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Abstract

Nitrogen (N) is the most important nutrient for growth, fruit yield, and quality of citrus plants. In order to reduce both the requirements for costly nitrogen fertilizers and environmental pollution of soil and water, the improvement of the nitrogen use efficiency (NUE) on citrus plants is fundamental in sustainable agriculture. In this chapter, a critical overview on the definitions of NUE and its components, nitrogen uptake (NUpE), and nitrogen utilization efficiency (NUtE) was provided, together with current knowledge and future challenges to understand and manipulate NUE in citrus plants. Further, the different N fertilizer use strategy in combination with irrigation to increase the NUE in citrus species was explained. The nitrogen content, the removal and the partitioning among the citrus organs, and the N availability in citrus soils provided a comprehensive picture of the N economy in citrus trees and soil orchards, and the basis of the NUE. However, an important approach for improving the NUE in citrus plants was to understand the regulation of the morpho-physiological and molecular mechanisms controlling plant nitrogen economy such as nitrogen uptake, translocation, assimilation, and remobilization. This approach accompanied by new techniques in molecular biology, root biology, plant-soil interactions, and modeling will provide an accurate criteria to discriminate between the nitrogen-efficient and inefficient citrus plants. Finally, the future challenges for improving NUE in citrus species considering both the “agronomic” and “physiological” approaches were discussed.

Keywords

Nitrogen uptake efficiency • Nitrogen utilization efficiency • Rootstock • Root architecture • Root morphology • Nitrate uptake transport system • Ammonium uptake transport system

16.1 Introduction

Nitrogen (N) is one of the most important nutrients for plant growth and development, and it is considered to be a major yield-limiting factor for many crops. As a constituent of

proteins, nucleic acids, and secondary products, N consists of 1.50–6.00% of the dry weight of many crops (Benton 1998). Higher plants acquire N from the soil mainly in the mineral forms such as ammonium (NH_4^+) and nitrate (NO_3^-) and also in the organic form such as urea and amino acids. Because of the essential role of nitrogen in crop production, over the past six decades, high N fertilizer dose was applied allowing to double the agricultural food production worldwide to meet a growing population but, on the other hand, causing many environmental problems. Indeed, the intensive use of N fertilizers in agriculture (ninefold increase) due mainly to a low price of N fertilizers, progressively caused a major detrimental

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impact on the biosphere such as eutrophication of freshwater (London 2005) and marine ecosystems (Beman et al. 2005) and an increase of the N oxides and toxic ammonia into the atmosphere (Ramos 1996; Stulen et al. 1998). Therefore, the challenge for the next years will be to develop a highly productive agriculture which preserves the quality of the environment and reduce the human risk development, thus an “eco-efficient agriculture.” As reported by Keating et al. (2010), eco-efficient agriculture consists “more agricultural output, in terms of quantity and quality, for less input of land, water, nutrients, energy, labor or capital.” This concept translates to the nitrogen means “a better use of the nitrogen for the crop growth and yield” or the “nitrogen use efficiency” (NUE).

In this chapter, we reported some aspects controlling the NUE in the citrus plants, the most economically important evergreen fruit crop in the world. Since nitrogen is the most important nutrient for citrus cultivation, fruit yield, and quality (Alva and Tucker 1999; Dasberg et al. 1984; Embleton and Jones 1978; Tucker et al. 1995), generally, the citrus farmer applied a heavy N fertilization which, combined with a not suitable N management practices, caused a severe groundwater contamination mainly attributed to NO_3^- leaching (Alva and Tucker 1999; Davies 1996; Embleton and Jones 1978; Embleton et al. 1986; Ramos et al. 2002). This has become a major environmental problem in Florida and Spain citrus production regions (Lamb et al. 1999; Fernández et al. 1998). Further, an application of N fertilization in excess in citrus production caused an increase of soil acidification (He et al. 1999; Cantarella et al. 2003) and ammonia volatilization (Cantarella et al. 2003) and a reduction of fruit quality (Legaz and Primo-Millo 1988).

In particular, in the first paragraph, the N partitioning among the plant organs of the seedlings and mature trees, bearing and no-bearing trees, and the N distribution between the plant and nursery and orchards soils were reported. Successively, starting from a general definition of NUE, a specific NUE definition together with data from experimental studies for citrus plants was provided. Further, other definitions such as “nitrogen uptake efficiency” (NUpE), “nitrogen utilization efficiency” (NUtE), “fertilizer use efficiency” (FUE), and “fertilizer N recovery” (FNR) were also reported. The fourth paragraph detailed on the NUE improvement in the citrus plants with particular focus on the growth and production responses to the rate, type, time, and frequency of fertilizer N application, soil type, and their interactions with irrigation management. Then, the nitrogen-efficient and -inefficient citrus rootstocks and the criteria adopted to facilitate the screening of citrus genotypes for improved efficiency were detailed. Further, the morphophysiological basis for improving the NUE such as the nitrate and ammonium transport systems, kinetic and energetics, and root morphological and architectural traits were analyzed.

16.2 Nitrogen Status of the Citrus Plant and Soil Nursery and Orchards

Nitrogen is an essential nutrient to sustain high growth in young citrus tree and plant vigor, fruit yield, and quality, in mature ones.

Nitrate and ammonium are the main source of nitrogen for citrus species, showing a seasonal uptake peaking during the periods of active shoot growth (Maust and Williamson 1994; Weinbaum et al. 1984). Generally, after absorption, the nitrate is translocated to the shoot in inorganic form, while ammonium was firstly reduced to amino acids, mainly glutamate, in roots and then translocated to the aboveground (Kato 1981, 1986). The N acquisition is needed to recover the N lost in harvested fruits, abscised fruitlets and flowers, senescent leaves, pruning wood, and root turnover. Further, it is necessary to replenish the N reserves used for ensuring the formation of new developing organs during the early stage of vegetative growth. For example, Chapman (1968) reported that 40 tons of orange discharged 47.2 kg of N, and Alva et al. (1998) pointed out a removal of 52.8, 56.2, 66.8, and 67.4 lb of N in 500 boxes of fruits (fresh wt basis) of Hamlin, Parson Brown, Valencia, and Sunburst orange varieties, respectively. Therefore, the citrus plants exhibited a different N status and partitioning among the different organs during the annual cycle. Table 16.1 (modified from Legaz et al. 1995) reported the N content (% with respect to the total tree) of the different organs in 3-year-old Valencia Late orange tree grafted on Troyer citrange during an annual cycle. In particular, Legaz et al. (1995) underlined the greatest N content in leaves (33.2–41.4%) and, at lesser extent, in roots (30.5–37.2%), considering the main organs of N reserves in citrus plants.

