

Impact of nitrogen fertilization and rootstock genotype on growth and fruit quality of Nules clementine

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This study focuses on understanding the impact of varying nitrogen rates on the growth, fruit production, and quality of clementine Nules variety trees grafted onto Flhorag and Carrizo citrange rootstocks and cultivated at the Sidi Allal Tazi Experimental Station of INRA Morocco, in clay soil, over five seasons. A split-plot design with three blocks of repetitions (two trees per bloc per treatment, n=6) was employed in this experiment. The main plot varied the nitrogen doses, while the subplot involved the genotype of rootstock. Three fertilizer treatments with N rates (T₀: control (native nutrient), T₁: 270, T₂: 540 in g/tree), with 135 P₂O₅ and 270 K₂O g/tree added to citrus trees. The research indicated that both nitrogen rate and rootstock genotype significantly influenced vegetative growth, yield, and fruit quality of Nules clementine. Increased nitrogen levels enhance vegetative growth, especially in Carrizo citrange rootstock, evidenced by greater tree height, canopy diameter, and volume. Significant and positive correlation was found between canopy volume and fruit yield, across both rootstock highlighting the importance of proper nitrogen management in citrus cultivation. Carrizo citrange was proved to be a more efficient rootstock for Nules variety in contrast to tetraploid Flhorag. Higher nitrogen rates with Carrizo citrange result in better yield and fruit quality (size, juice content, and solid soluble content) than with tetraploid Flhorag. Optimal yield and fruit quality in Nules clementine are achieved using 540g of N/tree/year when grafted on Carrizo citrange. The recommended NPK rate for achieving high yield and optimal fruit quality of Nules clementine was 540 N-135 P₂O₅-270 K₂O g/tree/year.

Keywords: Citrus, rootstocks, nutritional requirements, fertilizer application, yields, fruit quality.

INTRODUCTION

Citrus crops are the most produced fruit in the world, covering more than 129,000 ha and yielding around 2.4 million tons per year in Morocco (FAO, 2020). Recent decades have seen an increase in citrus plantations driven by rising demands for local consumption and export. Despite this growth, Moroccan citrus productivity remains low compared to other Mediterranean countries (FAO, 2020). Most current fertilization programs rely on outdated local recommendations or practices from countries with different citrus-growing conditions. This calls for more studies on optimal nutrient fertilizer management and the best citrus rootstock for maximizing yield and optimal fruit quality.

Citrus crops require balanced nutrition for growth and development, with nitrogen (N), phosphorus (P), and potassium (K) being crucial (Ghosh, 1990; Obreza and

Morgan, 2008). Nitrogen, in particular, is a key component in mineral fertilizers, significantly influencing tree growth, appearance, and fruit production/quality (Zekri and Obreza, 2003a,b). However, nitrogen deficiency or excess can lead to various growth and environmental issues. This highlights the importance of a balanced NPK fertilization program (Alva *et al.*, 2006a; Cantarella *et al.*, 2003; Obreza and Rouse, 1993). The nitrogen deficiency leads to decrease plant growth. The typical symptom is observed on the old leaves due to the translocation of nitrogen from the old to the developing young leaves (Alva *et al.*, 1998; Koo and Young, 1977). The old leaves, therefore, become yellow and fall off in the time, so the branches become dry and die from the top. This causes poor production of a small-size fruits of citrus (Osotsapa, 2001; Ritenour *et al.*, 2003).

Nitrogen (N) is the most important nutrient in the plant. It is a crucial and primary element of amino acid, proteins, enzyme



and chlorophyll (Alva *et al.*, 2008, 2006b; Cantarella *et al.*, 2003; Osotsapa, 2001; Quaggio *et al.*, 2000; Storey and Treeby, 2000; Warren *et al.*, 2000; Feungchan, 1995; Domingo *et al.*, 1992; Hewitt, 1984). Several studies have been reported that the nitrogen supply has a significant effect on citrus fruits quality (Alva *et al.*, 2008; Alva *et al.*, 2006c; Quaggio *et al.*, 2000; Omari *et al.*, 2020a and b; He *et al.*, 2003; Obreza and Rouse, 1993; Cameron and Dennis, 1986; Dasberg *et al.*, 1983; Sahota and Arora, 1981). Omari *et al.*, (2023) demonstrated that N rate and rootstocks genotype affected significantly plant growth, marked by increased plant height, rootstock and scion stem diameters, diameter and shoot length, relative water content (RWC), as well as leaf chlorophyll, proline and leaf nitrogen content of the Clementine Sidi Aissa trees. Liao *et al.*, (2019), reported that yield, fruit quality, enzyme activity, and enzyme-related gene expression were considerably lower at low than at high-N supply of citrus cultivar Huangguogan (*Citrus reticulata* × *Citrus sinensis*).

The utilization of a balanced NPK fertilization program positively influenced the yield, fruit quality, and both physical and chemical properties of different cultivars of citrus trees (Omari *et al.*, 2020a; Quaggio *et al.*, 2014; Tsakelidou *et al.*, 2007; Chen *et al.*, 1999; Zayan *et al.*, 1989). The recent findings of Omari *et al.* (2020a) and Huang *et al.* (2000) further validate the advantages of determining an optimal NPK fertilization approach to achieve maximum yield.

Previous research has also demonstrated that the use of different citrus rootstocks can affect both the yield and fruit quality parameters of various citrus scions (Omari *et al.*, 2020a,b; Omari *et al.*, 2012; Castle *et al.*, 1993; Fallahi and Rodney, 1992; Fallahi *et al.*, 1991; Laboren *et al.*, 1991 and Saunt, 1990). Numerous research projects have shown that rootstocks significantly influence scion varieties in various aspects such as plant size, physiological traits, productivity, and leaf nutrient content. Ultimately, using the right rootstock can enhance both the yield and quality of citrus groves (Yulianti and Agisimanto, 2023). Rootstock type affect tree size and vigor, precocity (early flowering and fruiting), fruit yield and quality of citrus (Hayat *et al.*, 2022). Numerous studies have examined how rootstocks can modify or enhance the functioning of citrus scion cultivars (Qureshi *et al.*, 2023). Rootstocks play a crucial role in affecting the tree's growth encompassing vegetative and reproductive development, mineral absorption, and the physiological behavior of scion varieties.

In this context, the utilization of tetraploid somatic hybrid in citrus breeding offers a novel approach to enhance yield, fruit quality, and nutrient use efficiency. Tetraploid somatic hybrids, such as Flhorag, have shown promising results in initial studies, outperforming traditional rootstocks in certain aspects of growth, yield, and fruit quality parameters (Dambier *et al.*, 2011). Indeed, a research study was carried out on a tetraploid somatic hybrid named Flhorag, a product

of protoplast fusion between *C. reticulata* cv 'Willow leaf' and *Poncirus trifoliata* cv 'Pomeroy', developed by CIRAD (Dambier *et al.*, 2011). The experiment was conducted at the Afourer experimental station in Morocco, which has soil characteristics suitable for citrus cultivation. The performance of Flhorag was evaluated against two well-known rootstocks from the INRA/CIRAD germplasm collection, famous for their resistance to CTV: the Volkamer lemon (*C. volkameriana*) and Carrizo citrange (*P. trifoliata* × *C. sinensis*).

The research focused on the influence of these rootstocks on the growth, productivity, and fruit quality of Valencia sweet oranges grafted onto them. After eight years, significant variations in growth characteristics like tree height, canopy size, and the diameters of both rootstock and scion were observed. Among these, trees grafted on *C. volkameriana* displayed the largest canopy and trunk sizes, while those on Flhorag were the smallest. In terms of fruit production over two seasons (2007 and 2008), *C. volkameriana* led in total fruit weight per tree each year, surpassing both Flhorag and Carrizo citrange. However, Flhorag excelled in terms of production efficiency and fruit quality, notably in juice content and average fruit weight. Despite these variances in growth and productivity, the grafts showed similar compatibility across all rootstocks, as evidenced by comparable scion-to-rootstock diameter ratios. Their distinctive genetic composition might enable superior adaptation to varying soil and climatic conditions, along with more effective nutrient utilization, addressing the gaps in current citrus cultivation practices.

However, there remains a significant gap in research regarding the comprehensive benefits and applications of tetraploid somatic hybrid in citrus cultivation especially when it grafted by clementine variety and under Gharb (north of morocco) soil and climate conditions. Detailed studies are needed to understand their interaction with different nutrient levels, especially nitrogen, and how they affect overall tree growth, fruit production, and quality.

This study aims to explore the potential of tetraploid somatic hybrids, specifically the Nules variety grafted onto Flhorag and Carrizo citrange rootstocks, in enhancing citrus production and fruit quality under the soil and climatic conditions of the Gharb region in northern Morocco. The primary hypotheses are: (1) Nules variety when grafted onto tetraploid somatic hybrids rootstock will demonstrate superior growth, yield, and fruit quality compared to Carrizo citrange rootstock under the same conditions. (2) An optimized nitrogen fertilization program can be developed for this clementine variety, which will maximize production and fruit quality while minimizing environmental impacts.

