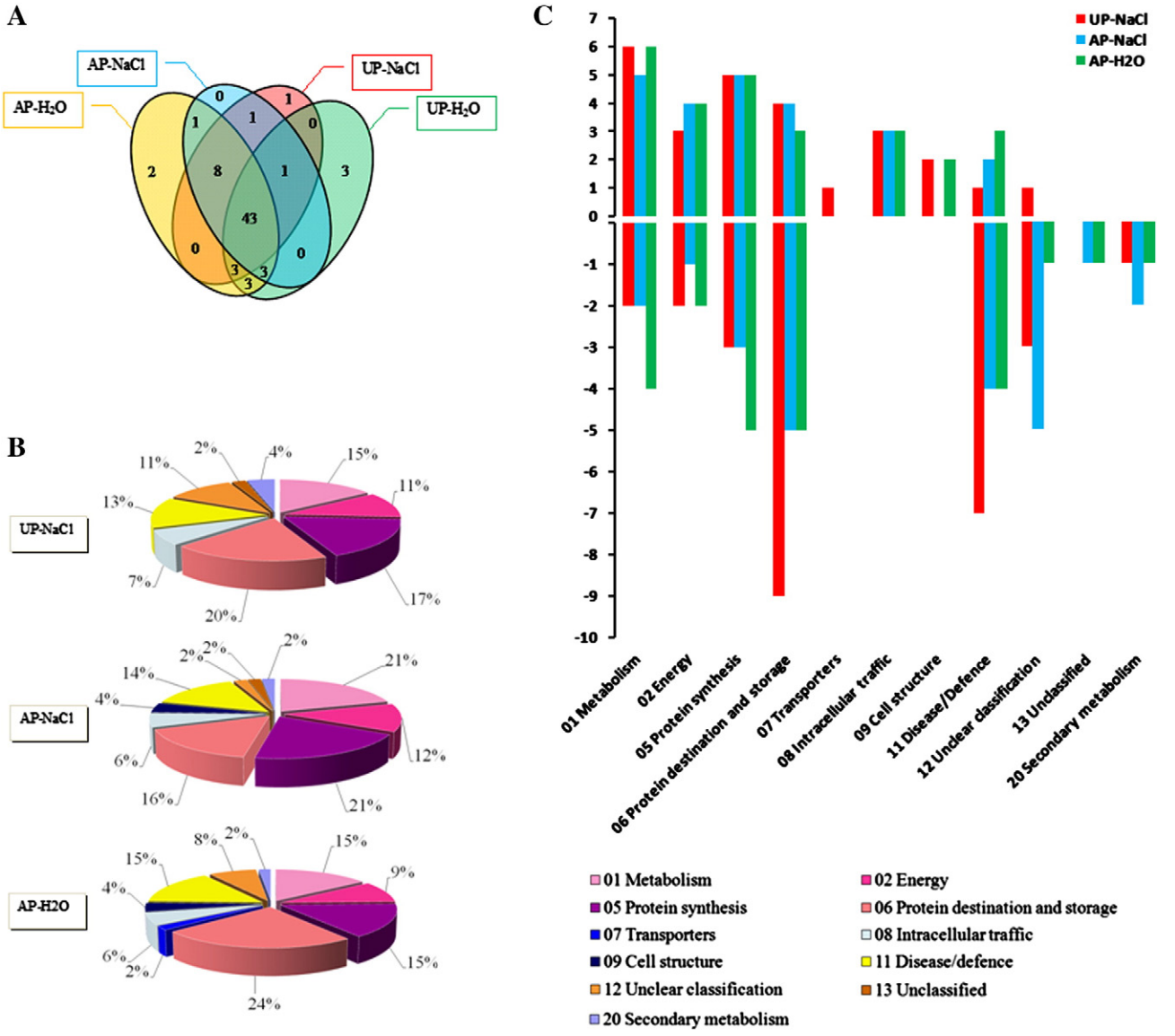

Fig. 2 – Metabolic proteome signature in embryo-ST of germinating primed and unprimed wheat seeds under salt stress as compared to control (UP-H_2O). A. Venn diagram based on the differentially accumulated proteins in primed and unprimed stressed seeds compared with controls; B. Functional classification of proteins according to Bevan et al. [31]; C. Functional distribution of proteins.

component analysis Supplementary Fig. S1), where the main factor contributing to PC1 was UP-NaCl treatment.

In embryo-ST, however, hierarchical clustering of protein accumulation profiles resulted in two major clusters, with the first consisting of proteins less abundant compared to control and the second of proteins whose abundance increased in embryo-ST of the both AsA-primed and unprimed wheat seeds during germination under salt stress conditions as compared to untreated, unstressed control (Fig. 5).

4. Discussion

High salinity is a major abiotic stress in agriculture worldwide. Seed germination is a pivotal developmental process in the plant life cycle and the most critical under salt stress conditions [1]. Salinity may affect seed germination osmotically, by decreasing the uptake of water by seeds, and ionically, by facilitating the accumulation of ions that are toxic to embryonic activity [35]. Presumably the effect of osmotic stress on imbibition, due to salinity, is the main cause for delaying germination. Similarly, salinity also increased the ABA level and decreased the level of gibberellins in germinating seeds [36]. Recently, it has been demonstrated that AsA plays a role in germination particularly under stress conditions by mediating the antagonism between ABA and gibberellins [22,23]. Consistent with this, our results revealed that AsA may play an important role in increasing the salt tolerance or mitigating the adverse effect of salinity (Fig. 3)

Table 2 – Embryo-surrounding tissues proteome of unprimed and ascorbate-primed wheat seeds during germination under saline and non-saline conditions.

