

## ON-LINE SUPPLEMENTARY MATERIAL

Pavoković D., Horvatić A., Tomljanović I., Balen B., Krsnik-Rasol M.: Sugar beet cells' cellular and extracellular events taking place in response to drought and salinity. Acta Bot. Croat., DOI: 10.37427/botcro-2023-008.

**On-line Suppl. Tab. 1.** Salt-responsive (300 mM NaCl) cellular proteins of sugar beet N cell line identified by MALDI-TOF/TOF MS. Accession No. – accession number from NCBI database, MW – protein molecular weight in kDa, Prot. pI – protein isoelectric point, Prot. score – protein score from Peptide Mass Fingerprint (PMF), Total ion score – protein ion score from MS/MS, FunCat - a functional annotation scheme for systematic classification of proteins from whole genomes. The Gene Ontology (GO) analysis was derived through Uniprot hit accessions for all protein identifications according to categories which describe Biological process, Molecular function, and Cellular compartment. \*20 cellular transport, transport facilitation and transport routes, \*30 cellular communication/signal transduction mechanism. Arrows indicate proteins that were up- (↑) or down-regulated (↓) compared to control on 2-DE gels.

Spot no.	Protein name	Accession no.	MW (kDa)	Prot. pI	Prot. score	Total ion score	Fold change	FunCat	Biological process	Molecular function	Cellular component
22	Malate dehydrogenase, cytoplasmic [ <i>Beta vulgaris</i> ]	gi 11133601	35.4	5.89	280	254	1.48 ↑	01 metabolism	malate metabolic process	L-malate dehydrogenase activity	cytoplasm
25	putative quinone reductase, partial [ <i>Vitis vinifera</i> ]	gi 37724581	17.6	5.59	205	205	1.62 ↑	01 metabolism	response to oxidative stress	NAD(P)H dehydrogenase (quinone) activity	cytoplasm
27	malate dehydrogenase, mitochondrial [ <i>Eucalyptus gunnii</i> ]	gi 473206	36.4	8.72	115	115	1.52 ↑	01 metabolism	malate metabolic process	L-malate dehydrogenase activity	mitochondrion
31	aldolase superfamily protein [ <i>Arabidopsis thaliana</i> ]	gi 15226185	42.3	8.19	138	118	0.58 ↓	01 metabolism	fructose 1,6-bisphosphate metabolic process	fructose-bisphosphate aldolase activity	chloroplast
32	fructose-bisphosphate aldolase [ <i>Secale cereale</i> ]	gi 226316439	38.8	6.39	208	173	2.28 ↑	01 metabolism	glycolytic process	fructose-bisphosphate aldolase activity	cytoplasm
5	putative ATP synthase beta subunit [ <i>Oryza sativa</i> Japonica Group] - mitochondrial	gi 56784992	45.2	5.26	543	498	0.67 ↓	02 energy	proton motive force-driven ATP synthesis	ATP binding	mitochondrion
11	transitional endoplasmic reticulum ATPase [ <i>Arabidopsis thaliana</i> ]	gi 11265361	93.5	5.37	368	338	0.47 ↓	02 energy	autophagosome maturation	ATP binding	endoplasmic reticulum
26	AtpB (Fragment) [ <i>Jensenia decipiens</i> ] - chloroplast	gi 61398932	2.5	9.5	24	-	1.63 ↑	02 energy	proton motive force-driven ATP synthesis	ATP binding	chloroplast
34	ATPase, V1 complex, subunit B protein [ <i>Arabidopsis thaliana</i> ]	gi 15233891	54.2	5.03	354	346	0.60 ↓	02 energy	ATP metabolic process	ATP binding	vacuole
35	23kDa polypeptide of the oxygen evolving complex of photosystem II [ <i>Sonneratia caseolaris</i> ]	gi 146454488	24.9	5.68	69	69	1.38 ↑	02 energy	photosynthesis	calcium ion binding	chloroplast
8	mitochondrial HSO70 2 [ <i>Arabidopsis thaliana</i> ]	gi 15242459	72.9	5.63	53	38	0.25 ↓	32 cell rescue, defense and virulence	response to unfolded protein	ATP binding	mitochondrion

