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Seasonal Dynamic of Mineral Macronutrients in Three Varieties of Clementine (*Citrus Reticulate*) Leaves

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ABSTRACT
The nutrient composition in terms of macronutrients of three different varieties (Early season "Orogrande", season "Nules" and late season "Nour") of clementine leaves (Citrus reticulata) was monitored at monthly intervals during three years in order to provide information for the development of nutrition management guidelines more suited to local conditions of southern Morocco. Nutrient concentrations at the sampled leaves for all the three elements, nitrogen (N), phosphorus (P) and potassium (K), changed seasonally for all tested varieties. Seasonal dynamic of those three elements doesn't differ significantly between years, particularly during the spring-summer period. Results of statistical analysis show a significant variability between the tested varieties in term of leaf nitrogen and potassium concentrations. However, no significant differences between tested varieties were recorded in term of the leaf phosphorus concentration.
Keywords – Macronutrients, Leaf concentration, Seasonal dynamic, Citrus

I. INTRODUCTION

In Morocco, the citrus production and export sector plays a very important social and economic role and it is considered as a main branch of the national economy. Citrus plantings cover approximately 85,000 ha with an annual production of 1,7 million tons of which 90% is used for fresh consumption either in the domestic or the export markets [1]. Due to its impact on foreign exchange revenues (about $350 million annually) and job creation in rural areas and the economy in general, this sector has received a great deal of support from the Moroccan government [2]. In the past several years, new citrus plantings have been growing at an average of about 2,000 hectares per year. This growth rate was deemed insufficient by the industry to keep up with the demand. Under the "Green Plan", the government of Morocco has set an ambitious strategy for citrus production in Morocco; Total citrus production is projected to grow from its initial level of 1.7 million MT to 2.9 million MT by 2018, through expanding citrus-planted areas by 20,000 hectares and renewing old plantation of 30,000 hectares [2]. However, this sector have to face many unfavorable production conditions (especially climate changes, losses in soil fertility, water scarcity and salinization... etc.) and a very narrow external market demanding quality products and resulting from an important competition with many countries such as Spain, Egypt and Turkey. Therefore, it has become very important to streamline the entire production factors (mainly orchard fertilization) based on scientific studies. Ensuring adequate levels of mineral nutrients is important to maintaining the health and production of citrus trees. The implementation of a citrus orchard guiding fertilization program should be based on a diagnosis of a nutrient status of citrus trees. Owing to the fact that the leaf tissue testing is the most important valuable tool which allows accomplishing this goal, particularly with respect to mobile nutrient such as nitrogen (N) and potassium (K), and micronutrients such as copper, iron, manganese, and zinc [3]. The mineral content of plant parts, in particular leaves, is used to identify nutrient deficiencies, excesses or imbalances within a crop. The nutritional status of citrus trees, particularly nitrogen (N), phosphorus (P) and potassium (K), influences citrus fruit quality as well as crop yield [4]. Seasonal change in the mineral nutrient status of citrus leaves is well established [5,6] and the effect of factors such as rootstock on leaf mineral nutrient composition have been reported in detail be some authors [7]. Characterization of the seasonal dynamics of leaf nutrient is necessary to develop criteria and standards defining the optimum nutrient status for both yield and fruit quality. Macro and micro-nutrient deficiencies and toxicities result in a wide assortment of undesirable alterations in the appearance of horticultural products [8]. In general, these alterations in macro and micronutrients are in correlations to fruit shape and size [9]; this author showed that high percentage of nitrogen dominated by ammoniac form results in smaller orange fruit, however, Phosphorus deficiency results in large 'Valencia' orange fruit [10,11] while zinc and iron deficiencies decrease citrus fruit size [12]. Although, critical leaf concentration standards for almost all nutrients are well established for citrus orange [13,14], however, there is few research results related to citrus clementine leaf nutrient dynamic and standards in Morocco. The aim of the present work was to characterize and compare the seasonal dynamic of three macronutrients (nitrogen, phosphorus and potassium) in three different varieties of citrus clementine (early, medium and late season variety) under a same standard fertilization and irrigation program over a period of three years. The results of this work can be used as preliminary norms for the interpretation of tree nutrients status for high commercial clementine orchards. These results can also provide useful information for the development of citrus nutrition management guidelines more suited to local conditions of the southern Morocco.