This research aims to fill the existing gap in current knowledge, providing insights into the effectiveness of tetraploid somatic hybrid in citrus cultivation, and ultimately



contributing to the advancement of sustainable agricultural practices in the citrus industry.

MATERIALS AND METHODS

Plant material and growth conditions: A field experiment was conducted in the Experimental station of Sidi Allal Tazi, INRA Morocco (34° 31' 07.8" N, 006° 14' 42.0" W) on Nules trees budded on Flhorag and Carrizo citrange rootstock planted at 5.0×3.0 m spacing with 667 trees/ha and planted in 2010. Flhorag is a produced by fusion between protoplasts of *C. reticulata* cv 'Willow leaf' and *Poncirus trifoliata* cv 'Pomeroy', obtained by CIRAD (Ollitrault *et al.*, 1996). Agronomical behavior of Flhorag has been evaluated in Morocco, in comparison with two traditional rootstocks: Volkamer lemon (*C. volkameriana*), and Carrizo citrange (*P. trifoliata* x *C. sinensis*) introduced from the INRA/CIRAD germplasm bank.

The soil of the experimental site of Nules is clay (Table 1). Standard cultural practices for Nules trees were used with drip irrigation and chemical weed control. Water pH was 7.84, while electrical conductivity was 0.964 mS/cm.

Table 1. Soil characteristics of the Experimental station of Sidi Allal Tazi, INRA Morocco

Depth	pH (water)	pH (KCl 1 N)	Organic matter (%)	P ₂ O ₅ (ppm)	K ₂ O (ppm)	CE (dS/m)
0-60	8.87	8.28	2.15	35.55	983.60	0.245

The experimental site, as indicated by the agrochemical soil data in table, reflects specific characteristics important for citrus crops. The phosphorus level (P₂O₅ = 35.55 ppm) observed in the soil is generally considered to be in the medium range for soil fertility (Srivastava and Singh, 2004; Alva *et al.*, 2006c). This level may be sufficient, but it might require careful monitoring. However, Potassium level (K₂O=963.60 ppm) is considered to be a high level. Given these data points, the experimental site appears well-suited for citrus cultivation, especially for balanced nutrition management practices.

One year after planting, formative pruning began to shape the trees, focusing on developing a strong, healthy branch structure to support a future canopy and substantial fruit yield. This pruning continued annually to mold the trees to the desired size and form. Additionally, maintenance pruning was carried out each year at the end of winter to balance vegetative growth and fruit development, ensuring proper airflow and sunlight within the canopy. This process, also known as fruiting pruning, involved thinning out the branches and foliage, and removing any weak, dry, or diseased ones (Matias *et al.*, 2023). These practices were consistently applied each year, but the pruning never exceeded 10% of the tree canopy until 2019, when the trees had reached a height of

3 meters. In the winter of 2019, a special medium and modified recovery pruning was conducted to address the oversized of the canopies, involving the removal of some medium-sized branches and parts of the canopy to rejuvenate it, in line with the density parameters (5m x 3m) used in our studies.

The experiment was conducted using a split-plot ANOVA with three replicated blocks and with two trees per treatment per blocs (n=6), where nitrogen doses were applied as the main plot (T₀, T₁ and T₂) and the rootstock type was applied as the subplot (Flhorag and Carrizo citrange). According to Gomez and Gomez (1984), the experimental trees were organized into three distinct blocks. The replications are spread across different blocks. In each replication, main-plot treatments are first randomly assigned to the main plots followed by a random assignment of the subplot rootstock genotype (two trees) within each main plot. Three nitrogen rates were applied to citrus tree in field condition over five seasons (2016-2017, 2017-2018, 2018-2019, 2019-2020, and 2020-2021), namely Control (native nutrient, T₀), application of 270 g/tree/year (T₁), application of 540 g/tree/year (T₂). Nitrogen, phosphorus and potassium were applied as Ammonium nitrate (33.5%), Triple Super Phosphate (0-45-0) and Potassium Sulphate (0-0-50), respectively.

The split-plot design was chosen due to its suitability for experiments in which some factors are applied at larger scales (main plot) and others at smaller scales (sub-plots) (Altman and Krzywinski, 2015). In this case, Nitrogen levels are the main factor and applied at a larger scale, affecting the entire plot. Rootstock genotype, on the other hand, is applied at a smaller scale, making it a sub-plot factor. The split-plot design is ideal for this hierarchical structure of factors. This design strikes a balance between experimental constraints, such as logistical considerations for applying different nitrogen levels, managing multiple rootstock genotypes, and accounting for variations in environmental conditions over the years. By adopting this split-plot experimental design, we aim to comprehensively assess the combined and individual impacts of nitrogen levels, rootstock genotype, and years/seasons on the selected response variables, providing a robust foundation for meaningful scientific conclusions and recommendations for horticultural practices.

Fruit quality characteristics: Fruit quality was determined for the 2016-2017 until 2020-2021 harvests.

Juice content (%) is extracted by a rotary extractor and the juice content was expressed as a percentage as given by the formula:

$$\text{Juice content (\%)} = \frac{(\text{weight of juice extracted from 10 fruits} \times 100)}{\text{Total weight of 10 fruits}}$$

Solid soluble content (*Brix) measured using a digital refractometer.

Total juice acidity is obtained according to the following formula:



$$A = \frac{Vs}{10}$$

(Vs: Volume of solution of the NaOH (ml) used for the titration and 10: Volume (ml) of juice used).

Ripening index (RI) was calculated as the ratio of Solid soluble content (°Brix)/ titratable acidity (%).

Agronomic and morphological characters:

Mean fruit weight (g) is determined by measuring total weight of the 10 fruits per tree.

Fruit size (mm): mean diameter of ten fruits sample was recorded.

Citrus Color Index: The fruit color was evaluated at the harvest time using a Chromameter 400/410 Minolta, (Japan). Thus, three replicates of five fruit per treatment were measured and three different readings were obtained along the equatorial circumference of each fruit. The CIE $L^*a^*b^*$ color scale was adopted (McGuire, 1992) and the citrus color index (CCI) was calculated according to Jiménez- Cuesta *et al.*, (1981):

$$CCI = \frac{1000 \times a^*}{L^* \times b^*}$$

Where, CCI = citrus color index, a^* = red-green color value, b^* = yellow-blue color value, L^* = lightness. The CCI is a comprehensive indicator for color impression with positive values for red, negative values for blue-green, and 0 for an intermediate mixture of red, yellow, and blue-green. Lightness (L^*) value ranges from 0 to 100 in which higher values indicate lighter color intensity (McGuire, 1992)

Trunk cross sectional area (TCSA): of the trees at the height of 20 cm above the soil level was measured at the beginning of the experiment and at fall. The relative TCSA growth was measured according to Forey *et al.* (2016).

Canopy volume: Tree height and canopy diameter were measured using a ruler, whereas the girth of the trunk was determined 15cm above and below the grafting union so the scion to stock ratio was calculated. The tree canopy volume was estimated according to Turrell's formula (1946).

Fruit Yield (kg/tree): Annually, in November the trees were harvested and total fruit weight was measured (in kg/tree), than the yield was monitored over a five-year period (2016-2017 until 2020-2021).

Cumulative yield (kg/tree) was calculated as sum of annual yield for 2016-2017 through 2020-2021(five-year cumulative yield).

SPAD (portable chlorophyll meter): The SPAD-502 meter is employed to evaluate leaf chlorophyll content which is recognized for its speed, affordability, and non-invasive approach. This device includes two light-emitting diodes (LEDs) and a silicon photodiode receptor. It quantifies leaf transmittance within the red (650 nm) and infrared (940 nm) regions of the electromagnetic spectrum. Based on the transmittance values, a relative SPAD-502 meter reading, ranging from 0 to 99, is generated. This value is directly related to the chlorophyll content within the examined sample

(Markwell *et al.* 1995; Uddling *et al.* 2007). For each tree, a total of 10 leaves were chosen for these measurements.

Statistical analysis: The mean values (mean± SE) of the data were analyzed using a split-plot ANOVA (three-way ANOVA). The GLM procedure in Statistical Analysis System (SAS) software was employed for this purpose. The main factor in the design was the year (seasons), which consisted of five levels representing the main plots. The second factor was the Nitrogen rate, comprising three levels and representing the subplots. The third factor was the rootstock genotype, which had two levels and represented the sub-subplots. To compare the means of the treatment groups, Duncan's multiple-range test with a significance level of $P \leq 0.05$ was utilized and the results were interpreted according to Gomes and Gomez (1984). Additionally, the linear relationships between the study parameters were evaluated using bilateral Pearson correlation.

RESULTS

Vegetative growth: The study on Nules clementine revealed that nitrogen fertilization and rootstock genotype significantly affect vegetative growth. Key parameters like tree height, canopy diameter, volume, and rootstock diameter varied notably with nitrogen levels. Trees grafted onto Carrizo citrange under a high nitrogen regime (540 g/tree) exhibited the greatest growth in terms of height, canopy dimensions, trunk cross-sectional area (TCSA), and chlorophyll content (SPAD), while those grafted on tetraploid Flhorag trees showed the least growth. The data underscores the strong correlation between nitrogen fertilization, rootstock type, and the growth dynamics of Nules clementine trees.

