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Nitrate Use-efficiency: a Morphological Analysis of the Above- and Below-ground Functional Traits in Two Citrus Rootstocks

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Abstract: An increased knowledge of the potential morpho-physiological mechanisms of the nitrate use efficiency (NUE) of the crops is fundamental for reducing the excessive input of the N-fertilizers maintaining an acceptable plant yield and environmental health. The aim of this work was to identify above- and below-ground morphological traits that improve nitrate acquisition efficiency in two citrus rootstocks, Sour Orange (*Citrus aurantium* L.) (SoO) and Sweet Orange (*Citrus sinensis* L.) (SwO), characterized as nitrate-use-efficient and –inefficient. Root length, tip numbers, leaf area and stem height, as functional traits, root mass ratio, leaf mass ratio and stem mass ratio, as allocation parameters, and specific root length, leaf mass per area and specific stem ratio, as cost parameters, were evaluated. Further, the functional, allocation and cost parameters of different root orders were analyzed. The results of this work revealed that, at low nitrate availability, SoO seedlings exhibited an above- and below-ground traits more competitive for the acquisition of this anion: wider leaf area, higher total length and tip numbers of the root system accompanied by higher total length of the 2nd order respect to the SwO. Further, the "competitive structure" of the SoO was reached by an increased biomass allocation and its better use efficiency towards the above- and below-ground parts of the seedlings. In conclusion, at the low nitrate level, the morphological changes of the below- and above-ground traits of the SoO could represent the mechanisms which define SoO as nitrate-use-efficient enhancing its capacity to absorb nitrate from soil environment.

Key words: Nitrate, citrus rootstock, leaf morphology, root morphology, root orders

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Introduction

Nitrate, the predominant nitrogen form in aerobic soils, is the most limiting nutrient of plant growth and productivity. In the last decades, a massive input of N fertilizer to maintain high nitrate level and increase the productivity in cropping systems has been applied. However, owing to the losses of N fertilizer by run-off and /or leaching into the environment, nitrate represents the major source of pollution in aquatic ecosystems (London, 2005; Beman *et al*., 2005), drinking waters (Carpenter *et al*., 1998) and caused $15.9 billion annual loss by fertilization of the cereal crops (Raun and Johnson, 1999). Therefore, the optimization of the N-fertlizer use appears to be fundamental for both minimise the nitrate pollution and guarantee high plant productivity and human health. In this respect, the selection of genotypes with high nitrogen use efficiency (NUE) or "aggressive nitrate acquisition" could contribute to a substantial reduction in N-fertiliser inputs with consequently environmental and economic advantages allowing high yields to be maintained (Lynch, 1998; Hirsch and Sussman, 1999).

Until now, considerable efforts have been made to select high N-use efficient genotypes and identify the plant traits that improve nitrate acquisition. For the below-ground of the plant, the root length was closely associated with higher NUE in wheat lines (Liao *et al*., 2004; 2006) and with high capability to acquire nitrate and less leaching in maize (Wiesler and Horst, 1993; 1994). Furthermore, highly branched root architectures

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were correlated with higher induction of the nitrate uptake in wheat (Sorgonà and Cacco, 2002) while the root proliferation responded to localized N-enriched soil patches in barley (Drew, 1975; Drew and Saker, 1975) and Arabidopsis (Zhang *et al*., 1999). However, considering that the root system is constituted by different root types/orders which are distinct genetically, developmentally and functionally (Zobel, 1995; Waisel and Eshel, 2002), it is still not clear which type of roots or root traits could be advantageous for the plant adaptation in a low N environment.

Moreover, the capacity to acquire nitrate by the root are sustained by the above-ground activity of plant traits, such as leaf area and/or stem height which could be involved in nitrate use efficiency. Indeed, an alteration of the leaf expansion was observed in response to the N availability in herbaceous species (Ryser and Lambers, 1995; Walch-Liu et al., 2005, Tian *et al*., 2007). Further, nitrogen deprivation caused starch accumulation in leaves, and an increase of the photosyntate allocation towards the roots resulting in a decline in the shoot:root ratios (Rufty *et al*., 1988).

However, both the below- and above-ground traits, such as root length, leaf area and stem height, depended on the dry mass (allocation parameters) whose optimal partitioning to different organs resulted in the plant growth maximization (Bazzaz and Grace, 1997). Nevertheless, the biomass allocation towards the above- and below-ground parts of the plant are considered to constrain each other suggesting that cost parameters, such as leaf mass per area (LMA), stem height per mass (specific stem height, SStH) and the root length per mass (specific root length, SRL), which better express how efficiently this biomass is used, could be considered fundamental in the plant efficiency for the nitrate acquisition.

Indeed, the low-LMA plant species showed an improvement of the light capture and a high rate of photosynthesis per unit leaf nitrogen (Poorter *et al*., 2009). The specific root length is usually associated with fast root elongation (Eissenstat, 1991) and high nutrient uptake capacity (Reich *et al*., 1998). Furthermore, the SRL was the plant trait which discriminate the annual species characterized by fast resource acquisition from the perennial considered as conservative-resource species (Roumet *et al*., 2006).

Most of the studies on the NUE and its morpho-physiological mechanisms were generally referred to the herbaceous species and, in particular, to cereal crops while few informations concerning these aspects have been conducted on citrus species, tree crops of economic importance in Mediterranean countries. In this respect, in the present study, a comparison of the morphological above- and below-ground traits of two citrus rootstocks, Sour Orange and Sweet Orange, with contrasting nitrate use efficiency (Sorgonà *et al*., 2006), in response to different nitrate availability was carried out. In particular, the following aspects were assessed: 1) which morphological changes in root length, leaf area and stem height, can be responsible to the difference in NUE , 2) which allocation and the cost parameters affected the root length, leaf area and stem height, 3) which root architecture adapted to low nitrate availability and, finally 4) how the morphological changes among the root orders could be correlated with the NUE.

Material and methods

Plant Material and Germination:

Sour Orange (*Citrus aurantium* L.) (SoO) and Sweet Orange (*Citrus sinensis* L.) Osbeck) (SwO) seeds were surface-sterilised for 20 min in 20% sodium hypochlorite solution and germinated according to Chilembwe *et al*. (1992). The seeds, soaked in aerated deionized water at 35° C for 2 d, were then placed on germination paper moistened with 1 mM $CaSO_4$ in a growth chamber maintained at 24° C and 70% relative humidity in darkness. When 80% of the seeds were germinated, the seedlings were placed in the light with a 14-h photoperiod (photon flux rate of 300 µmol m^{-2} s^{-1}) under the same environmental conditions.

