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

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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 μ g 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 other 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. 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. 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, 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-81 it was observed that seedlings grown both on the 1-g in-flight centrifuge and μ g 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.
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.

| Lipid metabolism | | | |
|---|---|---|---|
| A4GNA8 | AT4G25970 | 0.549 | phosphatidylserine decarboxylase 3 |
| Q9Z7P6 | AT4G38690 | 0.554 | 1-phosphatidylinositol phosphodiesterase-related protein |
| Q8LDN5 | | 0.585 | endomembrane-associated protein |

| Transporters | | | |
|---|---|---|---|
| P23586 | AT1G11260 | 0.370 | sugar transporter 1 |
| Q8LGU1 | AT3G21250 | 0.456 | multidrug resistance-associated protein 6 |
| Q5GZ72 | AT5G16150 | 0.523 | Plastidic glucose transporter 4 |
| Q5XH7 | AT5G62670 | 0.553 | H(+) - ATPase 11 |
| Q4FB9 | AT4G39850 | 0.559 | ABC transporter D family member 1 |
| Q5E45 | 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 | 0.326 | 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 | SOS ribosomal protein L12–3 |
| P56802 | ATCG00750 | 0.525 | ribosomal protein S11 |
| Q9VC7 | AT5G30510 | 0.578 | small subunit ribosomal protein S1 |

| Chloroplastic proteins | | | |
|---|---|---|---|
| Q94956 | 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 bisphosphate carboxylase small chain 2B |

| Chaperones | | | |
|---|---|---|---|
| Q7XAR9 | AT5G42480 | 0.346 | chaperone DnaJ-domain containing protein |
| P42763 | AT1G76180 | 0.417 | dehydrin ERD14 |

| Miscellaneous | | | |
|---|---|---|---|
| Q95K7 | AT1G70850 | 0.218 | MLP-like protein 34 |
| Q5D07 | AT3G51420 | 0.469 | strictosidine synthase-like 4 protein |
| Q95L9 | AT2G36870 | 0.512 | xyloglucan:xyloglucosyl transferase |
| P25856 | 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)

| UNIPROT  | AGI          | Ratio  | Annotation                                      |
|----------|--------------|--------|------------------------------------------------|
| Q94BW0   | AT3G16400    | 0.534  | Nitrile-specifier protein 1                     |
| Q95DM9   | AT3G16400    | 0.546  | cytosolic O-acetylserine(thiol)lyase             |
| Q09LF5   | AT1G70410    | 0.551  | β carbonic anhydrase 4                          |
| Q94CE4   | AT1G70410    | 0.562  | tubulin β                                       |
| P12411   | AT1G75780    | 0.565  | Glyceraldehyde-3-phosphate dehydrogenase GAPC2  |
| Q8L6Z6   | AT1G34400    | 0.583  | cytochrome P450 71B5                            |
| Q9LT39   | AT3G20820    | 0.587  | leucine-rich repeat-containing protein           |

Unknown proteins

| UNIPROT  | AGI          | Ratio  | Annotation                                      |
|----------|--------------|--------|------------------------------------------------|
| Q9LYM7   |              | 0.143  | putative protein                               |
| Q9LHA3   | AT3G28720    | 0.314  | uncharacterized protein                        |
| Q9SVW4   | AT4G18070    | 0.347  | putative protein                               |
| Q94F20   | AT5G24640    | 0.422  | uncharacterized protein                        |
| Q9S537   | 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                        |
| Q80821   | AT2G41470    | 0.592  | unknown protein                                |

B. Proteins over-represented in space conditions. Ratio (ISS 1 g / ground 1 g) > 1

| UNIPROT  | AGI          | Ratio  | Annotation                                      |
|----------|--------------|--------|------------------------------------------------|
| 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            |
| Q9X05    | 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                         |
| Q95EI4   | AT5G58290    | 1.731  | regulatory particle triple-A ATPase 3           |
| O81148   | AT3G22110    | 1.724  | proteasome subunit α type-4                     |
| Q9C9CQ   | AT1G73990    | 1.717  | signal peptide peptidase                       |

