*Article*

# Approach to Petiole Sap Nutritional Diagnosis Method by Empirical Model Based on Climatic and Growth Parameters

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**Abstract:** The aim of this work is to evaluate the relationship between the nutrient concentration in petiole sap and different agronomic and climatic variables for a tomato crop grown in a greenhouse in Mediterranean conditions. In addition, the persistence of the nutrient concentration in petiole sap was investigated with the aim of determining the sampling period that implies the best trade-off nutritional information. The experiment consisted of the selection of 20 sampling points inside the greenhouse. The samples of petiole, fully expanded leaf, and soil solution samples were collected weekly from 86 to 163 days after transplanting. Chloride, $NO_3^--N$, $H_2PO_4^--P$, $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ concentrations were determined in petiole sap and soil solution obtained by suction cups. Nitrogen, P, K, Cl, and Na concentrations were also determined in leaf. Finally, the petiole sap nutritional diagnosis method is the highest sensitive nutritional diagnosis method which compares soil solution and nutrient leaf content related to yield, and the statistical analysis performed in this research demonstrates that crop evapotranspiration (ETc), vapor pressure deficit (DPV), and leaf area index (LAI) are considered the most significant variables that allow the development of these empirical prediction models regarding nutrient concentration in petiole sap.

**Keywords:** crop evapotranspiration; leaf analysis; leaf area index; petiole sap; soil solution; vapor pressure deficit (VPD)

## 1. Introduction

The method of petiole sap analysis is used for the determination of crop nutritional status since 1920 [1,2] and is carried out on fresh material, giving a semiquantitative evaluation of the extractable nutrients that are present in soluble inorganic forms in the plant just at the sampling moment [3].

Xylem and phloem sap plus apoplastic solution are used to determine the nutrient concentration in a crop [4], but in order to establish a crop a nutritional diagnostic is required for comparison with

the sufficiency ranges of this crop [5]. The sufficiency ranges are commonly used to interpret plant nutrient composition as well as nutrient deficiency, sufficiency, luxury consumption, or excess [6].

There are several factors which can modify the nutrient sap concentration, such as fertilizer application, light intensity, phenological stage, and sampling position [2,7–9]. Regarding fertilizer application, Ikeda [10] reported a significant decrease in sap concentration of $NO_3^-$, $H_2PO_4^-$, $Ca^{2+}$, and $Mg^{2+}$ in tomato grown in nutrient solution after 10 days without nutrient solution. Considering the light intensity, Leyva et al. [11] found that in a tomato crop in the cycle of autumn–winter, when the days are shorter and the light intensity is lower than in summer months, there is a decrease in the synthesis of carboxylates and soluble carbohydrates used as osmolytes in plants resulting in a higher uptake of $NO_3^-$ to be used as osmolytes. Cadahía [12] found a significant variation in the concentration of $Cl^-$, $NO_3^-$-N, $H_2PO_4^-$-P, $SO_4^{2-}$-S, $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ in sap throughout the different phenological stages of a tomato crop. Garcia and Azuara [13] reported a high variation between sampled leaves selected (young leaves, fully grown young leaves, mature leaves, and older leaves), reporting that the concentration of $H_2PO_4^-$–P was higher in the young leaf because this ion is located in places with maximum metabolic activity. Similiarly, Llanderal et al. [14] reported that the selection of sampled leaves (fully grown young leaves, mature leaves, and older leaves) did not modify the $Ca^{2+}$, $Cl^-$, $SO_4^{2-}$S, and $Na^+$ concentrations, whereas $NO_3^-$-N, $K^+$, $Mg^{2+}$, and $H_2PO_4^-$-P concentrations showed great variability due to the selection of the sample leaf, therefore it is necessary to be careful with the sample selection.

The wide variability of sufficiency ranges in tomato proposed by different researchers includes: $NO_3^-$-N (700–1210 mg $L^{-1}$), $K^+$ (3500–5000 mg $L^{-1}$) [2], $PO_4H_2^-$-P (35–300 mg $L^{-1}$), $Ca^{2+}$ (280–1420 mg $L^{-1}$), $Mg^{2+}$ (190–2000 mg $L^{-1}$) [12], $Cl^-$ (750–4500 mg $L^{-1}$), and $Na^+$ (50–400 mg $L^{-1}$) [15] which can be related to the different factors aforementioned. Therefore, the aim of this work is to evaluate different parameters in relation with the nutrient concentration in petiole sap in a tomato crop in a greenhouse cultivated in Mediterranean conditions and to propose an empirical model in order to determine the nutrient concentration in petiole sap.

