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Supplementary Material

The effect of simulated microgravity on the *Brassica napus* seedling proteome

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Directory

Table S1 Instrument settings applied for ESI-Orbitrap-MS experiments S-3

Table S2 MaxQuant settings for database search and label-free relative quantification S-5

Table S3 The effect of simulated microgravity on the development of seedlings S-7

Table S4 Protein concentrations and recoveries obtained for *B. napus* seedlings by the phenol extraction procedure S-8

Figure S1 Effect of clinorotation on the seedling root length S-9

Figure S2 Effect of seed planting density on root length S-10

Figure S3 SDS-PAGE electropherogram of individual protein samples S-11

Figure S4 SDS-PAGE electropherogram of tryptic protein digests S-12

Table S1 Instrument settings applied for ESI-Orbitrap-MS experiments

Parameter	Setting
MS conditions	
Ionization mode	Positive
ESI emitter	outer/internal diameter 360/30 μm, 40 mm long (Thermo Fisher Scientific, Bremen, Germany)
Analyzer	Orbitrap
Resolution	120000
Ion spray voltage (IS)	1900 V
S-lens voltage	20 V
Ion transfer tube temperature	275 °C
Automatic gain control (AGC) target	$2*10^5$
Maximum injection time	50 ms
Mass to charge ratio (*m/z*) range	300 – 1500
MS/MS conditions	
Fragmentation	Collision activated dissociation
Analyzer	Orbitrap
Resolution	15000
Isolation width	2.0 *m/z*
Charge state rejected	1+, > 6+
Fragmentation	HCD
HCD collision energy	27%
Automatic gain control (AGC) target	$5*10^4$

Maximum injection time	200 ms
Dynamic exclusion repeat count	1
Dynamic exclusion duration	60 s
Dynamic exclusion mass width	± 2 ppm

Table S2 MaxQuant settings used for database search and label-free relative quantification

Parameter	Setting
Search engine/Program	Andromeda/MaxQuant
Protease	Trypsin
Missed cleavage sites	3
Modification	Mass increment (Da) / amino acids
Carbamidomethyl	+57.021 / C (fixed)
Oxidation	+15.995 / M (variable)
Multiplicity	1
Match type	Match from and to
First search peptide tolerance (ppm)	20
Main search peptide tolerance (ppm)	4.5
Isotope match tolerance (ppm)	2
Centroid much tolerance (ppm)	8
Minimal score for re-equilibration	70
Intensity threshold (counts)	100
Charge	2 - 7
LFQ	none
Maximal number of modifications per peptide	3
Decoy mode	Revert
Special amino acids	KR
Maximal peptide mass	4600
Minimal peptide length	7
Max. peptide length for unspecific search	25
PSM FDR/Protein FDR	0.05/0.05

Site decoy fraction	0.05
Minimal peptide length/peptides	7/1
Min. razor plus unique peptides	1
Min. score/delta score for modified peptides	40/6
Min. ratio count	1
Peptides for quantification	Unique plus razor
FTMS MS/MS much tolerance (ppm)	20
FTMS MS/MS de novo tolerance (ppm)	10
FTMS MS/MS deisotoping tolerance (ppm)	7
FTMS top peaks per 100 Da	12

Table S3 The effect of 3-D clinorotation on the development of *B.napus* seedlings

Treatment/duration	Percentage of seedlings (%) with		Percentage of non-germinated seeds (%)
	normal morphology	abnormal morphology	
Controls, 24 h	89 ± 6	7 ± 4	4 ± 2
Controls, 48 h	86 ± 5	10 ± 4	4 ± 2
Clinorotation, 24 h	84 ± 7	13 ± 5	3 ± 2
Clinorotation, 48 h	79 ± 4 *	18 ± 4 *	3 ± 2

The seeds were germinated on 0.8 % agar for 24 h or 48 h in dark under 1.0 g or in a two- axial clinostat. After the completion of the treatment, the seeds were transferred on wet filter paper and grown till the age of eight days at 22^{o}C. The morphological analysis was performed according the standards of the International Seed Testing Association (ISTA). The values are the means ± standard deviations (n = 25 seeds per replicate). Asterisk denotes significant difference between the static controls and plants, clinorotated for 48 h, at the confidence level of $p \leq 0.05$.

