Journal of Experimental Biology and Agricultural Sciences, June - 2021; Volume – 9(3) page 294 – 304

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

INFLUENCES OF THE SOIL PHYSICO-CHEMICAL CHARACTERISTICS ON THE MINERAL COMPOSITION OF THE MOROCCAN STRAWBERRY TREE (*Arbutus unedo* L)

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Received – April 28, 2021; Revision – June 03, 2021; Accepted – June 24, 2021
Available Online – June 25, 2021

DOI: http://dx.doi.org/10.18006/2021.9(3).294.304

KEYWORDS

Strawberry

Correlation

Leaves nutrients

Soil

Physic-chemical properties

Morocco.

ABSTRACT

Leaf mineral composition reflects the complex interaction between soil, climate, and plant genetic and epigenetic background in terms of organic and inorganic composition. The present study aimed to evaluate the effect of seven soil compositions on the mineral contents of Strawberry leaves (*Arbutus unedo*) under Moroccan conditions. The obtained results demonstrated a significant positive correlation between the soil nitrogen, phosphorus, and potassium levels and their levels in the Strawberry plant leaves. Also, a positive relation was reported between the soil organic matter and the level of manganese and phosphorus in leaves. Moreover, a significant negative correlation was demonstrated between the soil organic matter and the plant pH level. These correlations were confirmed by the principal component analysis (PCA) which demonstrated the presence of three principal components accounting for 54.42% of the total variations, reflecting the important proportions of the various patterns of mineral and organic traits among accessions. In PCA, the most discriminative traits were clustered in PC1 and PC2. These findings may highlight the specific mineral needs for the *A. unedo* plant for its optimal cultivation and subsequent domestication in Morocco.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

1 Introduction

The combination of organic and inorganic soil content is essential for plant growth, productivity, and nutritional quality (Condit et al., 2013; Rawat et al., 2019). The patterns of nutrient absorption and use by plants can be affected by several environmental and adaptive factors. As such, plants can adapt to a nutrient-limited environment by changing their root structures increasing their total surface area for nutrients acquisition.

Mineral nutrients are vital for plant's growth, they play a major role in many biochemical processes, their growth, and their resistance against several diseases (Kalsoom et al., 2020). Based on the plant requirement, 14 minerals are considered essential, such as macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, and Sulphur) and micronutrients (iron, manganese, zinc, chlorine, copper, molybdenum, boron, and nickel) (Brady & Weil, 2008; Adnan et al., 2020; Adnan et al., 2021). Nitrogen is the most important mineral absorbed by the plant from the soil and is highly required for body growth, leaf morphology, metabolic amino-acid synthesis, and plant nutritional quality (Toor et al., 2021). Like nitrogen, phosphorus is also important for plant growth, as it plays a vital role in the biosynthesis of proteins, nucleic acids, sugar, and adenosine triphosphate (ATP) productions, covering the plant's energy needs (Balkwill et al., 1988). Micronutrients such as zinc and iron play a main role in plant biotic processes, biochemical pathways, and several metabolic activities, namely the regulation of plant respiration, photosynthesis, sulfates, and nitrate reduction (Grotz & Guerinot, 2006).

According to Tan et al. (2005) Alloway (2008) deficiency in soil nutrients, in particular; nitrogen, phosphorus, potassium, and zinc can contribute to almost 27% of agricultural production loss worldwide (Tan et al., 2005; Alloway, 2008). Cross interactions between soil and plant in terms of mineral and organic molecules may reflect the ecological system changing (Stein et al., 2017).

The Strawberry tree is an evergreen tree, widely distributed in the Mediterranean-Atlantic area, mainly in southern Europe, northern Africa, Ireland, Palestine, and Macaronesia (Canarias) where it is usually used in gardening and landscaping. The height of the plant may reach up to 3m and it is well known to be resistant to harsh environmental conditions such as drought, coldness, and other hard edaphic conditions (Navarro et al., 2007; Celikel et al., 2008; Gomes & Canhoto, 2009). The *A. unedo* is characterized as a dominant species as it regenerates rapidly after natural disasters such as the forest fires that occurred many times during the last two decades (Arnan et al., 2013; Francos et al., 2016).

To improve our understanding related to the effect of soil composition on the chemical variation of the strawberry plants under the Moroccan ecosystem. The current study investigated the possible correlations between these parameters and determined the level of traits variation associated with soil provenance and how it affects the mineral composition and its distribution in strawberry plants.

2 Materials and Methods

2.1 Plant and soil sampling

Total 120 strawberry leaves (500g) and topsoil samples (0-20cm from the surface) were collected between November and December from 15 sites over seven different Moroccan regions (Figure 1). Strawberry tree populations were taxonomically identified by the National Scientific Institute of Rabat (Department of Botany and Plant Ecology, Morocco). Collected voucher specimens were registered at the Herbarium of National Institute of Agriculture Research (INRA) and in the National Herbarium of the Scientific Institute of Rabat under the RAB111500 code.

