Comparing Morphological Plasticity of Root Orders in Slow- and Fast-growing Citrus Rootstocks Supplied with Different Nitrate Levels

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INTRODUCTION

Competition among roots for below-ground resources is a major factor that influences spatial and temporal distribution of plant species in many natural and semi-natural ecosystems through its influence on acquisition of nutrients in different agro-ecosystems (Gerry and Wilson, 1995; Cahill, 1999; Rajaniemi et al., 2003). Within soil, nutrients are distributed unevenly both in space and time (Caldwell, 1994) and, consequently, plants have developed mechanisms to modify their root morphology and physiology in response to availability of nutrients. Such plant plasticity is strongly associated with many functional root traits including root elongation (Cahill and Casper, 2000) and its spatial influence (Casper et al., 2003), root architecture (Fitter et al., 2002), mycorrhizal symbiosis (Smith and Read, 1997) and uptake capacities (Caldwell et al., 1985). Studies on plasticity of functional root traits involved in nutrient acquisition have focused mainly on root length without considering such ‘morphological components’ as biomass allocation, specific root length, root fineness, and tissue density that affect root length. The plasticity of the above components in response to nitrate supply was studied in each root order of two co-generic citrus rootstocks, namely the fast-growing Citrus jambhiri ‘Rough Lemon’ (RL) and the slow-growing Citrus reshni ‘Cleopatra Mandarin’ (CM). Methods Morphological traits of individual root orders of CM and RL, grown at different nitrate levels (NO$_3$-N at 0-1, 0-5, 1 and 10 mM) were examined by using an image-specific analysis system. Key Results At high nitrate levels, the root length ratio, root mass ratio and, to a lesser degree, specific root length, root fineness and tissue density of tap and 1st-order laterals in both CM and RL were reduced. In 2nd-order laterals, however, the same treatment led to increased values of each morphological trait in CM but decreased values of the same traits in RL. At low nitrate supply, CM exhibited longer tap roots whereas RL developed longer 2nd-order laterals. These effects were due to root mass ratio and, to a lesser extent, specific root length. Conclusions Biomass allocation was the main component of nitrate-induced changes in root length ratio. The 2nd-order laterals were more sensitive to nitrate availability than the tap root and 1st-order laterals. At low nitrate availability, RL displayed longer 2nd-order lateral roots and lower root plasticity than CM. This suggests a different root growth strategy among citrus rootstocks for adapting to nitrate availability: RL invests in 2nd-order laterals, the preferred zone for acquiring the nutrient, whereas CM responds with longer tap roots.

Key words: Root morphology, root orders, phenotypic plasticity, nitrate, Citrus jambhiri, Citrus reshni.

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either by increasing biomass allocation to the roots, as demonstrated under a low supply of nitrogen (Ryser and Lambers, 1995; Sorgonà et al., 2005) or by increasing root fineness and/or reducing root tissue density, leaving biomass allocation unchanged (Ryser, 1998). In particular, in slow-growing grasses, high root tissue density, associated with a larger proportion of vascular tissue and vessels showing small diameters and thick cell walls (Wahl and Ryser, 2000) confers on roots a higher capacity to transport nutrients. In woody species, on the other hand, this capacity is determined by SRL (Comas et al., 2002; Comas and Eissenstat, 2004). Such kinds of adjustments, defined as root morphological plasticity, allow plants to adapt to non-uniform distribution of soil resources (Sultan, 2000).

So far, most studies on plasticity of root morphology in response to availability of nitrate have been carried out on the whole root system without considering the contribution of different root orders, which are distinct genetically, developmentally and functionally (Zobel, 1995; Waisel and Eshel, 2002). Furthermore, the limited data available on the behaviour of root orders in response to nitrate availability (Zhang et al., 1999; Linkohr et al., 2002; Sorgonà et al., 2005) do not cover the ‘morphological components’ of root length. Therefore, the present work addressed the following two questions. (1) Which components, among biomass allocation and/or root shape (SRL, root fineness and tissue density), determine changes in length of each root order in response to nitrate supply? (2) Which root order is the more sensitive and/or plastic in response to changing nitrate supply?