No	Identified Proteins	Entry Name	MW (kDa)	P-Value	Functional Category	Functional Description	R1	R2	R3
1	Fructan 1-exohydrolase w1	1FEH1_WHEAT	67	<0.0001	01 Metabolism	01.05 Sugars/ Polysaccharides	N	I	N
2	V-type proton ATPase catalytic subunit A Fragment	VATA_MAIZE	62	<0.0001	07 Transporters	07.22 Transport ATPases	I	N	N
3	Alpha-amylase type B isozyme	AMY2_HORVU	47	<0.0001	01 Metabolism	01.05 Sugars/ Polysaccharides	D	N	N
4	Calmodulin	CALM_WHEAT	17	<0.0001	12 Unclear classification		D	D	D
5	HMG1/2-like protein	HMGL_WHEAT	17	<0.0001	12 Unclear classification		N	N	D
6	Defensin Tk-AMP-D4	DEF4_TRIKH	5	<0.0001	11 Disease/ Defense	11.02 Defense-related	N	D	N
7	Alpha-amylase type B isozyme	AMY6_HORVU	48	<0.0001	01 Metabolism	01.05 Sugars/ Polysaccharides	N	I	N
8	Beta-amylase	AMYB_WHEAT	57	<0.0001	01 Metabolism	01.05 Sugars/ Polysaccharides	N	N	I
9	Alpha-glucan phosphorylase, H isozyme	PHSH_WHEAT	94	<0.0001	01 Metabolism	01.05 Sugars/ Polysaccharides	I	I	I
12	Glucose-1-phosphate adenylyltransferase small subunit	GLGS_HORVU	56	<0.0001	06 Protein destination/storage	06.20 Storage proteins	I	N	N
17	60S ribosomal protein L38	RL38_ARATH	8	0.00015	05 Protein synthesis	05.01 Ribosomal proteins	I	D	I
20	Wheatwin-2	WHW2_WHEAT	16	0.00023	11 Disease/Defense	11.02 Defense-related	D	N	N
22	Clathrin heavy chain 1	CLH1_ORYSJ	193	0.00033	08 Intracellular traffic	08.07 Vesicular	I	I	I
24	Rubber elongation factor protein	REF_HEVBR	15	0.00037	13 Unclassified		N	D	D
27	Peroxisomal fatty acid beta-oxidation multifunctional protein	MFP_ORYSJ	78	0.00041	01 Metabolism	01.06 Lipid and sterol	I	I	I
29	Phosphoenolpyruvate carboxykinase [ATP]	PCKA_CUCSA	74	0.00045	02 Energy	02.02 Gluconeogenesis	I	I	I
30	Elongation factor 1-alpha	EF1A2_HORVU	49	0.00048	05 Protein synthesis	05.04 Translation factors	I	I	I
34	26S protease regulatory subunit 6B homolog	PRS6B_HELAN	47	0.00067	06 Protein destination/storage	06.13 Proteolysis	N	I	I
36	60S ribosomal protein L23a	RL23A_DAUCA	18	0.00082	05 Protein synthesis	05.01 Ribosomal proteins	D	D	D
38	60S acidic ribosomal protein P0	RLA0_ORYSJ	34	0.00095	05 Protein synthesis	05.01 Ribosomal proteins	I	I	I
42	Fructokinase-2	SCRK2_ORYSI	36	0.0010	01 Metabolism	01.05 Sugars/ Polysaccharides	D	D	D
43	Tubulin beta-2 chain	TBB2_ELEIN	50	0.0011	09 Cell structure	09.04 Cytoskeleton	I	I	N
44	60S ribosomal protein L12-1	RL121_ARATH	18	0.0012	05 Protein synthesis	05.01 Ribosomal proteins	D	D	D
45	DEAD-box ATP-dependent RNA helicase 52C	RH52C_ORYSJ	66	0.0012	12 Unclear classification		I	N	D
47	Serine carboxypeptidase 3	CBP3_WHEAT	55	0.0013	06 Protein destination/storage	06.13 Proteolysis	D	N	N
54	Sulfite oxidase	SUOX_ARATH	43	0.0022	11 Disease/Defense	11.06 Detoxification	D	D	D
55	Tubulin alpha-2 chain	TBA2_HORVU	50	0.0023	09 Cell structure	09.04 Cytoskeleton	I	I	N
56	40S ribosomal protein S13	RS13_MAIZE	17	0.0024	05 Protein synthesis	05.01 Ribosomal proteins	N	D	N
58	Uridine 5′-monophosphate synthase Fragment	UMPS_TOBAC	50	0.0034	01 Metabolism	01.03 Nucleotides	I	I	I
60	Peptidyl-prolyl cis-trans isomerase	CYPH_MAIZE	18	0.0036	06 Protein destination/storage	06.01 Folding and stability	D	N	D
63	Ubiquitin-activating enzyme E1 1	UBE11_WHEAT	117	0.0037	06 Protein destination/storage	06.13 Proteolysis	I	I	I
64	3-isopropylmalate dehydrogenase 2, chloroplastic	LEU32_ARATH	43	0.0037	01 Metabolism	01.01 Amino acid	N	D	N
65	Cysteine proteinase EP-B 1	CYSP1_HORVU	40	0.0037	06 Protein destination/storage	06.13 Proteolysis	D	N	N
68	Sucrose synthase 1	SUS1_ORYSJ	93	0.0039	11 Disease/Defense	11.05 Stress response	I	I	I
69	Alpha-galactosidase	AGAL_ORYSJ	46	0.0040	01 Metabolism	01.05 Sugars/ Polysaccharides	I	I	I
75	Alpha/beta-gliadin clone PW1215	GDA6_WHEAT	34	0.0049	06 Protein destination/storage	06.20 Storage proteins	N	D	N
77	Formate dehydrogenase 1, mitochondrial	FDH1_ORYSJ	41	0.0059	12 Unclear classification		D	N	N

