Plant and Soil (2006) 280:253–266
DOI 10.1007/s11104-005-3224-1
© Springer 2006

# Ara-rhizotron: An effective culture system to study simultaneously root and shoot development of *Arabidopsis*

Florence Devienne-Barret[1,†], Céline Richard-Molard[†], Michaël Chelle, Olivier Maury & Bertrand Ney
*INRA-INA P-G, Unité Environnement et Grandes Cultures, Centre de Recherches de Grignon, 78850, Thiverval-Grignon, France.* [1]*Corresponding author**

Received 4 April 2005. Accepted in revised form 14 September 2005

*Key words: Arabidopsis*, cultivation method, irrigation, population, rhizotron, root development, shoot development

**Abstract**

Studying *Arabidopsis thaliana* (L.) Heynh. root development *in situ* at the whole plant level without affecting shoot development has always been a challenge. Such studies are usually carried out on individual plants, neglecting competition of a plant population, using hydroponic systems or Agar-filled Petri dishes. Those both systems, however, present some limitations, such as difficulty to study precisely root morphogenesis or time-limited culture period, respectively. In this paper, we present a method of *Arabidopsis thaliana* (L.) Heynh. cultivation in soil medium, named ''Ara-rhizotron''. It allows the non-destructive study of shoot and root development simultaneously during the entire period of vegetative growth. In this system, roots are grown in 2D conditions, comparable to other soil cultures. Moreover, grouping several Ara-rhizotrons in a box enables the establishment of 3D shoot competition as for plants grown in a population. In comparison to a control culture grown in pots in the same environmental conditions, the Ara-rhizotron resulted in comparable shoot development in terms of dry mass, leaf area, number of leaves and nitrogen content.We used this new culture system to study the effect of irrigation modalities on plant development. We found that irrigation frequency only affected root partitioning in the soil and shoot nitrogen content, but not shoot or root growth. These effects appeared at the end of the vegetative growth period. This experiment highlights the opportunity offered by the Ara-rhizotron to point out tardy effects, affecting simultaneously shoot development and root architecture of plants grown in a population. We discuss its advantages in relation to root development and physiology, as well as its possible applications.

## Introduction

*Arabidopsis thaliana* (L.) Heynh. is largely used for genetic, molecular, and physiological studies. It is a valuable species to bridge ecophysiology and genetics if cultivated in a population, as in agricultural cropping systems. *Arabidopsis* is commonly used to study the regulations of root system development under environmental conditions (Linkohr et al., 2002; Malamy and Ryan, 2001; Zhang and Forde, 2000). It can be grown on numerous substrates (soil, greenhouse mixes, vermiculite, sand, etc.), and also hydroponically (Arteca and Arteca, 2000; Gibeaut et al., 1997; Lejay et al., 1999). Although those substrates allow more or less easily harvesting of the global root system, they do not permit observation of

* FAX No: +33-1-30-81-55-63.
E-mail: devienne@bcgn.grignon.inra.fr
[†]Both authors have contributed equally to this work.

root development *in situ*. Compared to substrate cultures, hydroponic culture has the advantage of being easier to sample root systems. But, even if root observations are not destructive, manipulations of plants can cause stress, which modifies plant growth and functioning (Bloom and Sukrapanna, 1990; Depege et al., 1997; Giridhar and Jaffe, 1988; Latimer, 1998; McKendree and Smith, 1990). In addition, the period of morphological study is limited because roots get into a tangle after about 15 days of culture. Moreover, because of the absence of mechanical constraints, root system morphogenesis is modified (Mian et al., 1993).

Agar plate culture has been developed for *Arabidopsis*, using vertically oriented Petri dishes containing a dilute nutrient agar medium to allow the dynamic study of the root development (Zhang and Forde, 1998). This culture system does allow a rapid analysis of the *Arabidopsis* root growth, but presents several disadvantages. Firstly, its size means experiments come to a halt when seedlings are 20 days old. Secondly, it is not adapted to the study of canopy interactions of a three-dimensional plant population. Thirdly, because it is a confining culture system, very strict control of some environmental factors, such as temperature, is difficult. Indeed, the microclimate conditions are very modified compared to those of cultures in an open environment (Buer et al., 2000).

In this paper, we present a culture system with soil substrate, called "Ara-rhizotron", which allows *in situ* root observations as well as destructive sampling during all of the vegetative growth period of *Arabidopsis*. In other species, such as maize (Jordan, 1992) or trees (Pagès, 1992), the rhizotron has often been used for root studies because it allows two-dimensional root development and facilitates root observations. In this study, we propose a culture system adapted from this rhizotron and compared plants grown in this system to plants grown in pots. In addition to the classical rhizotron, it allows the cultivation of *Arabidopsis* plants in a 3D population with competition for light. As an example of its application, we focus on the effects of irrigation conditions on root and shoot development, growth, and architecture of plants grown in Ara-rhizotron. We discuss the advantages of the Ara-rhizotron in relation to root development and physiology, as well as its possible applications.

## Materials and methods

### *"Ara-rhizotron" culture system description*

Dimensions of the Ara-rhizotron were designed for maximal root development of *Arabidopsis thaliana* (L.) Heynh. during vegetative growth period. The Ara-rhizotron is made of five 3 mm thick pieces: the back sheet and three border pieces are made of PVC whereas the front sheet is made of transparent polycarbonate (Figure 1a). Between the front and the back sheets, there is a 3 mm space filled with sifted greenhouse mix. The sheets are held together by clamps (13 mm large, ACLE No. 2, France). The Ara-rhizotron is 49 cm high, 24 cm wide, 1.3 cm thick including clamps with a usable space 47 cm high, 20 cm wide and 3 mm thick.

The Ara-rhizotrons are grouped seven to a box in order to grow the plants in a population (Figure 1b). In the box, the Ara-rhizotrons are set up at a 20° angle in relation to the box sides, with the polycarbonate transparent sheet – along which the root would grow – leaning towards the lower side. There is a 4-cm space between each Ara-rhizotron. A blue felt sheet covers each box to keep the roots in darkness and to provide support for the leaves. To ensure air renewal and to regulate the temperature in the box, seven air holes (3 cm in diameter), protected from light by the upper felt sheet, are cut along the two large sides of each box (3 cm from the top and 5 cm from the bottom) at the same relative location for each rhizotron.

The irrigation is set up by connecting tubing to the Ara-rhizotrons, using three connectors placed at the top of the Ara-rhizotron back sheet (Figure 1a). The nutrient solution is supplied to the Ara-rhizotrons by microirrigation, using multi-cassette peristaltic pumps (Watson Marlow, 205U, 0.06–42 mL $min^{-1}$). To maximize irrigation homogeneity, each connector is individually connected to one pumphead cassette. An irrigation flow from 7 to 13 mL $min^{-1}$ preserved soil structure and avoided leaf wetting. This culture system led to the normal development of *Arabidopsis* roots and shoot (Figure 1c).

