

Microsome-associated proteome modifications of *Arabidopsis* seedlings grown on board the International Space Station reveal the possible effect on plants of space stresses other than microgravity

Christian Mazars^{1,†,*}, Christian Brière^{1,†}, Sabine Grat¹, Carole Pichereaux², Michel Rossignol², Veronica Pereda-Loth³, Brigitte Eche³, Elodie Boucheron-Dubuisson⁴, Isabel Le Disquet⁴, Francisco Javier Medina⁵, Annick Graziana¹, and Eugénie Carnero-Diaz⁴

¹Université de Toulouse UPS; CNRS UMR5546 Laboratoire de Recherches en Sciences Végétales; Castanet-Tolosan, France; ²Fédération de Recherche 3450 Agrobiosciences Interactions et Biodiversités Plateforme Protéomique Génopole Toulouse Midi Pyrénées Institut de Pharmacologie et de Biologie Structurale IPBS CNRS; Toulouse France; ³GSBMS; Université de Toulouse; Toulouse France; ⁴Institut de Systématique, Evolution, Biodiversité; UMR 7205 ISYEB; Université Pierre et Marie Curie-CNRS-MNHN-EPHE; Paris, France; ⁵Centro de Investigaciones Biológicas CSIC; Madrid, Spain

[†]These authors contributed equally to the work.

Keywords: spaceflight, International Space Station, quantitative proteomics, label-free, *Arabidopsis thaliana*, membrane proteins, gravity

Growing plants in space for using them in bioregenerative life support systems during long-term human spaceflights needs improvement of our knowledge in how plants can adapt to space growth conditions. In a previous study performed on board the International Space Station (GENARA A experiment STS-132) we assessed the global changes that microgravity can exert on the membrane proteome of *Arabidopsis* seedlings. Here we report additional data from this space experiment, taking advantage of the availability in the EMCS of a centrifuge to evaluate the effects of cues other than microgravity on the relative distribution of membrane proteins. Among the 1484 membrane proteins quantified, 227 proteins displayed no abundance differences between μg and $1 g$ in space, while their abundances significantly differed between $1 g$ in space and $1 g$ on ground. A majority of these proteins (176) were over-represented in space samples and mainly belong to families corresponding to protein synthesis, degradation, transport, lipid metabolism, or ribosomal proteins. In the remaining set of 51 proteins that were under-represented in membranes, aquaporins and chloroplastic proteins are majority. These sets of proteins clearly appear as indicators of plant physiological processes affected in space by stressful factors others than microgravity.

Envisioning the use of plants as life support for long-term human spaceflights and space exploration still requires a serious commitment in studies devoted to the knowledge of plant behavior in a spatial environment in order to determine optimal conditions for their growth and development in this unusual environment.¹⁻³ During evolution, terrestrial plants have adapted to the ground gravity to optimize their development on earth, but they are still able to sense changes in the gravity vector and mount appropriate responses.⁴ But, beside the lack of gravity, the spatial environment introduces new conditions that could have an effect on plant behavior, such as confined culture conditions, cosmic radiations, lack of convection, gaseous environment,⁵⁻⁹ and maybe some others factors likely still

not suspected. Most of these factors are different or absent on ground and thus should also be considered in the interpretation of spaceflight experiments. For instance, during the Space Shuttle mission STS-81⁶ it was observed that seedlings grown both on the $1-g$ in-flight centrifuge and μg displayed a phenotype similar to seedlings exposed to high levels of ethylene (shorter seedlings, greater root hair density, and anomalous hook formation on hypocotyls). The ethylene hypothesis could be tested on the next space experiment (STS-84) where a similar phenotype was obtained for flight-grown seedlings using the $1-g$ in-flight centrifuge and seedlings grown on ground and exposed to ethylene. Such high suspected levels of ethylene could be confirmed by measurements in the cabin atmosphere

*Correspondence to: Christian Mazars; Email: mazars@lrsv.ups-tlse.fr

Submitted: 06/04/2014; Revised: 06/17/2014; Accepted: 06/18/2014; Published Online: 07/16/2014

Citation: Mazars C, Brière C, Grat S, Pichereaux C, Rossignol M, Pereda-Loth V, Eche B, Boucheron-Dubuisson E, Le Disquet I, Medina FJ, et al. Microsome-associated proteome modifications of *Arabidopsis* seedlings grown on board the International Space Station reveal the possible effect on plants of space stresses other than microgravity. *Plant Signaling & Behavior* 2014; 9:e29637; PMID: 25029201; <http://dx.doi.org/10.4161/psb.29637>

Table 1. Proteins identified by LC-MS/MS and whose abundances were significantly changed (P values < 0.05) in microsomal extracts of 12-d-old *Arabidopsis* seedlings grown under 1 g on board the International Space Station as compared with 1 g ground control

