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# Optimization of NPK levels of Clementine Sidi Aissa (*Citrus reticulata* Blanco) trees grafted on different citrus rootstocks

F.E. Omari[1], L. Beniken[1], A. Zouahri[2], R. Mrabet[3], H. Benaouda[1], R. Benkirane[4] and H. Benyahia[1]

[1]Regional Center of Agricultural Research of Kenitra, National Institute of Agricultural Research (INRA), N°14, Ave. Abou Temmam mailbox: 257 Kenitra, Morocco
[2]Regional Center of Agricultural Research of Rabat, National Institute of Agricultural Research (INRA), Ave. Mohamed Belarbi Alaoui mailbox: 6356 – Institutes, Rabat Morocco
[3]National Institute of Agricultural Research (INRA), Rabat- Morocco. Ave. Ennasr mailbox: 415 RP Rabat Morocco
[4]Plant Productions, Animal and Agro-Industry Laboratory, Ibn Tofail University, Faculty of Science of Kenitra, University Campus, mailbox 133 Kenitra, Morocoo
*Correspondence: fatimaezahra.omari@inra.ma

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**Abstract.** The present study aims to investigate the impact of various nitrogen concentrations on young Clementine Sidi Aissa citrus trees (*Citrus reticulata* Blanco), grafted on five citrus rootstocks namely Moroccan Carrizo citrange, French Carrizo citrange, Troyer citrange, *Citrus macrophylla*, and sour orange *(Citrus aurantium* L.). The experiment took place in greenhouses at the Experimental station of El Menzeh INRA-Morocco, with the young trees grown in containers. We applied five different nitrogen treatments (expressed as mg $L^{-1}$ of N-$P_2O_5$-$K_2O$): (0–0–0), (0–25–50), (25–25–50), (50–25–50), and (100–25–50). The split-plot experimental design was used with three replications.

The findings demonstrate that the nitrogen enrichment resulted in enhanced 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 and proline content. Optimal growth of the Clementine Sidi Aissa trees was observed under the 100–25–50 (mg $L^{-1}$ of N-$P_2O_5$-$K_2O$) treatment.

The study also found that leaf nitrogen concentration increased in line with the quantity of nitrogen added, whereas the percentages of phosphorous and potassium in the leaves decreased. The most significant growth increase across the majority of the studied parameters was noted in Clementine Sidi Aissa trees grafted on Moroccan Carrizo citrange and Troyer citrange rootstocks.

**Key words:** citrus, rootstock, mineral nutrition, leaf nutrient, vegetative growth.

## INTRODUCTION

Agricultural methods employed in the cultivation of citrus have undergone significant transformations over time. The focus has increasingly shifted towards enhancing the yield and quality of fruit using several strategies including the adoption of improved rootstocks, grafting techniques, and nutrient management (Alva et al., 2006a, 2006b, 2006c; Hawkesford et al., 2012). In citrus farming, the overall performance is largely dependent on the combination of rootstock and scion, which form the root and aerial parts of the plant, respectively. The rootstock exerts an influence on the scion's growth, impacting parameters such as photosynthesis (González-Mas et al., 2009), flowering, fruit quality (internal and external), canopy size, yield, and resistance to various factors (Forner-Giner et al., 2003; Castle et al., 2009; Kucukyumuk & Erdal, 2011; Parameshwar et al., 2018). It also affects an effective interaction between the rootstock and scion which is critical for the efficient translocation of water and minerals, promoting biomass production and resilience against biotic and abiotic stress, such as nutrient deficiency (Martínez-Ballesta et al., 2010; Schwarz et al., 2010).

Parameshwar et al. (2018) highlighted that the choice of rootstock significantly influences citrus growth, development, and crop yield. Different citrus rootstocks exhibit varying degrees of compatibility with different soil types, root dispersion patterns, and interactions with mycorrhizal fungi. Consequently, this variability leads to differences in content of the mineral elements in leaves of budded cultivars, ultimately impacting vegetative growth, fruit yield and quality. Within a budded tree, the type of rootstock also exerts an influence on various scion characteristics, including plant height, spread, and overall volume.

Enhanced vigor in citrus rootstocks' root systems leads to improved uptake of soil nutrients and water (Taylor & Dimsey, 1993; Lu et al., 2019). Thus, rootstocks influence on the photosynthetic capacity of the scion can play a crucial role in the overall performance of citrus plants, including vigor, crop load, and fruit characteristics (Jover, 2012). The type of rootstock used in a grafted tree significantly influences numerous features including leaf mineral components (Mattos Jr. et al., 2003; Toplu et al., 2008). Rootstocks play a vital role in the plant's capacity to absorb water and nutrients from the soil. Nutrient concentrations in grafted varieties can show variation even when cultivated under the same conditions. Therefore, understanding the influence of rootstocks on plant nutrient levels is essential for optimizing citrus fertilization programs.

Citrus cultivation today relies on composite trees that combine a rootstock and a scion. The scion draws water and mineral nutrients from the rootstock, which in turn relies on the scion for photosynthetic assimilation (Kocsis et al., 2012). Scions are selected based on fruit production-related criteria, such as size, yield, and quality parameters in grafted plants (Davis et al., 2008). Rootstocks are chosen for their disease and pest resistance, adaptability to pedoclimatic conditions, and agronomic performance when combined with the grafted variety. Iqbal et al. (1999) reported that the rootstock selection has a noteworthy impact on the mineral components found in the leaves of the kinnow mandarin grafted variety. Pestana et al. (2005) observed a significant variation in iron absorption based on the type of citrus rootstock.

Among various factors that impact citrus growth, including environmental conditions, management practices, and genetics, nutrient management, particularly nitrogen (N), is crucial for optimizing citrus production (Huang et al., 2021). Fertilization, particularly with Macronutrients encompassing elements of NPK, which plants necessitate in abundant amounts, serves as a significant procedural function for plant development and is essential for enhancing the fruit yield and quality (Hawkesford et al., 2012). The mineral status of citrus leaves markedly affects tree growth, fruit yield, and quality (Esteves et al., 2021). A balanced NPK amount plays a crucial role in tree structure and metabolic processes (Sinha & Tandon, 2020).

Nitrogen is vital for plant growth and development, involved in several physiological processes (Marschner, 2012; Sarvade et al., 2014). It plays a significant role in citrus trees' growth, canopy, fruit production, and quality, having a greater impact compared to other elements (Zekri & Obreza, 2003a, 2003b; Obreza et al., 2008). In cotton, Karydogianni et al. (2020) have shown that the combination of Urea, Nitrification Inhibitor (NI) and Urease Inhibitor (UI) led to the highest seed cotton yield and have a positively influenced the overall nitrogen uptake of the plant and notably enhanced the quality of the fiber.

Previous research has thoroughly examined diverse factors affecting the development of citrus fruits, encompassing environmental conditions, cultivation techniques, and genetic aspect (Davies & Albrigo, 1994). It has been shown that in young trees, nitrogen level could improve vegetative growth while reducing floral induction (Menino et al., 2003). Overabundance of nitrogen can promote excessive tree growth (Schumann et al., 2003; Alva et al., 2005a, 2005b), leading to the risk of groundwater contamination due to nitrate leaching from excess irrigation (Alva et al., 1998; He et al., 2000; Alva et al., 2006a, 2006b, 2008).