### *Chemical analyses of Ara-rhizotron soil*

To evaluate the redistribution of nitrate and water in the Ara-rhizotron soil, an irrigation experiment

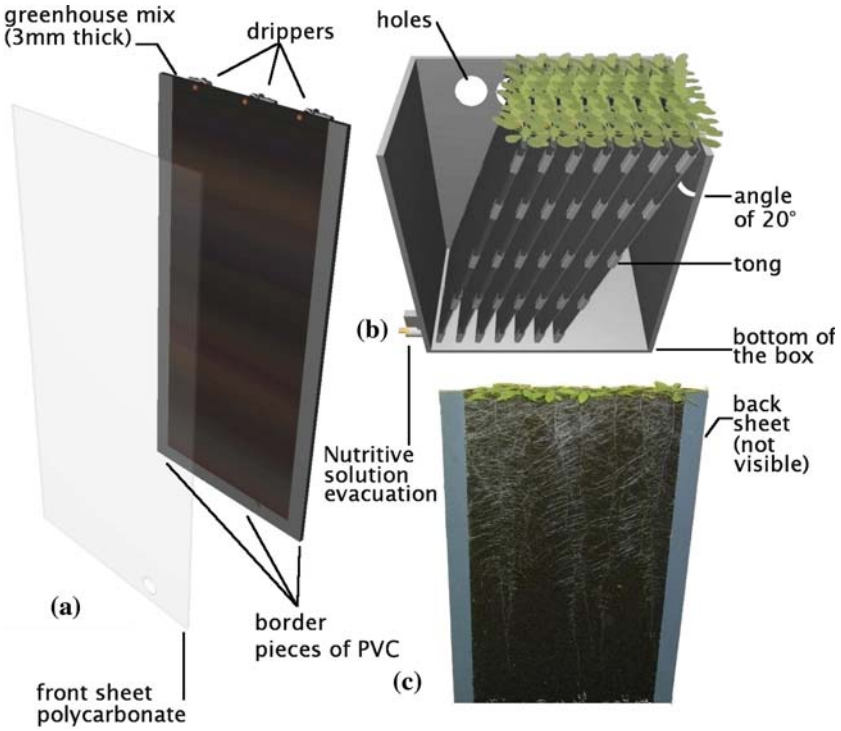


*Figure 1.* The rhizotron system called "Ara-rhizotron" for growing *Arabidopsis* plants in population. (a) Components used for the Ara-rhizotron including four pieces of PVC (one back sheet and three borders) and the front sheet of polycarbonate. The Ara-rhizotron is 49 cm high, 24 cm wide, and 1.3 cm thick with tongs for a usable space of 47 cm high, 20 cm wide and 3 mm thick. The irrigation system consists of three connectors independent placed at the back of the Ara-rhizotrons. (b) Cut away of the Ara-rhizotron box showing the disposition of the seven Ara-rhizotrons in each box, with an angle of 20° in relation to the vertical and separated from each other by 4 cm. The polycarbonate transparent sheets were on the lower face of the Ara-rhizotrons. (c) 33-day-old *Arabidopsis* plants grown in an Ara-rhizotron.

was carried out without plants for 10 days. Ara-rhizotrons (2 replicates) were filled with a homogenous greenhouse mix composed of blond and brown peat (1/1) containing 30% of organic matter and sifted at 3.93 mm (BASIC SUBSTRAT II, Stender GmbH, Germany). This substrate was provided with a pH ranging between 5.5 and 5.9 and with no added fertilizers. The N content of the substrate was 0.1% and 40 ppm of dry matter for organic and mineral nitrogen, respectively. The greenhouse mix was carefully compacted using a home-made tampboard to ensure similar and homogeneous bulk density ($0.093 \pm 0.0044$ kg $L^{-1}$) in each Ara-rhizotron. Moreover, each Ara-rhizotron was weighed before and after filling to check that the same quantity of greenhouse mix was poured. Twice a day, 5 mL per connector of a 5 m*M* $NO_3^-$ nutrient solution (4 m*M* $KNO_3$, 0.5 m*M* $Ca(NO_3)_2$, 0.25 m*M* $KH_2PO_4$, 0.5 m*M* $MgSO_4$, 0.2 m*M* NaCl, 10 mg $L^{-1}$ Fe-EDDHA) was delivered automatically for 1 min, resulting in a 30 mL per day irrigation. The second irrigation was scheduled 1 h after the first one.

Harvest of the greenhouse mix was carried out 24 h after the last irrigation to ensure soil drainage. The soil of each Ara-rhizotron was divided into 7 columns (A–G) and 5 strata. Columns A and G were 1.5 cm wide whereas columns B–F were 3.5 cm wide. Strata 2–5 were 10 cm deep. The first stratum was sub-divided into three sub-strata i.e., 1a (1.5 cm), 1b (2.5 cm) and 1c (3 cm) (Figure 3c). The connectors carrying the nutrient solution into the Ara-rhizotrons were located in columns B, D and F of sub-stratum 1a. Each soil sample was harvested separately and divided into two parts. To determine soil water content, the first portion was weighed before and

after a 2-day drying at 80 °C. Nitrate was extracted from the second portion of soil by first adding 8 mL of demineralized water to 1 g of soil. That was agitated for 30 min. After centrifugation (4 min, 4000×*g*), the nitrate content of the obtained supernatant was measured by continuous-flow colorimetry (Technicon, Bran&Luebbe, France), according to the Henriksen and Selmer-Olsen method (1970).

*Plant material and growth conditions*

To verify that Ara-rhizotron resulted in regular plant development, growth, morphogenesis, and N content, we carried out comparative experiments in Ara-rhizotrons and in pots. The control treatment in pots used procedures established by Loudet et al. (2003). Small rectangular joined pots (55 mm long, 60 mm wide, 60 mm high) (Puteaux, France) (Figure 2a) were filled with the same greenhouse mix as the one used in Ara-rhizotron. Careful attention has been paid to ensure a homogeneous soil density inside and between pots, equal to the bulk density in the Ara-rhizotrons ($0.115 \pm 0.0016$ kg $L^{-1}$). In both culture systems, greenhouse mix was fully wetted with demineralized water before sowing.

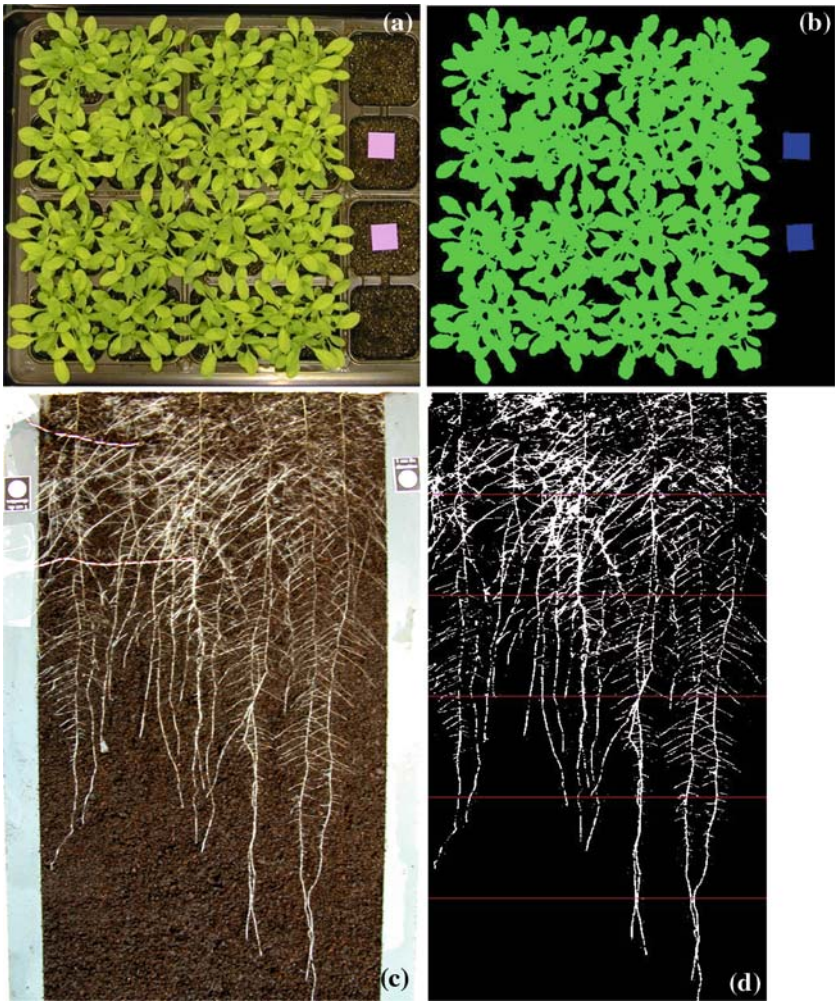


*Figure 2.* (a) Photo of the control culture in pots at 28 days after sowing. (b) The picture after the image analysis performed with AraLAI, a home-made C++ image analysis software (in green, the detected plant pixels, in blue the reference paper pixels). (c) Photo of the root part of a control Ara-rhizotron at 32 days after sowing. (d) The same picture after root detection performed with a home-made Java analysis software. Horizontal red lines indicate the soil layers in which the root density was quantified.

