**Scientific Journal of Faculty of Education, Misurata University-Libya, Vol. 9, No. 21, mar. 2023**
**Published online in March**
**المجلة العلمية لكلية التربية، جامعة مصراتة، ليبيا، السنة التاسعة، المجلد التاسع، العدد الحادي والعشرون، مارس 2023**
Issn :2710- 4141
**تاريخ النشر 2023/03/01** **تاريخ الاستلام 2023/01/29**

# Evaluation of some physiological traits of *Pistacia atlantica* grown in two areas in the Al-Jabal Al-Akhdar region, Libya

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**Abstract:**
Climate change has many consequences for plants, whether it be heat waves, increased flooding or drought. Besides these side effects of global warming, rising carbon dioxide concentrations and temperatures directly affect plant growth, reproduction, and resilience. According to the results recorded in this study, there are differences in some physiological properties that were investigated through this study such as photosynthetic pigments (chlorophyll *a*, *b* and carotenoids), soluble sugars, starch contents soluble proteins and minerals contents in *Pistacia atlantica* trees. By comparing plants collected from Balagrae and Derna regions the results showed slight differences in mineral contents in soil and plants alike, by comparing the results obtained from different samples. Overall, the study concluded that changes in physiological and chemical contents are mainly due to the effect of changes in temperature, pressure, locations, and soil types.

**Keywords:** *Pistacia atlantica* D.; Climate change; carotenoids; soluble proteins and nutrient imbalance

# تقييم بعض الصفات الفسيولوجية لنبات البطوم المزروع في منطقتين من الجبل الأخضر بليبيا

**خديجة مُحَمَّد علي المصراتية**
**قسم الأحياء–كلية التربية– جامعة مصراتة– ليبيا**

**الملخص:**
لتغيرات المناخ عواقب كثيرة على النباتات، سواء كانت موجات حرارية أو زيادة الفيضانات أو الجفاف. إلى جانب الآثار الجانبية للاحتباس الحراري، فإن ارتفاع تركيزات ثاني أكسيد الكربون ودرجات الحرارة يؤثر بشكل مباشر على نمو النبات وتكاثره ومرونته. وفقًا للنتائج المسجلة في هذه الدراسة، هناك اختلافات في بعض الخصائص الفسيولوجية التي تم التحقق منها من خلال هذه الدراسة مثل أصباغ التمثيل الضوئي (الكلوروفيل أ ، ب والكاروتينات)، السكريات القابلة للذوبان ومحتوي النشا والبروتينات الذائبة والمعادن في

أشجار نبات البطوم النامي في منطقة الجبل الاخضر، ومن خلال مقارنة النباتات التي تم جمعها من مناطق مختلفة من الجبل الاخضر هما درنة وبلغراي، أظهرت النتائج اختلافات طفيفة في المحتوى المعدني في التربة والنباتات على حد سواء، من خلال مقارنة النتائج التي تم الحصول عليها من عينات مختلفة، بشكل عام، خلصت الدراسة إلى أن التغيرات في المحتويات الفسيولوجية والكيميائية ترجع أساسًا إلى تأثير التغيرات في درجة الحرارة والضغط والمواقع وأنواع التربة.

**الكلمات المفتاحية**: نبات البطوم_ الجبل الاخصر –بلغراي – درنة– تغير المناخ– الكاروتينات– البروتينات القابلة للذوبان وعدم التوازن الغذائي.

## 1. Introduction

The study of predicting the potential geographical distribution of plant species is of great importance for the development of effective biodiversity conservation strategies (Lazo *et al.*, 2020), which is also an effective way to protect and manage some rare and endangered species (Fernandez *et al.*, 2019), recently climate change has become one of the main drivers of shifts in the geographical distribution of many plant species (Pacifici *et al.*, 2015). Changes in the geographic distribution of plants at local and regional levels affect vegetation composition, ecosystem function, and genetic diversity, which may make plants more vulnerable to continuous environmental change (Richardson AD *et al.*, 2010).

Plants are the main source of food and medicine for humans and all living things. Extensive attempts are still being made to define and classify many plant species and separate them into what is useful for use and what is harmful to be avoided and excluded. Evergreen, aromatic, and medicinal plants are nature's most abundant sources of important medicines, essential oils, and botanicals, and play an important role in alternative medicine and community preservation, particularly in rural areas. Various types of human and animal diseases are treated by medicinal and aromatic plants from all over the world. These medicinal and preventive uses have intrigued scientists, prompting them to increase their efforts to learn more about the anatomical structures and active chemicals (Radha R *et al.*, 2021).