The within-root system allocation (biomass allocation) and structural (SRL, fineness and tissue density) strategy, leads to a different level of efficiency in nitrate acquisition when the plant competes for this anion, and the choice serves to emphasize the concerted responses of different root orders and morphological traits as the basis of the plant’s adaptation to variation in the availability of nitrate. The theory of plant growth (Grime, 1977; Reich et al., 1997) predicts that slow-growing species promote resource conservation and stress tolerance, whereas fast-growing ones promote capture of soil resources. This latter functional ability is associated with greater root morphological plasticity (Fransen et al., 1998; Robinson and Van Vuuren, 1998; Čiamporová et al., 1998; Comas et al., 2002; Comas and Eissenstat, 2004; Wright and Westoby, 2000) and low tissue density, which permit a longer root system (Ryser, 1998). Therefore, the root morphology of different root orders of the slow-growing ‘Cleopatra Mandarin’ and the fast-growing ‘Rough Lemon’ were compared to identify the morphological traits of each root order closely related to its potential growth rate.

MATERIALS AND METHODS

Plant material and germination

Citrus jambhiri ‘Rough Lemon’ (RL) and C. reshni ‘Cleopatra Mandarin’ (CM) seeds were surface-sterilized for 20 min in 20% sodium hypochlorite solution and germinated according to Chilembwe et al. (1992). The seeds were soaked in aerated deionized water at 35 °C for 2 d and then placed on germination paper moistened with 1 mM CaSO₄ in a growth chamber maintained at a temperature of 24 °C and relative humidity of 70% in the dark. When 80% of the seeds germinated, the seedlings were maintained under the same environmental conditions with a 14-h photoperiod.

Growth conditions and treatment

The experimental design comprised completely randomized blocks with five replications. Each replicated block consisted of the two rootstocks and four levels of nitrate concentration. Since the block effect was non-significant (P > 0.05), the data were re-analysed as a 2 (rootstocks) × 4 (nitrate levels) factorial experiment with 5 replications per treatment.

Forty seedlings (2 rootstocks × 4 nitrate concentrations × 5 replications), 57 d old, selected for uniform size, were transplanted into 3-L pots (one plant per pot) filled with perlite (Perlite s.r.l., Milano). The seedlings were grown in a greenhouse for 75 d. Natural light was supplemented with 300 μmol m⁻² s⁻¹ photosynthetic photon flux density (PPFD), artificial illumination, which yielded, at midday, an average of 1800 μmol m⁻² s⁻¹ PPFD on sunny days and 300 μmol m⁻² s⁻¹ PPFD on cloudy days. During growth of the seedlings, the temperature ranged from 24 °C to 34 °C. The seedlings were watered daily with 1 L of modified Hoagland solution (Hoagland and Arnon, 1950): 2.5 mM K₂SO₄, 2 mM MgSO₄, 1 mM KH₂PO₄, 10% (w/v) micronutrients and 10% (w/w) FeEDTA. To obtain the four nitrate concentrations, namely 0-1, 0.5, 1 and 10 mM – the representative range of nitrate concentration commonly found in soil solution (Barber, 1995) – nitrate was added to the nutrient solution as Ca(NO₃)₂ and, to balance the calcium, 4.95, 4.75, and 4.5 mM CaSO₄ was also added. The pH of nutrient solutions was adjusted to 6.0 with 0-1 N KOH.

Root morphology and biomass allocation

After 75 d of the treatment, when the seedlings were 132 d old, one seedling for each rootstock and nitrate level was collected and separated into shoot and root. Shoot dry weight (Wₛ, g) was measured after drying in an oven at 70 °C for 48 h. The roots were divided into three orders: tap roots, emerging directly from the seed; 1st-order lateral roots formed from the tap root; and 2nd-order lateral roots emerging from the 1st-order roots. Each root was stained with 0-1% (w/v) toluidine blue O for 5 min and then scanned at a resolution of 300 dpi (WinRhizo STD 1600, Instruments Régent Inc., Canada) for determining the length of tap root (L₁, cm), the total length of the 1st- (L₇, cm) and 2nd-order lateral roots (L₄, cm), the volume of the tap root (V₁, cm³), and the total volume of 1st- (V₇, cm³) and 2nd-order lateral roots (V₄, cm³) by the WinRhizo Pro v. 4.0 software package (Instruments Régent Inc.). Then, dry weights of the tap root (Wₛ, g) and the total dry weight of 1st-order (W₁, g) and 2nd-order lateral roots (W₄, g) were measured after
drying in an oven at 70 °C for 48 h. The total root dry weight \(W_R\) was the sum of \(W_T\), \(W_I\) and \(W_{II}\), and the plant dry weight \(W_P\) was obtained by the sum of \(W_R\) and \(W_S\).