The vegetative growth of Nules clementine was significantly affected by the nitrogen rate and citrus rootstock genotype. The results indicate a significant effect of nitrogen rate on various parameters such as tree height, canopy diameter, canopy volume, diameter of the rootstock, diameter of the variety, TCSA (Trunk cross-section area) rootstock, TCSA variety, canopy projectional unit area and SPAD (Table 2).

Data in Table 2 and Figs. 1, 2, 3 and 4 clearly show that varying macro fertilization (N) was followed by a significant difference on vegetative growth parameters. There was a gradual promotion on all parameters with increasing levels of N. Significant differences on such parameters were observed between treatments.

The maximum values of tree height (2.98±0.06m) (Fig. 1), canopy diameter (3.12±0.09m) (Fig. 2), canopy volume (30.35±1.31m³) (Fig. 3), canopy projectional unit area (9,79±0,27m²) (Fig. 4) were registered on Carrizo trees under T₂ treatment (540 gram of N per tree). While the tetraploid Flhorag control trees (T₀) was produced the minimum values in all seasons of tree height (2.47± 0.02m), canopy diameter (2.57±0.12m), canopy volume (17.08±1.54m³) and canopy



Table 2. Effect of N rate and citrus rootstocks on diameter of the rootstock and diameter of the variety of Nules clementine.

	Diameter of the rootstock (mm)			Diameter of the variety (mm)		
	Nules/Flhorag			Nules/Flhorag		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
2016	17.50±2.25	20.80±1.62	23.00±0.70	15.17±1.33	21.70±1.55	22.50±0.96
2017	66.40±3.84	72.73±3.79	90.60±4.38	56.08±6.62	68.95±5.33	76.92±1.78
2018	79.51±1.50	89.79±7.09	96.22±3.87	63.97±4.96	73.82±6.17	83.17±4.06
2019	85.25±6.04	91.86±2.52	101.55±3.41	71.68±4.04	79.13±1.10	84.39±2.16
2020	84.88±4.88	90.19±2.29	95.70±1.67	74.17±6.83	81.57±1.77	82.15±3.47
Mean	66.71±6.99 a	73.07±5.75 a	81.41±5.61 a	56.21±6.06 a	65.03±4.77 a	69.82±4.56 a
	Nules/Carrizo citrange			Nules/Carrizo citrange		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
	2016	18.17±1.01	26.17±0.71	26.33±2.24	16.67±0.33	23.75±1.41
2017	77.85±3.92	78.57±2.70	88.08±2.86	65.60±2.22	71.42±2.33	81.05±3.99
2018	93.60±4.42	108.10±3.69	111.94±6.28	79.24±5.98	87.69±3.95	88.34±4.28
2019	95.06±1.32	107.87±5.27	111.83±3.05	80.97±6.18	88.78±2.04	92.71±3.41
2020	103.45±4.80	105.67±2.81	108.10±6.05	83.50±7.22	85.75±3.85	100.09±6.14
Mean	80.17±7.59 a	85.27±6.03 a	89.26±6.34 a	67.20±6.43 a	71.48±4.74 a	77.80±5.18 a

¹In each row for each parameter studied, values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Each value is the mean ±Standard Error.

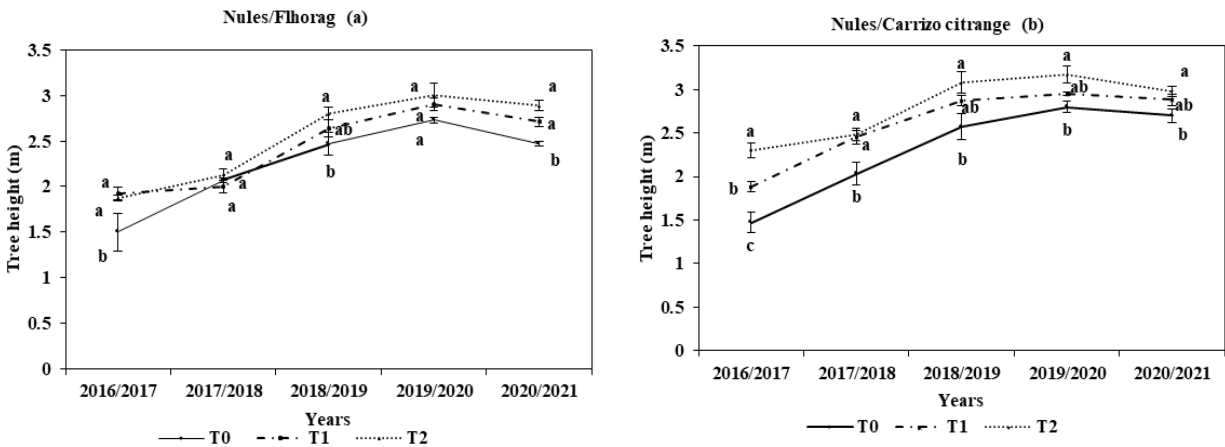


Figure 1. Effect of N rates and citrus rootstocks (Flhorag (a), Carrizo citrange (b)) on tree height (mean±SE) of Nules clementine. In each years, values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE).

projectional unit area ($8.06 \pm 0.38 \text{ m}^2$) at the end of the study period's (Fig. 1 to 4).

The growth of trees in the field showed an upward trend from year one to five. Specifically, the yearly average canopy volume for tetraploid Flhorag trees fluctuated between ($7.12 \pm 0.59 \text{ m}^3$) in 2016 and ($27.13 \pm 2.10 \text{ m}^3$) in 2020. Similarly, for Carrizo citrange trees, the canopy volume altered from ($9.87 \pm 1.21 \text{ m}^3$) in 2016 to a significantly larger ($30.35 \pm 1.31 \text{ m}^3$) in 2020, as illustrated in Figure 3. Canopy projectional unit area varied from ($5.97 \pm 0.21 \text{ m}^2$) in 2016 to ($9.37 \pm 0.36 \text{ m}^2$) in 2020 of tetraploid Flhorag trees and from

($6.28 \pm 0.28 \text{ m}^2$) to ($9.79 \pm 0.27 \text{ m}^2$) of Carrizo citrange trees (Fig. 4).

For Nules clementine, the diameter of the rootstock ($89.26 \pm 6.34 \text{ mm}$) and diameter of the variety ($77.8 \pm 5.18 \text{ mm}$) were found to be the highest for Carrizo citrange trees of T₂ (540 N g/tree) than other treatments for the averages of 5 years. However, control trees of tetraploid Flhorag (T₀) gave the lowest averages values of diameter of the rootstock ($66.71 \pm 6.99 \text{ mm}$) and diameter of the variety ($56.21 \pm 6.06 \text{ mm}$) as shown in Table 2.



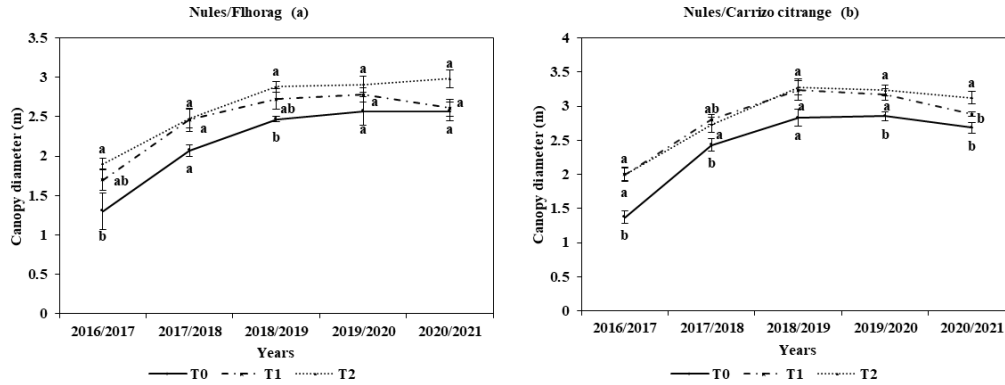


Figure 2. Effect of N rates and citrus rootstocks (Flhorag (a), Carrizo citrange (b)) on canopy diameter (mean±SE) of Nules clementine. In each years, values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (\pm SE).

