Plant Cell Physiol. 40(4): 396–405 (1999)

Effect of Horizontal Clinorotation on the Root System Development and on Lipid Breakdown in Rapeseed (*Brassica napus*) Seedlings

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Seedlings of *Brassica napus* were cultivated on a slowly rotating clinostat (1 rpm) or in the vertical control for 5 d. The root growth, the cotyledonary reserves and the transport of ^{14}C-labeled sucrose from cotyledons to root system were studied in both cultural conditions. The biomass (fresh weight) of the root system was 35% higher in the horizontally clinorotated seedlings than in the controls. This increase was correlated with a greater degradation of reserve lipids and faster accumulation of sucrose in the cotyledons. The activity of isocitrate lyase, one of the two enzymes necessary to conversion of lipids into glucids, was also greater in the cotyledons of clinorotated seedlings. The labeling distribution of ^{14}C in the cotyledons, the hypocotyl and the root system after 30, 60 and 120 min of application of ^{14}C-labeled sucrose on the cotyledons showed higher translocation of the cotyledonary sucrose to the root system of clinorotated seedlings. In addition, we studied the effects of clinorotation on the biomass of the excised root system (of 10 d old seedlings) cultivated in a medium containing 1% sucrose. The horizontally clinorotated root system grew more than that of the controls.

These results showed that the horizontal clinorotation acted on the root system growth and provoked a higher sucrose translocation from source to sink, i.e. from cotyledons to root system.

Key words: *Brassica napus* — Carbohydrate metabolism — ^{14}C-labeled sucrose translocation — Oil seed — Root system — Slowly rotating clinostat.

Several authors have shown that microgravity in space flight influences growth, development and metabolism in higher plants. The investigations of Brown et al. (1995), Merkys et al. (1984) and Parfenov et al. (1979) revealed an increase in the initiation of the lateral roots and in the growth of the primary roots. Additional studies in space by Levine et al. (1990) confirmed this finding and reported an increase in the development of the root system. However, a number of authors showed a decrease in plant development in space (Briarty et al. 1995, Cowles et al. 1984, Kiss et al. 1998). According to Kiss et al. (1998) the slower growth and morphological changes observed in flight seedlings of *Arabidopsis thaliana* may be due to the effects of ethylene present in the spacecraft. In addition, several workers have suggested that the slower growth rate of plants in space is due to microgravity affecting convection and, thus, gas exchange in plants (Iversen et al. 1996, Porterfield et al. 1997).

Microgravity also causes other metabolic modifications, for example, the reduction in starch content in various organs and species. Johnson and Tibbitts (1968) showed significantly lower starch and higher soluble sugar concentrations in pepper leaves in plants grown on board Biosatellite II. Abilov et al. (1986) and Aliyev et al. (1987) observed a decrease in starch content and in the number of starch grains in leaves of pea plants grown on board Salyut-7 in microgravity. Decrease in starch content was also observed in roots by Moore et al. (1987) in maize and by Volkmann et al. (1986) in *Lepidium sativum*. These authors also reported an increase in the volume of lipid bodies in the maize columella cells of space-grown plants. Thus, similar to other environmental factors (light, temperature, drought, salt, etc.) (Kuiper 1985), microgravity can produce quantitative and qualitative changes in plant lipid composition.

The slowly rotating clinostat was used by several authors in order to alter gravity conditions. Dedolph et al. (1965) reported that *Avena* seedlings developed a longer root system under horizontal clinorotation than in the vertical control. More recently, Driss-École et al. (1994) and Hilaire et al. (1996) also reported an increase in the development of the root system in different species on the clinostat (root length, fresh and dry weights). We have suggested that clinorotation induces a continuous slight stimulation of the root growth as a consequence of change in the hormonal balance in primary root tips (Aarrouf et al. 1999).

As in space, metabolic modification was observed on the clinostat. Thus, a decrease in starch content was shown by Hensel and Sievers (1980) in *Lepidium sativum* roots. Brown and Piastuch (1994) demonstrated that the amount of starch was lower in the cotyledons of soybean in horizontally clinorotated plants whereas the sucrose content was unchanged compared to the controls. These authors

suggested that the decrease in starch content on the clinostat was related to the activity of the ADP pyrophosphorylase which was lower than that found in the vertical control and not to any other starch metabolic enzymes measured. In addition, Brown and Piastuch (1994) found that soybean seedlings exposed to 6 d of clinorotation contained 16% more total lipids in cotyledons than in the control plants. These studies have indicated a gravity-dependent relationship between starch and lipids.

In these space and slowly rotating-clinostat experiments, the results showed an increase in lipid content and a decrease in starch content in different species and organs. However, the slowly rotating-clinostat is unable to simulate the conditions of space but it is considered to be able to compensate for the unilateral influence of gravity (Hoson et al. 1997).

In pea seeds, the cotyledons are storage organs which regulate the early development of seedlings (Rost and Toriyama 1991). Hinchee et and Rost (1986) and Wightman and Thimann (1980) demonstrated that the development of the root system in seedlings is dependent on the cotyledons because they provide auxin and nutritional factors to the roots that are essential for lateral root emergence. In the oil seeds such as *Brassica napus*, the metabolism of the storage lipids into carbohydrates during seed germination depends on the glyoxylate cycle activation (Tolbert 1981). Two enzymes characterize this cycle: the malate synthase (EC 4.1.3.2) and the isocitrate lyase (EC 4.1.3.1). This latter enzyme separates isocitrate into glyoxylate and succinate. The succinate is then converted into carbohydrates via gluconeogenesis.

Rapeseed represents a particularly interesting plant material because in this species the carbohydrate metabolism is strongly linked to the lipid metabolism, via the gluconeogenesis process, during the germination and post-germination of seeds. Thus, the objectives of this study were to determine the effect of horizontal clinorotation on: 1—the degradation of the lipid reserves in the cotyledons of rapeseed seedlings; 2—the translocation of ^{14}C-labeled sucrose from cotyledons to root system; 3—the lipid and carbohydrate metabolisms in roots; 4—the fatty acid composition in both cotyledons and root system; 5—the growth of the isolated root system.