Table 2 (*continued*)

No	Identified Proteins	Entry Name	MW (kDa)	P-Value	Functional Category	Functional Description	R1	R2	R3
78	Guanine nucleotide-binding protein subunit beta-like protein A	GBLPA_ORYSJ	36	0.0061	11 Disease/Defense	11.02 Defense-related	N	I	I
80	Regulator of ribonuclease-like protein 1	RRAA1_ARATH	18	0.0062	12 Unclear classification		D	N	D
81	23.6 kDa heat shock protein, mitochondrial	HS23M_ORYSJ	24	0.0069	06 Protein destination/storage	06.01 Folding and stability	D	D	D
82	Malate synthase, glyoxysomal	MASY_MAIZE	62	0.0069	02 Energy	Glyoxylate pathway	N	I	I
83	16.9 kDa class I heat shock protein 2	HS16B_WHEAT	17	0.0074	06 Protein destination/storage	06.01 Folding and stability	I	N	I
86	Gamma-gliadin B	GDBB_WHEAT	33	0.0082	06 Protein destination/storage	06.20 Storage proteins	D	D	N
88	70 kDa peptidyl-prolyl isomerase	FKB70_WHEAT	62	0.0093	06 Protein destination/storage	06.01 Folding and stability	D	N	D
91	Triosephosphate isomerase, cytosolic	TPIS_MAIZE	27	0.011	02 Energy	02.01 Glycolysis	D	D	D
92	Adenosine kinase 2	ADK2_ARATH	38	0.012	01 Metabolism	01.03 Nucleotides	N	D	N
94	Lactoylglutathione lyase	LGUL_SOLLC	21	0.014	20 Secondary metabolism		N	D	D
95	40S ribosomal protein S15a-4	R15A4_ARATH	15	0.015	05 Protein synthesis	05.01 Ribosomal proteins	D	I	I
96	Superoxide dismutase [Mn] 3.1, mitochondrial	SODM1_MAIZE	26	0.015	11 Disease/Defense	11.06 Detoxification	D	N	N
98	Phosphoenolpyruvate carboxylase 2	CAPPC_FLATR	111	0.016	02 Energy	02.02 Gluconeogenesis	I	I	I
102	40S ribosomal protein Sa-1	RSSA1_ARATH	32	0.017	05 Protein synthesis	05.01 Ribosomal proteins	I	I	I
104	Chaperone protein ClpB2, chloroplastic	CLPB2_ORYSJ	109	0.018	06 Protein destination/storage	06.01 Folding and stability	D	D	D
105	Ferredoxin–NADP reductase, root isozyme 2, chloroplastic	FNRR2_ARATH	43	0.020	02 Energy	02.20 E-transport	D	D	D
108	Chaperone protein dnaJ 2	DNAJ2_ARATH	46	0.022	06 Protein destination/storage	06.01 Folding and stability	D	D	D
115	Importin subunit alpha-1a	IMA1A_ORYSJ	58	0.024	08 Intracellular traffic	08.01 Nuclear	I	I	I
116	Probable N-acetyl-gamma-glutamyl-phosphate reductase, chloroplastic	ARGC_ORYSJ	45	0.024	01 Metabolism	01.01 Amino acid	I	N	D
118	Putative heat shock protein 1 Fragment	HSP01_PSEMZ	2	0.025	06 Protein destination/storage	06.01 Folding and stability	D	I	D
119	Probable LL-diaminopimelate aminotransferase, chloroplastic	DAPAT_ORYSJ	50	0.026	01 Metabolism	01.01 Amino acid	I	D	N
120	40S ribosomal protein S27-1	RS271_ARATH	9	0.026	05 Protein synthesis	05.01 Ribosomal proteins	I	I	N
122	Defensin Tk-AMP-D1.1	DEF11_TRIKH	5	0.027	11 Disease/Defense	11.02 Defense-related	D	D	D
130	Cationic peroxidase SPC4	PER1_SORBI	38	0.032	11 Disease/Defense	11.06 Detoxification	N	N	D
132	60S ribosomal protein L24	RL24_HORVU	18	0.035	11 Disease/Defense	05.01 Ribosomal proteins	N	D	D
133	N-carbamoylputrescine amidase	AGUB_ORYSJ	33	0.035	20 Secondary metabolism	20.5 Amines	D	N	D
134	Calnexin homolog	CALX_HELTU	61	0.036	06 Protein destination/storage	06.01 Folding and stability	I	N	I
136	GTP-binding protein SAR1	SAR1_TOBAC	23	0.038	08 Intracellular traffic	08.07 Vesicular	I	I	I
137	Probable calcium-binding protein CML7	CML7_ORYSJ	17	0.039	12 Unclear classification		D	N	D
138	L-ascorbate peroxidase 1, cytosolic	APX1_ORYSI	27	0.040	11 Disease/Defense	11.06 Detoxification	D	I	D
141	Isocitrate lyase	ACEA_GOSHI	65	0.045	02 Energy	Glyoxylate pathway	I	I	I
145	Peroxiredoxin-2C	PRX2C_ORYSJ	17	0.048	11 Disease/Defense	11.06 Detoxification	D	D	N

Embryo-surrounding tissues proteins found to be differentially expressed in unprimed and ascorbate-primed wheat seeds during germination under saline and non-saline conditions, as described in Materials and methods section and identified by MS, with relative expression ratios. Ratio salinity/control (R1), ratio ascorbate-priming/control (R2), ratio salinity × ascorbate-priming/control (R3) are reported, where the proteins that were decreased in abundance are indicated with D, not changed proteins with N, and the proteins that were increased in abundance with I.