Based on the measurements above, the following parameters were calculated for each root order:

\[
\begin{align*}
RLR &= \frac{L}{W_P} \text{ (cm g}^{-1}) \\
RMR &= \frac{W}{W_P} \text{ (g g}^{-1}) \\
SRL &= \frac{L}{W} \text{ (cm g}^{-1}) \\
F &= \frac{L}{V} \text{ (cm cm}^{-3}) \\
TD &= \frac{W}{V} \text{ (g cm}^{-3})
\end{align*}
\]

where RLR is the root length ratio, which expresses the root order’s potential for the acquisition of below-ground resources; the RMR is the root mass ratio, which indicates the relative biomass allocated to the root; the SRL, F and TD are the specific root length, fineness and tissue density, respectively, which represent the structural root parameters. While \(L\), \(W\) and \(V\) indicated the length, the dry weight and the volume of each root orders, respectively.

As reported by Ryser and Lambers (1995), the following relationships obtain among the above parameters:

\[
RLR = RMR \times SRL \quad (6)
\]

\[
SRL = \frac{F}{TD} \quad (7)
\]

The number of the 1st- \((N_I)\) and the 2nd-order lateral roots \((N_{II})\) was counted directly from the images of each root order. The average length of the 1st- \([L_I = L_I/N_I\] \text{ (cm)}\) and the 2nd-order lateral roots \([L_{II} = L_{II}/N_{II}\] \text{ (cm)}\) was also calculated.

For each rootstock and nitrate concentration, five seedlings were collected at the time of transplanting \((t_1 = 57 \text{ d})\) and at the end of the nitrate treatment \((t_2 = 132 \text{ d})\) to calculate the relative growth rate \((RGR, \text{ g g}^{-1} \text{ d}^{-1})\) according to Hunt (1982):

\[
RGR = \frac{(\ln W_{P2} - \ln W_{P1})}{(t_2 - t_1)} \quad (8)
\]

where \(W_{P1}\) and \(W_{P2}\) are the plant dry weight at \(t_1\) and \(t_2\), respectively.

**Statistical analysis of data**

The effects of rootstocks and nitrate levels on RGR were tested by two-way ANOVA. The data were checked for deviations from normality and homogeneity of variances prior to analysis. Tukey’s post hoc test comparison was applied to test the effect of the rootstock for each nitrate level at \(P < 0.05\).

Two-way ANOVA was performed for each parameter of root morphology and biomass allocation to test the effects of rootstock, nitrate level and rootstock × nitrate treatment interaction. In order to correct for allometric effects (Coleman et al., 1994), the ln-transformed plant dry weight \((\ln W_P)\) was used as a covariate in analysing the parameters of root morphology and biomass allocation when significant correlations between \(\ln W_P\) and these root parameters were found. The data were checked for deviations from normality and homogeneity of variances prior to analysis and the necessary transformations were carried out.

The effects of rootstocks and nitrate levels on the number and average length of the 1st- and 2nd-order lateral roots were tested by two-way ANOVA. The data are checked for deviations from normality and homogeneity of variances prior to analysis. Tukey’s post hoc test comparison was applied to test the effect of rootstock for each nitrate level and of nitrate level for each rootstock at \(P < 0.05\).

The phenotypic plasticity index (PPI) was calculated as described by Valladares et al. (2002). In particular, for each root trait \((Rtr)\), rootstock \((Ro)\) and root order \((Rty)\), the PPI was determined as the difference between the maximum and the minimum values among the four nitrate levels divided by the maximum value:

\[
PPI = \left[\frac{(\text{max value} - \text{min value})}{\text{max value}}\right] \quad (9)
\]

Means (± s.e.) of PPI were calculated for five individual seedlings for each root trait, rootstock and nitrate level. Then, the effects of the rootstock, root order and root trait on the PPI were tested by three-way ANOVA. The data were checked for deviations from normality and homogeneity of variances prior to analysis.

Statistical analysis was conducted using the Systat v. 8.0 software package (SPSS Inc., Evanston, IL, USA).