Materials and Methods

Plant culture—Seeds of *Brassica napus* were treated with 85% ethanol for 5 min, surface sterilized for 10 min in calcium hypochlorite (4%) and rinsed 3 times (10 min) in sterile water.

Tubes were filled with 25 ml of 0.3% (w/v) phytagel with Heller's salts (Flow Laboratories, U.K.). The seeds were aseptically placed in 160 tubes (1 seed/tube) and these tubes were put on two rotating devices. The seeds clung to the surface of the medium and did not move during clinorotation. The tubes were not hermetically sealed. On one device the seedlings were rotated about a vertical axis at 1 rpm (vertical controls) and, on the second about a horizontal axis (horizontal clinostat) at the same velocity. It must be noted that at this rotation speed, the seedlings were subjected to a maximal centrifugal acceleration of $1.7 \times 10^{-4} \times g$ which was lower than the threshold acceleration for gravitropic response (Shen-Miller et al. 1968). The clinostat and the vertical roller were placed in a separated chamber illuminated by white light Philips tubes (long duration, New Generation. 18W/865), providing a photon flux of 432 μmol m^{-2} s^{-1} at the level of the surface of the medium with a 16-h day-cycle, at 27°C. After 5 d of growth, the fresh weight of the shoot system and of the root system was determined. Then, each root system was placed carefully on a large glass plate and photographed for morphometric analysis. The number of emerged lateral roots and the length of the primary and secondary roots were measured on photographs by the means of a PC 486 (Compaq) computer equipped with an image digitizing card MVP AT (MATROX) (SAMBA 2005, Alcatel-TITN, Grenoble, France). for each condition 18 plants were analyzed.

In the experiments where the shoot system was removed, 1% (w/v) sucrose was added to the culture medium as a carbohydrate source of energy in order to compensate for the lack of cotyledons and maintain the growth of the excised root system (MacLeod and Thompson 1981). The cotyledons and hypocotyl were removed after 10 d of culture in the stationary vertical position, and the root system was then cultivated on the horizontal clinostat or in the controls for 15 d. After this period, the fresh weight of each root system was determined. for each condition 16 plants were analysed.

Extraction and measurement of lipids—After 5 d of culture, 700 mg of cotyledons and 300 mg of roots were obtained from horizontally clinorotated and the control plants. Lipids were extracted with chloroform-methanol (Bligh and Dyer 1959). For quantification on a mass basis, an appropriate amount of an internal standard (heptadecanoic acid) was added to the lipids. Saponification of total lipids and methylation of their fatty acids were carried out with methanolic NaOH and boron trifluoride-methanol (Metcalfe et al. 1966). Fatty acid methyl esters were analysed and quantified by gas liquid chromatography in a VARIAN 3300 gas chromatograph coated with carbowax 20 M (0.32 mm i.d. × 50 m).

Extraction and measurement of soluble sugars, starch and chlorophylls—For analysis of starch, soluble sugars and chlorophylls, 900 mg of cotyledons and 400 mg of roots were obtained from horizontally clinorotated and vertical control plants after 5 d of culture. These compounds were extracted with methanol/chloroform/water according to Darbelley et al. (1997). The chlorophorm-phase, containing the chlorophylls, was brought to dryness at 45°C in a rotatory evaporator, dissolved in 80% (v/v) acetone and then chlorophyll *a* and *b* contents were measured spectrophotometrically by the method of Arnon (1949).

The methanol-water phase, containing the soluble sugars, was rotatory evaporated to dryness at 45°C and then resolubilized in water. The sucrose was determined by chromatography using a dionex HPLC system. The concentration of the whole soluble sugars was determined colorimetrically by the anthrone-sulfuric acid method of McCready et al. (1950) with glucose as standard.

Starch was quantitated by digestion of the insoluble pellet with amyloglucosidase solution at 50°C for 24 h (Haissing and Dickson 1979). The mixture was centrifuged at 3,000 × *g* for 20 min at 4°C and the glucose present in the resulting supernatant was determined by chromatography using Dionex HPLC system

(Darbelley et al. 1997).

Protein extraction and enzyme assays—Cotyledons and root system (200 mg) were harvested, frozen in liquid nitrogen and the proteins were extracted in 2 volumes grinding medium containing 100 mM phosphate buffer (pH 6.9), 3 mM $MgCl_2$ and 3 mM dithiothreitol (Faure and Aarrouf 1994). The supernatant was used to determine isocitrate lyase activity and total protein assays. Isocitrate lyase activity was measured in the cotyledons according to Dixon and Kornberg (1959). For 1.2 ml of final volume, the reaction medium contained 87 mM phosphate buffer (pH 7.9), 4.6 mM dithiothreitol, 8.7 mM $MgCl_2$, 10 mM phenylhydrazine and 50 μl enzymatic extract. The reaction was initiated by adding 13 mM sodium isocitrate. The isocitrate lyase activity was evaluated by measuring absorbency at 324 nm and by using a 17,000 $M^{-1}\,cm^{-1}$ molecular absorption coefficient for the glyoxylate-phenyhydrazone complex. Proteins were measured according to the method by Bradford (1976) using a bovine serum albumin standard curve. All measurements were carried out at least in triplicate.

Transfer of ^{14}C-labeled sucrose through the plant—In order to study translocation of sucrose from cotyledons to root system, 5 μl containing 14.8 kBq of ^{14}C-labeled sucrose solution (Specific activity: 20.9 Gbq mM^{-1}, Amersham, Bucks, U.K.) was applied on the two cotyledons of 5 day-old seedlings cultivated on the clinostat or in vertical position. After 30, 60 and 120 min, the seedlings were cut and the cotyledons, the hypocotyl and the root system were separated for determining the ^{14}C labeling in these organs. After a short wash of the cotyledons in sterile water to eliminate ^{14}C-sucrose which was not incorporated, each organ was digested and decolorized in a solution containing 0.1 ml of 70% (v/v) perchloric acid and 0.1 ml of 30% (v/v) H_2O_2 and then placed in a water bath at 60°C for 30 min with agitation (Giaquinta 1977). The radioactivity (dpm) was determined by liquid scintillation spectroscopy.

Statistical test—In this study, the means were followed by their confidence interval at the 5% level and Student's test and analysis of variance (ANOVA) were used to compare the data of horizontally clinorotated seedlings with those of the controls.