Table S4 Protein concentrations and recoveries obtained for *B. napus* seedlings by the phenol extraction procedure

Sample	Protein concentrations (mg/mL)		Average concentration (mg/mL)	Protein yields (mg/g fresh weight)	Total line density (AU) in SDS-PAGE
	Replicate 1	Replicate 2			
Experiment 1					
Control 24 h- replicate 1	18.9	22.7	20.8	6.62	43100
Control 24 h- replicate 2	10.9	14.2	12.6	3.78	45800
Control 24 h- replicate 3	18.9	16.3	17.6	5.31	43200
Clinorotation 24 h- replicate 1	15.6	18.5	17.1	5.03	42800
Clinorotation 24 h- replicate 2	19.8	21.2	20.5	5.98	43500
Clinorotation 24 h- replicate 3	12.8	14.8	13.8	3.99	41700
Control 48 h- replicate 1	12.1	14.9	13.5	4.06	41900
Control 48 h- replicate 2	8.7	12.3	10.5	3.09	41100
Control 48 h- replicate 3	15.4	21.1	18.3	5.29	39800
Clinorotation 48 h- replicate 1	13.4	16.8	15.1	4.39	40400
Clinorotation 48 h- replicate 2	10.5	14.6	12.6	3.71	40600
Clinorotation 48 h- replicate 3	12.4	13.2	12.8	3.79	37900
Experiment 2					
Control 24 h- replicate 1	14.3	14.5	14.4	2.15	39300
Control 24 h- replicate 2	19	17.7	18.4	5.36	39000
Control 24 h- replicate 3	7.4	7.8	7.6	2.21	37800
Clinorotation 24 h- replicate 1	6.5	4.7	5.6	1.68	36000
Clinorotation 24 h- replicate 2	9.1	11.2	10.2	2.99	35800
Clinorotation 24 h- replicate 3	12.9	11.7	12.3	3.60	36800
Control 48 h- replicate 1	24.4	23.7	24.1	7.00	37700
Control 48 h- replicate 2	16.7	18.8	17.8	5.20	39000
Control 48 h- replicate 3	14.3	16.4	15.4	4.54	40500
Clinorotation 48 h- replicate 1	24.1	24.4	24.3	7.21	40000
Clinorotation 48 h- replicate 2	22.5	21.6	22.1	6.54	39400
Clinorotation 48 h- replicate 3	13.6	14.6	14.1	4.25	38600