Growth Conditions and Treatment:

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

Twenty seedlings (2 rootstocks x 2 nitrate concentrations x 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 mmol m^{-2} s^{-1} photosynthetic photonic flux density (PPFD) by artificial illumination, which yielded, at midday, an average of 1800 mmol m^{-2} s^{-1} PPFD on sunny days. During growth period, 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 K_2SO_4, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 10% (w/v) micronutrients and 10% (w/v) FeEDTA. In order to obtain the two nitrate concentrations, namely 0.1 and 10 mM, the anion was added to the nutrient solution as $Ca(NO_3)_2$ and, the balance of the calcium, was also achieved by the addition of 4.95 mM $CaSO_4$. The pH of nutrient solutions was adjusted to 6.0 with 0.1 N KOH.

After 75 d of the treatment, one seedling (132 d old) for each rootstock and nitrate level was collected and separated into leaves, stem and root system for the following analysis.

Morphology, Allocation and Cost Parameters of the Aboveground Functional Traits:

To measure the leaf area (LA) (cm^2) and the stem height (StH) (cm), the leaves and stem were scanned and analysed using the WinRhizo Pro Version 4.0 Software Package (Instruments Régent Inc., Canada). Finally, they were placed in an oven at 70° C for 2 days to determine leaf (LDW, g) and stem (StDW, g) dry weight. The plant dry weight (PDW, g) was given by the sum of LDW, StDW and root dry weight (RDW), this latter determined as reported in the following paragraph.

Based on the above measurements, the following allocation and cost parameters of the aboveground functional traits were calculated:

$LMR = LDW/PDW \quad (g\ g^{-1})$
$StMR = StDW/PDW \quad (g\ g^{-1})$
$LMA = LDW/LA \quad (g\ cm^{-2})$
$SStH = StH/StDW \quad (cm\ g^{-1})$

where LMR and StMR were the leaf mass ratio and stem mass ratio which indicated the relative biomass allocated to the leaves and stem, respectively; LMA and SStH were the leaf mass per area and the specific stem height and represented the biomass cost for constructing the leaf area and the stem height, respectively.

Morphology, Biomass Allocation and Cost Parameters of the Belowground Functional Traits:

The root system was divided into three root orders: tap root, 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) toludine 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 (L_T, cm), the total length of the 1st (L_{I°, cm) and 2nd order lateral roots (L_{II°, cm), the volume of the tap (V_T, cm^3), the total volume of 1st (V_{I°, cm^3) and 2nd order lateral roots (V_{II°, cm^3) by the WinRhizo Pro v. 4.0 software package (Instruments Régent Inc.). Then, dry weights of the tap root (DW_T, g) and the total dry weight of 1st order (DW_{I°, g) and 2nd order lateral roots (DW_{II°, g) were measured after drying in an oven at 70°C for 48 h. The root dry weight (RDW, g) was the sum of DW_T, DW_{I° and DW_{II°.

Based on the measurements above, the following allocation and cost parameters of the belowground functional traits were calculated:

$RMR_T = DW_T/PDW \quad (g\ g^{-1})$
$RMR_{I^\circ} = DW_{I^\circ}/PDW \quad (g\ g^{-1})$
$RMR_{II^\circ} = DW_{II^\circ}/PDW \quad (g\ g^{-1})$
$SRL_T = L_T/DW_T \quad (cm\ g^{-1})$
$SRL_{I^\circ} = L_{I^\circ}/DW_{I^\circ} \quad (cm\ g^{-1})$
$SRL_{II^\circ} = L_{II^\circ}/DW_{II^\circ} \quad (cm\ g^{-1})$

where RMR_T, RMR_{I° and RMR_{II° were the tap, 1st and 2nd order lateral root mass ratio which indicated the relative biomass allocated to the tap, 1st and 2nd order lateral root, respectively; the SRL_T, SRL_I and SRL_{II} were the specific tap, 1st and 2nd order lateral root length, respectively, which represented the biomass cost for constructing an unit of root length of each root order.

The number of the 1st (TN_{I°) and the 2nd order lateral roots (TN_{II°) was counted directly from the images of each root order. The average length of the 1st- [$AVL_{I^\circ} = L_{I^\circ}/N_{I^\circ}$] (cm) and the 2nd-order lateral roots [$AVL_{II^\circ} = L_{II^\circ}/N_{II^\circ}$] (cm) were also calculated. The total length (TLR) and tip number of the whole root system (TTN) were calculated as the following:

$TLR = L_T+L_{I^\circ}+L_{II^\circ} \quad (cm)$
$TTN = TN_{I^\circ}+TN_{II^\circ} \quad (cm)$

Statistical Analysis of Data:

Two-way ANOVA was performed for the morphological, allocation and cost parameters to test the effects of rootstock, nitrate level and rootstock x nitrate treatment interaction. The data were checked for deviations from normality and homogeneity of variances prior to analysis and the necessary transformations were carried out. Tukey's post hoc test comparison was applied to test the effect of rootstock for each nitrate level at P< 0.05.

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

Results:

The above-ground functional traits, leaf area and stem height, increased to the increase of the nitrate concentration although differently between the two citrus rootstocks (Tab 1). In particular, the Sour Orange exhibited a higher leaf area and stem height respect to the Sweet Orange (Tab 1).

The below-ground functional traits i.e. the total length and tip number of the whole root system and the total length of the different root orders were analysed. The TLR was increased by an increase of nitrate concentrations, showing a different response between the citrus rootstocks: the TLR of the SoO was higher than SwO, at both nitrate levels (Tab 1). This behavior was also observed in the total length of the 1st and 2nd order lateral root, L_{I° and L_{II°, while, it was not evident in the tap root, where SwO showed, at 0.1 mM nitrate but not at higher level, a longer tap root than SoO (Tab.1). Table 2 showed that the number and the average length of the 1st order lateral roots, didn't vary between SoO and SwO, although the higher nitrate level raised the AVL_{I°. On the contrary, SoO produced a greater number of the 2nd order lateral root, especially at low nitrate level, than SwO and, also evidenced a higher average length, AVL_{II°, at both nitrate concentrations (Tab.2). The number of tips in the whole root system, TTN, was different between the citrus rootstocks and subjected to the GxN interaction: the SoO exhibited greater root tip number than SwO at 0.1 mM nitrate only (Tab.2).