Nitrogen requirements for citrus have been found to vary widely, depending on factors such as tree age, rootstock, graft variety, and environmental conditions (Marschner, 2012; Mesquita et al., 2016; Carranca et al., 2018, Omari et al., 2020a). However, the optimal nutritional management of citrus seedlings have not yet fully understood, and there is a lack of recommendations on application of fertilizers in nursery conditions.

Most research on nitrogen management in citrus has focused on trees grown in fields (Nguyen & Tai, 2020; Omari et al., 2020b; Wassel et al., 2022); but studies on the production of citrus seedling under greenhouses conditions (nurseries) remain insufficient. It is imperative to understand the specific nutrient requirement of young citrus seedlings under such controlled environmental conditions.

Despite the agricultural importance of Clementine Sidi Aissa, research addressing the specific NPK requirements for trees grafted onto various citrus rootstocks remains notably scarce. While existing studies have focused on the general nutritional requirements of citrus trees, they often overlook to study the interaction scion/rootstock that arise from grafting scion on varying rootstocks. Indeed, prior studies have explored the impact of various rootstocks on citrus growth and nutrient uptake (Rameeh et al., 2019), but very few have aimed to identify the most suitable NPK doses for specific graft combinations. However, information regarding the optimal nutritional management of young citrus trees is lacking, leading to an inadequacy of precise fertilizer recommendations for nursery conditions. Consequently, the existing gap in knowledge significantly impedes the efforts to develop precise fertilization approaches aimed at optimizing the growth, yield, and fruit quality of citrus crop.

In light of the aforementioned research gap, this study hypothesizes that the optimization of NPK levels for Clementine Sidi Aissa plants will exhibit significant variations depending on the type of citrus rootstock employed. We propose that adopting a sustainable nutrient management strategy to meet crop needs throughout a growing season, considering the interactions between the scion and rootstock will result in superior vegetative growth, increased fruit yield, and elevated fruit quality compared to conventional fertilization methods.

This study aims to address this gap by investigating the effects of different nitrogen doses on the vegetative growth and nutritional status of young citrus trees grafted on selected rootstocks in greenhouse conditions. The objective is to determine the optimal nitrogen doses that result in optimal plant growth and adequate foliar nutrient concentrations. This will help in determining the most appropriate NPK doses for each grafting combination for young Clementine Sidi Aissa seedlings. Such information can assist citrus farmers in fine-tuning their nutrient management strategies to suit specific grafting combinations and achieve optimal growth and yield results.

## MATERIAL AND METHODS

### Plant material and growth conditions

The experiment was conducted in a greenhouse at the Experimental station El Menzeh (CRRA-Kenitra, INRA-Morocco), located 10 km north of Kenitra at an altitude of 25m and at a latitude of 34°64N.

The study was carried out on 3-year-old seedlings of the clementine variety Sidi Aissa grafted on five citrus rootstocks: Carrizo citrange B2 28608 (French), Troyer citrange B2 31655, *Citrus macrophylla*, Carrizo citrange (Morocco) and Sour orange (*Citrus aurantium* L.). The studied rootstocks come from the collection of germplasm and from the citrus seed park of the El Menzeh experimental station of the National Institute of Agricultural Research (INRA) of Kenitra in Morocco. These rootstocks were chosen for their importance in new citrus plantations and the Sidi Aissa variety was used as a reference for small fruits.

These grafted plants were grown in 10L plastic containers containing a substrate composed of a mixture of peat and sand at 1:2 ratio. This substrate have a pH of 7.09 and containing 2.25% organic matter, 267.57 mg of $P_2O_5$ $kg^{-1}$ and 268.11 mg of $K_2O kg^{-1}$.

The doses of fertilizer elements are provided from the nutrient solution (Maust & Williamson, 1994) which contains (in mg $L^{-1}$) (120 of Ca ($CaSO_4.2H_2O$); 40 Mg of ($MgSO_4.7H_2O$); 0.5 Mn ($MnCl_2.4H_2O$); 0.02 Mo ($NaMoO_4.2H_2O$); 0.02 Cu ($CuSO_4.5H_2O$); 0.05 Zn of ($ZnSO_4.7H_2O$); 0.5 B of ($BO_3$) and 5 Fe of (NaFeEDTA)). The source products used for NPK fertilizing elements are $NH_4NO_3$ for nitrogen, $H_3PO_4$ for phosphorus and $K_2SO_4$ for potassium. Five treatments of NPK fertilization, measured in milligrams per liter of Nitrogen-Phosphorus-Potassium (N-$P_2O_5$-$K_2O$), were evaluated: Treatment 0 ($T_0$) with 0–0–0, Treatment 1 ($T_1$) with 0–25–50, Treatment 2 ($T_2$) with 25–25–50, Treatment 3 ($T_3$) with 50–25–50, and Treatment 4 ($T_4$) with 100–25–50.

The plants were irrigated twice a week with the nutrient solution at the rate of 500 mL per containers throughout the study period.

The experiment was carried out using a split-plot design with three blocs replications, including the dose of NPK fertilizing elements as the main plot and the rootstock the subplot. Factor 1 (the main plot): N-$P_2O_5$-$K_2O$ doses at five levels ($T_0$, $T_1$, $T_2$, $T_3$ and $T_4$). Factor 2 (the subplot): Five-level of rootstock (*Citrus macrophylla*, Carrizo citrange (Morocco), Troyer citrange B2 31655, Carrizo citrange B2 28608 (French), Sour orange (*Citrus aurantium* L.).

**Measured parameters**

**Morphological parameters**

Plant Height was periodically measured with a measuring tape graduated in 1mm precision. The measurement were taken from the base of the stem to the shoot apical. Rootstock stem diameter at 5 cm below the grafting point and diameter of scion stem at 5 cm above the grafting point were measured by a digital caliper at 0.001 mm precision. Diameter and length of the four shoots (young shoots) marked per containers were measured using a caliper to 0.001 mm precision and a tape measure graduated to 1 mm accuracy at every 15 days throughout the trial period. Number of leaves of the four marked shoots per containers was counted biweekly throughout the trial period.

**Physiological parameters**

**The relative water content (RWC %):** One leaf per rootstock and per treatment was cut with petiole and weighed immediately to determine the fresh weight ($P_f$). These leaves are put in test tubes filled with distilled water (submerged petiole), the tubes are placed in the dark for 24 hours, then weighed again to obtain the weight at saturation (Psat). Then, the samples are put in an oven for 24 hours at 80°C, the dry weight (Psec) is thus obtained. The relative water content is calculated according to the following formula (1):

$$RWC\ (\%) = 100*(P_f - P_{sec}) / (P_{sat} - P_{sec}) \quad (1)$$

**Biochemical parameters**

**Concentration of total chlorophyll in the leaves:** To determine the concentration leaf in chlorophyll, we used the method of Arnon (1949) described by Esposti et al. (2003). Thus, the chlorophyll content is calculated using the following Eq. 2:

$$Ch\ total\ (mg\ L^{-1}) = 8.02\ (D.O_{663}) + 20.20\ (D.O_{645}) \quad (2)$$

**Proline content:** Proline determination was performed following the method described by Bates et al. (1973) based on the reaction of proline with ninhydrin. The values obtained are converted into proline content (mg $g^{-1}$ MF) from a standard curve, the relation of which is as follows (3):

$$Y = 0.1043*X \quad (3)$$

With: Y – represents the absorbance; X – represents the sodium concentration (mg $g^{-1}$ MF).