**RESULTS**

**Root order morphology in response to nitrate supply**

Nitrate affected the RLR of different root orders differently (Table 1 and Fig. 1). In particular, the RLR of the 1st-order laterals \((RLR_I)\) decreased with increasing nitrate level \((-30\% \text{ and } -21\% \text{ for } RL \text{ and } CM, \text{ respectively}; \text{ Table 1 and Fig. 1B})\). Although no statistical differences were observed along the nitrate range for RLR of the tap root \((RLR_T)\) and RLR of 2nd-order lateral \((RLR_{II})\) (Table 1), the significant interaction rootstock \((Ro) \times N\) (Table 1) indicated that increased supply of nitrate \(a\) reduced \((-45\%) \text{ RLR}_{T} \text{ in CM but not in RL and } b\) increased \(RLR_{II} \text{ in CM (+81\%) and reduced it (-58\%) in RL (Fig. 1A and C).}\)

RLR is the product of two components, namely root mass ratio, i.e. biomass allocation towards roots, and specific root length, i.e. root length per unit dry mass. Increasing the nitrate level significantly reduced biomass allocation to the tap root, i.e. root mass ratio of tap \((RMR_{T})\) (Table 1 and Fig. 2A), but had no effect on root mass ratio of the 1st- \((RMR_I)\) and 2nd-order lateral roots \((RMR_{II})\) (Table 1). However, the opposite trend was observed with root mass ratio of the 2nd-order laterals in response to nitrate...
supply among the citrus rootstocks masked the actual influence of this anion on this trait (significant interaction Ro × N, P = 0.006; Table 1 and Fig. 2C).

Specific root length of tap root (SRLT) showed a slight decrease up to 1 mM nitrate while the pattern was just the opposite for 2nd-order lateral roots (SRL2) (Fig. 3A and C). Although nitrate did not influence SRL of the 1st-order laterals (SRL1), a weak significant interaction Ro × N was reported (P = 0.03; Table 1). Indeed, as nitrate supply increased, this parameter increased slightly in RL and decreased in CM (Fig. 3B).

Specific root length is the ratio of two components, namely fineness, i.e. root length per unit volume, and tissue density, i.e. root biomass per unit volume. The fineness of the tap root (F1) and 1st- (F2) and 2nd-order lateral roots (F3) was not influenced by nitrate level (Table 1 and Fig. 4) but tissue density was sharply modified. Indeed, tissue density of the tap root (TD1) declined with increasing nitrate level in RL but not in CM (significant interaction P = 0.002; Table 1 and Fig. 5A). Increasing nitrate concentration had no effect on tissue density of the 2nd-order lateral roots (TD2) either in CM or in RL (Table 1 and Fig. 5C); however, tissue density of the 1st-order lateral roots (TD1) was differentially affected in the two citrus rootstocks: the tissue density increased in CM (+17%) and decreased in RL (−20%) (Table 1 and Fig. 5B).

Root length ratio of different root orders showed marked differences between the two rootstocks. Under low nitrate concentrations, CM exhibited a higher RLR of the tap than RL (significant interaction Ro × N; Table 1 and Fig. 1A) which, instead, displayed two-times longer 2nd-order laterals compared with CM (significant interaction Ro × N, P = 0.004, Table 1 and Fig. 1C). The length of the 1st-order laterals was similar in both rootstocks (Table 1 and Fig. 1B).

Table 1 shows that RL produced a greater number of 1st-order laterals than CM at all the nitrate levels with no significant difference in average length between the two. Conversely, the average length and number of 2nd-order laterals, especially at the lowest nitrate levels, were higher in RL than in CM (Table 3).

At all the nitrate levels, CM allocated more biomass to the tap root (high RMR) than RL (Fig. 2A) but rootstock had no effect on biomass allocation to 1st- and 2nd-order lateral roots (Table 1 and Fig. 2B, C). This was due to the opposite patterns of RMR between the two rootstocks, particularly in the case of 2nd-order laterals, as demonstrated by the highly significant interaction (P = 0.006) (Table 1 and Fig. 2C). Indeed, at low nitrate levels, RL allocated more biomass towards 1st-order laterals (+40%) and 2nd-order laterals (+85%) than CM (Fig. 2C, D).

Specific root length of the tap root was higher (+50%) in CM at all nitrate levels (Table 1 and Fig. 3A) whereas in RL, it was higher in the 1st- and 2nd-order laterals at 1 mM and 0.1 mM nitrate, although the latter observation is not supported by statistical analysis (significant interaction Ro × N; Table 1 and Fig. 3B, C).