Table 1: Morphology of the above- and below-ground functional traits of Sour Orange (SoO) and Sweet Orange (SwO) seedlings grown on two nitrate levels for 75 days. Bars indicate s.e. (n=5). Significant effects of citrus genotypes (G), nitrate levels (N) and their interaction are presented in Statistics as F-values and level of significance (*P<0.05, **P<0.01, ***P<0.001) as estimated by a two-way ANOVA

Functional traits	SoO		SwO		Statistics
	0.1 mM	10 mM	0.1 mM	10 mM	
Leaf area (cm^2)	38.7 (4.8)	79.6 (2.4)	23.3 (3.5)	71 (7.3)	G: F = 6.03* N: F = 82.48**
Stem height (cm)	6.12 (0.4)	7.6 (0.1)	5.3 (0.4)	6.8 (0.2)	G: F = 7.11* N: F = 26.31***
Total length of the whole root system (cm)	143 (23)	171 (10)	94 (6)	135 (22)	G: F = 6.00*
Total length of the tap root (cm)	26.7 (3.8)	29.3 (0.5)	34.8 (1.8)	27.7 (2.1)	GxN: F = 4.18*
Total length of the 1st order lateral root (cm)	79 (13.5)	98 (5.9)	52 (4.8)	80 (14.8)	G: F = 4.23* N: F = 4.81*
Total length of the 2nd order lateral root (cm)	37 (7.1)	43 (10.6)	7 (2.4)	27 (7.7)	G: F = 9.63**

Table 2: Tip numbers of whole root system and number and average length of the 1st and 2nd order lateral root of Sour Orange (SoO) and Sweet Orange (SwO) seedlings grown on two nitrate levels for 75 days. Bars indicate s.e. (n=5). Significant effects of citrus genotypes (G), nitrate levels (N) and their interaction are presented in Statistics as F-values and level of significance (*P<0.05, **P<0.01, ***P<0.001) as estimated by a two-way ANOVA.

Functional traits	SoO		SwO		Statistics
	0.1 mM	10 mM	0.1 mM	10 mM	
Tip number of whole root system (n.)	71.7 (5)	51.6 (5)	35.4 (2.9)	44.4 (4.7)	
Number of 1st order lateral root (n.)	30 (5.3)	24 (4.1)	26 (4)	22 (3)	
Number of 2nd order lateral root (n.)	33 (5)	28 (7)	9 (3)	22 (4)	G: F = 8.22* GxN: F = 3.26*
Average length 1st order lateral root (cm)	2.7 (0.1)	4.5 (0.7)	2.2 (0.4)	3.7 (0.5)	N: F = 11.67**
Average length 2nd order lateral root (cm)	1.1 (0.2)	1.6 (0.1)	0.6 (0.1)	1.2 (0.2)	G: F = 9.77** N: F = 12.17**

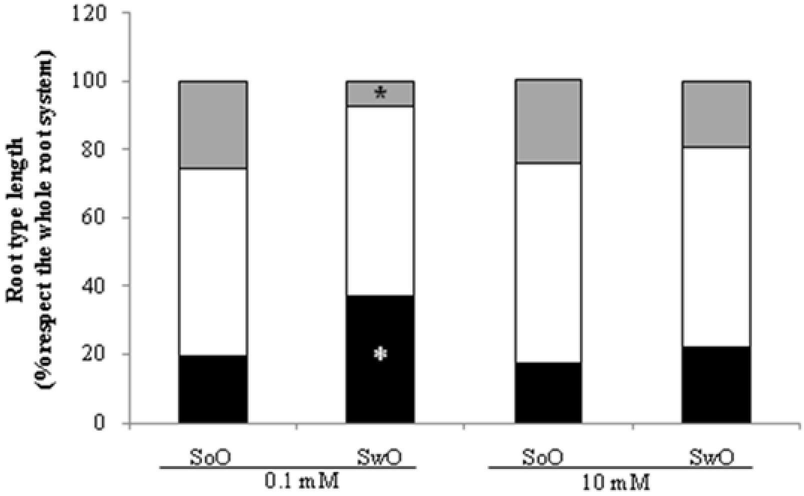


Fig. 1: Root architecture of Sour Orange (SoO) and Sweet Orange seedlings (SwO) grown on two nitrate levels for 75 days. Tap root (?), lateral 1st order ( ) and lateral 2nd order root (?). Bars indicate s.e. (n=5). The asterisks indicated significant difference among the root types at $p<0.05$ (Tukey's test).