*Arabidopsis thaliana* (L.) Heynh. wild type seeds, ecotype Shahdara (References N929 and CS929, respectively in the Nottingham Arabidopsis Stock Center and Arabidopsis Biological Resource Center catalogs) were stratified in the dark at 4 °C for 72 h in 0.12% agar solution in water. Then, they were sown in the following way. In each pot, six holes forming a circle were made, whereas seven holes in a line were made along the top of each Ara-rhizotron. In both culture systems, seed position was determined so that the future plants would be 2.7–3 cm apart, resulting in a final density of about 1260 plants $m^{-2}$. Approximately five seeds per position were introduced using an automatic pipette. Seeds were maintained in darkness until germination, which occurred 2 days after sowing (DAS). Seven days after sowing, only one seedling per position was retained, while the others were removed, resulting in six homogeneous seedlings per pot or seven per Ara-rhizotron.

Every 2 days, the base of the pots was immersed for 2 h in a nutrient solution containing 15 m*M* nitrate (8 m*M* $KNO_3$, 3.5 m*M* $Ca(NO_3)_2$, 0.25 m*M* $KH_2PO_4$, 0.5 m*M* $MgSO_4$, 0.2 m*M* NaCl, 10 mg $L^{-1}$ Fe-EDDHA), an ample nutrition under our growth conditions (unpublished data). The volume of nutrient solution available to the plants was around 4.5 mL per plant and per day. Irrigation of the Ara-rhizotrons was carried out automatically twice a day for 1 min with the same nutrient solution. This amounted to a volume of 30 mL per day, i.e., about 4.3 mL per plant and per day. The irrigation flow was 5 mL $min^{-1}$. Both irrigation procedures occurred during the light period and provide 15 mL to the soil. They were spaced at a 1-h interval.

To study the effect of irrigation conditions on plant development, two additional irrigation modalities were applied to plants cultivated in Ara-rhizotrons. The first modality (M1) was identical to the above-mentioned one (called CT) except that the flow was 10 mL $min^{-1}$. To maintain a total irrigation volume of 30 mL per day, the irrigation duration was therefore reduced to 30 s. For the second modality (called M2), nutrient solution was trickled into the Ara-rhizotron: 5 s irrigations were carried out 16 times a day during the light period (i.e., every 30 min) with a flow of 22.5 mL $min^{-1}$, resulting in a supply of 1.875 mL per irrigation. For both modalities, the total irrigation volume for a day was 30 mL.

All experiments were conducted in the same growth chamber (Strader, France), equipped with HPI-T PLUS lamps (400 W, Philips, Netherlands) providing Photosynthetically Active Radiation (PAR) of 295 $\mu$mol $m^{-2}$ $s^{-1}$. Plants were grown for up to 35 days, corresponding to the vegetative growth period, under short day conditions with a 16/8-h photoperiod. The day and night air temperatures were 21 and 18 °C, respectively. Air temperatures below the plant leaves and soil temperatures at 2-cm depth were measured with copper–constantan thermocouples (TYPE T, Thermo Electric, NL) and recorded as a 30-min average with a C21x data logger (Campbell Scientific Ltd., UK). The humidity fluctuated between 65%, during the day, and 85%, during the night. To avoid excessive soil surface dryness, which is lethal in the early stages of growth, humidity was maintained with a continuous water spray in the growth chamber during the first 7 days of growth.

*Shoot development analysis*

Shoot development analysis entailed destructive measurements of the shoot organs and, in the case of the comparison of the different irrigation modalities, a non-destructive tracking of photosynthetic leaf area (PLA). For each destructive sampling, total N content of the leaves was analysed.

The regular, destructive samplings of plants were made at 21, 25, 28, 30, 32 and 35 days after sowing (about 2 days before floral bud emergence). The sampling procedure was designed to limit border effects: (i) within a Ara-rhizotron, by taking the five middle plants, (ii) between Ara-rhizotrons, by continually using the same Ara-rhizotron as the outer one forever excluded from harvest, and (iii) for the control culture in pots, by discarding the plants around the outside edges of the joined pots and harvesting only six plants in the middle. For each plant, the number of visible leaves was noted. The minimum visible leaf area was estimated to be 2 $mm^2$. In addition, at 21, 28, 32 and 35 DAS, the total leaf area per plant was estimated by planimetry measurement using an area meter (LI3100, Licor, USA). Then, the fresh leaves were lyophilized, weighed and

ground into a fine powder. The total nitrogen of the leaves was determined using a flash combustion technique based on the Dumas principle, involving an automatic element analyzer (Carlo Erba ANA 1500). At each date and for each culture, the results are the mean of each separate plant.

Photosynthetic leaf area was estimated by taking photos of growing plants in the nadir direction at 28, 32 and 35 days after sowing. The calculation of PLA from the photos was carried out by counting the number of pixels corresponding to the leaves and those corresponding to paper squares acting as surface references. The latter were cut out of a sheet of paper and placed among the plants (Figure 2b). These calculations were performed by "AraLAI", a home-made C++ image analysis software based on colour thresholding and linear filtering (Gonzales and Woods, 1992).

*Root development analysis in Ara-rhizotron culture*

Root development analysis was only carried out on the Ara-rhizotron treatments and consisted of two types of measurements: a non-destructive tracking of root development and regular destructive samplings of plants during all of the vegetative growth period.

The non-destructive tracking of root development entailed total root length measurements and lateral root counts. The root systems of the seven plants of the same Ara-rhizotron were analysed at 15, 18, 21, 24, 26 and 28 days after sowing with a scanner connected to a computer using version 2002 WINRHIZO PRO software package (Regent Instruments, Inc., Canada). This software estimated total root length, primary root length, and number of laterals until 28 days after sowing. After this date, the image analysis of individual plants became difficult with this software because the root systems had become very entangled. Because of this difficulty, the root systems of the seven plants included in the same Ara-rhizotron were also traced on a transparent sheet at 8, 11, 15, 18, 21, 24, 31 and 35 days after sowing with different colours (one colour per day). The tracing was analysed plant by plant with WINRHIZO PRO at 31 and 35 DAS to complete the data set. For each date, results are the mean of the seven individual plants.

In the case of the irrigation treatment experiment, we calculated the density of roots in the soil from Ara-rhizotron pictures at 32 DAS. The calculation for each soil layer entailed dividing the number of root pixels by the number of layer pixels; the root pixels being determined by grey-scale thresholding. It was done with a home-made Java image analysis software (Gonzales and Woods, 1992).

Concerning the destructive samplings of plants, the plants sampled at 21, 25, 28, 30, 32 and 35 days after sowing for shoot development analysis were also used to study root development. The root systems were disentangled and the soil was removed by carefully cleaning with a water sprayer. Then, the roots were patted dry with paper towels. Plant sampling, and measurement of dry biomass and total N content of roots were carried out using the same procedure as for the shoot.