Results

Development of the clinorotated seedlings—Brassica napus seedlings grown for 5 d on the horizontal clinostat exhibited a faster development than in the controls (Fig. 1). The number of lateral roots and the primary root length were significantly greater in the former (Table 1). The biomass (fresh weight) of the root system, and to a lesser extent also that of the shoot, was greater on the horizontal clinostat than in the controls (Table 1). Similar results were obtained by Driss-École et al. (1994) on *Veronica arvensis*.

Effect of the horizontal clinostat on metabolites and chlorophylls in cotyledons—Metabolites were measured in the cotyledons of *Brassica* seedlings grown for 5 d on the horizontal clinostat and compared to the controls. The levels (in μg (mg FW)$^{-1}$) of starch, soluble sugars, proteins and chlorophylls did not differ significantly between the two culture conditions (Table 2). In contrast, the lipid level in horizontally clinorotated cotyledons was significantly less while sucrose level was significantly higher than in the controls (Fig. 2). The proportions of palmitic, oleic, linoleic and linolenic acids in total fats were not sig-

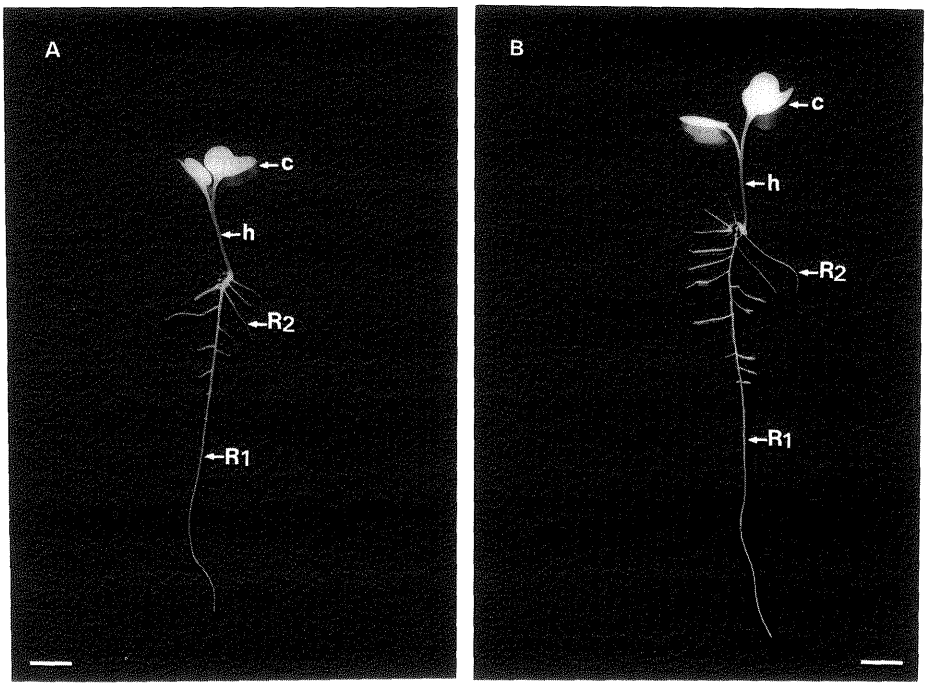

Fig. 1 Representative seedlings of *Brassica napus* grown for 5 d, (A) in the controls and (B) on the horizontal clinostat. c, cotyledons; h, hypocotyl; R1, primary root; R2, secondary root. Bar represents 1 cm.

Table 1 Average number of lateral roots, primary root length and the biomass of root system and the shoot system (stem and cotyledons) of *Brassica napus* seedlings grown 5 d on the horizontal clinostat or in the controls

	Controls	Horizontal clinostat	Percent change	t test
Average number of the lateral roots	10.3±1.8	14.9± 2.1	44%	3.32 (S)
Average length of the primary root (mm)	87.1±5.5	104.7±10.5	20%	3.18 (S)
Biomass of the root system (mg)	17.8±2.0	24.1± 1.8	35%	4.74 (S)
Biomass of the shoot system (mg)	76.0±5.7	87.8± 6.0	15%	2.98 (S)

The means (n=18) are followed by their confidence interval at the 5% level. S, significant.

nificantly affected by horizontal clinorotation (Table 3).

Isocitrate lyase activity in cotyledons—The higher sucrose levels found in cotyledons of horizontally clinorotated seedlings compared to the controls could be the result of stronger degradation of reserve lipids during seedling growth on the clinostat. In order to confirm this hypothesis, the activity of the isocitrate lyase, which is one of the two enzymes characterizing the glyoxylate cycle, was studied in the cotyledons of horizontally clinorotated seedlings and in the controls.

Figure 3 shows that isocitrate lyase activity decreased progressively during the first 5 d of growth in the horizontally clinorotated seedlings and in the controls. A maximal isocitrate lyase activity in the cotyledons was obtained after 2 d of culture in both conditions and thereafter declined. At days 2 and 3, enzyme activity was significantly higher (at the 5% level, t=3.85 and 4.43 respectively) in the horizontally clinorotated seedlings than in the controls. This result was in agreement with the hypothesis that the increase in sucrose level in the cotyledons could be due to the higher degradation of the storage lipids on the horizontal clinostat and could depend on the glyoxylate cycle activation, at least at the beginning of the germination. At day 4 isocitrate lyase was significantly lower at the 5% level (t=3.52) in the cotyledons of horizontally clinorotated seedlings compared to the controls because the lipid degradation had taken place earlier on the clinostat.

It was also possible that the increase of sucrose content in the cotyledons on the horizontal clinostat was due to the fact that this sugar was less translocated. However, this was in contradiction with a faster development of the seedlings in this cultural condition.

^{14}C-labeled sucrose translocation from the cotyledons to root system—The results showed that incorporation of ^{14}C-labeled sucrose in the cotyledons was fast and increased as function of time, and that the horizontal clinorotation did not affect this incorporation (Fig. 4, Table 4). In the hypocotyl, it seemed that the clinorotated plants had more ^{14}C labeling than the controls. However, the ANOVA results demonstrated that this increase was not significant ($F_{(1,18)}=4.28$). In the case of the root system, the ^{14}C labeling increased continually as function of time and was higher on the clinostat than in the controls. In all cases, there was no significant interaction between the two factors, the increase of ^{14}C labeling was due to the duration of their incorporation or to clinorotation.