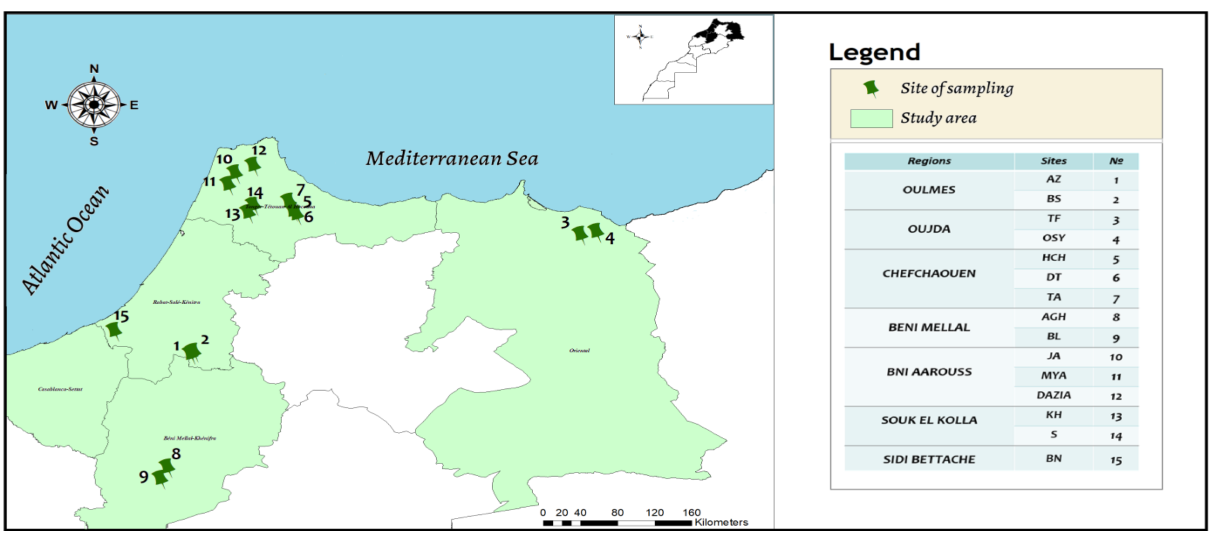

Figure1 Plant and soil sampling in November-December during the year 2018

2.2 Physico-chemical Analyses of soil

Topsoil samples were dried in the open air, then crushed and sieved to remove plant debris larger than 2 mm. An aliquot of finer diameter is reserved for nitrogen and organic matter analysis. The Air-dried soil samples are used to determine the physico-chemical composition by analyzing pH (water), pH (KCl), organic matter (OM), nitrogen (N), available phosphorus (P_2O_5), exchangeable potassium (K_2O), granulometry, and mineral elements.

The pH of soil samples was measured by the potentiometric method using a pH meter (Mettler Toledo Seven Easy-728 Metrohm) (Mweetwa et al., 2016); total nitrogen was quantified by macro Kjeldahl method (Bremner, 1965); Organic matter percentage was determined by Walkley and Black method (Laghrour et al. 2016); Phosphorus (P_2O_5) content was determined by using the Olsen P method using an acidified solution of ammonium molybdate (Tévez & dos Santos Afonso, 2015); the mixture absorption was measured using a UV-visible spectrophotometer (JENWAY6405 Model) at 825 nm; sodium (Na^+) and exchangeable potassium (K_2O) were determined using flame photometer model CL 378 (Mweetwa et al., 2016); soil texture and percentages of $CaCO_3$ were calculated using the Robinson pipette method (Pétard, 1993) and the level of trace mineral contents, including iron (Fe^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), and manganese (Mn^{2+}) were identified using atomic absorption spectrometry in an air-acetylene flame.

2.3 Mineral composition and total nitrogen content of strawberry leaves

The collected strawberry leaves were dried in a ventilated oven at 36°C for 72 hours before being crushed to a fine powder, and total nitrogen content was quantified by using the Kjeldahl method (AOAC, 2011). While the total mineral content was analyzed by incineration of 1g of dried leaf samples for 6 hours at 500°C and then extracted with hydrochloric acid (Harris & Marshall, 2017); mineral elements as well as Fe, Mn, Zn, and Cu were determined by atomic absorption spectrophotometry (SAA) of the air-acetylene flame; and sodium (Na+), potassium (K+), and phosphorus (P) contents were measured using the same method described before for topsoil analysis.

2.4 Statistical analysis

Statistical analysis was performed using SAS Software GenStat 18th. Data were analyzed by one-way ANOVA followed by Bonferroni posthoc test ($P<0.05$). The correlation between topsoil physicochemical properties (pH, salts mineral, MO, Nitrogen, and traces elements) and the leaves' nutrients contents (pH, salts mineral, Nitrogen, and traces elements) was analyzed using Pearson's correlation test and PCA. Results were expressed as mean ±standard error mean (SEM).

3 Results and Discussion

Statistical analysis of the physicochemical data of strawberry leaf populations and their corresponding soil sample demons showed a significant effect on all the mineral organic parameters ($p<0.001$). These results may reflect the effect of climate and environmental conditions on plant contents and explain strawberry distribution all over the Mediterranean area (Temuçin, 1993; Vallicrosa et al., 2021).