*Statistical analysis*

Analyses of variance for aerial and root biomass, nitrogen content, number of leaves, and leaf area were carried out with the general linear procedure of SAS statistical package (SAS Institute Inc., 1999) using the model $Yij = Vi + Nj + VNij + eijk$ where V*i* was the culture system effect, N*j* the date effect, and VN*ij* was the interaction between culture system and date. This implies the use of the Bonferroni test.

## Results

*Benchmark of the Ara-rhizotron: comparison with pot culture*

*Nutrient solution distribution*

The nutrient solution was evenly distributed throughout the Ara-rhizotron soil. A slight vertical gradient was observed in soil water content (Figure 3a) with a variation of 2.6% between the first and the fifth strata, probably due to gravity. The nitrate content of the soil was identical for strata 2–5 (Figure 3b). In contrast, a large heterogeneity was observed for the first stratum, with the

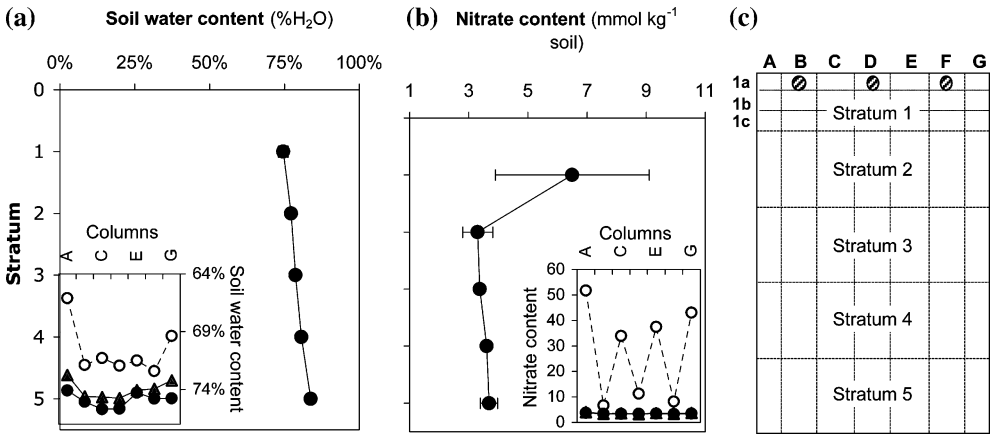


*Figure 3*. Variation of soil water content (a) and nitrate content (b) of the greenhouse mix in the Ara-rhizotron. Twice a day, 5 mL per connector (hatched circles, c) of a 5 mM $NO_3^-$ nutrient solution were delivered automatically for 1 min, resulting in a 30 mL per day irrigation. Experiment was carried out without plants for 10 days. Harvest of the greenhouse mix was carried out by dividing the Ara-rhizotron soil into 7 columns (A–G) and 5 strata (c). The first stratum was sub-divided into three sub-strata (1a, 1b, and 1c). Results are presented as the mean of the 7 columns for each stratum. Inserts in (a) and (b) explicit soil water and nitrate content for the 7 columns of the three sub-strata 1a (open circles), 1b (grey triangles) and 1c (closed circles). Error bars indicate standard deviation of mean ($n = 7$–14).

mean nitrate content about 2-fold higher. A detailed study of the first stratum (insert Figure 3b) indicated that the heterogeneity was due to the first centimetres of soil (stratum 1a) where very high nitrate content was recorded, especially in columns A, C, E and G. This accumulation of nitrate in the parts without connectors in the first sub-stratum is consistent with the lower soil water content of those parts (see insert Figure 3a), suggesting that local desiccation occurred in the soil surface leading to elevated concentration of nitrate due to capillarity attraction and evaporation of nutrient solution.

*Microclimate conditions*

Heterogeneity of PAR inside the culture chamber was very limited, PAR was equal to $295 \pm 20$ $\mu$mol $m^{-2}$ $s^{-1}$. Similar air and soil temperatures were measured in Ara-rhizotrons and pots: for pots, the average temperatures during the light period were equal to 23 °C in the air and 24 °C in the soil; for Ara-rhizotron, they were both equal to 23.5 °C. The soil temperature fluctuations were more important in the Ara-rhizotrons (the maximal variations around the mean were 1.5 °C) than in pots (maximal variations around the mean were 0.5 °C), probably due to the higher occurrence of irrigation, which cooled the soil more frequently and limited the soil temperature stability.

*Shoot growth and development analysis*

Shoot growth of plants cultivated in Ara-rhizotrons, when compared to plants grown in pots, showed that the Ara-rhizotron did not modify growth: shoot biomass was different between plants grown on pots and plants grown in Ara-rhizotron only at 35 days after sowing ($P = 0.0001$) (Figure 4a). There was also no developmental difference since the number of leaves did not vary between the plants grown under the two cultivation conditions (Figure 4b). The only significant difference of total leaf area between plants grown on potting media and plants grown in Ara-rhizotrons was at 31 days after sowing ($P = 0.0018$) (Figure 4c). But this difference no longer existed at 35 days after sowing.

Total N content declined slowly over time during the vegetative growth period (Figure 6a). There was no significant N content difference between plants grown in pots and plants grown in Ara-rhizotrons, except for the last sampling for which total N content of plants grown in Ara-rhizotrons was slightly higher than that of plants grown in pots ($P = 0.0001$).

*Root growth and development analysis*

In the Ara-rhizotron, root dry weight increased over time, very slowly during the first 20 days after sowing. From 21 to 35 DAS, it increased sharply (Figure 5a). Total root length (Figure 5b)

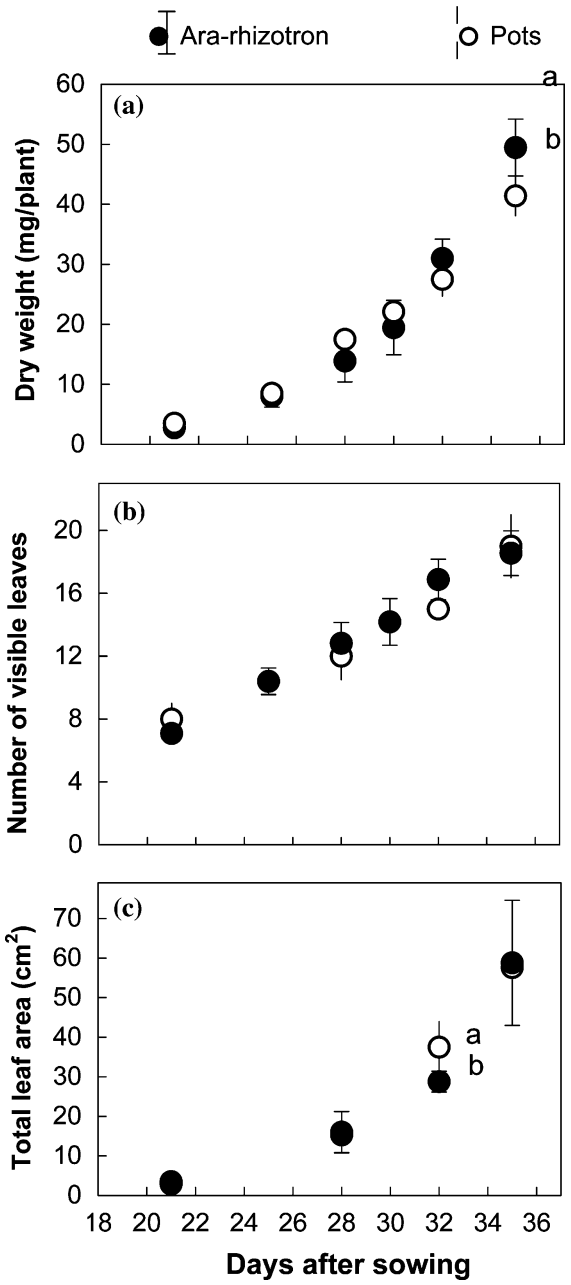


*Figure 4*. Aerial development of *Arabidopsis* plants grown in pots filled with greenhouse mix (open circles) or in Ara-rhizotron filled with the same substrate (closed circles) during the vegetative growth period. (a) Dry weight of the shoot. (b) Number of visible leaves for each plant. Leaves were counted when their size reached at least 2 $mm^2$. Cotyledons were not considered. (c) Total leaf area estimated by planimetry. Error bars indicate standard deviation of mean ($n = 5$ or 6). Using ANOVA with $P = 0.001$, the letters a and b indicate significant difference between plants grown in pots and plants grown in Ara-rhizotron.

