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Nutritional Efficiency and Morphophysiological Aspects With Growth in the 'Okinawa Roxo' Peach Rootstock

Aline das G. Souza[1], Cristina W. Ritterbusch[1], Renata D. Menegatti[1], Oscar J. Smiderle[2] & Valmor J. Bianchi[1]

[1] Federal University of Pelotas, Capão do Leão, RS, Brazil

[2] Brazilian Agricultural Research Corporation, Boa Vista, RR, Brazil

Correspondence: Renata D. Menegatti, Institute of Biology, Department of Botany, Federal University of Pelotas, Campus Universitário, S/N., Caixa Postal 345, 96010-900 Capão do Leão, RS, Brazil. Tel: 55-959-9904-6104. E-mail: renata.d.menegatti@gmail.com

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Abstract

The aim of this study was to evaluate the efficiency of nutrient uptake, accumulation, distribution and use, and its relationship with growth variables, chlorophyll content, and root and shoot dry-weight partitioning in the 'Okinawa Roxo' peach rootstock, submitted to different nutrient solutions and substrates. The experimental design was completely randomised, with four treatments and five replications. The treatments were T1: Ns1 = Souza et al. nutrient solution (2011), applied to a sand substrate; T2: Ns2 = Hoagland and Arnon (1950), applied to a sand substrate; T3: Commercial Substrate + H_2O; and T4: Control, Sand + H_2O. Ninety days after transplanting (DAT), morphological, physiological and nutritional parameters were determined in the 'Okinawa Roxo' rootstock, together with the correlation between nitrogen concentration and nitrogen balance index, and the chlorophyll content and index. The greatest zinc content was detected in the shoots of the 'Okinawa Roxo' plants, at more than 80% of the accumulated total. Treatments T1 and T2 (nutrient solutions Ns1 and Ns2 respectively) made it possible to obtain 'Okinawa Roxo' plants with the best morphophysiological characteristics, being ready for grafting three months after transplanting in a greenhouse. The use of the Ns1 nutrient solution resulted in greater efficiency in the uptake and use of the nutrients nitrogen (N), potassium (K) and iron (Fe). The N and Total Chlorophyll concentrations in the leaves of the red-leaved peach tree can be estimated indirectly with a chlorophyll meter, and are an accurate indication of the nutritional status of the plant in relation to nitrogen content.

Keywords: *Prunus persica*, rootstocks, seedling production, nutrient partioning, mineral nutrition

1. Introduction

Brazil is one of the largest fruit producers in the world, with soil and climate characteristics suitable for the development of fruit farming using species of temperate, subtropical and tropical climates (Brazilian Fruit Yearbook, 2017). Thus, since the 1980s, there have been technological advances in plant production systems for several fruit species in the country (Pereira & Kavati, 2011).

Due to this modernisation of the sector and the importance of plants quality in the success and profitability of the orchard, nurserymen have invested in production and management technology (Peticila et al., 2017; Granatstein et al., 2016), seeking to introduce the use of containers, new substrates, environments with plastic covering (Picolloto et al., 1997), irrigation systems, and organic (Reeve et al., 2017) and mineral fertilisers (Peticila et al., 2017), with the aim of producing rootstocks and grafted plants of high quality.

These advances contrast with the production of *Prunus persica* plants in Brazil, where according to Fischer et al. (2016), the traditional method of rootstock production still generally predominates, in which sowing is still carried out in the field, using a mixture of seeds of scion cultivars obtained from the peach-processing industry.

Faced with this scenario, several studies have sought to evaluate genotypes selected for use as rootstock, such as the studies carried by Fischer et al. (2016), Rocha et al. (2016), Almeida et al. (2015), and Mayer et al. (2015). However, there is still little knowledge of the nutritional requirements and the physiological and growth responses of plants of these genotypes in production systems in protected environments using different substrates and mineral fertiliser.

Fertilising plants with nutrient solutions has been increasingly used in the nutritional management of fruit species, with a view to optimising the use of inputs and plant growth under different cropping systems (Pradeepkumar et al., 2017; Wang et al., 2017; Miloševic et al., 2013). Nutrient solutions also have a wide application in studies of plant physiology, with the aim of studying and correlating the presence and concentration of mineral nutrients with the processes that coordinate plant growth (Abrisqueta et al., 2011; Baldi et al., 2014).

In order for a plant to develop and express its maximum productive potential, the correct supply of nutrients through fertilisation is necessary (Savvas et al., 2017). Epstein and Bloom (2006) add that much effort has been invested in formulating nutrient solutions, but there is little information on the concentration and/or proportion of salts that would be suitable for all crops.