**On-line Suppl. Tab. 1. continued**

9	putative lactoylglutathione lyase [ <i>Brassica rapa</i> ] (Glyoxalase I)	gi 157890952	31.8	5.37	115	115	0.52 ↓	32 cell rescue, defense and virulence	methylglyoxal catabolic process to D-lactate	lactoyl glutathione lyase activity	cytoplasm
14	60 kDa chaperonin subunit beta - garden pea chloroplast	gi 2506277	62.9	5.85	344	343	0.53 ↓	32 cell rescue, defense and virulence	response to unfolded protein	ATP binding	chloroplast
15	Stromal ascorbate peroxidase [ <i>Spinacia oleracea</i> ]	gi 2832921	39.5	8.46	174	156	2.00 ↑	32 cell rescue, defense and virulence	response to oxidative stress	heme binding	chloroplast
19	Stromal ascorbate peroxidase [ <i>Spinacia oleracea</i> ]	gi 2832921	39.5	8.46	227	205	3.85 ↑	32 cell rescue, defense and virulence	response to oxidative stress	heme binding	chloroplast
23	Heat shock protein hsp70 [ <i>Saussurea medusa</i> ]	gi 56554815	70.8	5.11	471	440	0.45 ↓	32 cell rescue, defense and virulence	response to unfolded protein	ATP binding	cytoplasm
28	Glutathione S-transferase 6 [ <i>Brassica juncea</i> ]	gi 31790105	23.9	7.15	51	38	1.48 ↑	32 cell rescue, defense and virulence	detoxification	glutathione transferase activity	cytoplasm
29	ascorbate peroxidase [ <i>Spinacia oleracea</i> ]	gi 310587	27.6	5.41	103	95	1.14 ↑	32 cell rescue, defense and virulence	response to oxidative stress	heme binding	cytoplasm
7	bZIP transcription factor [ <i>Micromonas pusilla</i> CCMP1545]	gi 303274372	31.4	9.55	44	-	1.59 ↑	11 transcription	positive regulation of transcription by RNA polymerase II	DNA binding	nucleus
21	PREDICTED: proteasome subunit alpha type-1-A [ <i>Ricinus communis</i> ]	gi 255538698	30.3	4.89	246	237	0.19 ↓	14 protein fate	proteasomal protein catabolic process	hydrolase activity	cytoplasm
18	PREDICTED: NADH-cytochrome b5 reductase-like protein [ <i>Ricinus communis</i> ]	gi 255566959	35.4	8.87	57	44	1.89 ↑	20 cellular transport <sup>#</sup>	sterol biosynthetic process	cytochrome-b5 reductase activity, acting on NAD(P)H	endoplasmic reticulum
1	RACK1 [ <i>Phaseolus vulgaris</i> ]	gi 258406674	35.6	7.6	116	94	0.65 ↓	30 cellular communication*	cell cycle	ribosome binding	nucleus
20	PREDICTED: UMP-CMP kinase 3 [ <i>Vitis vinifera</i> ]	gi 225454048	23.5	5.14	60	27	1.82 ↑	01 metabolism	'de novo' pyrimidine nucleobase biosynthetic process	ATP binding	cytoplasm
3	hypothetical protein [ <i>Oryza sativa</i> Japonica Group]	gi 46805573	21.1	12	40		5.81 ↑	30 cellular communication*	unknown	unknown	cytoplasm
13	Nucleic acid-binding, OB-fold-like protein [ <i>Arabidopsis thaliana</i> ]	gi 1063685975	15.1	9.04	43	35	1.51 ↑	-	fork protection and fork restart	unknown	nucleus

**On-line Suppl. Tab. 2.** Mannitol-responsive (600 mM mannitol) cellular proteins of sugar beet N cell line identified by MALDI-TOF/TOF MS. Accession No. – accession number from NCBI database, MW – protein molecular weight in kDa, Prot. pI – protein isoelectric point, Prot. score – protein score from Peptide Mass Fingerprint (PMF), Total ion score – protein ion score from MS/MS, FunCat - a functional annotation scheme for systematic classification of proteins from whole genomes. The Gene Ontology (GO) analysis was derived through Uniprot hit accessions for all protein identifications according to categories which describe Biological process, Molecular function, and Cellular compartment. \*20 cellular transport, transport facilitation and transport routes, \*30 cellular communication/signal transduction mechanism. Arrows indicate proteins that were up- (↑) or down-regulated (↓) compared to control on 2-DE gels.

