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Research Article

Nutritional standards through Integrated Differential Diagnosis (IDD) in pomegranate (*Punica granatum* L.)

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Abstract

The foliar analysis is a useful tool to detect the nutritional status of plants, predict future problems in the reproductive cycles of fruit production and implement preventive and corrective measures to achieve a mineral balance in fruit trees, obtaining higher productivity. There is little information available on nutrient sufficiency ranges in pomegranate leaf and the literature reported differs due to diverse cultivation practices, cultivars, and agroecological conditions. Therefore, the objective of this work was to determine the mineral content of 'Wonderful' pomegranate leaves for each the nutritional elements and the cation balance, and to establish the nutritional standards using Integrated Differential Diagnosis (IDD). The mineral content of the pomegranate leaves was determined and based on the results, nutritional standards for macro elements, cation balance and microelements were generated through the IDD, which evaluate whether there are mineral and/or physiological imbalances. The macro elements in the cultivation of the pomegranate were the following: NO_3 3747.5 μg g^{-1}, Total Nitrogen 1.35%, P 0.14%, K 0.88%, Ca 1.65%, Mg 0.19%, Na 0.006%. For the cation balance, the optimal values were: (Ca+Mg)/K 4.48 mEq 100 g^{-1}, Ca/(K+Mg) 2.13 mEq 100 g^{-1}, Mg/(K+Ca) 0.16 mEq 100 g^{-1}, Ca/Mg 5.06 mEq 100 g^{-1}, K/Mg 1.36 mEq 100 g^{-1}. Finally, the optimal values for the microelements were: Fe 59.5 μg g^{-1}, Mn 31.4 μg g^{-1}, Zn 6.3 μg g^{-1}, Cu 6.1 μg g^{-1}. The values obtained for the micro and macro elements were like those reported. In the case of cation balance and foliar nitrate content, they have not been previously reported. The IDD is a promising and effective auxiliary tool to characterize the nutritional status of pomegranate trees and, in turn, an alternative to conventional methods of nutritional diagnosis with the advantage of establishing a nutritional deficiency (soil-plant relationship) or a physiological imbalance (relation plant - climate) for this crop.

Keywords: cation balance; cv. 'Wonderful'; leaf analysis; leaf standards; mineral composition

Introduction

Pomegranate (*Punica granatum* L.) presents great expectations for its cultivation due to its profitability and its adaptability to development in arid zones and with few water requirements, being able to develop and produce in conditions in which other fruit trees would not do so profitable (Moreno, 2010). There are more than 1,000 cultivars of *Punica granatum*, originating in the Middle East and extending throughout the

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Mediterranean, to eastern China and India, as well as in the southwestern United States, California, and Mexico (Çam *et al.*, 2009). Data on total area and world production are currently not reported due to the rapid increase in cultivation in recent years. In 2014, an approximate production of 3 million tons (t) was estimated and by 2017 the estimated production was 3.8 million tons (Karapetsi *et al.*, 2021).

In 2020, it was reported that México has a planted area of 1,262 hectares (ha), of which 1,146.25 ha are harvested for a total production of 8,769.36 t. The Chihuahua state has a planting extension for pomegranate cultivation of 45 ha, of which 40 ha are harvested with a production of 615 t in the year 2020 (SIAP, 2020).

In the pomegranate, as in other fruit crops, the growth, yield and quality of the fruits are influenced by the dynamics of the nutrients (Maity *et al.*, 2017; Zahedi *et al.*, 2019). Therefore, monitoring the nutrient content in the plant throughout the season is essential to define the optimal conditions for the crop, as well as to determine the moment and the amount of nutrients that the plant needs (Maity *et al.*, 2019). Due to the impact of nutrients on the production and quality of crops, it is necessary that fertilization systems are designed exclusively for the specific requirements of the crop. Thus, it should indicate where, how, how much, and at what time to apply the different fertilizers (Chater *et al.*, 2019), in order to have effective fertilization that contributes to increasing the quality and production of crops.

There are adequate diagnostic tools to avoid nutrient shortages or excesses. Leaf analysis is a tool that helps to accurately identify the need for fertilization; this type of analysis is based on the positive relationship between the doses of nutrients supplied, the nutrient content in the leaf and the crop yield (Chater *et al.*, 2019). Leaf analysis can be useful to assess the nutritional status of plants if adequate diagnostic procedures are available from the analytical data obtained from leaf analysis (Bhargava and Chadha, 1993). In this case, the Integrated Difference Diagnosis (IDD) is a tool that helps interpret these nutritional analyses through the prediction of nutritional and/or physiological imbalances. IDD is based on enzyme kinetics and generates a scale in which values less than 0.71 indicate a nutritional deficiency (ND), which refers to a soil-plant relationship and is generally corrected with the provision of nutrients. However, values greater than 2.0 show a physiological imbalance (PI), which speaks of a plant-climate relationship and can be caused by an imbalance in nutrition management. In addition, an optimal nutrition range of 0.84-1.19 is mentioned (Uvalle, 1993, 1995; Uvalle and Chávez, 1994; Yáñez, 1998; Soto-Parra *et al.*, 2003). In the literature, examples of physiological imbalances have been reported that were diagnosed using IDD, such as the relationship of cations in the quality of the apple (Mancera-López, 2018); apple rosette (Sánchez *et al.*, 2001); abiotic stress (Soto *et al.*, 2020) and physiological disorders associated with calcium (Soto *et al.*, 1999), etc. Therefore, the nature of nutritional or physiological imbalances can be clarified through the physiological foundations of IDD.