Ribosomal proteins

| UNIPROT  | AGI          | Ratio  | Annotation                                      |
|----------|--------------|--------|------------------------------------------------|
| Q8RW4T   | 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)

| Accession | Protein Name          | Ratio  | Function                                      |
|-----------|-----------------------|--------|-----------------------------------------------|
| 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 5a-1                    |
| Q9M339    | AT3G53870             | 1.880  | 40S ribosomal protein S3-2                    |
| B9DGN3    | AT2G27710             | 1.871  | 60S acidic ribosomal protein P2-2              |
| Q9C9C      | AT1G74050             | 1.862  | 60S ribosomal protein L6-3                    |
| B9DP6     | AT3G25520             | 1.856  | ribosomal protein                              |
| Q9LRX8    | AT3G24830             | 1.836  | 60S ribosomal protein L13a-2                  |
| Q9LZH9    | AT3G62870             | 1.834  | 60S ribosomal protein L7a-2                   |
| Q9SI7F    | 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

| Accession | Protein Name          | Ratio  | Function                                      |
|-----------|-----------------------|--------|-----------------------------------------------|
| O04485    | 2.830 Strong similarity to S. pombe leucyl-tRNA synthetase (gbjZ73100) |
| O9CZ52    | AT1G10840             | 1.887  | translation initiation factor eIF-3 subunit 3 |
| O9FL55    | AT4G11420             | 1.769  | translation initiation factor eIF-3 subunit 10 |
| O04630    | AT5G26830             | 1.704  | threonyl-tRNA synthetase                      |

Transcription regulation

| Accession | Protein Name          | Ratio  | Function                                      |
|-----------|-----------------------|--------|-----------------------------------------------|
| 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

| Accession | Protein Name          | Ratio  | Function                                      |
|-----------|-----------------------|--------|-----------------------------------------------|
| 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 |
| Q9FN9C    | AT5G43970             | 1.923  | mitochondrial import receptor subunit TOM22–2  |
| Q9SKX0    | 1.904 ABC transporter C family member 13             |
| Q9SLO5    | AT2G05620             | 1.788  | protein PROTON GRADIENT REGULATION 5           |
| Q9CBG9    | AT1G30400             | 1.752  | ABC transporter C family member 1              |
| O04619    | AT4G01100             | 1.704  | adenine nucleotide transporter 1               |

ATPases

| Accession | Protein Name          | Ratio  | Function                                      |
|-----------|-----------------------|--------|-----------------------------------------------|

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**Plant Signaling & Behavior**
| Protein ID | Accession | Fold Change | Function |
|------------|-----------|-------------|----------|
| 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 M125 |
| 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**

| Protein ID | Accession | Fold Change | Function |
|------------|-----------|-------------|----------|
| Q9FKT8 | AT5G56090 | 2.544 | cytochrome c oxidase subunit XV assembly protein |
| Q9SS88 | AT1G80230 | 2.470 | cytochrome c oxidase subunit Vb |
| Q95L99 | AT1G22450 | 1.825 | cytochrome C oxidase 6B |

**TCA cycle**

| Protein ID | Accession | Fold Change | Function |
|------------|-----------|-------------|----------|
| O82662 | AT2G0420 | 2.362 | Succinyl-CoA ligase (GDP-forming) subunit β |
| O82663 | AT5G66760 | 1.921 | succinate dehydrogenase [ubiquinone] flavoprotein subunit 1 |
| Q95I98 | AT2G05710 | 1.718 | aconitate hydratase 2 |
| P20115 | AT2G44350 | 1.694 | citrate synthase 4 |

**Sugar metabolism**

| Protein ID | Accession | Fold Change | Function |
|------------|-----------|-------------|----------|
| Q9C8Y9 | AT1G66280 | 2.257 | β-glucosidase 22 |
| Q9C525 | AT1G66270 | 2.150 | β-glucosidase 21 |
| Q94OS | AT4G29000 | 1.681 | aldose 1-epimerase family protein |