## 2. Materials and Methods

### 2.1. Plant Material and Experimental Conditions

This study was carried out in an Almeria-type greenhouse (1024 m²) located in the "UAL-ANECOOP; Research Center" in a culture system called "mulching sandy soil", which is the standard cropping system of the area, constituted by a layer of grave-sand (granulometry variable compound of particles smaller than 1 mm up to 5 mm), an intermediate layer of organic matter (it is usually placed in layer or mixed whole or in part with the topsoil), and natural soil (variable texture which can vary from clay to sandy loam, or the autochthonous soil) [16]. The tomatoes (*Solanum lycopersicum* cv. "Ramyle RZ F1") had a planting density of 2 plants per m² in a crop cycle during autumn-winter (15/08/2012 to 25/01/2013). The nutrients were applied by means of fertigation with a trickle-feed distribution irrigation system with a flow of 3 L $h^{-1}$ per emitter. The nutrient solution contained (in mmol $L^{-1}$): $Cl^-$ 6.44, $NO_3^-$ 13.00, $H_2PO_4^-$ 1.26, $SO_4^{2-}$ 6.82, $K^+$ 7.62, $Ca^{2+}$ 5.50, $Mg^{2+}$ 3.90, $Na^+$ 7.54, an electrical conductivity (EC) of 3.40 dS $m^{-1}$, and a pH value of 5.80 and was applied from the first day of planting. The nutrient solution derived from tap water (pH, 7.7, EC 1.9 dS $m^{-1}$, $Cl^-$ 6.44, $NO_3^-$ 0.36, $SO_4^{2-}$ 5.36, $K^+$ 0.28, $Ca^{2+}$ 3.19, $Mg^{2+}$ 3.90, and $Na^+$ 7.54 mmol $L^{-1}$) and phosphoric acid ($H_3PO_4$), nitric acid ($HNO_3$), potassium nitrate ($KNO_3$), potassium sulphate ($K_2SO_4$), and calcium nitrate ($Ca(NO_3)_2$) supplies. The total water applied during the experimental period was 244.3 L $m^{-2}$ modified weekly according to the water requirements of the plant, ranging from 0.9 to 2.1 L $m^{-2}$ $day^{-1}$ over the entire growth period.

### 2.2. Experimental Design and Parameters Assessed

The experimental design consisted of the selection of 20 sample points inside the greenhouse (Figure 1). One sample of petiole and leaf of fully young leaves in the upper part and soil solution

(SS) per sample point were collected weekly from 86 (October) to 163 (January) days after transplanting (DAT) with a total of 240 samples. Additionally, ripe fruits of the 20 plants were harvested weekly from 107 to 163 DAT and weighed to determine the yield.
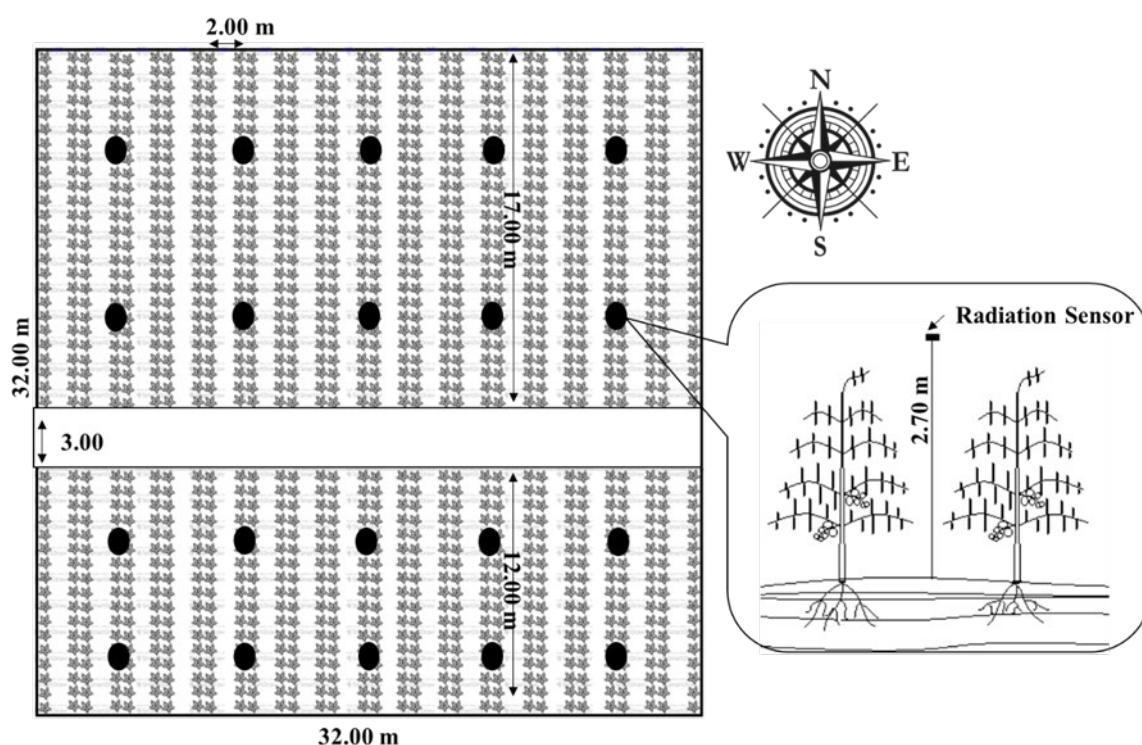


**Figure 1.** Location of sample points inside the greenhouse.

**Plant parameters:** Leaf area index (LAI) was estimated using the equation proposed by Medrano et al. [17] for tomato crop (Equation (1)):

$$LAI = (a + b)/((1 − \exp( − (DAT − c) × d)) \tag{1}$$

where a = −0.46, b = 5.1, c = 43.2, d = 19.1, and DAT = days after transplanting.