On the other hand, numerous studies have described the chemical characteristics of various pomegranate cultivars, however, few have been carried out on 'Wonderful', ignoring the importance of this cultivar since it is considered the standard cultivar for the industry in countries such as the United States and Israel (Chater and Garner, 2019). Therefore, it is necessary to implement innovative methodologies that help producers to optimize yield and profitability for agricultural sustainability. Hence, the objective of this work is mainly focused on establishing nutritional standards for the 'Wonderful' pomegranate cultivation through the IDD.

Materials and Methods

Collection of leaf samples

Leaf samples of 'Wonderful' pomegranates were taken on October 11, 2019, collected completely randomly in eight different lots located in the Coyame area (Latitude: 28.6353, Longitude: 106.089 28° 38′ 7″ North, 106° 5′ 20″ West) in the state of Chihuahua, México. Five points were considered within each orchard, aligned to the center and the four cardinal points. Once the central tree to be sampled was defined, five trees were spaced in each of the central, north, southeast, and west directions, in which around 30 leaves were collected, thus forming the composite samples, such that each sample represents five trees, for each sampling point, and five repetitions for each lot.

Soil and water characteristics

In the case of agronomic management, it is given in a rustic way, since no type of nutrient is provided to the soil, irrigation is given every 15 to 21 days, with pruning generally to obtain propagation material. Table 1 shows the properties of the soil in each of the plots, as well as the characteristics of the irrigation water (Table 2).

Table 1. Soil characteristics

Plot	Texture	E.C	$CaCO_3$	O.M	NO_3	P	pH	K	Ca	Mg	Na	Cu	Fe	Mn	Zn
1	Sandy clay loam	0.5	19.4	1.8	90.0	8.47	7.85	587.5	2875.0	300.0	475.0	0.3	1.0	1.7	1.2
2	Sandy clay loam	2.0	20.5	1.6	86.3	7.3	7.7	537.5	3025.0	250.0	762.5	0.1	0.5	2.3	0.2
3	Loamy sandy	1.6	20.5	0.7	105.0	15.1	7.6	437.5	2375.0	162.5	325.0	0.1	0.5	2.7	0.2
4	Sandy clay	1.9	19.4	1.6	90.0	5.8	7.8	512.5	2887.5	212.5	387.5	0.2	0.5	2.6	0.4
5	Sandy clay loam	1.8	18.3	1.4	97.5	7.5	7.7	312.5	2712.5	162.5	787.5	0.2	0.7	2.1	0.1
6	Loamy sandy	2.9	19.4	1.4	90.0	9.0	7.7	412.5	2525.0	175.0	537.5	0.1	0.7	2.0	0.3
7	Sandy loam	4.5	21.2	1.4	136.9	11.1	7.8	350.0	3075.0	262.5	1850.0	0.1	0.8	2.3	0.2
8	Sandy loam	3.0	29.9	1.3	112.5	9.9	7.8	262.5	2700.0	150.0	1275.0	0.1	0.8	2.0	0.1

Data are presented in: Electrical conductivity (E.C. mmhos cm); Calcium carbonates ($CaCO_3$ %), Organic matter (O.M. %); NO_3, P (kg ha^{-1}); K, Ca, Mg, Na, Cu, Fe, Me, Zn (mg kg^{-1}).

Table 2. Water characteristics

Cations	Anions	SAR 2.19	SP 4.58
Ca 4.65	$CaCO_3$ 0.0	SP 40.12	%PrS 52.22
Mg 0.46	HCO_3 5.00	PSP 93.99	pH 7.65
Na 3.50	SO_4 0.0	RSC - 0.11	E.C. 995
K 0.12	Cl 4.58	ES 3.72	Classification: C2 S1

The data is presented in: cations and anions (mEq L), Sodium Adsorption Ratio (SAR), Sodium Percentage (SP), Possible Sodium (PSP), Residual Sodium Content (RSC), Effective Salinity (ES), Potential salinity (PS), Percentage of precipitable sodium (%PrS), Electrical conductivity (E.C.), Classification C2 S1 (Medium salinity with little sodium, good for irrigation)

Determination of mineral content

For each of the composite samples, a triple washing with common water, water with 4 N hydrochloric acid and finally with distilled water, followed by the leaf samples were dried in a drying oven at 30 °C and subsequently ground in a mill type Willey was finally weighed in for chemical analysis.