For a long time, the leaf tissue analysis was a useful tool to evaluate citrus N status by comparing the actual leaf N concentration with the critical one established from previous studies (Jones and Embleton 1969; Malavolta 1992; Terblanche and Du Plessis 1992; Hanlon et al. 1995; Quaggio et al. 1998; Kohli et al. 1998). The optimum level of N leaf content was between 25 and 27 g kg^{-1} for orange (Koo et al. 1984; Alva et al. 2006), 22–23 g kg^{-1} for grapefruit (He et al. 2003), and 27–29 g kg^{-1} for Clementine mandarin trees (Hammami et al. 2010). The N leaf removal from the critical leaf concentration standards caused a nitrogen deficiency or excess status in the citrus plants.

The N-deficient citrus trees exhibited the following characteristics: (1) slow, weak and stunted growth, and irregular flushes; (2) decrease and sparse in flowering and fruiting; and (3) yellowing of the foliage, the most important visual symptom of the N starvation (Spiegel-Roy and Goldschmidt 1996; Davies and Albrigo 1994; Zekri and Obreza 2003). The chlorosis firstly appeared on the older leaves which became completely pale, while the new leaves are small,

Table 16.1 N content (% with respect to the total tree) of diverse organs of Valencia Late orange tree (3 years old) grafted on Troyer citrange at different growth stages

	Dormancy (%)	Flowering (%)	Fruit set (%)	Midsummer flush (%)	Early autumn flush (%)
Reproductive organs	0.0	4.2	3.0	2.6	6.1
Leaves	39.0	33.2	36.9	39.3	41.4
Twigs	0.0	2.1	2.4	2.8	3.4
Old branches + trunk	24.0	23.3	21.9	21.6	18.6
Roots	37.0	37.2	35.8	33.7	30.5

Modified from Legaz et al. (1995)

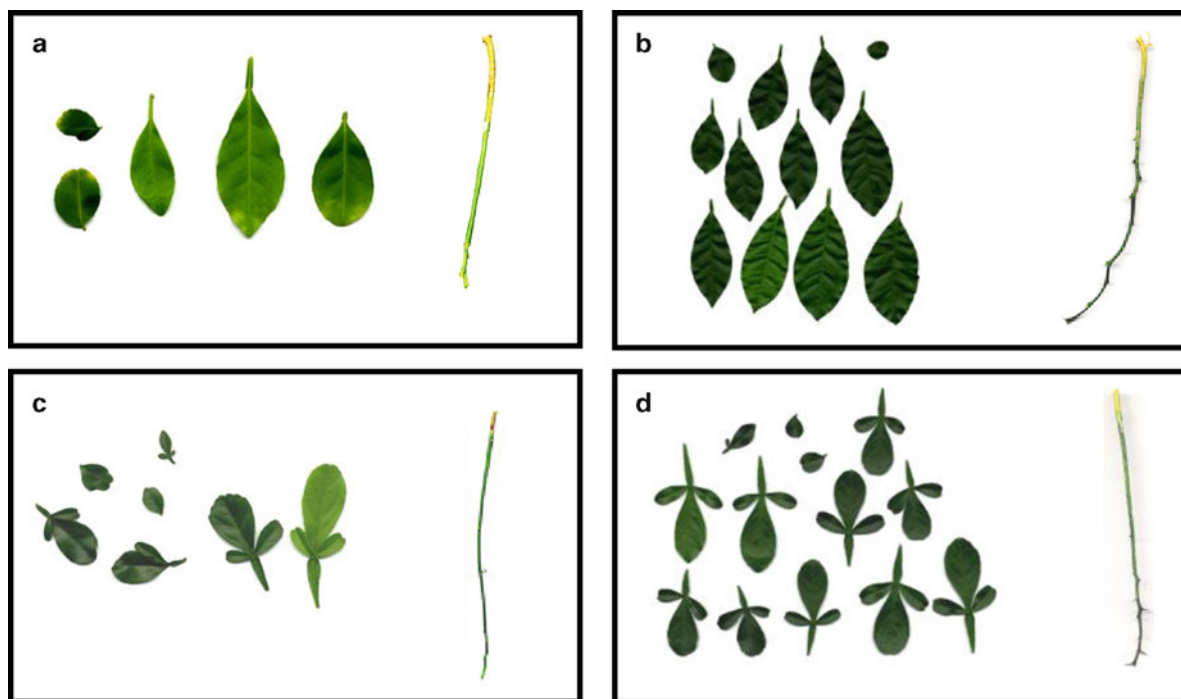


Fig. 16.1 Leaves and stems of Volkamer lemon (a and b) and Carrizo citrange (c and d), 120 days old, grown with two different nitrate concentrations: 5 μM (a and c) and 1,000 μM (b and d). To note (1) the

yellowing of the old leaves in (a) and (c) and (2) the higher number of leaves in (b) and (d)

thin, and fragile. The leaves and stems of Volkamer lemon and Carrizo citrange seedlings, 120 days old, grown at two different nitrate concentrations (5 and 1,000 μM) are further shown (Fig. 16.1) (Sorgonà, unpublished data). Both citrus rootstocks, at low nitrate levels, exhibited a reduced number of leaves whose older leaves showed yellowing. Further, the stems of the N-starved citrus rootstocks appeared also chlorotic and lesser taller than those grown with 1,000 μM nitrate (Sorgonà, unpublished data).

The N excess also hampered the growth and production of citrus plants. Nitrogen fertilization over the recommendation rates such as 225 $\text{kg ha}^{-1} \text{ year}^{-1}$ for young orange trees and 280 $\text{kg ha}^{-1} \text{ year}^{-1}$ for mature trees (Obreza and Morgan 2008) caused a reduction of fruit yield and quality. Schumann

et al. (2003) observed that the high rate of N fertilizer (240 $\text{kg ha}^{-1} \text{ year}^{-1}$) produced a reduction of fruit number and yield, juice and soluble yield, and fruit mass of Hamlin orange trees. Further, Koo (1988) showed that the increase of N fertilization rates caused a reduction of fruit size, weight, and peel thickness of orange fruits. Recently, Hammami et al. (2010) demonstrated that N rates in excess of 192 $\text{kg ha}^{-1} \text{ year}^{-1}$ caused fruit yield reduction in Clementine mandarin.