Hoagland and Arnon nutrient solution is the most commonly used for investigating nutritional problems in plants, and is the basis for the formulation of other commercial nutrient solutions (Marles, 2017). According to Souza et al. (2017) the use of a complete nutrient solution, as formulated by Souza et al. (2011), together with substrates, is a promising technique in the initial formation of peach rootstocks in a greenhouse.

An strategy for identifying the nutritional requirements of the species is to determine the concentration that would promote the best uptake, distribution and use of nutrients and their effects on growth variables (Elsadr & Sherif, 2016; Baldi et al., 2014; Abrisqueta et al., 2011), the levels of photosynthetic pigments, and the effects of dry-weight partitioning on the mineral composition of the roots, stems and leaves of the plants (Zhu et al., 2017; Elsadr & Sherif, 2016; Baldi et al., 2014).

There are no studies of the chemical and physiological attributes of the red-leaved cultivars of the peach tree found in the literature, and ‘Okinawa Roxo’ is a read leaf peach rootstock with great potential for use in Brazil (Souza et al., 2016; Souza et al., 2017), a fact which stimulated the development of the present study.

Accordingly, the aim of this study was to evaluate the efficiency of nutrient uptake, accumulation, distribution and use in rootstocks of the ‘Okinawa Roxo’ cultivar submitted to different nutrient solutions and substrates, as well as aspects of growth variables, photosynthetic pigment content and dry weight partitioning.

2. Method

Peach [*Prunus persica* (L.) Batsch] rootstock cultivar ‘Okinawa Roxo’, obtained from seeds, was used as plant material. Mature fruit was harvested, and the pulp immediately removed from the endocarp, which was dried in the shade after washing.

To obtain the plants, the endocarp was broken and the seeds stratified in moist cold at 7 ºC for 21 days, as described by Picollotto et al. (2007). Sowing was then carried out in polystyrene trays of 72 cells (114 cm^3 per cell), containing a substrate composed of soil + vermiculite + medium sand + Bioplant® commercial substrate (1:1:1:1), whose chemical composition is shown in Table 1.

Table 1. Chemical composition of the substrates used for the production of peach rootstocks

Substrate	OM[**]	V	Al	H+Al	SB	CEC	P	K	Ca	Mg	Zn	Fe	Mn
	------- % -------		------------ $cmol_c\ dm^{-3}$ -----------				------------- $mg\ dm^{-3}$ -------------				------- $\mu g\ dm^{-3}$ ------		
SS[*]	2.12	78.00	0.01	1.00	1.83	1.78	1.89	7.80	0.42	0.10	1.62	5.12	3.12
Sand[**]	0.00	67.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.00
Bioplant®	20.5	72.00	0.17	8.10	20.56	2.90	776.48	1.410	12.22	4.72	0.00	0.00	0.00

Note. *SS = Substrate used when sowing = 25% soil + 25% vermiculite + 25% sand + 25% commercial substrate; [**]100% sand; **OM: Organic Matter; V: Base saturation; SB: Sum of Bases; CEC: Cation Exchange Capacity.

The present study was conducted between September to December 2015 at dependences of Federal University of Pelotas (UFPel), in Capão do Leão, Brazil. The plants were kept on benches in a greenhouse at average temperatures ranging from 18 °C to 36 °C, night and day respectively. At 23 days after sowing, the seedlings had a mean height of 15±3 cm, and were transplanted into plastic bags containing 2.5 L of the following substrates: a) 100% sand; b) 100% Bioplant® commercial substrate. The manually irrigated as needed with three weekly irrigations of 50 mL of the nutrient solutions shown in Table 2.

Table 2. Chemical composition of the nutrient solutions described by Hoagland and Arnon (1950) and by Souza et al. (2011)

Nutrient	Hoagland & Arnon	Souza
---------- mg L^{-1} nutrient solution ----------		
N	210.01	223.8
P	31.0	61.9
K	234.6	326.2
Ca	200.4	221.3
Mg	48.6	49.2
S	64.2	68.7
---------- µg L^{-1} nutrient solution ----------		
B	300	498
Cu	20	39
Fe	3022	5000
Mn	502	419
Zn	50	114

The experimental design was completely randomised with four treatments and five replications of five plants each, giving a total of 25 plants per treatment. The experiment was unifactorial, comprising the following treatments: T1: Ns1 = nutrient solution Souza et al. (2011) applied to a substrate of medium sand; T2: Ns2 = Hoagland and Arnon (1950) applied to a substrate of medium sand; T3: H_2O applied to Bioplant® commercial substrate; T4: Control, H_2O applied to a substrate of medium sand. Every 15 days the 'Okinawa Roxo' plants were evaluated for height (using a millimeter rule) and stem diameter (10 cm above the substrate, with the aid of digital callipers).