A. Proteins under-represented in space conditions. Ratio (ISS 1 g / ground 1 g) < 1			
UNIPROT	AGI	Ratio	Annotation
Lipid metabolism			
A4GNA8	AT4G25970	0.549	phosphatidylserine decarboxylase 3
Q9SZP6	AT4G38690	0.554	1-phosphatidylinositol phosphodiesterase-related protein
Q8LDH5		0.585	endomembrane-associated protein
Transporters			
P23586	AT1G11260	0.370	sugar transporter 1
Q8LGU1	AT3G21250	0.456	multidrug resistance-associated protein 6
Q56ZZ7	AT5G16150	0.523	Plastidic glucose transporter 4
Q53XH7	AT5G62670	0.553	H(+)-ATPase 11
Q94FB9	AT4G39850	0.559	ABC transporter D family member 1
Q9SE45	AT2G38760	0.587	annexin D3
Aquaporins			
Q41975	AT4G17340	0.219	putative aquaporin TIP2-2
Q08733	AT1G01620	0.306	aquaporin PIP1-3
P61837	AT3G61430	0.352	aquaporin PIP1-1
Q41951	AT3G16240	0.373	aquaporin TIP2-1
P43287	AT2G37170	0.418	aquaporin PIP2-2
Q39196	AT4G00430	0.506	putative aquaporin PIP1-4
Q8LAA6	AT4G23400	0.526	putative aquaporin PIP1-5
Proteases, proteasome			
O23712	AT1G47250	0.257	proteasome subunit α type-1-B
Q42044	AT2G45180	0.532	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
Ribosomal proteins			
P36212	AT3G27850	0.510	50S ribosomal protein L12-3
P56802	ATCG00750	0.525	ribosomal protein S11
Q93VC7	AT5G30510	0.578	small subunit ribosomal protein S1
Chloroplastic proteins			
Q949S6	AT1G14345	0.371	NAD(P)-linked oxidoreductase-like protein
P56778	ATCG00280	0.391	photosystem II 44 kDa protein
Q8H124	AT2G34460	0.454	NAD(P)-binding Rossmann-fold-containing protein
Q9FL44	AT5G07020	0.510	proline-rich family protein
P10797	AT5G38420	0.518	ribulose biphosphate carboxylase small chain 2B
Chaperones			
Q7XAR9	AT5G42480	0.346	chaperone DnaJ-domain containing protein
P42763	AT1G76180	0.417	dehydrin ERD14
Miscellaneous			
Q9SSK7	AT1G70850	0.218	MLP-like protein 34
Q9SD07	AT3G51420	0.469	strictosidine synthase-like 4 protein
Q9S JL9	AT2G36870	0.512	xyloglucan:xyloglucosyl transferase
P25858	AT3G04120	0.527	glyceraldehyde-3-phosphate dehydrogenase, cytosolic
Q38814	AT5G54770	0.530	thiazole biosynthetic enzyme

Table 1. Proteins identified by LC-MS/MS and whose abundances were significantly changed (P values < 0.05) in microsomal extracts of 12-d-old *Arabidopsis* seedlings grown under 1 g on board the International Space Station as compared with 1 g ground control (continued)

Q94BW0		0.534	At1G35160/T32G9_30
Q9SDM9	AT3G16400	0.546	Nitrile-specifier protein 1
Q0WLF5		0.548	cytosolic O-acetylserine(thiol)lyase
Q94CE4	AT1G70410	0.551	β carbonic anhydrase 4
P12411	AT1G75780	0.562	tubulin β
B9DGZ4	AT1G13440	0.565	Glyceraldehyde-3-phosphate dehydrogenase GAPC2
Q8L6Z6		0.583	cytochrome P450 71B5
Q9LT39	AT3G20820	0.587	leucine-rich repeat-containing protein
Unknown proteins			
Q9LYM7		0.143	putative protein
Q9LHA3	AT3G28720	0.314	uncharacterized protein
Q9SVW4	AT4G18070	0.347	putative protein
Q94F20	AT5G25460	0.422	uncharacterized protein
Q9SS37	AT3G10260	0.435	reticulon-like protein B8
Q9SLJ2	AT1G54410	0.502	dehydrin family protein
Q8L604	AT1G65230	0.502	uncharacterized protein
Q96316	AT3G60280	0.540	uclacyanin 3
Q9FKA5	AT5G39570	0.559	uncharacterized protein
O80821	AT2G41470	0.592	unknown protein
B. Proteins over-represented in space conditions. Ratio (ISS 1 g / ground 1 g) > 1			
UNIPROT	AGI	Ratio	Annotation
Proteases, Proteasome			
Q84WU8	AT2G29080	2.721	cell division protease ftsH-3
Q0WQQ6	AT5G23540	2.343	26S proteasome non-ATPase regulatory subunit 14
Q6EMB6	AT1G20200	2.217	26S proteasome regulatory subunit N3
Q9XI05	AT1G21720	2.166	proteasome subunit β type-3-A
Q6XJG8	AT4G28470	2.117	26S proteasome regulatory subunit S2 1B
O81149	AT1G53850	2.075	proteasome subunit α type-5-A
Q8W4A0	AT3G02200	1.987	Proteasome component (PCI) domain protein
Q9FGM0	AT5G53170	1.928	cell division protease ftsH-11
Q42134	AT3G14290	1.919	proteasome subunit α type-5-B
P42742	AT3G60820	1.891	proteasome subunit β type-1
Q2V3D4	AT4G31300	1.885	proteasome subunit β type-6
O24412	AT5G05780	1.872	26S proteasome regulatory subunit N8
O81062	AT2G03120	1.831	minor histocompatibility antigen H13
P57681	AT5G63910	1.776	prenylcysteine oxidase
Q9SEI4	AT5G58290	1.731	regulatory particle triple-A ATPase 3
O81148	AT3G22110	1.724	proteasome subunit α type-4
Q9C9C0	AT1G73990	1.717	signal peptide peptidase
Ribosomal proteins			
Q8RWT4	AT2G42710	2.481	ribosomal protein 0.1/L10 family protein
P59233	AT3G62250	2.240	ubiquitin-40S ribosomal protein S27a-3
P49692	AT2G47610	2.117	60S ribosomal protein L7a-1