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**Table 1.** Two-way ANOVA analysis (F- and P-value) for the root morphological parameters of different root orders of the citrus rootstocks (‘Rough Lemon’ and ‘Cleopatra Mandarin’) grown under different nitrate concentrations (0.1, 0.5, 1 and 10 mM) for 75 d

<table>
<thead>
<tr>
<th>Root orders</th>
<th>Rootstock (Ro) (d.f. = 1)</th>
<th>Nitrate (N) (d.f. = 3)</th>
<th>Plant dry weight (d.f. = 1)</th>
<th>Ro × N (d.f. = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root length ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap*</td>
<td>F (P)</td>
<td>0.50 (0.48)</td>
<td>1.69 (0.18)</td>
<td>40.88 (&lt;0.001)</td>
</tr>
<tr>
<td>I order*</td>
<td>F (P)</td>
<td>0.001 (0.97)</td>
<td>5.53 (0.004)</td>
<td>2.27 (0.14)</td>
</tr>
<tr>
<td>II order*</td>
<td>F (P)</td>
<td>1.69 (0.20)</td>
<td>0.24 (0.86)</td>
<td>0.005 (0.94)</td>
</tr>
<tr>
<td>Root mass ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap</td>
<td>F (P)</td>
<td>11.12 (0.002)</td>
<td>28.29 (&lt;0.001)</td>
<td>0.17 (0.68)</td>
</tr>
<tr>
<td>I order</td>
<td>F (P)</td>
<td>4.58 (0.04)</td>
<td>1.02 (0.39)</td>
<td>–</td>
</tr>
<tr>
<td>II order*</td>
<td>F (P)</td>
<td>1.43 (0.24)</td>
<td>0.058 (0.98)</td>
<td>0.079 (0.78)</td>
</tr>
<tr>
<td>Specific root length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap</td>
<td>F (P)</td>
<td>7.95 (0.008)</td>
<td>4.58 (0.009)</td>
<td>43.54 (&lt;0.001)</td>
</tr>
<tr>
<td>I order</td>
<td>F (P)</td>
<td>0.007 (0.93)</td>
<td>0.102 (0.96)</td>
<td>–</td>
</tr>
<tr>
<td>II order</td>
<td>F (P)</td>
<td>0.7 (0.41)</td>
<td>4.01 (0.01)</td>
<td>–</td>
</tr>
<tr>
<td>Fineness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap</td>
<td>F (P)</td>
<td>5.41 (0.03)</td>
<td>4.08 (0.15)</td>
<td>41.68 (&lt;0.001)</td>
</tr>
<tr>
<td>I order</td>
<td>F (P)</td>
<td>0.0009 (0.97)</td>
<td>0.57 (0.64)</td>
<td>0.53 (0.47)</td>
</tr>
<tr>
<td>II order</td>
<td>F (P)</td>
<td>0.69 (0.41)</td>
<td>0.35 (0.79)</td>
<td>2.66 (0.11)</td>
</tr>
<tr>
<td>Tissue density</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap</td>
<td>F (P)</td>
<td>3.67 (0.06)</td>
<td>5.95 (0.002)</td>
<td>8.94 (0.005)</td>
</tr>
<tr>
<td>I order</td>
<td>F (P)</td>
<td>1.65 (0.20)</td>
<td>0.57 (0.64)</td>
<td>0.1 (0.75)</td>
</tr>
<tr>
<td>II order</td>
<td>F (P)</td>
<td>1.32 (0.26)</td>
<td>0.63 (0.60)</td>
<td>4.61 (0.04)</td>
</tr>
</tbody>
</table>

The P-values are given within brackets. The natural logarithm of plant dry mass was used as the covariate. The dashes indicate that no significant correlation between lnWy and root morphological parameters was found and the covariate was not used in the analysis of variance. The block effect was not significant and is not shown. The degrees of freedom (d.f.) are given within brackets in the column titles. Transformations: *square root; †natural logarithm.
At all nitrate levels, the tap root was thinner (+46 %) in CM (Table 1 and Fig. 4A) but the two rootstocks did not differ in the fineness of their higher order lateral roots (Table 1 and Fig. 4B, C), although a slightly significant interaction Ro × C2 was observed for FI and FII (root fineness of 1st- and 2nd-order laterals, respectively) (Table 1). Indeed, at low nitrate concentrations, CM showed thinner 1st-order laterals compared with RL, with the opposite occurring for the 2nd-order laterals (Fig. 4B, C).