**Content of the mineral elements in leaves:** Once collected, the leaves are washed with distilled water, dried in an oven at a temperature of 60 to 65 °C for 3 days and then ground using a grinder. The shredded leaves thus obtained were analyzed at the soil chemical and physical analysis Laboratory of the Research Unit on the Environment and the Conservation of Natural Resources (INRA-Rabat). The mineral analyzes focused on N, P, K mineral elements.

**Statistical analysis**

Values are presented as mean ± SE (standard error). Data were subjected to analysis of variance (ANOVA) using the GLM procedure SAS statistical software (SAS Institute Inc., NC, USA, version 9). Two classification factors were used, the nitrogen dose factor and the rootstock factor. We utilized Duncan's multiple-range test to compare the differences in averages between treatments and rootstocks, with a significance level set at $P \leq 0.05$.

## RESULTS AND DISCUSSION

**Effect of NPK nutrient doses and rootstocks on citrus plant growth**

Statistical evaluation demonstrated a very highly significant effect of fertilizer treatment on plants height of the Sidi Aissa variety ($P < 0.0001$). The highest average height of the plants is recorded under $T_3$ (50–25–50) mg $L^{-1}$ with an average value of 109.73cm, on the other hand the lowest value was observed under control with an average value of 80.03cm (Fig. 1).

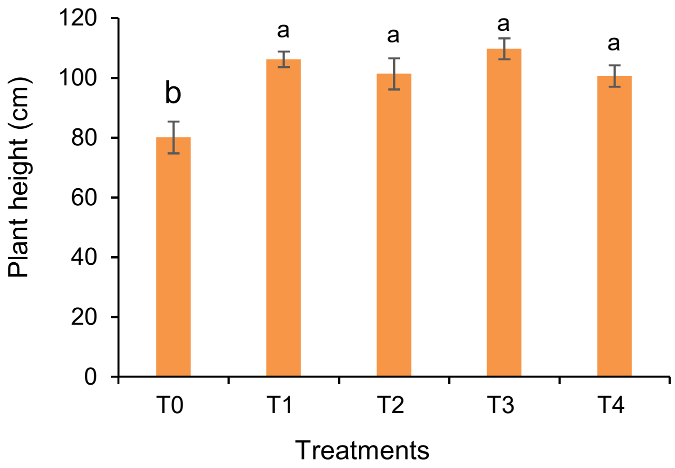


**Figure 1.** Effect of NPK doses of fertilizer elements on plant height of Sidi Aissa variety.
[*Values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE)].

Statistical analysis revealed a significant impact of NPK doses ($P = 0.0105$) and rootstock ($P = 0.0002$) on the rootstock stem diameter of Sidi Aissa trees. The highest rootstock stem diameter was noted in the plants under $T_4$ (100N- 25$P_2O_5$- 50$K_2O$) mg $L^{-1}$

(18, 12 mm) (Fig. 2). The highest rootstock stem diameter is observed in Troyer citrange B2 31655 (18.83 mm), while the lowest value is recorded in sour orange (*Citrus aurantium L.*) (16.77 mm) (Fig. 2).

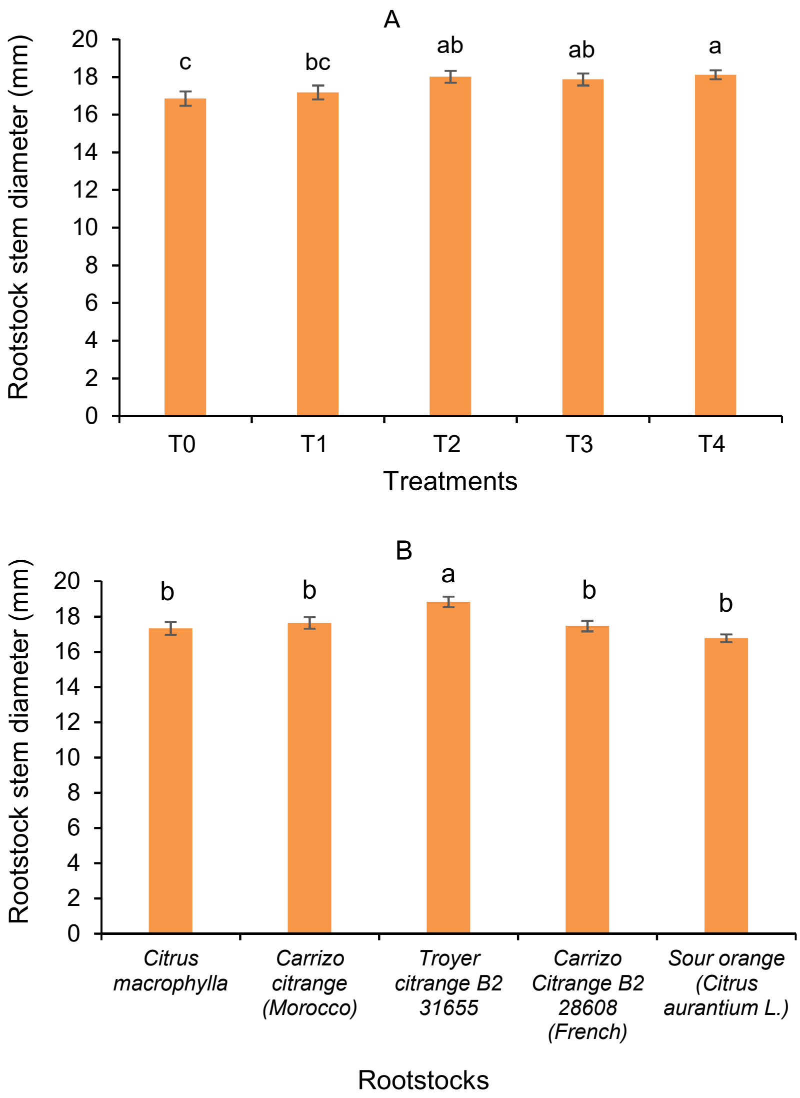


**Figure 2.** Effect of NPK doses (A) and citrus rootstocks (B) on rootstock stem diameter of Sidi Aissa variety.

[*Values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE)].

Statistical analysis revealed a significant impact of the rootstock on the scion stem diameter ($P = 0.0239$) of Sidi Aissa trees. The largest main scion stem diameter was observed in the rootstocks *Citrus macrophylla* (13.88 mm) and Troyer citrange B2 31655 (13.60 mm) (Fig. 3).