At 90 days after transplanting (DAT), the chlorophyll index (CHLI) and the nitrogen balance index (NBI) were determined using a Dualex chlorophyll meter. Between 09:00 and 11:00 each morning, measurements were taken on two completely expanded leaves located on the apical third of each plant. Immediately after taking the readings, the leaves were detached and placed in polystyrene boxes containing ice, and taken to the laboratory to determine the chlorophyll content. To do this, leaf discs were collected to make up a sample of approximately 1.5 g fresh weight, which was finely ground in 80% acetone in the absence of light. The extracts were filtered through filter paper and collected in 25 ml volumetric flasks, and the volume topped up at the end of filtration. The supernatants were analysed with an Agilent UV-Vis spectrophotometer, readings being taken at 645 nm (A_{645}) and 663 nm (A_{663}) against a blank containing 80% acetone. The chlorophyll *a* and *b*, and the total chlorophyll content were calculated using the equations established by Arnon (1949).

The other variables evaluated at 90 DAT were the root and shoot dry weight, with the aim of assessing assimilate partitioning. The shoots and roots were separated and packed into paper bags, and placed in a drying oven at 70 °C for 72 hours. After drying, the samples were weighed on a 0.01g precision balance to determine shoot dry weight (MSPA), root-system dry weight (RDW), and by summing these, the total dry weight of the plant (TDW). In order to measure the quality of the plants for use as rootstock, the formula proposed by Dickson et al. (1960) was used to define the Dickson Quality Index (DQI).

The roots and shoots were then ground in a Wiley mill with a 20-mesh screen, and the macro- and micronutrient concentrations were analysed as per Malavolta et al. (1997). The content of each nutrient was calculated by multiplying the dry weight of each part of the plant by the concentration of each nutrient (Bataglia et al., 1983). The following indices were also calculated: (a) uptake efficiency = (total nutrient content of the plant)/(root-system dry weight), as per Swiader et al. (1994); (b) transport efficiency = [(nutrient content of the shoots)/(total nutrient content of the plant)] × 100, as per Li et al. (1991); (c) use efficiency = (total dry matter produced)^2/(total nutrient content of the plant), in macronutrients (mg plant^{-1}) and micronutrients (µg plant^{-1}) respectively, as per Siddiqi and Glass (1981).

Possible differences among treatments were verified by analysis of variance (ANOVA). The variables that showed significant differences were submitted out Tukey's test at 5% probability using the Sisvar software (Ferreira, 2011). Pearson correlation analysis was performed between the concentrations of nitrogen, chlorophyll *a* and *b*, and total chlorophyll, the chlorophyll index and the nitrogen balance index, using the R software (2014).

3. Results and Discussion

At 90 days after transplanting (DAT), the plants from treatment T1 (nutrient solution-Sn1) presented the highest mean values for height, reaching 123.0 cm (Table 3). In the production sector for peach tree plants, it is interesting that rootstocks show rapid growth in both height and stem diameter, since in Brazil grafting is performed almost exclusively by active budding or vegetative bud (Mayer et al., 2017). Stem diameter in particular determines whether the rootstocks are suitable for grafting as early as possible, and should be between 5.0 and 10 mm according to the production standards for peach rootstocks (Brazil, 2005).

Table 3. Mean values for height (H, cm), stem diameter (SD, mm), root-system dry weight (RDW, g), shoot dry weight (SDW, g), total dry weight (TDW) and Dickson quality index (DQI), determined in 'Okinawa Roxo' rootstocks under different forms of cultivation, at 90 days after transplanting

Treatment	H	SD	SDW	RDW	TDW	DQI
T1 (Ns1)**	123.0a*	7.33a	53.94a	20.28a	74.22a	4.89a
T2 (Ns2)	93.6b	5.28b	42.18b	16.81b	58.99b	3.90b
T3	64.0c	4.28c	28.38c	10.81c	39.19c	3.52b
T4	18.3d	1.97d	2.88d	1.64d	4.52d	1.79c
CV (%)	3.71	8.62	12.89	11.91	11.30	9.92

Note. *Mean values followed by the same letter in a column do not differ by Tukey's test ($p \leq 5\%$). ** T1 (Ns1) Souza et al. nutrient solution (2011) in medium sand; T2 (Ns 2) Hoagland and Arnon solution (1950) in medium sand; T3 (Commercial substrate + water); T4 (Sand + water).

The mean stem diameter of the plants ranged from 1.97 to 7.33 mm (Table 3). The lowest mean value for stem diameter was obtained with treatment T4 (1.97 mm) followed by T3 (4.28 mm). Peach rootstocks suitable for grafting should have a minimum diameter of 5.0 mm (Brazil, 1984). Therefore, according to Brazil (1984), treatments T3 and T4 did not meet the required standards during this period.