Table 1. Proteins identified by LC-MS/MS and whose abundances were significantly changed (P values < 0.05) in microsomal extracts of 12-d-old *Arabidopsis* seedlings grown under 1 g on board the International Space Station as compared with 1 g ground control (continued)

P60040	AT2G01250	2.113	60S ribosomal protein L7-2
Q9M352	AT3G53740	2.090	60S ribosomal protein L36-2
P59230	AT2G27530	2.030	60S ribosomal protein L10a-2
Q9SKZ3	AT2G32060	1.986	40S ribosomal protein S12-2
Q08682	AT1G72370	1.971	40S ribosomal protein Sa-1
Q9M339	AT3G53870	1.880	40S ribosomal protein S3-2
B9DGN3	AT2G27710	1.871	60S acidic ribosomal protein P2-2
Q9C9C5	AT1G74050	1.862	60S ribosomal protein L6-3
B9DHP6	AT3G25520	1.856	ribosomal protein
Q9LRX8	AT3G24830	1.836	60S ribosomal protein L13a-2
Q9LZH9	AT3G62870	1.834	60S ribosomal protein L7a-2
Q9SIP7	AT2G31610	1.817	40S ribosomal protein S3-1
P51418	AT2G34480	1.809	60S ribosomal protein L18a-2
P42733	AT5G23740	1.794	40S ribosomal protein S11-3
P57691	AT3G11250	1.754	60S acidic ribosomal protein P0-3
Q9FF90	AT5G23900	1.727	60S ribosomal protein L13-3
Q8LD46	AT2G39460	1.724	60S ribosomal protein L23a-1
Protein synthesis			
O04485		2.830	Strong similarity to <i>S. pombe</i> leucyl-tRNA synthetase (gb Z73100)
Q9C5Z2	AT1G10840	1.887	translation initiation factor eIF-3 subunit 3
Q9LD55	AT4G11420	1.769	translation initiation factor eIF-3 subunit 10
O04630	AT5G26830	1.704	threonyl-tRNA synthetase
Transcription regulation			
Q8LEZ4	AT1G19520	3.020	pentatricopeptide repeat-containing protein
Q94AH9	AT4G25630	2.660	rRNA 2'-O-methyltransferase fibrillarin 2
O04379	AT1G48410	2.367	protein argonaute
Q9FZ48	AT1G16890	1.949	ubiquitin-conjugating enzyme E2 36
Transport			
Q9C5H6	AT3G59360	5.597	UDP-galactose transporter 6
Q8H0U4	AT2G16950	2.456	transportin 1
Q84L08	AT5G19980	2.297	golgi nucleotide sugar transporter 4
Q84WD8		2.195	putative Na ⁺ dependent ileal bile acid transporter
Q84WD8		2.195	putative Na ⁺ dependent ileal bile acid transporter
Q8L9P5		2.142	putative peroxisomal membrane carrier protein
Q8GX78	AT4G00370	2.089	putative anion transporter 2
Q39232	AT1G71880	2.051	sucrose transport protein SUC1
Q9LHG9	AT3G12390	1.953	Nascent polypeptide-associated complex subunit α -like protein 1
Q9FNC9	AT5G43970	1.923	mitochondrial import receptor subunit TOM22-2
Q9SKX0		1.904	ABC transporter C family member 13
Q9SL05	AT2G05620	1.788	protein PROTON GRADIENT REGULATION 5
Q9C8G9	AT1G30400	1.752	ABC transporter C family member 1
O04619	AT4G01100	1.704	adenine nucleotide transporter 1
ATPases			

Table 1. Proteins identified by LC-MS/MS and whose abundances were significantly changed (P values < 0.05) in microsomal extracts of 12-d-old *Arabidopsis* seedlings grown under 1 g on board the International Space Station as compared with 1 g ground control (continued)