II. MATERIALS AND METHODS

2.1 Experiment site and design

The experiment was carried out in a commercial citrus orchard located in southern Morocco at the biggest citrus production area of the country at Taroudant district (lon: 30.632518°E, lat: -8.414420°N). The experiment was conducted on three sites of five years old trees (age at the beginning of the experiment) using three different varieties (Early season "Orogrande", season "Nules" and late season "Nour") of clementine citrus (*Citrus reticulata*) grafted on "Sour orange" (*Citrus aurantium*) rootstock.

Each site is planted with only one of the three varieties and divided into four experimental plots (four repetitions). The blocks had the same row orientation at 6x4m spacing (416 trees/ha).

2.2 Soil, Water and Climate conditions

The three sites were located on adjacent blocks and established on a loamy clay soil with high pH and high soil potassium, magnesium calcareous content (see soil chemical analysis in Table 1).

Table 1: Soil chemical analysis

	Site 1	Site 2	Site 3
pH	8.3	8.3	8.4
EC (1/5 extract: mS/cm)	0.24	0.21	0.23
Organic matter (%)	0.94	0.92	0.87
Total N (g/Kg)	0.93	0.87	0.79
C/N ratio	5.90	6.10	6.30
Total calcareous (%)	14.50	13.90	16.10
Active calcareous (%)	8.60	7.3.00	9.40
P_2O_5 (ppm)	95.00	103.00	117.00
K_20 (ppm)	248.00	231.00	289.00
MgO (ppm)	477.00	508.00	430.00
CaO (ppm)	7951.00	7720.00	8672.00
Na (ppm)	24.00	37.00	41.00

Site 1: Nules Site; 2: Orogrande; Site 3: Nour

Irrigation water is pumped from underground source. It is characterized by medium salinity and high pH value and hardness. This water contains high content of calcium; magnesium and sulfur that can satisfy the citrus tree needs during all the annual cycle considering a total irrigation water volume per year of 8000 m3/ha (see chemical analysis of irrigation water in Table 2).

Table 2: Irrigation water chemical analysis

pH	7.6	Na^+ (mg/L)	33
EC (mS/cm)	1.05	NO_3^- (mg/L)	39
NH_4^+ (mg/L)	0.1	$H_2PO_4^-$ (mg/L)	0.1
K^+ (mg/L)	1.7	SO_4^{2-} (mg/L)	172
Ca^{2+} (mg/L)	110	HCO_3^- (mg/L)	278
Mg^{2+} (mg/L)	54	Cl^- (mg/L)	44

The climate is Mediterranean semi-arid with very low and irregular rainfall (100 to 150mm/year) distributed between late autumn and early spring. Temperatures are slight cold in the winter and very high in the summer associated sometimes with very low air humidity.

2.3 Fertilization and irrigation

The irrigation was performed using double drip lines irrigation system with 75 cm spaced emitters that generate a flow of 4l/h/emitter.

Irrigation and fertilization management were made within a fertigation station through electro-valves. Daily reference evapo-transpiration ETo was calculated using the formula of [15]. To avoid water loss, net maximum irrigation dose was determined referring to physical properties of the soil. All the three sites received the same standard fertilization program usually used by local citrus farmers and adapted depending with experiment site local conditions (see fertilization program in Table 3). This program is performed using four chemical fertilizers (ammonium nitrates, mono-ammonium phosphate, potassium nitrates and sulfates salts) mixed with water through drip irrigation. The trees micronutrients requirement was satisfied by four foliar applications of a commercial mixture of chelated micronutrients (Fe 5%, Zn 3%, Mn 3%, B 2%, Cu 1% and Mo 0,2%) on February, March, May and July at a rate of 4Kg/Ha/application.

Table 3: Annual fertilization program applied during the three years of experimentation

	N	P_2O_5	K_2O
		(Kg/ha/year)	
Vegetative growth – Flowering (Jan – Mar)	50	20	25
Fruit set – End of fruit cell division (Apr – Jun)	70	25	50
Fruit enlargement – Fruit ripening (Jul – Oct)	70	15	70
Harvesting – Vegetative growth	30	10	15
Total	220	70	160

2.4 Leaves sampling and analysis

Leaf samples were collected at monthly intervals during all the three years of the experimentation from five representative trees of each plot. The third leaf of ten non-bearing shoots were taken at random around the selected trees at heights of 1,5 to 2,0 m above the ground [3]. Once at the laboratory, sampled leaves were washed using detergent solution and several rinses by deionized water [16], dried at 80°C for 48h, grounded, ashed at 550°C for 5 h and then submitted to the chemical analysis. Leaf total nitrogen was determined by the semi-micro K method [17]; phosphorus concentrations were determined by UV-Vis spectrophotometer and potassium using Atomic-absorption Spectrophotometer.