In treatment T1 (Ns1), plant diameter was 41.61% greater than in treatment T3 (commercial substrate) (Table 3), demonstrating the importance of adequate nutrient application via nutrient solution in the production of rootstocks of the 'Okinawa Roxo' cultivar, regardless of the substrate used.

At 180 days after transplanting, Picolloto et al. (2007) recorded a mean height and diameter of 46.6 cm and 5.0 mm in 'Okinawa' and 'Capdeboscq' peach rootstocks respectively, grown in a greenhouse in plastic bags (4.5 L) containing commercial substrate (Plantmax®). Commercial substrates have several compositions, and may contain coconut fibre, pine bark, manure, sawdust, vermiculite, rice husks, ash, agricultural gypsum, calcium carbonate, magnesium, magnesium thermophosphate (yoorin) and additives (fertilisers). Even when containing mineral elements in their composition, commercial substrates do not generally promote the satisfactory growth of peach rootstocks, possibly because the physiological and biochemical processes associated with plant growth and development are limited due to the reduced availability and rapid depletion of nutrients during the rapid growth phase.

Research to establish the best formulation of a nutrient solution suitable for all crops has intensified, with the aim of achieving high morphophysiological and nutritional quality (Furlani et al., 1999). These parameters were clearly seen in the present study, since the shoot dry weight (SDW), root-system dry weight (RDW) and total dry weight (TDW) of the 'Okinawa Roxo' plants showed the highest mean values with use of the Ns1 nutrient solution, followed by the Ns2 nutrient solution, even in an inert substrate such as sand (Table 3).

Differences were found between treatments for the macro- and micronutrient content, and were greater in both the shoots and roots of the plants from T1 (nutrient solution-Ns1) (Table 4), which is consistent with the greater increase in shoot and root dry weight of the plants (Table 3). Overall, T1 also resulted in better seedling quality, with a DQI of 4.89, superior to T2 and T3, which did not differ from each other (Table 3).

Table 4. Mean values for macro- (mg plant^{-1}) and micronutrients (µg plant^{-1}) in the shoots and root system, determined in 'Okinawa Roxo' rootstocks under different forms of cultivation, at 90 days after transplanting

Treatment	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	----------- macronutrient content mg plant^{-1} -----------						-------- micronutrient content µg plant^{-1} --------				
*Shoots***											
T1 (Ns1)	414.11a *	44.41a	317.06a	94.04a	82.90a	28.66a	554.29a	50.74a	2280.58a	666.18a	430.33a
T2 (Ns2)	309.65b	30.96b	242.16b	74.19b	51.18b	16.85b	341.94b	34.10b	1806.65b	359.86b	196.82b
T3	198.28c	21.00c	181.55c	15.13c	23.95c	6.68c	95.98c	16.00c	414.01c	45.17c	144.91c
T4	1.90d	1.82d	1.12d	0.85d	0.67d	0.27d	0.22d	0.62d	0.15d	0.25d	0.36d
**CV %	14.17	12.9	13.82	13.45	13.7	13.4	14.14	13.2	13.74	13.86	13.97
*Root system***											
T1 (Ns1)	203.03a	23.59a	170.53a	51.64a	57.31a	19.37a	217.69a	37.87a	2607.97a	223.30a	54.77a
T2 (Ns2)	151.28b	18.75b	103.55b	27.86b	28.79b	8.78b	158.83b	21.24b	1290.72b	228.53b	38.04b
T3	92.45c	12.23c	71.58c	11.37c	11.89c	3.37c	60.48c	6.37c	258.83c	35.92c	23.33c
T4	3.26d	2.19d	2.71d	1.02d	0.80d	1.22d	1.16d	0.25d	0.23d	0.83d	0.22d
***CV %	10.38	8.36	9.98	10.27	10.01	11.15	10.01	10.40	10.45	10.83	10.41

Note. Mean values followed by the same letter in a column do not differ by Tukey's test ($p \leq 5\%$). **T1 (Ns1); T2 (Ns2); T3 (Commercial substrate + water); T4 (Sand + water). Where, N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulphur; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc.

In general, the lowest macro- and micronutrient content was seen in treatments T3 and T4 (Table 4). Despite the plants from T3 having a low nutrient content in the shoots, there were no visual symptoms of leaf deficiency. It is possible that 'hidden hunger' was present with this treatment, characterised when plants are deficient in a specific nutrient without presenting visible symptoms, the deficiency being detrimental to plant growth (Meriño-Gergichevich et al., 2017). This was evidenced by the smaller growth of the 'Okinawa Roxo' plants in T3 when compared to T1 (Ns1) and T2 (Ns2).