Upon imbibition, embryonic cells switch from quiescence to highly active metabolism [10]. Consistent with this, comparatively to our previous study where we examined the effect of seed priming on the metabolic proteome of quiescent durum wheat seeds [18], in this study we have identified more than two-fold metabolic proteins (Table 1, Supplementary material Table S1).

4.1. *Metabolic proteome of embryo as affected by salinity and AsA-priming*

In this study, when compared between stressed and/or treated and control samples, the abundance of 167 proteins was significantly changed (Table 1, Supplementary material Table S1). Among these, proteins belonging to the "metabolism and energy", "protein destination/storage", "cell structure" and "disease/defense related-proteins" categories are highly represented (about 75%, Fig. 1B).

As shown by clustering analysis (Fig. 4), this discussion will focus mainly on proteins the abundance of which was differently affected by AsA-priming and salt stress (clusters II and III), in attempt to explain the improved tolerance exhibited by AsA-primed wheat seeds toward salinity. However, given the fact that the genotype selected for the present study is sensitive to NaCl at germination stage (Fig. 3), the proteins that were decreased in abundance by NaCl application will be more considered [37,38]. Therefore, the change in protein abundance will be discussed in the context of previously well-defined biochemical and metabolic aspects of salt tolerance.)

4.1.1. *Metabolism*

Forty five proteins belonging to "metabolism" category were differentially accumulated in response to salt stress and/or AsA-priming (Table 1; Supplementary material Table S1); of these, 32 were decreased and 13 were increased in abundance (Fig. 1). AsA-priming, however, significantly affects the abundance of 24 proteins in presence or absence of NaCl salinity. Proteins involved in amino acids metabolism are the most affected class with 18 proteins. Methionine metabolism plays a central role in seed germination (for review sees Rajjou et al., [39]). Methionine plays a multiple levels role in cellular metabolism not only as substrate for protein synthesis, or initiation of mRNA translation, but also as a regulatory molecule in the form of S-adenosylmethionine (SAM). Proteomic analysis of *Arabidopsis thaliana* germinating and developing seedlings has shown that methionine synthase and SAM synthetase accumulate differentially during seed germination [10], and the specific inhibitor of methionine biosynthesis, D, L-propargylglycine, strongly inhibits seed germination [40]. Previously it has been reported that overexpression of the genes involved in methionine biosynthesis or methionine supplementation enhances salt tolerance [41]. More recently, investigating how the methionine biosynthetic pathway is regulated under saline conditions at the transcriptional level in *A. thaliana* plants at germination and early growth stages, Ogawa and Mitsuya [42] have been found that the expression of homocysteine methyltransferase (HMT) and methionine methyltransferase (MMT) genes in S-methylmethionine (SMM) cycle had altered toward increasing Met production by the presence of NaCl. Consisting with these findings, our results revealed that at least six proteins involved in methionine metabolism (proteins no. 20, 34, 128, 137, 175, 283) were affected by salinity. Interestingly, methionine S-methyltransferase (protein no. 137) was increased but the SAM synthesis-related proteins (proteins no. 20, 34, 175) were decreased in abundance. SAM serves as a precursor for many metabolites including glycinebetaine, methylated polyols, polyamines and ethylene which

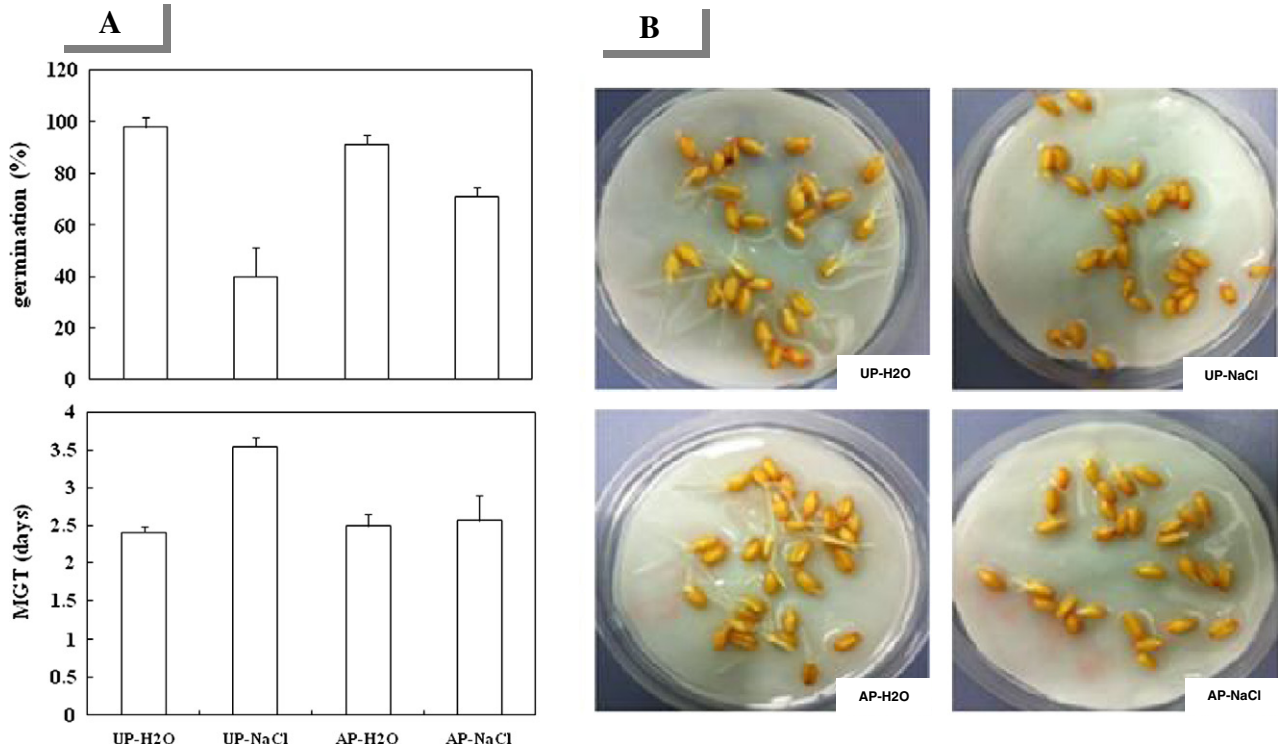

Fig. 3 – Effect of NaCl on wheat seed germination. A. final germination percentage (%) and mean germination time (day). B. Wheat seeds at 42 hours post imbibition. Results are presented as the average of three replicates ± SE.