Spot no.	Protein name	Accession no.	MW (kDa)	Prot. pI	Prot. score	Total ion score	Fold change	FunCat	Biological process	Molecular function	Cellular component
34	Malate dehydrogenase, cytoplasmic	gi 11133601	35.41	5.89	586	570	1.57 ↑	01 metabolism	malate metabolic process	L-malate dehydrogenase activity	cytoplasm
35	Malate dehydrogenase, mitochondrial	gi 1170898	36.44	8.72	106	106	1.55 ↑	01 metabolism	malate metabolic process	L-malate dehydrogenase activity	mitochondrial matrix
43	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	gi 548533	61.03	5.98	123	123	0.47 ↓	01 metabolism	glucose catabolic process	2,3-bisphosphoglycerate-independent phosphoglycerate mutase activity (iPGAM)	cytoplasm
46	glyceraldehyde-3-phosphate dehydrogenase [ <i>Beta vulgaris</i> ]	gi 125662890	36.65	6.77	574	559	0.42 ↓	01 metabolism	glucose metabolic process	NAD binding	mitochondrial matrix
56	glyceraldehyde-3-phosphate dehydrogenase [ <i>Capsicum annuum</i> ]	gi 18072805	44.40	8.9	177	177	0.43 ↓	01 metabolism	glucose metabolic process	NAD binding	mitochondrial matrix
10	ferredoxin--NADP reductase, root isozyme [ <i>Zea mays</i> ]	gi 195640470	41.80	8.86	76	37	1.68 ↑	02 energy	photosynthesis	ferredoxin-NADP+ reductase activity	chloroplast
31	oxygen evolving complex 33 kDa photosystem II protein [ <i>Nicotiana tabacum</i> ]	gi 30013657	35.18	5.63	344	323	1.63 ↑	02 energy	photosynthesis	oxygen evolving activity	chloroplast
19	Mn-superoxide dismutase II [ <i>Helianthus annuus</i> ]	gi 166029873	25.36	7.1	270	258	1.82 ↑	32 cell rescue, defense and virulence	cellular response to oxidative stress	manganese ion binding	mitochondrial matrix
42	glutathione S-transferase F3 [ <i>Arabidopsis thaliana</i> ]	gi 15227063	24.11	6.25	78	62	1.57 ↑	32 cell rescue, defense and virulence	defense response to infection	glutathione binding	cytoplasm
45	heat shock 70 protein [ <i>Spinacia oleracea</i> ]	gi 2654212	72.33	5.54	316	294	0.59 ↓	32 cell rescue, defense and virulence	protein folding, response to stress	ATP binding	cytoplasm
49	heat shock protein hsp70 [ <i>Saussurea medusa</i> ]	gi 56554815	70.82	5.11	512	479	0.42 ↓	32 cell rescue, defense and virulence	protein folding, response to stress	ATP binding	cytoplasm

**On-line Suppl. Tab. 2. continued**

54	stromal ascorbate peroxidase [ <i>Spinacia oleracea</i> ]	gi 2832921	39.52	8.46	278	262	1.94 ↑	32 cell rescue, defense and virulence	cellular response to oxidative stress	heme binding	cytoplasm
24	telomerase reverse transcriptase catalytic subunit [ <i>Arabidopsis thaliana</i> ]	gi 5852939	130.47	9.62	38		1.59 ↑	10 cell cycle and DNA processing	telomere maintenance	metal ion binding	nucleus
39	condensin complex component [ <i>Micromonas commoda</i> ]	gi 255072357	143.04	5.53	59		2.40 ↑	10 cell cycle and DNA processing	meiotic cell cycle	ATP binding	nucleus
48	putative glycine-rich RNA binding protein-like [ <i>Solanum tuberosum</i> ]	gi 77416975	17.63	8.48	42	42	2.52 ↑	11 transcription	-	RNA binding	membrane
27	potassium channel beta subunit, partial [ <i>Platanus x hispanica</i> ]	gi 110681470	12.17	8.62	113	95	2.99 ↑	20 cellular transport*	unknown	K <sup>+</sup> transport	membrane
36	putative metallophosphatase [ <i>Lupinus luteus</i> ]	gi 52353232	70.96	6.1	100	100	0.40 ↓	30 cellular communica- tion*	response to low phosphate in cell	acid phosphatase activity	vacuole
55	CBS domain-containing protein CBSX3, mitochondrial [ <i>Arabidop- sis lyrata</i> subsp. <i>lyrata</i> ]	gi 297811195	22.72	9.12	60	59	1.58 ↑		cell redox homeostasis	ligand binding	cytoplasm
12	PREDICTED: probable aldo-keto reductase 4 [ <i>Vitis vinifera</i> ]	gi 731390561	37.52	6.11	146	137	1.51 ↑	01 metabolism	alleviation cellular stress	carbonyls reduction or reverse oxidation reactions	cytoplasm
20	unknown [ <i>Medicago truncatula</i> ]	gi 217075801	52.01	6.21	126	120	0.48 ↓	01 metabolism	histidine biosynthetic process	histidinol dehydroge- nase activity	chloroplast
11	predicted protein [ <i>Physcomitrella patens</i> subsp. <i>patens</i> ] (probable NADH dehydrogenase [ubiqui- none] 1 alpha subcomplex subunit 5, mitochondrial)	gi 168051724	15.36	5.24	92	92	1.83 ↑	02 energy	transcriptional regulators	NADH-ubiquinone oxidoreductase-related	nucleus
14	PREDICTED: ubiquitin receptor RAD23c isoform X2 [ <i>Vitis vinifera</i> ]	gi 225423953	41.92	4.73	120	120	1.44 ↑	10 cell cycle and DNA processing	nucleotide-excision repair	damaged DNA binding	cytoplasm
25	PREDICTED: uncharacterized protein LOC100266186 [ <i>Vitis vinifera</i> ]	XP_010663768	21.43	9.45	58		0.52 ↓	10 cell cycle and DNA processing	unknown	unknown	membrane