**Climatic conditions:** The climatic parameters inside the greenhouse were determined with 4 dry and wet bulb air temperature models (Nutricontrol, Murcia, Spain) by means of wire resistance thermometers in aspirated boxes (temperature and relative humidity) and 20 pyranometers (models CS300, Campbell Scientific., Barcelona, Spain) in order to determine the photosynthetically active radiation (PAR) at a height of 2.40 m. Crop evapotranspiration (ETc) was estimated using the equation proposed by Bailey et al. [18] (Equation (2)):

$$T = A × f_1 (LAI)R+B × LAI × DPV \tag{2}$$

where T (mm day$^{-1}$) is the crop transpiration, R is the solar radiation (MJ m$^{-2}$ day$^{-1}$), DPV is vapor pressure deficit (kPa), A and B (mm day$^{-1}$ kPa$^{-1}$) are both crop coefficients, which must be determined experimentally for each crop. The factor $f_1$ is a dimensionless exponential function that considers the interception of the radiation by the canopy as a function of an extinction coefficient (k) (Equation (3)):

$$T = 1e^{\wedge}((−k.LAI)) \tag{3}$$

**Suction cups:** Each suction cup was placed 10 cm from the neck of the plant with 15 cm of depth in order to extract the soil solution to a vacuum of −70 kPa, according to the methodology described by Lao et al. [19]. The nutrient parameters measured in the soil solution were as follows: pH and EC with a pH meter (model Milwaukee pH 52; Rocky Mount, NC, USA) and EC meter (model Milwaukee CE 66; Rocky Mount, NC, USA), respectively. Anions (Cl$^-$, NO$_3^-$, and H$_2$PO$_4^-$) and cations (K$^+$, Ca$^{2+}$, Mg$^{2+}$, and Na$^+$) concentrations were determined by HPLC (model 883 Basic IC Plus, anions

ion exchange column model Metrosep A SUPP 4, cations ion exchange column model Metrosep C4 100, IC conductivity detector range (0–15,000 μS cm$^{-1}$), Metrohm, Herisau, Switzerland), as described by Csáky and Martínez-Grau [20].

**Petiole sap:** Samples of fully developed young leaves were selected and cleaned using a damp cloth cleaved in fractions of 0.5 cm which were frozen at –16 °C for 24 h. Petiole sap (xylem and phloem sap plus the apoplastic, cytosolic, and vacuolar solution) extraction was performed using a hydraulic press, according to the methodology described by Cadahía [21]. In the petiole sap, Cl$^-$, NO$_3$$^-$-N, H$_2$PO$_4$$^-$-P, Na$^+$, K$^+$, Ca$^{2+}$, and Mg$^{2+}$ concentrations were determined by high-performance liquid chromatography (model 883 Basic IC Plus, anions ion exchange column model Metrosep A SUPP 4, cations ion exchange column model Metrosep C4 100, IC conductivity detector range (0–15,000 μS cm$^{-1}$), Metrohm, Herisau, Switzerland), as described by Csáky and Martínez-Grau [20].

**Leaf analysis:** The uppermost, fully expanded leaves were sampled. Samples were washed with distilled water and then dried to constant weight at 60 °C in a NüveE FN500 oven. After drying, leaf samples were then ground and divided into two subsamples: one was used to determine the soluble ionic forms (NO$_3$$^-$, SO$_4$$^{2-}$, and Cl$^-$) extracted with water and analyzed by HPLC (high-performance liquid chromatography, model Metrohm 883 Basic IC Plus) as described by Csáky and Martínez-Grau [20] and the other subsample was digested with sulfuric acid (H$_2$SO$_4$, 96%) in the presence of hydrogen peroxide (H$_2$O$_2$) at 300 °C to determine organic N and total P concentration colorimetrically. Organic N [22] was quantified at 630 nm and total P by the molybdo-vanadate method at 430 nm [23]. Total N was calculated as the sum of organic N and NO$_3$$^-$-N concentration. Total K$^+$ and Na$^+$ were directly measured by flame spectrophotometry [24] using a Jenway PFP7 flame photometer.

*2.3. Statistical Analysis*

The data obtained were analyzed and the average, minimum, and maximum values of all measured parameters were estimated in climatic variables and nutrient concentrations in the sap, soil solution, and leaf analysis. With the aim of developing functional relationships between each nutrient concentration in sap and the climatic variables and nutrient concentrations in the soil solution, a multivariate regression analysis was carried out.

To simplify the model and select the independent variables that most significantly affect the sap concentrations, a stepwise regression was performed. The stepwise regression procedure successively adds or removes variables from the regression with the aim of obtaining the simplest model containing only significant predictors, but without excluding useful variables.

