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Influence of Simulated Microgravity on Growth, Yield, and Quality of Leafy Vegetables: Lettuce and Rocket

C. M. Rivera[1)], A. Battistelli[2)], S. Moscatello[2)], S. Proietti[2)], Y. Rouphael[1)], M. Cardarelli[1)] and G. Colla[1)]
([1)]Dipartimento di Produzione Vegetale, Università della Tuscia, Viterbo, Italy and [2)]Istituto di Biologia Agro-ambientale e Forestale, Porano, Italy)

Summary

A closed hydroponic system (Porous Tube Plant Nutrient Delivery System) combined with a horizontal uniaxial clinostat has been designed and built-up to grown leafy vegetables under simulated microgravity conditions. Two research activities were carried out to investigate the effects of clinorotation on growth, yield, and quality of rocket seedlings (*Eruca sativa* Mill.) and lettuce plants (*Lactuca sativa* L.), which may constitute a source of healthy fresh food for the astronauts during space missions. The percentage of germination of rocket was affected by clinorotation with the highest value observed in the control treatment. The seedling fresh weight of the control treatment, was 19 % higher compared to that of the clinorotation treatment while the opposite trend was observed for chlorophyll a/b ratio. Clinorotation did not influence the quality (total carbohydrates, triglycerides, carotenoids and vitamin C) of the rocket seedlings. Shoot fresh and dry weight, leaf area, specific leaf dry weight, root dry weight and shoot:root ratio were lower in the clinorotated lettuce plants than those grown in the control treatment. Quality parameters (chlorophyll, total carbohydrates and vitamin C) of lettuce plants were negatively affected by clinorotation. Lettuce genotype comparison under simulated microgravity showed that 'Mortadella di primavera' should be adopted for space experiments due to the better growth and productivity.

Zusammenfassung

Der Einfluss simulierter Mikro-Schwerkraft auf Wachstum, Ertrag und Qualität von Blattgemüse: Kopfsalat und Rukola. Es wurde ein geschlossenes hydroponisches System (Pflanzennahrung über einen porösen Schlauch) kombiniert mit einem einachsigen Klinostaten entwickelt und gebaut, um Blattgemüse unter simulierter Mikro-Schwerkraft zu kultivieren. In zwei Versuchen wurden die Wirkungen der Klinostaten-Rotation auf das Wachstum, den Ertrag und die Qualität von Rukola-Sämlingen (*Eruca sativa* Mill.) und Kopfsalatpflanzen (*Lactuca sativa* L.), die für Astronauten auf Weltraummissionen eine potentielle Quelle für Frischnahrung darstellen, untersucht. Die Keimrate von Rukola wurde durch die Klinostaten-Rotation beeinflusst und der höchste Wert bei der Kontrolle erzielt. Das Sämlingsfrischgewicht der Kontrolle war 19 % höher als beim Klinostaten, wohingegen beim Chlorophyll a/b-Verhältnis der gegensätzliche Trend beobachtet wurde. Die Klinostaten-Rotation hatte keinen Einfluss auf die Qualität (Gesamtkohlenhydrate, Trigyceride, Karotinoide und Vitamin C) der Rukola-Sämlinge. Das Frisch- und Trockengewicht der Sprosse, die Blattfläche, das spezifische Trocken- und Wurzeltrockengewicht sowie das Spross:Wurzel-Verhältnis war bei den Salatpflanzen auf dem Klinostaten niedriger als bei den Kontrollpflanzen. Die Qualitätsparameter (Chlorophyll, Gesamtkohlenhydrate und Vitamin C) der Salatpflanzen wurden durch die Klinostaten-Rotation negativ beeinflusst. Ein Vergleich unterschiedlicher Salatsorten in simulierter geringer Schwerkraft zeigte, dass 'Mortadella di primavera' auf Grund von besserem Wachstum und Qualität für die Weltraumexperimente ausgewählt werden sollte

Key words. clinostat – fresh food – hydroponics – quality – seedlings – space missions