The main cause of N deficiency or excess in citrus trees depended on the N availability in the soil. Low N availability in both citrus orchards and nursery resulted from N losses mainly due to nitrate leaching and ammonium volatilization. Syvertsen and Smith (1996) reported that N losses from 4-year-old grapefruit trees grown in lysimeters averaged

11% and 20% for Volkameriana lemon and Sour orange rootstocks, respectively. Lea-Cox and Syvertsen (1996) observed nitrate leaching between 10% and 40% in Sour orange and Volkamer lemon seedlings (22 weeks old), grown in pot systems. Further, Lea-Cox et al. (2001) observed that the <30% and >50% of the total N applied were leached as ^{15}N nitrate at soil layers below 30 cm in Redblush grapefruit (4 years old) grafted on Volkamer lemon and Sour orange, respectively, grown for 29 days in tanks. Finally, in Valencia sweet orange (6 years old) orchard, the N inorganic content in the 20–60-cm soil depth layer accounted for up to 56 kg ha⁻¹ (average of 4 years of fertilizer application) with annual N application rate of 260 kg ha⁻¹ year⁻¹ (Cantarella et al. 2003). Beside the leaching of nitrate, the volatilization of ammonium is a component of N losses from the soil. Indeed, NH_4^+ volatilization, between 26% and 44% was observed after the urea fertilizer application in field cultivated with Valencia sweet orange (10 years old) (Cantarella et al. 2003).

How the N status of the citrus plants and citrus soil in orchards and nursery is modified by different biotic and abiotic factors such as rootstocks, irrigation, doses and timing of N fertilizer application, and irrigation will be discussed later.

16.3 Nitrogen Use Efficiency Definitions

The word “efficiency” generally indicates “the level of output per unit of input.” Plant system “efficiency” defines the “growth, physiological activity, yield, or harvested yield (output) per unit of land, water, nutrient, or energy (input).” While focusing the attention on the specific nutrient such as nitrogen, the term “nitrogen use efficiency” defines as “the plant growth, physiological activity, yield or harvested yield per unit of nitrogen.” Even within this simple ratio, “nitrogen use efficiency” has been defined in many ways in diverse context (Clark 1990; Blair 1993) which are grouped in “agronomic” and “physiological” terms. With regard to agronomic terms, the NUE definition emphasized the productivity including “the plant biomass or yield or harvested yield per unit of available N in the soil” (together with the N residual present in the soil other than that applied by fertilizer) (Caradus 1990; Moll et al. 1987; Saric 1982; Saurbeck and Helal 1990) or “the plant biomass or yield or harvested yield per unit of nitrogen applied” (only fertilizer applied) (Balingar et al. 1990; Blair 1993; Thung 1988) (g of plant dry weight per mg of nitrogen or kg yield per kg fertilizer). With regard to the internal nutrient plant requirement, the NUE definition fell into the physiological group being defined as “plant biomass produced per unit nitrogen absorbed” (Balingar et al. 1990; Gerloff and Gabelman 1983; Glass 1989) or “amount of harvestable product per unit of N absorbed” (Moll et al. 1987) (g plant dry weight per mg of nitrogen or kg of yield per g of nitrogen).

Summarized experimental data of different citrus NUE pointing out their wide variability are shown (Table 16.2) because of genotypes, age, season, scion/rootstock, and experimental setup. In spite of this wide NUE variability, a common consideration should be done: the NUE increased with the plant aging. Indeed, the NUE (g plant DW g⁻¹ N applied) of orange trees budded on Carrizo citrange were 5.25, 24.1, and 54.9 after the first, second, and third years, respectively (values extrapolated from Menino et al. 2007). In terms of fruit yield per fertilizer applied, the NUE showed a temporal pattern: the young (3–5 years old) trees of Ambersweet orange on Swingle citrumelo exhibited an average of 101.3 kg fruit yield per kg⁻¹ fertilizer, while mature trees (8–10 years old) reached 382.5 kg fruit yield per kg⁻¹ (extrapolated data from Morgan et al. 2009). Finally, similar results were obtained from Davies and Zalman (2002) in the Rohde Red Valencia orange grafted on different citrus rootstocks.

Nitrogen use efficiency is however a complex trait that according to Moll et al. (1982) can be dissected into “nitrogen uptake efficiency” (NUpE) and “nitrogen utilization efficiency” (NUtE) (Table 16.2). The NUpE referred to the ability of the plant to remove N from soil, and it was defined as “the nitrogen absorbed in the plant or in the yield per unit of N supplied or applied” (Maust and Williamson 1994). Generally, the NUpE was expressed as mg N per g N applied but, for a better comparison, was also reported as % of nitrogen respect to the N applied by fertilizer which was also termed as “fertilizer N recovery” (FNR) or “fertilizer use efficiency” (FUE). Syvertsen and Smith (1996) estimated the FNR of Redblush grapefruit grafted on Volkamer lemon and Sour orange to be 61% and 52%, respectively, averaged over the 2-year period and N rates. Furthermore, Scholberg et al. (2002) pointed out that the NUpE values in citrus rootstocks (Swingle citrumelo and Volkamer lemon, 10 weeks old), calculated by the difference of the N leaching losses between tree tank and no tree tank, ranged between 16.6% and 83.1% in relation to N rates and N residence times. However, this technique did not consider the N losses due to volatilization or immobilization from the soil (no tree tank). Conversely, the advantage of ^{15}N -labeled fertilizer technique was the ready N identification and estimation which improved the estimation of the nitrogen uptake efficiency in citrus plants. Wallace (1953) firstly used this technique in citrus plants, and afterward several authors benefited from the ^{15}N tracer for estimating the N uptake and remobilization. By this technique, Quiñones et al. (2007) reported that the NUpE values for Navelina orange (8 years old) on Carrizo citrange, in lysimeters, ranged between 62.7% and 75.1%. More recently, Boaretto et al. (2010) estimated the NUpE averaged in 36% and 52% for orange and lemon trees (3 years old), respectively, both grafted on Swingle citrumelo. Similar values ranging between 25% and 80% were obtained not lately in mature trees grown in the field (Dasberg et al. 1984;