One of the main nutrients responsible for plant growth is nitrogen (N). Restriction of this nutrient reduces growth, as it is part of the structure of amino acids, proteins, nucleic acids, enzymes, vitamins, photosynthetic pigments and their by-products. Nitrogen also plays a part in such processes as ionic absorption, respiration, and cell multiplication and differentiation (Barlog et al., 2017). Irrespective of the treatment, it was found that N and K are the most necessary nutrients during the initial growth and development phase of 'Okinawa Roxo' plants (Table 4).

According to Souza et al. (2015), the ideal range for total N content in peach seedlings is from 509.94 to 617.24 (mg plant^{-1}), which agrees with the present research. Perennial fruit species of the genus Prunus, such as the peach tree [*Prunus persica* L. (Basthc)], accumulate N in the form of free proteins or amino acids, as well as non-structural carbohydrates that include starch and soluble sugars (sucrose, glucose, fructose and sorbitol) (Haider et al., 2018). Such molecules play an essential role in vegetative growth, providing structural components for the formation of new cells and consequently maximum biomass production.

As regards the total phosphorus (P) content, only the plants from treatments T1 (Sn1) and T2 (Sn2) (Table 4) showed values within the range (P = 49.53 to 69.61 mg plant^{-1}) considered suitable for the crop (Souza et al., 2015). An adequate P content is fundamental to plant metabolism, allowing rapid and intense root growth (Ullah et al., 2017; Laakso et al., 2017), especially due to its low mobility and its functional importance, where it participates in the formation and integrity of the cell membranes involved in the production and transfer of cellular energy involving ATP, as well as in the metabolism of carbohydrates (Buchanan et al., 2000).

In the production of plants on a commercial scale, a well-formed root system favours the uptake of nutrients and water, providing better plant development when transplanted to the field (Cabeza et al., 2017; Shen et al., 2016). Considering the importance of the roots in fixing and supporting the plants, the information available in the literature on biomass production and the nutritional aspects of the root system in the peach tree are scarce (Mayer et al., 2015).

With respect to the quality of the root system in the commercial substrate (T3), the roots were more fragile, breaking easily when removed from the substrate for chemical analysis of the macro- and micronutrients. This can be attributed to insufficient levels of the nutrients P, K and Ca in the plants from T3 affecting the quality and development of new roots (Higuchi & Hara, 2017). The deficiency of such nutrients as Ca may present serious restrictions to root development, since this nutrient has limited internal redistribution due to its low mobility (Vliet & Giller, 2017).

Ca was the third most-accumulated nutrient in the 'Okinawa Roxo' plants, with a maximum content of 94.04 mg plant^{-1} in the shoots and 51.64 mg plant^{-1} in the roots (Table 4). The accumulated Ca content in the plants from T1 (Ns1) was greater than in the other treatments at 90 DAT, with accumulation values 21.10% greater in the shoots and 46.04% in the roots compared to the plants from T2 (Ns2) (Table 4); it can be said that the greater accumulation of each nutrient is due to the higher concentration of nutrients in Ns1 in relation to Ns2 (Table 2) and to the high uptake of Ca by the crop.

Ca is strongly related to the integrity of the membranes and cell walls, and to root growth (Vliet & Giller, 2017). It also acts as a secondary messenger, integrating the complex system for transducing the signals initiated by the binding of plant hormones to their receptors (Silva et al., 2017). Among the most common calcium-binding proteins are calmodulin and the calcium-dependent protein kinases (Vliet & Giller, 2017).

It is also worth noting that Ca and Mg are the activators of several enzymes, some of them related to the absorption of other nutrients; an example is Mg, which activates the enzymes (ATPases) that act in the formation of H^+ gradients, directly influencing the transport of phosphorus and other nutrients into the cells (Xiong et al., 2015), as well as transport in and out of the vacuole. A fundamental difference between these nutrients is their mobility within the plant. Whereas Mg can be easily translocated via the phloem, Ca is an element with low mobility in the plant (Marles, 2017). The Mg absorbed by the roots and accumulated in the shoots can be remobilised to meet the nutritional needs of younger tissue, when its availability in the rhizosphere is reduced (Singh et al., 2016).

In this respect, it can be shown that the Mg content in the plants from T1 (Ns1) was greater than in the other treatments (Table 4), with accumulation values 38.26% higher in the shoots and 49.76% in the roots in relation to T2 (Ns2). Furthermore, the Mg content in treatments T2, T3 and T4 did not meet the ideal range (142.0 mg plant^{-1}) for the peach plants (Souza et al., 2015). According to Rigon et al. (2012), an adequate Mg content contributes to high photosynthetic rates and consequently better plant adaptation when planting in the field.