Introduction

The plants play a fundamental role in the human exploration of space. The concept of advanced life support (ALS) in space is based on the intrinsic relationships among plants and animals in natural earthly ecosystems. In this sense, plants should exchange matter and energy cyclically in a bioregenerative closed system (MONJE et al. 2003). Despite intensive scientific efforts done in the past decades looking to materialize this concept, the state of art is still in the early stage. The principal drawback is tied up to the economical and technical limitations that redoubt the opportunities to perform experiments under real microgravity conditions (μG). The implementation of the International Space Station (ISS), as scientific laboratory, would have a "catalyst" role in the development of this scientific field (FERL et al. 2002). The preliminary activities that looked to prepare the experiments in orbit, in-

volved the accurate selection of plant genotypes, which have to respond to the specific requirements of ALS concept (STANKOVIC 2001). In this way, the preparation trials must reproduce, as close as possible, the "environmental" conditions of spatial aircrafts or ISS scenarios (FU et al. 1996). All growth conditions (e.g. light and temperature) can be simulated at the earth level using special growth chambers. The most usual technique employed to simulate the μG in plant experiments is the clinorotation, which consists in rotating the plants at low angular velocity aiming to disorient them similarly to what occurs under real μG (HOSON et al. 1997; KRAFT et al. 2000). Clinorotation has been widely used for *in vitro* and seedling studies (PAOLICCHI et al. 2002; ARONNE et al. 2003). However, seedling studies focused mainly on physiological and genetical aspects of germination and growth without taking into consideration the potential of seedlings as source of healthy fresh food for the astronauts during long-term space missions. Moreover, information is still lacking on growing plants under simulated microgravity conditions (clinorotation) due to the difficulties of supplying adequately nutrients and water to the root apparatus up to advanced phenological stages. Thinking to grow plants up to the cotiledonary stage, *in vitro* culture results were inappropriate and hydroponics becomes an obligate practice. Hydroponic systems which release the nutrient solution through a microporous material (WRIGHT et al. 1988; KOONTZ et al. 1990; JOHNSON et al. 1996; STEINBERG et al. 2000) could be the most suitable systems for growing plants under simulated microgravity conditions.

Starting from the above considerations, the aim of the present research was to evaluate the quanti-qualitative traits (growth, yield and quality) of rocket seedlings and lettuce plants under simulated microgravity conditions.

Material and Methods

Clinorotation system and growth conditions

A uniaxial clinostat was used to simulate the microgravity conditions. The rotatory movement was generated by an asynchronous single-phase motor plus planetary reduction unit with electronic velocity regulator (0.4–7.0 rpm) (Mini Motor, Italy). Rotation rate was 1.5 rpm. The movement was transmitted through a roller chain to six 50-cm long growth modules supported by an aluminum structure and placed within a growth chamber. In all experiments, the growth chamber was programmed to maintain a 12 h photoperiod with corresponding 23 °C light/18 °C night and 65 % RH. Average photosynthetic photon flux at canopy level was of 450 μmol m^{-2} s^{-1}.

Rocket experiment

In the seedling experiment, rocket (*Eruca sativa* Mill. cv. 'Rucola coltivata a foglia lobata', SAIS, Italy) was selected as the best species for producing seedlings during space mission due to the brief productive cycle (5 days) and the high lipid content of the seeds.

Seeds of rocket were surface sterilized with a solution containing 80 % of ethanol. After sterilization (10 min), the seeds were washed two times with sterile distilled water. Just after sterilization, seeds were sown on a solidified agar gel (10 g L^{-1}) in Petri dishes (Ø 10 cm). Each dish contained 70 seeds. Dishes were then closed with parafilm to maintain adequate environmental conditions for seed germination (moisture, sterility). Three dishes were mounted on each growth module (GM) of the clinostat previously described. Two treatments (presence and absence of clinorotation) were compared in a randomised complete block design with three replicates. Rocket seedlings were counted and weighted after 5 days from sowing and were oven dried at 80 °C for dry matter determination. One sample of seedlings per plot was used for quality analysis. Analysis of carbohydrates (glucose, fructose, sucrose) and starch were performed through an enzyme linked spectrophotometric assay as described by JONES et al. (1977), including the modification of ANTOGNOZZI et al. (1996). The extraction and determination of vitamin C was performed using the protocol of KAMPFENKEL et al. (1995). Total carotenoids were measured through a spectrophotometer using the procedure described by LICHTENTHALER and WELLBURN (1983). Triglycerides (expressed as glycerol) determinations were performed using the protocol of EGGSTEIN and KUHLMANN (1974).