P83484	AT5G08690	2.239	ATP synthase subunit β -2
P56758	ATCG00150	2.028	ATP synthase CF0 A subunit Chloroplastic
Q04613	ATMg00640	1.852	ATP synthase protein MI25
B9DI55	AT3G09840	1.827	cell division control protein 48-A
P92549		1.807	ATP synthase subunit α , mitochondrial
Q96252	AT5G47030	1.695	ATP synthase subunit delta'
CytC Oxidase			
Q9FKT8	AT5G56090	2.544	cytochrome c oxidase subunit XV assembly protein
Q95SB8	AT1G80230	2.470	cytochrome c oxidase subunit Vb
Q957L9	AT1G22450	1.825	cytochrome C oxidase 6B
TCA cycle			
O82662	AT2G20420	2.362	Succinyl-CoA ligase [GDP-forming] subunit β
O82663	AT5G66760	1.921	succinate dehydrogenase [ubiquinone] flavoprotein subunit 1
Q9SIB9	AT2G05710	1.718	aconitate hydratase 2
P20115	AT2G44350	1.694	citrate synthase 4
Sugar metabolism			
Q9C8Y9	AT1G66280	2.257	β -glucosidase 22
Q9C525	AT1G66270	2.150	β -glucosidase 21
Q940G5	AT4G25900	1.681	aldose 1-epimerase family protein
Chaperones			
Q8L7B5	AT2G33210	2.321	chaperonin CPN60-like 1
Q94K05	AT3G03960	2.268	TCP-1/cpn60 chaperonin family protein
Q93ZM7	AT3G13860	1.771	chaperonin CPN60-like 2
P29197	AT3G23990	1.702	chaperonin CPN60
Oxidoreductases			
Q8VYP0	AT3G08950	2.510	electron transport SCO1/SenC-like protein
Q9SUM3	AT4G30210	2.117	NADPH-cytochrome P450 reductase 2
O05000		2.091	NADH-ubiquinone oxidoreductase chain 2
Q93ZX2		2.013	putative cytochrome P450 protein
Q9SA85	AT1G30700	1.889	FAD-binding and BBE domain-containing protein
Q9FKV0	AT5G44380	1.809	FAD-binding and BBE domain-containing protein
Q9M5K3	AT1G48030	1.803	dihydrolipoyl dehydrogenase 1
Q9LK96	AT3G15090	1.793	GroES-like zinc-binding alcohol dehydrogenase family protein
Trafficking			
Q8VZB4		2.177	GDP dissociation inhibitor
Q9M0Y8	AT4G04910	2.026	vesicle-fusing ATPase
O49048	AT1G77140	1.997	vacuolar protein sorting-associated protein 45-like protein
Q95N35	AT4G18800	1.898	RAB GTPase homolog A1D
Q7X659	AT2G17790	1.813	protein VPS35A
O80915	AT2G38360	1.782	PRA1 family protein B4
Q8RXF8	AT5G27540	1.682	MIRO-related GTP-ase 1
Lipid metabolism			
Q9T053	AT4G11850	2.960	phospholipase D gamma 1

Table 1. Proteins identified by LC-MS/MS and whose abundances were significantly changed (P values < 0.05) in microsomal extracts of 12-d-old *Arabidopsis* seedlings grown under 1 g on board the International Space Station as compared with 1 g ground control (continued)

Q6NQI8	AT1G13580	2.214	LAG1 longevity assurance homolog 3
Q96242	AT5G42650	2.145	allene oxide synthase
Q9C8P0	AT1G34430	1.976	pyruvate dehydrogenase E2 component
P33207	AT1G24360	1.964	3-oxoacyl-[acyl-carrier-protein] reductase
Q56WD9	AT2G33150	1.961	3-ketoacyl-CoA thiolase 2
Q9LM02	AT5G13710	1.939	cycloartenol-c-24-methyltransferase
Q9LTV6	AT3G12800	1.914	peroxisomal 2,4-dienoyl-CoA reductase
Q9LSQ0	AT3G16910	1.874	acyl-activating enzyme 7
C0Z3A0		1.793	AT3G06850
Q9ZPI5	AT3G06860	1.688	enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase
Q8LAZ1		1.681	very-long-chain fatty acid condensing enzyme CUT1
Auxin metabolism and trafficking			
Q9FZ33	AT1G54990	2.909	protein auxin RESPONSE 4
Q9SRU2	AT3G02260	2.471	Auxin transport protein BIG
Q9S9W2	AT4G05530	2.449	dehydrogenase/reductase SDR family member 4
Q0WLB5	AT3G08530	1.744	Clathrin, heavy chain
Cell Wall			
Q1JPL7	AT1G11580	2.304	bifunctional pectinesterase 18/rRNA N-glycosylase
Q8LB19	AT5G15650	2.054	reversibly glycosylated polypeptide 2
Miscellaneous			
A8MQP6	AT4G10480	5.282	Nascent polypeptide-associated complex subunit α -like protein 4
P41088	AT3G55120	4.096	chalcone-flavonone isomerase 1
Q8LA39		2.744	prohibitin-like protein
Q9C4Z6	AT1G48630	2.674	receptor for activated C kinase 1B
Q56XG6	AT5G11200	2.482	DEAD-box ATP-dependent RNA helicase 56
Q8VZM1	AT1G80410	2.303	tetratricopeptide repeat-containing protein
Q8RXR5		2.265	putative poly(A)-binding protein
Q9C5S7		2.226	Gamma carbonic anhydrase 2
Q9C5J8	AT5G19620	2.180	outer envelope protein
Q9SPK5	AT1G50480	2.143	Formate-tetrahydrofolate ligase
Q9ZU52	AT2G01140	2.123	fructose-bisphosphate aldolase, class I
Q9STI1	AT4G12300	2.118	cytochrome P450, family 706, subfamily A, polypeptide 4
Q9LG23	AT1G55890	2.101	pentatricopeptide repeat-containing protein
Q9C5J7	AT5G56630	2.098	6-phosphofructokinase 7
Q9LV28	AT3G18130	2.097	receptor for activated C kinase 1C
Q680A5	AT2G42910	2.092	ribose-phosphate pyrophosphokinase 4
Q8LFT2	AT2G14120	2.036	dynamamin-related protein 3B
Q9FF10	AT5G07830	2.036	Heparanase-like protein 1
Q9SIL6	AT2G20530	1.997	prohibitin 6
Q0WRX8	AT5G23300	1.971	dihydroorotate dehydrogenase
Q0WWL2		1.859	glutamine synthetase like protein
Q8LC49		1.846	putative signal sequence receptor, α subunit (SSR- α)
Q9FHX0	AT5G42150	1.846	Glutathione S-transferase family protein