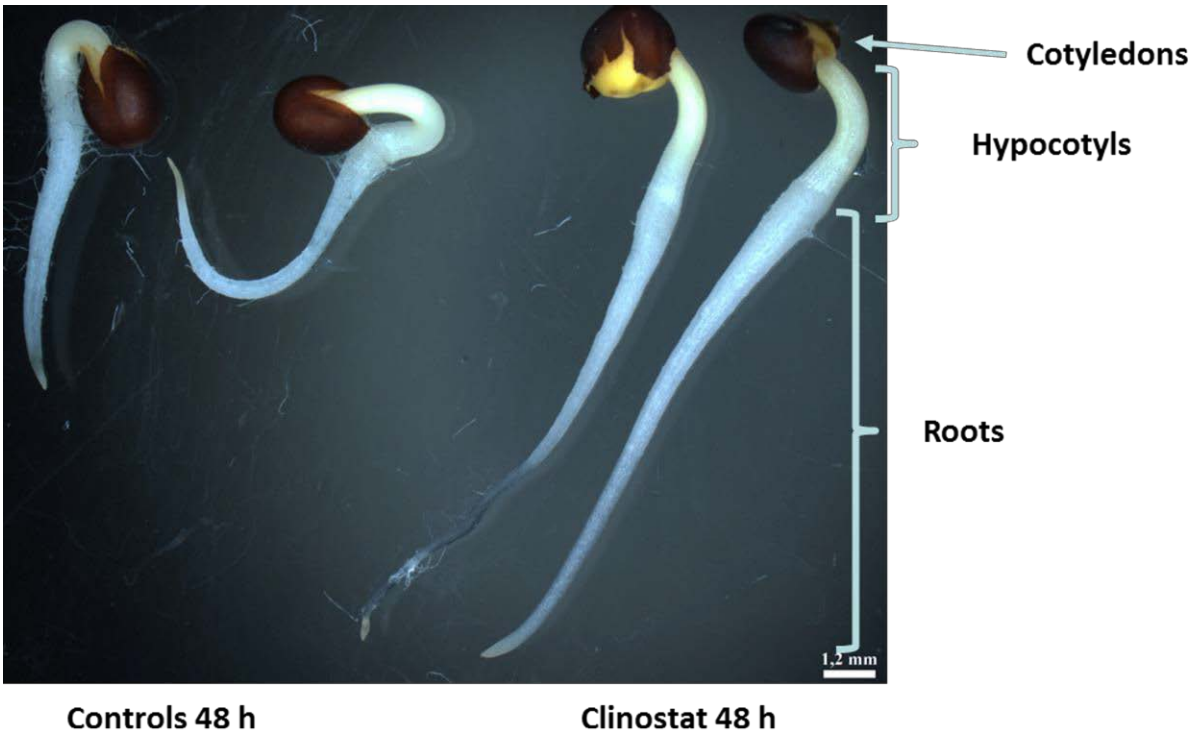

Figure S1 *B. napus* seedlings growing for 48 h under static control conditions (left) and in two-axial clinostat (right). The roots of the microgravity-treated plants have higher length of the root hair and elongation zone.