Lettuce experiments

A hydroponic porous approach, initially proposed by DRESCHEL and SAGER (1989) and known as Porous Tube Plant Nutrient Delivery System (PTPNDS), has been adopted for lettuce experiments due to the tubular shape and its ability to maintain the water content in the substrate, which are fundamental characteristics for the application of clinorotatory movement. The GM consists in a hydrophilic microporous polyethylene tube (Porex Technologies, Germany) with 8–20 μm of pore size, 38.1 mm external diameter and 6.1 mm of wall thickness inside a 6-cm internal diameter solid polyvinyl chloride (PVC) pipe. The external PVC tube of each growing unit had a row of 9 holes (Ø 0.15 cm) 5 cm apart in which small rockwool capsules were placed, where the seeds were sown (Fig. 1). The design created an almost totally enclosed root zone between the outer PVC shell and the inner microporous tube.

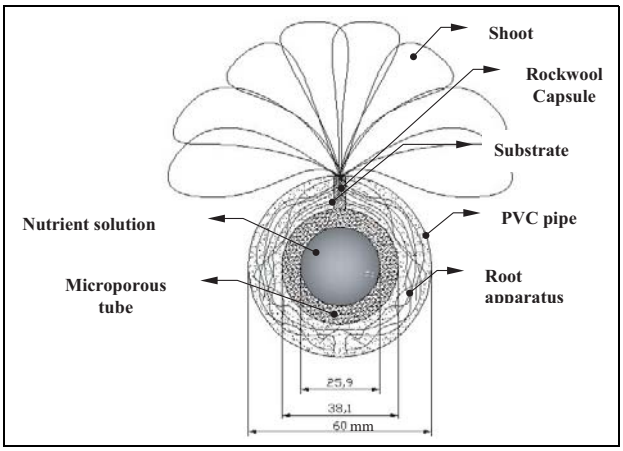


Fig. 1. Cross section of the growth module unit.

The root zone was completely filled with perlite (Ø 1–2 mm) having the following physical and chemical properties: bulk density 105 kg m^{-3}, pH 6.5, EC 0.03 dS m^{-1}, CEC 0.87 meq 100 g^{-1}, and easily available water 13 % v/v. Nutrient solution flowed through the microporous tubes at 180 cm^3 min^{-1} under slight negative pressure (–0.59 kPa) which was maintained by use of a siphon. The siphon adopted was previously described by TIBBITTS et al. (1995). Negative pressure inside the microporous tube was in the optimal range (–0.5 to –1.5 kPa) as reported by previous studies (DRESCHEL and SAGER 1989; CAO and TIBBITTS 1996; STEINBERG and HENNINGER 1997). Through capillary action the nutrient solution fills the entire tube porosity wetting the substrate where the plants develop their root apparatus. Because the system was operated in a siphon mode, it was extremely sensitive to air bubbles breaking the suction. We connected the porous tubes in series so that any air bubble entering the system were pushed through the tubes and were unlikely to collect in the corners of a manifold. In addition, a positive pressure inside the system was generated daily to eliminate any air bubbles. During the experiment, the nutrient solution pressure within the tubes was daily monitored and adjusted using piezometres located at the beginning and the end of the porous tubes.

Two lettuce experiments were carried out: the first one aimed to test the functionality of the system and the second one to assess the effects of clinorotation on quanti-qualititative traits of three lettuce cultivars. In experiment 1, two treatments (presence and absence of clinorotation) were compared in a randomised complete block design with three replicates using a lettuce cultivar (*Lactuca sativa* L. cv. 'Rosario' - SAIS, Italy). There were six GMs (2 clinorotation treatments × 3 replicates) each of them represented an experimental unit containing nine plants. In the second experiment, two clinorotation treatments (presence and absence) × three lettuce cultivars (cv. 'Mortadella di primavera' 'Bionda degli ortolani' and 'Rosario' - SAIS, Italy) were tested in a split plot design with three replicates. The main plot was the clinorotation treatment, while the cultivar treatment was at the subplot level. There were six GMs, each of them represented the main plot (presence or absence of clinorotation) containing nine plants; the subplot was represented by three consecutive plants of the same GM. Seeds were sown directly on the rockwool capsules of the GM's. Three seeds per holes were sown and at cotyledonal stage seedlings were removed leaving one plant per hole. In both experiments, the nutrient solution used contained (mg L^{-1}): N-NO_3 (196), N-NH_4 (14), S-SO_4 (30), P- H_2PO_4 (31), K (234), Ca (160), and Mg (48), Fe (0.6), Mn (0.5), B (0.5), Cu (0.02), Zn (0.05), and Mo (0.01). The EC of the solution was 1.8 dS m^{-1} and the pH was 6.0. To prevent large fluctuations in EC, pH and ionic concentrations, a relatively high volume of nutrient solution per plant (5 L) was recirculating in all treatments. In both experiments, plants were harvested 40 days after sowing and the following measurements were made: fresh and dry weight of shoot and roots (only in the experiment 1) and leaf area (using an electronic area meter, Delta-T Devices Ltd, Cambridge, UK).