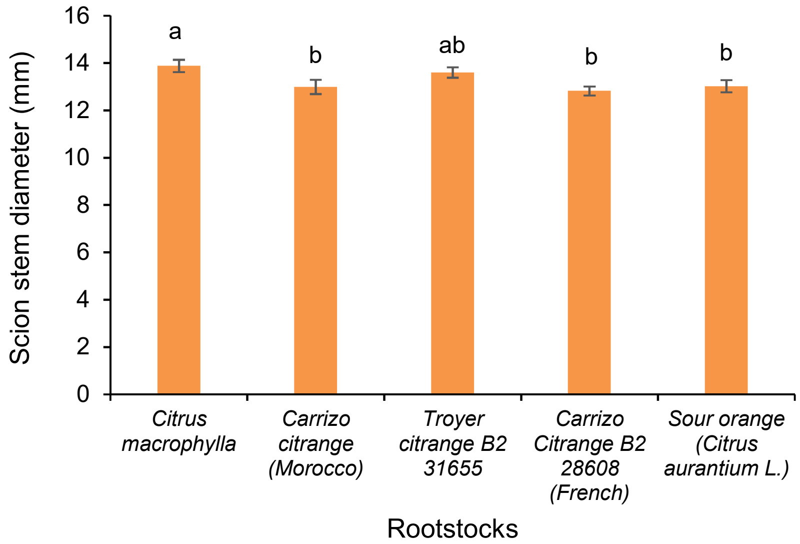


**Figure 3.** Effect of citrus rootstocks on scion stem diameter of Sidi Aissa.
[*Values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE)].

Statistical analysis revealed a significant impact of fertilizer treatments ($P = 0.0048$) and rootstock ($P < 0.0001$) on shoot diameter. The highest shoot diameter is noted in plants at $T_4$ (100N-25$P_2O_5$-50$K_2O$) (2.26 mm) (Fig. 4). The highest average shoot diameter was observed in sour orange (*Citrus aurantium* L.) and Carrizo citrange (Morocco) (2.27 mm) (Fig. 4).

A significant impact of NPK doses ($P < 0.0001$) and the rootstock ($P < 0.0001$) was showed on an average length of the young shoot, and a significant effect of the interaction fertilizer Treatments x Rootstock ($P = 0.0109$). Carrizo citrange (Morocco) showed the highest average length of the young shoot of Sidi Aissa (17.00 cm) in $T_4$ (100N- 25$P_2O_5$- 50$K_2O$), while the lowest value was noted in Carrizo citrange B2 28608 (French) (7.39 cm) under $T_0$ (Table 1).

**Table 1.** Effect of NPK doses and rootstocks on the average length of young shoot (cm) of clementine Sidi Aissa

| Rootstocks | Treatments N-$P_2O_5$-$K_2O$ (mg $L^{-1}$) | | | | |
|---|---|---|---|---|---|
| | T0 | T1 | $T_2$ | $T_3$ | $T_4$ |
| *Citrus macrophylla* | 8.82 ± 1.19b* | 9.54 ± 0.57b | 9.93 ± 0.74b | 10.17 ± 0.99b | 14.17 ± 1.96a |
| Carrizo Citrange (Morocco) | 10.11 ± 0.76b | 9.25 ± 0.66 b | 11.08 ± 0.83b | 13.69 ± 0.92a | 17.00 ± 1.46a |
| Troyer Citrange B2 31655 | 8.56 ± 0.73b | 11.73 ± 0.65a | 10.63 ± 0.68b | 10.00 ± 1.08b | 14.25 ± 1.05a |
| Carrizo Citrange B2 28608 (French) | 7.39 ± 0.58b | 10.92 ± 0.67ab | 10.54 ± 1.00b | 12.54 ± 0.96a | 13.75 ± 1.51a |
| Sour orange (*Citrus aurantium* L.) | 13.26 ± 1.22a | 11.57 ± 0.69a | 15.71 ± 1.19a | 14.29 ± 0.83a | 16.79 ± 1.12a |

[*The means of the same column assigned by the same letter do not differ significantly at the 5% level according to Duncan's test. Each value is the mean ±Standard Error].

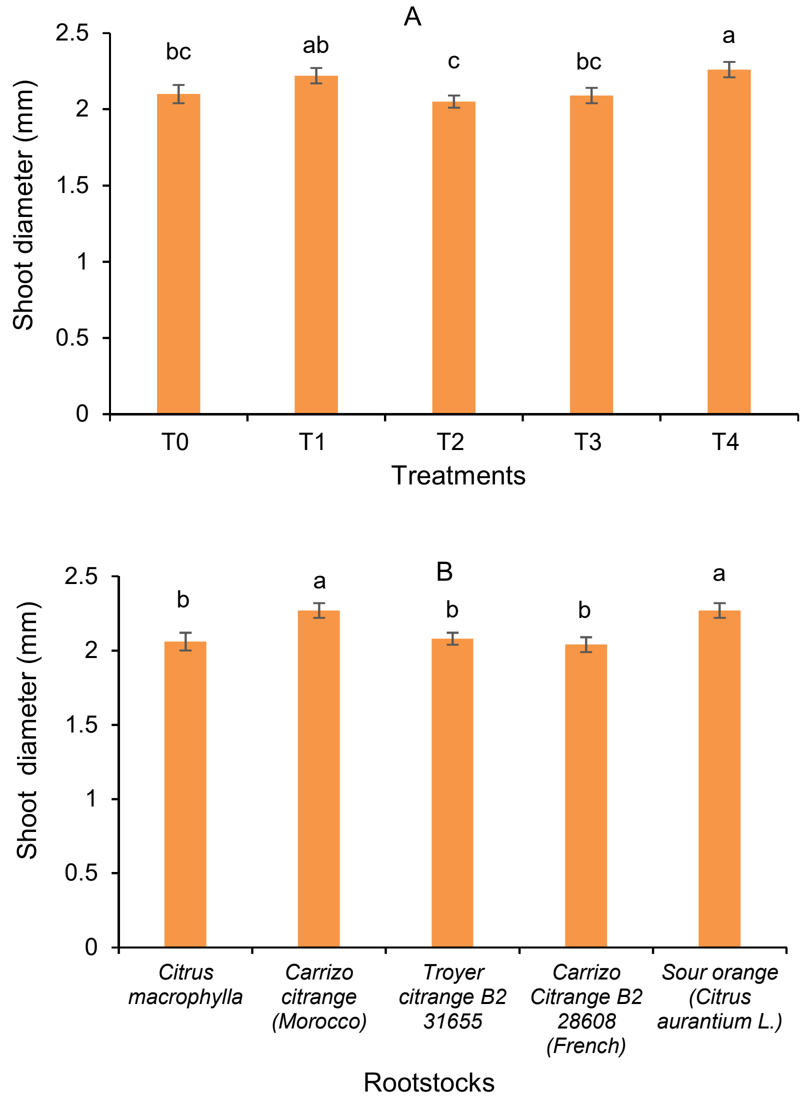


**Figure 4.** Effect of NPK doses (A) and citrus rootstocks (B) on young shoot diameter of Sidi Aissa variety.

[*Values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE)].

A significant impact of the fertilizer treatments ($P < 0.0001$), the rootstock ($P = 0.0061$) and the interaction Fertilizer Treatments x Rootstocks ($P = 0.0051$) was shown on the average number of leaves per young shoot of Sidi Aissa variety. Under $T_4$ ($100N\text{-}25P_2O_5\text{-}50K_2O$) the highest average number of leaves per young shoot was recorded for Carrizo citrange B2 28608 (French) and Sour orange (*Citrus aurantium* L.) which is 20 leaves, whereas the lowest value was noted in the Troyer citrange B2 31655 (6 leaves) under the control treatment $T_0$ (Table 2).