At 0.1 mM and 0.5 mM nitrate, TDT was slightly higher in RL (Table 1 and Fig. 5A). Conversely, CM showed a higher value of TDI only at 1 mM nitrate, yielding a significant interaction (Table 1 and Fig. 5B). The tissue density of 2nd-order laterals did not vary with the rootstock (Table 1 and Fig. 5C).

**Root order morphological plasticity of citrus rootstocks**

The PPI was evaluated in order to understand the different intensities of response to nitrate availability between root orders, root traits and citrus rootstocks. Among root traits, RLR and RMR were more plastic than fineness and tissue density, which exhibited the lowest PPI (Fig. 6). Regardless of rootstock, the 2nd-order laterals were the most plastic (Fig. 6). However, the significant interaction Rty × C2Rtr (P < 0.05; Fig. 6), indicated that the PPI of the 2nd-order lateral roots was higher for RLR and RMR and, at a low statistical significance, for tissue density as well. CM showed more plastic root morphology (Fig. 6), essentially due to the 2nd-order laterals (significant interaction Ro × Rty, P < 0.001), for all root traits.
DISCUSSION

Root order morphology in response to nitrate supply

The root system comprises different root orders or classes, which respond differently to environmental cues (Waisel and Eshel, 2002). Understanding the specific morphological responses of each root class to nitrate supply could help to predict plant distribution and adaptation in response to nitrogen availability. In the present work, nitrate availability affected the RLR of each root order differently: increasing nitrate supply reduced the RLR in 1st-order laterals whereas it remained apparently unaffected in tap roots and 2nd-order laterals. However, the significant R × N interaction for RLR of the tap root and 2nd-order laterals masked the actual effect of nitrate on these root orders. Furthermore, the 2nd-order laterals were found to be the most sensitive to nitrate supply, as indicated by their PPI (averaging 0.785), which was higher than that of tap roots (0.53) or of 1st-order laterals (0.385). This sensitivity may be the mechanism behind the ecological role of the 2nd-order laterals, and that of higher root orders generally, of increasing the plant’s ability to take up water and nitrate, as reported by Lazof et al. (1992) and Peterson and Enstone (1996).

However, the question of which ‘morphological components’ among biomass allocation and/or SRL and its components contributes the most to variation in RLR of each root order in response to nitrate availability remains open. The present results suggest that the change in RLR of each root order in response to nitrate availability could be due to the variation in RMR both quantitatively (in terms of the degree of variation) and qualitatively...
(in terms of the direction of variation), more than those in SRL and its components. Such an idea is also reinforced by the higher PPI of RMR in both the citrus rootstocks (0.59 in RL and 0.50 in CM) than that of SRL (0.36 in RL and 0.50 in CM). Such differences are even more evident in the 2nd-order laterals for which the PPI values for RMR (0.83 in CM and 0.67 in RL) are almost double those for SRL (0.45 in CM and 0.38 in RL). Taken together, the above results suggest that (a) RMR is the morphological parameter responsible for nitrate-induced variation in RLR and, once again, (b) the most plastic response is observed in the 2nd-order laterals. Hence, N-deficiency preferentially alters the partition of root biomass (RMR) rather than such shape of biomass as SRL and its components, namely root fineness and tissue density. In support of the central role of RMR, it has been reported that in N-deficient plants, by increasing sink strength and photosynthate export, the root to shoot ratio is shifted in favour of the former and that decreasing nitrate levels and/or increasing carbohydrate concentration are the putative signals that drive such a shift (Reynolds and D’Antonio, 1996; Améziane et al., 1997).

Finally, the greater sensitivity of the 2nd-order lateral roots to availability of nitrate, in terms of RMR, poses another question: Why do higher order roots display a greater variation than the tap root in biomass allocation? The source/sink relationships described by Poiseuille’s equation allows biomass partitioning among the root branches to be predicted within a given root system by a combination of the branching order and the distance of a branch from the source (Farrar and Williams, 1991). Indeed, Aguirrezabal et al. (1993) showed in sunflower that roots of lower branching orders have the priority in carbon allocation, which ensures that the tap root elongates faster than the laterals. In this respect, it could be supposed that, in response to nitrate supply, the 2nd-order laterals modify their RMR value (higher plasticity) more than the tap root and the 1st-order laterals, both of which have a higher priority in biomass allocation.

