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Nutrient Passage in Different Grafted Lemon Trees †

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Abstract: Spain is one of the most important producer of lemon fruits in the world (1,250,000 Tm in 2020/2021). Besides, about 80% of the Spanish production of lemons is located in the arid southeast, where fertirrigation is important to ensure the highest productivity [1]. The aim of the present study was to determine the content of nutrients present in two differently grafted lemon trees *(Citrus x limon)* of the Verna variety, located in a drip irrigation farm in Librilla (Region of Murcia, Spain). The first one grafted in a Sweet orange rootstock *(Citrus x sinensis)*, and the second one in a Bitter orange rootstock *(Citrus x aurantium)*. Both in 40-year-old trees grown in conventional agricultural practices. The Bitter orange rootstock *(Citrus x aurantium)* favoured the appearance of the "Miriñaque"(in spanish) or protuberance of the trunk at the union of the graft. While it did not appear in the Sweet orange rootstock *(Citrus x sinensis)* [2]. For the analysis, fresh samples of old leaf, young leaf and root were collected from 5 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. Also, 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 x limon)* of the Verna variety. The first one grafted in a Sweet orange rootstock *(Citrus x sinensis)* and the second on a Bitter orange rootstock *(Citrus x aurantium)*. Direct grafting of the lemon tree *(Citrus x limon)* into Bitter orange *(Citrus x aurantium)* as rootstock are widely extended in traditional lemon-growing areas with both organic and conventional methods in the South East of Spain.

However, when trees get older, direct grafted into Bitter orange lemon trees live much less and present more problems than those grafted on Sweet orange trees. If the average life of a healthy lemon tree is about 70 years, that of a lemon tree grafted on Bitter orange rootstock *(Citrus x aurantium)* is about 45 years [2].

Also, a protuberance in the trunk appeared caused by a lack of affinity or physiological incompatibility between lemon tree and Bitter orange, producing unfavourable medium-term effects on sap circulation. At first instance, 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 [2].

It is a proven fact that the intermediate wood of Sweet orange *(Citrus sinensis)* resolves the incompatibility between lemon tree and Bitter orange *(Citurs x aurantium)*, due to its intermediate situation in terms of genetic kinship between the two, showing affinity to both varieties [2]. Therefore, the aim of this work was to compare the mineral nutrient uptake and transport between both rootstocks and the grafted *(Citrus x limon)* of the Verna variety.

Figure 1. *Citrus x aurantium* (with Protuberance) y *Citrus x sinensis* (no Protuberance)**.**

2. Methods

The lemon trees were located in the area of Librilla (Region of Murcia, Spain), in the semi-arid South East of Spain. Five different trees were selected within each type of root stock *(Citrus x sinensis and Citrus x aurantium)*, (40-year-old trees) grown in conventional agricultural practices. From each tree, 500 g of old leaf, new leaf and root samples 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) [3]. Abbreviations are provided in Table 1. Calculation of whole tree biomass. It was estimated from small samples as reported by Carvajal et al. [3].

Table 1. Abbreviations and meaning.

ABBREVIATIONS	Meaning
New leaf no miri	New leaf of *Citrus x limon* grafted on *Citrus sinensis*
Old leaf no miri	Old leaf of *Citrus x limon* grafted on *Citrus sinensis*
New leaf miri	New leaf of *Citrus x limon* grafted on *Citrus x aurantium*
Old leaf miri	Old leaf of *Citrus x limon* grafted on *Citrus x aurantium*
Root no miri	Root of *Citrus sinensis*
Root miri	Root of *Citrus x aurantium*

3. Results and Discussion

The results obtained by inductively coupled plasma (ICP) analysis (Table 3) 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 on Sweet orange rootstock. The rest of the nutrients showed no significant differences.

New and old leaves of Verna lemon on Sweet orange rootstock showed higher amounts of Fe, Mn and Zn than new and old leaves of Verna lemon on Bitter orange rootstock. In addition, the rest of the nutrients showed no apparent significant differences.; All of this can be seen in Table 1.

Mineral analysis of leaves (Table 2), showed that calcium is 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 no 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 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 leaf, old leaf.

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

	Ca (g/100g)	±SE	Fe (mg/Kg)	±SE	K (g/100g)	±SE	Mg (g/100g)	±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

Mineral analysis of root (Table 3), 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 x aurantium).*

With regard to Table 4, we can see that both the total biomass and the annual biomass, both expressed in kg per tree, were higher in the lemon tree grafted on Sweet orange rootstock (*Citrus sinensis*) than lemon tree grafted on Bitter orange rootstock *(Citrus x aurantium)*. Another data to highlight is that lemon production was higher in *Citrus sinensis* with 200 Kg per tree compared to 85 Kg per tree in *Citrus x aurantium.*

Table 3. Mineral analysis of roots from lemon grafted trees. Data are average of 5 trees.

	Ca (g/100g)±SE	Fe (mg/Kg)±SE	K (g/100g)±SE	Mg (g/100g)±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 ± 69	0.28 ± 0.06	0.174 ± 0.01	43.24 ± 6.8	0.33 ± 0.04	18.01 ± 2.72

Table 4. 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

The study revealed that this protuberance in the trunk (miri) that appeared in *Citrus x limon* grafted on *Citrus x 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 in *Citrus sinensis*, these two factors are higher than in the lemon tree grafted in *Citrus x 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)", that compares the lemon tree grafted in *Citrus x aurantium* against the lemon tree grafted in *Citrus macrophylla* [4].

According to study of Chang-PinChun [5], in which the concentration of mineral elements (Cd) in different types of citrus rootstocks was compared, the rootstock *Citrus x aurantium* was the one with the highest absorption rate. They compared the concentration of Cd in roots and shoots revealing that *Citrus x aurantium* provided higher concentration in shoots compared to the others. This fact suggested that our rootstock in lemon (*Citrus x limon*) presented low affinity rate, thus, reducing the transport of elements from the root to the leaves [5].

Also, if we compare the concentration of the root samples of the lemon tree with Bitter orange rootstock with the samples of the lemon tree grafted on Sweet orange rootstock we observed higher concentration in the first ones. However, not all of them showed higher concentration in leaves of *Citrus x lemon* grafted on *Citrus sinensis.* Therefore, it could indicate that the fertilization need to be higher for obtained 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 cell-to-cell passage of water affecting nutrients.

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