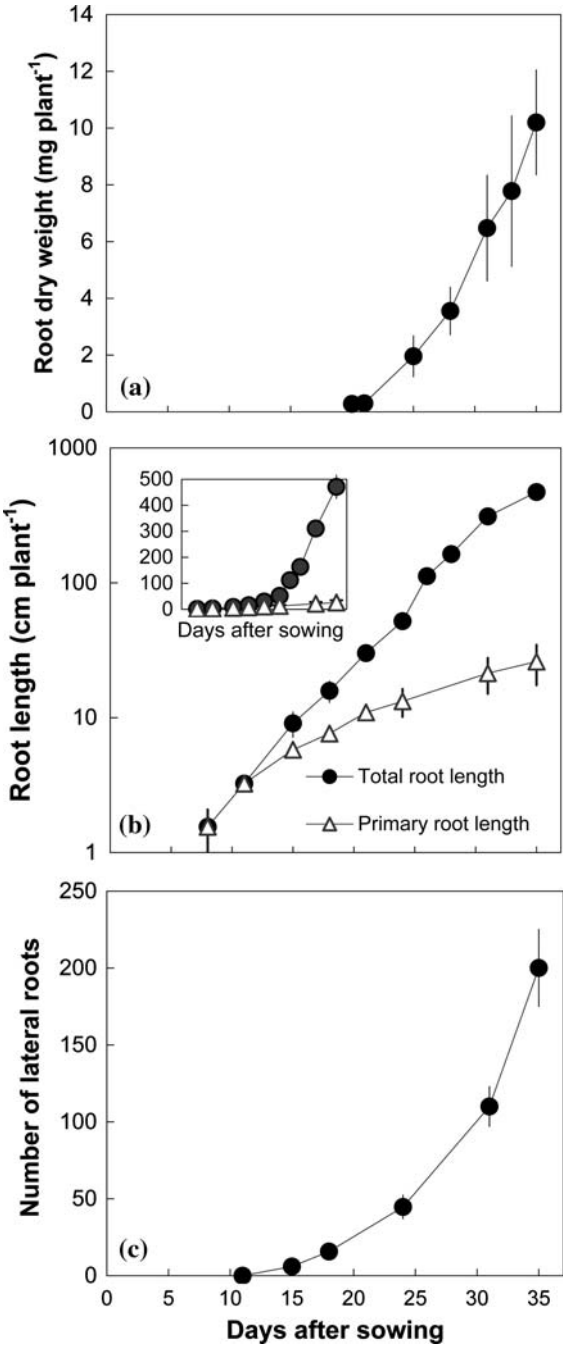


*Figure 5*. Root development of *Arabidopsis* plants grown in Ara-rhizotrons filled with greenhouse mix. (a) Dry weight of the roots. (b) Time evolution of total root length (closed circles) and primary root length (open triangles) in logarithmic scale, during the vegetative growth period. Insert: time evolution of the same variables at the beginning of vegetative growth period in natural scale. (c) Time evolution of the number of lateral roots. Error bars indicate standard deviation of the mean ($n = 5$).

increased regularly on a logarithmic scale. The insert in Figure 5b shows it had a quasi-exponential pattern on a natural scale: it increased very slowly until 15 DAS and very quickly after that

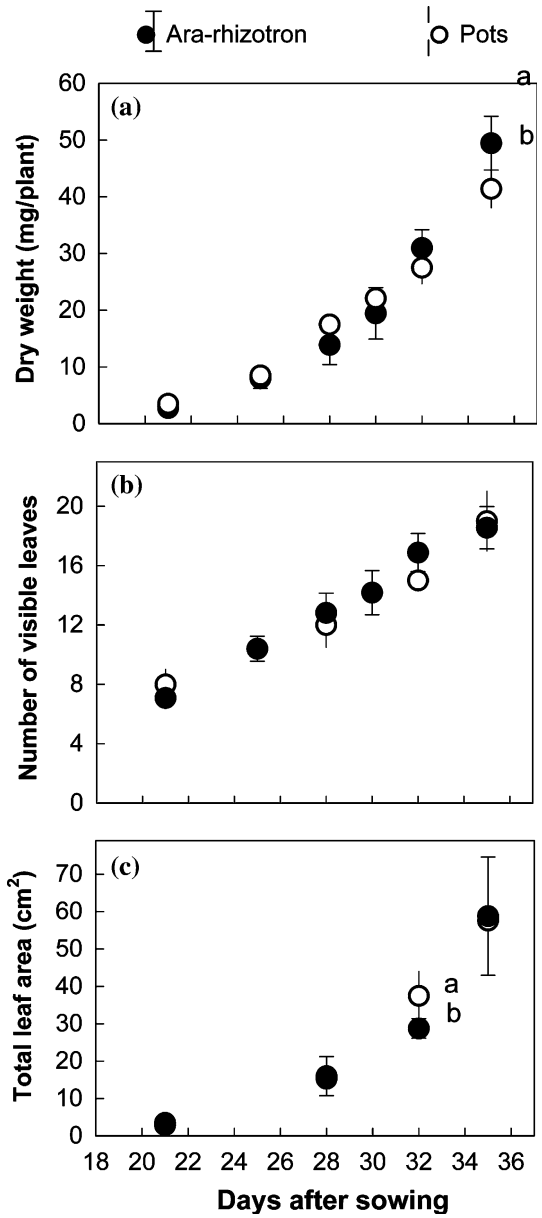


*Figure 6*. Total N concentration of shoot (a) and roots (b) of *Arabidopsis* plants during the vegetative growth period. *Arabidopsis* populations were grown in pots (open circles) or in Ara-rhizotron (closed circles) filled with greenhouse mix. Error bars indicate standard deviation of mean ($n = 5$ or 6). Using ANOVA results with $P = 0.001$, the letters a and b indicate significant difference between plants grown in pots or in Ara-rhizotron.

date. The primary root length increased slowly and linearly during the entire experiment (Figure 5b). Lateral roots began to appear about 12 days after sowing and their number per plant increased slowly until 15 days after sowing. This number increased very substantially after this date (Figure 5c). These results showed that the major part of the root morphogenesis processes took place 15–20 days after sowing.

Root N content varied from 3.6% to 4.8% of dry matter, with no tendency towards decreasing, as was observed for shoot N content (Figure 6b).