There are few studies in the domestic and international literature of the supply of sulphur (S) to peach plants (Bakić et al., 2017; Muhammad et al., 2018; Mayer et al., 2015; Souza et al., 2014). It was found that the S content of the shoots ranged from 0.27 to 28.66 mg plant^{-1}. According to Raij (1991), an adequate S content in the shoots of subtropical fruit, is generally between 10.0 and 40 mg plant^{-1}. In the present study, the S content was within the range established by Raij (1991), except in T4 (Table 4). S applied in suitable doses can favour better plant growth and development due to its participation in the conversion of non-protein nitrogen into protein nitrogen (Yilmaz & Gökmen, 2016).

As regards micronutrients, Smiderle et al. (2017) showed that iron (Fe) is the micronutrient most accumulated by plants at the initial growth phase, followed by manganese (Mn). However, under the conditions of the present study, the mean Fe content was within the ideal range (2945.00 to 4292.33 μg plant^{-1}) for *Prunus persica* (Souza et al., 2015), except in the plants from T3 and T4, where it was below the ideal range (Table 4). It is known that Fe plays an important role in the activation of enzymes, acting as a prosthetic group (Jucoski et al., 2016). In addition, Fe catalyses chlorophyll biosynthesis, being one of the enzymes responsible for its formation (Yilmaz & Gökmen, 2016).

Only the plants from T1 (Sn1) had a Cu and Mn content within the range suitable for *Prunus persica.* The other treatments did not provide an adequate Cu content for the peach, as per Souza et al. (2015).

The highest zinc (Zn) content was detected in the shoots of the 'Okinawa Roxo' plants, at more than 80% of the total accumulated in the plant (Table 4). Despite the importance of Zn to crops in general (Mehraj et al., 2017; Wasaya et al., 2017), there is little information in the literature on the use of this micronutrient in the production of peach rootstocks. When applied in suitable doses, in addition to maintaining the structural integrity of the cell membrane (Tiecher et al., 2017), Zn can favour the production of vigorous plants (Hidoto et al., 2017) due to its direct participation in the synthesis of the amino acid, tryptophan, the precursor of indoleacetic acid (IAA), a plant growth regulator related to cell growth and stretching.

Distinct plant responses for nutrient-uptake efficiency were seen in relation to the treatments (Table 5), with the maximum values for uptake efficiency of the macro- and micronutrients recorded in the plants from T1 (Table 5). The smallest values for macro- and micronutrients were seen in T4, and can be explained by the lack or even absence of nutrients essential for the growth and development of the plants during cultivation, as it is an inert substrate. When nutrient concentrations are low, or nutrients are unavailable, plants tend to reduce their uptake, even though they may be able to extract them from the substrate and use them more efficiently (Schiattone et al., 2017).

Table 5. Absorption efficiency in 'Okinawa Roxo' rootstock under different forms of cultivation, at 90 days after transplanting

Treatment	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
*Absorption efficiency**											
	--------------- mg nutrient/g root dry weight ----------------						--------- µg nutrient/g root dry weight ----------				
T1 (Sn1)	30.43a	3.35a	24.04a	7.18a	6.91a	2.37a	38.07a	4.37a	241.05a	43.86a	23.92a
T2 (Sn2)	27.42b	2.95b	20.57b	6.07b	4.76b	1.52b	29.79b	3.29b	184.26b	35.00b	13.97b
T3	26.89b	3.07a	23.42a	2.45c	3.32b	0.93c	14.47c	2.07c	62.24c	7.50c	15.56b
T4	3.15c	2.45b	2.34c	1.14d	0.90c	0.91c	0.84d	0.53d	0.23d	0.66d	0.35c
*CV (%)	9.62	6.23	4.72	3.31	3.89	4.02	12.3	8.56	12.99	9.52	7.45
*Transport efficiency***											
	--- % ---										
T1 (Sn1)	67.10a	67.31a	67.03 a	65.55a	59.13a	59.67a	71.80a	57.26b	56.65a	74.90a	88.71a
T2 (Sn2)	67.18a	72.34a	70.05a	72.70a	64.00 a	65.74a	68.28a	61.62b	58.33a	64.16a	83.80a
T3	68.20a	63.20b	71.72a	57.09b	66.82a	66.47a	61.34b	71.52a	61.53a	55.70b	86.13a
T4	36.82b	45.39c	29.24b	45.45c	45.58b	18.12b	4.09c	71.26a	39.47c	23.15c	62.06b
**CV (%)	14.17	12.9	13.82	13.45	13.7	13.4	14.14	13.2	13.74	13.86	13.97
*Use efficiency****											
	--------- (dry weight)^{2}g/mg accumulated nutrient ---------						--- (dry weight)^{2}g/µg accumulated nutrient ----				
T1 (Sn1)	8.92a	81.01a	11.60a	37.81a	39.19a	114.69c	7.14c	62.17a	1.13c	6.19b	11.36b
T2 (Sn2)	7.05b	70.00b	9.98b	34.10b	43.51a	135.77b	6.95c	62.88a	1.12c	5.91b	14.82b
T3	5.28c	46.22c	6.07c	17.96c	42.85a	152.82a	9.82b	68.66a	2.28b	18.94a	9.13b
T4	3.95d	5.09d	5.03c	10.93d	13.90b	13.71d	14.80a	23.48b	57.36a	18.92a	35.22a
***CV (%)	6.23	5.59	6.89	8.76	5.01	8.97	4.12	5.21	7.41	3.69	7.41