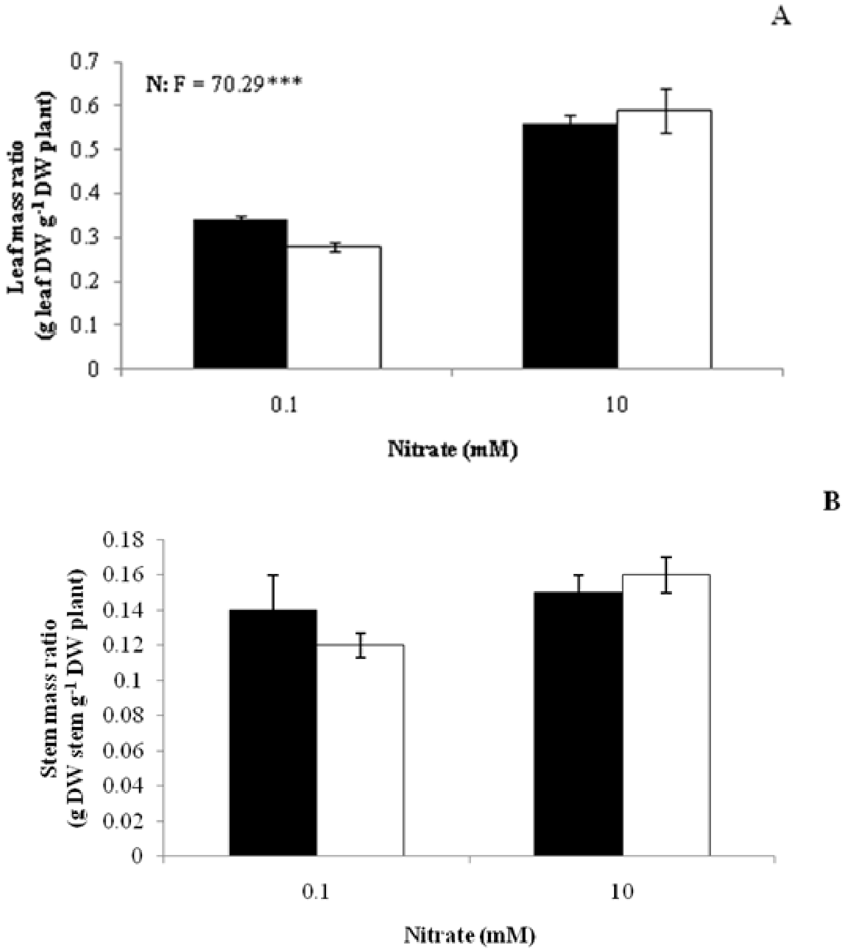


Fig. 2: Allocation parameters of the aboveground functional traits (A: leaf area, B: stem height) of Sour Orange (¢) and Sweet Orange seedlings (£) grown on two nitrate levels for 75 days. Bars indicate s.e. (n=5). Significant effects of citrus genotypes (G), nitrate levels (N) and their interaction are presented as F-values and level of significance (*$P<0.05$, **$P<0.01$, ***$P<0.001$) as estimated by a two-way ANOVA.

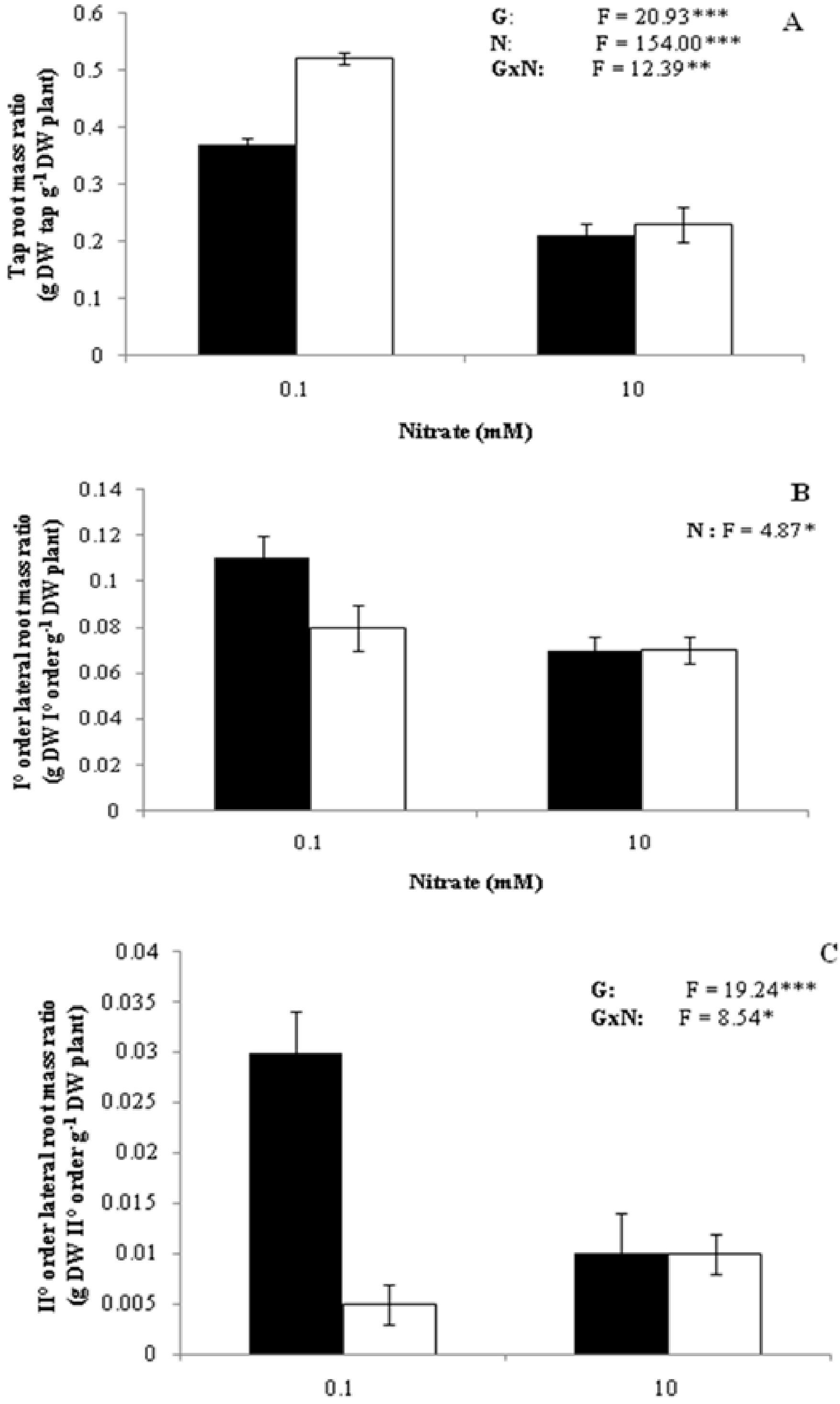


Fig. 3: Allocation parameters of the belowground functional traits (A: tap root, B: 1st order lateral root, C: 2nd order lateral root) of Sour Orange (¢) and Sweet Orange seedlings (£) grown on two nitrate levels for 75 days. Bars indicate s.e. (n=5). Significant effects of citrus genotypes (G), nitrate levels (N) and their interaction are presented as F-values and level of significance (*P<0.05, **P<0.01, ***P<0.001) as estimated by a two-way ANOVA.