Table 16.2 An update of nitrogen use efficiency (NUE), nitrogen uptake efficiency (NUpE), and nitrogen utilization efficiency (NUtE) values reported or extrapolated from literature for the citrus plants

Citrus plants (rootstocks or scion/rootstocks)	Age (year old)	Experimental treatments	References	NUE (g plant dry weight g ⁻¹ N applied)	NUpE (% of N absorbed with respect to the N applied)	NUtE (g plant dry weight mg ⁻¹ N absorbed)
Hamlin orange/ Swingle citrumelo	6	Fertilizer type	Mattos et al. (2003)	81.6–108.8 ^a	25.5–39.4	0.276–0.320 ^a
Valencia orange/ Swingle citrumelo; Lisbon lemon/ Swingle citrumelo	3	Species N application	Boaretto et al. (2010)	28.4–40.6 ^a	29.2–53.8	0.079–0.078 ^a
Newhall navel orange/Carrizo citrange	1–2	N rates Fertigation frequency	Weinert et al. (2002)	–	2.2–5.3 10.9–23.5	–
Rohde Red Valencia orange/ Swingle citrumelo	3	Rootstocks	Davies and Zalman (2002)	5.8–327.7 ^b	–	–
Rohde Red Valencia orange/ Carrizo citrange	4	Age				
Rohde Red Valencia orange/ <i>Citrus volkameriana</i>	5	N rates				
Hamlin orange/ Cleopatra mandarin	25	N application	Alva et al. (2006)	283.9–681.2 ^{b,c}	–	–
Clementine mandarin/ Sour orange	25	Different NK ratio	Hammami et al. (2010)	147.0–235.6 ^{b,c}	–	–
Swingle citrumelo	30 weeks old	Type of fertilizers	Dou and Alva (1998)	6.9–16.7 ^a	–	–
Cleopatra mandarin						
Swingle citrumelo	5 months old	Fertigation frequency	Melgar et al. (2010)	26.1–90.0	–	–
Pera sweet orange/ Rangpur lime	4	–	Boaretto et al. (2006)	76.2 ^a	20–27	–
Swingle citrumelo	8 months old	Type of amendments	Syvertsen and Dunlop (2004)	51.9 ^a –65.1 ^a	55–81	–
Valencia orange/ Rough lemon	36	Type of fertilizers	Alva et al. (1998)	252.8–267.8 ^{a,b,c}	–	–
Cleopatra mandarin	16 weeks old	Salinity stress	Lea-Cox and Syvertsen (1993)	–	13.8–14.4	27.2–32.4 ^a
Volkamer lemon						
Hamlin orange/ Swingle citrumelo	4–5	Fertigation frequency and wetting pattern	Syvertsen and Sax (1999)	53.0–114.5 ^{a,b}	12–44 ^{a,d}	–
Hamlin orange/ Swingle citrumelo	6–7	Fertigation frequency	Syvertsen and Jifon (2001)	32.1–79.6 ^{a,b}	24.1–41.5 ^d	–
Redblush grapefruit/ Volkamer lemon	4	Rootstocks	Syvertsen and Smith (1996)	–	18–83 ^d	–
Redblush grapefruit/ Sour orange		N rates				
Navelina/Carrizo citrange	8	N application	Quiñones et al. (2007)	–	62.7–75.1 ^e	–

(continued)

Table 16.2 (continued)

Citrus plants (rootstocks or scion/rootstocks)	Age (year old)	Experimental treatments	References	NUE (g plant dry weight g ⁻¹ N applied)	NUpE (% of N absorbed with respect to the N applied)	NUtE (g plant dry weight mg ⁻¹ N absorbed)
Navelina/Carrizo citrange	8	N application	Quiñones et al. (2003)	274.9–328.2 ^a	64–75 ^c	6.13–6.25 ^{a,e}
Cleopatra mandarin	14–16 weeks old	Rootstocks	Lea-Cox and Syvertsen (1996)	40–100	26.8–60.0 ^f	–
Swingle citrumelo		N rates				
Sour orange						
Volkamer lemon						
Orange/Carrizo citrange	2	Age	Menino et al. (2007)	5.2–54.9 ^b	6–30 ^f	0.087–0.183 ^{a,c}
Redblush grapefruit/ Sour orange	4	Rootstocks N rates	Lea-Cox et al. (2001)	24–155 ^a	14.9–42.2 ^e	–
Redblush grapefruit/ Volkamer lemon						
Swingle citrumelo	10 weeks old	Rootstocks	Scholberg et al. (2002)	–	16.6–83.1 ^d	–
Volkamer lemon		N rates				
		Time of residence of N				
Rough lemon	132 days old	Rootstocks	Sorgonà et al. (2006)	–	24–80 ^f	0.04–0.09
Sweet orange						
Cleopatra mandarin						
Sour orange						

^aExtrapolated from experimental data

^bNUE in terms of fruit yield: kg fruit yield kg⁻¹ fertilizer

^cReferred to ha of soil

^dThe NUpE was calculated as the difference between the N leached from tank without tree with that in presence of tree

^eNUpE measured by ¹⁵N technique

^fNUpE calculated as total nitrogen accumulation divided by root dry weight

Feigenbaum et al. 1987; Weinbaum and Van Kessel 1998) and citrus rootstock seedlings (Lea-Cox and Syvertsen 1996). The young nonbearing citrus plant, conversely, exhibited lower values of FUE by <5% (Weinert et al. 2002) and <6% (Menino et al. 2007) in the first year after transplantation, while Lea-Cox et al. (2001) reported FNR values ranging from 14.9% to 39.3% in bearing Redblush grapefruit, 4 years old. Probably, these contrasting results could be due to the stronger influence of the fruit loading as sink on the nitrogen uptake. The NUpE was also defined as total nitrogen accumulation per unit of root dry weight (Elliot and Laüchli 1985). In this respect, Sorgonà et al. (2006) showed the NUpE values ranging from 24 to 80 mg N accumulated per g^{-1} root dry weight in different citrus rootstocks grown in pot at different nitrate levels.