**Chaperones**

| Protein ID | Accession | Fold Change | Function |
|------------|-----------|-------------|----------|
| Q8L785 | 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**

| Protein ID | Accession | Fold Change | Function |
|------------|-----------|-------------|----------|
| Q8VYP0 | AT3G08950 | 2.510 | electron transport SCO1/SenC-like protein |
| Q95UM3 | AT4G30210 | 2.117 | NADPH–cytochrome P450 reductase 2 |
| O0S5000 | AT4G66270 | 2.091 | NADH-ubiquinone oxidoreductase chain 2 |
| Q93ZK2 | AT2G39000 | 2.013 | putative cytochrome P450 protein |
| Q95A8S | AT1G30700 | 1.889 | FAD-binding and BBE domain-containing protein |
| Q9FVV2 | AT3G44380 | 1.809 | FAD-binding and BBE domain-containing protein |
| Q9NM53 | AT1G48030 | 1.803 | dihydrolipoyl dehydrogenase 1 |
| Q9L9K6 | AT3G15090 | 1.793 | GroES-like zinc-binding alcohol dehydrogenase family protein |

**Trafficking**

| Protein ID | Accession | Fold Change | Function |
|------------|-----------|-------------|----------|
| Q8VZB4 | AT4G04910 | 2.177 | GDP dissociation inhibitor |
| Q9MOY8 | AT4G04910 | 2.026 | vesicle-fusing ATPase |
| Q9O48 | AT1G77140 | 1.997 | vacuolar protein sorting-associated protein 45-like protein |
| Q9SN35 | AT4G18800 | 1.898 | RAB GTPase homolog A1D |
| Q7X6S9 | AT2G17790 | 1.813 | protein VP535A |
| Q80915 | AT2G38360 | 1.782 | PRA1 family protein B4 |
| Q8RXF8 | AT5G27540 | 1.682 | MIRO-related GTPase 1 |

**Lipid metabolism**

| Protein ID | Accession | Fold Change | Function |
|------------|-----------|-------------|----------|
| Q9TOS3 | 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)

| Accession | SwissProt | Fold change | Description |
|-----------|-----------|-------------|-------------|
| Q6NQ8     | AT1G13580 | 2.214       | LAG1 longevity assurance homolog 3 |
| Q96242    | AT5G42650 | 2.145       | alene 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 |
| C02Z4A0   |           | 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**

| Accession | SwissProt | Fold change | Description |
|-----------|-----------|-------------|-------------|
| Q9FZ33    | AT1G54990 | 2.909       | protein auxin RESPONSE 4 |
| Q9SRU2    | AT3G02260 | 2.471       | Auxin transport protein BIG |
| Q9SW92    | AT4G05530 | 2.449       | dehydrogenase/reductase SDR family member 4 |
| QOWL5     | AT3G08530 | 1.744       | Clathrin, heavy chain |

**Cell Wall**

| Accession | SwissProt | Fold change | Description |
|-----------|-----------|-------------|-------------|
| Q1JPL7    | AT1G1580  | 2.304       | bifunctional pectinesterase 1B/RNA N-glycosylase |
| Q8LB19    | AT5G15650 | 2.054       | reversibly glycosylated polypeptide 2 |

**Miscellaneous**

| Accession | SwissProt | Fold change | Description |
|-----------|-----------|-------------|-------------|
| A8MQP6    | AT4G10480 | 5.282       | Nascent polypeptide-associated complex subunit α-like protein 4 |
| P41088    | AT3G55120 | 4.096       | chalcone–flavonone isomerase 1 |
| Q8L3A9    |           | 2.744       | prohibitin-like protein |
| Q9CAZ6    | 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 |
| Q8XR5     |           | 2.265       | putative poly(A)-binding protein |
| Q9CS57    |           | 2.226       | Gamma carbonic anhydrase 2 |
| Q9CSJ8    | AT5G19620 | 2.180       | outer envelope protein |
| Q9PKS5    | AT1G50480 | 2.143       | Formate–tetrabhydrofolate ligase |
| Q9ZUS2    | 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 |
| Q9CSJ7    | AT5G56630 | 2.098       | 6-phosphofructokinase 7 |
| Q9LV28    | AT3G18130 | 2.097       | receptor for activated C kinase 1C |
| Q6BOA5    | AT2G42910 | 2.092       | ribose-phosphate pyrophosphokinase 4 |
| Q8LFT2    | AT2G14120 | 2.036       | dynamin-related protein 3B |
| Q9F10     | AT5G07830 | 2.036       | Heparanase-like protein 1 |
| Q9SL6     | AT2G20530 | 1.997       | prohibitin 6 |
| Q0WRX8    | AT5G23300 | 1.971       | dihydroorotate dehydrogenase |
| Q0WUL2    |           | 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)