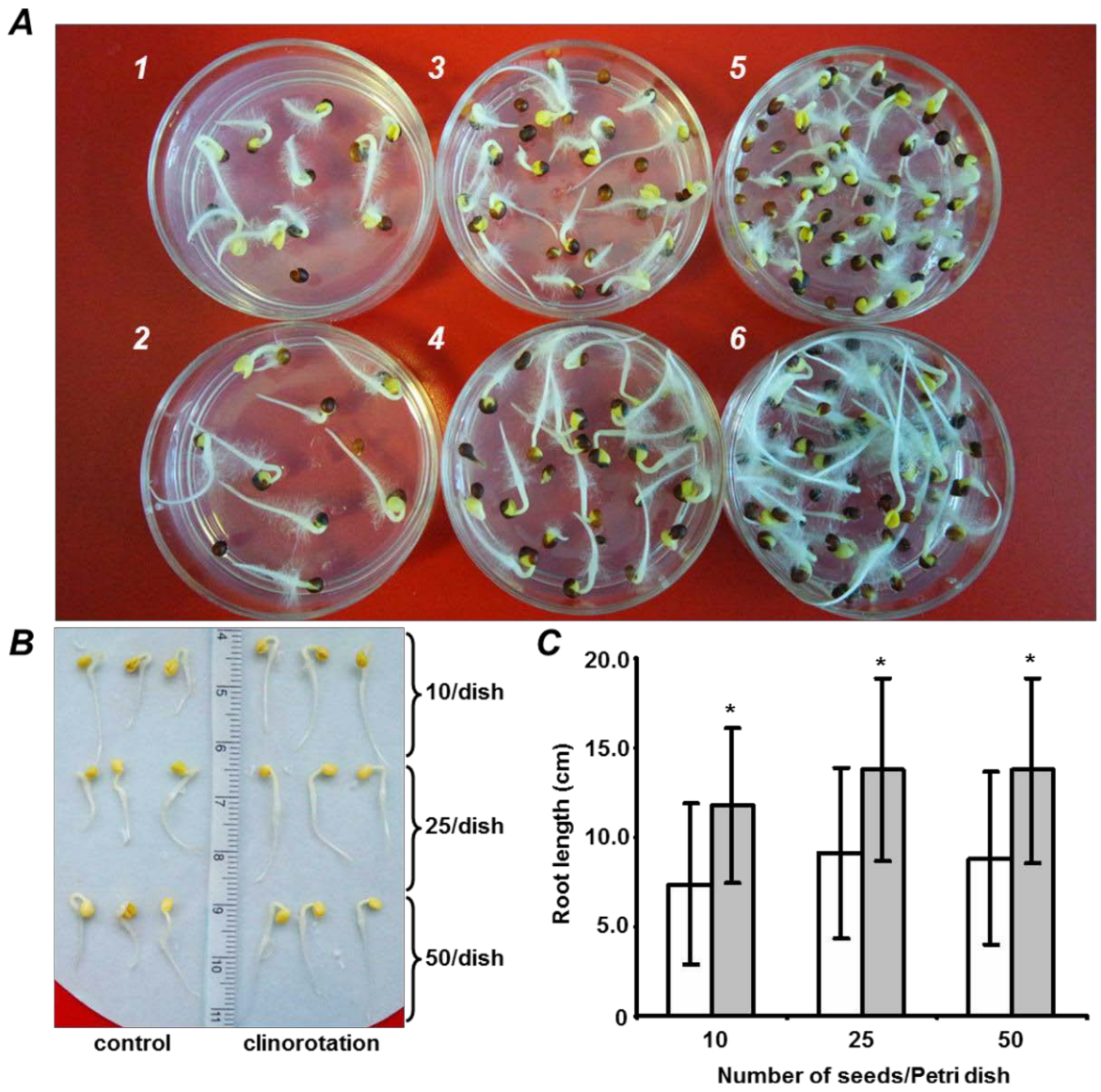

Figure S2 *B. napus* seeds planted in the density of 10 (A1 and 2), 25 (A3 and 4) or 50 (A5 and 6) seeds per Petri dish, and grown for 48 h under the static control conditions (A1, 3 and 5) and in two-axial clinostat (A2, 4 and 6). The root length of individual seedlings was measured (B) and inter-group comparison was performed (C). The difference between clinorotated seedlings and corresponding controls was significant at the confidence level of $p \leq 0.01$ (marked with asterisk).

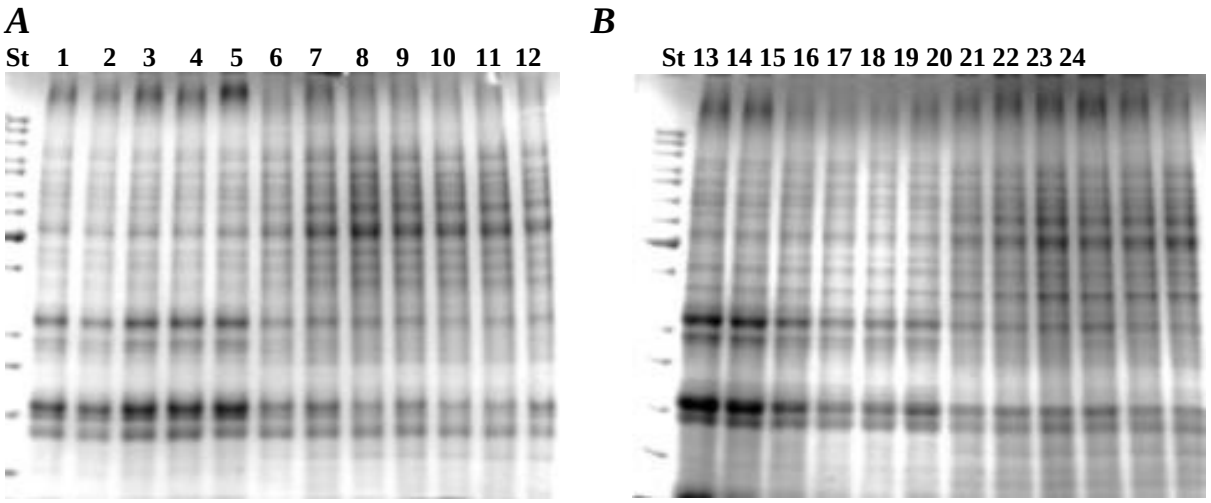

Figure S3 SDS-PAGE electropherogram of individual protein samples (5 µg) isolated from one (1 – 6 and 13 – 18)- and two (7 – 12 and 19 – 24)-day old *B. napus* seedlings harvested in the experiments 1 (A) and 2 (B) after growing in two-axial clinostat (4 – 6, 10 – 12, 16 – 18, and 22 – 24) and under the static control conditions (1 – 3, 7 – 9, 13 – 15, and 19 – 21). The gels were stained with colloidal Coomassie Blue G250 for 2 h, washed for 24 h with at least five changes of water and documented with ChemiDoc.