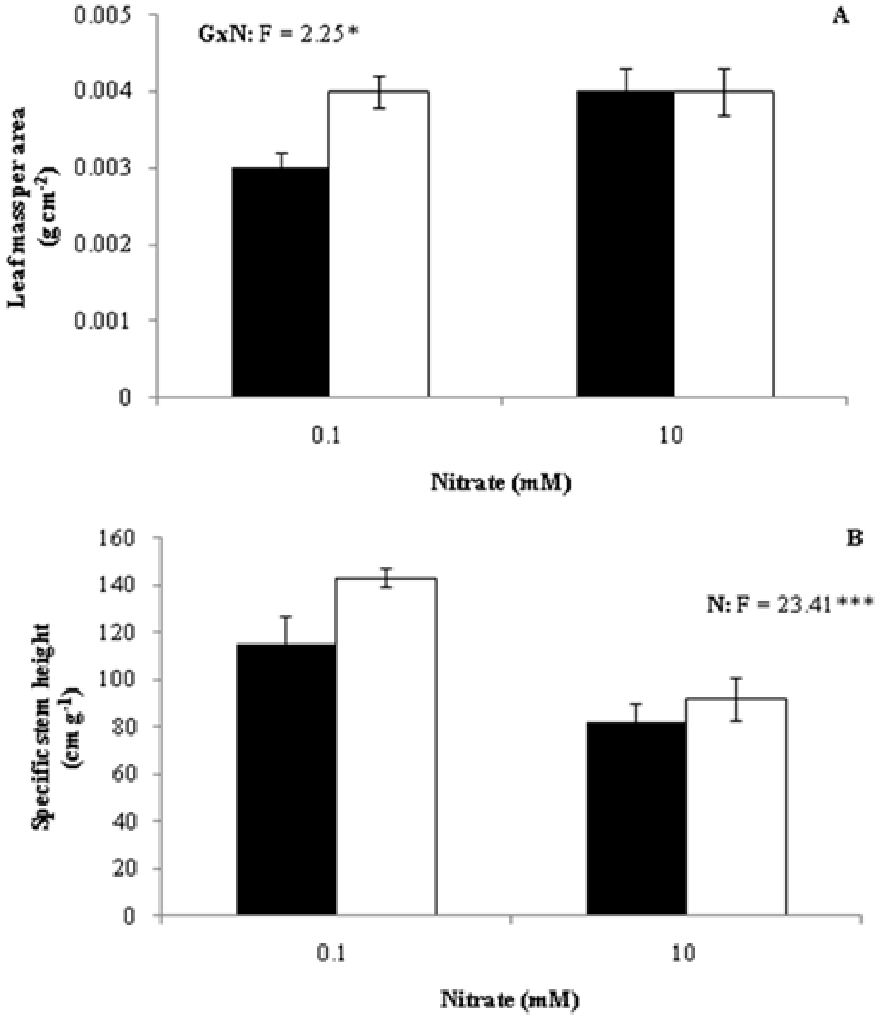
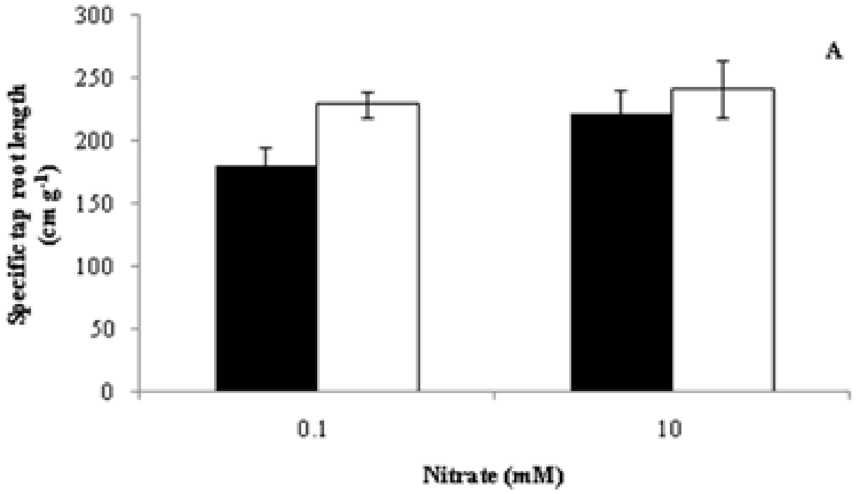


Fig. 4: Cost parameters of the aboveground functional traits (A: leaf area, B: stem height) of Sour Orange (¢) and Sweet Orange seedlings (£) grown on two nitrate levels for 75 days. Bars indicate s.e. (n=5). Significant effects of citrus genotypes (G), nitrate levels (N) and their interaction are presented as F-values and level of significance (*P<0.05, **P<0.01, ***P<0.001) as estimated by a two-way ANOVA.



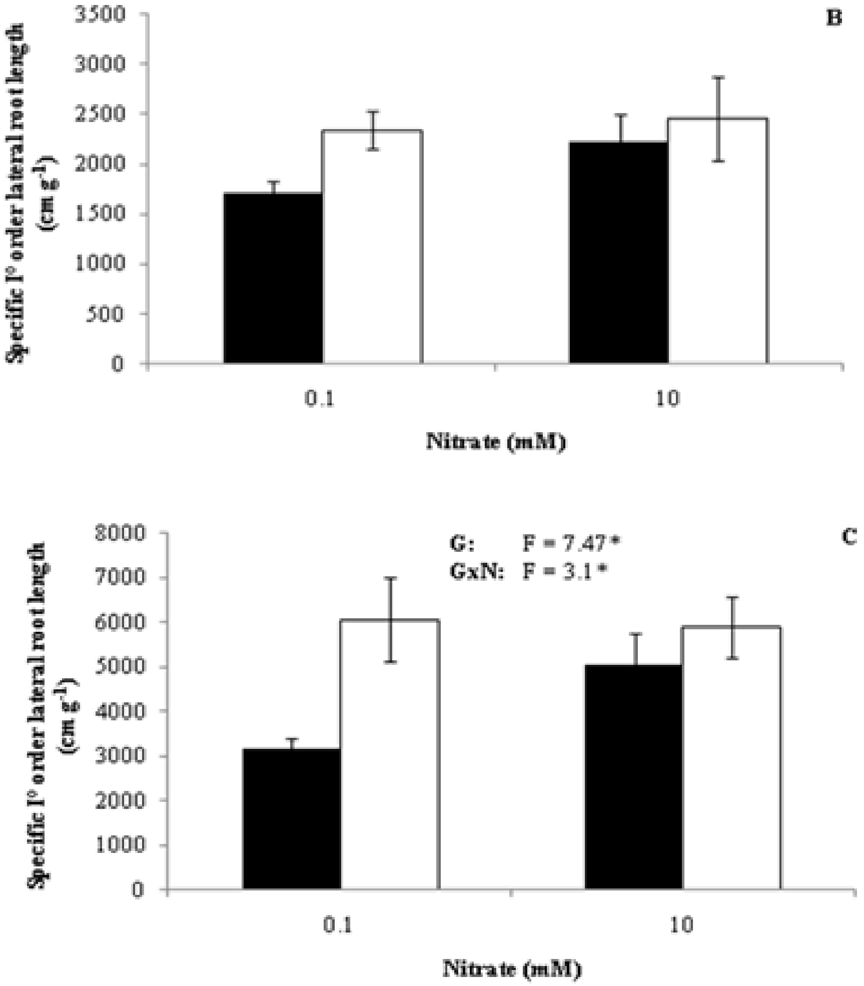


Fig. 5: Cost parameters of the belowground functional traits (A: tap root, B: 1st order lateral root, C: 2nd order lateral root) of Sour Orange (¢) and Sweet Orange seedlings (£) grown on two nitrate levels for 75 days. Bars indicate s.e. (n=5). Significant effects of citrus genotypes (G), nitrate levels (N) and their interaction are presented as F-values and level of significance (*P<0.05, **P<0.01, ***P<0.001) as estimated by a two-way ANOVA