### *Study of the effect of irrigation type on plant development and growth*

The comparative observation of the plant phenotypes (Figure 7a) indicated that the pattern of irrigation altered the morphogenesis of shoot and root parts. For the roots, the quantification of the root density at 32 DAS (Figure 7b) showed that the root profiles were similar for the three irrigation treatments, except in the top stratum where the M2 treatment (16 irrigations per day) had a density two times higher than CT and M1 ($P = 0.0015$). For the shoot, the effect on morphogenesis was quantified *via* the photosynthetic leaf area (Figure 8d), which was 1.3 times lower for the M2 modality compared to the control or M1 plants at the end of vegetative growth. The total leaf area was also affected similarly (data not shown). In contrast, there was no significant difference for growth between the plants of the three irrigation treatments, neither for the shoot (Figure 8a) nor for the root (Figure 8b). Shoot development was not affected either, since the number of visible leaves was the same in all three treatments (Figure 8c). Besides the effect on morphogenesis, the irrigation modality influenced N content of the shoot (Figure 8e). From 32 DAS, shoot N content of the M2 plants (16 irrigations per day) was significantly lower compared to the control plants ($P = 0.0003$). At 35 DAS, they contained 1.5% less nitrogen in the shoot than the control plants ($P = 0.00001$). In contrast, root N concentrations of the CT and M2 plants were not significantly different ($P = 0.38$) at 32 DAS (Figure 8f). As for the others variables, the M1 modality plants did not significantly differ from the control irrigation ones concerning N content.

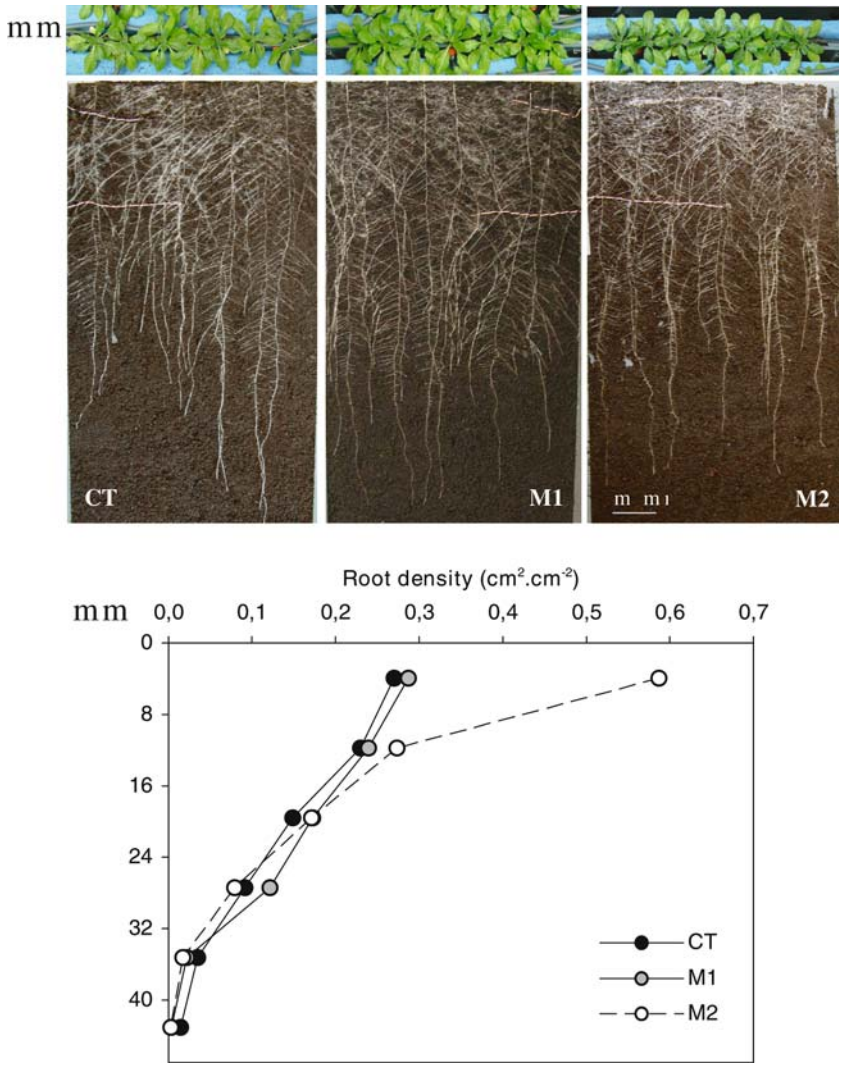


*Figure 7*. Effect of irrigation modalities on shoot and root morphogenesis in *Arabidopsis*. Plants were grown in Ara-rhizotrons for 35 days and were submitted to three types of irrigation. The control treatment (CT) entailed in 2 irrigations of 1 min per day, with a flow of 5 ml $min^{-1}$. The M1 treatment was similar to the CT, except that the duration of each irrigation was 30 s and the flow rate was 10 ml $min^{-1}$. In the M2 treatment, one irrigation of 5 s was carried out every 30 min during the light period with a flow of 22.5 ml $min^{-1}$, so that the nutrient solution trickled into the Ara-rhizotron. All treatments resulted in a 30 mL supply per day. Plants were harvested at 28, 32 and 35 days after sowing (DAS). (a) Phenotype of root and shoot observed in the Ara-rhizotron at 32 DAS. (b) Profiles of root densities at 32 DAS, quantified from the pictures presented in (a), using a home-made Java software. Error bars indicate standard deviation of the mean ($n = 3$). Using ANOVA results with $P = 0.001$, the letters a and b indicate significant differences between irrigation treatments.

## Discussion

The objective of creating the Ara-rhizotron was to provide a growing system to dynamically study the root development of plants for which growing features were equivalent to those of plants grown in potting media. As regards the shoot part of the plants, growth, development,