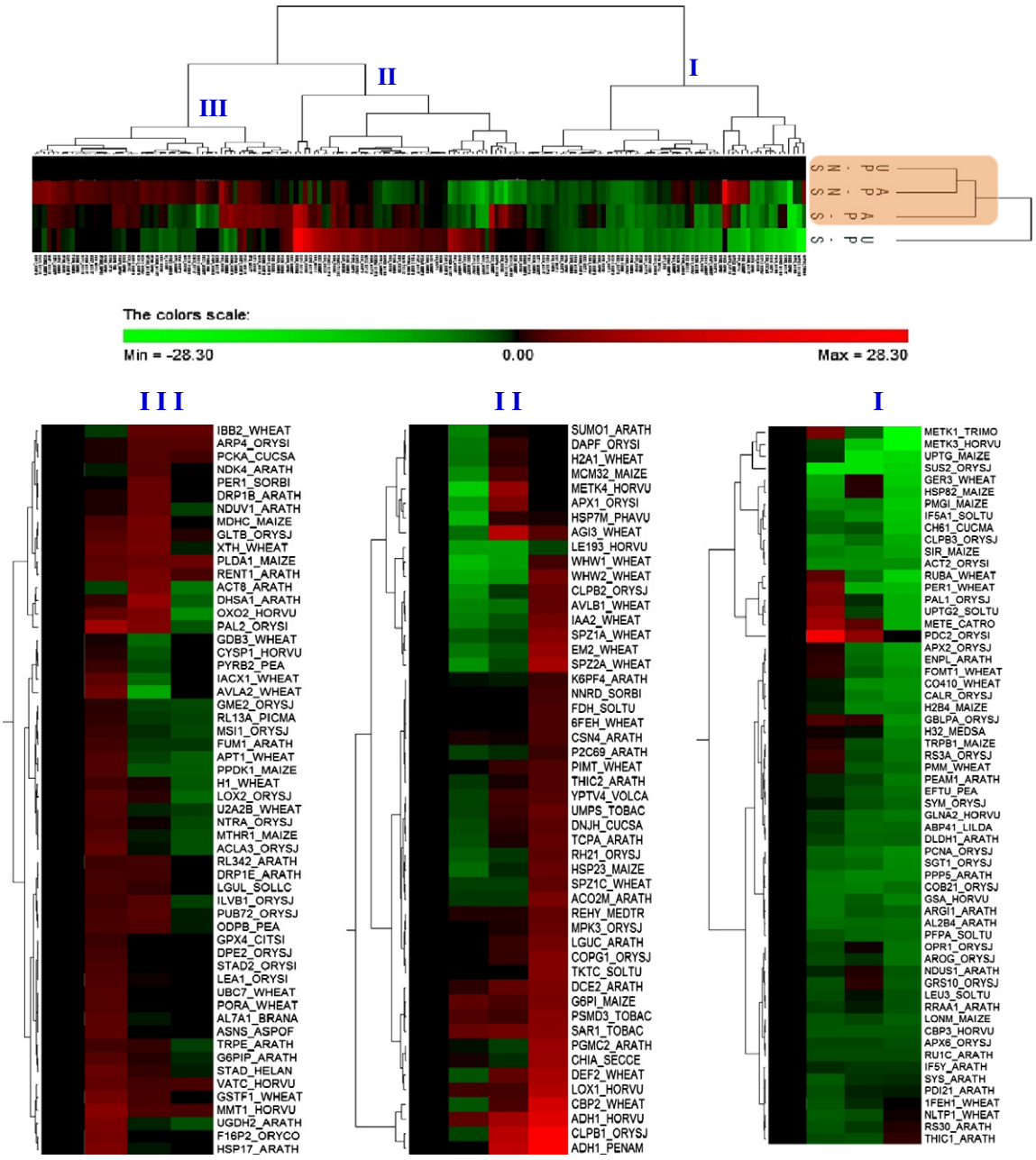

Fig. 4 – Hierarchical clustering of accumulation patterns of differentially regulated proteins in Wheat Embryo. Log2 based fold changes after exponential transformation of the quantitative values was used to create the heatmap. Differences in protein accumulation are shown in color as per the lower scale.

accumulate in response to salt stress in a wide range of plants [43]. Taking into account the salt-sensitivity of the present genotype, it is not surprising to find that salinity decreases the abundance of SAM synthesis-related proteins. Similar results were reported in tomato [44]. Also, it has been reported that salinity decrease the production of ethylene in cucumber [45] and many other species [43] during germination.