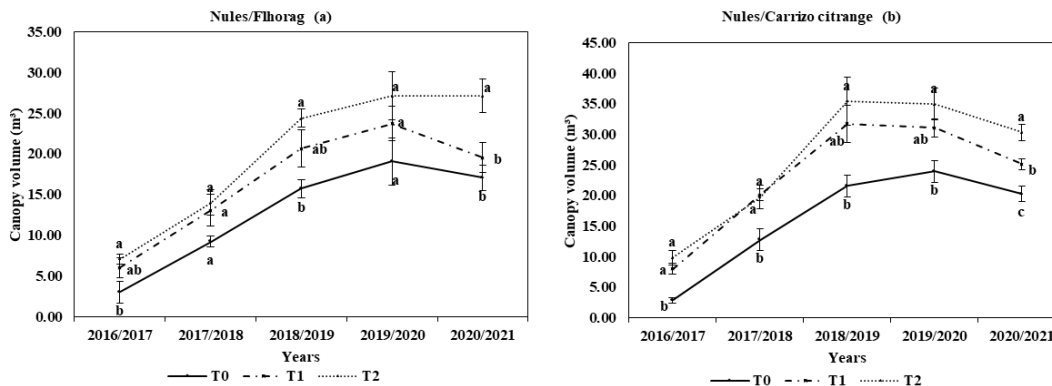


Figure 3. Effect of N rates and citrus rootstocks (Flhorag (a), Carrizo citrange (b)) on canopy volume of (mean±SE) of Nules clementine. In each years, values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (\pm SE).

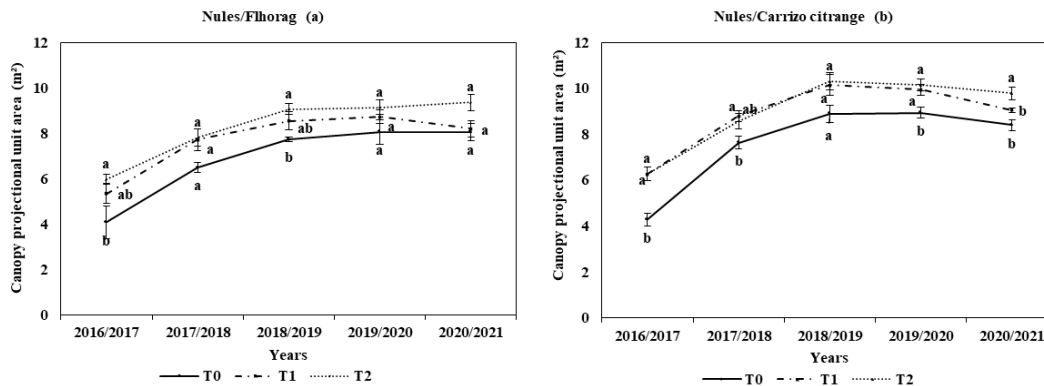


Figure 4. Effect of N rates and citrus rootstocks (Flhorag (a), Carrizo citrange (b)) on canopy projectional unit area (mean±SE) of Nules clementine. In each years, values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (\pm SE).

The trunk cross-sectional area (TCSA) is typically deemed to have a strong correlation with both the height of a tree and its

canopy volume (Westwood and Roberts, 1970). Carrizo citrange tree of T₂(540 N g/tree) had the highest TCSA



Table 3. Effect of N rate and citrus rootstocks on TCSA (Trunk cross-sectional area) rootstock and TCSA variety of Nules clementine.

	TCSA (Trunk cross-sectional area) rootstock (cm ²)			TCSA variety (cm ²)		
	Nules/Flhorag			Nules/Flhorag		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
2016	2.49±0.58	3.48±0.54	4.17±0.26	1.83±0.30	3.77±0.50	4.01±0.34
2017	34.86±4.03	42.00±4.23	65.21±6.25	25.39±5.49	38.23±6.34	46.60±2.18
2018	49.69±1.86	64.91±9.89	73.30±5.73	32.52±5.08	44.00±7.15	54.97±5.18
2019	57.66±7.81	66.47±3.64	81.44±5.21	40.61±4.43	49.21±1.38	56.11±2.90
2020	56.96±6.32	64.04±3.25	72.04±2.48	43.94±7.63	52.35±2.32	53.48±4.61
Mean	40.33±5.82 b	48.18±5.37 ab	59.23±5.54 a	28.86±4.45 b	37.51±4.01 ab	43.03±3.95 a
	Nules/Carrizo citrange			Nules/Carrizo citrange		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
2016	2.61±0.29	5.40±0.28	5.64±0.98	2.18±0.09	4.51±0.56	5.86±1.09
2017	47.84±4.76	48.77±3.28	61.26±4.00	33.88±2.26	40.27±2.64	52.22±5.17
2018	69.11±6.4	92.30±6.30	99.96±11.46	49.88±7.53	61.01±5.46	62.00±6.22
2019	71.01±1.96	92.48±8.79	98.59±5.28	52.39±7.85	62.06±2.72	67.96±4.87
2020	84.60±7.79	88.00±4.51	93.21±10.17	55.98±9.03	58.33±5.38	80.16±9.36
Mean	57.71±7.31 a	65.39±6.74 a	71.73±7.36 a	40.67±5.56 a	45.24±4.36 a	53.64±5.35 a

¹ In each row for each parameter studied, values with the same letter are not significantly different (Duncan test, p ≤ 0.05). Each value is the mean ±Standard Error.

Table 4. Effect of N rate and citrus rootstocks on SPAD of Nules clementine.

	Nules/Flhorag			Nules/Carrizo citrange		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
2016	44.27±3.74	58.92±3.79	61.05±1.68	39.97±1.08	51.82±3.99	59.43±2.73
2017	66.40±1.54	71.40±1.96	73.00±1.88	68.30±1.33	73.53±3.34	77.07±2.19
2018	50.60±3.11	68.52±0.96	75.17±1.90	53.83±1.59	71.95±2.36	75.02±1.48
2019	59.73±1.02	67.86±2.70	72.60±1.53	65.33±2.82	70.12±2.56	76.48±1.54
2020	59.67±4.80	66.86±3.76	70.43±2.16	60.08±3.60	65.62±1.56	74.98±1.09
Mean	56.13±2.03 b	66.71±1.47 a	70.45±1.19 a	57.44±2.56 c	66.61±1.93 b	72.6±1.46 a

¹ In each row for each parameter studied, values with the same letter are not significantly different (Duncan test, p ≤ 0.05). Each value is the mean ±Standard Error.

rootstock (71.73±7.36cm²) and TCSA variety (53.64±5.35cm²), whereas tetraploid Flhorag control trees (T₀) had the lowest TCSA rootstock (40.33±5.82cm²) and TCSA variety (28.86±4.45cm²) with significant differences (Table 3).

Overall average of SPAD varied from (56.13±2.03) to (70.45±1.19) of tetraploid Flhorag trees and from (57.44±2.56) to (72.6±1.46) of Carrizo citrange trees. The maximum value of SPAD (72.6±1.46) was registered on C. citrange trees under T₂ (540 gram of N per tree) (Table 4).

Yield: The yield of Nules clementine trees exhibited significant variations across different treatments, particularly in relation to nitrogen application and rootstock type. Yield consistently increased with higher nitrogen levels, with the most substantial improvements observed in trees receiving the T₂ (540 g N/tree). Over a five-year period, trees grafted onto Carrizo citrange, particularly those receiving the T₂ nitrogen treatment, achieved significantly higher total yields and yield per canopy projectional unit area compared to those grafted

onto tetraploid Flhorag rootstock. The study also confirmed a significant and positive correlation between canopy volume and fruit yield, highlighting that as the canopy volume expanded with increased nitrogen treatment, fruit yield correspondingly increased.

Yield means of Nules clementine trees showed significant differences among the treatment. Yield was progressively increased with increasing levels of N, the application of the treatment T₂ (540 N g/tree) was very beneficial for improving yield than other treatment (65.23±1.84 on Carrizo citrange and 58.44±2.17 on tetraploid Flhorag rootstock). Carrizo citrange had the greatest yields for 2016 to 2020 (Table 5).

For the total yield harvested over a five-year period, Carrizo citrange demonstrated a significantly higher production (326.17±9.18 kg/tree) on the trees fertilized with T₂ (540 N g/tree) than tetraploid Flhorag in Nules. Tetraploid Flhorag had lower cumulative yield (181.67±14.24 kg/tree) than Carrizo citrange rootstock in T₀ (control) (Fig.5).



Table 5. The annual yield of Nules clementine trees on two rootstocks (Flhorag (a), Carrizo citrange (b)) (2016/2017-2020/2021).

		Nules/Flhorag		
		T ₀	T ₁	T ₂
Yield (kg/tree)	2016/2017	31.67±4.41b	45.00±2.74a	54.17±2.01 a
	2017/2018	40.00±11.55b	65.40±6.57a	70.00±5.16 a
	2018/2019	26.67±3.33b	37.00±6.24ab	49.17±2.39 a
	2019/2020	55.00±2.89b	68.00±3.74ab	75.00±5.48 a
	2020/2021	28.33±1.67c	35.00±1.58b	45.00±1.29 a
Average Yield (Kg/tree)		36.33±2.85 c	50.08±2.48b	58.44±2.17a
		Nules/Carrizo citrange		
Yield (kg/tree)	2016/2017	40.00±2.89b	48.33±2.11b	62.50±4.43 a
	2017/2018	58.33±4.41a	75.83±8.98a	77.83±4.32 a
	2018/2019	28.33±1.67b	38.33±1.67b	55.00±4.47 a
	2019/2020	57.50±2.50c	70.83±3.27b	83.33±4.22 a
	2020/2021	33.75±2.39b	40.83±2.39ab	47.50±2.14 a
Average Yield (Kg/tree)		44.37±1.14 c	54.83±2.36b	65.23±1.84a

¹Mean separation within columns by Duncan test ($p \leq 0.05$). In each row, values with the same letter are not significantly different. Each value is the mean ±Standard Error.