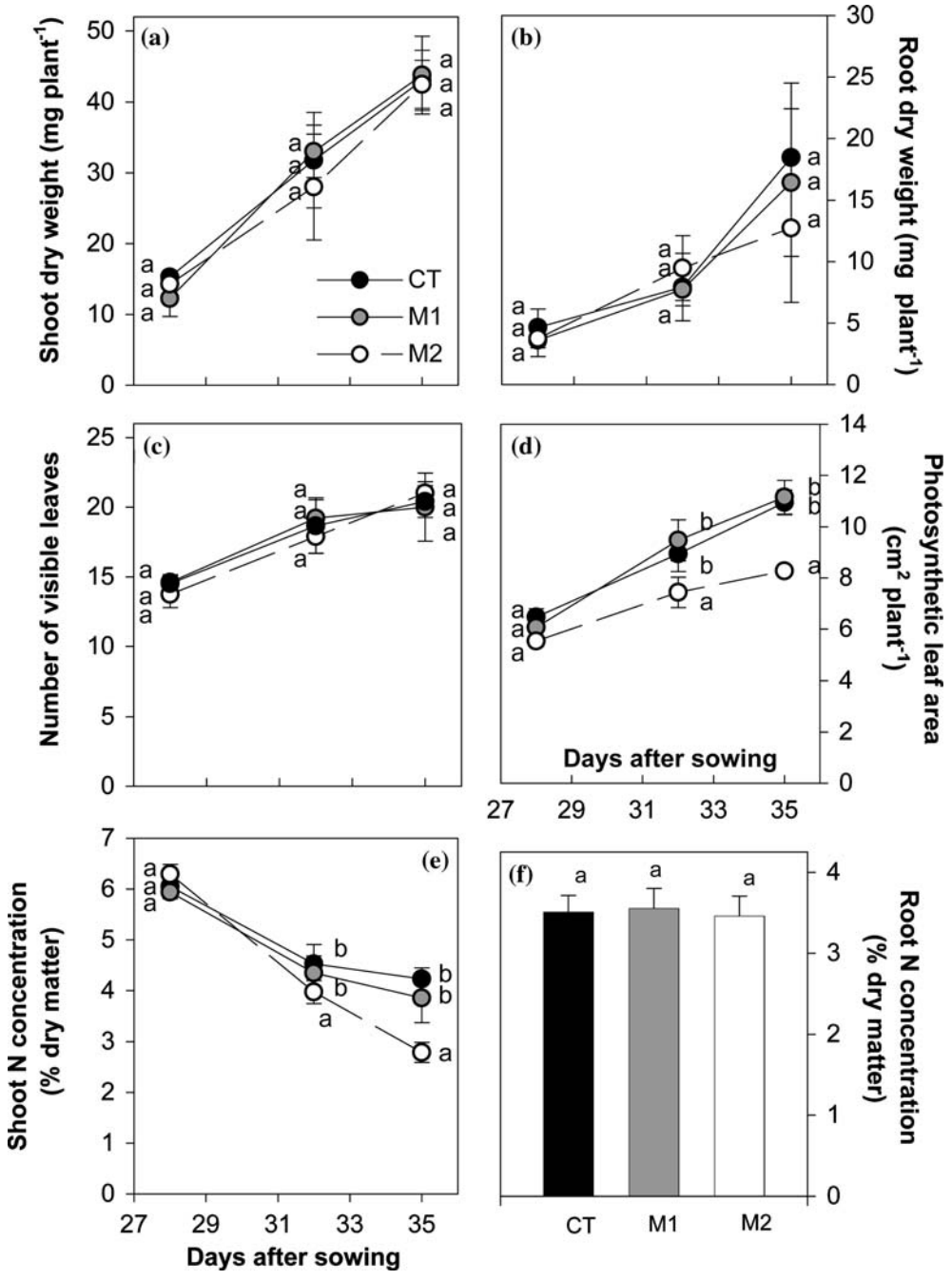


*Figure 8*. Effect of irrigation modalities on plant growth and development, characterized by time evolution of (a) shoot dry weight, (b) root dry weight, (c) number of visible leaves, (d) photosynthetic leaf area, (e) shoot N concentration and (f) root N concentration at 32 days after sowing. Error bars indicate standard deviation of the mean ($n = 3$–10). Using ANOVA results with $P = 0.01$, the letters a and b indicate significant differences between irrigation treatments.

and N content were overall not modified by the culture system in comparison to the control pot culture (Figures 4 and 6). These two culture systems provided similar culture conditions. Moreover, when several Ara-rhizotrons were grouped in a box with small intervals between them (Figure 1), plants grew in dense canopy where there was competition for light between

neighbouring plants, as in pot cultures. Equally important, the microclimates of the two culture systems were very similar.

Compared to pots, the Ara-rhizotron allows an easy recovery of a clean, entire root system ready for tissue analysis, but, above all, it allows the complete study of the root morphology and morphogenesis *in situ* (number and position of laterals, root length, appearance and disappearance of roots, measurements of diameters, kinetics, etc.) without any destructive sampling. Our measurements showed, under constant nutritive conditions, a continuous increase of the total root length, essentially due to lateral roots length increase (Figure 5). Lateral root length and number increased greatly after 20 DAS, simultaneously to total leaf area, confirming that root elongation depends on carbon availability in the plant (Freixes et al., 2002). Our root length data from 8 to 15 DAS (5.78 cm $plant^{-1}$, 5.6–14.6 mm $d^{-1}$) were consistent although a little higher than those obtained by Zhang and Forde (1998) who measured a primary root length of 4 cm $plant^{-1}$ at 14 DAS and by Freixes et al. (2002) who measured a primary root elongation rate varying from 4 to 11 mm $d^{-1}$ depending on the irradiation treatment. The differences were probably due to the substrate temperature (in average, 21 °C in Petri dishes compared to 23.5 °C in the Ara-rhizotron).

We tested our new culture system to determine if the type of irrigation, in terms of duration, flow and frequency, influenced plant development and morphogenesis. With this aim in mind, we provided the same volume of nutrient solution per day to plants but according to various modalities. Plants cultivated with CT and M1 irrigation did not differ significantly (Figures 7 and 8), indicating that under ample N nutrition conditions, duration and flow of irrigation had no effect on plant growth, development, architecture, as already observed by Xu et al. (2004) on barley, or on nitrogen content. In contrast, increasing irrigation frequency strongly altered plant morphology (Figure 7a). The pattern of irrigation affected the root system architecture resulting in a 2-fold increase in soil area used by roots in the first soil layer for M2 plants (Figure 7b). This was probably explained by the different water and nutrient availability in this layer: the higher flow rates through the soil in the CT and M1 treatments immediately after irrigation resulted in rapid movement of nutrient solution (water and nutrients) past the roots near the surface towards the lower layers before it was taken up. In grapefruit trees, Swietlik (1992) also observed that trickle irrigation favoured root partitioning into the upper soil layers, compared with flood irrigation. In sugar beet, root length density was increased with short irrigation intervals (Camposeo and Rubino, 2003). However, in those studies, water volumes brought to the plants varied among the irrigation treatments, which did not allow to discriminate between an effect of irrigation frequency and irrigation volume.

However, root expansion over a greater soil area was not enough to sustain nitrogen absorption, since shoot N content was significantly less in M2 plants (Figure 8e). The shoot of M2 plants were limited in nitrogen from 32 DAS compared to control plants, probably because of a lower N uptake rather than a higher distribution of N towards roots (Figure 8b, f). At 32 DAS, N quantity taken up by roots of M2 plants was 0.7-fold lower compared to control plants (1.33 mg for M2 plants vs. 1.89 mg for CT plants, $P = 0.004$), which was confirmed by $^{15}N$ influx measurements at 32 DAS (data not shown). It is important to notice that the decrease in nitrogen uptake was not due to a lack of water because excess nutrient solution was drained out through the bottom of the Ara-rhizotron over the entire experimental period (data not shown). The decrease in shoot N content was accompanied by a marked decrease in leaf area (Figure 8d). This suggests that the limitation of internal nitrogen availability impaired leaf elongation as already known for others species (Greenwood, 1976). Nevertheless, no effects on root or shoot biomass accumulation came to light (Figures 8a, b), suggesting that the reduction in the leaf area had no consequences on photosynthetic activity and carbon accumulation.

The results obtained when comparing pot culture to Ara-rhizotron culture, or when comparing the different types of irrigation, greatly emphasized the advantages of the Ara-rhizotron since it pointed out features or differences impossible to characterize with other plant culture systems. For example, as shown above, irrigation frequency affected specifically root architecture but not biomass accumulation, something that

would not have been evidenced with hydroponics or pot cultures. Moreover, we noticed that the effect of irrigation modalities on plant development occurred late in the vegetative growth (Figure 8), and we observed important modifications of root development during the fourth week after sowing under our culture conditions (Figure 5). It seems therefore relevant to study the root development during the entire vegetative growth of plants. With the Ara-rhizotron, we were able to study the plants for 35 DAS.

If attention is paid to provide the same amount and type of nutrition, the Ara-rhizotron did not modify the culture conditions when compared to the pot culture. The two systems mimic real soil conditions, with diffusion and mass flow fluxes due to the soil material and the root system. On the other hand, greenhouse mix used in the Ara-rhizotron raises the problem of mineralization of the organic nitrogen present in the substrate and of mineral retention of the soil. For nitrate, which is a mobile ion, this problem was partly regulated by abundant irrigation, the excess of solution draining off the nitrate ions present in the soil before irrigation. Our results showed that the design of the system allowed a highly homogeneous and precise distribution of water and nitrogen throughout the Ara-rhizotron (Figure 3) associated with a total renewal of the soil solution at each irrigation since drainage was observed over the entire culture period (data not shown). However, the mineralization flux should be fully evaluated under our experimental conditions in order to perfectly manage nitrate nutrition of plants.