Salinity affects also the glutamate/glutamine metabolism, by up-regulating glutamate decarboxylase (GDA, protein no. 45) and down-regulating glutamate-1-semialdehyde 2,1-aminomutase (GSA, protein no. 167) and glutamine synthase (GS, protein no. 70). However, in addition to the up-regulation of asparagine synthetase (AS, protein no. 280) and ferredoxin-dependent glutamate synthase (Fd-GOGAT; protein no. 244), AsA seemed to mitigate the effects of salinity. Previously, it has been reported that exogenous L-Glu evoked ethylene release from imbibed seeds and attenuated the reduction in ethylene production induced by salinity [45]. The enzyme Fd-GOGAT (EC 1.4.7.1) catalyzes an essential step in the pathway of glutamate biosynthesis. The enzyme AS (EC 6.3.5.4) catalyzes the adenosine triphosphate (ATP)-dependent transfer of the amino group of glutamine to a molecule of aspartate to generate glutamate and asparagine [46]. Thus, in addition to the alleviation of the negative effects of NaCl on glutamate synthesis-related

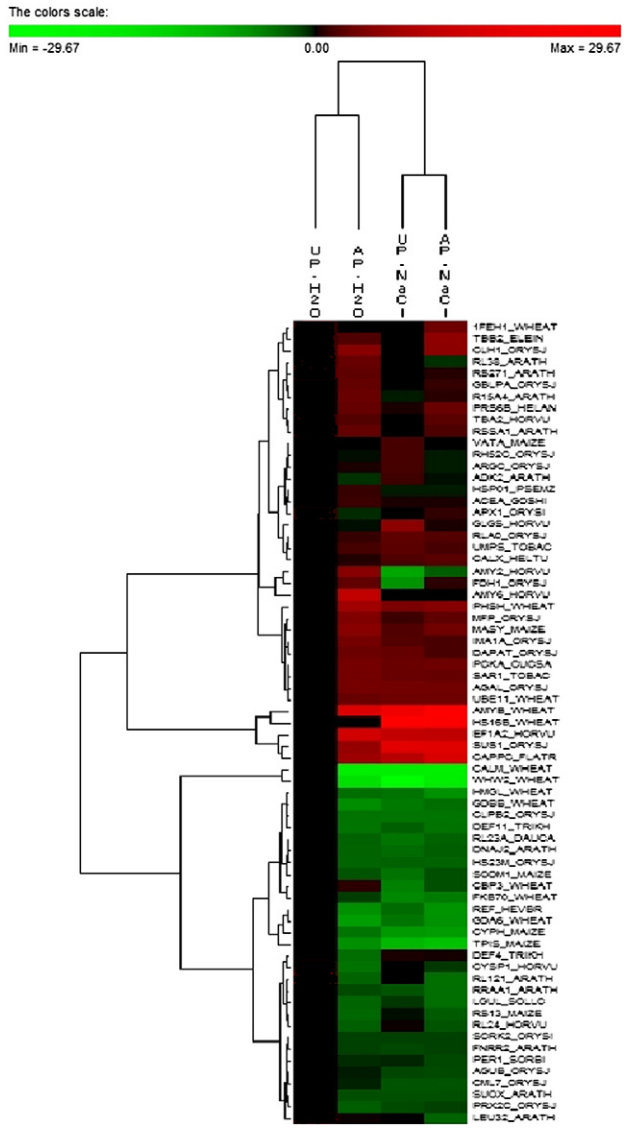

Fig. 5 – Hierarchical clustering of accumulation patterns of differentially regulated proteins Wheat embryo-surrounding tissues. Log2 based fold changes after exponential transformation of the quantitative values was used to create the heatmap. Differences in protein accumulation are shown in color as per the upper scale.

enzymes, the induction of the last two enzymes could provide the embryo with glutamate required for the proline synthesis which is a common response to salt stress [47]. This is in agreement with the increase of proline content in wheat seedlings issued from AsA-primed seeds (data not presented).

On the other hand, three proteins involved in tryptophan biosynthesis (proteins no. 67, 260, 297) were found to be decreased in abundance in UP-NaCl but not in AP-NaCl sample (Table 1; Supplementary material Table S1). Tryptophan (Trp) is an essential amino acid in plants that plays a major role in the regulation of plant development and defense responses. A tryptophan synthase β chain 1 (TSB1, protein no. 67) is responsible for the final step of Trp synthesis pathway. Previously, it has been reported that the over-expression of TSB1 in *A. thaliana* and tomato confers tolerance to cadmium, which is associated with low lipid peroxidation [48]. Anthranilate synthase (protein no. 260) is the key enzyme for Trp synthesis, which is the main substrate to produce auxin via a single intermediate, indole-3-pyruvic acid [49,50]. Recently, it has been postulated that auxin plays major role as an integrator of the activities of multiple phytohormones to control plant growth in response to environmental stress [51], suggesting that the up-regulation of anthranilate synthase could play a role in overcoming growth inhibition by salinity.

Nine proteins involved in nucleotides metabolism and cofactors biosynthesis were found to be significantly affected by salinity and AsA-priming treatments (Table 1; Supplementary material Table S1). It is widely recognized that embryos from dry seeds of durum wheat are completely devoid of AsA, while the de novo biosynthesis of AsA starts in the wheat embryos after 8–10 h of germination [52]. Salinity leads to the reduction in AsA content [53], thus pre-treatment with appropriate concentrations of AsA leads to improves salt tolerance in wheat plants, as showed in the present study (Fig. 3) and in other previous works [54,55]. So, it is not surprising that, in our results, at least three proteins involved in AsA biosynthesis (proteins no. 142, 191, 235, 258) were decreased in abundance by salinity, while AsA pre-treatment mitigates the adverse effect of NaCl on AsA metabolism. Among these proteins, the GDP-d-mannose 3′,5′-epimerase (GME, EC 5.1.3.18, pro. 258), which converts GDP-d-mannose to GDP-l-galactose, was generally considered to be a central enzyme of the major AsA biosynthesis pathway in higher plants [56]. Over-expression of GME genes leads to AsA accumulation and improves oxidative stress, cold, and salt tolerance of tomato plants [57].