Table 1. Proteins identified by LC-MS/MS and whose abundances were significantly changed (P values < 0.05) in microsomal extracts of 12-d-old *Arabidopsis* seedlings grown under 1 g on board the International Space Station as compared with 1 g ground control (continued)

Q94JQ3		1.828	At4g32520/F8B4_220 Serine hydroxymethyl transferase
Q9C7N2	AT1G29690	1.762	MAC/Perforin domain-containing protein
O04331	AT5G40770	1.752	prohibitin 3
Q8LPR8	AT5G22640	1.749	MORN (Membrane Occupation and Recognition Nexus) repeat-containing protein
P56786	ATCG01280	1.723	Ycf2
Q52T38	AT5G20350	1.716	S-acyltransferase TIP1
Q9SYB5	AT1G61790	1.699	Probable dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 3B
Unknown			
Q9LY25		3.071	putative protein
Q5XEU8	AT3G15000	3.050	cobalt ion binding protein
Q9FYF7	AT1G67360	2.836	Rubber elongation factor protein (REF)
Q9LNS1		2.578	F1L3.2
Q858F1	AT2G36885	2.568	uncharacterized protein
Q9LV60	AT5G48540	2.506	cysteine-rich repeat secretory protein 55
O82251	AT2G47840	2.440	uncharacterized protein
Q9C6M1	AT1G25520	2.376	putative transmembrane protein
Q9SZV4	AT4G30010	2.168	uncharacterized protein
Q0WPB8		2.165	hypothetical protein
Q9SN96	AT5G59613	2.142	uncharacterized protein
Q38842	AT4G00860	2.043	uncharacterized protein
Q9FF91	AT5G23890	2.023	uncharacterized protein
Q9ASX8	AT1G27760	2.015	Interferon-related developmental regulator domain-containing protein
P56785		1.961	hypothetical protein Arthcp087
Q8LAS5		1.947	unknown
O81814		1.858	src2-like protein
Q9C9Z2	AT3G08640	1.856	uncharacterized protein
Q3EBX0	AT2G21660	1.853	glycine-rich RNA-binding protein 7
Q9ZUX4	AT2G27730	1.842	copper ion binding protein
Q9C803	AT1G33490	1.826	uncharacterized protein
Q0WLE7		1.821	hypothetical protein
Q9FE06	AT5G64260	1.811	protein EXORDIUM like 2
Q9XIR5		1.793	similar to translational activator
Q9LM25	AT1G18260	1.786	Hr.3 like protein
Q0WL65		1.765	hypothetical protein
Q9LZ82	AT5G04430	1.763	binding to TOMV RNA 1L (long form) protein
Q8LB46		1.759	light induced protein like
C0SVD5	AT3G44310	1.752	unknown
O65660	AT4G39730	1.743	Lipase/lipoxygenase, PLAT/LH2 family protein
Q9SI32	AT2G04940	1.704	scramblase-related protein
Q9SFB1	AT3G08030	1.699	uncharacterized protein
Q9M898	AT3G02420	1.696	uncharacterized protein
Q9LK72	AT3G16530	1.692	legume lectin-like protein