**Table 2.** Effect of NPK doses and rootstocks on the average number of leaves per young shoot of plants of Sidi Aissa clementine

| Rootstocks | Treatments $N\text{-}P_2O_5\text{-}K_2O$ (mg $L^{-1}$) | | | | |
|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| *Citrus macrophylla* | 9 ± 1.58ab* | 8 ± 0.87a | 11 ± 1.33a | 8 ± 0.78b | 13 ± 2.07b |
| Carrizo Citrange (Morocco) | 11 ± 1.38a | 10 ± 1.41a | 9 ± 1.50ab | 9 ± 0.98ab | 13 ± 1.31b |
| Troyer Citrange B2 31655 | 6 ± 0.57b | 9 ± 1.15a | 8 ± 1.06b | 9 ± 1.17ab | 15 ± 1.67ab |
| Carrizo Citrange B2 28608 (France) | 8 ± 1.05ab | 10 ± 0.94a | 10 ± 1.18ab | 9 ± 0.86ab | 20 ± 1.85a |
| Bigaradier (*Citrus aurantium* L.) | 12 ± 2.25a | 9 ± 0.94a | 9 ± 0.87ab | 11 ± 1.17a | 20 ± 2.29a |

[*The means of the same column assigned by the same letter do not differ significantly at the 5% level according to Duncan's test. Each value is the mean ±Standard Error].

NPK doses has a significant impact on plants height of Sidi Aissa variety. Omari et al. (2012) showed that the nitrogen dose significantly affects the height growth of citrus rootstock plants. The height of the plant increases with the increase in dose of nitrogen applied from 0 to 5 mM. The highest values were noted in the plants having received the dose 5mM of nitrogen (*Citrus macrophylla* with 30.68 cm, *Citrus volkameriana* B2 28613 with 29.58 cm and Citrumelo 4475 AB6 A4 with 28.79 cm). Ouma (2006) demonstrated that the nitrogen dose has a highly significant effect on the height growth of citrus plants (Rough lemon). Thus, this author showed that citrus plants responded positively to increasing nitrogen doses. The height of the stem increased from 44 to 65cm by increasing the dose of nitrogen from 6 to 24g. The positive effect of nitrogen on growth parameters has also been reported in orange plants under nursery conditions (Maust & Williamson, 1994; Guazzelli et al., 1996; De Campos Bernardi et al., 2000). Kakabouk et al. (2020) have been reported that varying Nitrogen treatments significantly influenced the root density, surface area, and volume of camelina plants, and the highest impact was seen at 100 days post-treatment with 90 ppm Nitrogen, affecting plant leaf area, growth, yield, and 1,000 seed weight. Al-Jilihawi & Merza (2020) reported that treating lemon saplings with mineral NPK at a concentration of 2.0 g $L^{-1}$ significantly boosted stem diameter, total leaf area, shoot and root dry weight.

The highest rootstock stem diameter of the plant was noted under $T_4$ ($100N\text{-}25P_2O_5\text{-}50K_2O$) mg $L^{-1}$ in Troyer citrange B2 31655. The largest main scion stem diameter of the variety was observed in *Citrus macrophylla* and Troyer citrange B2 31655. This result agrees with what was found in the work of Esposti et al. (2003) conducted on seedlings of two citrus varieties grafted onto different rootstocks, which reported that increasing the nitrogen dose significantly affected growth (expressed in stem height, collar diameter, leaf, stem and root dry biomass, number of leaves per plant and leaf area) and leaf chlorophyll content.

The highest shoot diameter is noted in plants at $T_4$ ($100N\text{-}25P_2O_5\text{-}50K_2O$) in sour orange (*Citrus aurantium* L.) and Carrizo citrange (Morocco). Carrizo citrange (Morocco) showed the highest average length of the young shoot of Sidi Aissa in $T_4$ ($100N\text{-}25P_2O_5\text{-}50K_2O$). Our results are in line with Lu et al. (2004) who showed that young shoot length of citrus seedlings decreased by 21.3% in the absence of nitrogen in the nutrient solution and that by just 0–6.1% when the seedlings were been treated without the application of potassium and phosphorus, respectively. On the other hand, these researchers reported that the growth of citrus plants increases accordingly with rising N dose. Also, Maust & Williamson (1991) showed that Nitrogen dose applied to citrus plants in the nursery has a highly significant effect on shoot length. The shoot length of 'Hamline' variety grafted on two rootstocks (Cleopatra mandarin and Carrizo citrange) responded significantly to the increase in the nitrogen dose. The maximum shoot length recorded on Cleopatra mandarin and Carrizo citrange rootstock (96.30 cm and 78.46 cm, respectively) under 100 ppm and 50 ppm, respectively. Alkhafaji & Khalil (2019) reported that lemon trees grafted on *Citrus aurantium* L. exhibited greater plant height, whereas trees grafted on *Citrus volkameriana* demonstrated higher rates in rootstock, scion diameter, and shoot number.

A significant effect of fertilizer treatments and rootstock on the average number of leaves per young shoot of Sidi Aissa variety has demonstrated. Our results are in agreement with those reported by Ouma (2006) who showed that the dose of nitrogen has a highly significant effect on the growth of citrus plants (Rough lemon) and the number of leaves per plant increased from 155 to 167 from 6 to 24 g of N/container.

Shaban & Mohsen (2009) revealed that the rootstock has a highly significant effect on the number of leaves per plant, growth (stem height), leaf area, root length, fresh and dry biomass, chlorophyll content and NPK leaf concentration. Thus, the maximum values of these parameters were observed in *Citrus volkameriana* compared to Sour orange which they recorded the minimum values for these parameters.

Chaudhry & Naveed (1992) studied the effect of rate of nitrogen application and their application date on leaf formation and yield of sweet lemon (*Citrus limettioides*). The nitrogen dose has a highly significant effect on number of leaves per shoot. Increasing the dose of nitrogen from 0.5 to 1.5 kg of N tree$^{-1}$ significantly improved the number of leaves per shoot from 8.2 to 10.

Another experiment conducted by Hafez (2006) highlighted the effect of different rootstocks on growth parameters in citrus. Thus, he compared the vegetative parameters of six citrus rootstocks. Troyer citrange has the highest stem height (31.1cm) and the lowest is observed in *Citrus volkameriana* (13.2cm). Lime rangpur had the highest number of leaves per plant (45.4), while *Citrus volkameriana* and Sour orange had the lowest number of leaves (14.8). The maximum leaf area is recorded in Troyer citrange (8.2 cm$^2$) and the lowest is observed in Sour orange and *Citrus volkameriana* (4.6 cm$^2$).

**Effect of NPK doses and rootstocks on Relative Water Content (RWC), total chlorophyll concentration and total proline content in the leaves**

Statistical analysis revealed a significant impact of the fertilizer treatments ($P = 0.0183$) on Relative Water Content of Sidi Aissa clementine leaves. The highest Relative Water Content value was recorded in the plants treated with $T_4$ (100N-25$P_2O_5$-50$K_2O$) treatment with a percentage of 86.07%. On the other hand, the lowest value was noted in the control $T_0$ with a percentage of (66.27%) (Fig. 5).

Statistical analysis revealed a significant impact of fertilizer treatments on leaf chlorophyll content ($P < 0.0001$). The Sidi Aissa clementine plants treated with $T_4$ (100N-25$P_2O_5$-50$K_2O$) represent the highest value of the total chlorophyll content (14.70 mg L$^{-1}$). Whereas, the lowest value (6.57 mg L$^{-1}$) was observed under control (Fig. 6).