Seven proteins involved in lipids metabolism were found to be changed in abundance (Table 1; Supplementary material Table S1). Acyl-[acyl-carrier-protein] desaturase acts as the first enzyme in the conversion of stearic acid to an unsaturated fatty acid. Unsaturated fatty acids are involved in membrane fluidity and normal functioning of critical integral membrane proteins [58]. Previously, it has been reported that over-expressed Acyl-ACP desaturase improved freezing tolerance significantly [59]. Because unsaturated fatty acids are the favored target of reactive oxygen species (ROS), the higher levels of unsaturated fatty acids might decrease the possibility of ROS damage to membrane lipids [60]. In agreement, while one Acyl-ACP desaturase (protein no. 184) was found to be decreased in abundance by salinity, two acyl-ACP desaturases (proteins no. 184, 121) were found to be increased in abundance by AsA-priming, a situation which may lead to an increase in unsaturated fatty acid content and enhances membrane resistance towards ROS.

4.1.2. *Energy*

Sixteen proteins belonging to “energy” category were found to be changed in abundance, with eight of them decreased in abundance by salinity. Interestingly, AsA supply do not only partially or completely alleviates the adverse influences of salinity upon these proteins, but also increases the abundance of many of them. An early event during germination is the resumption of energy metabolism. Mitochondrial respiration and energy production are processes which resume very

rapidly following imbibition of dormant seeds [61]. However, such processes may be affected by salinity. For instance, in durum wheat, it has been reported that succinate-dependent oxidative phosphorylation was significantly damaged by salinity, which may be related to the stress-induced alteration in inner mitochondrial membrane permeability, as indicated by changes in $\Delta\Psi$ profiles [62]. In agreement, four mitochondrial/chloroplastic proteins involved in electron transport (proteins no. 78, 102, 192 and 215) were found to be decreased in abundance by salinity (Table 1, Supplementary material Table S1). In the plant mitochondria, electron transfer along the respiration chain is coupled to the formation of ATP, and the redundant electron leads to the formation of ROS if the ATP synthesis is blocked [63]. Glycolysis provides intermediates for energy supply upon seed germination. In our study, 4 enzymes involved in glycolysis/Krebs cycle pathway were decreased by salinity but increased in abundance by AsA pretreatment (Table 1, Supplementary material Table S1). In line with this, it has been reported that enzymes involved in the Krebs cycle were decreased in abundance in cucumber subjected to salt stress [64]. In such circumstance plant cells inevitably undergo fermentation to fulfill the demand for energy which increases under salinity stress conditions [43,65]. Previously, it has been revealed that stress conditions such as cold, desiccation, and salinity leads to increased alcohol dehydrogenase transcripts [66]. In agreement, our results revealed that, at least, four proteins involved in fermentation, namely alcohol dehydrogenase (proteins no. 37, 69), formate dehydrogenas (protein no. 1), pyruvate decarboxylase isozyme 2 (protein no. 5) and aldehyde dehydrogenase family 2 member B4 (protein no. 40), were increased in abundance by salinity (Table 1, Supplementary material Table S1). Thus, the enhancement of glycolysis/ATP synthesis could increase the tolerance to salinity in wheat. In agreement, the over-expression of a cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene leads to a high germination rate of rice seeds under salt stress [57].

4.1.3. *Protein synthesis/cell growth and cell structure*

Protein synthesis during seed germination is generally decreased under salt stress conditions [7]. In agreement, among the nine protein synthesis-related proteins identified in our study, six of them (proteins no. 127, 129, 157, 164, 198 and 248) were found to be decreased in abundance by salinity. In contrast, the effect of NaCl was partially or completely alleviated by AsA-priming (Table 1, Supplementary material Table S1). It is recognized that during germination the translation machinery reforms rapidly following imbibition [67]. In line with this, three proteins were identified as translation initiation factors (proteins no. 129, 157 and 248). These results support the presumption that the rapid translation of stored transcripts is the main mechanism whereby the processes of early germination get underway [14,68]. In addition, by analyzing the transcriptome of wheat embryo, Wilson et al. [14] found that some of the mRNAs that accumulated in the embryo during germination, encoded carbohydrate metabolizing enzymes. Some of them are obviously involved in the synthesis of new cell walls. Consistent with this, two enzymes (proteins no. 17 and 111) involved in cell wall biogenesis were found to be decreased by salinity but increased in abundance by AsA-pre-treatment. In similar way, two enzymes (proteins no. 91 and 148) involved in cell division were also decreased by salinity and increased in abundance by AsA-priming. These findings obviously suggested that AsA attenuated salt stress-induced cell growth arrest (Fig. 3).

4.1.4. *Protein destination and storage*

Wheat germination was accompanied by an increase in mobilization of storage proteins [69]. Salinity, however, delay the mobilization of storage proteins [70]. In agreement, the quantitative analysis of germinating embryos under salinity showed that they are more abundant in proteins belonging to "protein destination and storage" category than embryos germinated in the absence of salt stress (Fig. 1; Table 1, Supplementary material Table S1). Likewise, salinity increases the abundance of three serpin Z proteins (proteins no. 63, 132 and 138), which are likely to use their irreversible inhibitory mechanism in the inhibition of proteinase capable of breaking down seed storage proteins [71]. Also, salinity decreases the abundance of a Bowman-Birk type proteinase inhibitor II-4 (protein no. 152) and two ubiquitination-related proteins (proteins no. 49 and 224), each of which belonging to a unique protein degradation system utilized by eukaryotes to efficiently degrade detrimental cellular proteins and control the entire pool of regulatory components [72]. By contrast, most of the effects of NaCl were mitigated by AsA-priming (Table 1, Supplementary material Table S1). On the other hand, under control conditions, AsA pretreatment induces the decrease in abundance of seventeen proteins, most of which involved in the protection, repair of damaged proteins, such as chaperons and heat shock proteins (proteins no. 104, 145, 238, 253 and 228), protein disulfide-isomerase (protein no. 295), serpin Z proteins (proteins no. 132 and 138) and proteases inhibitors (protein no. 152). Similar results were obtained in our previous study [18].