An autocorrelation analysis was also performed with the temporal series of nutrient concentrations in petiole sap. The autocorrelation function is a measure of the correlation between observations of a time series that are separated by "k" time units. This autocorrelation analysis can be useful for detection if the concentration of a specific nutrient is dependent on the concentrations of previous sampling intervals. When a specific nutrient shows no temporal autocorrelation, it means that its concentration varies rapidly and randomly during the sampling period which can be regarded as a measure of the mobility of the nutrient in the sap. This autocorrelation analysis can be useful to establish the modeling of the nutrient considering its temporal persistence and establish appropriate sampling intervals.

The statistical analyses were conducted using the Statgraphics Centurion XVI© (Statpoint Technologies, Inc. Warrenton, VA, USA) software with different confident levels ($p < 0.05$ and $p < 0.01$, respectively).

**3. Results**

*3.1. Growth Conditions of the Experiment*

The climatic conditions during the experiment and the parameters used to evaluate the development of the plant are shown in Table 1. The ranges for the climatic parameters were: ETc from

1.17 to 2.43 mm day$^{-1}$, global radiation inside the greenhouse from 4.03 to 8.63 MJ m$^{-2}$ day$^{-1}$, temperature from 10.50 to 17.14 °C, relative humidity from 71.63 to 96.78%, vapor pressure deficit (VPD) absolute values from 0.21 to 0.53 kPa. The ranges for plant parameters considered were: LAI from 4.15 to 4.63 and yield from 7.2 to 9.4 kg m$^{-2}$. Other growth parameters estimated, such as plant height and relative growth rate, did not correlate with the other parameters (data not shown).

**Table 1.** Average, maximum, and minimum values of the climatic parameters (crop evapotranspiration (ETc) (mm day$^{-1}$), global radiation (Rad) (MJ m$^{-2}$ day), temperature (°C), relative humidity (%), vapor pressure deficit (VPD) (kPa), and plant parameters (leaf area index (LAI) and yield). N = 240 samples.

| | | Climatic Parameters | | | | Plant Parameters | |
|---|---|---|---|---|---|---|---|
| | ETc (mm day$^{-1}$) | Rad. (MJ m$^{-2}$ day$^{-1}$) | T$^{a}$ (°C) | RH (%) | VPD (kPa) | LAI (m$^2$ m$^{-2}$) | Yield (kg m$^{-2}$) |
| Min | 1.17 | 8.03 | 10.50 | 71.63 | 0.21 | 4.15 | 7.2 |
| Max | 2.43 | 6.63 | 17.14 | 96.78 | 0.53 | 4.63 | 9.4 |
| Ave | 1.79 | 4.78 | 14.51 | 85.43 | 0.32 | 4.52 | 7.9 |

Table 2 shows the average, maximum, and minimum values of the different parameters analyzed in soil solution, petiole sap, and leaf.

**Table 2.** Average, maximum, and minimum of soil solution values (pH and electrical conductivity (EC) (dS m$^{-1}$)) and concentrations (Cl$^-$, NO$_3$−, H$_2$PO$_4^-$, K$^+$, Na$^+$, Ca$^{2+}$, and Mg$^{2+}$ (mmol L$^{-1}$)), petiole sap concentrations (Cl$^-$, NO$_3^-$-N, H$_2$PO$_4^-$-P, Na$^+$, K$^+$, Ca$^{2+}$, and Mg$^{2+}$ (mg L$^{-1}$)), and leaf content (N, P, K, S, Na, and Cl (mg g$^{-1}$ DW)). N = 240 samples.

| | | | | Soil Solution (mmol L$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | EC (dS m$^{-1}$) | Cl$^-$ | NO$_3^-$ | H$_2$PO$_4^-$ | Na$^+$ | K$^+$ | Ca$^{2+}$ | Mg$^{2+}$ |
| Min | 7.43 | 3.15 | 8.91 | 12.22 | 0.18 | 9.71 | 6.24 | 7.65 | 6.57 |
| Max | 7.75 | 4.41 | 10.12 | 23.33 | 0.20 | 11.63 | 13.63 | 8.73 | 8.22 |
| Ave | 7.59 | 3.62 | 9.46 | 18.24 | 0.22 | 10.51 | 9.22 | 8.15 | 7.31 |
| | | | | Petiole Sap (mg L$^{-1}$) | | | | | |
| | - | - | Cl$^-$ | NO$_3^-$-N | H$_2$PO$_4^-$-P | Na$^+$ | K$^+$ | Ca$^{2+}$ | Mg$^{2+}$ |
| Min | - | - | 1041 | 1037 | 26.33 | 508.9 | 4000 | 486.1 | 1275 |
| Max | - | - | 1202 | 1408 | 185.3 | 608.1 | 4869 | 640.9 | 1965 |
| Ave | - | - | 1118 | 1247 | 72.83 | 552.2 | 4441 | 552.2 | 1674 |
| | | | | Leaf (mg g$^{-1}$ DW) | | | | | |
| | - | - | Cl | N | P | Na | K | - | - |
| Min | - | - | 6.29 | 33.5 | 3.42 | 4.58 | 46.3 | - | - |
| Max | - | - | 6.83 | 39.2 | 5.38 | 7.38 | 54.6 | - | - |
| Ave | - | - | 6.54 | 36.3 | 4.27 | 5.81 | 50.8 | - | - |