The different total length and tip number of the root orders determined a diverse variation of the root architecture between the citrus rootstocks, in response to nitrate availability, especially at low nitrate level. Indeed, at 0.1 mM nitrate, the root system of the SoO was composed by a greater number of 2nd order lateral roots than SwO which in turn exhibited a longer tap root (Fig. 1). However, these differences disappeared at 10 mM nitrate (Fig. 1).

The biomass allocation towards leaves and stem was not different between the citrus rootstocks but the former parameter was affected by nitrate level (Figure 2). Indeed, the leaf mass ratio, and less markedly stem mass ratio, increased with the increase of the nitrate concentration. On the other hand, the biomass allocation towards the tap and the 2nd order lateral roots was sharply influenced by genotype x nitrate interaction. Indeed, at 0.1 mM nitrate, whereas the SwO allocated more biomass towards the tap root, the SoO addressed the biomass towards the 2nd order lateral roots (Figure 3A and C). The biomass allocation towards the 1st order lateral root was influenced by nitrate level decreasing with the increase of the nitrate concentrations (Figure 3B).

Figure 4 showed the variation of cost parameters of the aboveground functional traits between the citrus rootstocks and in response to the nitrate levels. Although, the leaf mass per area appeared to be not modified by the nitrate concentrations, a significant interaction GxN was evident. Indeed, Sour Orange exhibited a smaller leaf area per mass than Sweet Orange at low nitrate availability (Fig. 4A). Conversely, the specific stem height was significantly changed by nitrate treatment which determined a decrease of this trait (Fig. 4B).

The specific tap and 1st order lateral root length was not affected by either nitrate treatment or citrus rootstocks, while that relative to the 2nd order lateral root was subjected to the GxN interaction (Figure 5A and B). In particular, the specific 2nd order lateral root length of the SoO was lesser than that of the SwO, at 0.1 mM nitrate (Figure 5C).

Discussion:

The objective of this study was to evaluate the morphological traits which confer a higher ability to compete for the low nitrate availability in soil comparing two citrus rootstocks characterized by contrasting N-use efficiency (Sorgonà et al., 2006). Since the plant's capacity to respond to N fluctuation is associated with both leaf and root morphological changes (Ryser and Lambers, 1995; Walch-Liu *et al*., 2005, Tian *et al*., 2007), an integral analysis of these functional traits were carried out.

The leaf area and the stem height determine the amount of the light captured by plant which in turn, is traduced in "energy" and/or "structural biomass" conferring a different ability of species/genotypes in the nitrogen acquisition (Tian *et al*., 2007). In particular, leaf area, a functional trait associated with the plant capacity to acquire aboveground resources (Lambers and Poorter, 1992), was affected by N supply in both herbaceous (Trapani and Hall, 1996, Vos and van der Putten, 1998; Tian et al., 2007) and woody species (Cruz *et al*., 1997). According to these results, the data confirmed a positive and marked relationship between leaf area-nitrate supply in both citrus seedlings. However, the comparison of leaf area response to the nitrate provision between the citrus rootstocks, indicated that Sour Orange exhibited a greater leaf area than Sweet Orange, especially at 0.1 mM nitrate (+66%). These results indirectly suggested that SoO, producing more biomass by greater light interception, could better support the requirement for the nitrate acquisition than SwO. Furthermore, the light capture also depends by the plant height which is set by the stem height. Similarly but less evident to the leaf area, the stem height was increased by the increase of nitrate treatment and was higher in the Sour Orange compared to Sweet Orange.

The below-ground functional trait which best describes the capacity of the root system to explore the soil and to acquire soil resource, is the root length (Ryser, 1998). The root length is strictly associated with different plant's competitive ability for the nitrate acquisition (Hodge *et al*., 1999; Robinson *et al*., 1999) and its role in this process has been already reported in several species (Brady *et al*., 1995, Sattelmacher *et al*., 1993; Sullivan *et al*., 2000). The genetic variability of the root length in response to the nitrate availability was already demonstrated in citrus rootstocks comparing the slow-growing, Cleopatra Mandarin, with the fast-growing, Rough Lemon (Sorgonà *et al*., 2007) and the high-vigorous, Volkamer Lemon, with the less-vigorous, Carrizo Citrange (Sorgonà *et al*. 2005). The genetic differences of the root length between Sour Orange and Sweet Orange, defined as nitrate-use-efficient and –inefficient rootstocks, respectively, (Sorgonà *et al*., 2006) has been pointed out here. The root system of the SoO was characterized by higher total length, at both nitrate levels, confirming the important role of this functional trait for the nitrate use efficiency of citrus rootstock. Further, the SoO showed a double of tip number in its root system respect to the Sweet Orange. Considering that the apex of the root axes of citrus seedlings were more efficient to absorb the nitrate (Sorgonà *et al*., 2010) and the root systems with high tip numbers exhibited a high induction of the nitrate uptake in both herbaceous and woody species (Sorgonà and Cacco, 2002), the results suggested that the SoO root system was more competitive for the nitrate acquisition than that of SwO.

The root system of the higher plant comprises diverse root orders or classes which differently respond to environmental cues (Waisel and Eshel, 2002). Little information are available on the response of root orders to nitrate provision (Zhang *et al*., 1999; Linkhor *et al*., 2002; Sorgonà *et al*., 2005). Recent results on citrus seedlings indicated that 2nd order lateral roots were more sensitive to nitrate availability than tap and 1nd order lateral roots (Sorgonà *et al*., 2007). The data revealed that while the SwO showed a longer tap root, especially at low nitrate level, SoO exhibited a higher total length of 1st and 2nd order lateral roots. Since the 2nd-order laterals of the citrus species 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), this different behavior between citrus rootstocks could have a prominent adaptive significance conferring to SoO a more competitive capacity for nitrate acquisition, especially at scarce soil nitrate levels. Furthermore, the change in total length of the 2nd order lateral roots of SoO was due to an higher root number than average length. The increase of the number of the lateral roots was pointed out as fundamental functional trait for the competition for nitrate in nitrate-rich patchy soils (Hodge *et al*., 1999) and phosphorus ion (Zhu and Lynch, 2004).