3.1 Plant nutrients and pH value

Results related to the salts mineral, nitrogen, trace elements contents, and pH values were presented in figure 2. All results demonstrated significant differences in all mineral and organic contents among the studied regions and samples ($p<0.001$). The level of strawberry leaves pH in all samples collected from different regions revealed an acidic characteristic with an average value of 4.82 and the content of mineral salts was reported between 0.06-0.13% for phosphorus, 0.27-0.52% for potassium, and 0.021-0.047% for sodium. The levels of nitrogen were the highest among all other minerals, and their values were comprised between 0.86-1.16%. For micronutrients, iron (Fe) and zinc (Zn) content were the highest with ranging between 10.33- 29.79 mg/100g and 9.32-14.51 mg/100g respectively, followed by manganese (Mn) with a content level of 1.17-5.06 mg/100g and least the copper (Cu) ranging between 0.12 and 1.21 mg/100g. Plant's mineral content data reported in the current study were similar to those obtained by Navarro García et al. (2011) despite the small variation in nitrogen and potassium levels 0.86% and 0.14%, *vs.* 1.10% and 0.35%, respectively, and in phosphorus and sodium contents 0.16% and 0.22%, *vs.*0.09 and 0.03%, respectively (García et al., 2011). On another hand element levels reported in earlier studies were similar to the current study (25mg/100g of iron; 2.7mg/100g of manganese, and 8mg/100g of zinc). The availability of these nutrients in leaves at these levels can be explained by the slightly basic character of the soil, as high acidity levels may inhibit nutrients uptake (Kamalu et al., 2014).

3.2 Physico-chemical properties of topsoil

As depicted in figures 3 & 4, particle size analysis revealed a differential growth of strawberry trees and it depending on the soil composition and texture. Based on these parameters topsoil is classified into; clayey-sandy soil (For the BA region, TF site from OUJ region, AGH and S sites from BM and SQ region, respectively), heavy clay soil (BL site from BM, TA from CH), clayey soil (HCH and DT sites from CH region, KH site from SQ region, OUL and SB regions) and sandy-clayey loam soil (OSY site from OUJ region). The pH values of the topsoil samples were ranged from 4.37 to 7.79 with the highest and lower pH levels found in the CH and BM regions, respectively.