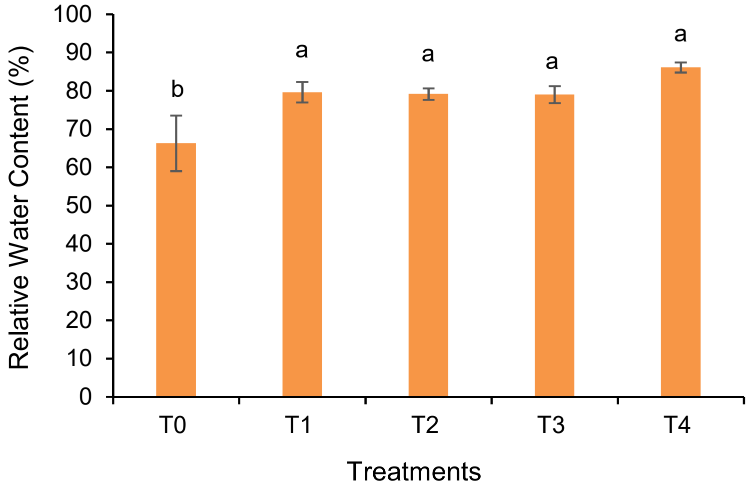


**Figure 5.** Effect of NPK doses on average Relative Water Content (RWC) of Sidi Aissa variety. [*Values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE)].

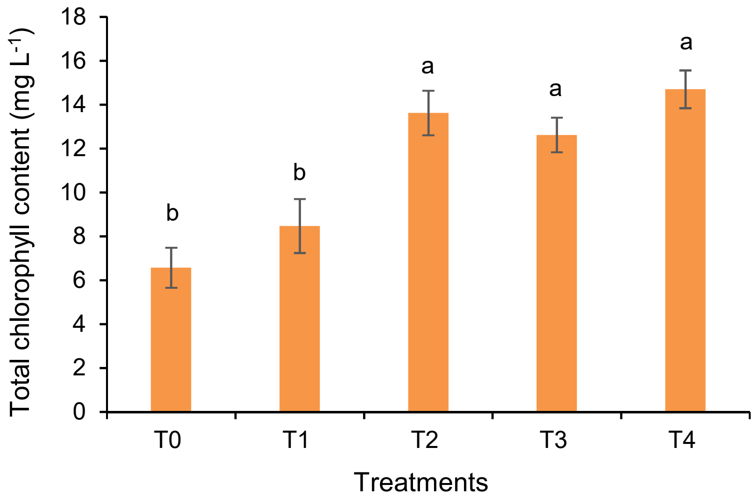


**Figure 6.** Effect of NPK doses on leaf total chlorophyll concentration of Sidi Aissa variety. [*Values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE)].

A significant effect of the fertilizer treatments ($P < 0.0001$), the rootstock ($P < 0.0001$) and the interaction of fertilizer doses x Rootstock ($P < 0.0001$) on the leaf content in proline. The highest proline content was recorded in *Citrus macrophylla* (0.10 mg $L^{-1}$) under the $T_3$ (50 N-25$P_2O_5$-50$K_2O$), while the lowest value was noted in Troyer citrange B2 31655 (0 mg $L^{-1}$) under the control $T_0$ (Table 3).

**Table 3.** Effect of NPK doses and rootstocks on the total proline content (mg $g^{-1}$) in the leaves of Sidi Aissa plants

| Rootstocks | Treatments N-$P_2O_5$-$K_2O$ (mg $L^{-1}$) | | | | |
|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| *Citrus macrophylla* | 0.02 a* | 0.02 a | 0.04 a | 0.10 a | 0.07 b |
| Carrizo citrange (Morocco) | 0.01 bc | 0.02 a | 0.05 a | 0.04 c | 0.06 b |
| Troyer citrange B231655 | 0.00 c | 0.03 a | 0.04 b | 0.05 b | 0.07 b |
| Carrizo citrange B2 28608 (French) | 0.01 b | 0.02 a | 0.03 d | 0.05 b | 0.04 c |
| Sour orange (*Citrus aurantium* L.) | 0.01 b | 0.01 a | 0.04 c | 0.04 c | 0.07 a |

[*The means of the same column affected by the same letter does not differ significantly at the 5% level according to Duncan's test. Each value is the mean ±Standard Error].

NPK dose showed significant impact on Relative Water Content of Sidi Aissa clementine leaves. The highest Relative Water Content was recorded in the plants treated with 100N-25$P_2O_5$-50$K_2O$. Omari et al. (2012) reported that the application of nitrogen doses significantly affects the relative water content (RWC). The RWC values increase with increasing of nitrogen dose from 0 to 5 mM and that the rootstocks *Citrus volkameriana* B2 28613 (90.48%), sour orange (*Citrus aurantium* L.) (91.76%), and *Citrus macrophylla* (91.80%) showed the highest percentages of relative water content under treatments $T_1$ (0Mm of N), $T_2$ (1mM of N) and $T_3$ (5 mM of N), respectively.

Sidi Aissa clementine plants treated with $T_4$ (100N-25$P_2O_5$-50$K_2O$) represent the highest value of total chlorophyll leaf content (14.70 mg $L^{-1}$). Omari et al. (2012) reported that the application of nitrogen doses and rootstock significantly affects the concentration of total chlorophyll in the leaves of citrus rootstocks and that the 5 mM dose of N constitutes the treatment which recorded the highest value (11.06 mg $L^{-1}$) in *Citrus macrophylla* rootstock. Bondada & Syvertsen (2003) reported that the nitrogen dose significantly affects the concentration of chlorophyll in the leaves of the Cleopatra mandarin. The increase of nitrogen dose induced an increase in the concentration of chlorophyll in the leaf tissues of this rootstock. A positive and highly significant correlation was established between the applied nitrogen fertilizer and the total chlorophyll concentration of the leaves of Cleopatra mandarin plants. The increase of nitrogen dose induced a significant rise of the concentration of total chlorophyll in the leaves of 'Hamlin' variety grafted on the citrumelo Swingle. The change from 0 to 218 g of nitrogen per tree caused an increase in chlorophyll of 0.05 to 0.65 mmol $m^{-2}$ in the leaves of 'Hamlin' variety (Bondada & Syvertsen, 2005).

The highest proline content was recorded in *Citrus macrophylla* rootstock under the $T_3$ (50N-25$P_2O_5$-50$K_2O$). Omari et al. (2012) reported that the proline content of leaves of citrus rootstocks in total soluble sugars was markedly affected by the nitrogen dose. The latter increases in parallel with the increase in the nitrogen dose from 0 to 5 mM and that Carrizo citrange recorded the highest value of the sugar content under the dose of 5 mM of nitrogen.