Data were analyzed using MINITAB statistical software version 15.1.1.0. Treatment means were separated by Tukey's test at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

3.1 Seasonal changes

Nutrient concentrations at the sampled leaves for all the three elements, nitrogen (N), phosphorus (P) and potassium (K) changed seasonally for all tested varieties. Seasonal dynamic of those three elements doesn't differ significantly between years, particularly during the spring-summer period.

- Nitrogen concentration

During the three experimental years, as shown by Month

Figure 1, there was a decrease in mean leaf nitrogen concentration from a maximum at the beginning of the vegetative growth of the new shoots and last during all winter and spring to reach a minimum at the end of fruit cell division by the end of spring season. This decline in leaf nitrogen concentration is followed by an increase in late summer and autumn to reach its maximum level after fruit harvest before the beginning of the successive vegetative growth. Leaf nitrogen concentrations during the annual cycle were more or less in the optimum range as described by [18] except the period between May to June of rapid fruit growth where those levels remain under the optimum range. These results seems to indicate that the rapid decline of nitrogen (repeated during the three years experiment) can be explained by the insufficient availability of this element in the root zone before cell division stage which is known to require important nitrogen quantities in biomass production process. The lack of nitrogen in the root zone during the period of fruit cell division can be explained by a misallocation in the supply of this essential element along the citrus annual cycle and not a lack of the total annual nitrogen supply for the orchard. This can be justified by high leaf concentrations of this element along the cycle outside of this stage. Consequently, it is essential to support high nitrogen requirements of the fruit cell division by additional supplies of this major element before the beginning and during this critical stage following fruit set.

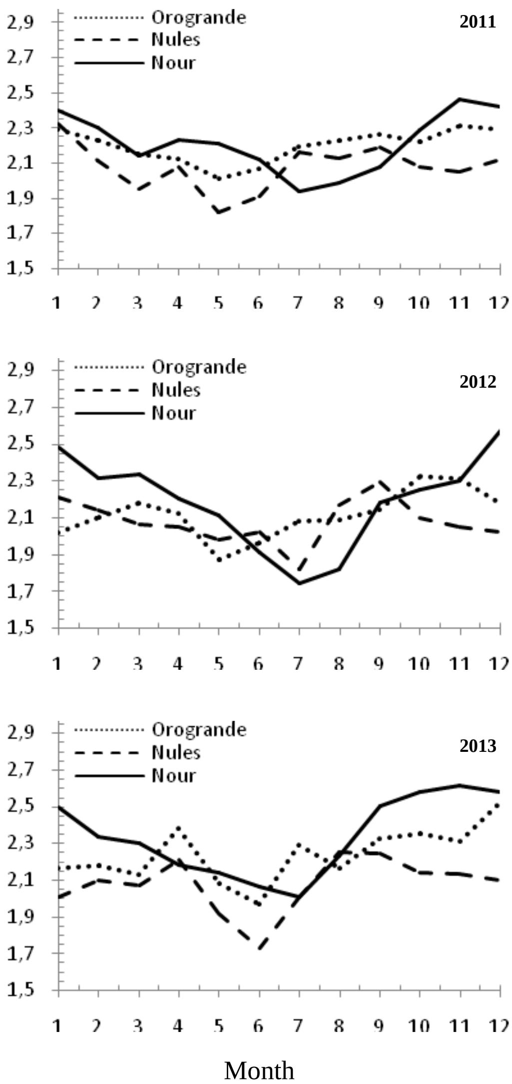


Figure 1: Annual changes of leaf nitrogen concentration in dry matter presented per month.