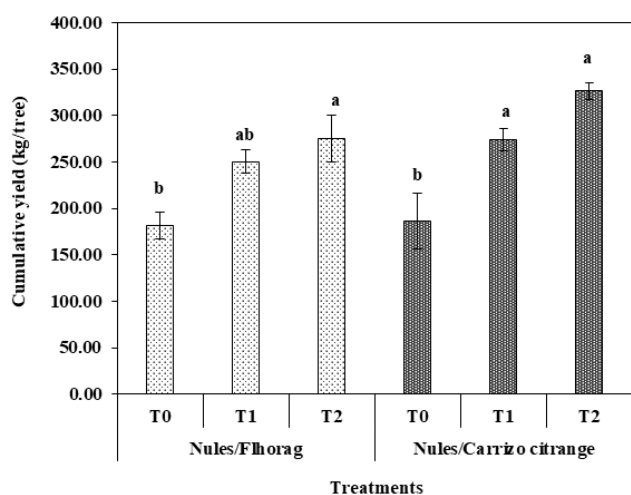


Figure 5. The cumulative yield (kg/tree) (mean±SE) of Nules clementine trees on two rootstocks (Flhorag, Carrizo citrange) (2016/2017-2020/2021). In each years, values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE).

The parameters of growth and production allowed the estimation of yield per canopy projectional unit area (kg/m^2) for the Nules variety. This was influenced by nitrogen fertilizer rates in the years 2017, 2018, and 2020. Yield/canopy projectional unit area (kg/m^2) varied from (3.45 ± 0.45) to (9.13 ± 0.90) of tetraploid Flhorag trees and from (3.21 ± 0.30) to (10.10 ± 0.93) of Carrizo citrange trees. The maximum Yield/canopy projectional unit area (10.10 ± 0.93) was registered on Carrizo citrange trees under T₂ (540 N g/tree) (Fig. 6).

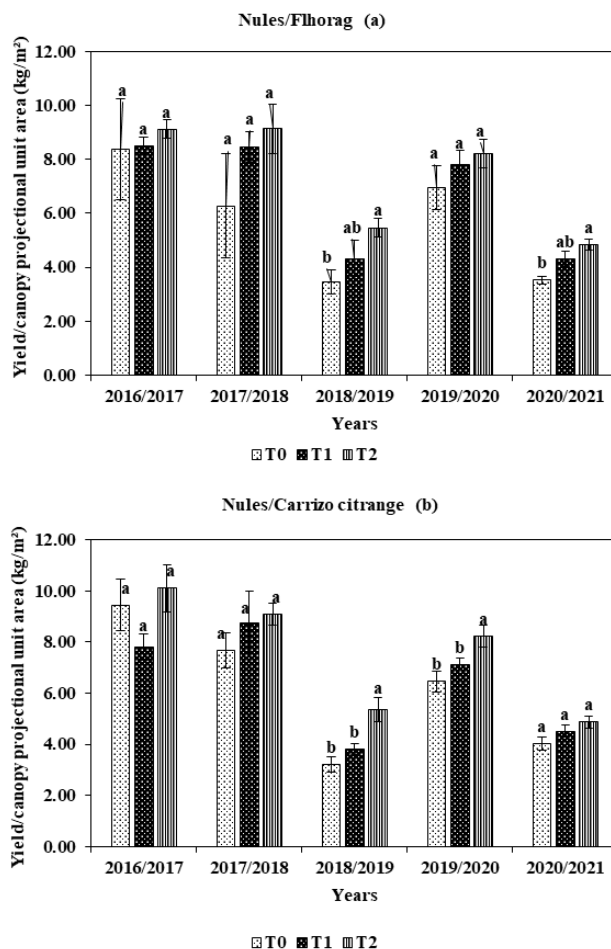


Figure 6. Yield/canopy projectional unit area (kg/m^2) (mean±SE) of Nules clementine trees on two rootstocks (Flhorag (a), Carrizo citrange (b)) (2016/2017-2020/2021). In each years, values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE).

Results regarding the effect of citrus rootstocks on yield clearly show that yield was varied according to citrus rootstocks genotype. Nules clementine trees grown on Carrizo citrange rootstock produced higher yield than the trees on tetraploid Flhorag rootstock. Under such promising treatment, yield reached $83.33 \pm 4.22 \text{ kg}/\text{tree}$ (Table 5). Flhorag had less cumulative yield than Carrizo citrange (Fig. 6).

Since N rate affected tree growth according rootstock genotype, a significant and positive linear correlation between canopy volume and fruit yield was verified (Fig. 7 and 8).

Nutrient is essential for the growth of citrus trees, and even more so for improving their fruit yield. For both rootstock, as the canopy volume expands with the application of nitrogen



treatments, a corresponding increase in fruit yield is observed (as seen in Figures 7 and 8). Elevating the nitrogen rate from 0 to 540g per tree leads to a substantial increase in fruit yield from 22.01 to 47.36 kg per tree (as shown in Figure 9). The N rate influenced tree growth, canopy volume and fruit yield. The fruit yield has showed a positive and significant linear response to increasing nitrogen rate as observed in (Fig. 9).

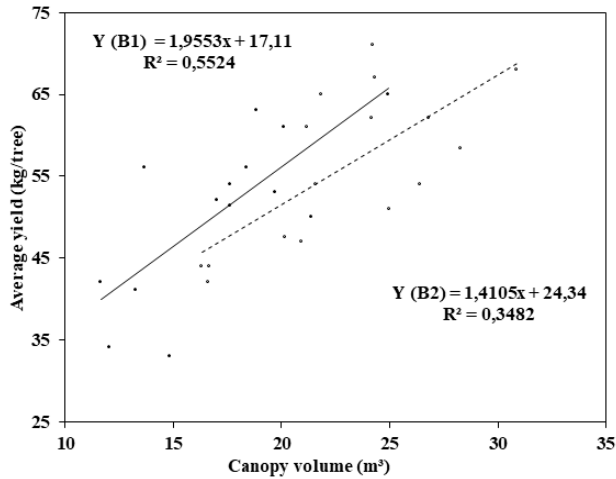


Figure 7. Fruit yield of Nules clementine trees on two rootstocks related to canopy volume (B1: Flhorag and B2: Carrizo citrange rootstock).

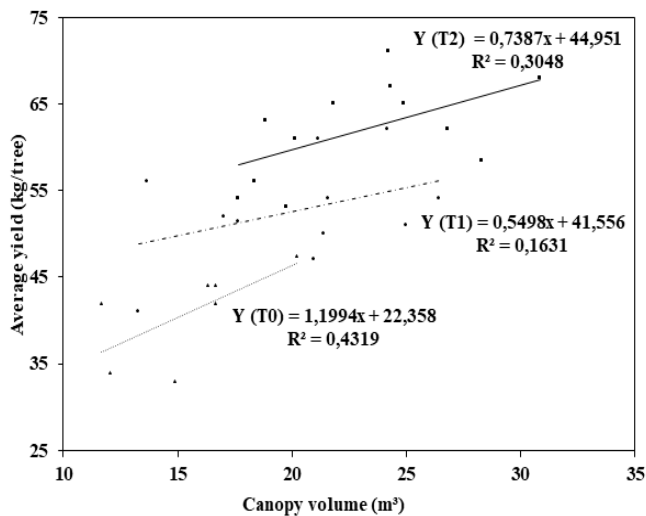


Figure 8. Fruit yield of Nules clementine trees related to canopy volume (T₀: Control (native nutrient), T₁: 270, T₂: 540 of N g/tree/year).

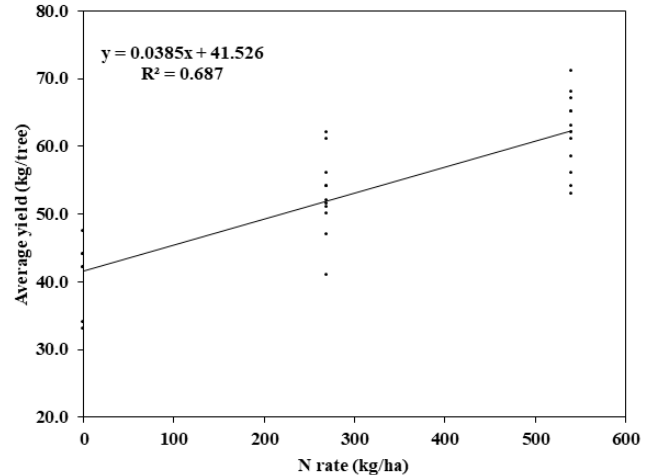


Figure 9. Fruit yield of Nules clementine trees related to Nitrogen rate.