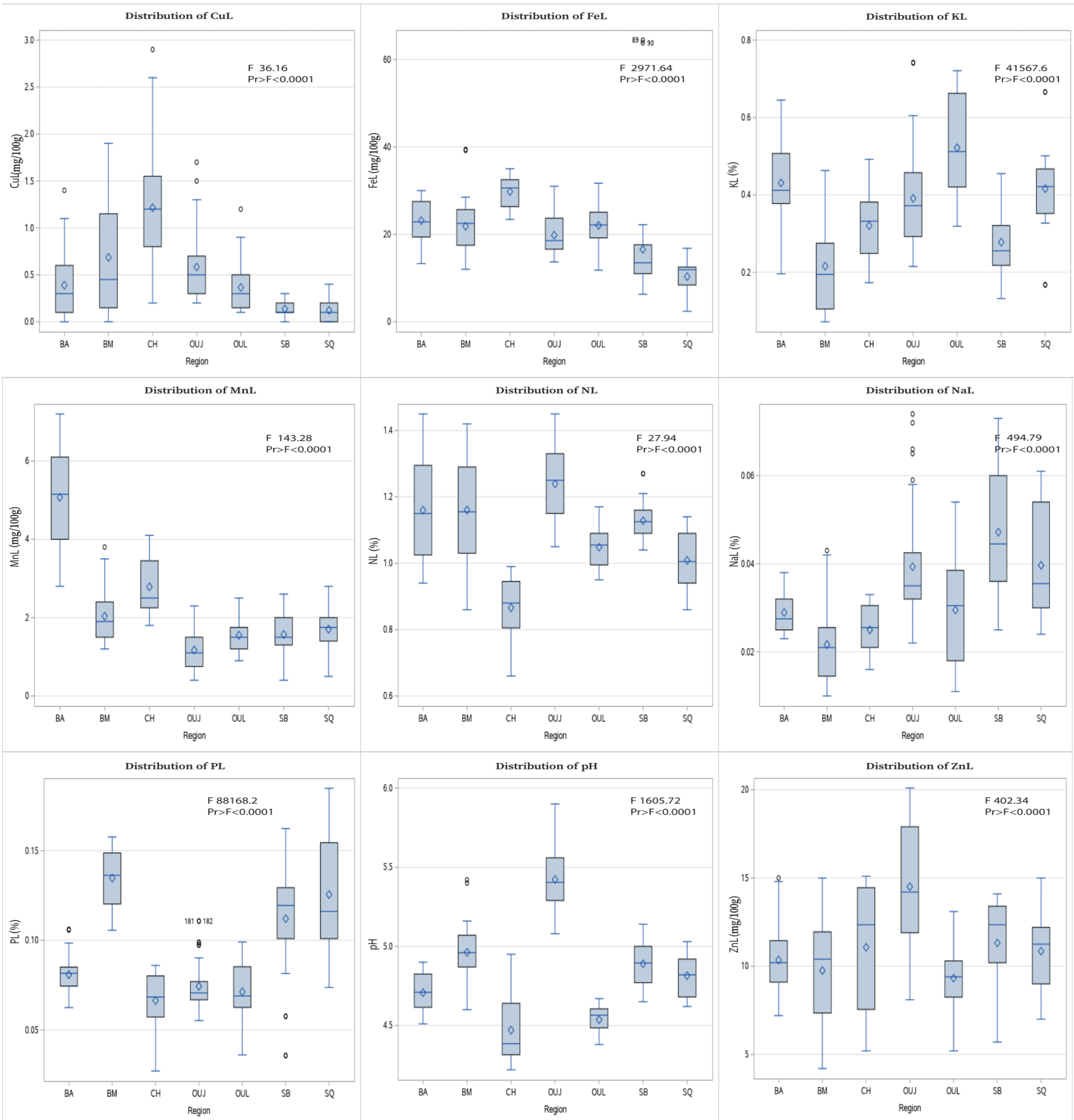

Figure 2 Nutrients contents and pH value of leaves of *A. unedo* plants at the different wild Moroccan grown areas. All data are reported as mean±ESM, alongside p and F value obtained by the one-way ANOVA test. Samples are distributed according to the posthoc test.

To the best of our knowledge, only a few studies depicted the effect of soil chemical composition on the mineral composition of *A. undo*'s under their natural habitat. The level of Calcium carbonate ($CaCO_3$) and organic matter contents fluctuate in a very wide variation between 0 to 23.3% and 3.11 to 27.01% respectively. All analyzed samples from surveyed regions demonstrated significant variability in the chemical components ($p<0.001$) mainly in phosphorus (4.47-15.75%), potassium (20.6-41.78%), sodium (0.07-0.14%), and nitrogen (0.04-0.25%), as well as trace elements (Figure 3). Mineral element contents were relatively high in topsoil in comparison to their levels in the leaves. This might be used as an indicator of the plant capacity of absorbing and storing these elements. The major mineral elements found in the topsoil were iron and zinc at 64.55 - 212.7mg/100g and 2.21 - 105.5mg/100g, respectively, (15% and 34% higher than their concentration in leaves). However, copper (8.32-58.83 mg/100g) and manganese (2.74-8.40 mg/100g) were relatively low (Figure 3).