- Phosphorus and potassium concentration

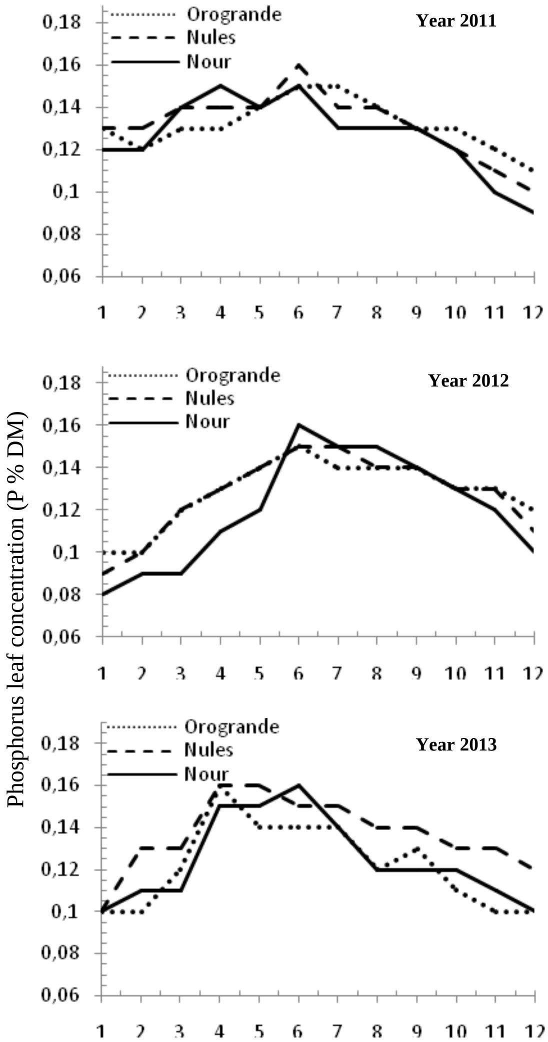
Mean leaf phosphorus and potassium concentrations followed similar seasonal patterns but differ completely from the nitrogen ones. As shown in Months

Figure 2 and Months

Figure 3, potassium and phosphorus leaf concentrations increased during winter and spring to reach the maximum levels by June and July at the end of fruit cell division and the beginning of fruit enlargement. As the season progresses, phosphorus and potassium leaf concentrations declined progressively until fruit were harvest at late autumn.

On the basis of leaf nutrient guidelines for citrus trees [13], leaf phosphorus and potassium concentrations of the clementine trees in our experiment were adequate or high (above 0,12% for phosphorus and 1% for potassium) for much of the annual cycle (March to October). However, those values were low during the vegetative growth before flowering and during the fruit ripening. Therefore, in one side, the high demand for phosphorus due to summer wood lignifications and the tree reserves accumulation is necessary for the following season fruiting, in the other side, the high demand for potassium, by the clementine fruit at ripening, coupled with low leaf potassium concentrations suggest that phosphorus and potassium latent deficiencies may occur to fruits colour disorder, bearing alternate phenomenon and lead to the harvest delay. However, respecting the standard citrus fertilization program used by local farmers, phosphorus and potassium are well distributed during all the citrus annual cycle for orchard. Moreover, the experiment soil which contains high levels of these two elements was supposed to cover a large part of trees requirements. Consequently, this phenomenon could be ascribed mainly by the phosphorus bioavailability related to high temperature and its impact on the activation of soil micro-organisms (mainly mycorrhizae) responsible of phosphorus release and its easy uptake by tree roots [19]. Thus, it is recommended to supply phosphorus to citrus trees through foliar applications during all periods of the annual cycle with low air and soil temperature in order to maintain an adequate leaf concentration of this element.

Regarding potassium, low leaf contents during fruit ripening and vegetative growth could be explained mainly by a misallocation in the supply of potassium along the citrus annual for the orchard as in the case of nitrogen previously cited. Thus, it is very important to support high potassium requirements of the fruit enlargement and ripening by additional supplies of this element before the beginning and during these stages.