Fruit quality variables: The study on Nules clementine fruit quality revealed that both nitrogen fertilization and rootstock genotype significantly impact various quality parameters. Trees grafted onto Carrizo citrange rootstock, especially with higher nitrogen treatment (540 g/tree), produced fruits with superior qualities such as heavier weight, larger diameter, higher juice content, fruit color index and improved solid soluble content compared to tetraploid Flhorag rootstock. The study also demonstrated a linear increase in acidity and solid soluble content with rising nitrogen levels, underscoring the influential role of nitrogen in enhancing fruit quality in Nules clementines.

The fruit quality variables were influenced by the nitrogen rate and rootstock genotype in Nules clementine. The heaviest fruits were obtained from Carrizo citrange (135.86±4.6g) trees under T₂ (by application of 540 gram of N per tree per year), whereas the lowest was from tetraploid Flhorag (102.77±4.1g) than Carrizo citrange rootstock in T₀ (control) (Table 6).

Nules's fruit diameter was affected by the N treatment and the rootstock. Application of 540 gram of nitrogen per tree yielded significant improvements in fruit size compared to improving fruit diameter compared to using T₁ (270 N g/ tree) treatment, demonstrating its high effectiveness. The highest values were observed on Carrizo citrange tree under T₂ (69.59±1.42 mm) while the minimum values were observed on tetraploid Flhorag trees control (T₀) (59.79±1.04 mm) (Table 6).

It is clear that from data in Tables 7, 8 and 9, varying N rate had a considerable effect on the juice content and the total acidity, Solid soluble content, Ripening Index and fruit color of Nules clementine examined in our study. Juice content ranged from (42.2±0.81%) on tetraploid Flhorag trees of T₀ to (51.72±0.7%) on Carrizo citrange trees of T₂ (540 N g/tree) (Table 7). The Solid soluble content ranged from (9.85±0.23)



Table 6. The effects of N fertilization and two citrus rootstocks on pomological characters: fruit weight and fruit diameter of Nules clementine (2016-2020).

	Fruit weight (g)			Fruit diameter (mm)		
	Nules/Flhorag			Nules/Flhorag		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
2016	92.99±1.71	112.29±4.17	134.00±2.31	57.42±2.26	61.31±0.15	64.51±0.81
2017	81.00±0.84	83.52±2.35	85.42±2.50	55.17±0.58	56.81±0.18	58.47±0.43
2018	118.33±4.41	131.00±4.64	137.25±5.22	63.15±1.73	64.98±0.98	68.18±0.97
2019	118.33±3.33	122.00±3.74	127.50±4.61	64.03±0.17	65.3±0.7	66.67±0.87
2020	103.2±5.14	127.94±2.80	138.38±6.17	59.17±1.00	69.5±1.34	74.41±1.32
Mean	102.77±4.10 b	118.67±3.80 a	128.21±4.12 a	59.79±1.04 c	64.44±0.98 b	67.69±1.10 a
	Nules/Carrizo citrange			Nules/Carrizo citrange		
2016	101.14±3.13	124.39±0.78	168.77±9.23	61.24±1.98	61.25±0.45	62.16±0.21
2017	82.76±0.76	84.71±1.11	88.29±3.47	56.58±0.51	58.52±0.24	61.07±0.94
2018	124.50±3.23	133.50±6.55	140.83±2.71	63.86±1.87	66.85±1.18	70.45±0.66
2019	112.50±5.20	126.67±5.11	135.00±3.65	63.76±1.17	65.39±0.51	67.49±0.64
2020	118.43±3.91	128.62±6.15	139.08±2.58	65.60±2.04	66.32±1.11	78.79±2.35
Mean	109.63±3.75 c	123.33±3.94 b	135.86±4.60 a	62.57±0.98 b	64.61±0.72 b	69.59±1.42 a

¹ In each row for each parameter studied, values with the same letter are not significantly different (Duncan test, p ≤ 0.05). Each value is the mean ±Standard Error.

Table 7. The effects of N fertilization and two citrus rootstocks on juice content, total acidity of Nules clementine (2016-2020).

	Juice content (%)			Acidity (%)		
	Nules/Flhorag			Nules/Flhorag		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
2016	39.79±2.36	42.10±0.4	46.85±0.62	0.64±0.02	0.86±0.01	0.93±0.01
2017	40.53±1.02	41.37±0.51	44.19±1.18	0.94±0.01	1.08±0.02	1.19±0.04
2018	44.69±2.32	46.59±0.65	48.42±1.19	0.9±0.03	0.96±0.03	1.05±0.03
2019	42.20±1.54	45.08±1.24	51.64±1.37	0.79±0.00	0.94±0.02	1.09±0.03
2020	43.76±0.38	45.75±0.46	50.24±1.11	0.75±0.01	0.85±0.01	0.97±0.02
Mean	42.20±0.81 c	44.64±0.54 b	48.95±0.72 a	0.81±0.03 c	0.93±0.02 b	1.04±0.02 a
	Nules/Carrizo citrange			Nules/Carrizo citrange		
2016	42.96±1.02	46.12±0.74	52.02±2.31	0.67±0.01	0.89±0.01	0.96±0.05
2017	42.38±0.48	43.26±1.04	45.96±0.10	1.02±0.01	1.12±0.01	1.29±0.01
2018	45.29±0.80	48.56±0.34	51.28±1.05	0.94±0.01	1.18±0.01	1.28±0.04
2019	43.35±0.87	48.00±1.02	52.57±1.18	0.95±0.03	1.06±0.02	1.21±0.01
2020	45.68±0.97	47.61±0.66	54.04±0.94	0.76±0.01	0.90±0.02	0.96±0.02
Mean	44.07±0.47 c	47.22±0.47 b	51.72±0.7 a	0.87±0.03 c	1.04±0.03 b	1.15±0.03a

¹ In each row for each parameter studied, values with the same letter are not significantly different (Duncan test, p ≤ 0.05). Each value is the mean ±Standard Error.

on Flhorag trees of T₀ to (11.63±0.24) on Carrizo citrange trees of treatment T₂ (Table 8).

Total acidity of Nules Clementine was also higher in fruits of Carrizo citrange (1.15±0.02 %) and tetraploid Flhorag (1.04±0.002%) trees of T₂ (540 N g/tree) than in the other treatments (Table 7). Whereas, Ripening Index of Nules Clementine was higher in fruits of Carrizo citrange (12.16±0.5) and tetraploid Flhorag (12.4±0.41) trees of control (T₀) than in the other treatments (Table 8).

The fruit color is affected by the rootstock genotype and the nitrogen rate. The l* color for the fruits of the trees T₂ (540 N

g/tree) grafted on Carrizo citrange has the highest l* value (61.91 ±0.52), compared to the others treatments. Fruits obtained on control trees (T₀) grafted on tetraploid Flhorag has the lowest values (57.56 ±1.84) compared to the others (Table 9).

The a* color of Nules Clementine was also higher in fruits of Carrizo citrange (25.94±2.02) and tetraploid Flhorag (26.02±1.7) trees of T₂ (540 N g/tree) than in the other treatments. Whereas, b* color was lower in fruits of tetraploid Flhorag (49.9±3.13) trees of control T₀ than in the other treatments (Table 9).



Table 8. The effects of N fertilization and two citrus rootstocks on Solid soluble content and Ripening Index of Nules clementine (2016-2020).

	Solid soluble content (SSC)			Ripening Index (RI)		
	Nules/Flhorag			Nules/Flhorag		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
2016	8.40±0.26	9.10±0.12	8.90±0.26	13.20±0.39	10.58±0.25	9.58±0.39
2017	9.73±0.03	10.33±0.26	10.40±0.17	10.37±0.08	9.59±0.41	8.72±0.17
2018	10.03±0.26	10.94±0.27	11.33±0.24	11.12±0.24	11.49±0.53	10.83±0.39
2019	10.10±0.06	10.6±0.09	11.13±0.15	12.74±0.11	11.35±0.29	10.21±0.26
2020	10.97±0.19	11.72±0.28	12.68±0.14	14.55±0.22	13.85±0.39	13.15±0.33
Mean	9.85±0.23 b	10.7±0.20 a	11.2±0.25 a	12.40±0.41 a	11.62±0.35 ab	10.83±0.34 b
	Nules/Carrizo citrange			Nules/Carrizo citrange		
2016	8.57±0.09	9.70±0.17	9.47±0.15	12.72±0.14	10.89±0.27	9.88±0.51
2017	10.13±0.07	10.53±0.18	11.13±0.13	9.95±0.07	9.41±0.21	8.61±0.02
2018	10.70±0.30	11.30±0.16	11.68±0.29	11.45±0.40	9.57±0.19	9.15±0.29
2019	10.13±0.32	11.20±0.11	11.57±0.18	10.77±0.62	10.57±0.24	9.55±0.19
2020	11.75±0.26	12.58±0.11	12.98±0.19	15.51±0.29	14.02±0.46	13.57±0.42
Mean	10.36±0.26 b	11.3±0.20 a	11.63±0.24 a	12.16±0.50 a	11.08±0.39 ab	10.38±0.41 b

¹ In each row for each parameter studied, values with the same letter are not significantly different (Duncan test ($p \leq 0.05$)). Each value is the mean ±Standard Error.