Reserve metabolism in the root system of the clinorotated seedlings—As the horizontal clinostat influenced the development of the root system it was necessary to determine whether the content of metabolites in these organs on the horizontal clinostat was different from that of the controls. The levels of lipids, starch, sucrose, soluble sugars and proteins (fresh weight basis) of the root system did not differ significantly between both culture conditions

Table 2 Starch, soluble sugars, protein and chlorophyll (*a, b*) contents (μg (mg FW)$^{-1}$) in the cotyledons of *Brassica napus* seedlings cultivated on the horizontal clinostat or in the control for 5 d

	Controls	Horizontal clinostat	t test
Starch	0.62±0.32	0.58±0.20	0.30 (NS)
Soluble sugars	4.05±1.38	3.89±1.09	0.21 (NS)
Proteins	16.69±1.07	15.22±1.35	1.83 (NS)
Chlorophylls	0.48±0.10	0.53±0.10	0.98 (NS)

The means (n=3) are followed by their confidence interval at the 5% level. NS, not significant.

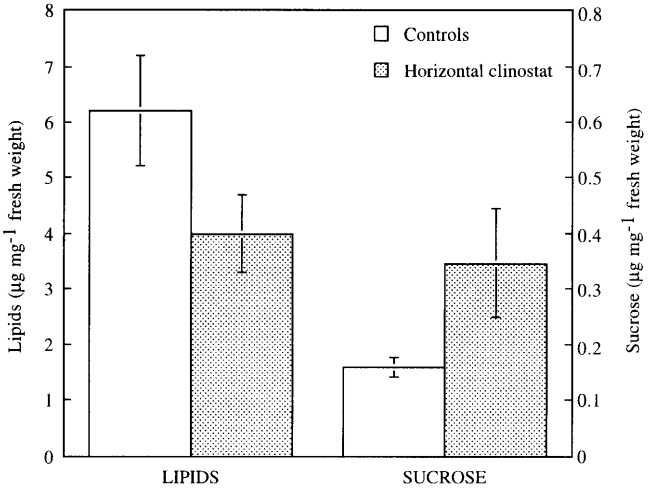

Fig. 2 Lipid and sucrose contents in 5 day-old *Brassica napus* cotyledons of seedlings grown on the horizontal clinostat or in the controls. Bars represent confidence interval at the 5% level (n=4-6).

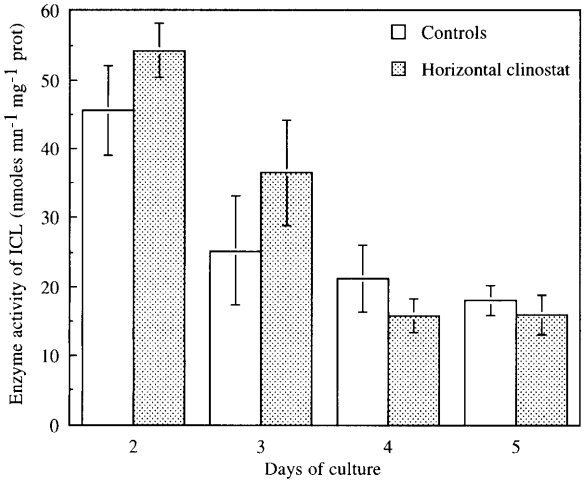

Fig. 3 Isocitrate lyase activity in cotyledons of *Brassica napus* during 5 d of culture on the vertical clinostat or in the controls. Difference is significant (at the 5% level) after 3 and 4 d of culture (t tests, n=3).

(Table 5). Fatty acid composition in the root system was also unaffected by horizontal clinorotation as shown in Table 6.

Effect of the clinostat on the biomass of the isolated root system—The development of the root system of *Brassica* seedlings was faster on the horizontal clinostat compared to the vertical controls. This result might be due to the greater degradation of the lipid reserves in the horizontally clinorotated cotyledons and the greater translocation of sucrose toward the root system. In order to determine whether the clinostat could act on the root system development independently of storage lipid metabolism in the cotyledons, root development was studied in absence of the metabolites supplied by the shoot system: the cotyledons and the hypocotyl were removed from 10 day-old plants grown in phytagel with 1% sucrose in the stationary vertical position (Fig. 5A, B). The isolated root systems were then cultivated for 15 d in the vertical controls or on the horizontal clinostat (Fig. 5C, D). The root system continued to grow in both conditions (see arrows in Fig. 5), but its biomass was significantly greater on the horizontal clinostat than in the controls (Table 7), which showed that clinorotation acted on the root development.

Discussion

When seeds of *Brassica napus* were cultivated on the horizontally rotating-clinostat, several changes occurred. An increase in the overall development of the root system was observed. The same result was obtained in different species cultivated in microgravity (Brown et al. 1995, Gallegos et al. 1995) or on the clinostat (Driss-École et al. 1994). We found that in 5 day-old seedlings, this increase in the biomass of the root system was due to more abundant and greater growth of the lateral roots as well as a somewhat greater elongation of the primary root. In contrast, other studies have shown that growth is decreased or not affected by spaceflight (Briarty et al. 1995, Kiss et al. 1998)

Table 3 Fatty acid composition (in percentages of total lipids) in the cotyledons of *Brassica napus* seedlings cultivated on the horizontal clinostat or in the controls for 5 d

	Controls	Horizontal clinostat	t test
Palmitic acid	12±5	12± 5	0.11 (NS)
Oleic acid	28±9	21±13	1.07 (NS)
Linoleic acid	20±3	19± 3	0.30 (NS)
Linolenic acid	39±7	47±10	1.59 (NS)

The means (for n=6) are followed by their confidence interval at the 5% level. NS, not significant.