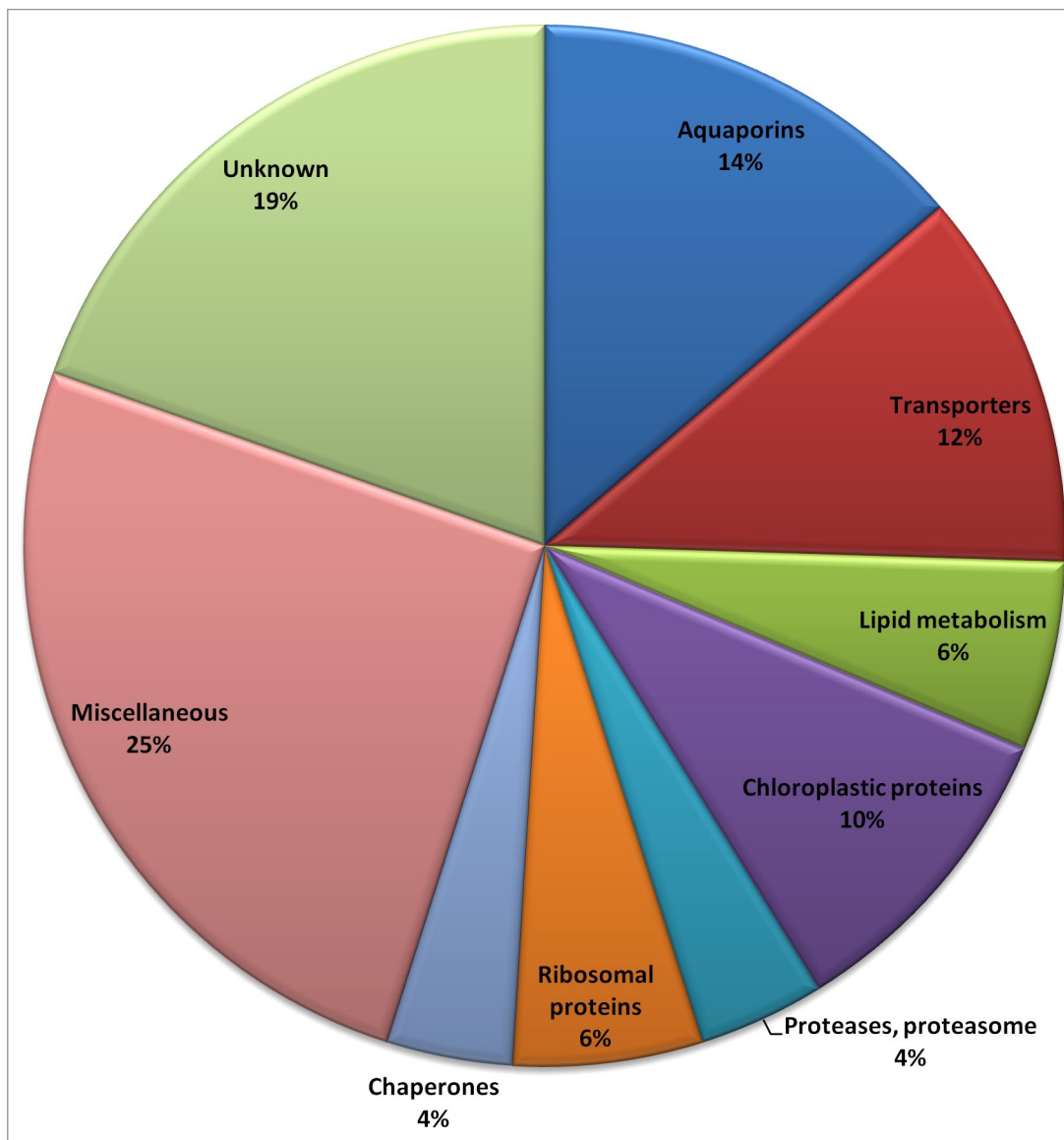


Figure 1A. Repartition of proteins significantly under-represented in microsomal extracts of 12-d-old *Arabidopsis* seedlings grown under 1 *g* on board the ISS as compared with 1 *g* ground control. Proteins were distributed into functional categories according to UNIPROT annotations.

by NASA reporting levels as high as 1.1–1.6 $\mu\text{l l}^{-1}$ during this STS-84 mission⁷. Similarly the group of Ferl evaluated the effects of the lack of convection-driven gas movement on space-induced hypoxia⁸.

Recently, in the frame of GENARA-A, an experiment hosted by the International Space Station (STS-132 - ULF-4 05/14/2010), we analyzed the effect of microgravity on the membrane proteome of *Arabidopsis thaliana* seedlings grown under either μg or 1 *g* conditions in the European Modular Cultivation System (EMCS).¹⁰ Using LC-MS/MS analysis and UNIPROT annotations we sorted out functional groups of proteins that were found to be significantly less or more abundant in membranes during microgravity conditions.¹⁰ In this previous analysis, in order to assess the effect of microgravity alone, a highly stringent filter was used: A protein was considered to be suitable for analysis only if its abundance in membranes did

not significantly change between 1 *g* in space and 1 *g* on ground in the same culture conditions, i.e., EMCS growth conditions.

In contrast, in the present report, we took advantage of the presence of a centrifuge in EMCS to focus on proteins whose abundance in membranes was not dependent upon gravity, i.e., quantitative changes were not significantly different between microgravity and 1-*g* conditions on board ISS. This set of membranes proteins that is not responsive to a change in gravity in space was then further analyzed to evaluate possible effects of the other environmental conditions found on board ISS (radiations, gases, vibrations, lack of convection...). For this purpose the relative abundances of these proteins in membranes were compared between 1 *g* obtained on a centrifuge in ISS and 1 *g* condition achieved on Earth.