| Protein ID  | Description                                                                 |
|-------------|------------------------------------------------------------------------------|
| Q94JQ3      | 1.828 At4g32520/F8B4_220 Serine hydroxymethyl transferase                   |
| Q9C7N2      | 1.762 MAC/Perforin domain-containing protein                                |
| O04331      | 1.752 prohibitin 3                                                          |
| Q8LPR8      | 1.749 MORN (Membrane Occupation and Recognition Nexus) repeat-containing protein |
| P56786      | 1.723 Ycf2                                                                  |
| Q52T38      | 1.716 S-acyltransferase TIP1                                                |
| Q95YB5      | 1.699 Probable dolichyl-diphosphooligosaccharide–protein glycosyltransferase subunit 3B |
| Q9LY25      | 3.071 putative protein                                                       |
| Q5XE08      | 3.050 cobalt ion binding protein                                             |
| Q9FYF7      | 2.836 Rubber elongation factor protein (REF)                                |
| Q9LNP1      | 2.578 F1L3.2                                                                |
| Q8SF1       | 2.568 uncharacterized protein                                                |
| Q9LV60      | 2.506 cysteine-rich repeat secretory protein 55                              |
| O82251      | 2.440 uncharacterized protein                                                |
| Q9C6M1      | 2.376 putative transmembrane protein                                         |
| Q95ZV4      | 2.168 uncharacterized protein                                                |
| Q9PB8       | 2.165 hypothetical protein                                                   |
| Q0S96       | 2.142 uncharacterized protein                                                |
| Q3R42       | 2.043 uncharacterized protein                                                |
| Q9FF91      | 2.023 uncharacterized protein                                                |
| Q9ASX8      | 2.015 Interferon-related developmental regulator domain-containing protein   |
| P56785      | 1.961 hypothetical protein Arthcp087                                        |
| Q8LASS5     | 1.947 unknown                                                                |
| O81814      | 1.858 src2-like protein                                                      |
| Q0C9Z2      | 1.856 uncharacterized protein                                                |
| Q3EBX0      | 1.853 glycine-rich RNA-binding protein 7                                     |
| Q9ZUX4      | 1.842 copper ion binding protein                                             |
| Q9C803      | 1.826 uncharacterized protein                                                |
| Q0WLE7      | 1.821 hypothetical protein                                                   |
| Q9FE06      | 1.811 protein EXORDIUM like 2                                                |
| Q9X1R5      | 1.793 similar to translational activator                                     |
| Q9LM25      | 1.786 Hr.3 like protein                                                      |
| Q0WL65      | 1.765 hypothetical protein                                                   |
| Q9L2Z82     | 1.763 binding to TOMV RNA 1L (long form) protein                            |
| Q9LB46      | 1.759 light induced protein like                                              |
| C05VD5      | 1.752 unknown                                                                |
| O655560     | 1.743 Lipase/lipoxygenase, PLAT/LH2 family protein                           |
| Q95I32      | 1.704 scramblase-related protein                                             |
| Q95FB1      | 1.699 uncharacterized protein                                                |
| Q9MB98      | 1.696 uncharacterized protein                                                |
| Q9LK72      | 1.692 legume lectin-like protein                                             |

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by NASA reporting levels as high as 1.1–1.6µl l⁻¹ during this STS-84 mission. Similarly the group of Ferl evaluated the effects of the lack of convection-driven gas movement on space-induced hypoxia.

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 µ g or 1 g conditions in the European Modular Cultivation System (EMCS). 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. 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...
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 oxidase, 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. This suggests that flavonoids might also be involved in the response to other radiations such as cosmic rays.
large set of proteins with protease activity could also be linked
with the above-mentioned stress knowing that proteolytic activi-
ties 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.

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