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The genus Pistacia belongs to the Anacardiaceae, a cosmopolitan family that comprise about 70 genera and over 600 species.The species of the genus Pistacia are evergreen or deciduous resin-bearing shrubs and trees which are characterized as xerophytic trees and growing to 8–10 m tall (Pourreza M *et al.*, 2008).

Atlantica is deciduous trees or shrub, 5-7 m tall with dense globose crown. Leaves imparipinnate, leaflets 2-4 paired. very variable ,orbicular -ovate, ovate-oblong or lanceolate, 2-4.5 x 0.5-1.8 cm, obtuse, emucronate, glabrous, dark green above, pale green beneath, margin ciliolate, rachis uniformly very narrowly winged: petioles puberulent. flowers somewhat brownish arranged on long branches arranged on long branches. drupes obovoid or sub-globose, 5-8 x 5-6 mm, reddish at first ,brownish (Pourreza M *et al.*, 2008).

Scientific findings have revealed that different parts of this plant exhibit broad pharmacological activities, such as antioxidant properties (Radha R *et al.*, 2021), antimicrobial (Pourreza M *et al.*, 2008), antiviral (Özçelik B *et al.*, 2005), anticholinesterase (Vinutha B *et al.*, 2007), antiinflammatory (Minaiyan M *et al.*, 2015), antinociceptive (Orhan I *et al.*, 2006), antidiabetic (Ahmed ZB *et al.*, 2018),antitumor(Rahman HS *et al.*,2018), antihyperlipidemic (Hosseini S *et al.*, 2020), and hepatoprotective activities (Parvardeh S *et al.*, 2002), and also their beneficial effects in gastrointestinal disorders (Minaiyan M *et al.*, 2015).

The current study aimed for the first time to highlight the relationship and interactions between environmental factors and some physiological characteristics of *Pistacia atlantica* collected from two different regions in Al Jabal Al Akhdar (Balagray and Derna).

## 2. Materials and methods

### 2.1. Study site

Al Jabal Al Akhdar is a limestone plateau 760 to 870 m above sea level with an undulating surface which tips gently to the south stretches between the longitudes 20°, 35′ E to 23°,15′ E and latitudes 30°, 58′ N to 32° 56′ N. Balagrae is located in Al-Jabal Al-Akhdar region about 12 km south of Al-Baida, 32° 70– 32° 77 N 21° 70 – 21° 68 E, 522.5 m height above sea level.

Derna is a Libyan city located on the northeastern coastal strip of the country. It is located at 32.45 longitude and 22.40 latitude. It is bordered to the north by the Mediterranean Sea and to the south by a series of green mountain ranges. The city bisects the stream of the valley into two parts, and this valley is called Wadi Derna, and it is one of the large valleys known in Libya. Derna area has moderate climatic conditions dominant year. They were chosen as study sites because they are the most diverse forests in Al-Jabal Al-Akhdar (El-Barasi *et al.*, 2013).

### 2.2. Plants Sampling

Plants from Al-Jabal Al-Akhdar area (two deferent regions Balagrae and Derna) from the beginning of October 2022.were selected in this study. Leaves of the studied plants were separated, then washed several times with distilled water and then dried in the dark for 3 weeks. The dried samples were grinded and stored in polyethylene bottles until the start of the analysis.

### 2.3. Soil Samples

Soil samples (approximately 5 cm) were collected from a depth of 0-25 cm from the area under investigation and combined into a pooled sample in order to analyze its nutritional content.

### 2.4. Estimation of photosynthetic pigment contents:

Leaf samples (0.3 g) of studied plants were homogenized in acetone 85% (v/v) following (Arnon, D, 1949) method. Extract was centrifuged at 5,000 rpm for 15 min and absorbance was recorded at 646 and 663 nm for chlorophyll (*a* and *b*) estimation and at 470 nm for carotenoids. Pigment content was calculated (mg $g^{-1}$ DW) according to the following formulae as reported by (Lichtenthaler H *et al.*, 1983).

$$\text{Mg Chlorophyll } a/\text{ g tissue} = 12.25\ A_{663} - 2.79\ A_{646}$$

$$\text{Mg Chlorophyll } b/\text{ g tissue} = 21.21\ A_{646} - 5.1\ A_{66}$$

$$\text{Carotenoids} = (1000\ A_{470} - 1.8\ \text{Chl } a - 85.02\ \text{Chl } b)$$

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### 2.5. Soluble sugars and starch extraction and estimation