In conclusion, although based on a single experiment, the present work highlights the relevant contribution of 2nd-order lateral roots in making the whole root system more plastic in response to nitrate availability. Therefore, the plant’s ability to modify its 2nd-order lateral roots helps in optimizing the structures devoted to capturing resources, thus increasing the plant’s adaptive efficiency to the nitrate availability.

### Table 2. Relative growth rate (g g⁻¹ W₀ d⁻¹) of the ‘Rough Lemon’ and the ‘Cleopatra Mandarin’ rootstock grown at different nitrate concentrations for 75 d

<table>
<thead>
<tr>
<th>Nitrate (mM)</th>
<th>‘Rough Lemon’</th>
<th>‘Cleopatra Mandarin’</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.027 ± (0.001)ᵃ</td>
<td>0.020 ± (0.002)ᵇ</td>
</tr>
<tr>
<td>0.5</td>
<td>0.033 ± (0.0009)ᵃ</td>
<td>0.024 ± (0.001)ᵇ</td>
</tr>
<tr>
<td>1</td>
<td>0.034 ± (0.0006)ᵃ</td>
<td>0.026 ± (0.001)ᵇ</td>
</tr>
<tr>
<td>10</td>
<td>0.032 ± (0.001)ᵃ</td>
<td>0.025 ± (0.002)ᵇ</td>
</tr>
</tbody>
</table>

Different letters in a row indicate a significant difference at P < 0.05 among the rootstocks (Tukey’s test). Values within brackets indicate the standard error (n = 5).

#### Root order morphology of citrus rootstocks

RL and CM showed marked differences in their RGR (averaging 0.031 g g⁻¹ W₀ for RL and 0.024 g g⁻¹ W₀ for CM) and nitrate use efficiency (Sorgona et al., 2006), although the lengths of their entire root system were similar (data not shown). Since root length is a key trait for acquiring soil resources (Ryser, 1998), how do similar root lengths explain the differences in plant growth rate and nitrate use efficiency between the two citrus rootstocks? Such apparent contradiction might be explained by analysing the contribution of different root orders to the

![Figure 5](image-url)
whole root system. In this respect, RL showed a higher RLR and a larger number of 2nd-order laterals with respect to CM, which, on the contrary, showed a higher RLR of the tap root, especially at low nitrate levels. In other words, the slow-growing CM increased its rooting depth at the expense of root spread, compared with the fast-growing RL, which invested more in higher-order laterals. This difference may have an important functional role in terms of nitrate uptake since the laterals are characterized by high nitrate absorption capacity (Lazof et al., 1992). In Citrus species, investing in even higher orders may have a prominent adaptive significance, since the 2nd-order laterals have a larger number of passage cells (Eissenstat and Achor, 1999), and are the preferred sites of water and nutrient uptake (Peterson and Enstone, 1996), compared with low root orders. Therefore, the structurally different root systems of CM and RL may account for diverse functional and adaptive efficiencies to nitrate availability, making the root system of the latter more competitive for nitrate acquisition, especially at scarce soil nitrate levels.

Table 3. Number (n) and average length (cm) of the 1st- and the 2nd-order lateral roots of ‘Rough Lemon’ and ‘Cleopatra Mandarin’ rootstock grown at different nitrate concentrations for 75 d

<table>
<thead>
<tr>
<th>Nitrate (mM)</th>
<th>0.1</th>
<th>0.5</th>
<th>1.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of 1st-order lateral root (n)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Rough Lemon’</td>
<td>84 ± 7.8a</td>
<td>89 ± 5.1a</td>
<td>110 ± 3.9a</td>
<td>79 ± 13a</td>
</tr>
<tr>
<td>‘Cleopatra Mandarin’</td>
<td>30 ± 4.6a</td>
<td>32 ± 3.4b</td>
<td>22 ± 3.3b</td>
<td>24 ± 5.5b</td>
</tr>
<tr>
<td><strong>Average 1st-order lateral root length (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Rough Lemon’</td>
<td>2.38 ± 0.17a</td>
<td>2.83 ± 0.12a</td>
<td>2.7 ± 0.14a</td>
<td>2.76 ± 0.09a</td>
</tr>
<tr>
<td>‘Cleopatra Mandarin’</td>
<td>1.65 ± 0.18a</td>
<td>2.13 ± 0.3a</td>
<td>2.92 ± 0.3a</td>
<td>3.3 ± 1.13a</td>
</tr>
<tr>
<td><strong>Number of 2nd-order lateral root (n)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Rough Lemon’</td>
<td>68 ± 10a</td>
<td>114 ± 13a</td>
<td>78 ± 4.5a</td>
<td>39 ± 11a</td>
</tr>
<tr>
<td>‘Cleopatra Mandarin’</td>
<td>3.8 ± 2.3a</td>
<td>3.8 ± 2.2b</td>
<td>16.4 ± 5.8b</td>
<td>17.4 ± 6.2a</td>
</tr>
<tr>
<td><strong>Average 2nd-order lateral root length (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Rough Lemon’</td>
<td>0.97 ± 0.04a</td>
<td>1.12 ± 0.09a</td>
<td>1 ± 0.06a</td>
<td>1.26 ± 0.07a</td>
</tr>
<tr>
<td>‘Cleopatra Mandarin’</td>
<td>0.31 ± 0.19b</td>
<td>0.8 ± 0.2a</td>
<td>1.19 ± 0.19a</td>
<td>0.9 ± 0.25a</td>
</tr>
</tbody>
</table>