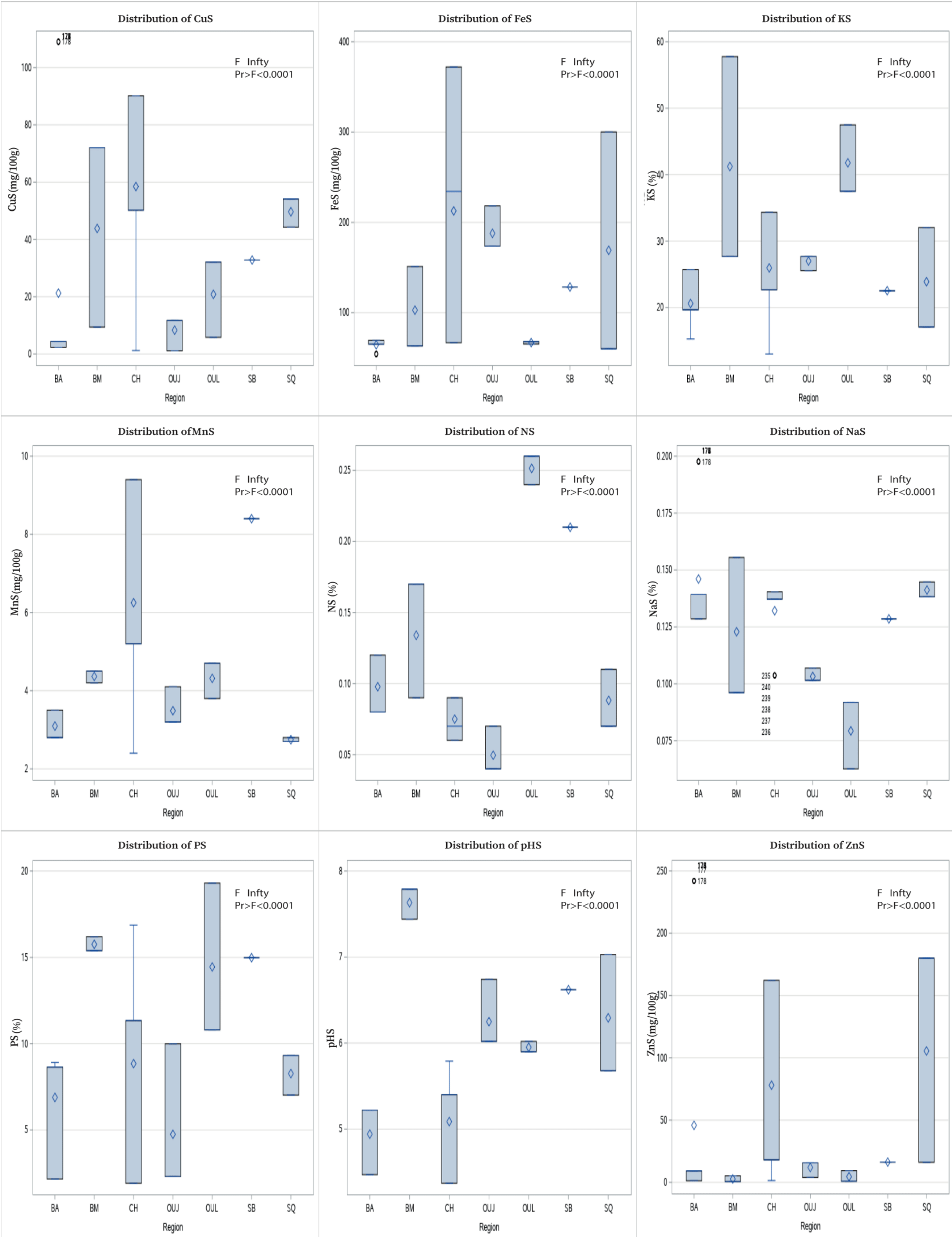

Figure 3 Mineral salts, Nitrogen content in trace elements in Soils. All data are reported as mean±ESM, alongside p and F value obtained by the one-way ANOVA test. Samples are distributed according to the posthoc test

As depicted in Figure 4, the texture of topsoil samples analysis proved that the strawberry preferably grows in clayey (42.5%) and clayey-sandy (41.6%) and a lower rate in heavy clayey and sandy-clayey-loam soils (10% and 5.8%, respectively). Soil pH analysis revealed that 77.2% of strawberry trees grew under acidic soil conditions with a pH of 4.37 - 6.74, while the remaining 20.8% grew on a slightly alkaline soil (pH value varied from 7.03 to 7.79). These data contrasted Başlar et al. (2002) findings, those who reported that strawberry trees from Turkey grow mainly on basic topsoil samples with pH values ranging between 6.78 and 7.80 as such clayey-loam, loamy, and sandy-clayey-loam (56.25%, 31.25%, and 12.50%, respectively) (Başlar et al., 2002). The level of calcium carbonate content ($CaCO_3$) in topsoil samples may also affect the growth of strawberry, as it has been demonstrated by Başlar et al. (2002) that 43.3% concentration of $CaCO_3$ (<1%) in the topsoil poorly supporting the strawberry growth, 40% non-calcareous (1 - 16% $CaCO_3$) and 16.6% is moderately calcareous topsoil ($CaCO_3$ > 16%) (Başlar et al., 2002). The amount of organic matter may vary and it is depending on the soil's regional characteristics. The obtained results showed that the BM region had the highest organic matter content (27.01%), while the CH region had the lowest level (3.11%). This variation among samples collected from different regions and locations is explained by the fact that organic matter contents are influenced by several factors including climatic conditions, vegetative stages of plants, particle sizes, and microclimate of soil (El Oumlouki et al., 2018). It was also proved that organic matter alongside the soil structure, plays the main role in reducing the dry matter of soil and in the diminution of pH value, responsible for the acidic character of soils over time (Banning et al., 2008).

In the case of nitrogen, most of the studies are in agreement with the nitrogen value reported in this study (Figure 4) while the nitrogen level value reported by Başlar et al., (2002) was slightly higher (0.52%) than the current study (0.12±0.001%). This topsoil nitrogen is generally originated from the decomposition of organic matter, particularly in tropical areas' soils (Ikpe et al., 2003). Alongside nitrogen, Başlar et al. (2002) found low levels of phosphorus and potassium (0.07% and 0.04% respectively) in the topsoil samples collected for his study while in the current study these two elements were reported at a higher concentration. The level of phosphorus, potassium, and sodium in the current study was reported 10.52±0.34%, 29.00±071%, and 0.12±0.001% respectively.