Months

Figure 2: Annual changes of leaf phosphorus concentration in dry matter per month

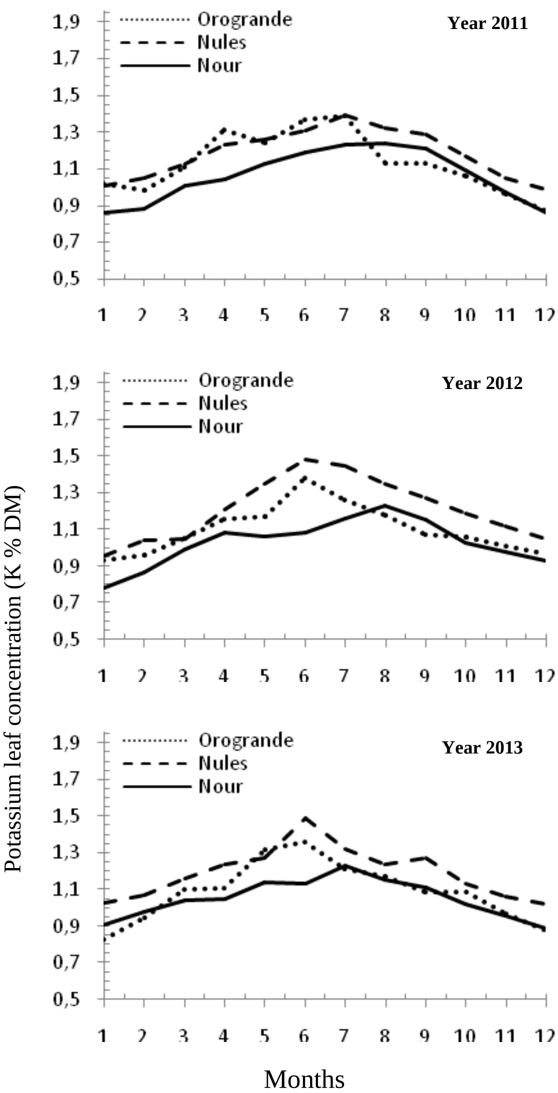


Figure 3: Annual changes of leaf potassium concentration in dry matter per month

3.2 Variety effect

Data presented in figures 1, 2 and 3 illustrate different trends of the three tested varieties regarding the dynamic of leaf nitrogen, phosphorus and potassium concentrations during the three experiment years. Results of statistical analysis show a significant variability between the tested varieties in term of leaf nitrogen and potassium concentrations. However, no significant differences between tested varieties were recorded in term of the leaf phosphorus concentration.

- Leaf nitrogen concentration

Values of leaf nitrogen concentrations of the three tested varieties presented in figure 1 show different trend curves. Statistical analysis reveals the presence of two homogeneous groups. In the first one, the late season variety “Nour” appear to have the capacity to accumulate higher concentrations of leaf nitrogen than the early and medium season varieties “Orogrande and Nules” in the second group even at the same soil conditions and fertilization program. These results, can perfectly explain the high tree vigor of “Nour” and the medium vigor of “Orogrande” and “Nules” varieties. Consequently, this result should be taken into consideration when developing fertilization programs especially for “Nour” variety in order to control more tree vigor, succeed a balance between leaves and fruiting and finally to better control the phenomenon of bearing alternate known in this last variety.

Furthermore, the “Nour” leaf nitrogen concentration curve records its maximum decline always one month after the rest of varieties (July for Nour and June for Orogrande and Nules). This can be explained by its endogen characteristics in terms of lateness, which also gives it a delay in the timing of cell division compared to the other tested varieties.

- Leaf phosphorus concentration

Values of leaf phosphorus concentrations presented in figure 2 show similar trends between the three tested varieties. Statistical analysis confirms that there are no significant differences between tested varieties in term of the leaf phosphorus concentration.

- Leaf potassium concentration

Values of leaf potassium concentration of the three tested varieties presented in figure 3 show different trend curves. Statistical analysis reveals the presence of two homogeneous groups. In the first group, the early and medium season varieties “Orogrande and Nules” appear to have the capacity to accumulate higher concentrations of leaf potassium than the late season varieties “Nour” in the second group even at the same soil conditions and fertilization program. On the basis of leaf nutrient guidelines for citrus trees [13], leaf potassium concentrations of “Orogrande” and “Nules” are at normal rates during all the annual cycle. Therefore, those values are low and not sufficient in the case of “Nour” trees mainly during fruit ripening and vegetative growth. Thus, it is very important to support high potassium requirements of this variety mainly at vegetative growth, fruit enlargement and ripening by additional supplies of this element before the beginning and during these stages. As in the case of nitrogen, this result should be taken into consideration when developing fertilization programs for “Nour” variety in order to control more tree vigor, keep equilibrium between leaves and fruiting and finally to better control the phenomenon of bearing alternate very known for this variety.