In both experiments, shoots of all treatments were used for quality analysis (carbohydrates, proteins, vitamin C and nitrates). Chlorophyll, carbohydrates (glucose, fructose, sucrose and starch), vitamin C, and proteins were determined using the same methods reported in the rocket experiment. Quantification of proteins was performed according to the principle of protein-dye binding (BRADFORD 1976). The nitrate concentration was determined by enzymatic assay (BEUTLER and WURST 1986).

Statistical analysis

All data were statistically analysed by ANOVA using the SPSS software package (SPSS 10 FOR WINDOWS 2001). Duncan's multiple range test was performed at P=0.05 measured on each of the significant variables.

Results and Discussion

Rocket

The percentage of germination was significantly affected by clinorotation with a higher value observed in the control treatment in comparison to the percent of germination recorded under simulated microgravity conditions (Table 1). No morphological alterations of epygeal part of rocket seedlings (e.g. cotyledons) were observed in simulated microgravity conditions as reported for maize seedlings grown in space (UEDA et al. 1999) and for bean grown under simulated microgravity (ARONNE et al. 2003). Moreover, roots of rocket seedlings were also observed in aerial space of the Petri dishes. The seedling fresh weight of the control treatment was 19 % higher compared to those of the clinorotation treatment (Table 1). However, no significant

Table 1. Effect of clinorotation on percent of germination, fresh weight, dry weight, and dry matter of rocket seedlings. Values are the means of three replicate samples. In each column, values followed by different letter correspond to significantly different values at P=0.05.

Clinorotation	Germination (%)	Fresh wt. (mg seedling^{-1})	Dry wt. (mg seedling^{-1})	Dry matter (%)
Presence	65.20 b	13.47 b	1.27 a	9.42 a
Absence	93.30 a	16.02 a	1.40 a	8.79 a
Significance[1]	**	*	ns	ns

[1]ns, *, **: not significant or significant at P≤0.05 and 0.01 respectively.

difference was recorded on the seedling dry weight (avg. 1.33 mg seedling^{-1}) and seedling dry matter (avg. 9.1 %); differences on seedling fresh weight were probably due to the effect of water uptake. The chlorophyll a/b ratio was significantly ($P<0.05$) higher in the clinorotation treatment (2.30) than in the control treatment (1.85). Compared with other species (SHIMAZU et al. 2001), rocket seemed to be more sensitive to clinorotation as shown by the reduction in germination, seedling fresh weight, and by the increasing of the chlorophyll a/b ratio. No significant difference between the treatments were observed on chlorophyll (avg. 255.1 μg g^{-1} f. wt), total carbohydrates (avg. 8.2 mg g^{-1} f. wt), triglycerides (avg. 0.7 mg g^{-1} f. wt), carotenoids (avg. 76.6 μg g^{-1} f. wt) and vitamin C (avg. 57.6 μg g^{-1} f. wt). Phytochemical components (e.g. carotenoids, chlorophyll) and other substance like vitamins, such as ascorbic acid, are well know to be responsible for the protective effect (e.g. anti-carcinogenic, immunity-supporting, anti-bacterial, anti-fungal, anti-viral, cholesterol-lowering, anti-thrombotic and anti-inflammatory effects) against human diseases (SCHREINER 2004). Therefore, raising the human intake of these health-promoting compounds by increasing the proportion of seedlings into the diet is desirable especially for astronauts who are exposed to a stressful environment. The lack of differences on quality parameters between control and clinorotated seedlings suggested that rocket seedlings can be considered an important source of healthy fresh food for astronauts during space missions.