## On-line Suppl. Tab. 2. continued

1	probable myosin heavy chain [imported] [ <i>Arabidopsis thaliana</i> ]	gi 25408221	141.92	4.82	76		1.72 ↑	16 protein with binding function or cofactor requirement	defense response to infection	binds phospholipid vesicles	endoplasmic reticulum
21	unknown [ <i>Picea sitchensis</i> ]	gi 116781285	25.48	9.11	89	89	1.73 ↑	32 cell rescue, defense and virulence	cellular response to oxidative stress	thioredoxin peroxidase activity	cytoplasm
30	hypothetical protein SELMO- DRAFT_143805 [ <i>Selaginella moellendorffii</i> ]	gi 302765483	30.14	6.71	82	82	1.95 ↑	42 biogenesis of cellular components	monosaccharide biosynthetic process	3-deoxy-8-phos- phooctulonate synthase activity	cytoplasm
22	predicted protein [ <i>Micromonas commoda</i> ]	gi 255075951	41.01	4.82	14	14	0.62 ↓		defense response to fungus	hydrolase activity	extracellular
37	predicted protein [ <i>Physcomitrella patens</i> subsp. <i>patens</i> ]	gi 168024045	93.13	9.69	57		0.56 ↓		nuclear gateway traffic	Zn-finger in Ran binding protein and others	nuclear membrane
47	hypothetical protein POPTR_0017s08550g [ <i>Populus trichocarpa</i> ]	gi 566212203	69.97	9.44	65		0.34 ↓		cell wall modification	4 iron, 4 sulfur cluster binding	Casparian strip
51	unknown		0.00				2.52 ↑		unknown	unknown	unknown
53	unknown		0.00				1.64 ↑		unknown	unknown	unknown

**On-line Suppl. Tab. 3.** Salt- (300 mM NaCl) and mannitol-responsive (600 mM mannitol) extracellular proteins of sugar beet N cell line identified by MALDI-TOF/TOF MS. Accession No. – accession number from NCBI database, MW – protein molecular weight in kDa, Prot. pI – protein isoelectric point, Prot. score – protein score from Peptide Mass Fingerprint (PMF), Total ion score – protein ion score from MS/MS, FunCat - a functional annotation scheme for systematic classification of proteins from whole genomes. The Gene Ontology (GO) analysis was derived through Uniprot hit accessions for all protein identifications according to categories which describe Biological process and Molecular function.

Spot no.	Protein name	Accession no.	MW (kDa)	Protein pI	Protein score	Total ion score	FunCat	Biological process	Molecular function
E1	beta-D-xylosidase 4 [ <i>Arabidopsis thaliana</i> ]	gi 15237736	84.3	7.75	68	60	42 biogenesis of cellular components	arabinan catabolic process	alpha-L-arabinofuranosidase activity
E2	<i>Arabidopsis thaliana</i> unknown protein (At1g41830) mRNA, complete cds	gi 22136093	60.5	9.37	102	86	42 biogenesis of cellular components	cell tip growth	copper ion binding
E3	AT1G76160 [ <i>Arabidopsis thaliana</i> ]	gi 222423158	60.0	8.49	106	87	42 biogenesis of cellular components	root growth	copper ion binding
E4	Chitinase precursor [ <i>Beta vulgaris</i> subsp. <i>vulgaris</i> ]	gi 75315114	28.3	8.59	482	446	32 cell rescue, defense and virulence	carbohydrate metabolic process	chitin binding
E5	–						unknown	unknown	unknown