Table 4 Two way analysis of variance (ANOVA; N=24) of ^{14}C labeling in the cotyledons, hypocotyl or root system

	FC	Ft	Fi
Cotyledons	3.78 (NS)	15.69 (NS)	0.40 (NS)
Hypocotyl	4.29 (S-NS)	1.47 (NS)	0.16 (NS)
Root system	6.51 (S)	11.37 (S)	0.06 (NS)

The first factor is culture condition (clinostat or vertical control), the second factor is time (30, 60, 120 min). Fc (F for culture condition), Ft (F for time) and Fi (F for interaction between culture condition and time) have to be compared to the limit of significance at the 5% level (depending upon the degrees of freedom): F(1,18)=4.41, F(2,18)=3.55 and F(2,18)=3.55 respectively. S, significant; NS, not significant, S-NS, at the limit of significance.

or clinorotation (Hensel and Iversen 1980, Legué et al. 1992, Perbal and Driss-École 1994). However, in these studies, the analyses were conducted on different species at different phases of development and in different culture conditions. According to Claassen and Spooner (1994) these controversial results were due to the fact that the effects of microgravity on root growth are depending upon the duration of exposure to space. The root growth was generally greater when in microgravity for long periods of time (Cowles et al. 1984, Levine et al. 1990, Porterfield et al. 1997). In the same way, several studies on clinostat showed a greater development of the roots after a long duration culture (Dedolph et al. 1965, Driss-École et al. 1994, Hilaire et al. 1996). Taken altogether these data indicated that clinostat at least mimic the action of microgravity on the development of the root system.

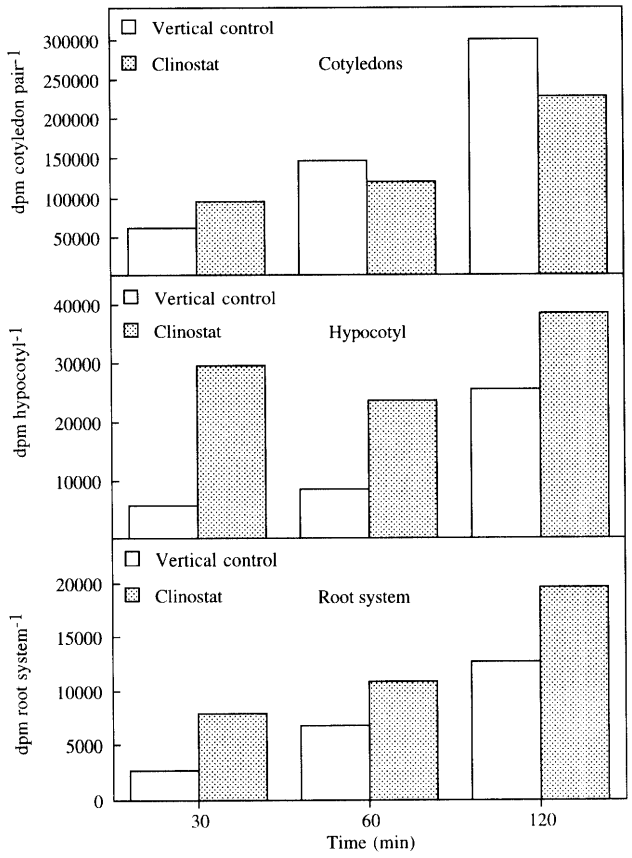

Fig. 4 Labeling distribution of ^{14}C after accumulation for 30, 60 and 120 min of exogenously supplied ^{14}C-sucrose in the cotyledons, hypocotyl and root system of 5 day-old seedlings grown on the clinostat or in the vertical position (n=4).

A carbohydrate source of energy is necessary for maintaining cell proliferation for initiation and emergence of lateral roots (Van't Hof and Kovacs 1972). Before acquisition of autotrophy the cotyledons are the source of carbohydrates and metabolites necessary to seedling growth (Murin 1976). Total or partial removal of the cotyledons reduces or suppresses lateral root development (Hinchee and Rost 1986, Wightman and Thimann 1980).

Brown and Huber (1987, 1988) reported an accumulation of starch in soybean cotyledons during the first days of seedlings growth. However, when exposed to horizontal clinorotation for 6 d, these organs contained less starch than the controls (Brown and Piastuch 1994). Hilaire et al. (1996) reported an increased root growth under horizontal clinorotation and suggested that this phenomenon may be related to the lower starch concentration due to enhanced carbon translocation from cotyledons.

In the present study, we found that the lipid content was lower in the cotyledons of seedlings exposed to horizontal clinorotation than in the vertical controls. An opposite change was observed for sucrose concentration. In contrast, the starch, soluble sugar (except sucrose), protein and chlorophyll contents did not differ statistically in both culture conditions. Tripathy et al. (1996) reported a decrease in photosynthetic activity in wheat seedlings grown in microgravity compared to the controls. These data suggested that the higher levels of sucrose found in the cotyledons of horizontally clinorotated plants could be the result of a greater degradation of storage lipids. Isocit-