The total nitrogen concentration (TN) was determined by the Micro-Kjeldahl method. For the determination of micro and macro elements (Sodium (Na), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), Calcium (Ca), Potassium (K), Magnesium (Mg)), a by triadic digester mixture and atomic absorption spectrophotometry using a Perkin Elmer® equipment (model AAnalyst 100). For phosphorus (P), it was determined by means of triacid digestion, ammonium metavanadate molybdate and concentrations in a UV spectrophotometer (Model Spectronic® Genesys 5).

For the determination of nitrates (NO_3) the following was carried out: 0.2 g of the sample was weighed, placed in plastic bottles, 25 ml of distilled water were added and stirred for 10 min, filtered and 2.5 ml of water were taken. The sample was filtered in a beaker, 1 ml of $CaCO_3$, 0.5 ml of 30% H_2O_2 were added and left in the oven at 30 °C overnight, the next day 1.5 ml of phenoldisulfonic acid were added, 25 ml of distilled water and 10 ml of ammonium hydroxide (1:1), the samples are made up to 100 ml with distilled water and finally they were read in the spectrophotometer at 425 n.m.

Integrated Differential Diagnosis

The Integrated Differential Diagnosis (IDD) is made up of three concepts: 1) Diagnosis, which is defined as the knowledge of the difference between two situations, one that is considered ideal, and the other that is expected to behave in a different way. 2) Differential, conceptualized in making contrasts to its minimum expression to understand the nature of its deviation between both situations and 3) Integrated, focused as a whole, where certain areas of knowledge actively participate (physiology and plant nutrition, biochemistry, etc), which help to broaden and deepen the knowledge of the Water-Soil-Plant-Atmosphere factors, to identify the causes that limit the production or productivity of the fruit and vegetable activity (Uvalle-Bueno, 1995). The physiological foundation of IDD in plants is described hereafter.

We have the mechanisms of enzyme kinetics, the principles of catabolism and the synthesis of energy-rich phosphate compounds:

1) Energy charge equation. Cells contain an infinite number of energy-storing compounds, particularly adenosine phosphates (AMP, ADP, and ATP) that can be present as high-energy or low-energy compounds. Thus, a cell can be said to be "fully charged" when all its adenylates are present as ATP. Simply when all the ATP is hydrolyzed to AMP, the cell is "fully discharged." It can be deduced that the energy charge will have a value equal to 1 when all the cellular AMP and ADP have been converted into ATP. It is possible to approach this condition, when, for example, the cell performs its oxidative phosphorylation (involves oxidation-reduction reactions) at an accelerated rate at a time when few biosynthetic reactions are carried out. The maximum number of high-energy phosphate bonds will be available, and the energy charge will be equal to 1.5, when all adenosine compounds appear as ADP, which means that the adenyl system contains half of the high-energy bonds. The energy charge will have a value equal to zero, that is, the adenylic system will lack energy-rich structures, when ATP and ADP have been converted into AMP. Studies carried out on the energy load indicate that: ATP = 0.5 – 2.5 g kg of weight in a constant biological system.

2) Free activation energy (G). It is the amount of energy needed to bring all the molecules of 1 mol of substances at a given temperature, to the transition state, at the top of the activation barrier. When the value of G is negative, in biological processes there is a release of inorganic P, and an opposite element (antagonistic) is found that immobilizes it, therefore, it is not available for the plant.

3) Q_{10} Generally has a value of 2. Enzymatic reactions differ from chemical ones because the increase in speed with respect to temperature is interrupted between 45 and 50 °C, since a denaturation of proteins (enzymes) occurs; therefore, the Q_{10} of an enzyme for ranges above 40 °C will generally be much less than 2.0 (Lehninger, 1991).

4) Enzyme kinetics (Michaelis-Menten equation). The general principles of chemical reaction kinetics apply to enzyme-catalyzed reactions, but enzyme-catalyzed reactions also exhibit a characteristic feature not seen in non-enzymatic reactions: substrate saturation. The smaller Km is, the greater its affinity with enzymes, therefore, its depletion is faster; and vice versa, the higher the value of Km, the lower its affinity, so its decay is slower.

Development of critical values of IDD. Y describes the nutritional situation; Z describes the physiological situation. Energy charge values (EC = 1), Michaelis-Menten constant ($Km = \frac{1}{2}$) y $Q_{10} = 2$; describe nutritional deficiency (DF): $Z = (CE^{*}Q_{10}) = 2.0$, the equilibrium situation corresponding to X = 1.0, the intensity of the shift towards a nutritional disorder or physiological disorder is inversely exponential (Figure 1). The optimal nutritional range is between $0.71 \leq Y \leq 1.0$, and the optimal physiological range is between $1.0 \leq Z \leq 1.19$. It can be said that an equilibrium situation exists when:

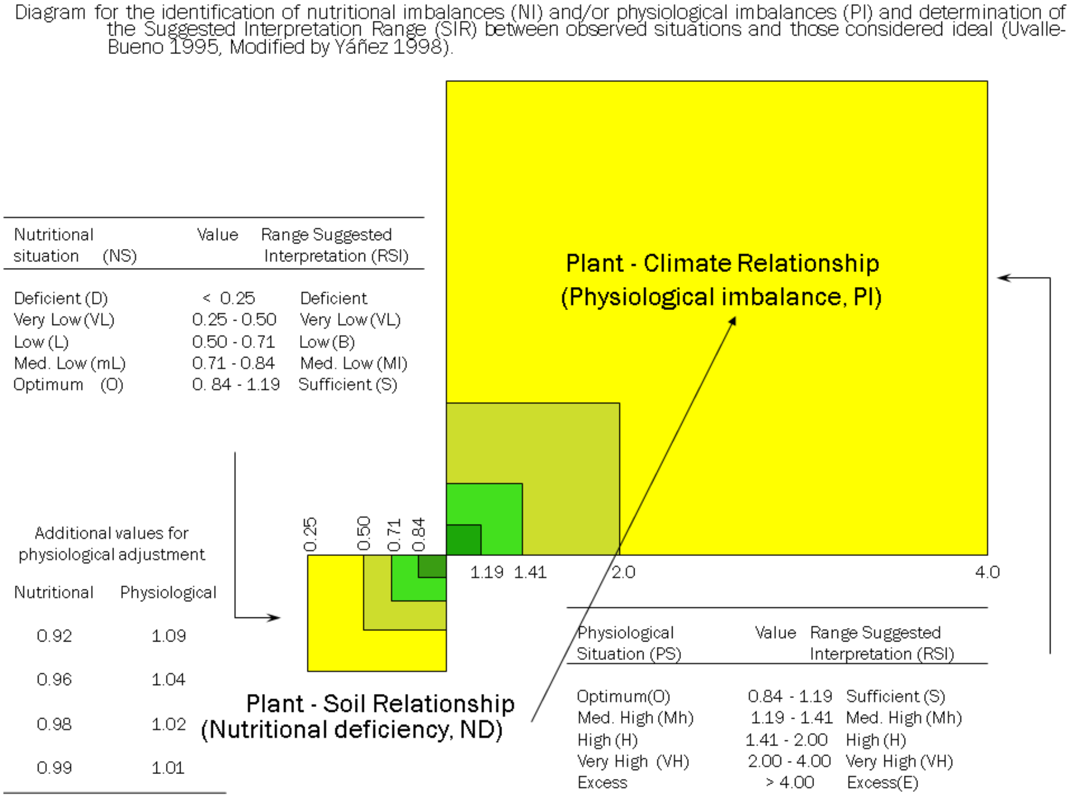
$$X = (1.0 \text{ when there is only ATP, CE} = 1.0\text{, Km} = \frac{1}{2}\text{, } Q_{10} = 2) \tag{1}$$

$$Y = \text{describes the nutritional situation; } Y = (X) * (\text{Km}) \tag{2}$$

$$Y = (1.0) * \left(\frac{1}{2}\right) = 0.5 \text{ you have a nutritional imbalance} \tag{3}$$

$$Z = \text{describe the physiological state; } Z = (Q_{10}) * (X) \tag{4}$$

$$Z = (2) * (1) = 2.0 \text{ there is a physiological imbalance} \tag{5}$$



Nutritional situation (NS)	Value	Range Suggested Interpretation (RSI)
Deficient (D)	< 0.25	Deficient
Very Low (VL)	0.25 - 0.50	Very Low (VL)
Low (L)	0.50 - 0.71	Low (B)
Med. Low (mL)	0.71 - 0.84	Med. Low (Ml)
Optimum (O)	0. 84 - 1.19	Sufficient (S)

Additional values for physiological adjustment

Nutritional	Physiological
0.92	1.09
0.96	1.04
0.98	1.02
0.99	1.01

Physiological Situation (PS)	Value	Range Suggested Interpretation (RSI)
Optimum(O)	0.84 - 1.19	Sufficient (S)
Med. High (Mh)	1.19 - 1.41	Med. High (Mh)
High (H)	1.41 - 2.00	High (H)
Very High (VH)	2.00 - 4.00	Very High (VH)
Excess	> 4.00	Excess(E)

Figure 1. Diagram for the identification of nutritional imbalances (NI) and/or physiological imbalances (PI) and determination of the Suggested Interpretation Range (SIR) between observed situations and those considered ideal (Uvalle-Bueno, 1995, modified by Yáñez, 1998)

Generation of nutritional standards through the IDD

For each nutrient analysed and the ratio of cations, seven levels, values or mineral concentrations were obtained: Deficient (D), Very Low (VL), Low (L), Sufficient (S), High (H), Very High (VH) and Excess (E), based on the IDD, defined as the seven possible ranges in which nutrients can be in a tissue, related to relative growth, crop production or its quality (Soto *et al.*, 2003). Given the importance of the mineral cation ratio, the following milliequivalent ratios were determined: (Ca+Mg)/K, Ca/(Mg+K), Mg/(K+Ca), Ca/Mg and K/Mg (Mancera Lopez *et al.*, 2018). In this way, the average of each nutrient and cation ratio was multiplied by each of the critical values, generating the mineral interval for each category with the product of the antecedent and present critical value for that category. For each mineral range, the arithmetic mean (average of the lower limit and upper limit) was obtained, with the mean of the sufficient interpretation range being the reference for all nutrients and mineral ratios (Table 3) (Mancera López *et al.*, 2018).