Note. Mean values followed by the same letter in a column do not differ by Tukey's test ($p \leq 5\%$). **T1 (Ns1); T2 (Ns2); T3 (Commercial Substrate + Water); T4 (Sand + Water). Where: N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulphur; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc.

As it is a genetically controlled factor, the nutritional efficiency of a given species and/or genotype can be an aid to breeding programs through which it is possible to select species with a greater capacity to absorb and transport nutrients when under limiting conditions for growth (Rosolem et al., 2017; Retamal-Salgado et al., 2017).

Transport efficiency is an indication of the ability of the plant to transport nutrients from the roots to the shoots and incorporate the absorbed nutrient into the biomass (Xavier & Natale, 2017). Under the conditions of the present study, the nutrient solutions of T1 (Sn1) and T2 (Sn2) were most suitable for the genetic potential of the 'Okinawa Roxo' plants, showing similar nutrient transport efficiency, greater than seen in the plants from T3 and T4 (Table 5).

Therefore, optimising nutritional efficiency is of great importance, since in general, fertilisers contribute approximately 30% to the total cost of seedling production (Marles, 2017). Currently in Brazil, few studies characterise plants as to nutrient use efficiency; the few results available in the domestic and international literature refer mainly to cereal production (Beche et al., 2014).

For stone-fruit trees, just as in the production of peach rootstocks, there are no studies on the use efficiency of nutrients from nutrient solutions or substrates. In general, plants of the 'Okinawa Roxo' cultivar from T1 (Ns1) were more efficient in the use of N, P, K and Ca, which can be attributed to a number of factors, such as the higher concentration of nutrients in T1 (Ns1) (Table 2) and by the formation of ion pairs between the Ca and nitrate (NO_3^-).

The higher nitrate content contributes to the dynamics of the cationic nutrients in the soil, since the NO_3^- anion is considered an accompanying ion of Ca_2^+, Mg_2^+, K^+ and Al_3^+ (Marles, 2017) and thus may improve the absorption and use of these nutrients, the results of which can be seen in the morphometric characteristics of the plants, such as a greater investment in shoot growth, and early grafting, as recorded in the present study.

This relationship between canopy growth and N, K and Ca use efficiency was also seen by Xavier and Natalle (2017), where uptake efficiency was the most important parameter in explaining variations in total dry weight production in rootstock of the starfruit (*Averrhoa carambola* L.).

From the results of the present study, it could be inferred that there was no significant difference in micronutrient use efficiency between Sn1 and Sn2, this fact demonstrates the involvement of such micronutrients as Cu, Mn and Fe in electron transport during photosynthesis, on the greater growth in height of the plants.

In order to carry out photosynthesis, superior plants depend on light absorption and the significant presence of carotenoids and chlorophylls a and b in the leaves to control carbohydrate metabolism in the chloroplast and cytosol via the chemicals ATP and NADPH (Falqueto et al., 2017). Chlorophylls are related to the photosynthetic efficiency of plants, and consequently to their growth and adaptability to different forms of cultivation (Rigon et al., 2012).

For the variables, chlorophyll a and b and total chlorophyll, it should be pointed out that the 'Okinawa Roxo' plants from T1 (Ns1) showed an increase of 28.43% in total chlorophyll content in relation to T2 (Ns2), and 55.0% in relation to the plants from T4, despite being a cultivar with red leaves (Table 6). It should be noted that the plants from T1 and T2 had the highest values for N concentration ([N]), Nitrogen Balance Index (NBI), chlorophyll index (CHLI), chlorophyll *a* (CHLa, μg/mL), chlorophyll *b* (CHLb, μg/mL) and total chlorophyll (CHLT μg/mL), when compared to T3 and T4, with all the treatments being different (Table 6).