The term “nitrogen utilization efficiency” or “NUE” indicates “the ability of the plant to use N to produce biomass or yield or harvested yield.” Often called the “nutrient efficiency ratio,” it was evaluated by the total plant dry weight divided by nitrogen absorbed (g plant dry weight or kg yield per mg N absorbed) (Balingar et al. 1990; Gerloff and Gabelman 1983; Glass 1989; Moll et al. 1987). Lea-Cox and Syvertsen (1993) observed that the young Cleopatra mandarin and Volkamer lemon (16 weeks old) exhibited greater NUtE values by 32.4 and 27.2 g dry weight per mg N absorbed, respectively, than more mature (9 years old) and grafted citrus plants (Navelina orange on Carrizo citrange), whose NUtE ranged between 6.13 and 6.25 g dry weight per mg N absorbed (Quiñones et al. 2003).

The NUE, NUpE, and NUtE definitions have been also referred to the different plant organs providing a “partitioning of nitrogen efficiency” within the citrus plants. The N recovery efficiency measurement in the different plant organs aimed at estimating and monitoring the fate and transformations of N applied through the ^{15}N -enriched fertilizer in the soil-plant citrus system. In particular, the studies were focused on (1) the identification of plant organ where the ^{15}N fertilizer was mostly allocated, (2) the recognition of plant organ with the stronger N demand (sink organs), and (3) the influence of N rate and seasonal application, fertilizer types, rootstocks, and plant age on ^{15}N distribution patterns among the plant organs. For example, the N provision from March to June generally caused a preferential N allocation in young organs (Kubota et al. 1976; Akao et al. 1978; Lea-Cox et al. 2001; Martínez et al. 2002; Quiñones et al. 2005, 2007), while a delay of the N application time, during late fall or winter, determined a higher N recovery in the old tissues (Legaz et al. 1983; Quiñones et al. 2005, 2007). The higher N recovery in young tissue than older ones in the orange trees on Carrizo citrange did not vary with increase of the plant age (Menino et al. 2007).

16.4 Improving the NUE in the Citrus

In the past 25 years, the experimental studies for improving NUE and/or NUtE, maintaining optimal citrus fruit yield and quality and minimizing nitrate leaching below the root zone, have been focused on two main aspects: (1) to develop the optimal “N and irrigation best management practices,” i.e., the influence of the rate, type, time, and frequency of application of N fertilizer, soil type and their interactions with irrigation; (2) to provide information on the morphological, physiological, and molecular mechanisms that defined NUE-related traits which are associated with N-efficient citrus rootstocks.

16.4.1 Best Management Practices

A substantial work was carried out to define several strategies of a rational N fertilization in citrus trees aimed at maximizing the NUE or NUpE and, in parallel, reducing the N leaching losses in the soil.

An important first result was that the N status of the citrus trees affected both the NUE and the NUpE, i.e., the efficiency with which nitrogen was absorbed by its root system. Indeed, a negative correlation between total N plant content and the NUE (g DW g^{-1} N applied) in different citrus rootstocks (Sour orange and Volkamer lemon), grown at different N rates, was observed by Lea-Cox and Syvertsen (1996). Similar behavior was subsequently confirmed by Lea-Cox et al. (2001) with the same citrus rootstocks grafted with Redblush grapefruit. Furthermore, the N-starved conditions were positively correlated with NUpE which was higher than that of the N-sufficient trees. In fact, Dasberg (1987) showed that the N-deficient citrus trees exhibited a 57% NUpE which instead reached only a 40% value in trees grown at high N levels. This NUpE response to the N status of mature citrus trees was also evident in citrus rootstock seedlings. Indeed, at N rates of 18, 53, and 105 mg week^{-1} , Sour orange and Volkamer lemon (14 weeks old) exhibited 51%, 47% and 27%, and 50%, 49% and 32% of NUpE, respectively (Lea-Cox and Syvertsen 1996). These results suggested that the N uptake interacted with the N reserves to meet the N requirements for the growth and yield of the citrus plants.

A second result was that in relation to the different forms of N fertilizers applied to the soils, a variability in the NUE- or NUpE-related citrus responses was observed. Mattos et al. (2003) showed a higher NUpE in Hamlin orange (6 years old) supplied with the ammonium nitrate (39.4%) than the urea fertilizer (25.5%). An improvement of citrus NUE by fertigation management with respect to dry granular fertilizer

application was also reported by Dasberg et al. (1988), Alva and Paramasivam (1998), Alva et al. (1998) and Quiñones et al. (2005). Further, Alva et al. (2006) demonstrated that NUE, expressed as an increment in fruit yield (kg fruit kg⁻¹ N applied) of the Hamlin orange tree grafted on Cleopatra mandarin, was greater with the N applied as fertigation or water-soluble granules than with a mix of these fertilizers. Although, several authors indicated that the controlled-release N fertilizers (CRF) (both resin- and sulfur-coated N organic and inorganic forms) enhanced the growth and yield of citrus trees compared to that dry and soluble N fertilizers (Koo 1986; Dou and Alva 1998; Schumann et al. 2003; Morgan et al. 2009). However, the effects of CRF on the NUE are still lacking. Some studies reported a reduction of N losses in the soils with the CRFs fertilizers application, suggesting an indirect effect to improve the N uptake efficiency (Koo 1986; Alva and Tucker 1993; Dou and Alva 1998).

Finally, a third important result was to develop an optimal combination of irrigation and N management to improve N uptake efficiency of citrus trees. Drip irrigation determined a higher fertilizer N recovery (75.1%) of Navelina orange grafted onto Carrizo citrange with respect to flooding irrigation (62.7%) (Quiñones et al. 2007). A moderate irrigation rate increased the yield of young and mature Ambersweet orange trees with respect to lower one (Morgan et al. 2009). By fertigation, it was also possible to manage the frequency of N application which in turn positively influenced the NUE and NUpE. Quiñones et al. (2003, 2005, 2007) observed a higher NUE with 66 split application by drip irrigation with respect to five applications by flood irrigation in Navelina orange trees. Similar results were pointed out by Scholberg et al. (2002), Alva et al. (2006), Boman (1996), and Morgan et al. (2009), although several authors pointed that citrus rootstock seedlings (Melgar et al. 2010) and mature trees (Syvertsen and Jifon 2001; Weinert et al. 2002) showed no significant relationship between N application frequency by fertigation and NUE and NUpE.