Different letters among the rootstocks for each root parameter indicate a significant difference at P < 0.05 (Tukey’s test). Values within brackets indicate the standard error (n = 5).

Conversely, at a non-limiting nitrate level (10 mM), CM and RL show a significantly different behaviour as far as the tap root is concerned, but not that of the 1st- and 2nd-order laterals, in all morphological traits. This difference probably reflects the different functional roles of root orders: the tap root, as the embryonic root, is strongly dependent on the genotype while the 1st- and 2nd-order laterals, as post-embryonic roots, are involved in nutrient acquisition and,
hence, more sensitive to such environmental conditions as nutrient stress.

Which ‘morphological components’ drives the variation in length of different root orders in the two rootstocks? Under low nitrate availability (0·1 mm and 0·5 mm), the greater elongation of 2nd-order laterals in RL is essentially due to the variation of RMR (85 % and 95 % of the differences among the rootstocks at 0·1 mm and 0·5 mm nitrate, respectively) rather than SRL (only 65 % at 0·1 mm nitrate). Furthermore, the change in RMR (37–59 %) exceeds that in SRL as a determinant of the longer tap root of CM. These results are strengthened by the higher PPI of RMRII (0·68 in RL and 0·83 in CM) than that of SRLII (0·38 in RL and 0·45 in CM) and of RMR (0·44 in RL and 0·46 in CM) than that of SRL (0·44 in RL and 0·37 in CM). Hence, RMR, once again, is more important than SRL in determining a higher RLR of tap roots and 2nd-order laterals in CM and RL, the slow- and fast-growing rootstocks, respectively. Nevertheless, RL allocates greater biomass to higher order laterals, the primary function of which is resource acquisition. Furthermore, since the 2nd-order laterals require a lower biomass investment per unit of root length, the increased length of these root orders in RL, the fast-growing rootstock, imposes a lower cost in terms of biomass allocation at the whole root system level when compared with CM, the slow-growing rootstock.

Another aspect that differentiates between the fast- and slow-growing species at the level of the whole root system is the higher RMR and lower tissue density in fast-growing species of grasses (Ryser and Lambers, 1995; Wahl and Ryser, 2000) but not in woody species (Wright and Westoby, 2000; Comas et al., 2002). Such discrepancy may be traced to the fact that they estimated the ‘average’ RMR and tissue density, which may well have masked the high morphological variability among different root orders, especially in woody species, which have abundant sclerenchymatic tissue in their roots. Indeed, the fast-growing RL allocates more biomass towards the 2nd-order laterals, typically showing low tissue density, which results in lower RMR and tissue density at the whole root level compared with the slow-growing CM (data not shown). In this respect, RL seems to be able to allocate biomass optimally within its root system and to use this biomass better than CM. Hence, evaluating RMR and tissue density of different root orders, rather than analysing the whole root system, provides a clearer picture of the different strategies used by the two fast- and slow-growing species – which come to light only when single root orders are evaluated – only reflect the slow- and fast-growing habits of the two citrus rootstocks studied here. This finding could acquire a more general significance only when more slow- and fast-growing woody species are studied, as reported on temperate tree species by Comas et al. (2002): ‘variation in root traits between fast- and slow-growing species was probably related to differences in their strategies for acquiring soil resources because the greatest variation was found in first and second-order roots’.

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LITERATURE CITED