Table 9. The effects of N fertilization and two citrus rootstocks on the fruit coloration (CCI) of Nules clementine.

Variable	Fruit Color Index					
	Nules/Flhorag			Nules/Carrizo citrange		
	T ₀	T ₁	T ₂	T ₀	T ₁	T ₂
I*	57.56±1.84 b	59.40±0.96 ab	61.13±0.61 a	61.07±0.94 a	61.39±0.69 a	61.91±0.52 a
a*	10.67±4.54 b	15.64±2.95 b	26.02±1.70 a	19.52±3.17 a	22.82±2.27 a	25.94±2.02 a
b*	49.90±3.13 b	53.42±1.87 ab	57.60±1.01 a	56.49±1.64 a	57.40±1.25 a	58.52±0.93 a
CCI*	2.23±1.52 b	4.15±0.98 b	7.21±0.48 a	5.32±0.80 a	6.13±0.62 a	7.01±0.52 a

¹ In each row for each parameter studied, values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Each value is the mean ±Standard Error.

The CCI* fruits of Nules trees fertilizer under T₂ (540 N g/tree) and grafted on the Carrizo citrange and tetraploid Flhorag rootstocks has the highest CCI* value (7.01±0.52 and 7.21±0.48), compared to the others treatments. Fruits obtained on control trees (T₀) grafted on tetraploid Flhorag have the lowest values (2.23±1.52) (Table 9).

The Nitrogen fertilization influenced fruit quality variables, the acidity (%) and Solid soluble content (SSC) of Nules clementine fruits. They have a significant linear response to N fertilization as observed (Fig. 10 and 11). Acidity (%) and Solid soluble content (SSC) rise up with an increase in Nitrogen rate for Nules from 0.80 to 1.10 % for Acidity (%) (Fig. 10).

Citrus rootstocks genotype resulted a significant difference in most parameters of fruit quality. The fruit weight, fruit diameter, juice content, solid soluble content, total acidity and fruit color of Nules clementine fruits were greater for Nules trees grafted on Carrizo citrange rootstock than on tetraploid Flhorag rootstock (Table 6, 7, 8 and 9).

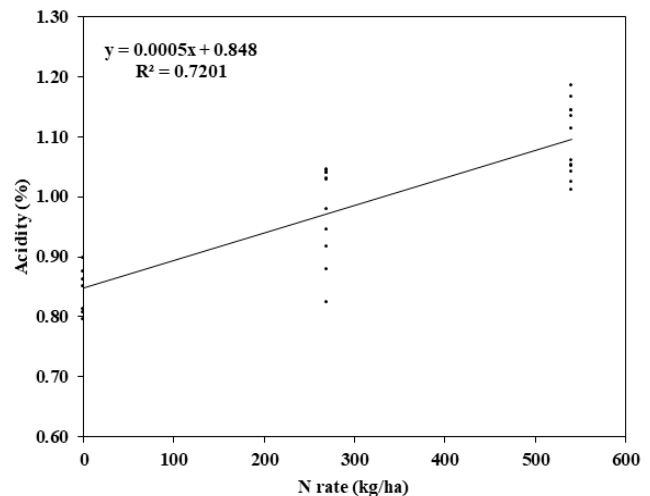


Figure 10. Acidity (%) of Nules clementine fruits related to Nitrogen rate.



Table 10. Pearson’s correlation matrix between vegetative growth, quality and yield variables of Nules clementine.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)
(1) N rate	1.00																			
(2) Tree height	0.63	1.00																		
(3) Canopy diameter	0.57	0.85	1.00																	
(4) SPAD	0.89	0.56	0.54	1.00																
(5) Canopy projectional unit area	0.57	0.85	1.00	0.54	1.00															
(6) Diameter of the variety	0.45	0.76	0.71	0.45	0.71	1.00														
(7) Diameter of the rootstock	0.37	0.79	0.73	0.35ns	0.73	0.92	1.00													
(8) TCSA variety	0.44	0.75	0.70	0.45	0.70	0.99	0.92	1.00												
(9) TCSA rootstock	0.38	0.79	0.73	0.37	0.73	0.90	0.99	0.92	1.00											
(10) Yield	0.83	0.73	0.70	0.78	0.70	0.64	0.60	0.64	0.61	1.00										
(11) Canopy volume	0.59	0.92	0.97	0.54	0.97	0.70	0.74	0.71	0.75	0.72	1.00									
(12) Yield/ canopy projectional unit area	0.68	0.36	0.19ns	0.64	0.19ns	0.31ns	0.27ns	0.33ns	0.29ns	0.82	0.27ns	1.00								
(13) Fruit weight	0.75	0.65	0.71	0.70	0.71	0.53	0.48	0.50	0.46	0.75	0.69	0.46	1.00							
(14) Fruit diameter	0.73	0.65	0.71	0.65	0.71	0.47	0.41	0.44	0.40	0.65	0.72	0.33ns	0.85	1.00						
(15) Juice content	0.84	0.81	0.78	0.74	0.78	0.66	0.64	0.67	0.65	0.87	0.83	0.60	0.76	0.79	1.00					
(16) Acidity	0.85	0.76	0.75	0.79	0.75	0.64	0.61	0.65	0.63	0.86	0.78	0.60	0.72	0.62	0.88	1.00				
(17) Solid soluble content (^o Brix)	0.63	0.73	0.84	0.62	0.84	0.56	0.55	0.53	0.52	0.66	0.82	0.24ns	0.78	0.88	0.80	0.64	1.00			
(18) Ripening Index	-0.60	-0.36ns	-0.26ns	-0.53	-0.26ns	-0.33ns	-0.30	-0.36	-0.34ns	-0.57	-0.31ns	-0.58	-0.28ns	-0.06ns	-0.46	-0.76	0.00ns	1.00		
(19) Fruit Color Index (CCI)	0.40	0.44	0.32ns	0.34ns	0.32ns	0.34ns	0.39	0.34ns	0.40	0.45	0.38	0.42	0.36ns	0.28ns	0.54	0.42	0.44	-0.17ns	1.00	
(20) Cumulative yield	0.72	0.51	0.56	0.65	0.56	0.34ns	0.28ns	0.37	0.32ns	0.85	0.59	0.75	0.60	0.50	0.67	0.74	0.45	-0.57	0.25ns	1.00

Correlation analysis: To compare such different treatments of Nules variety on Carrizo citrange and tetraploid Flhorag rootstocks, a correlation matrix was established based on Pearson’s correlation coefficients (r) and the variables studied (Table 10 and 11). The correlation analysis of Nules clementine variety on Carrizo citrange and tetraploid Flhorag rootstocks revealed several significant relationships among various growth and fruit quality parameters. Key findings include a positive and significant correlation between yield and yield/canopy projectional unit area ($r=0.82$, $p<0.0001$) and SPAD chlorophyll content ($r=0.78$, $p<0.0001$). As hypothesized, yield was strongly linked with cumulative yield ($r=0.85$, $p<0.0001$). While tree height has a same relationship with canopy volume ($r=0.92$, $p<0.0001$). Fruit weight and fruit diameter had a highly positive correlation ($r =0.85$, $p<0.0001$). In contrast, a negative correlation was found between Ripening Index and Fruit Color Index (CCI) ($r=-0.17$, $p=0.3798$). Similarly, a negative correlation was also found between Acidity and Ripening Index ($r=-0.76$, $p<0.0001$). Additionally, TCSA variety and TCSA rootstock were strongly positively correlated too ($r=0.92$, $p<0.0001$) (Table 10).

DISCUSSION

Vegetative growth: Our study demonstrates the significant impact of nitrogen fertilization and rootstock selection on vegetative growth in Nules clementine. The present research illustrates how nitrogen fertilization and the choice of citrus rootstock, specifically for Nules clementine, significantly influence various vegetative growth aspects. This effect was evident in tree height, canopy dimensions, rootstock and variety diameters, trunk cross-sectional area (TCSA) for both

rootstock and variety, canopy projectional unit area, and SPAD readings.

It was observed that increasing nitrogen levels led to a noticeable improvement in these growth parameters. The study by [Westwood and Roberts \(1970\)](#) supports the strong correlation between TCSA and tree height and canopy volume. Particularly, Carrizo citrange trees treated with a specific nitrogen dose (540 N g/tree) exhibited the highest TCSA values for both rootstock and variety. Comparable TCSA measurements were also noted in 'Clementine' mandarin grafted onto *C. volkameriana* ([Georgiou, 2002](#)). These findings align with those from [Bassal, \(2009\)](#) study, which reported that trees budded on Carrizo citrange and 'Swingle' citrumelo had greater heights than those grafted onto Cleopatra mandarin. [Kaplankiran et al. \(2005\)](#) also reported similar observations. The larger canopy volumes in Carrizo citrange-grafted trees, compared to other rootstocks, are believed to contribute to these results.