Lettuce

The PTPNDS combined with a uniaxial clinostat was successfully tested in the first lettuce experiment. In experiment 1, all growth parameters were significantly affected by clinorotation (Table 2). Shoot fresh and dry weight were 31 % and 84 % respectively higher in the plants of control treatment in comparison to the clinorotated plants. Moreover, leaf area, specific leaf dry weight, root dry weight and shoot:root ratio were significantly lower in the clinorotated plants by 22 %, 30 %, 37 % and 14 %, respectively than those grown in the control treatment. No significant difference among treatments was observed for total protein (avg. 5.90 mg g^{-1} f. wt) and nitrate concentrations (avg. 588 mg kg^{-1} f. wt) (Table 3), while the concentrations of chlorophyll, total carbohydrates and vitamin C were influenced by clinorotation and were significantly lower by 29, 70 and 40 %, respectively, as compaired to control plants (Table 3). Finally, the chlorophyll a/b ratio was higher in the clinorotation treatment (1.69) than in the control treatment (1.57). In experiment 2, except for specific dry weight of leaves and roots, no significant interaction between genotype and clinorotation was observed for growth parameters and quality characteristics (Tables 4 and 5). The effects of clinorotation on quanti-qualitative traits of lettuce grown in the second experiment followed a similar trend of experiment 1 with higher values recorded on plants grown in the absence of clinorotation in comparison to those grown under microgravity simulation.

The results recorded in our experiments are in line with those reported by MIYAMOTO et al. (1995, 2001) who observed that simulated microgravity conditions on clinostat enhanced the loss of chlorophyll in oat leaf segments due to alterations of the physiological processes of leaf senescence (e.g. protease activity) and the dynamics of endogenous plant hormone levels (e.g. ABA). Overall, the data indicated that the clinorotated plants have less photosynthetised than the control ones

Table 2. Effect of clinorotation on yield and growth parameters of lettuce. Values are the means of three replicate samples. In each column, values followed by different letter correspond to significantly different values at P=0.05.

Clinorotation	Shoot			Leaf area	Specific leaf dry wt	Root dry wt	Shoot:Root dry wt ratio
	fresh wt (g plant^{-1})	dry wt (g plant^{-1})	dry matter (%)	(cm^2 plant^{-1})	(mg cm^{-2})	(g plant^{-1})	
Presence	15.81 b	1.10 b	6.94 b	443.50 b	2.46 b	0.56 b	1.96 b
Absence	20.75 a	2.03 a	9.74 a	568.48 a	3.53 a	0.89 a	2.27 a
Significance[1]	*	***	**	*	***	**	*

[1]ns, *, **, ***: not significant or significant at P≤0.05, 0.01 and 0.001 respectively.

Table 3. Effect of clinorotation on quality parameters of lettuce. Values are the means of three replicate samples. In each column, values followed by different letter correspond to significantly different values at P=0.05.

Clinorotation	Chlorophyll (μg g^{-1} f. wt)	Carbohydrates (mg g^{-1} f. wt)	Proteins (mg g^{-1} f. wt)	Vitamin C (μg g^{-1} f. wt)	Nitrates (mg kg^{-1} f. wt)
Presence	360.60 b	7.10 b	5.98 a	162.14 b	618.12 a
Absence	508.83 a	24.21a	5.82 a	270.21 a	557.31 a
Significance[1]	***	***	ns	*	ns

[1]ns, *, ***: not significant or significant at P≤0.05 and 0.001 respectively.

as observed by TRIPATHY et al. (1996) under real microgravity conditions. Nevertheless, the reduction of assimilation is clearly noticed from the less content of carbohydrates, directly connected to the photosynthetic activity.

At harvest, the clinorotated plants showed a lower insertion angle of leaves than those observed in the control treatment group showing an epinastic response, which is the major growth of the superior part of the petiole in relation to the inferior one (TAIZ and ZEIGER 2002). Such phenomenon seems to be caused by the modification of the hormonal equilibrium of auxin and ethylene whereas auxin plays a promoter role in the ethylene synthesis, which would directly be responsible of this phenomenon. Therefore, it is known that such phenomenons happen, in many cases, as an answer to stressful events and that they can involve messages exchanging between radical apparatus and aerial part through a direct influence on the ethylene metabolism (BRADFORD and YANG 1980). Moreover, the prolonged rotation on the clinostat is reported to be disruptive in that it causes ultrastructural alterations in the columella cells including digestion of middle lamellae which is considered a sign of premature senescence (HENSEL and SIEVERS 1980)

In experiment 2, the genotypes of lettuce differed significantly in growth, yield, and quality. Cultivar 'Mortadella di primavera' exhibited the highest productivity

Table 4. Mean effect of genotype and clinorotation on yield and growth parameters of lettuce. In each column, values followed by different letter correspond to significantly different values at P=0.05.