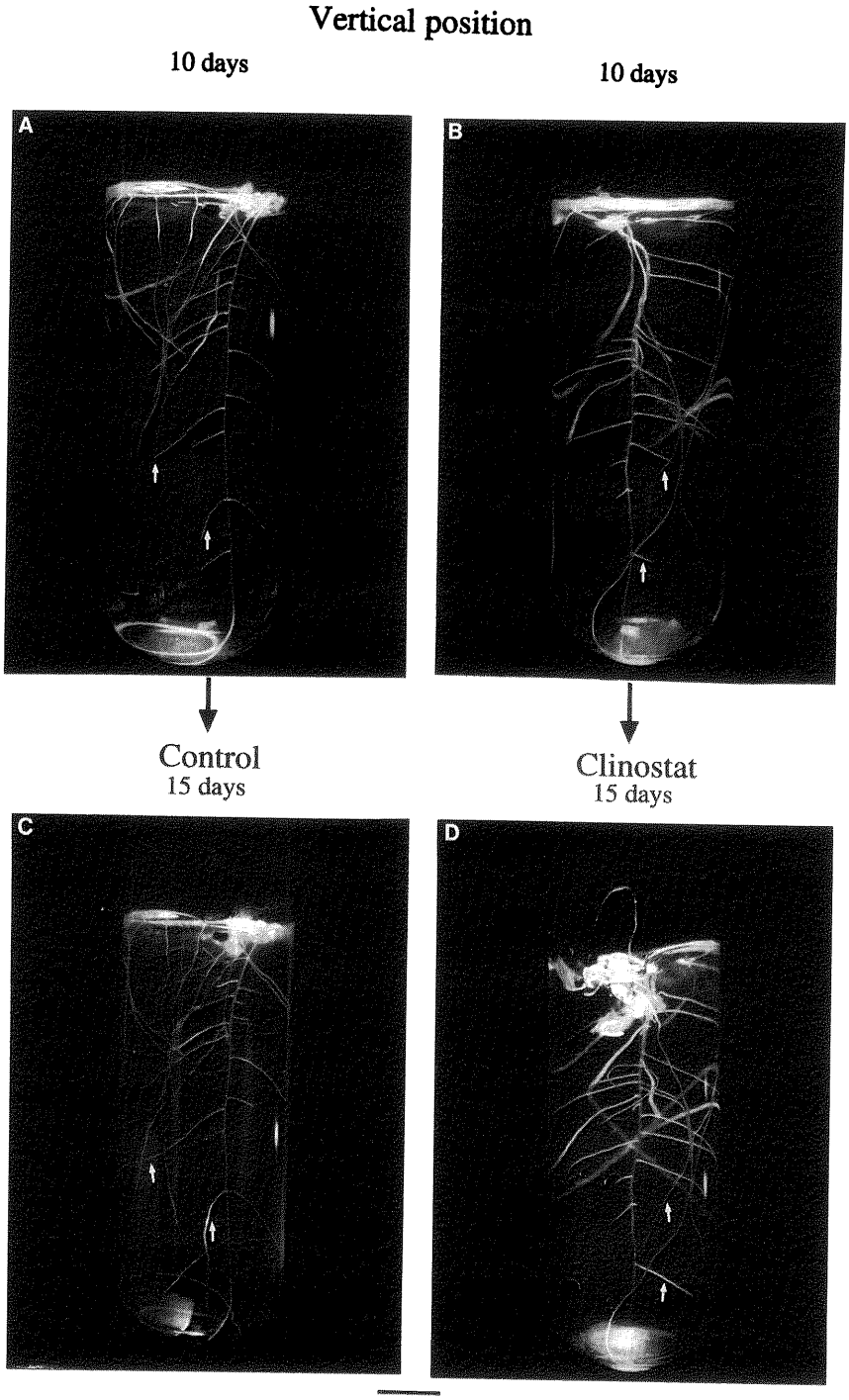

Fig. 5 Representative root systems (A and B) of *Brassica napus* seedlings grown for 10 d in the stationary vertical position (without rotation). The shoot system of the seedlings was removed and the detached root systems were either rotated vertically (C) or on the horizontal clinostat (D) at 1 rpm for a further 15 days. Bar = 1 cm, arrows show roots where growth can be easily followed.

rate lyase activity was enhanced in cotyledons of clinorotated seedlings during the first days, confirming this view. Inversely to the results obtained for soybean seedlings (Brown and Piastuch 1994) the increase in sucrose concentration, the decrease in lipid content and the stability of starch concentration on the clinostat may have taken place by a different pathway of reserve mobilization during seed germination and early growth of rapeseed seedlings.

Table 5 Lipid, starch, sucrose, soluble sugars and protein contents (μg (mg FW)$^{-1}$) in the root system of *Brassica napus* seedlings cultivated on the horizontal clinostat or in the controls for 5 d

	Controls	Horizontal clinostat	t test
Lipid	1.18±0.26	1.09±0.16	0.11 (NS)
Starch	0.09±0.02	0.08±0.01	1.07 (NS)
Sucrose	0.18±0.02	0.16±0.04	0.11 (NS)
Soluble sugars	1.98±0.73	1.62±0.16	0.30 (NS)
Proteins	6.28±0.77	4.99±1.08	1.59 (NS)

The means (n=3) are followed by their confidence interval at the 5% level. NS, not significant.

The translocation of the sucrose from cotyledons to the growing root during seedling development was often investigated according to the concept of "Source and Sink" (Giaquinta 1983, Ho 1988, Orlich et al. 1998). Thus, we studied the effect of the clinorotation on this phenomenon and our results showed a higher translocation of ^{14}C-labeled sucrose from cotyledons to the root system, i.e. source and sink, of clinorotated seedlings compared to the controls. We demonstrated that the horizontal clinorotation induced a rapid degradation of lipids and an increase sucrose concentration in the cotyledons of *Brassica* seedlings in order to assume the faster development of the root system. However, despite the higher sucrose translocation, we found a higher sucrose concentration in the clinorotated cotyledons. It seemed that clinorotation could have a direct effect on the lipids degradation and the increase in sucrose concentration, i.e., a direct action on the source of metabolites.

We did not observe any difference in the metabolite content between horizontally clinorotated and control root systems. These data led us to suggest that in the root system of clinorotated seedlings there was a rapid use of the metabolites (as sucrose) mobilized from the cotyledons.

When the isolated root systems of 10 day-old seedlings were cultivated 15 d in a medium containing 1% sucrose, the results showed that the horizontal clinorotation enhanced the development of these organs. The enhancement of the root system development is therefore independent of mobilization of reserves from the cotyledons.

Different environmental factors may alter plant lipid composition (Fouzia et al. 1995). For example, Williams et al. (1992) reported that low temperature induced desaturation of fatty acids in leaves of *Brassica napus*. A similar result was obtained by Ashworth and Christiansen (1981) in the wheat root in which the level of linolenic acid increased and the level of linoleic acid decreased. These investigators suggested that the change in fatty acid composition could increase the fluidity of membranes and may influence other membrane-associated processes (active transport, water permeability). We did not find any difference in the fatty acid composition of the root system and the cotyledons of horizontally clinorotated seedlings or in the controls. The effect of horizontal clinorotation on the root system is thus not the same as that of other environmental stresses (as temperature, light, salt, drought, etc.).

In conclusion, there was clear evidence that clinorotation was responsible for the increase of the root development and of the translocation of sucrose from cotyledons to root system. Moreover, the conversion of lipid reserves into carbohydrates may be an effect of clinorotation by the control of the activity of the glyoxylate cycle

Table 6 Fatty acid composition (in percentages of total lipids) in the root system of *Brassica napus* seedlings cultivated on the horizontal clinostat or in the controls for 5 d

	Controls	Horizontal clinostat	t test
Palmitic acid	27±1	25±3	1.08 (NS)
Oleic acid	2±2	6±5	1.78 (NS)
Linoleic acid	48±3	42±6	1.97 (NS)
Linolenic acid	21±4	24±6	0.85 (NS)

The means (for n=6) are followed by the confidence intervals at the 5% level. NS, not significant.