Table 3. Relationship between physiological and nutritional situations

	Physiological situation					Nutritional situation				
X	0.0625	0.25	0.50	0.71	0.84	1.19	1.41	2.00	4.00	16.0
Direct	$\leftarrow X^2$					$X^2 \rightarrow$				
Inverse	16.0	4.0	2.0	1.41	1.19	0.84	0.71	0.50	0.25	0.0625
Additional			0.92	0.96	0.98	1.02	1.04	1.08		

Results and Discussion

Leaf analysis is a nutritional diagnostic tool used in fruit production. The collection period depends on the species and the sampling objective. The result of the foliar analysis indicates the percentage content of nutrients present in the leaf in most fruit species (Soto *et al.*, 2008).

Table 4. Range nutritional sufficiency for macronutrients in pomegranate

Range		Deficient (D)	Very low (VL)	Low (L)	Sufficient- Optimum (S -O)	High (H)	Very high (VH)	Excess (E)
IDD		0.25	0.5	0.71	0.84 - 1.19	1.41	2.00	4.00
Nutrient	Mean							
NO_3	3692.2	< 1846.1	1846.1-2621.5 2233.7	2621.5 - 3101.4 2861.4	3101.4 - 4393.7 **3747.5**	4393.7 - 5206.0 4799.8	5206.0 - 7384.4 6295.1	> 7384.4
NT	1.33	< 0.67	0.68 - 0.95 0.81	0.96- 1.12 1.03	1.13 - 1.59 **1.35**	1.60 - 1.88 1.73	1.89 - 2.67 2.27	> 2.68
P	0.14	< 0.07	0.08 - 0.10 0.08	0.11- 0.11 0.11	0.12 - 0.16 **0.14**	0.17 - 0.19 0.18	0.20 - 0.27 0.23	> 0.28
K	0.87	< 0.44	0.45 - 0.62 0.53	0.63- 0.73 0.67	0.74 - 1.04 **0.88**	1.05 - 1.23 1.13	1.24 - 1.74 1.48	> 1.75
Ca	1.63	< 0.82	0.83-1.16 0.99	1.17- 1.37 1.26	1.38 - 1.94 **1.65**	1.95 - 2.30 2.12	2.31 - 3.26 2.78	> 3.27
Mg	0.19	< 0.10	0.11-0.13 0.11	0.14-0.16 0.15	0.17-0.23 **0.19**	0.24-0.27 0.25	0.28-0.38 0.32	> 0.39
Na	0.006	< 0.003	0.004-0.004 0.004	0.005-0.005 0.005	0.006 - 0.007 **0.006**	0.008 - 0.008 0.008	0.009-0.012 0.010	> 0.013

Data is presented in NO_3 (μgg^{-1} DW), NT, P, K, Ca, Mg, Na (%). Generated by Integrated Differential Diagnostics (IDD). The mean of each nutrient was multiplied by each of the critical values, generating the nutritional interval for each category with the product of the antecedent and present critical value for that category.