IV. CONCLUSION

According the previous results, leaf nutrient concentrations (N, P and K) changed seasonally for all tested varieties. Seasonal dynamic of those three elements doesn't differ significantly between the three years of experiment. However, significant variability exists between the tested varieties in term of leaf nitrogen and potassium concentrations. Moreover, no significant differences between tested varieties were recorded in term of the leaf phosphorus concentration. Thus, the results of the present work provide interesting information for the development of nutrition management guidelines more suited to local conditions of southern Morocco.

REFERENCES

[1] MAPM, 2013. La filière agrumicole, *Contrat programme pour la mise à niveau*, ministère de l'agriculture et de la pèche maritime du Maroc, 2009.

[2] I. El Honsali. Moroccan citrus annual report. *Global agricultural information network*, Rabat US embassy, OM1212, 2012.

[3] TA. Obreza, AK. Alva, EA. Hanlon & RE. Rouse. *Citrus grove leaf tissue and soil testing, sampling*, analysis and interpretation. Univ. of Florida. Coop. Ext. Ser. Bull., SL115, 1992, 1- 4.

[4] G.I. Moss. *The role of potassium in determining fruit quality of sweet orange*. Aust. J. Exp. Agric. Anim. Husbandry 12, 1972, 195±202.

[5] W.W. Jones, E.R. Parker. *Seasonal trends in mineral composition of Valencia orange leaves*. Proc. Am. Soc. Hortic. Sci. 57, 1951, 101±103.

[6] C.K. Labanauskas, W.W. Jones, T.W. Embleton,. *Seasonal changes in concentrations of micronutrients (zinc, copper, boron, manganese, and iron)* in leaves of Washington navel orange. Proc. Am. Soc. Hortic. Sci. 74, 1959, 300±307.

[7] B.K. Taylor, R.T. Dimsey. *Rootstock and scion effects on the leaf nutrient composition of citrus*. Aust. J. Exp. Agric. 33, 1993, 363±371.

[8] H.M. Seyed, R. Majid. *Seasonal changes of mineral nutrients and phenolics in pomegranate (Punica granatum L.) fruit*. Scientia Horticulturae 111, 2007, 120–127.

[9] A. Bar-Akiva, V. Hiller, J. Patt,. *Effects of foliar application of nutrients on increasing of 'Valencia' orange*. HortScience 10, 1968, 69–70.

[10] T.W. Embleton, W.W. Jones, C.K. *Labanauskas. Leaf analysis and phosphorus fertilization of oranges.* Calif. Citrogr. 56, 1971, 101–103.

[11] T.W. Embleton, W.W. Jones, R.G. Platt. *Plant nutrition and citrus fruit crop quality and yield*. HortScience 10, 1975, 45–50.

[12] W.W. Jones, P.F. Smith. *Nutrient deficiencies in citrus.* In: Sprague, H.B. (Ed.), Hunger Signs in Crops. Mckay Co., New York, 1964, 359-414.

[13] E.A. Hanlon, T.A. Obreza, A.K. Alva,. *Tissue and Soil Analysis.* In: Nutrition of Florida Citrus Trees, Tucker, D.P.H., University of Florida, Gainesville, Florida, 1995, 13-20.

[14] A.K. Alva, S. Paramasivam, T.A. Obreza, A.W. Schumann,. *Nitrogen best management practice for citrus trees: I. Fruit yield*, quality and leaf nutritional status. Sci. Hortic., 107, 2005, 233-244.

[15] Allen R. G., Periera L. S., Raes D., Smith M., *Crop evapotranspiration: guideline for computing crop water requirement*. FAO, Rome, Italy, 301 (1998).

[16] AK. Alva, DPH. Tucker. *Surface contamination of citrus leaves for macro and micronutrients analysis*. Proc. Fla. State Hort. Soc. 110, 1997, 86-88.

[17] JM. Bremner. Nitrogen - Total P. 1085-1121. In D.L. *Sparks et al. (ed.) Methods of soil analysis*. Part 3. SSSA Book Ser. 5. SSSA and ASA, Madison, WI. 1996.

[18] HD. Chapman. *Leaf and soil analysis in citrus orchards*, Manual 25, Calif. agric., exper. Sta., 1960, 1-53.