16.4.2 Nitrogen-Efficient and -Inefficient Citrus Rootstocks: Root Morphology and Nitrogen Uptake Mechanisms

In its last review on citrus rootstocks, Castle (2010) argued that "...citrus rootstocks bring many advantages and profitability to commercial enterprise... citrus rootstocks are the sole determining element that allows citrus to be grown in particular circumstances," and he concluded "...as the knowledge base increases, perhaps new rootstocks designed in response to particular concerns could more readily be produced..." Synthesizing Castle's opinion and correlating them with the NUE in citrus species, we may assert that (1) the rootstocks are the main subject for enhancing the

fitness of citrus plants to different N soil availabilities and (2) the morpho-physiological and molecular mechanisms of rootstocks are essential for the improvement of NUE in citrus plants.

The genotypic variability of citrus rootstocks, collected by Wutscher (1989), induced a different leaf nitrogen content on scion component. Wutscher also grouped the rootstocks in high- and low-induced N levels: Rough lemon, Sweet orange, Rusk citrange, Alemow and Rangpur lime, the high N level inducer rootstocks, and Sour orange, Trifoliolate orange, Cleopatra mandarin, and grapefruit, the lower ones, whereby these results underlined that there is a different N acquisition capacity among the citrus rootstocks responsible consequently of a diverse citrus NUE. Syvertsen and Smith (1996) observed that N uptake efficiency of Redblush grapefruit budded on Volkamer lemon, high vigorous rootstock, was 61% averaged over the 2-year period and N rates, while that on Sour orange, low vigorous rootstock, was 52% only. Lea-Cox et al. (2001) confirmed that the stronger-induced vegetative growth rootstock absorbed the ¹⁵N more than lower vigorous ones, showing the higher N uptake efficiency. However, this NUpE pattern was not observed on citrus rootstock seedlings (Scholberg et al. 2002).

Commonly, the single value of NUpEs or NUEs was used for comparing the behavior of different citrus rootstocks at diverse treatments (rate, type, time, and frequency of application of N fertilizer) with the aim to individuate the nitrogen-efficient and -inefficient citrus rootstocks. Gourley et al. (1994), comparing various criteria for defining crop NUE, demonstrated that single-value terms of NUE were not suitable, especially under low nutrient input, to discriminate between nitrogen-efficient and -inefficient germplasms. They suggested that the nutrient efficiency classification should take into account the plant performance either in presence and absence of the nutrient considered, and they proposed that "a well defined response curves are required for nutrient efficient differences to be determined." This approach enabled to estimate the maximum yield at non-limiting nutrient availability (α) and the nutrient concentration at which half-maximum yield is achieved (β) (Fig. 16.2), essential indices for determining nutrient efficiency in crop germplasms. Indeed, equivalent α and different β defined efficient/inefficient genotypes, while different α indicated genotypes with higher/lower genetic potential (Fig. 16.2) which as sustained by Gourley et al. (1994) exhibited "a greater overall genetic potential...due to factors other than those mechanisms specifically associated with nutrient acquisition..." However, these theoretical criteria were applied on different herbaceous germplasms (*Trifolium repens* L.) in response to different phosphorus levels (Gourley et al. 1994). More recently, Sorgonà et al. (2006), adopting the Gourley's criteria, characterized the nitrate efficiency in citrus rootstocks and compared the results with other nitrogen efficiency

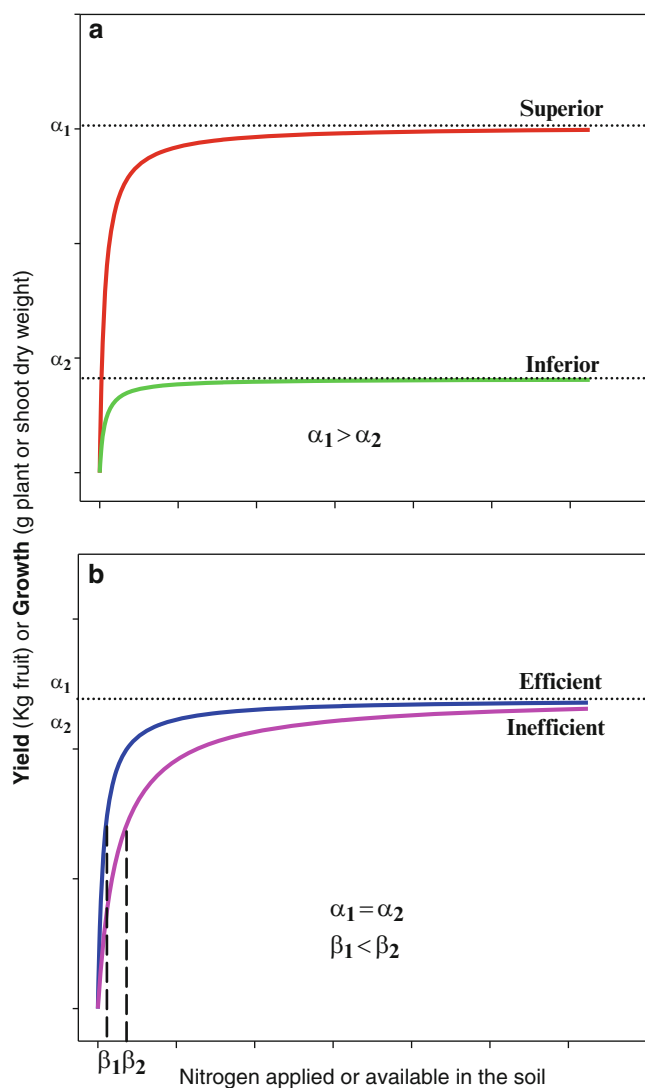


Fig. 16.2 Gourley's criteria adopted by Sorgonà et al. (2006) to identify the nitrogen efficiency in citrus rootstocks. (a) The maximum yield or growth obtained at non-limiting N availability (α) is different between the superior and inferior potential genetic of citrus genotypes. (b) The α 's values are equal, while the β 's values are different: the value of β of nitrogen-efficient citrus genotypes is lower than that of nitrogen-inefficient (Adapted from Gourley et al. 1994)

definitions. In agreement with Gourley et al. (1994), they demonstrated that (1) the estimation of the α and β indices by shoot dry weight response curves in response to increasing nitrate availability permitted, in a less ambiguous way, to discriminate the nitrate-efficient and inefficient citrus rootstocks, and (2) in addition to Gourley's criteria, they proposed the use of total leaf area response curves, parameter estimated by nondestructive techniques instead of shoot dry weight curves, for the early characterization of citrus rootstocks with high NUE. Assuming these criteria, it was possible to define the Rough lemon and Cleopatra mandarin as the rootstocks with superior and inferior genetic potential,

respectively, while Sour orange and Sweet orange as the nitrate-efficient and -inefficient citrus rootstocks, respectively (Sorgonà et al. 2006). However, it will be needed to verify these results on the grafted citrus rootstocks for a wide and practical application.