Yield: The five-year yield analysis revealed a positive correlation between nitrogen fertilization and fruit yield, particularly in Carrizo citrange. This rootstock when fertilized with a specific nitrogen treatment (540 N g/tree), yielded considerably more than Flhorag in the Nules region. This yield enhancement was found to be directly proportional to the increase in nitrogen levels highlighting the pivotal role of nitrogen in enhancing fruit set and yield. Among the various treatments, this specific nitrogen application (540 N g/tree) was the most effective in boosting yield. The period from 2016 to 2020 saw Carrizo citrange achieving the highest yield per canopy projection unit area.

The effect of nitrogen fertilization on fruit yield corroborates with findings from previous research ([Omari et al. 2020 a](#) and [b](#), [Sarooshi et al. 1991](#), [Fawzi et al. 1990](#) and [Zayan et al. 1989](#)). These studies underline a strong positive relationship



between nitrogen fertilization rate and overall fruit yield. Increasing nitrogen levels was observed to significantly enhance fruit set in citrus trees, thereby increasing the amount of fruit produced per canopy volume. These observations are consistent with the findings of [Du Plessis and Koen in 1984](#), who noted maximum fruit yield in orange trees at specific nitrogen and potassium levels (225 kg/ha of N + 310 kg/ha of K).

Previous studies on Coorg mandarin demonstrated that fruit yield could be improved by adjusting nitrogen rates, especially when grafted on Rangpur lime and trifoliolate rootstocks. The optimal application of 668g N/tree for trees grafted on Rangpur lime and 623g N/tree for those budded on trifoliolate stocks results in the highest fruit yield of Coorg mandarin. The NPK recommended fertilizer rate for achieving higher yields with good fruits quality was 670g N-100g P-400g K (per tree) for citrus scion budded on vigorous rootstocks and 625g N-100g P-400g K (per tree) for those grafted on dwarf rootstocks ([Anjaneyulu and Murthy 1984](#)). The 2018-2019 and 2020-2021 citrus growing seasons saw a decline in yields due to a combination of environmental, biological, and management factors. Adverse weather conditions like droughts, excessive rainfall, and unusual temperatures adversely affected citrus tree health and productivity. Problems such as root rot and growth pattern disruptions were noted. Pest challenges, particularly from whiteflies, were significant, leading to increased pesticide use. Orchard management issues, such as poor practices in irrigation, pruning, and pest control, coupled with the natural aging of trees, also contributed to this yield decline. The broader context of global climate change further influenced these outcomes.

Yield variations in citrus trees were also linked to the type of rootstock used. Nules clementine trees grown on Carrizo citrange rootstock showed higher yields compared to those on Flhorag rootstock. This contradicts previous findings by [Georgiou \(2000\)](#) regarding sour orange and Carrizo and Troyer citranges, and supports [Temiz \(2005\)](#) findings about Carrizo citrange's high yield for the Nova variety. [Reese and Koo \(1975\)](#) study also indicated that the highest yield for orange varieties, when grafted onto Rough lemon rootstock, was found at a Nitrogen rate of 202 kg per hectare.

The study also highlighted the rootstock genotype's influence on the tree's response to nitrogen fertilization, as noted in works by [Mattos \(2000\)](#) and [Quaggio et al. \(2004\)](#). Trees grafted on different rootstocks showed varying responses to nitrogen, suggesting that the rootstock's genotype plays a pivotal role in the tree's nutrient uptake and growth. [Mattos \(2000\)](#) found that sweet orange trees budded on Cleopatra rootstock exhibited a significantly higher response to N fertilization compared to those grafted on Rangpur lime rootstock ([Quaggio et al. 2004](#); [Davies and Albrigo, 1994](#)). This suggests that the rootstock genotype likely plays a

crucial role in determining the citrus trees' response to nitrogen fertilization, rather than the citrus cultivars.

Finally, the study revealed a direct correlation between canopy volume and fruit yield. Both Flhorag and Carrizo citrange rootstocks showed increased fruit yields with higher nitrogen levels, indicating the importance of appropriate fertilization management in citrus orchards for optimal fruit yield and quality. This is in line with [Mattos, \(2000\)](#) observations on the positive impact of increased nitrogen and potassium rates on fruit yield in trees grafted onto Rangpur lime or Cleopatra mandarin rootstocks.

Fruit quality variables: In the study of Nules clementine, various quality aspects of the fruit were observed to be affected by the applied nitrogen (N) rate and the genotype of rootstock. Specifically, administering 540 grams of nitrogen per tree per year was found to substantially enhance fruit characteristics such as size, weight, juice content, acidity, soluble solids, ripening index, and color (measured as CCI). Both acidity percentage and soluble solid content (SSC) showed a noticeable linear response to the applied nitrogen levels. This was particularly evident in the treatment referred to as T2, which combined 540 grams of N, 135 grams of P₂O₅, and 270 grams of K₂O per tree, leading to a marked improvement in the quality of Nules clementine fruits, irrespective of rootstock. The noticeable improvement in fruit size, juice content, and other quality parameters underlines the importance of balanced nutrition in citrus.

This enhancement in fruit quality is attributable to the crucial role played by nitrogen, phosphorus, and potassium in the synthesis and transfer of carbohydrates, which ultimately enhances fruit maturity. The beneficial influence of NPK on citrus fruit quality supported by previous research conducted by [Omari et al. \(2020a and b\)](#), [Huang et al. \(2000\)](#), and [Chen et al. \(1999\)](#) and is in line with observations made by [Huchche et al. \(1998\)](#). Various research, including works by [Reese and Koo \(1975\)](#), [Stewart et al. \(1961\)](#), [Stewart and Wheaton \(1965\)](#), and [Koo \(1979\)](#), have documented significant improvements in Total Soluble Solids (TSS) content in oranges with increasing nitrogen rates. However, studies like those of [Reitz and Koo \(1960\)](#), [Smith et al. \(1968\)](#), and [Deszyck et al. \(1958\)](#) have reported varying trends.

The genotype of the citrus rootstock also plays a significant role in determining the chemical characteristics of the fruits. For instance, Nules clementine fruits on Carrizo citrange rootstock were found to have better quality parameters compared to those on Flhorag rootstock, aligning with [Castle et al. \(1993\)](#) research. Similarly, [Demirkeser et al. \(2009\)](#) noted larger fruit size and weight in Nova mandarin grafted on Troyer and Carrizo citrange rootstocks than on sour orange rootstock, similar results founded by [Maazoun et al., \(2022\)](#) for Tunisian Maltese orange highlighting that C35 citrange rootstock resulted in higher fruit size and weight when compared to sour orange and *C. volkameriana* rootstocks.



Furthermore, the coloration of Nules clementine fruits, specifically their CCI* value, was observed to be influenced by both rootstock genotype and nitrogen application. Fruits from trees receiving the T2 treatment and grafted on Carrizo citrange and Flhorag rootstocks exhibited the highest CCI* values. This is in accordance with studies by Legua *et al.* (2013, 2011), which found optimal color in Lane late fruits on *Citrus macrophylla* and *Citrus volkameriana* rootstocks.

In contrast, other studies, such as those by Filho *et al.* (2007), reported no significant differences in juice quality based on rootstock genotype for Fallglo and Sunburst mandarin cultivars. Additionally, they observed that fruit weight and juice content were not influenced by the rootstock type, corroborating earlier research by the same authors and Demirköser *et al.* (2009) concerning Satsuma and Robinson mandarins.

Our investigation primarily concentrated on specific rootstocks and a set nitrogen level. To enhance our understanding of their effects on vegetative growth, it would be beneficial for future research to examine a more diverse array of rootstocks and nutrient combinations. Although this study highlights the significance of nitrogen, it does not entirely take into account the environmental and management factors. Incorporating these elements in future research would contribute to more comprehensive and sustainable cultivation methods.

Conclusion: The results of this study reveal the significant effect of N rates and rootstocks genotype on vegetative growth, yield and fruit quality of Nules clementine. The research demonstrates that increased nitrogen levels, particularly at 540 g/tree, notably enhance vegetative growth, as seen in the augmented tree height, canopy diameter, and volume, and trunk cross-sectional area (TCSA), especially in Carrizo citrange rootstock. The findings also reveal a noteworthy linear relationship between canopy volume and fruit yield, underscoring the importance of appropriate nitrogen management in citrus cultivation.

Carrizo citrange seem to be a more efficient rootstock for Nules variety than tetraploid Flhorag under experiment growing conditions. This rootstock, under higher nitrogen rates, also showed superior performance in yield and fruit quality, including size, juice content, and solid soluble content, compared to the tetraploid Flhorag rootstock. Nules trees grafted on Carrizo citrange produce a higher yield and the optimal fruit quality using 540g of N/tree/year. Selecting the right rootstock and optimizing nitrogen fertilization are crucial for enhancing both the quantitative and qualitative aspects of Nules clementine production.

In conclusion, the optimal NPK rate for achieving optimal yield and fruit quality of Nules clementine was 540 N-135 P₂O₅-270 K₂O g/tree/year. This study provides valuable insights for farmers and citrus producers to improve the growth, yield, and quality of Nules clementine fruit trees in

similar soil and climatic conditions. These findings adds to the expanding knowledge base on citrus farming, emphasizing the importance of choosing the right rootstocks and managing nutrients effectively.

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