We report here that large families of proteins were affected by others parameters than gravity in space. Among the proteins

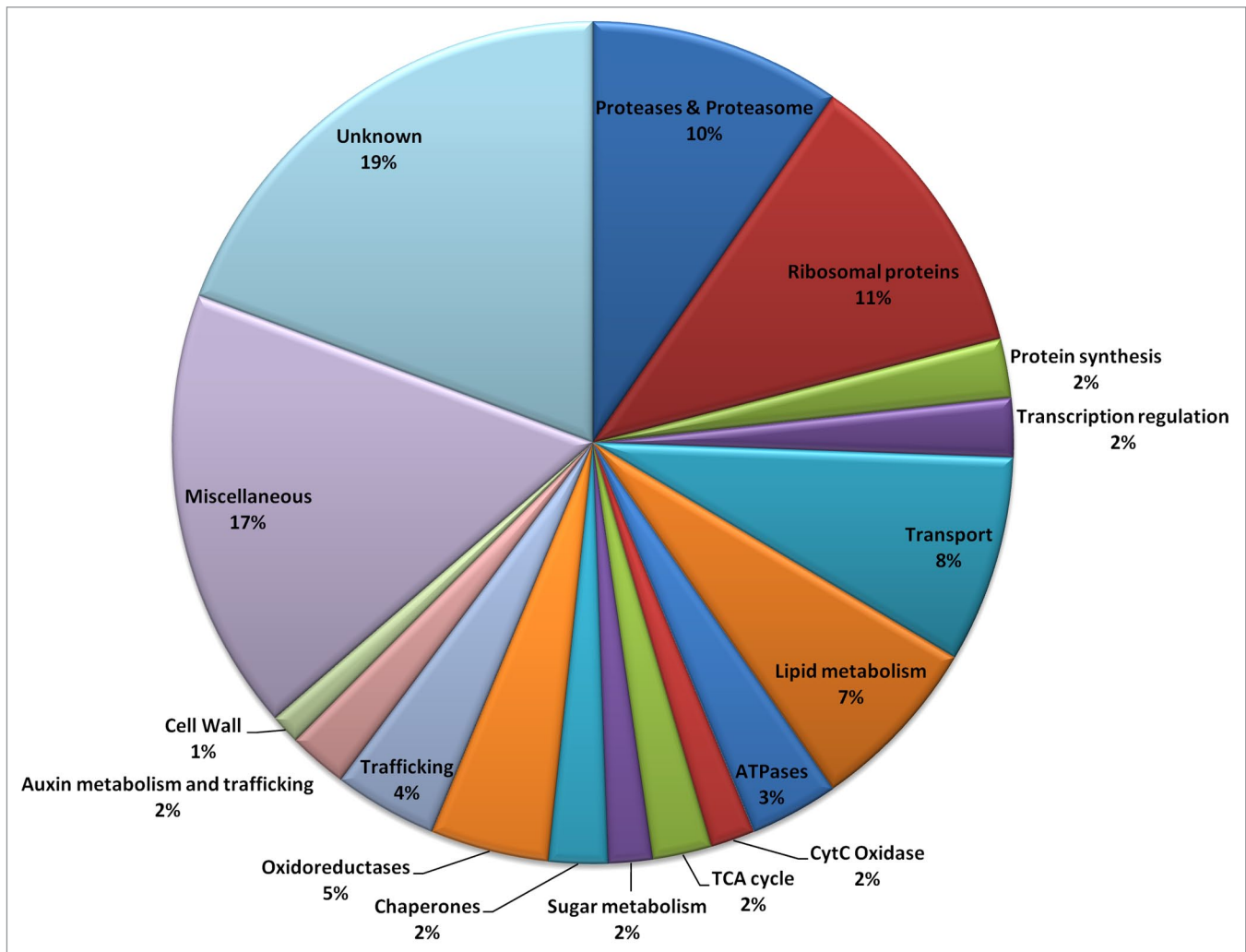


Figure 1B. Repartition of proteins significantly over-represented in microsomal extracts of 12-d-old *Arabidopsis* seedlings grown under 1 g on board the ISS as compared with 1 g ground control. Proteins were distributed into functional categories according to UNIPROT annotations.

whose abundances in cell membranes were not changed by microgravity in the ISS (1202 from 1484 quantified proteins with at least 2 peptides; P value > 0.05), 227 proteins were found to be significantly (P value < 0.05) either more or less abundant in cell membranes of seedlings grown under 1 g in space as compared with seedlings grown on 1-g ground (all other culture conditions being equal). Among these, more than three quarters (176 proteins) were over-represented while only one quarter (51 proteins) was under-represented in membranes (Table 1). With a few exceptions the fold-change between the two conditions was close to 2 with a maximum of about 5.

Figure 1 displays a repartition of these proteins grouped into functional categories using UNIPROT database annotations. In the set of 51 proteins under-represented in space conditions (Fig. 1A; Table 1A) aquaporins constituted one of the main groups and were among the more under-represented, with a mean fold-change of about 3. Transporters and chloroplastic proteins formed two other important groups. We found also in this set a few ribosomal proteins and some proteins involved in the lipid metabolism. Other proteins of this set were

miscellaneous proteins with diverse (25%) or unknown (19%) functions.