Identification of citrus genotypes with different nitrogen efficiencies generally includes investigation of potential morphological, physiological, and molecular mechanisms involved. Nitrogen-efficient genotypes usually exhibited above- and below-ground traits which conferred them an improved and "aggressive" nitrogen acquisition from N-deficient soils. The understanding of their architectural, morphological, physiological, and molecular mechanisms involved in response to low nitrogen availability will make it possible to genetically manipulate the plant to improve its nitrogen use efficiency. The root nitrogen acquisition capacity depends on root biomass, morphology, age, and proliferation and on nitrogen transport mechanisms, but it was correlated to shoot and leaf structural and biochemical features also. For a better understanding of these processes, we will split the NUE-related above- and below-ground traits of citrus rootstocks into three parts: root architecture and morphology, root nitrogen transport, and above-ground structures.

In general, root morphology and architecture are important plant traits for nutrient uptake efficiency (Sattelmacher et al. 1993; Lynch 1995), and several studies, mainly on cereal species, demonstrated the close relationships between the root performances and plant growth and yield (for review and references therein, see Hirel et al. 2007; Garnett et al. 2009). Furthermore, some positive correlations among QTLs for N-uptake and root morphology and architecture clearly underlined the importance of an efficient root system in N acquiring to increase the NUE (Coque et al. 2008). The citrus rootstocks exhibited a genetic variability in root morphology and architecture: Rough lemon, Sour orange, and Cleopatra mandarin showed a vigorous and spreading root system; Sweet orange and Orlando tangelo was compacted; and Carrizo citrange was poorly developed (Castle and Youtsey 1977).

In order for this root variability to be exploited to improve the nitrogen acquisition of the citrus species, it was necessary to understand how the root morphology and architecture of citrus rootstocks responded to change of soil nitrogen availability. Sorgonà et al. (2005) showed that Volkamer lemon and Carrizo citrange seedlings modified their root morphology and architecture in relation to the nitrate availability. In particular, at low nitrate supply ($5 \mu\text{M}$), Volkamer lemon allocated more biomass toward the root, increasing the length of the tap and first-order lateral roots and was lesser branched than Carrizo which, on the other hand, exhibited a higher length of second-order lateral roots and a pronounced root proliferation. However, at the high nitrate level ($1,000 \mu\text{M}$), this effect disappeared. These first results indicated that the citrus rootstocks showed a root morphological



Fig. 16.3 Volkamer lemon (120 days old) grown at two nitrate supplies, 5 μM (left seedling) and 1,000 μM (right seedling). Note the different root architectures: herringbone-like (left) and dichotomous-like (right)

plasticity in response to nitrate supply which was obtained by a within-root modification of the morphology. How much was the root plasticity and which root order was more plastic in N-efficient citrus rootstocks in response to the change of nitrogen supply were discussed later by Sorgonà et al. (2007). In particular, they evidenced that (1) the second-order was more responsive than tap and first-order lateral roots, (2) the biomass allocation more than structural parameters (root fineness and tissue density) was the “morphological components” that drives the length variation of different root orders, and (3) the slow- and fast-growing citrus rootstocks adopted a different root morphological strategy to the soil nitrate changes. Indeed, Cleopatra mandarin, slow-growing rootstock, exhibited a root system highly plastic, characterized by long tap root and poor branching; conversely, Rough lemon, fast-growing rootstock, invested on the length of second-order lateral roots and on the root proliferation, especially at low nitrate availability (Sorgonà et al. 2007). All these morphological traits defined a different root architecture more responsive to the modification of N availability, highly able to explore the soil environment and, consequently, to acquire nitrogen from the soil.

Generally, the root architecture of citrus rootstocks can vary within two extreme types: the herringbone system, with branching confined to the main axis, and the dichotomous type with more random branching at low and high N availability, respectively. Figure 16.3 shows the shifting of root architecture of Volkamer lemon seedlings grown at two nitrate levels (5 and 1,000 μM) from herringbone (seedling on the left) to dichotomous (seedling on the right). Further, root architecture is correlated with soil resource exploitation efficiency: in low-fertility soils, the herringbone-like struc-

ture is more efficient in nutrient acquisition, but more expensive to construct than dichotomous root architecture (Fitter and Stickland 1991; Fitter et al. 1991). The different root architecture of Volkamer and Carrizo, herringbone- and dichotomous-like structure, respectively, grown at low N availability, revealed their different root strategy efficiency for taking up the nitrate. In particular, Carrizo citrange exhibited an optimal root architecture to acquire the nitrate in N-deficient soil (Sorgonà et al. 2005). In-depth study on the root architecture responses to N availability on Rough lemon and Cleopatra mandarin, superior and inferior genetic potential for nitrate acquisition, and Sour orange and Sweet orange, N-efficient and -inefficient rootstocks, was conducted (Sorgonà et al. 2007). Rough lemon and Sweet orange exhibited a higher degree of root architecture plasticity in response to different soil N levels, shifting from a herringbone-like to dichotomous-like root architecture at low nitrate and high nitrate levels, respectively. Conversely, Cleopatra mandarin and Sweet orange showed a lesser plastic root architecture in response to the nitrate availability (Sorgonà et al. 2007).