Table 7 Fresh weight of the detached root systems of *Brassica napus* seedlings grown for 15 d on the horizontal clinostat or in the control after removal of the aerial part of the seedlings

	Controls	Horizontal clinostat	Percent change	t test
FW (mg)	29.7±4.5	36.5±5.3	23%	2.1 (S)

The means (for=16) are followed by the confidence interval at the 5% level. S, significant.

enzymes (such as isocitrate lyase). Thus, clinorotation may stimulate root development by enhancing both the source and sink avtivities.

This work was supported by the Centre National des Etudes Spatiales (CNES, 927-34S). We thank Dr. M. Tepfer for the gift of seeds of *Brassica napus* and Professor A. Pavia for his support in carbohydrate analysis.

References

Aarrouf, J., Schoëvaërt, D., Maldiney, R. and G. Perbal, G. (1999) Changes in hormonal balance and meristematic activity in primary root tips on the slowly rotating clinostat and their effect on the development of the rapeseed root sytem. *Physiol. Plant.* (in press).

Abilov, Z.K., Alekperov, U.K., Mashinskiy, A.L., Fadeyeva, S.I. and Aliyev, A.A. (1986) The morphological and functional state of the photosynthetic system of plant cell grown for varying periods under space flight conditions. *USSR Space Life Science Digest* 8: 15-16.

Aliyev, A.A., Abilov, Z.K., Mashinskiy, A.L., Ganiyeva, R.A. and Ragimova, G.K. (1987) The ultrastructure and physiological characteristics of the photosynthesis system of shoots of garden peas grown for 29 days on the Salyut-7 space station. *USSR Space Life Science Digest* 10: 15-16.

Arnon, D.I. (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* 24: 1-15.

Ashworth, E.N. and Christiansen, M.N. (1981) Effect of temperature and BASF 13338 on the lipid composition and respiration of wheat roots. *Plant Physiol.* 67: 711-715.

Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Bioch. Physiol.* 37: 103-106.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.

Briarty, L.G., Maher, E.P. and Iversen, T.H. (1995) Growth, differentiation and development of *Arabidopsis thaliana* under microgravity conditions. *In* Biorack on Spacelab IML-1. European Space Agency. Edited by Mattok, C. pp. 141-154. ESA Publications Division, Estec, Noordwijk, The Netherlands.

Brown, C.S., Hilaire, E.M., Guikema, J.A., Piastuch, W.C., Johnson, C.F., Stryjewsky, E.C., Peterson, B.V. and Vodermark, D.S. (1995) Metabolism, ultrastructure and growth of soybean seedlings in microgravity. *ASGSB Bull.* 9: 93.

Brown, C.S. and Huber, S.C. (1987) Photosynthesis, reserve mobilization and enzymes of sucrose metabolism in soybean (*Glycine max*) cotyledons. *Physiol. Plant.* 47: 225-228.

Brown, C.S. and Huber, S.C. (1988) Reserve mobilization and starch in soybean (*Glycine max*) cotyledons in relation to seedling growth. *Pysiol. Plant.* 72: 518-524.

Brown, C.S. and Piastuch, W.C. (1994) Starch metabolism in germinating soybean cotyledons is sensitive to clinorotation and centrifugation. *Plant Cell Environ.* 17: 341-344.

Claassen, D.E. and Spooner, B.S. (1994) Impact of altered gravity on aspects of cell biology. *Int. Rev. Cytol.* 156: 301-373.

Cowles, J.R., Scheld, H.W., Lemay, R. and Peterson, C. (1984) Growth and lignification in seedlings exposed to eight days of microgravity. *Ann. Bot.* 54: 33-48.

Darbelley, N., Razafindramboa, N., Chambost, J.P. and Pavia, A. (1997) Light effects on α-amylase activity and carbohydrate content in relation to lipid mobilization during the seedling growth of sunflower. *J. Plant Res.* 110: 347-356.

Dedolph, R.R., Naqvi, S.M. and Gordon, S.A. (1965) Effect of gravity compensation on the geotropic sensitivity of Avena seedlings. *Plant Physiol.* 40: 961-965.

Dixon, G.H. and Kornberg, H.L. (1959) Assays methods for key enzymes of the glyoxylate cycle. *Biochem. J.* 72: 3.

Driss-École, D., Cottignies, A., Jeune, B., Corbineau, F. and Perbal, G. (1994) Increased mass production of *Veronica arvensis* grown on a slowly rotating clinostat. *Environ. Exp. Bot.* 34: 303-310.

Faure, O. and Aarrouf, J. (1994) Metabolism of reserve products during development of somatic embryos and germination of zygotic embryos in grapevine. *Plant Sci.* 96: 167-178.

Fouzia, N., Marzouk, B. and Cherif, A. (1995) Sodium chloride effect on the evolution of fatty acid composition in developing rape seedlings. *In* The Plant Lipid Metabolism. Edited by Kader, J.C. and Mazliak, P. pp. 435-437. Kluwer Academic publishers, The Netherlands.

Gallegos, G.L., Odom, W.R. and Guikema, J.A. (1995) Effect of microgravity on stress ethylene and carbon dioxide production in *sweet clover* (*Melilotus alba* L.) *J. Gravit. Physiol.* 2: 155-156.

Giaquinta, R.T. (1977) Phloem loading of sucrose. pH dependence and selectivity. *Plant Physiol.* 59: 750-755.

Giaquinta, R.T. (1983) Phloem loading of sucrose. *Annu. Rev. Plant Physiol.* 34: 347-387.

Haissing, B.E. and Dickson, R.E. (1979) Starch measurement in plant tissue using enzymatic hydrolysis. *Physiol. Plant.* 47: 151-157.

Hensel, W. and Iversen, T.-H. (1980) Ethylene production during clinostat rotation and effect on root geotropism. *Z. Pflanzenphysiol.* 97: 343-352.