Treatment	Shoot			Leaf area	Specific leaf dry wt	Root dry wt	Shoot : Root dry wt ratio
	fresh wt (g plant^{-1})	dry wt (g plant^{-1})	dry matter (%)	(cm^2 plant^{-1})	(mg cm^{-2})	(g plant^{-1})	
Genotype (G)							
'Mortadella di Primavera'	32.29 a	2.22 a	6.81 b	697.33 a	3.04 a	0.62 a	3.80 a
'Bionda degli Ortolani'	5.71 b	0.44 c	7.64 a	268.75 b	2.13 b	0.12 c	3.91 a
'Rosario'	12.25 b	0.89 b	7.04 ab	329.25 b	2.42 b	0.23 b	3.78 a
Clinorotation (C)							
Presence	13.31 b	0.83 b	6.52 b	284.68 b	2.10 b	0.21 b	3.32 b
Absence	20.19 a	1.54 a	7.81 a	392.12 a	2.96 a	0.43 a	4.34 a
Significance[1]							
C	***	***	***	***	***	***	ns
G	*	***	*	*	***	***	*
C×G	ns	ns	ns	ns	**	***	ns

[1]ns, *, ***: not significant or significant at P≤0.05 and 0.001 respectively.
S : R: Shoot : Root

Table 5. Mean effect of genotype and clinorotation on quality parameters of lettuce. In each column, values followed by different letter correspond to significantly different values at P=0.05.

Treatment	Chlorophyll (μg g^{-1} f. wt)	Carbohydrates (mg g^{-1} f. wt)	Proteins (mg g^{-1} f. wt)	Nitrates (mg kg^{-1} f. wt)
Genotype (G)				
'Mortadella di primavera'	393.70 a	11.72 ab	4.81 b	747.53 b
'Bionda degli Ortolani'	217.84 c	14.35 a	6.66 a	296.87 c
'Rosario'	325.86 b	8.48 b	5.96 a	1046.75 a
Clinorotation (C)				
Presence	286.87 b	8.08 b	5.43 b	425.98 b
Absence	338.41 a	14.96 a	6.24 a	968.21 a
Significance				
C	***	**	**	***
G	*	***	*	***
C×G	ns	ns	ns	ns

[1]ns, *, **, ***: not significant or significant at P≤0.05, 0.01 and 0.001 respectively.

both on fresh and dry weight basis followed by the cultivar 'Rosario', while the lowest crop performance was observed on 'Bionda degli ortolani'. Moreover, the most productive cultivar has shown a percentage of dry matter slightly less than those of the other two, while values of the specific leaf dry weight and root dry weight followed a similar trend of cultivar productivity.

The highest chlorophyll concentration was observed in cultivar 'Mortadella di primavera', while the highest total carbohydrates were recorded in 'Bionda degli ortolani' and 'Mortadella di primavera'. Moreover, the lowest protein concentration was observed on 'Mortadella di primavera'. Finally the highest nitrate concentration was recorded on 'Rosario' followed by 'Mortadella di primavera' and finally on 'Bionda degli ortolani'. Great variability among cultivars of lettuce on nitrate concentration, has also been observed by ESCOBAR-GUTIÉRREZ et al. (2002). High amounts of nitrates in vegetables have been reported as a negative quality trait since it is a potential threat to human health by causing, indirectly, methaemoglobinaemia in infants and stomach cancer in adults (WALKER 1990). Since leafy vegetables are the main source of dietary nitrate, the European Union established maximum limits for nitrate content in lettuce and spinach in 1997. However, ADDISCOTT and BENJAMIN (2004) pointed out that the contribution of nitrates to potential human carcinogenicity and the magnitude of the associated risk has not been demonstrated yet. Moreover, ADDISCOTT and BENJAMIN (2004) suggested a positive role of nitrates on human health since nitrates are reduced by microbes to nitrites, which generates in the stomach nitric oxide which acts against *Salmonella* and *Escherichia coli* and other microorganisms that cause gastroenteritis.

To summarize, we can conclude, that the PTPNDS combined with a uniaxial clinostat is a valuable tool for growing leafy vegetables till adult stage under simulated microgravity conditions. Experimental results have also showed that the negative effects of clinorotation on growth, yield, and quality were more pronounced on lettuce plants than on rocket seedlings. Finally, our data suggest that 'Mortadella di primavera' should be adopted for space experiments due to the highest growth and productivity.

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Addresses of authors: C. M. Rivera, Y. Rouphael, M. Cardarelli and G. Colla (corresponding author), Dipartimento di Produzione Vegetale, Università della Tuscia, 01100 Viterbo, and A. Battistelli, S. Moscatello, and S. Proietti, Istituto di Biologia Agro-ambientale e Forestale, CNR, 05010 Porano, Italy, e-mail: giucolla@unitus.it.