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Nutrient Passage in Differentially Grafted Lemon Trees †

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Abstract: Spain is one of the most important producers of lemon fruits in the world (1,250,000 Tm in 2020/2021). In addition, about 80% of the Spanish production of lemons is located in the arid southeast, where fertirrigation is important to ensure the highest productivity. The aim of the present study was to determine the content of nutrients present in two differently grafted lemon trees (*Citrus* × *limon*) of the Verna variety, located on a drip-irrigation farm in Librilla (Region of Murcia, Spain). The first one was grafted in a Sweet orange rootstock (*Citrus* × *sinensis*), and the second one was grafted in a Bitter orange rootstock (*Citrus* × *aurantium*). Both were in 40-year-old trees grown in conventional agricultural practices. The Bitter orange rootstock (*Citrus* × *aurantium*) favoured the appearance of the 'Miriñaque' (in Spanish) or protuberance of the trunk at the union of the graft, whereas this did not appear in the Sweet orange rootstock (*Citrus* × *sinensis*). For the analysis, fresh samples of old leaf, young leaf, and root were collected from five different trees for each rootstock. Once processed after having been weighed, dried, and ground, they were analysed by inductively coupled plasma (ICP) analysis (Optima 3000, PerkinElmer). The result showed that the growth in aerial biomass was higher in Verna grafted on Sweet orange rootstock. The root samples of the lemon tree with Bitter orange rootstock contained a higher amount of Fe, Mn, and Zn than the samples of the lemon tree with Sweet orange rootstock. The rest of the nutrients did not show significant differences. The new and old leaves of Verna in Sweet orange rootstock showed a higher amount of Fe, Mn, and Zn than the new and old leaves of Verna in Bitter orange rootstock. Additionally, the rest of the nutrients did not show significant differences. The study revealed that this protuberance in the trunk prevents the passage of these elements from the root to the aerial part of the trunk. This is probably related to the cell-to-cell passage.

Keywords: lemon tree; nutrients; biomass; Miriñaque; protuberance; rootstock; elements

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1. Introduction

The aim of this study was to determine the content of nutrients present in two differently grafted lemon trees (*Citrus* × *limon*) of the Verna variety. The first one was grafted onto a Sweet orange rootstock (*Citrus* × *sinensis*) and the second onto a Bitter orange rootstock (*Citrus* × *aurantium*). Direct grafting of the lemon tree (*Citrus* × *limon*) onto Bitter orange (*Citrus* × *aurantium*) as a rootstock is a widely used practice in traditional lemon-growing areas with both organic and conventional methods in southeastern Spain.

However, as trees grow older, lemon trees directly grafted into Bitter orange rootstock live much less and present more problems than those grafted into Sweet orange rootstock. If the average life of a healthy lemon tree is about 70 years, that of a lemon tree grafted on Bitter orange rootstock (*Citrus* × *aurantium*) is about 45 years [1].

Additionally, a protuberance in the trunk appeared (Figure 1), caused by a lack of affinity or physiological incompatibility between the lemon tree and Bitter orange, producing unfavourable medium-term effects on sap circulation. Initially, the union between the tissues produces a good weld without any symptoms of rejection, but the ability to function properly once the two plant materials are joined is evidently low, thus resulting in a protrusion at the junction of the two as a result of a malfunction with cumulative effects over time [1].

Figure 1. *Citrus × aurantium* (with Protuberance) and *Citrus × sinensis* (no Protuberance).

It is a proven fact that the intermediate wood of Sweet orange (*Citrus sinensis*) resolves the incompatibility between the lemon tree and Bitter orange (*Citrus × aurantium*), due to its intermediate situation in terms of genetic kinship between the two, showing affinity to both varieties [1]. Therefore, the aim of this research was to compare the mineral nutrient uptake and transport between both rootstocks and the grafted (*Citrus × limon*) of the Verna variety.

2. Methods

The lemon trees were located in the area of Librilla (Region of Murcia, Spain), in the semi-arid southeastern Spain. Five different trees were selected within each type of rootstock (*Citrus × sinensis* and *Citrus × aurantium),* (40-year-old trees) grown in conventional agricultural practices. From each tree, 500 g samples of old leaf, new leaf, and root were collected in situ. Once collected, the samples were transferred to the laboratory for processing. They were weighed, dried in an oven at 60 °C for 4 days, and after being ground, they were analysed by an inductively coupled plasma (ICP) analysis (Optima 3000, PerkinElmer) [2]. Abbreviations are provided in Table 1. Calculation of whole tree biomass was estimated from small samples, as reported by Carvajal et al. [2].

Table 1. Mineral analysis of leaves from lemon grafted trees. Data are average of 5 trees.

	Ca (g/100 g) ± SE	Fe (mg/Kg) ± SE	K (g/100 g) ± SE	Mg (g/100 g) ± SE	Mn (mg/Kg) ± SE	Mo (mg/Kg) ± SE	Zn (mg/Kg) ± SE
New leaf no miri	2.693 ± 0.231	118.140 ± 18.821	1.510 ± 0.017	0.288 ± 0.017	32.393 ± 2.256	0.253 ± 0.009	17.590 ± 1.754
Old leaf no miri	4.430 ± 0.462	211.760 ± 29.545	0.933 ± 0.029	0.307 ± 0.029	38.887 ± 2.866	0.130 ± 0.026	17.267 ± 1.072
New leaf miri	2.490 ± 0.186	70.227 ± 6.019	1.463 ± 0.110	0.264 ± 0.006	25.517 ± 0.148	0.273 ± 0.048	17.183 ± 1.599
Old leaf miri	4.753 ± 0.761	149.111 ± 25.541	0.969 ± 0.213	0.370 ± 0.050	38.720 ± 4.028	0.191 ± 0.099	19.460 ± 3.428

3. Results and Discussion

Mineral analysis of leaves (Table 1) showed that calcium was higher in old leaves than in new leaves. However, there were no significant differences between no miri and miri. Fe and Mo were also higher in old leaves than in new leaves, but Fe was found greatly reduced in trees with miri in both new and old leaves, while Mo was not changed. K concentration was oppositely higher in new leaves than in old leaves but with no differences between no miri and miri. Mn was reduced in old leaves with miri, compared with old leaves with no miri, but there were no differences between both old leaves. Zn was not altered among all trees. In general, Fe was found the microelement with the highest concentration, followed by Mn and Zn in new and old leaves.