Hensel, W. and Sievers, A. (1980) Effects of prolonged omnilateral gravistimulation on the ultrastructure of statocytes and on the gravireponse of roots. *Planta* 150: 338-346.

Hilaire, E.M., Peterson, B.V., Guikema, J.A. and Brown, C.S. (1996) Clinorotation affects morphology and ethylene production in soybean seedlings. *Plant Cell Physiol.* 37: 929-934.

Hinchee, A.W. and Rost, T.L. (1986) The control of lateral development in cultured pea seedlings. I. The role of seedling organs and plant growth regulators. *Bot. Gaz.* 147: 137-147.

Ho, L.C. (1988) Metabolism and compartimentation of imported sugars in sink organs in relation to sink strength. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39: 355-378.

Hoson, T., Kamisaka, S., Masuda, Y., Yamashita, M. and Buchen, B. (1997) Evaluation of the three-dimentional clinostat as a simulator of weightlessness. *Planta* 203: S187-S197.

Iversen, T.H., Ødegaard, E., Beisvåg, T., Johnsson, A. and Rasmussen, O. (1996) The behaviour of normal and agravitropic transgenic roots of rapeseed (*Brassica napus* L.) under microgravity conditions. *J. Biotechnol.* 47: 137-154.

Johnson, S.P. and Tibbitts, T.W. (1968) The liminal angle of a plagiotropic organ under weightlessness. *Bioscience* 18: 655-661.

Kiss, J.Z., Katembe, W.J. and Edelmann, R.E. (1998) Gravitropism and development of wild-type and starch-deficient mutants of *Arabidopsis* during spaceflight. *Physiol. Plant.* 102: 493–502.

Kuiper, P.J.C. (1985) Environmental changes and lipid metabolism of higher plants. *Physiol. Plant.* 64: 118–122.

Legué, V., Driss-École, D. and Perbal, G. (1992) Cell cycle and differentiation in lentil roots grown on a slowly rotating clinostat. *Physiol. Plant.* 84: 386–392.

Levine, H.G., Kann, R.P. and Krikorian, A.D. (1990) Plant development in space: observations on root formation and growth. *In* Proceedings of the 4th European Symposium on Life Sciences Research in Space. Edited by David, V. pp. 503–508. Trieste (Italy) 28 May–1 June. Noordwijk, The Netherlands, ESA SP-307.

MacLeod, R.D. and Thompson, A. (1981) Some effects of sucrose concentration on primordium development in excised primary roots. *Ann. Bot.* 49: 291–301.

McCready, R.M., Guggolz, J., Silviera, V. and Owens, H.S. (1950) Determination of starch and amylose in vegetables. *Anal. Chem.* 22: 1156–1158.

Merkys, A.J., Laurinavichius, R.S. and Shvegzhdene, D.V. (1984) Plant growth, development and embryogenesis during Salyut-7 flight. *Adv. Space Res.* 4: 55–63.

Metcalfe, J.D., Schmitz, A.A. and Palka, J.R. (1966) Rapid preparation of fatty acids esters from lipids for gas chromatographic analysis. *Anal. Chem.* 38: 514–515.

Moore, R., McClelen, C.E., Fondren, W.M. and Wang, C.L. (1987) The influence of microgravity on root-cap regeneration and the structure of columella cells in *Zea mays*. *Amer. J. Bot.* 74: 216–221.

Murin, A. (1976) Effect of removal of cotyledons on mitotic cycle in seedling roots of *Vicia faba* L. *Nucleus* 19: 98–101.

Orlich, G., Hofbrückl, M. and Schulz, A. (1998) A symplasmic flow of sucrose contributes to phloem loading in *Ricinus* cotyledons. *Planta* 206: 108–116.

Parfenov, G.P., Platonova, R.N., Tairbekov, M.G., Zhvalikovskaya, V.P. and Mozgovaya, I.E. (1979) Biological experiments carried out aboard the biological satellite Cosmos-936. *Life Sci. Space Res.* 17: 297–299.

Perbal, G. and Driss-École, D. (1994) Sensitivity to gravistimulus of lentil seedling roots grown in space during the IML 1 Mission of Spacelab. *Physiol. Plant.* 90: 313–318.

Porterfield, D.M., Matthews, S.W., Daugherty, C.J. and Musgrave, M.E. (1997) Spaceflight exposure effects on transcription, activity and localization of alcohol dehydrogenate in the roots of *Arabidopsis thalian*. *Plant Physiol.* 113: 685–693.

Rost, T.L. and Toriyama, H.A. (1991) Comparative analysis of the root systems of light- and dark-grown seedlings of pea (*Pisum sativum* cv. Alaska). *Plant Sci.* 75: 117–121.

Shen-Miller, J., Hinchman, R. and Gordon, S.A. (1968) Thresholds for georesponse to acceleration in gravity-compensated avena seedlings. *Plant Physiol.* 43: 338–344.

Tolbert, N.E. (1981) Metabolic pathways in peroxisomes and glyoxysomes. *Annu. Rev. Bioch.* 50: 133–157.

Tripathy, B.C., Brown, C.S., Levine, H.G. and Krikorian, A.D. (1996) Growth and photosynthetic responses of wheat plants grown in space. *Plant Physiol.* 110: 801–806.

Van't Hof, J. and Kovacs, C.J. (1972) Mitotic cycle regulation in the meristem of cultured roots: the principal control point hypothesis. *In* Some Effects of Sucrose Concentration on Primordium Development in Excised Primary Roots. *Ann. Bot.* 49: 291–301.

Volkmann, D., Behrens, H.M. and Sievers, A. (1986) Development and gravity sensing of cressroots under microgravity. *Naturwissenschaften* 73: 438–441.

Wightman, F. and Thimann, K.V. (1980) Hormonal factors controlling the initiation and development of lateral roots. I. Sources of primordia-inducing substances in the primary root of pea seedlings. *Physiol. Plant.* 49: 13–20.

Wiliams, J.P., Khan, M.U. and Wong, D. (1992) Low temperature-induced fatty acid desaturation in *Brassica napus*: thermal deactivation and reactivation of process. *Biochem. Biophys. Acta* 1128: 275–279.

(Received July 14, 1998; Accepted February 1, 1999)