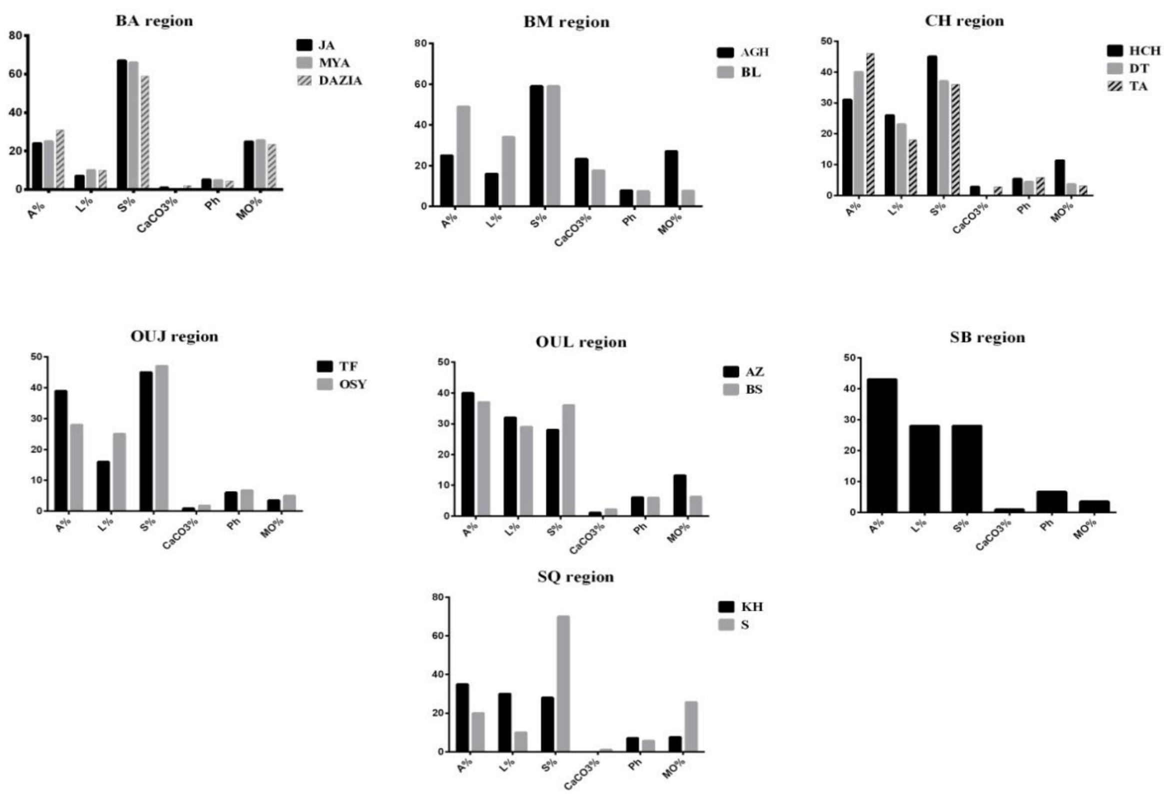

Figure 4 Physical properties and pH value of topsoil (pH and MO measurements were presented as mean value ± SE). A: Clayey; L: Loam; S: Sandy; MO: Organic matter (n=120).

Results of trace mineral elements in survey soil illustrated in figure 3 are shown significant variability in soil samples. In the current study higher average values of iron (133.2±5.45mg/100g), zinc (32.71±3.9mg/100g), and copper (31.98±1.87 respectively) was reported, while in the case of manganese lower (4.74±0.13mg/100g) value was reported. Despite the main role of copper in several physiological processes of the plant (respiration, distribution, and fixation of nutrients), it was reported that a concentration of 100mg/kg of copper in the soil is considered a critical value because the same level inhibits plants growth by affecting their photosynthesis (Maksymiec & Baszyński, 1996; Kamunda et al., 2016).

3.3 Correlation between the topsoil physicochemical properties and leaves nutrients contents

As summarized in table1, the concentration of nitrogen in topsoil was not correlated to its corresponding concentration of the leaves, a similar result was also reported by Başlar, et al. (2002) those who demonstrated a negative correlation between soil nitrogen content and plant potassium level using regression analysis**.** On the another hand topsoil nitrogen concentration was positively correlated to phosphorus level of the leaves (r=0.15; p<0.01) while it was negatively correlated to the rest of the elements levels of the leaves; pH, Cu, Zn, and Mn (r= -0.30;p<0.001, r= -0.29; p<0.001, r= -0.24; p<0.001 and r= -0.12; p<0.05, respectively). These correlation results between soil physicochemical data and strawberry foliar nutrient concentrations suggested that the concentration of mineral contents in the leaves may not necessarily depend on their level in the topsoil. This subsequently applies that plants may not have the capacity to store these elements and/or it may be using other resources such as their proper metabolites.

Phosphorus in soil was positively correlated with phosphorus levels of leaves (r=0.46; p<0.001), and negatively correlated with potassium, pH, zinc and Mn (r= -0.24;p<0.001, r= -0.19; p<0.01, r= -0.31; p<0.001, and r= -0.16; p<0.01, respectively). It has been shown that the available phosphorus level in the plant is strictly related to phosphorus levels in the soil (Randhawa & Arora, 2000). Topsoil potassium level was not significantly correlated with its level in leaves. However, it was positively correlated with Fe (r=0.20; p<0.001) and negatively with Zn and Mn (r= -0.30; p<0.001and r= -0.21; p<0.001 respectively). While, soil pH values were positively correlated with phosphorus (r=0.59; p<0.001), pH (r=0.43 p<0.001) and nitrogen (r=0.19; p<0.001) levels of *A. unedo* leaves. These results are corroborated by a recent study that demonstrated a positive correlation (p<0.01) between the pH of the soil and different nutrients in the mustard plant (Kumar et al., 2017). Moreover, they confirm the constant report of Osayande et al. (2014), those who suggested the major role of soil pH in the interactions and interferences of uptake and use of nutrients by plants (Osayande et al., 2014).