Thanks to its versatility, the Ara-rhizotron can be used for various applications. Firstly, plants can be grown isolated or in a population allowing for density studies. Secondly, the Ara-rhizotron can be filled with different substrates, such as sand or inert substrates. This can be useful to study the effect of different mechanical constraints on root growth, or when the substrate must not supply mineral nutrients to the plants. Thirdly, thanks to the peristaltic pumps, which provide very precise volumes, and to the three independent irrigation points of the Ara-rhizotron, it is very easy to supply variable mineral nutrition to generate either temporal or spatial heterogeneity and study its effect on root morphogenesis. A horizontal split-root system may also be established with the Ara-rhizotron, by separating different horizontal zones in the substrate with wax. With a little more engineering, it is possible to modify the system so that different nutrient solutions can be laterally injected into the various zones and the roots can easily grow through the wax which has little mechanical resistance to root growth, but which is impervious to the solution. This would present a real advantage over a vertical split-root system usually used in root growth and physiological studies (Chaillou et al., 1994; Dunbabin et al., 2001; Saravitz et al., 1994; Tang et al., 1990). Indeed, in natural conditions, the largest spatial heterogeneity of nutrient distribution is found vertically down towards the soil (and not horizontally along the topsoil) because the mineralization of organic matter occurs mainly in the first 30 cm of soil (Mary et al., 1996). Since the root develops mainly in the vertical direction, a horizontal split-root system seems to be the most appropriate system to study the effect of nutrient spatial heterogeneity on root morphogenesis and development.

As a conclusion, the Ara-rhizotron is a convenient and accurate tool for root studies. While Agar-plate culture system does remain convenient for a rapid screening of a large number of genotypes, the Ara-rhizotron culture system is particularly useful for whole plant and whole culture-cycle studies.

## Acknowledgements

We would like to thank Suzette Tanis-Plant and Norbert Brugière for their critical reading of earlier versions of the manuscript. We are grateful to Alain Fortineau and Florence Lafouge for additional technical assistance and to Philippe Huet for his help with statistics analysis. This work was funded by the INRA 'Ecogène' grant.

## References

Arteca R N and Arteca J M 2000 A novel method for growing *Arabidopsis thaliana* plants hydroponically. Physiol. Plant. 108, 188–193.

Bloom A J and Sukrapanna S S 1990 Effects of exposure to ammonium and transplant shock upon the induction of nitrate absorption. Plant Physiol. 94, 85–90.

Buer C S, Masle J and Wasteneys G O 2000 Growth conditions modulate root-wave phenotypes in *Arabidopsis*. Plant Cell Physiol. 41, 1164–1170.

Camposeo S and Rubino P 2003 Effect of irrigation frequency on root water uptake in sugar beet. Plant Soil 253, 301–309.

Chaillou S, Rideout J W, Raper C D J and Morot-Gaudry J -F 1994 Responses of soybean to ammonium and nitrate supplied in combination to the whole root system or separately in a split-root system. Physiol. Plant. 90, 259–268.

Depege N, Thonat C, Coutand C, Julien J L and Boyer N 1997 Morphological responses and molecular modifications in tomato plants after mechanical stimulation. Plant Cell Physiol. 38, 1127–1134.

Dunbabin V, Rengel Z and Diggle A 2001 *Lupinus angustifolius* has a plastic uptake response to heterogeneously supplied nitrate while *Lupinus pilosus* does not. Aust. J. Agr. Res. 52, 505–512.

Freixes S, Thibaud M C, Tardieu F and Muller B 2002 Root elongation and branching is related to local hexose concentration in *Arabidopsis thaliana*. Plant Cell Environ. 25, 1357–1366.

Gibeaut D M, Hulett J, Cramer G R and Seemann J R 1997 Maximal biomass of *Arabidopsis thaliana* using a simple, low-maintenance hydroponic method and favorable environmental conditions. Plant Physiol. 115, 317–319.

Giridhar G and Jaffe M J 1988 Thigmomorphogenesis: XXIII. Promotion of foliar senescence by mechanical perturbation of *Avena sativa* and four other species. Physiol. Plant. 74, 473–480.

Gonzales R C and Woods R E 1992 Digital Image Processing. Addison Wesley Pub. Co, Reading MA 793 pp.

Greenwood D J 1976 Nitrogen stress in plants. Adv. Agron. 28, 1–35.

Henriksen A and Selmer-Olsen A R 1970 Automatic methods for determining nitrate and nitrite in water and soil extracts. Analyst 95, 514–518.

Jordan M O 1992 Les rhizotrons peuvent-ils être utilisés pour l'étude de la ramification des racines primaires nodales du maïs (*Zea mays* L)?. Agronomie 12, 3–14.

Latimer J G 1998 Mechanical conditioning to control height. Hort. Technol. 8, 529–534.

Lejay L, Tillard P, Lepetit M, Olive F D, Filleur S, Daniel-Vedele F and Gojon A 1999 Molecular and functional regulation of two $NO_3^-$ uptake systems by N- and C-status of *Arabidopsis* plants. Plant J. 18, 509–519.

Linkohr B I, Williamson L C, Fitter A H and Leyser H M O 2002 Nitrate and phosphate availability and distribution have different effects on root system architecture of *Arabidopsis*. Plant J. 29, 751–760.

Loudet O, Chaillou S, Merigout P, Talbotec J and Daniel-Védèle F 2003 Quantitative trait loci analysis of nitrogen use efficiency in *Arabidopsis*. Plant Physiol. 131, 345–358.

Malamy J E and Ryan K S 2001 Environmental regulation of lateral root initiation in *Arabidopsis*. Plant Physiol. 127, 899–909.

Mary B, Recous S, Darwis D and Robin D 1996 Interactions between decomposition of plant residues and nitrogen cycling in soil. Plant Soil 181, 71–82.

McKendree W L and Smith R C 1990 Inhibition by white light of $^{86}Rb^+$ absorption in the root apex of corn. Plant Physiol. 93, 767–771.

Mian M A R, Natziger E D, Kolb F L and Teyker R H 1993 Root growth of wheat genotypes in hydroponic culture and in the greenhouse under different soil moisture regimes. Crop Sci. 33, 283–286.

Pagès L 1992 Mini-rhizotrons transparents pour l'étude du système racinaire de jeunes plantes. Application à la caractérisation du développement racinaire de jeunes chênes (*Quercus robus*). Can. J. Bot. 70, 1840–1847.

Saravitz C H, Chaillou S, Musset J, Raper C D J and Morot-Gaudry J -F 1994 Influence of nitrate on uptake of ammonium by nitrogen-depleted soybean: is the effect located in roots or shoots?. J. Exp. Bot. 45, 1575–1584.

Swietlik D 1992 Yield, growth, and mineral nutrition of young 'Ray Ruby' grapefruit trees under trickle or flood irrigation and various nitrogen rates. J. Am. Soc. Hort. Sci. 117, 22–27.

Tang C, Robson A D and Dilworth M J 1990 A split-root experiment shows that iron is required for nodule initiation in *Lupinus angustifolius* L. New Phytol. 115, 61–67.

Xu G, Levkovitch I, Soriano S, Wallach R and A. S 2004 Integrated effect of irrigation frequency and phosphorus level on lettuce: P uptake, root growth and yield. Plant Soil 263, 297–309.

Zhang H and Forde B G 1998 An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. Science 279, 407–409.

Zhang H M and Forde B G 2000 Regulation of *Arabidopsis* root development by nitrate availability. J. Exp. Bot. 51, 51–59.

*Section editor: H. Lambers*