*3.2. Petiole Sap Nutrients, Climatic Parameters, and Nutrients in Soil Solution*

The main reasons for the great variability between nutrient concentration in the petiole sap may be ascribed to climatic conditions, plant growth, and nutrient concentration in the soil solution. In order to determine these relationships, correlation coefficients between all parameters in petiole sap were calculated (Table 3). There was a high positive correlation between ETc and nutrient concentrations in petiole sap for all nutrients. Additionally, a negative correlation was determined between global radiation and NO$_3^-$-N concentration in sap. Nevertheless, no correlation was found between temperature and nutrient concentrations in petiole sap, only Ca$^{2+}$ concentration showed a moderately negative correlation with relative humidity (RH). Similarly to ETc, DPV showed a high positive correlation with all the nutrients studied in the petiole sap. On the other hand, LAI showed

a high (Cl⁻, NO₃⁻-N, Ca²⁺ and Mg²⁺) and moderate (H₂PO₄⁻-P, Na⁺ and K⁺) positive correlation with the nutrients in the sap. Finally, the only significant correlation between the soil solution and nutrients in sap was in $NO_3^-$-N ($r$ = 0.68).

**Table 3.** Correlation coefficients (r) between petiole sap concentrations (Cl⁻, NO₃⁻-N, H₂PO₄⁻-P, Na⁺, K⁺, Ca²⁺, and Mg²⁺) (mg L⁻¹) and the climatic parameters (ET_c (mm day⁻¹), global radiation (MJ m⁻² day⁻¹), temperature (°C), relative humidity (RH) (%) and vapor pressure deficit (VPD) (kPa), LAI (−), and soil solution (SS) nutrient concentrations (Cl⁻, NO₃⁻, H₂PO₄⁻, SO₄²⁻, K⁺, Na⁺, Ca²⁺, and Mg²⁺) (mmol L⁻¹).

| Parameters | ETc | Rad. | Temp. | RH | VPD | LAI | SS |
|---|---|---|---|---|---|---|---|
| Cl⁻ | 0.88* | −0.06 | −0.29 | −0.30 | 0.83* | −0.84* | −0.15 |
| NO₃⁻−N | 0.85* | −0.61* | −0.32 | −0.20 | 0.80* | −0.84* | 0.68* |
| H₂PO₄⁻-P | 0.87* | 0.32 | 0.25 | 0.18 | 0.75* | −0.74* | 0.06 |
| Na⁺ | 0.68* | 0.05 | 0.30 | 0.06 | 0.53* | −0.69* | 0.11 |
| K⁺ | 0.65* | 0.27 | 0.24 | 0.38 | 0.63* | −0.52* | −0.21 |
| Ca²⁺ | 0.85* | −0.31 | −0.34 | −0.69* | 0.87* | 0.79* | 0.12 |
| Mg²⁺ | 0.82* | −0.33 | 0.25 | −0.24 | 0.75* | 0.91* | 0.13 |

\* indicates significant differences at $p < 0.05$.

Table 4 shows the autocorrelation between nutrient concentrations in petiole sap over time, where the sap nutrient concentrations corresponding to a week are related to the sap nutrient concentrations after one week (1), or 2 weeks (2), 3 weeks (3), and 4 weeks (4). It is important to highlight that NO₃⁻-N, H₂PO₄⁻-P, K⁺, Ca²⁺, and Mg²⁺ show a moderate autocorrelation with the nutrient concentrations of the previous week (week 1) but no correlations are found for longer periods. Nevertheless, no significant autocorrelations are found for Cl⁻ and Na⁺ concentrations over time.

**Table 4.** Autocorrelation of nutrient concentrations in petiole sap over time (1: determination in 7 days (1 week), 2: determination in 14 days (2 weeks), 3: determination in 21 days (3 weeks), and 4: determination in 28 days (4 weeks). To estimate the nutrient concentration of the following week, consider the nutrient concentration of the previous week.

| | | Lag | | | Equation |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | |
| Cl⁻ | 0.11 | −0.18 | −0.11 | −0.24 | y = 0.1523x + 939.25 |
| NO₃⁻-N | 0.79* | 0.55 | 0.30 | −0.01 | y = 0.8893x + 112.76 |
| H₂PO₄⁻-P | 0.61* | 0.52 | 0.22 | −0.10 | y = 0.7148x + 27.994 |
| Petiole sap Na⁺ | 0.28 | 0.12 | 0.00 | −0.41 | y = 0.2808x + 393.98 |
| K⁺ | 0.68* | 0.42 | 0.22 | 0.03 | y = 0.5778x + 1977.2 |
| Ca²⁺ | 0.55* | 0.36 | 0.12 | −0.01 | y = 0.7393x + 131.47 |
| Mg²⁺ | 0.67* | 0.37 | 0.10 | 0.01 | y = 0.9023x + 108.30 |

\* indicates significant differences at $p < 0.05$.