There were significant positive correlations between soil organic matter levels and leaves manganese and phosphorus contents (the correlation coefficients were 0.55 and 0.26 respectively, where probability values were less than 0.001), while this parameter was negatively correlated with the pH of the studied plant part (significant correlation coefficient of -0.16; p<0.01). The significant and positive correlation between organic matter and phosphorus was highlighted by the importance of organic matter decomposition to produce humus which ensures phosphorus availability in soil (Voltr et al., 2021). Additionally, the cases between soil and leaf traces mineral elements contents among the investigated samples were not shown any significant correlation (p>0.05), except for some cases. The lack of numerous significant

Table 1 Correlation coefficient between soil physic-chemical properties and *A. unedo* leaves nutrients (n = 120)

Plant nutrients Soil properties	N	P	K	Na	pH	Cu	Zn	Mn	Fe
N	-0.075ns	0.156**	0.005ns	0.03ns	-0.30***	-0.29***	-0.24***	-0.12*	-0.11ns
P	-0.18**	0.46***	-0.24***	-0.05ns	-0.19**	0.004ns	-0.31***	-0.16**	-0.0009ns
K	0.09ns	0.16**	0.10ns	-0.15**	-0.05ns	0.12*	-0.30***	-0.21***	0.20***
Na	-0.07ns	0.18**	-0.05ns	0.03ns	-0.17**	-0.01ns	-0.17**	0.42***	0.05ns
Ph	0.19**	0.59***	-0.39***	-0.008ns	0.43***	-0.09ns	-0.04ns	-0.50***	-0.25***
MO	-0.04ns	0.26***	-0.06ns	-0.31***	-0.16**	-0.05ns	-0.12*	0.55***	-0.06ns
Cu	-0.42***	0.17**	-0.25***	-0.18**	-0.21***	0.10ns	-0.01ns	0.10ns	0.003ns
Zn	-0.33***	0.04ns	0.12*	0.07ns	-0.16**	0.08ns	0.01ns	0.22***	0.007ns
Mn	-0.16**	0.04ns	-0.31***	0.19**	-0.20***	-0.05ns	0.001ns	-0.19**	0.04ns
Fe	-0.06ns	-0.21***	-0.09ns	0.08ns	0.07ns	0.15*9	0.18**	-0.27***	0.10ns

* P<0.05, **P<0.01, ***P<0.001, ns: non-significant. All studied measurements are presented as mean values ± SE (n=120)

correlations between soil and leaves mineral composition can be explained by the transport of water to the lower soil levels, as a result, plant roots are not sufficiently penetrated to the soil to absorb a significant amount of nutrient, e.g. nitrogen is available in a potent level between 0-25cm, and it depends on the thickness of the soil layer (Nwachukwu et al., 2020).

3.4 Principal component analysis of topsoil and strawberry leaf chemical parameters

The results of principal component analysis (PCA) (Table 2) explained the elemental variability in the surveyed populations of *A. unedo*. The first three Principal Components had a cumulative variance of 54.42% of all the traits, with eigen values exceeds one; suggested that the classified traits within the axes have important effects on the overall variation. A scatter plot was drawn between the first and second principal components (PC1 and PC2) represented a clear pattern of *A. unedo* leaf samples distribution (Figure 5). They exhibit the chemical variation among the surveyed populations and explain their dispersion along both axes be aware that PC1 and PC2 contributed 21.37% and 18.19% of the total variation respectively.

The variables associated with the first three principal components are often useful to demonstrate differentiating accessions (Emmanouilidou & Kyriacou, 2017). However, according to the criterion of Raji (2002), the factor scores greater than 0.3 owns a significant effect on the overall variation shown in the present study. PCA of quantitative parameters shown that the first principal component (PC1) accounted for 21.37% of the overall variation was loaded by the majority of traits, except for the leaf potassium (K-L), leaf zinc (Zn-L), soil manganese (Mn-S), and soil iron (Fe-S) with factor scores of -0.19, -0.01, -0.034 and -0.01 respectively. Likewise, the second principal component (PC2) contributes 18.19% of the total variation and covers a large number of traits with factor scores greater than 0.3, except for leaf potassium (K-L), leaf zinc (Zn-L), soil nitrogen (N-S), soil phosphorus (P-S), soil sodium (Na-S) and soil pH (pH-S), whereas, PC3 was most loaded with leaf pH (pH-L) leaf zinc (Zn-L), soil nitrogen (N-S) and soil phosphorus (P-S).

All populations of *A. unedo* leaves are widely scattered in axes (Figure 5), exhibiting the chemical variation among the surveyed population and explaining the correlations between quantitative traits of leaf and provenance soil identified by Pearson correlation analysis. Regarding the clustering pattern presented in figure 5, *A. unedo* populations are organized into four distinct clusters. Cluster 1 covers all populations from BM, OUJ, OUL, and SB regions and one population (KH) from the SQ region, while cluster 2 had populations (Hch, Ta, and Dt) from the CH region, cluster 3 gathers populations from the BA region and finally, one population (S) from SQ region was formed a separate cluster (Cluster 4). Hence, PC1 and PC2 were loaded with the most discriminative traits, which are useful to enhance the *A. unedo* species domestication program in Morocco.