Table 4 shows the nutritional standards for pomegranate macronutrients, in this case, NO_3 deficiency is below 1846.1 $\mu g\ g^{-1}$, while the sufficiency range is 3101.4-4393.7 $\mu g\ g^{-1}$ with a mean of 3747.6 $\mu g\ g^{-1}$ and the excess occurs above 7384.4 $\mu g\ g^{-1}$. Mancera-López (2005) determined the ranges of sufficiency in two varieties of apple, where the maximum values of nitrates ranged between 1008.3-1428.4 $\mu g\ g^{-1}$, these data are below the values obtained here, so these differences may be due to the type of crop and the region. For TN, the deficiency occurs below 0.67%, the optimal mean is 1.35%, and the excess occurs when the content is greater than 2.66%. Savita *et al.* (2016) reports a sufficiency range of 0.91-1.66%, which agree with the results obtained in this research, while Chater and Garner (2018) mention a percentage of 1.44% and 2.0% in pomegranates of the 'Wonderful' variety in California, while Urlic *et al.* (2019) mention ranges of 1.41-1.67% in different varieties grown in Croatia. The P below 0.07% indicates deficiency in pomegranate and a sufficient-optimal range of 0.11-0.16% whose mean is 0.14%, while above 0.28% it is shown as an excess. This sufficient-optimal value agrees with what was reported by Chater *et al.* (2019) who report a range of 0.13-0.15%. In the case of Croatian cultivars ranges of 0.12-0.40% are mentioned (Urlić *et al.*, 2019). The K showed deficiency below 0.44%, an optimal mean of 0.88% in a range of 0.73-1.04% and an excess when the percentage is greater than 1.74%. Hasani *et al.* (2012), reports K levels between 0.85-1.0%, while Chater and Garner (2018) mention levels of 0.93 and 0.74% in pomegranate control plants. These data agree with those obtained through the IDD. In the case of Ca, a level lower than 0.82% predicts a state of deficiency, from 1.37-1.94% a range of sufficiency with an average pressure of 1.65% and an excess of this element above 3.26%. Savita *et al.* (2016) report a percentage of 1.81% obtained through the Integrated Diagnostic and Recommendation System (DRIS) which is within the sufficient-optimal range obtained by the IDD 1.37-1.94%. Mg below 0.10% indicates deficiency, 0.16-0.23% is its range of sufficiency with a mean of 0.19% and above 0.38% shows an excess. Savita *et al.* (2016) and Chater *et al.* (2019) report values above 0.30% while Urlić *et al.* (2019) mention values of 0.13-0.23%, which are similar to the IDD values. For Na, its deficiency values are below 0.003%, 0.005-0.007% its sufficient-optimal state with an average of 0.006% and above 0.012% a physiological imbalance due to excess. Holland *et al.* (2009) mention a percentage of 0.02%, these differences between the mentioned results may be due to the sampling time or the prevailing edaphoclimatic conditions in the crop (Hepaksoy *et al.*, 2000).

Table 5. Range nutritional sufficiency for cation balance in pomegranate

Range		Deficient (D)	Very Low (VL)	Low (L)	Sufficient- Optimum (S -O)	High (H)	Very High (VH)	Excess (E)
IDD		0.25	0.5	0.71	0.84 - 1.19	1.41	2.00	4.00
Nutrient	Mean							
(Ca+Mg)/K	4.41	< 2.21	2.21 - 3.13 2.67	3.13 - 3.70 3.42	3.70 - 5.25 **4.48**	5.25 - 6.22 5.73	6.22 - 8.82 7.52	>8.82
Ca/(K+Mg)	2.10	< 1.05	1.05 - 1.49 1.27	1.49 - 1.76 1.63	1.76 - 2.50 **2.13**	2.50 - 2.96 2.73	2.96 - 4.20 3.58	> 4.20
Mg/(K+Ca)	0.157	< 0.08	0.08 - 0.11 0.09	0.11 - 0.13 0.12	0.13 - 0.19 **0.16**	0.19 - 0.22 0.20	0.22 - 0.31 0.27	> 0.31
Ca/Mg	4.99	< 2.50	2.50 - 3.54 3.02	3.54 - 4.19 3.87	4.19 - 5.94 **5.06**	5.94 - 7.04 6.49	7.04 - 9.98 8.51	> 9.98
K/Mg	1.34	< 0.67	0.67 - 0.95 0.81	0.95 - 1.13 1.04	1.13- 1.59 **1.36**	1.59 - 1.89 1.74	1.89 - 2.68 2.28	> 2.68

Data is shown in mEq/100 g^{-1}. Generated by Integrated Differential Diagnostics (IDD). The mean of each nutrient was multiplied by each of the critical values, generating the nutritional interval for each category with the product of the antecedent and present critical value for that category.