**Effect of NPK doses and rootstocks on content of the mineral elements in leaves**

Statistical analysis revealed a significant impact of the fertilizer treatments and the rootstock on the leaf nitrogen content of Sidi Aissa variety ($P < 0.0001$) and a highly significant effect of the interaction between rootstocks and treatments ($P = 0.0049$). The nitrogen content of Sidi Aissa clementine leaves is clearly influenced by the nitrogen dose. Indeed, the leaf nitrogen concentration increases progressively and linearly with the increase in the nitrogen dose from 0 to 100 mg $L^{-1}$ in all the rootstocks studied (Table 4). Under the $T_3$ ($50N-25P_2O_5-50K_2O$) treatment, the concentration of the leaves of Sidi Aissa variety in nitrogen in all studied citrus rootstocks were in the optimal range according to the standards interpreting of leaf analysis of Hanlon et al. (1995). However, under the $T_1$ ($0N-25P_2O_5-50K_2O$) treatment the nitrogen content of the leaves is considered to be at deficient levels in the different Sidi Aissa/rootstock associations (Table 4). This decrease in leaf nitrogen concentration in $T_2$ **(**$25N-25P_2O_5-50K_2O$**)** may be related to the high phosphorus fertilization application.

The nitrogen concentration of Sidi Aissa clementine leaves is also influenced by the rootstock. In fact, a variability in leaf nitrogen concentration is clearly observed within the different rootstocks with respect to the same nitrogen dose (Table 4). *Citrus macrophylla* rootstock recorded the lowest leaf nitrogen concentration under the control, which is around 1.157%. While, sour orange (*Citrus aurantium* L.) with 3.487%,Carrizo citrange B2 28608 (French) with 3.303% and Carrizo citrange (Morocco) with 3.3% showed the highest nitrogen content of Sidi Aissa clementine leaves under the treatment $T_4$($100N-25P_2O_5-50K_2O$) (Table 4).

**Table 4.** Effect of NPK doses and rootstocks on leaf N content of Sidi Aissa

| Rootstocks | Leaf N content (%) | | | | |
|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| *Citrus macrophylla* | 1,157 ± 0.022c* | 1,307 ± 0.112b | 2,200 ± 0.231ab | 2,520 ± 0.127cd | 2,993 ± 0.118bc |
| Carrizo citrange (Morocco) | 1,470 ± 0.023b | 1,507 ± 0.118ab | 2,347 ± 0.089a | 2,910 ± 0.104ab | 3,300 ± 0.057ab |
| Troyer citrange B2 31655 | 1,160 ± 0.115c | 1,530 ± 0.017ab | 1,793 ± 0.003c | 2,713 ± 0.008bc | 2,843 ± 0.026c |
| Carrizo citrange B2 28608 (French) | 1,683 ± 0.003a | 1,877 ± 0.003a | 2,397 ± 0.118a | 3,113 ± 0.014a | 3,303 ± 0.003ab |
| Sour Orange (*Citrus aurantium* L.) | 1,393 ± 0.003b | 1,473 ± 0.274ab | 1,880 ± 0.069bc | 2,343 ± 0.014d | 3,487 ± 0.245a |

[*The means of the same column affected by the same letter does not differ significantly at the 5% level according to Duncan's test. Each value is the mean ±Standard Error].

A significant effect of the fertilizer treatments showed on the leaf phosphorus content ($P < 0.0001$). The phosphorus concentration of Sidi Aissa clementine leaves is clearly influenced by the nitrogen dose. A negative relationship and antagonism has been demonstrated between the phosphorus concentration of the leaves and the nitrogen dose applied and it is clearly noted that the phosphorus content of the leaves decreases with the increase in the nitrogen dose of $T_1$ ($0N-25P_2O_5-50K_2O$) to $T_4$ ($100N-25P_2O_5-50K_2O$) in all the rootstocks studied while remaining at optimal levels (Fig. 7).

The Sidi Aissa clementine plants treated with $T_1$ ($0N-25P_2O_5-50K_2O$) and $T_2$ ($25N-25P_2O_5$ - $50K_2O$) represent the highest value of the phosphorus content of the leaves (0.185% and 0.179%, respectively). Whereas, the lowest value was observed in the control plants $T_0$ with 0.126% of leaf phosphorus content (Fig. 7).

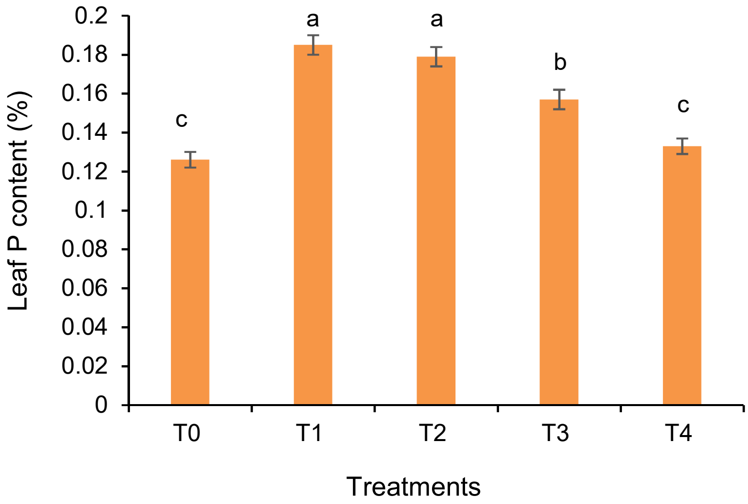


**Figure 7.** Effect of NPK doses and rootstocks on leaf P content of Sidi Aissa.
[*Values with the same letter are not significantly different (Duncan test, $p \leq 0.05$). Error bars represent standard errors of the mean (±SE)].

Statistical analysis revealed a significant impact of the fertilizer treatments ($P < 0.0001$), the rootstock ($P < 0.0001$) and the interaction of Fertilizer treatments x Rootstock ($P < 0.0001$). The leaf potassium content of Sidi Aissa is clearly influenced by the nitrogen dose. The leaf potassium concentration varies according to the interaction between the applied of N and K nutrients. A negative correlation was demonstrated between the dose of nitrogen applied and the leaf potassium concentration in Sidi Aissa plants. It is clearly noted that the leaf potassium content decreases with the increase in the dose of nitrogen from $T_1$ (0N-25$P_2O_5$-50$K_2O$) to $T_4$ (100N-25$P_2O_5$-50$K_2O$) in all the rootstocks studied while remaining at optimal levels sufficient for the proper development of young citrus plants (Table 5).

**Table 5.** Effect of NPK doses and rootstocks on leaf K content of Sidi Aissa

| Rootstocks | Leaf K content (%) | | | | |
|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| *Citrus macrophylla* | 1,167 ± 0.01a* | 2,333 ± 0.193b | 1,820 ± 0.104b | 1,710 ± 0.000b | 1,657 ± 0.078 a |
| Carrizo citrange (Morocco) | 1,000 ± 0.115b | 2,943 ± 0.003a | 2,270 ± 0.219a | 1,867 ± 0.014a | 1,543 ± 0.009ab |
| Troyer citrange B2 31655 | 1,160 ± 0.023a | 1,980 ± 0.011b | 1,787 ± 0.003b | 1,600 ± 0.058c | 1,217 ± 0.061c |
| Carrizo citrange B2 28608 (French) | 1,240 ± 0.023a | 2,860 ± 0.052a | 1,957 ± 0.020ab | 1,733 ± 0.037b | 1,363 ± 0.101bc |
| Sour Orange (*Citrus aurantium* L.) | 1,203 ± 0.02a | 2,733 ± 0.147a | 1,947 ± 0.032ab | 1,690 ± 0.058bc | 1,383 ± 0.049bc |

[*The means of the same column affected by the same letter does not differ significantly at the 5% level according to Duncan's test. Each value is the mean ±Standard Error].