The length and number of the diverse root orders produced different root architectures between citrus rootstocks. It is well known that differences on root system architecture can influence the competitive ability to acquire soil resources, the adaptive capacity on nutrient rich and poor soil environments (Fitter and Stickland, 1991), and, consequently, the productivity (Lynch, 1995). In particular, at low nitrate availability, the root system of the SwO was tap-rooted while that of the SoO was more fibrous, “optimal structure” to confer a potential competitiveness on nutrient limited environments. Indeed, simulation models predicted that the highly branched root architectures were efficient in the nitrate acquisition and could be considered as functional traits in the NUE of the higher plants (Dunbabin *et al*., 2003, 2004).

Both the above- and below-ground functional traits, i.e. leaf area, stem height and root length, depend on the dry mass partitioning in the diverse organs (allocation parameters) and, especially, how this biomass is efficiently used for constructing the structure devotes to capture the soil resources (cost parameters). Although the dry mass allocation towards the leaves was not different between the citrus rootstocks, the comparison of the leaf mass per area (LMA) indicated that SoO more efficiently used this biomass for constructing an unit of leaf area than SwO. In other words, SoO could be considered a low-LMA rootstock respect to SwO. The LMA was demonstrated to vary between and within the functional groups and, in particular, the low-LMA plant species showed fast resource acquisition and high rate of photosynthesis per unit leaf nitrogen (Poorter *et al*., 2009). This consideration could suggest that the low-LMA SoO could guarantee the carbohydrate supply more than SwO for sustaining energetically and structurally the root machinery for the nitrate acquisition. How this biomass was partitioning within the root system of the citrus rootstocks?

The within-root distribution of the biomass matched the length pattern of the different root orders of both citrus seedlings. Indeed, the dry mass was preferentially allocated towards the tap root and the 2nd order lateral roots in SwO and SoO, respectively, especially at low nitrate availability. This partitioning pattern of the biomass within the root systems was similar to that observed comparing the fast-growing Rough Lemon and the slow-growing rootstock Cleopatra Mandarin (Sorgonà *et al*., 2007) and the high-vigorous Volkamer Lemon and the low-vigorous rootstock Carrizo Citrange (Sorgonà *et al*., 2005). However, the costs for sustaining the increase of the total length of the 2nd order lateral roots in SoO were higher than SwO (lesser specific 2nd order lateral root length). These results indicated that SoO allocated more dry mass towards the 2nd order lateral roots but for a higher increase of the root number rather than the average length of this root order. These results suggested that the investment in the 2nd order lateral root represents the strategy utilized by citrus rootstocks for adapting to low nitrate availability

Conclusion:

The results of the present study indicated that the difference in nitrate-use-efficiency between the citrus rootstocks, especially at low nitrate availability, could be ascribed to the morphological variation of the functional traits of both the above- and below-ground parts. Indeed, SoO by an efficient use of the biomass allocated towards the leaves, exhibited a larger leaf area which could make available more carbon for the metabolic and structural costs of the root functions such as the nitrate acquisition. Furthermore, SoO showed a higher total length and tip numbers of the root system accompanied by higher total length of the 2nd order. This lateral rooting strategy could make the SoO more competitive for the nitrate acquisition respect to the SwO, especially at scarce soil nitrate levels. Furthermore, this study confirmed that in citrus seedlings 1) the 2nd order lateral are the root order more plastic in response to nitrate and responsible of the genetic difference in nitrate acquisition for citrus species and 2) both the dry mass allocation and the efficiency use of this biomass determine the above- and below-ground structure for the competition of the resource acquisition.

References

Bazzaz, F.A. and J. Grace, 1997. Plant resource allocation. Academic Press, San Diego, California, USA.

Beman, J.M., K. Arrigo and P.M. Matson, 2005. Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. Nature, 434: 211-214.

Brady, D.J., C.L. Wenzel, I.R.P. Fillery and P.J. Gregory, 1995. Root growth and nitrate uptake by wheat (*Triticum aestivum* L.) following wetting of dry surface soil. J. Exp. Bot., 46: 557-564.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharply and V.H. Smith, 1998. Nonpoint pollution of surface waters with phopshorus and nitrogen. Ecol. Applic., 8: 559-568.

Chilembwe E.H.C., W.S. Castle and D.J. Cantliffe, 1992. Grading, hydrating and osmotically priming seed of four Citrus rootstocks to increase germination rate and seedling uniformity. J. Am. Soc. Hort. Sci., 117: 368-372.

Cruz, C., S.H. Lips and M.A. Martins-Loução, 1997. Changes in the morphology of roots and leaves of carob seedlings induced by nitrogen source and atmospheric carbon dioxide. Ann. Bot., 80: 817-823.

Drew, M.C. and L.R. Saker, 1975. Nutrient supply and growth of seminal root system in barley. 2. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of root system. J. Exp. Bot., 26: 79-90.

Drew, M.C., 1975. Comparison of the effects of a localised supply of phosphate, nitrate, ammonium, potassium on the growth of the seminal root system, and the shoot, in barley. New Phytol., 75: 479–490.

Dunbabin, V., A. Diggle and Z. Rengel, 2003. Is there an optimal root architecture for nitrate capture in leaching environments?. Plant, Cell and Environment, 26: 835-844

Dunbabin, V., Z. Rengel and A. Diggle, 2004. Simulating form and function of root systems: efficiency of nitrate uptake is dependent on root system architecture and the spatial and temporal variability of nitrate supply. Functional Ecology, 18: 204-211.

Eissenstat, D.M. and D.S. Achor, 1999. Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. New Phytol., 141: 309-321.

Eissenstat, D.M., 1991. On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstock. New Phytol., 18: 63-68.

Fitter, A.H. and T.R. Stickland, 1991. Architectural analysis of plant root systems 2. Influence of nutrient supply on architecture in contrasting plant species. New Phytol., 118: 383-389.

Hirsch, R. and M. Sussman, 1999. Improving nutrient capture from soil by genetic manipulation of crop plants. Trends Biotechnol., 17(9): 356-361.

Hoagland, D.R. and D.I. Arnon, 1950. The water-culture method for growing plants without soil. California Agriculture Experimental Station Circular, No., 347: 32.