A stepwise regression approach for each nutrient was conducted in order to select the most fitted models with the minimum number of significant predictor variables. At each step of the stepwise procedure a new variable is entered or removed from the model based on the values of the partial F-test (F-value to enter or remove equal to 8). The regression coefficients and the independent variables selected by the stepwise regression procedure are shown in Table 5, along with the values of some goodness of fit statistics.

**Table 5.** Parameters of linear multiple regression between petiole sap (mg $L^{-1}$), climatic parameter (ETc (mm $day^{-1}$), global radiation (MJ $m^{-2}$ day), temperature (°C), relative humidity (%), vapor pressure deficit (VPD) (kPa), soil solution (mmol $L^{-1}$), and the concentration of petiole sap of the last week (mg $L^{-1}$) with C as the constant of the equation. Determination coefficient ($R^2$), adjusted determination coefficient (Adj $R^2$), F-ratio, and *p*-value of the stepwise regression for each nutrient are evaluated, (-) no significative correlation between parameters.

| Petiole Sap | Regression Coefficients | | | | | | | | | Statistics and Tests | | |
| | ETc | Rad. | Temp. | RH | VPD | LAI | SS | Petiole Sap (t−1) | C | $R^2$ (Adj $R^2$) | F-Ratio | *p*-Value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $Cl^-$ | 82.28 (17.27) | - | - | - | - | - | - | - | 964.96 (±30.86) | 76.42 (73.04) | 22.68 | <0.05 |
| $NO_3^-$-N | - | - | - | - | 1306.82 (±32.20) | - | - | - | 881.14 (±19.41) | 98.86 (98.48) | 240.25 | <0.05 |
| $H_2PO_4^-$-P | - | - | - | - | - | −701.28 (±66.56) | - | - | 3259.22 (±301.66) | 95.69 (94.82) | 111.01 | <0.01 |
| $Na^+$ | - | - | - | - | 69.29 (±26.71) | −91.06 (±32.36) | - | - | 940.39 (±144.93) | 79.95 (71.93) | 9.97 | <0.05 |
| $K^+$ | 148.64 (±40.67) | - | - | - | - | −1358.91 (±157.74) | - | - | 10240.80 (±740.18) | 97.39 (96.09) | 74.71 | <0.01 |
| $Ca^{2+}$ | - | - | - | - | 469.83 (±54.18) | - | - | - | 435.65 (±15.24) | 94.94 (94.68) | 75.18 | <0.01 |
| $Mg^{2+}$ | - | - | - | - | 399.63 (120.49) | 1469.45 (186.95) | - | - | −5072.22 (826.88) | 97.09 (95.63) | 66.80 | <0.01 |

All the adjusted models had a high goodness of fit since the coefficient of determinations ($R^2$) was moderate for $Cl^-$ and $Na^+$ and high for $NO_3^-$-N, $H_2PO_4^-$-P, $K^+$, $Ca^{2+}$, and $Mg^{2+}$, respectively. In five cases, their values were higher than 94% and only in two cases were lower than 80% (76.42% for $Cl^-$ and 79.95% for $Ca^{2+}$), which means that the adjusted models explain a high percentage of the nutrient petiole sap variability in all cases. In addition, the *p*-values for all the tests evaluated were less than 0.05, indicating that there is a statistically significant relationship between the variables involved in each model at the 95% confidence level. It is interesting to point out that the only significant variables are ETc, DPV, and LAI. In four cases ($Cl^-$, $NO_3^-$-N, $H_2PO_4^-$-P, and $Ca^{2+}$) it was necessary to use one significant predictor variable whereas in the other three cases ($Na^+$, $K^+$, and $Mg^{2+}$) it was necessary to use two significant predictor variables.

*3.3. Correlations between Different Diagnostic Methods and Yield*

Table 6 shows the correlation coefficient between nutrient concentration in soil solution, petiole sap concentration, and leaf content with the yield obtained in tomato crops. The concentration of $NO_3^-$-N, $H_2PO_4^-$P, and $K^+$ in petiole sap showed a high negative correlation with the yield ($r = -0.82$, $-0.77$, and $-0.91$). On the contrary, soil solution only showed a positive correlation between $K^+$ concentration and yield. With respect to leaf content, it was the same trend in petiole sap since N, P, and K showed a high negative correlation with the yield ($r = -0.69$, $-0.52$, and $-0.72$, respectively). Finally, it is important to point out that the petiole sap method shows the best coefficient of correlation with the yield compared to soil solution and leaf analysis.