Table 2 Factor scores, Eigen value, Total and cumulative variance of the first three principal component axes to variation in *A. unedo* leaf populations in Morocco.

Parameters	PC1	PC2	PC3
N-L	0.3267	0.432	-0.2803
P-L	0.4965	0.5495	0.18
K-L	-0.1914	0.2642	0.1157
Na-L	0.4736	0.331	-0.3614
pH-L	0.5541	0.3245	-0.5809
Cu-L	-0.3751	-0.6861	0.0792
Zn-L	-0.0102	0.0499	-0.8181
Mn-L	-0.7211	0.3646	0.1221
Fe-L	-0.5611	-0.5433	0.2191
N-S	0.3606	0.0589	0.6285
P-S	0.4279	-0.1345	0.6864
K-S	0.4925	-0.1848	0.4593
Na-S	-0.5391	0.4156	-0.1333
pH-S	0.8431	0.009	0.1168
MO	-0.3039	0.7512	0.2701
Cu-S	-0.451	0.3345	0.1366
Zn-S	-0.5563	0.537	0.0612
Mn-S	-0.034	-0.4167	-0.1014
Fe-S	-0.0135	-0.6441	-0.5282
Eigen value	4.0605	3.4562	2.8241
Total variance (%)	21.371	18.1906	14.8637
Cumulative variance (%)	21.371	39.5616	54.4253

PC: Principal component; L: Leaves; S: Soil

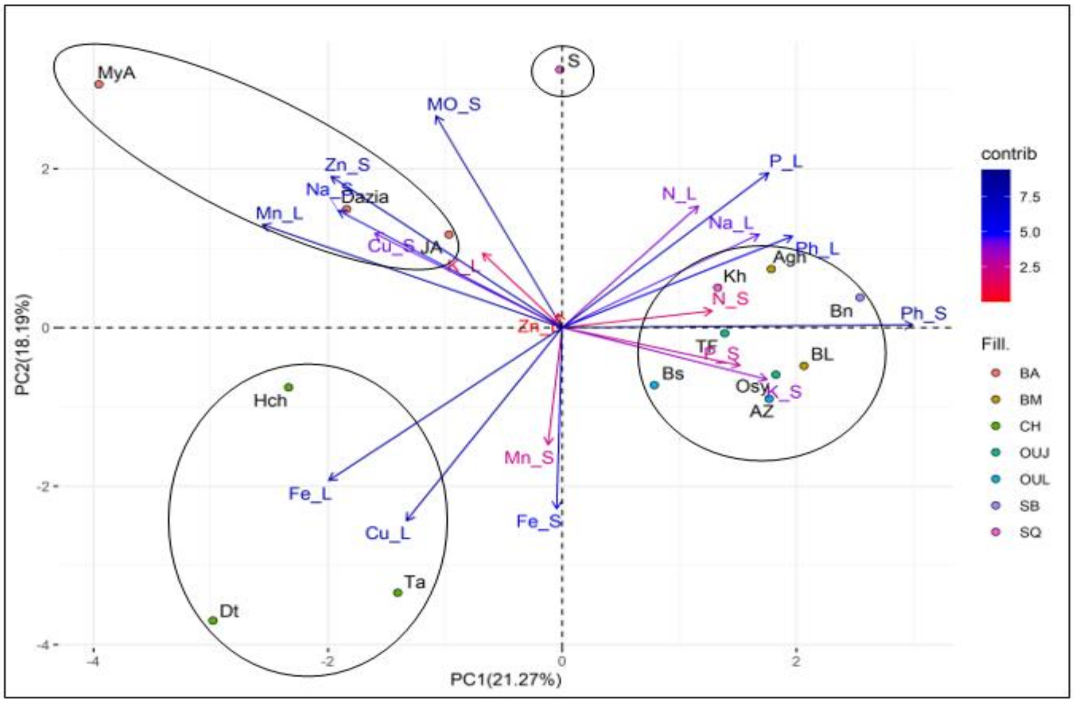

Figure 5 Distribution of *A. unedo* populations across Two Principal Components (PC1 and PC2) of 19 chemical traits

Conclusion

The results of the current study conclude that the resistance of *A. unedo* trees to harsh environmental conditions could be related to their ability to grow in several types of soils with different physicochemical properties. The soil-plant nutrients correlation analysis proved that plant nutrient is not directly related to the mineral composition of the soil, while several other factors such as organic matter contents in soil and soil pH and roots architecture are responsible for the uptake of the mineral salt element from the soil. This knowledge should be generalized by further research in other species native to the same studied areas; to give insights on the behavior development of shrubs belongs to the Mediterranean coastal.

Acknowledgements

The authors would like to thank the staff of the National Institute of Agricultural Research (INRA) (Rabat, Morocco) for their precious help.

Conflict of interest

No potential conflict of interest was reported by the authors

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