The results obtained by inductively coupled plasma (ICP) analysis (Table 2) showed that the root samples of the lemon tree with Bitter orange rootstock contained a greater amount of the elements Fe, Mn, and Zn than the samples of the lemon tree grafted onto Sweet orange rootstock. The rest of the nutrients showed no significant differences.

Table 2. Mineral analysis of roots from lemon grafted trees. Data are average of 5 trees.

	Ca (g/100 g) ± SE	Fe (mg/Kg) ± SE	K (g/100 g)±SE	Mg (g/100 g) ± SE	Mn (mg/Kg) ± SE	Mo (mg/Kg) ± SE	Zn (mg/Kg) ± SE
Root no miri	1.15 ± 0.12	619.72 ± 53	0.17 ± 0.04	0.113 ± 0.23	21.09 ± 3.5	0.51 ± 0.08	5.32 ± 0.98
Root miri	2.74 ± 0.10	1299.45	0.28 ± 0.06	0.174 ± 0.01	43.24 ± 6.8	0.33 ± 0.04	18.01 ± 2.72

New and old leaves of Verna lemon grafted onto Sweet orange rootstock showed higher amounts of Fe, Mn, and Zn than new and old leaves of Verna lemon grafted onto Bitter orange rootstock. In addition, the rest of the nutrients showed no apparent significant differences.

Mineral analysis of root (Table 2) showed that values were higher for Ca, Fe, K, Mg, Mn, and Zn in 'root miri' (*Citrus aurantium*) than for 'root no miri' (*Citrus sinensis*). On the contrary, the Mo element showed a higher concentration in 'root no miri' (*Citrus sinensis*) than 'root miri' (*Citrus* × *aurantium*).

With regard to Table 3, we can see that both the total biomass and annual biomass, expressed in kg per tree, were higher in the lemon tree grafted onto Sweet orange rootstock (*Citrus sinensis*) than lemon tree grafted onto Bitter orange rootstock (*Citrus* × *aurantium*). Other data to highlight is that lemon production was higher in *Citrus sinensis*, with 200 Kg per tree, compared with 85 Kg per tree in *Citrus* × *aurantium*.

Table 3. Total and annual biomass of the two types of rootstocks.

	Biomass Total (Kg/Tree) ± SE	Biomass Annual (Kg/Tree) ± SE	Lemon Annual (Kg/Tree) ± SE
Citrus aurantium	215.79 ± 35	224.96 ± 45	85 ± 10
Citrus sinensis	270.33 ± 51	404.43 ± 45	200 ± 10

4. Discussion and Conclusions

This study revealed that the protuberance in the trunk (miri) that appeared in *Citrus* × *limon* grafted onto *Citrus* × *aurantium* could block the passage of Fe and Mn from the root to the aerial part of the trunk. The type of grafting also influences the amount of biomass and production of the tree; in the lemon tree grafted onto *Citrus sinensis*, these two factors are higher than in the lemon tree grafted onto *Citrus* × *aurantium*, as is also demonstrated in the study 'Fino lemon clones compared with the lemon varieties Eureka and Lisbon On two rootstocks in Murcia (Spain)', which compares the lemon tree grafted onto *Citrus* × *aurantium* against the lemon tree grafted onto *Citrus macrophylla* [3].

According to a study by Chang-PinChun [4], in which the concentration of mineral elements (Cd) in different types of citrus rootstocks was compared, the rootstock *Citrus* × *aurantium* was the one with the highest absorption rate. They compared the concentration of Cd in roots and shoots revealing that *Citrus* × *aurantium* provided a higher concentration in shoots, compared with the others. This fact suggested that our rootstock in lemon (*Citrus* × *limon*) presented a low-affinity rate, thus reducing the transport of elements from the root to the leaves [4].

Additionally, when comparing the concentration of the root samples of the lemon tree with Bitter orange rootstock with the samples of the lemon tree grafted onto Sweet orange rootstock, we observed a higher concentration in the first ones. However, not all of them showed higher concentration in leaves of *Citrus* × *lemon* grafted onto *Citrus sinensis*. Therefore, this could indicate that the fertilisation needs to be higher to obtain similar values in the aerial part. However, this should be confirmed in future experiments. The blockage process that probably occurred in the grafting area is probably related to the cell-to-cell passage of water affecting nutrients.

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Abbreviations

New leaf no miri	New leaf of *Citrus* × *limon* grafted onto *Citrus sinensis*
Old leaf no miri	Old leaf of *Citrus* × *limon* grafted onto *Citrus sinensis*
New leaf miri	New leaf of *Citrus* × *limon* grafted onto *Citrus* × *aurantium*
Old leaf miri	Old leaf of *Citrus* × *limon* grafted onto *Citrus* × *aurantium*
Root no miri	Root of *Citrus sinensis*
Root miri	Root of *Citrus* × *aurantium*

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