Davarpanah *et al.* (2017) mentions that the changes that occur in the mineral concentrations in the leaves after fertilization are attributed to synergistic and antagonistic relationships between the nutrients, so in Table 5 we can see the cation balances for 'Wonderful' pomegranates, where in the case (Ca+Mg)/K shows a deficiency below 2.21 mEq 100 g^{-1}, a sufficiency of 3.70-5.25 mEq 100 g^{-1} with a mean of 4.48 mEq 100 g^{-1} and an excess greater than 8.82 mEq 100 g^{-1}. In apple stems, a maximum concentration of 1.39-1.96 mEq 100 g^{-1} is reported (Mancera-López, 2007). In this case, K applications can improve the yield and quality of the fruit. It is also reported that the foliar application of different forms of K increased the quantity and quality of the mandarin fruit (Davarpanah *et al.*, 2017). In addition, Khayyat *et al.* (2012), reports that applications of KNO_3 (500 mg L^{-1}) in pomegranate increased the diameter, weight and volume of the fruit compared to the control. Potassium plays an important role in different processes in plants, including photosynthesis, respiration, ion absorption and transport, protein synthesis, and enzyme activation (Navarro *et al.*, 2000). For the Ca/(K+Mg) ratio at a level of 1.05 mEq 100 g^{-1} it shows deficiency, while the sufficient-optimal range goes from 1.76-2.50 mEq 100 g^{-1} with a mean of 2.13 mEq 100 g^{-1} and higher than 2.96 -4.20 mEq 100 g^{-1} as very high, therefore, above this value excess is marked. In 'Golden Delicious' apple peduncles a maximum range of 0.39-0.50 mEq 100 g^{-1} is mentioned (Mancera-López, 2005), which in the case of pomegranate is well below the range of deficiency. Gransee and Führs (2013), mention that the high availability of Ca decreases the absorption of Mg and K, due to the antagonism between each other, for which deficiencies of Mg and K are often found in the production, while the nutritional disorders caused by Ca^{2+} deficiency, even without visible symptom appearances on foliage, can significantly limit crop yield. The presence of Ca guarantees the stability of the cellular structure and mechanical resistance, and it is an important component of plant tissue, participating in the protection of plant cells and in the regular process of maturation (Korkmaz *et al.*, 2016). In addition, disorders such as bitterness and cracking of the fruit have been associated with low levels of Ca ions. In this case, the cell walls have been recognized as the places where Ca undergoes cross-linking with acid pectin residues. Low-methylated pectin molecules cross-linked with Ca ions make cell walls more rigid and consequently increase tissue firmness (Saei e*t al.*, 2014). In turn, it has been reported that at the end of fruit growth, the rigidity of the peel increases, which the authors suggested as a result of greater lignification and degradation of pectin (Saei *et al.*, 2014). Singh *et al.* (1993) hypothesized that foliar nutrient sprays increase the elasticity and permeability of the fruit peel cell wall by decreasing pomegranate peel cracking. On the other hand, for Mg/(K+Ca) in a range of 0.08 mEq 100 g^{-1} indicates deficiency, 0.13-0.19 mEq 100 g^{-1} is considered as the optimal range with a mean of 0.16 mEq 100 g^{-1} of sufficiency and higher than 0.31 mEq 100 g^{-1} as excess, while Mancera-López (2005) reports a range of 0.13-0.19 mEq 100 g^{-1} both in peduncles and in apple peel and pulp, data that are similar to those obtained by the IDD for pomegranate leaves. In this case, mention is made of cationic competition, where Mg absorption is strongly influenced by the availability of other cations such as NH_4, Ca and K (Navarro *et al.*, 2000). Mg deficiency presents carbohydrate accumulation in the source leaves and reduced root growth due to restricted supply of carbohydrates to the roots. Altered carbohydrate partitioning can be considered as a latent deficiency affecting yield (Gransee and Führs, 2013). In turn, for the Ca/Mg ratio, below 2.50 mEq 100 g^{-1} was observed, it is considered deficient, 4.19-5.94 mEq 100 g^{-1} as the optimal range, while the mean of the sufficiency range is 5.06 mEq 100 g^{-1} and above 9.98 mEq 100 g^{-1} an excess is produced. Again, the comparison is made with what was reported in apple peduncles where the maximum reported value was 1.90-2.69 mEq 100 g^{-1} (Mancera-López, 2005). In mature leaves with low Ca concentration, the Ca/Mg ratio is around 0.8-0.9 mmol kg dry weight, while with high Ca level it is around 7 mmol kg dry weight. Therefore, a Ca/Mg nutritional imbalance can cause reduced plant growth, in this case Ca competes with Mg for binding sites on the plasma membrane and reduces the rate of Mg uptake (Navarro *et al.*, 2000). Finally, K/Mg showed that the deficiency is marked at 0.67 mEq 100 g^{-1}, the optimal range of 1.13-1.59 mEq 100 g^{-1} with its sufficient mean of 1.36 mEq 100 g^{-1} and above 2.68 mEq 100 g^{-1} it is interpreted as excess. Mancera-López, (2005) reports a value of 1.14-1.62 mEq 100 g^{-1} in 'Red Delicious' peduncles, which are similar to those observed in pomegranate leaves.

Table 6. Ranges of nutritional sufficiency for micronutrients in pomegranate

Range		Deficient (D)	Very Low (VL)	Low (L)	Sufficient- Optimum (S -O)	High (H)	Very High (VH)	Excess (E)
IDD		0.25	0.5	0.71	0.84 - 1.19	1.41	2.00	4.00
Nutrient	Mean							
Fe	58.6	< 29.3	29.3 -41.6 35.4	41.6- 49.2 45.4	49.2 - 69.7 **59.5**	69.7- 82.6 76.2	82.7 - 117.2 99.9	> 117.2
Mn	30.9	< 15.4	15.4- 21.9 18.7	21.9- 25.9 23.9	25.9- 36.7 **31.3**	36.7- 43.5 40.1	43.5 - 61.8 52.6	> 61.8
Zn	6.2	< 3.1	3.1 - 4.4 3.7	4.4 - 5.2 4.8	5.2 - 7.4 **6.3**	7.4 - 8.7 8.0	8.7 - 12.4 10.6	> 12.4
Cu	6.0	< 3.0	3.0 - 4.3 3.6	4.3 - 5.0 4.6	5.0 - 7.1 **6.1**	7.1 - 8.4 7.8	8.4 - 12.0 10.2	> 12.0

Data is presented in µg g^{-1} DW. Generated by Integrated Differential Diagnostics (IDD). The mean of ach nutrient was multiplied by each of the critical values, generating the nutritional interval for each category with the product of the antecedent and present critical value for that category.