The root capacity for N transport was widely studied at physiological and molecular level, mostly on herbaceous species (see reviews and reference therein Forde and Clarkson 1999; Tischner 2000), but first results can be drawn for citrus species. Nitrate and ammonium are the nitrogen forms mainly absorbed by citrus rootstocks; therefore, we focused on the transport systems of both ions. Like the herbaceous species, the citrus rootstocks take up the nitrate by at least two different transport systems, a low affinity (LATS) and high affinity transport system (HATS) (Cerezo et al. 1997, 2000; Sorgonà and Cacco 2002; Sorgonà et al. 2005, 2006). The LATS has a low affinity for nitrate and is activated by external nitrate concentrations higher than 1,000 μM in Troyer citrange and Cleopatra mandarin (Cerezo et al. 1997, 2000) or 200 μM in Sour orange (Sorgonà et al. 2010). The HATS showed a high affinity for nitrate, operating at external concentrations up to 1,000 μM (Cerezo et al. 1997) or 200 μM (Sorgonà et al. 2010). This nitrate transport system, more interesting in N-deficient soils, is highly regulated and made up by the constitutive (cHATS) and the inducible (iHATS) high affinity transport systems. The cHATS is constitutively expressed in NO_3^- -starved roots, mediating a constant nitrate uptake rate, while the iHATS is induced by NO_3^- and feedback regulated by downstream N metabolites (Sorgonà and Cacco 2002; Sorgonà et al. 2005, 2010). Sorgonà and Cacco (2002) showed that N-starved *Citrus volkameriana* seedlings exhibited a net nitrate uptake rate (NNUR) of cHATS by 0.085 $\mu\text{mol NO}_3^- \text{g}^{-1} \text{FW h}^{-1}$ which increased (induction phase of iHATS) reaching, after 24 h of nitrate contact, the complete induction with 0.29 $\mu\text{mol NO}_3^- \text{g}^{-1} \text{FW h}^{-1}$ NNUR. A subsequent feedback inhibition caused a decline of the NNUR (decay phase of iHATS).

Further, an estimate of the half-time ($t_{1/2}$) of NNUR, during the induction and inhibition phases, indicated that the nitrate transport system of *Citrus volkameriana* was induced in 10.3 h and inhibited after 46.8 h of contact with anion (Sorgonà and Cacco 2002). The authors demonstrated that the half-time of the induction phase was negatively correlated ($r^2=0.855$) with the number of root tip (Sorgonà and Cacco 2002), suggesting that the root systems of citrus rootstocks characterized by elevated numbers of root tips showed a rapid induction in NNUR. The role of root tip as NO_3^- -sensing region for the early soil exploration and belowground competition was recently confirmed by Sorgonà et al. (2010) which observed an earlier maximum induction of the iHATS of N-nitrate of the apical root segments of tap root compared to basal ones in Sour orange. The comparison of cHATS and iHATS pattern for nitrate among citrus rootstocks characterized by different root morphology and architecture could provide useful information on the diverse physiological basis of NUE. For example, Carrizo citrange, characterized by longer second-order lateral roots, pronounced root proliferation, and dichotomous-type architecture, showed a higher efficiency in nitrate uptake by higher full, and faster induction of the nitrate transport system than Volkamer lemon having a longer tap and first-order lateral roots and herringbone-type architecture (Sorgonà et al. 2005). These results further confirmed that the root systems of citrus rootstocks characterized by higher branching and proliferation were more “aggressive” for catching and taking up the nitrate in N-deficient soils and, hence, more efficient in nitrogen uptake.

Regarding the NH_4^+ , the regulation of the transport systems of this ion in citrus rootstocks was investigated by Cerezo et al. (2001). As well as the nitrate, the ammonium uptake in Troyer citrange showed a biphasic pattern characterizing by two different transport systems: a low affinity non-saturable (LATS) and a high affinity saturable (HATS). The V_{\max} and K_m , kinetic parameters of N-deficient Citrange troyer, were $12.5 \mu\text{mol g}^{-1} \text{root DW h}^{-1}$ and $170 \mu\text{M}$, respectively. Over 1 mM external NH_4^+ concentration, the influx of this ion increased linearly, indicating that it was operating the LATS. The HATS and LATS for ammonium in Troyer citrange were regulated in an opposite manner: the N-deficient condition increased the activity of the HATS and decreased that of the LATS, while under NH_4^+ supply, the activity of the LATS was stimulated and repressed that of HATS (Cerezo et al. 2001). The molecular mechanism of NH_4^+ transport system in citrus rootstock was studied by Camañes et al. (2007), which identifies and isolates the CitAMT1 highly homologous to ammonium transporter AMT1 of other plant species. They further studied the regulation of the NH_4^+ uptake by light conditions and C status (Camañes et al. 2007).

The capacity to acquire nitrogen by the root systems was sustained by the above-ground plant traits, such as leaf area and/or stem height which was correlated with nitrate use

efficiency. In herbaceous species, an alteration of leaf expansion in response to the N availability was observed (Ryser and Lambers 1995; Walch-Liu et al. 2005; Tian et al. 2007). In citrus species, Sour orange, nitrate-efficient rootstocks (Sorgonà et al. 2006), exhibited a greater leaf area and higher stem length than Sweet orange, nitrate-inefficient rootstock, especially at low nitrate availability (Sorgonà et al. 2011). This result suggested that the leaf area and stem height could be considered NUE-related traits in citrus rootstocks. However, the construction cost in terms of biomass of leaf area and the stem height could reduce the nitrogen efficiency of the citrus rootstock. In this respect, Sorgonà et al. (2011) found that Sour orange used more efficiently the biomass for constructing a unit of leaf area than Sweet orange, confirming that the former rootstock pointed out more adapted above-ground morphology for sustained an efficient nitrogen uptake from the soil.

16.5 Future Research

As detailed above, the improvement of NUE and its components, NUtE, and NUpE may be the primary goal over the next years to minimize the loss of N, reduce environmental pollution, and decrease the input cost in the citrus cultivation. In this respect, an exciting challenge will be to understand the following key aspects regarding either the impact of the agronomic management practices and citrus rootstock morpho-physiological and molecular mechanisms involved in NUE, NUtE, and NUpE:

1. The genotypic variability of the citrus rootstocks responses to different N regimes, especially to N limitations
2. The physiological basis of the citrus (species, rootstocks) responses to split application of N fertilizer during the growing season, and the interactive effect of the nitrogen with soil water status
3. The morpho-physiological and molecular traits (at development, growth, metabolic levels) controlling N use in citrus rootstocks, particularly in N limited availability, to develop, through molecular breeding and genetic engineering, citrus species with improved NUE

An increase of knowledge of these aspects together with the genomics, proteomics, and transcriptomic approaches will likely pave the way for engineering citrus rootstocks/scion combination able to give satisfactory economic yield under N-deficient soils or reduced N fertilizer inputs.

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