The enthrone method is a colorimetric method for determining the concentration of total sugars. Sugars react with the enthrone reagent under acidic conditions to yield a blue-green color which had a linear relationship with the sample amount of sugar. This method determines both reducing and non-reducing sugars due to the presence of the strongly oxidizing sulfuric acid (Riazi A *et al.*, 1985). Soluble sugars in leaf samples of (*P. atlantica*) studied plants were extracted following (Angelov, MN *et al.*, 1993). Method . Leaf (0.3 g) was milled and extracted with 15 ml of 80% (v/v) ethanol for several times in boiling water bath. The ethanol soluble fractions from each sample were pooled, dried, re solubilized in 10 ml of distilled water. Total soluble sugar was determined following (Riazi A *et al.*, 1985) method, in which 0.5 ml of the alcoholic extract was gently added in a test tube to 3.0 ml freshly prepared anthrone (150 mg of anthrone and 100 ml of 72% of $H_2SO_4$) and placed in a boiling water bath until stable color development. Color was read at 625 nm and standard sample of glucose (0.1 mg / ml) were treated alone with treatments for calculations.

Estimation of starch was carried out following (Hall, MB, 2013) method. Sample (0.1 g) from dried sugar free pellet was re suspended in 2.5 ml of distilled water and subsequently 3.5 ml of 52% (v/v) perchloric acid (PCA) was added to the residue after stirring the mixture, the content was centrifuged for 15 min at 4,000 rpm. The supernatant was decanted, collected and the whole procedure was repeated twice. Supernatant of each step was then hydrolysied poured and the total volume was made up to 15 ml with distilled water. After filtration, 1.0 ml of the aliquot of this filtrate was analyzed for starch content following the same procedure as that of total soluble sugars. Quantity of starch was calculated in terms of glucose equivalent. The quantity of starch was expressed mg glucose/g DW.

### 2.6. Estimation of total soluble proteins

Soluble protein was determined by using Folin - Ciocalteau reagent according to (Lawry OH *et al.*, 1951) assay and protein was expressed as mg protein / g DW.

### 2.7. Estimation of minerals in plant and Soil

For determination of nitrogen (N), phosphorus (P), calcium (Ca), potassium

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(K) in leaves and soil samples (0.5 g) were dried at 75 ºC, ground and digested by mixture of $H_2SO_4$-$H_2O_2$ as the procedure described by (Lachica M *et al.*, 1973). Total nitrogen was determined by microkjeldahl according to (Pregl, F, 1945) method with some modifications. The phosphorus content was determined colorimetrically according to the method of (Jackson, ML, 1958) potassium, and calcium ions were measured by flame-emission photometry according to (Brown JD et al., 1946).

## 2.8. Statistical analysis

All chemical analyses were performed in triplicate. All data was analyzed using a two-way general liner model design analysis of variance (ANOVA) with the factors of geographic region and gender. Tukey's multiple range tests was used to compare the treatment means (SAS 9.3). Non-parametric S pearman rank correlation coefficients of chemical components and environment characteristics were calculated using the SPSS software version 22.0

## 3. Results

The continuous climate change affected the vegetation cover in several ways by influencing plant growth, distribution, adaptive strategy and productivity, and this study focused on the some physiological characteristics of Pistacia atlantica trees from two different locations (Balagrae and Derna) of the two regions of Al-Jabal Al-Akhdar. Significant differences in total soluble sugars, starch and soluble proteins contents and Mineral contents were detected by sex and geographical area, the big difference is found in a few different locations.

## 3.1. Changes in photosynthetic pigments

Chlorophyll (Chl), which harvests, transfers, and converts sunlight, is the initiator of photosynthesis and is thus acknowledged as the best indicator of productivity. The samples from the Derna showed increased concentrations of Chlorophyll a and Chlorophyll b was record (0.431, 0.428) respectively compared to samples from the Balagray (Figure 1). On the other hand has been significantly increased by the Carotenoids also in Pistacia atlantica trees with locations Derna was recorded 0.733 (mg/g-1 FW) compared to samples from the Balagray 0.446 (Fig1).