Hodge, A., D. Robinson, B.S. Griffiths and A.H. Fitter, 1999. Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. Plant, Cell and Environment, 22: 811-820.

Zhu, J. and J.P. Lynch, 2004. The contribution of lateral rooting for phosphorus acquisition efficiency in maize seedlings. Functional Plant Biology, 31: 949-958.

Lambers, H. and H. Poorter, 1992. Inherent variation in growth rate between higher plants: A search for physiological causes and ecological consequences. Adv. Ecol. Res., 23: 187-261.

Liao, M.T., I.R.P. Fillery and J.A. Palta, 2004. Early vigorous growth is a major factor influencing nitrogen uptake in wheat. Functional Plant Biology, 31: 121-129.

Liao, M.T., J.A. Palta and I.R.P. Fillery, 2006. Root characteristics of vigorous wheat improve early nitrogen uptake. Australian J. Agric. Research, 57: 1097-1107.

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

London, J.G., 2005. Nitrogen study fertilizes fears of pollution. Nature, 433: 791.

Lynch, J.P., 1995. Root architecture and plant productivity. Plant Physiol., 109: 7-13.

Lynch, J.P., 1998. The role of nutrient-efficient crops in modern agriculture. J. Crop Prod., 1: 241-264.

Peterson, C.A. and D.E. Enstone, 1996. Functions of passage cells in the endodermis and exodermis of roots. Physiol. Plant., 97: 592-598.

Poorter, H., U. Niinemets, L. Poorter, I.J. Wright and R. Vilar, 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol., 182: 565-588.

Raun, W. R. and G. V. Johnson, 1999. Improving nitrogen use efficiency for cereal production. Agronomy Journal, 91: 357-363.

Reich, P.B., M.B. Walters, M.G. Tjolker, D.W. Vanderklein and C. Buschena, 1998. Photosynthesis and respiration rates depend on leaf and root moprhology and nitrogen concentration in nine boreal tree species differing in relative growth rate. Functional Ecology, 12: 395-405.

Robinson, D., A. Hodge, B. S. Griffiths and A. H. Fitter, 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. Proc. R. Soc. London B, 266: 431-435.

Roumet, C., C. Urcelay and S. Diaz, 2006**.** Suites of root traits differ between annual and perennial species growing in the field. New Phytol., 170: 357-368.

Rufty, T.M., S.C. Huber and R.J. Volk, 1988. Alterations in Leaf Carbohydrate Metabolism in Response to Nitrogen Stress. Plant Physiol., 88:725-730.

Ryser, P., 1998. Intra- and interspecific variation in root length, root turnover and the underlying parameters. In Variation in plant growth, Eds., Lambers, H., H. Poorter and M.M.I. Van Vuuren. Backhuys Publishers, Leiden, pp: 441-465.

Ryser, P. and H. Lambers, 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. Plant Soil, 170: 251-265.

Sattelmacher, B., J. Gerendas, K. Thoms, H. Bruck and N.H. Bagdady, 1993. Interaction between root growth and mineral nutrition. Environ. Exp. Bot., 33: 63-73.

Sorgonà, A., M. R. Abenavoli, P.G. Gringeri and G. Cacco, 2007. Comparing morphological plasticity of root orders in slow- and fast-growing citrus rootstocks supplied with different nitrate levels. Ann. Bot., 100: 1287-1296.

Sorgonà, A., M.R. Abenavoli and G. Cacco, 2005. A comparative study between two citrus rootstocks: effect of nitrate on the root morpho-topology and net nitrate uptake. Plant Soil, 270: 257-267.

Sorgonà, A., M.R. Abenavoli, P.G. Gringeri and G. Cacco, 2006. A comparison of nitrogen use efficiency definitions in Citrus rootstocks. Scientia Horticulturae, 109: 389-393.

Sorgonà, A. and G. Cacco, 2002. Linking the physiological parameters of nitrate uptake with root morphology and topology in wheat (*Triticum durum* Desf.) and in citrus rootstock (*Citrus volkameriana* Ten & Pasq). Can. J. Bot., 80: 494-503.

Sorgonà, A., G. Cacco, L. Di Dio, W. Schmidt, P.J. Perry and M.R. Abenavoli, 2010. Spatial and temporal patterns of net nitrate uptake regulation and kinetics along the tap root of *Citrus aurantium*. Acta Physiol. Plant., 32: 683-693.

Sullivan, W.M., Z. Jiang and R.J. Hull, 2000. Root morphology and its relationship with nitrate uptake in kentucky bluegrass. Crop Sci., 40: 765-772.

Tian, Q., F. Chen, F. Zhang and G. Mi, 2007. Genotypic difference in nitrogen acquisition ability in maize plants is related to the coordination of leaf and root growth. J. Plant Nutrition, 29(2): 317-330.

Trápani, and A.J. Hall, 1996. Effects of leaf position and nitrogen supply on the expansion of leaves of field grown sunflower (*Helianthus annuus* L.). Plant Soil, 184: 331-340.

Vos, J. and P.E.L. van der Putten, 1998. Effect of nitrogen supply on leaf growth, leaf nitrogen economy and photosynthetic capacity in potato. Field Crops Research, 59(1): 63-72.

Waisel, Y. and A. Eshel, 2002. Functional diversity of various constituents of a single root system. In Plant roots: the hidden half, Eds., Waisel, Y, A. Eshel and U. Kafkafi. New York, NY: Marcel Dekker, pp: 157-174.

Walch-Liu, P., S. Filleur, Y. Gan amd B.G. Forde, 2005. Signaling mechanisms integrating root and shoot responses to changes in the nitrogen supply. Photosynth Res., 83: 239-250.

Wiesler, F. and W.J. Horst, 1993. Differences among maize cultivars in the utilization of soil nitrate and the related losses of nitrate through leaching. Plant and Soil., 151: 193-203.

Wiesler, F. and W.J. Horst, 1994. Root growth and nitrate utilization of maize cultivars under field conditions. Plant and Soil., 163: 267-277.

Zhang, H., A. Jennings, P.W. Barlow and B.G. Forde, 1999. Dual pathways for regulation of root branching by nitrate. Proc. Nat. Acad. Sci. USA, 96: 6529-6534.

Zobel, R.W, 1995. Genetic and environmental aspects of roots and seedling stress. HortScience, 30: 1189-1192.

Zhu, J. and J.P. Lynch, 2004. The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays* L.) seedlings. Functional Plant Biology, 31: 949-958.