In general, foliar nutrient applications can decrease the division of pomegranate fruits by increasing the flexibility of the peel, since the accumulation of solutes such as K, Mg and Ca in the vacuole are necessary for the osmotic potential necessary for cell expansion (Marschner, 1995). Chater and Garner (2018) mention that foliar applications of $MgSO_4$ and KNO_3 resulted in significant changes in the nutrient concentrations of the leaves and significantly reduced the incidence of pomegranate cracking. Changes in the incidence of split fruit could be due to direct or indirect effects of nutrient availability affecting peel elasticity, suggesting that foliar nutrient applications could be a promising and feasible practice to mitigate fruit split in 'Wonderful' cultivar.

Table 6 shows the optimal ranges for the micronutrients in which Fe shows deficiency at 29.3 μg g^{-1}, from 49.2-69.7 μg g^{-1} its sufficiency range with its optimal average of 59.5 μg g^{-1} and above 117.2 μg g^{-1} excess. In Croatian pomegranates, sufficiency ranges of 38.9-58.8 μg g^{-1} are managed depending on the variety (Urlić *et al.*, 2019), while Chater *et al.* (2019) mentions an optimal range of 70.0-85.0 μg g^{-1}. For the IDD this would be in the classification from high to very high, may be due to the different study sites since the varieties are similar. On the other hand, Mn is deficient at 15.4 μg g^{-1}, 25.9-36.7 μg g^{-1} are its optimal-sufficient values with an average of 31.3 μg g^{-1}, while 43.5-61.8 μg g^{-1} is very high, so that, above these values is considered as excess. Holland *et al.* (2009) mentions an optimal percentage in Israel of 33 μg g^{-1}, which is within the sufficient-optimal range of 25.9-36.7 μg g^{-1} obtained by the IDD. For Zn, the deficiency is present below 3.1 μg g^{-1}, from 5.2-7.4 μg g^{-1} it shows its range of sufficiency, and its optimal mean is 6.3 μg g^{-1}, in addition, above 12.4 μg g^{-1} it is in excess. Urlic *et al.* (2019) mention an optimal level of 8.41 μg g^{-1} in 'Wonderful' pomegranates, in this case for the IDD this sufficiency level would be high, again it is mentioned that the differences could be due to the study site. For Cu below 3.0 μg g^{-1}, this element is in deficiency, from 5.0-7.1 μg g^{-1} it is in sufficient conditions with an average of 6.1 μg g^{-1} and in excess when its value exceeds 12.0 μg g^{-1}. Hasani *et al.* (2012) mentions a level of 6.5 μg g^{-1} in pomegranate control plants, and it is similar to what Chater *et al.* (2019) since it mentions a range of 4.5-7.0 μg g^{-1} Cu, just below that obtained by the IDD of 5.0-7.1 μg g^{-1}.

Conclusions

The Integrated Differential Diagnosis is a promising and effective auxiliary tool to characterize the nutritional status of the pomegranate crop, in addition, it is an alternative to the conventional methods of nutritional diagnosis with the advantage of establishing a classification of deficiency or excess of nutrients. The sufficiency obtained for the macro elements were the following: NO_3 3747.55 μg g^{-1}, N 1.35%, P 0.14%, K 0.88%, Ca 1.65%, Mg 0.19%, Na 0.006%. For the cation balance, the optimal values were: (Ca+Mg)/K 4.48 mEq 100 g^{-1}, Ca/(K+Mg) 2.13 mEq 100 g^{-1}, Mg/(K+Ca) 0.16 mEq 100 g^{-1}, Ca /Mg 5.06 mEq 100 g^{-1}, K/Mg 1.36 mEq 100 g^{-1}. Finally, the optimal values for the microelements were: Fe 59.5 μg g^{-1}, Mn 31.3 μg g^{-1}, Zn 6.3 μg g,$^{-1}$ Cu 6.1 μg g^{-1}. Most of the optimal values obtained by the IDD were similar to those reported by different authors. Therefore, the IDD contributes to the identification and prioritization of nutritional deficiencies and/or physiological imbalances and determines the strategy to be followed for their correction. In addition, it is a tool for the formulation of efficient crop fertilization strategies. This work must be complemented with soil standards and their empirical validity on other parameters of plant development, such as vigour, the ratio of fruiting shoots / vegetative shoots, as well as production and quality.

Authors' Contributions

Conceptualization: JMSP, RMYM; Methodology: NGTB, LCNM, RMYM; Validation: JMSP, NGTB; Formal analysis: JMSP; Investigation: NGTB, LCNM, RMYM; Data curation: JMSP; Funding acquisition: JMSP; Project administration: JMSP; Writing: NGTB, LCNM; Review and editing: NGTB, JMSP, LCNM; All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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