4.1.5. *Stress response*

ROS production by germinating seeds has often been regarded as a cause of stress that might affect the success of germination [73]. However, ROS such as H_2O_2 and NO can be beneficial or harmful for the germination, depending on the accumulation level within the embryonic cells [20]. The maintenance of the cellular ROS homeostasis requires a fine-tuned balance between ROS production and scavenging [74]. Therefore, antioxidant compounds and enzymes have been widely regarded as being of particular importance for the completion of the germination [73]. Wheat seed cells have varying protection mechanisms against oxidative stress that occurs during germination [19,20], but they seem to be disturbed by salinity [53]. Consistent with this, our results revealed that salinity decreases the abundance of several H_2O_2-scavenging enzymes, with three L-ascorbate peroxidases (proteins no. 41, 92 and 245) among them. By contrast, AsA-priming partially or completely alleviates the effects of salinity upon these enzymes. On the other hand, salinity up-regulates two enzymes involved in detoxification, namely 1-Cys peroxiredoxin (1-Cys Prx, protein no. 292) and glutathione S-transferase (protein no. 204). Seed germination and

seedling salt tolerance were improved after over-expression of GST in arabidopsis [75] and tobacco [76]. Previously, it has been suggested that the higher amount of oxidized glutathione (GSSG) in dry embryos compared to germinating seeds could contribute to prevent the germination process, since in dormant wheat embryos GSSG seemed to blocks protein synthesis [77]. 1-Cys Prx is localized in the nuclei of aleurone and scutellum cells [78]. This enzyme is a peroxidase specifically and highly produced in seeds and seems to be involved in the inhibition of germination particularly under salt, osmotic and oxidative stress conditions [79]. Interestingly, AsA-priming seems to decrease the abundance of the last two enzymes under stress conditions.

4.2. *Metabolic proteome of embryo-ST as affected by salinity and AsA-priming*

As expected, only 69 proteins were identified as changed in abundance in embryo-ST, thus reflecting a minor effect of salinity and AsA pre-treatments on these tissues. In agreement, Almansouri et al. [6] revealed that isolated embryos are more affected by salinity than whole seeds. Based upon the cluster analysis and the functional distribution of identified proteins only two protein categories represented the most important variation in proteins accumulation between the treatments. However, it is worth noting that the detection of a small number of storage proteins reflected the efficiency of the fractionation procedure adopted in this study, and indicating that the samples were not contaminated with one another.

In wheat, the non-gluten protein classes, albumins and globulins, represent a smaller percentage of total endosperm protein and have mainly metabolic activity or structural functions [26]. Even though wheat seed storage proteins were not considered in the present study, salinity down-regulates nine proteins involved in proteolysis and folding stability (proteins no. 81, 88, 104, 108, 60, 118, 86, 65 and 47). By contrast, AsA-priming mitigates partially or completely the effect of NaCl on six of them (Table 2, Supplementary material Table S2). As mentioned above, seed germination was accompanied by mobilization of storage proteins, which is decreased in salinity condition [70]. Also, seed imbibition was accompanied by the loss of desiccation tolerance which may explain the decrease in abundance of proteins induced by all the treatments examined.

Previously, it has been postulated that oxidative status is more pronounced in the embryos than in the endosperm [77]. In line with this, in embryo-ST, only eleven proteins belonging to the "disease/defense" category were found to be changed in abundance. Of them, seven proteins were decreased in abundance by salinity, most of which are involved in detoxification, such as superoxide dismutase [Mn] 3.1 (SOD, protein no. 96), L-ascorbate peroxidase 1 (APX, protein no. 138), sulfite oxidase (protein no. 54) and peroxiredoxin-2C (protein no. 145). In wheat, seed imbibition and germination are believed to be associated with enhanced cellular capacity to detoxify H_2O_2 [20]. For this detoxification the operation of AsA peroxidase together with the AsA-regenerating enzymes appears to be of particular importance [80]. These increases, particularly for AsA peroxidase, were much higher in the embryo than in the endosperm [80]. Consistent with this, compared to the embryo, in embryo-ST all the enzymes involved in ROS-scavenging were decreased in abundance by salinity. The negative effect of salinity on SOD activity was reported in several salt-sensitive species or varieties such as in rice [81], pea [82], and cowpea [83]. Thus, given the salt-sensitivity of the wheat genotype used in the present study, it is not surprising to find that salinity decreases the abundance of many ROS-scavenging enzymes such as SOD and APX. However, it is noteworthy that in absence of stress, AsA decreases considerably (9.5-fold) the abundance of peroxiredoxin-2C (protein no. 145). Similar results were obtained in Arabidopsis [84].

5. Conclusions

In this study, the impact of ascorbate priming upon wheat seed metabolic proteome during germination under saline and non-saline conditions was evaluated using shotgun proteomic approach. The results revealed that the decrease in germination induced by salinity was accompanied by a significant variation in metabolic protein abundance, which presumably explain the salt-induced dormancy of wheat seeds. However, given the protective effects of ascorbate on salinity damage, it is suggested that ascorbate pretreatment lead to partial reorganization of embryo-gene expression allowing germinating embryos/seedlings to survive early salt stress. Methionine, auxin (maybe other phytohormones) metabolism, ROS managing and signaling seem to play an important role in the modulation of this process.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jprot.2014.04.040.

Transparency document

Transparency Document associated with this article can be found, in the online version.

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