**Table 6.** Correlation coefficients (r) between nutrient concentration in soil solution ($Cl^-$, $NO_3^-$, $H_2PO_4^-$, $SO_4^{2-}$, $K^+$, $Na^+$, $Ca^{2+}$, and $Mg^{2+}$) (mmol $L^{-1}$), petiole sap concentration ($Cl^-$, $NO_3^-$-N, $H_2PO_4^-$-P, $SO_4^{2-}$-S, $K^+$, $Na^+$, $Ca^{2+}$, and $Mg^{2+}$) (mg $L^{-1}$), and leaf nutrient concentration (N, P, K, Na, and Cl (mg $g^{-1}$ DW)) with tomato yield.

| | Yield | | | |
|---|---|---|---|---|
| **Parameters** | **Petiole Sap** | **Soil Solution** | **Leaf** | |
| $Cl^-$ | 0.20 | −0.03 | Cl | 0.25 |
| $NO_3^-$-N | −0.82* | −0.02 | N | −0.69* |
| $H_2PO_4^-$-P | −0.77* | 0.05 | P | −0.52* |
| $Na^+$ | 0.23 | −0.23 | Na | 0.34 |
| $K^+$ | −0.91* | 0.68* | K | −0.72* |
| $Ca^{2+}$ | 0.16 | −0.26 | | |
| $Mg^{2+}$ | 0.25 | −0.25 | | |

* indicates significant differences at $p < 0.05$. ns indicates non-significant differences.

## 4. Discussion

Concerning climatic parameters, the range of ETc obtained in our trial (1.2 to 2.4 mm $day^{-1}$) was within the ranges proposed by Fernández et al. [25] and Baeza et al. [26], who established a minimum value of 0.5 and a maximum value of 2.4 mm $day^{-1}$. The range of global radiation in our experiment was from 6.7 to 8.0 MJ $m^{-2}$ $day^{-1}$ and was inside in the range of 5 to 11 MJ $m^{-2}$ $day^{-1}$ reported by Baeza et al. [26] for the production of tomato in Mediterranean greenhouses. The range of temperature during the experiment (10 to 17 °C) was in line with the findings reported by Baeza et al. [26] who established a range from 4 to 25 °C inside the greenhouse in a tomato crop. Finally, the average relative humidity in our experiment (85% of RH) was inside the range established by Baudoin et al. [27] (70%–90% of RH) for a tomato crop in the Mediterranean area.

As far as plant parameters are concerned, the values of LAI in our experiment ranged from 4.2 to 4.6. These values were inside the range (3.8 to 4.7) reported by different researchers such as Barraza et al. [28] and Medrano et al. [17]. Finally, the yield obtained in our experiment was lower compared to the production of this area with an average of 9.56 kg $m^{-2}$ [29]. This yield reduction can be due to the low daily radiation inside the greenhouse, since Baudoin et al. [27] recommended a minimum

daily radiation in tomato of around 8.5 MJ m$^{-2}$ day$^{-1}$. Similiarly, Sandri et al. [30] and Llanderal et al. [31] reported the same results in a tomato greenhouse crop.

The mean values for pH, EC, and nutrient concentrations in soil solution in our experiment (pH (7.5–8.1) and EC (2.1–4.1 dS m$^{-1}$) values and concentration of H$_2$PO$_4^-$ (0.1–0.2 mmol L$^{-1}$), K$^+$ (3.1–7.6 mmol L$^{-1}$), Ca$^{2+}$ (2.5–5.9 mmol L$^{-1}$), Mg$^{2+}$ (2.5–11.3 mmol L$^{-1}$), Cl$^-$ (1.3–13.2 mmol L$^{-1}$), and Na$^+$ (2.5–11.3 mmol L$^{-1}$) were inside the optimal ranges proposed by Lao et al. [19]. On the other hand, the range of NO$_3^-$ concentration was higher than the optimal range proposed by Lao et al. [19] (NO$_3^-$ 6.4–19 mmol L$^{-1}$). The higher NO$_3^-$ concentration found in our experiment can be related to an excessive nitrogen input which is well above the nutritional requirements of the crop.

The mean values of nutrient concentrations in petiole sap in our experiment were inside the optimal range proposed by Cadahía [12] for H$_2$PO$_4^-$-P (35–135 mg L$^{-1}$), K$^+$ (600–4590 mg L$^{-1}$), Ca$^{2+}$ (280–1420 mg L$^{-1}$), and Mg$^{2+}$ (190–200 mg L$^{-1}$) concentrations, and for Cl$^-$ and Na$^+$, the values in our experiment were inside in the optimal range proposed by Urrestarazu [15] (750–4500 and 50–400 mg L$^{-1}$, respectively). On the other hand, the range of NO$_3^-$-N concentration was higher than the optimal range proposed by Cadahía [12] (133–1000 mg L$^{-1}$). The high concentration of NO$_3^-$-N in sap over our trial could be a result of the excess of NO$_3^-$ in the soil solution (SS) due to the high supply of nitrogen fertilizers, which is in agreement with Fontes and Ronchi [32] who found a positive correlation between NO$_3^-$ concentration in soil solution and in petiole sap. Moreover, the high concentration of NO$_3^-$-N in sap over our trial could be due to the low light intensity inside the greenhouses because under low-light conditions, there is a decrease in the activity of nitrate reductase as reported by Llanderal [9].

The mean values of leaf nutrient concentration in our experiment were inside the optimal ranges for N (25–48), P (2.6–4.7), and K (16–31 mg g$^{-1}$ DW) proposed by Llanderal et al. [33] and higher than the optimal values proposed by Casas and Casas [34] for Cl (<5 mg g$^{-1}$) and Na (<1.8 mg g$^{-1}$ DW).