The main group of over-represented proteins (Fig. 1B; Table 1B), representing about 25% of this set, was associated to protein synthesis and degradation: ribosomal proteins 11%, protein synthesis 2%, proteins with a protease activity (10%), and transcription regulation 2%. Other important groups over-represented in membranes were composed of proteins involved in transport (9%), lipid metabolism (7%), and oxidoreductases (5%). Proteins associated to energy and basic metabolism (ATP synthase, Cytochrome C oxydase, TCA cycle, sugar metabolism) were also found over-represented in membranes. Interestingly, the most over-represented protein in space conditions was the UDP-galactose transporter 6 (fold-change 5.6) which has a sugar:hydrogen symporter activity. Another highly over-represented protein was a chalcone-flavonone isomerase (fold-change 4.1) which belongs to the biosynthetic pathway of flavonoids known to be involved in UV-filtration in higher plants.¹¹ This suggests that flavonoids might also be involved in the response to other radiations such as cosmic rays. The

large set of proteins with protease activity could also be linked with the above-mentioned stress knowing that proteolytic activities can help the cell to remove damaged/oxidized proteins. The unexpected decrease in aquaporins observed in plantlets grown in space conditions may be related to the different plant water status existing in the EMCS between the ISS and ground localization.

Altogether, our results show also that in space, factors other than microgravity, may affect various aspects of plant metabolism, such as protein and lipid metabolism or transport. Interestingly, although EMCS light conditions were the same in the ISS and on ground, differences were observed in the amount of chloroplastic proteins.

More experiments would be necessary to analyze more deeply the effects of the various factors that could modulate plant physiology in space conditions. These preliminary and purely descriptive results show that beside the major effects of microgravity on plant growth and development, which can be overcome using centrifugation, other uncontrolled factors that

are present in a space station (e.g., cosmic rays) may have non negligible effects on the physiology of plants and thus should be taken into considerations for long-term missions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors would like to thank the National Aeronautics and Space Administration (NASA) who successfully performed the spaceflight experiment; they also thank the astronauts for performing the required tasks on board the ISS. We acknowledge the Norwegian User Support and Operations Center team (NUSOC) for the ground and space preparation of the GENARA-A experiment and we thank the European Aeronautic Defense and Space Company (Astrium EADS) for the design and building of the hardware. We also thank the European Space Agency (ESA) and the Centre National d'Etudes Spatiales (CNES) for their scientific and financial support.

References

1. Ferl R, Wheeler R, Levine HG, Paul A-L. Plants in space. *Curr Opin Plant Biol* 2002; 5:258-63; PMID:11960745; [http://dx.doi.org/10.1016/S1369-5266\(02\)00254-6](http://dx.doi.org/10.1016/S1369-5266(02)00254-6)
2. Wolverson C, Kiss JZ. An update on plant space biology. *Gravit Space Res* 2009; 22:13-20
3. Paul AL, Wheeler RM, Levine HG, Ferl RJ. Fundamental plant biology enabled by the space shuttle. *Am J Bot* 2013; 100:226-34; PMID:23281389; <http://dx.doi.org/10.3732/ajb.1200338>
4. Kordyum EL. Plant cell gravisensitivity and adaptation to microgravity. *Plant Biol (Stuttg)* 2014; 16(Suppl 1):79-90; PMID:23731198; <http://dx.doi.org/10.1111/plb.12047>
5. Musgrave ME, Kuang A, Matthews SW. Plant reproduction during spaceflight: importance of the gaseous environment. *Planta* 1997; 203(Suppl):S177-84; PMID:9299797; <http://dx.doi.org/10.1007/PL00008107>
6. Kiss JZ, Katembe WJ, Edelmann RE. Gravitropism and development of wild-type and starch-deficient mutants of *Arabidopsis* during spaceflight. *Physiol Plant* 1998; 102:493-502; PMID:11541086; <http://dx.doi.org/10.1034/j.1399-3054.1998.1020403.x>
7. Kiss JZ, Edelmann RE, Wood PC. Gravitropism of hypocotyls of wild-type and starch-deficient *Arabidopsis* seedlings in spaceflight studies. *Planta* 1999; 209:96-103; PMID:10467035; <http://dx.doi.org/10.1007/s004250050610>
8. Paul AL, Daugherty CJ, Bihn EA, Chapman DK, Norwood KL, Ferl RJ. Transgene expression patterns indicate that spaceflight affects stress signal perception and transduction in *Arabidopsis*. *Plant Physiol* 2001; 126:613-21; PMID:11402191; <http://dx.doi.org/10.1104/pp.126.2.613>
9. Wolff SA, Coelho LH, Zabrodina M, Brinckmann E, Kittang AI. Plant mineral nutrition, gas exchange and photosynthesis in space: A review. *Adv Space Res* 2013; 51:465-75; <http://dx.doi.org/10.1016/j.asr.2012.09.024>
10. Mazars C, Brière C, Grat S, Pichereaux C, Rossignol M, Pereda-Loth V, Eche B, Boucheron-Dubuisson E, Le Disquet I, Medina FJ, et al. Microgravity induces changes in microsome-associated proteins of *Arabidopsis* seedlings grown on board the international space station. *PLoS One* 2014; 9:e91814; PMID:24618597; <http://dx.doi.org/10.1371/journal.pone.0091814>
11. Winkel-Shirley B. Biosynthesis of flavonoids and effects of stress. *Curr Opin Plant Biol* 2002; 5:218-23; PMID:11960739; [http://dx.doi.org/10.1016/S1369-5266\(02\)00256-X](http://dx.doi.org/10.1016/S1369-5266(02)00256-X)