Under the treatments $T_1(0N-25P_2O_5-50K_2O)$ and $T_2(25N-25P_2O_5-50K_2O)$ the leaf potassium concentration of Sidi Aissa in all citrus rootstocks studied was higher according to the interpretation standards of leaf analysis by Hanlon et al. (1995). However, under the $T_4$ treatment ($100N-25P_2O_5-50K_2O$) the leaf potassium content decreased significantly but remained within the optimal range in the Sidi Aissa plants/different rootstocks (Table 5).Carrizo citrange rootstock (Morocco) recorded the lowest leaf potassium concentration under the control treatment $T_0$(1%). Sour orange (*Citrus aurantium* L.) (2.733%), Carrizo citrange B2 28608 (French) (2.860%) and Carrizo citrange (Morocco) (2.943%) showed a higher leaf potassium content of Sidi Aissa clementine under $T_1$ ($0N-25P_2O_5-50K_2O$) (Table 5).

The leaf nitrogen content of Sidi Aissa clementine is clearly influenced by the nitrogen doses. The leaf nitrogen concentration increases linearly with the increase in the nitrogen dose from 0 to 100 mg $L^{-1}$ in all the rootstocks studied. These results are in agreement with those obtained by many researchers. Thompson & White (2004) reported that leaf nitrogen content of citrus trees was positively correlated with increasing applied nitrogen rate.

The nitrogen concentration of Sidi Aissa clementine leaves is also influenced by the rootstock. In fact, a variability in leaf nitrogen concentration is clearly observed within the different rootstocks with respect to the same nitrogen dose. Our results are in agreement with those reported by Jahromi et al. (2012) who reported that the type of rootstock has a significant influence on the total nitrogen concentration in the leaves of citrus varieties (Sweet lime, Mexican lime, Valencia and orange Washington navel, Kinnow mandarin and Orlando tangelos). The maximum total N concentration (2.12% dry matter) was in Sour orange and the lowest (1.77% dry matter) in Mexican lime and *C. volkameriana* rootstocks.

The type of rootstocks affects more than twenty horticultural properties, among these properties is the leaf mineral element concentration of citrus varieties and each particular scion receives a varying amount of the mineral elements from a special rootstock (Waqar et al., 2007). Rootstocks directly influence the capacity of the grafted plant to absorb water and nutrients from the soil (Richardson et al., 2003).

Sorgona et al. (2006) reported that the highest absorption and total nitrogen concentration is observed in Sour orange and weakest in sweet orange (Sweet orange). Thus, sour orange is very suitable for soils with a low nitrogen dose. On the contrary, Toplu et al. (2008) reported that the highest leaf nitrogen concentration is observed in Carrizo citrange and the lowest in sour orange.

Iqbal et al.(1999) evaluated the effect of rootstock type on the total N concentration of Kinnow Mandarin leaves, they found that the highest total N concentration was in the leaves of varieties grafted on the Citrumelo 4475 and Citrumelo 1452 rootstocks and the weakest in the Rough lemon and Yuma citrange rootstocks. Satsuma grafted on Carrizo citrange rootstock had a higher total N concentration in the leaves than on sour orange (Creste, 1995).

Leaf phosphorus concentration of Sidi Aissa clementine is clearly influenced by the nitrogen dose. A negative relationship and antagonism has been demonstrated between the phosphorus concentration of the leaves and the nitrogen dose applied and it is clearly noted that the phosphorus content of the leaves decreases with the increase in the nitrogen doses in all the rootstocks studied while remaining at optimal levels. Mattos et al. (2006) reported that there is an antagonism between N and P when the applied nitrogen fertilization is very high in young citrus trees. On the other hand, Sabbah et al. (1997) reported that leaf phosphorus content was not significantly affected by the amount of nitrogen applied to citrus plants. Tu et al. (2018) reported that the application of phosphorus (P) induced elevated levels of N, P, K and Mg in various parts of citrus plants, leading to a significant increase in the dry weight of roots, branches and leaves.

The leaf potassium content of Sidi Aissa is clearly influenced by the nitrogen dose. The leaf potassium concentration varies according to the interaction between the applied of N and K nutrients. A negative correlation was demonstrated between the dose of nitrogen applied and the leaf potassium concentration in Sidi Aissa plants. This decrease in foliar potassium concentration with increasing N fertilizer doses can be considered as an effect caused by vigorous plant growth induced by nitrogen application. Similar results were obtained by Sabbah et al. (1997) and Wassel et al. (2007) on citrus trees. Similarly, Robert et al. (1999) reported that as the $N/K_2O$ fertilizer ratio increases leaf potassium concentration decreases. De Campos Bernardi et al. (2000) reported that 4 months after transplanting the seedlings to the nursery, the maximum leaf potassium concentration was produced with the application of the nitrogen dose of 0.47 g $plant^{-1}$ and a dose of potassium of 4.67 g $plant^{-1}$. Toplu et al. (2012) observed notable variations in nutrient concentrations within the leaves of three Mandarin varieties (Okitsu, Clausellina, and Silverhill) when grafted on three different citrus rootstocks (Sour Orange, Troyer Citrange, and Carrizo Citrange). Specifically, varieties grafted on Carrizo Citrange demonstrated elevated levels of nitrogen and potassium in the leaves. Whereas those grafted on Troyer Citrange revealed higher leaves phosphorus and iron content. Yilmaz et al. (2018) reported that the rootstocks significantly affects the physiological properties and yield of Rio Red grapefruit. Specifically, the study found that all examined rootstocks (Carrizo citrange, citremon, sour orange, Swingle citrumelo, Troyer citrange and *Citrus volkameriana*) influenced the leaf chlorophyll concentrations. Moreover, notable rootstock effects were observed on the concentrations of several leaf minerals, with *C. volkameriana* having the highest leaf Zn concentration. However, there were no significant rootstock effects on leaf N, P, K, and Fe concentrations.

## CONCLUSION

The study investigated the impact of various fertilizer treatments on Sidi Aissa citrus plants. The increase in the nitrogen dose significantly affects the morphological, physiological and biochemical parameters of Sidi Aissa plants grafted on five citrus rootstocks. The optimal growth on plants height, rootstock stem diameter, number of leaves, shoot length and shoot diameter of the young plants of Sidi Aissa clementine was recorded under the treatment of $100N\text{-}25P_2O_5\text{-}50K_2O$ in mg $L^{-1}$. Plants treated with $100N\text{-}25P_2O_5\text{-}50K_2O$ mg $L^{-1}$ showed the highest Relative Water Content and total chlorophyll content. In addition, we have shown that there is variability within Sidi Aissa associations on different rootstocks with respect to the response to the dose of NPK nutrients. Sidi Aissa clementine plants grafted on Carrizo citrange (Morocco) and Troyer citrange B231655 rootstocks showed significant improvements in all studied parameters. Nitrogen leaf content of Sidi Aissa clementine increases proportionally with the increase in nitrogen dose from 0 to 100 mg $L^{-1}$ while the foliar content of phosphorus and potassium were reduced in Sidi Aissa plants on all the rootstocks studied. In the absence of mineral element deficiency or toxicity, it is advised to utilize a nutrition solution with a composition of 100 N-25 $P_2O_5$-50 $K_2O$ mg $L^{-1}$ to ensure optimal growth of citrus plants in the nursery.

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