The variability of nutrient concentrations in sap are associated with several factors, such as climatic parameters, leaf area index, nutrient concentrations in soil solution, and the previous nutrient concentrations in petiole sap. All nutrient concentrations in the petiole sap showed a positive correlation with the crop ETc, and this can be related to the reduction of the water content in the plant as suggested by Hochmuth [2]. No significant correlation between nutrient concentrations and temperature was found. The only significant correlation between RH and nutrient concentrations in sap was for Ca$^{2+}$. These results are in line with the findings reported by Armstrong and Kirkby [35] who established that under high relative humidity conditions (95%), the nutrient uptake of calcium by mass flow was restricted. No correlation between NO$_3^-$-N concentration and RH was found in our experiment and this could be due to the fact that NO$_3^-$-N concentration is independent of the RH conditions as proposed by Erica et al. [36].

No relationship between Cl$^-$, H$_2$PO$_4^-$-P, Na$^+$, K$^+$, and Mg$^{2+}$ concentrations in sap and RH is due to the fact that ions such as Cl$^-$, H$_2$PO$_4^-$-P, Na$^+$, K$^+$, and Mg$^{2+}$ are considered mobile in the phloem, so they can be deposited in plant organs or translocated [14,37].

The increase of nutrient concentration in sap could be related to the reduction of water content in the plant, since crop transpiration increases with increasing atmospheric vapor pressure deficit (VPD) [38]. In plant parameters, LAI has a negative correlation with Cl$^-$, NO$_3^-$-N, H$_2$PO$_4^-$-P, K$^+$, and Na$^+$ concentration in sap which can be due to the dilution factor related to biomass increase proposed by Opstad [39]. On the other hand, there was a positive correlation with Ca$^{2+}$ and Mg$^{2+}$ due to the accumulation of these ions over the crop cycle as reported by Llanderal [9]. No correlation between nutrient concentrations in sap and soil solution is due to the fact that the concentrations in soil solution were in the optimal range as proposed by Lao et al. [19]. Nevertheless, the physiological process such as nutrients uptake, bio-assimilation, and storage affect the nutrient concentrations in sap [9].

The significant autocorrelation over the crop cycle of NO$_3^-$-N, H$_2$PO$_4^-$-P, K$^+$, Ca$^{2+}$, and Mg$^{2+}$ concentration relates to the capacity of plants to regulate nutrient concentrations in response to changes in environmental conditions [40]. However, no significant autocorrelation of Cl$^-$ and Na$^+$ over the experimental period occured due to the regulation developed by some wild tomato species

through the accumulation of these ions into the vacuole for their osmotic regulation [41], which in turn can be diluted for a growth and succulence mechanism as proposed by Cuartero et al. [42].

Finally, comparing the different diagnostic methods established with the yield, our results report that the petiole sap method shows the best coefficient of correlation. The negative correlation in petiole sap ($NO_3^-$-N, $H_2PO_4^-$-P, and $K^+$) and leaf analysis (N, P, and K) can be due to the dilution factor as a consequence of the increase of growth and yield in the plant [31,43]. Moreover, the negative correlation can be due to the translocation of these nutrients to the fruit [44]. On the other hand, it is necessary to point out that the positive correlation between $K^+$ concentration in soil solution and yield is in line with the findings reported by Bugarín-Montoya et al. [45] who proposed the same positive correlation in tomato crop.

## 5. Conclusions

The method of petiole sap shows the best coefficient of correlation with the yield, compared with the different diagnostic methods established: soil solution and leaf nutrient concentrations; therefore, nutrient sap concentration can be recommended as the most sensitive nutritional diagnosis methods related to the expected yield.

The highest problem of sap diagnosis methods is the wide range of nutrient concentrations related to optimal nutrient status. In this paper, a model is proposed to determine the nutrient concentrations in petiole sap in response to climatic parameters, nutrients in soil solution, and growth. The ETc, DPV, and LAI are the most significant variables that allow the development of these empirical prediction models regarding nutrient concentrations in petiole sap. It is important to highlight that the reduction of the water content in the plant increases the concentration of all the nutrients in petiole sap.

The high autocorrelation of nutrient concentrations in sap for one week suggests that a longer sampling period is needed in order to analyze the nutrient change in sap, therefore we recommend that the best option is a sampling period of 15 days. Nevertheless, it is necessary for other experiments to further support and confirm these findings due to the scarcity of previous knowledge.

**Author Contributions:** Investigation, data curation, writing—original draft preparation, A.L.; formal analysis, investigation, P.G.-C., J.P.-A., J.I.C., M.L.S., and J.R.; conceptualization, supervision, project administration, M.T.L. All authors have read and agreed to the published version of the manuscript.

## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| ETc | Crop evapotranspiration |
| DAT | Days after transplanting |
| HPLC | High-performance liquid chromatography |
| LAI | Leaf area index |
| PAR | Radiation photosynthetically active |
| RH | Relative humidity |
| SS | Soil solution |
| VPD | Vapor pressure deficit |

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