Table 6. Mean values for nitrogen balance index (NBI), nitrogen concentration ([N]), chlorophyll index (CHLI), chlorophyll *a* (CHL*a*, μg/mL), chlorophyll *b* (CHL*b*, μg/mL) and total chlorophyll (CHLT μg/mL) in 'Okinawa Roxo' rootstocks under different forms of cultivation, at 90 days

Treatment	NBI	[N]	CHLI	CHL*a*	CHL*b*	CHLT
T1 (Ns1)**	32.18a*	30.82a	31.12a	20.55a	11.21a	31.76a
T2 (Ns2)	26.67b	24.31b	24.67b	15.48b	7.25b	22.73b
T3	18.00c	16.03c	17.09c	10.89c	6.62c	17.51c
T4	10.11d	8.02d	9.48d	5.10d	3.15d	8.25d
Mean	21.74	19.79	20.59	13.05	7.05	20.06
CV (%)	6.06	7.09	6.80	3.77	3.83	3.92

Note. *Mean values followed by the same letter in a column do not differ by Tukey's test ($p \leq 5\%$). ** T1 (Sn1); T2 (Sn2); T3 (Commercial Substrate + Water); T4 (Sand + Water).

Positive correlations were found for the variables associated with the levels of chlorophyll and N, ranging from moderate to high magnitude (Table 7). According to Souza et al. (2016), a correlation is considered of high magnitude when the correlation coefficient ranges from $0.8 \leq r < 1.0$. Positive correlations of high magnitude were obtained between the CHLI and the CHLT content of the leaves of the 'Okinawa Roxo' plants. The same occurred between the NBI and [N] in the leaves, showing that non-destructive measurement using a chlorophyll meter gives an excellent estimation of these variables, since a high correlation was also obtained between [N] and CHLI.

Table 7. Correlation matrix for the Pearson coefficients* between the variables, nitrogen balance index (NBI), nitrogen concentration ([N]), chlorophyll index (CHLI), chlorophyll *a* (CHL*a*, μg/mL), chlorophyll *b* (CHL*b*, μg/mL) and total chlorophyll (CHLT μg/mL) in 'Okinawa Roxo' rootstocks under different forms of cultivation, at 90 days

Variable	CHLI	CHL*a*	CHL*b*	CHLT	[N]
NBI	0.98^*	0.89^*	0.85^*	0.96^*	0.99^*
CHLI	-	0.86^*	0.79^*	0.99^*	0.97^*
CHL*a*	-	-	0.84^*	0.86^*	0.76^*
CHL*b*	-	-	-	0.71^*	0.71^*
CHLT	-	-	-	-	0.97^*
[N]	-	-	-	-	-

These estimates corroborate the usefulness of the chlorophyll meter as a tool for estimating or diagnosing the nutritional status of various crops (Xiong et al., 2015), for example, the results obtained with nectarine plants (Takle et al., 2017), peach trees (Souza et al., 2017) and apple trees (Tamburini et al., 2015).

[N] was positively correlated with the total chlorophyll content, showing that this can act as an indicator of [N] in the leaves of the peach, and making it possible to detect N deficiency earlier, even in the case of red-coloured leaves. A positive correlation of high magnitude was found between NBI and CHLI, which is mainly due to the fact that 50% to 75% of the N is present in cells of the mesophile associated with nitrite reductase enzymes (NR) in the chloroplasts (Singh et al., 2016). According to Gitelson et al. (2016), this enzyme is considered the key in regulating N metabolism, since the nitrate absorbed by the roots must be reduced to NH_4^+ before being incorporated into organic compounds in the shoots and/or root system.

In the present study, the correlations of moderate to high magnitude between these variables are of great importance for establishing a standard to aid in early N diagnosis, thereby allowing timely decision-making for possible N fertilisation. For this reason, the chlorophyll meter proved to be a suitable tool for use in precision agriculture.

In general, the results of the present study showed that adequate nutrition during the stage of rapid plant growth is important to allow the satisfactory physiological performance of the plants. As the plant is intended for grafting, it could be demonstrated that, even using inert or partially inert substrates such as sand, it is possible to have a growth pattern good enough for early grafting when using suitable nutrient solutions.

This implies a reduction in the time plants spend in the nursery, reducing production costs, an important characteristic for the production sector of stone-fruit rootstock. Considering that in the domestic and international literature there is no information on the mechanisms of uptake, translocation, remobilisation or nutrient use efficiency in the 'Okinawa Roxo' rootstock, the present study represents an important contribution of information as an aid in the nutritional management of the peach during seedling production.

4. Conclusions

Commercial substrate without the addition of nutrients is not indicated for the production of 'Okinawa Roxo' rootstock in plastic bags in a greenhouse.

The nutrient solutions Ns1 and Ns2 make it possible to obtain 'Okinawa Roxo' plants of excellent morphophysiological and nutritional characteristics, that are suitable for grafting three months after transplanting in a greenhouse.

The best absorption efficiency of macro- and micronutrients by 'Okinawa Roxo' rootstock is when using the Ns1 nutrient solution.

Use of the chlorophyll meter allows an adequate estimation of the nitrogen and total chlorophyll content in the red-leaved peach tree, and is a precise tool for indicating the nutritional state of the plant.

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