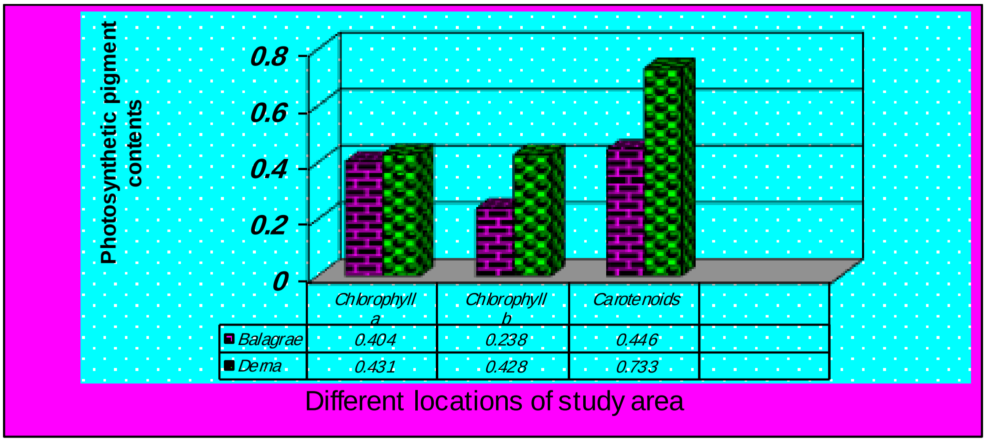


**Figure 1: Changes in photosynthetic pigments contents of *Pistacia atlantica* plants grown in Balagray and Derna regions.**

## 3.2. Changes in soluble sugars contents:

Te results of chemical composition analysis for all samples are summarized in the (Fig. 2) Significant divergences were observed in the chemical composition two different locations (Derna and Balagray) regions of Aljabal Alakhdar ($P< 0.05$) contents for gender andgeographic region. ANOVA results indicated that both geographic loca-tion had significanteffects on the total soluble sugars. Significant differences were found for total soluble sugars content between samples with the maximum and mini-mum soluble sugars recorded for the Pistacia atlantica leaves in locations (Derna) 1.165 and (Balagray) 0.648 (DW/g) regions, respectively.

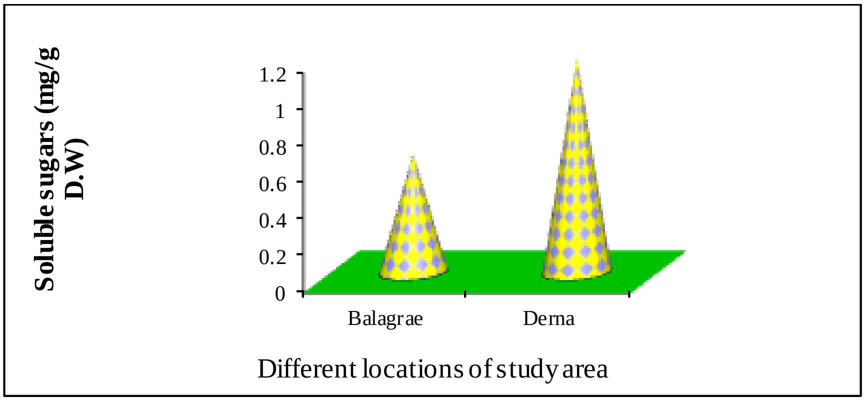


**Figure 2: Changes in soluble sugars contents of *Pistacia atlantica* plants grown in Balagray and Derna regions**

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تاريخ النشر 2023/03/01 تاريخ الاستلام 2023/01/29

### 3.3. Changes in starch contents

Significant differences were found for Starch content between samples ($P < 0.05$) with the maximum and minimum Starch contents recorded for the Derna (2.346%) and Balagray (1.539%) regions, respectively (Fig. 3.)

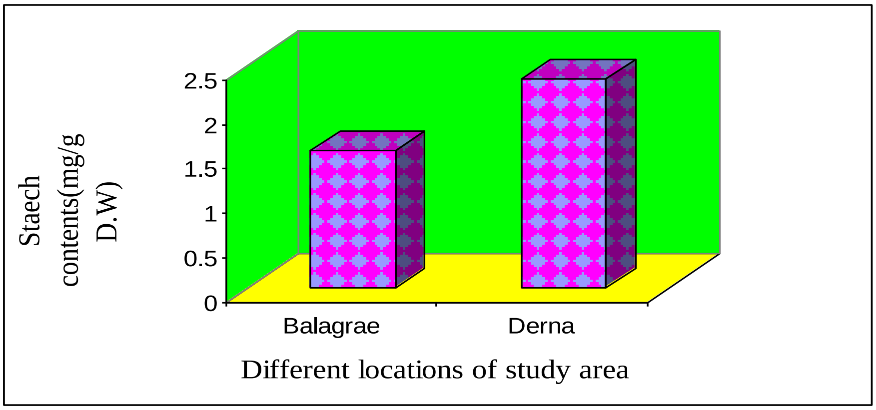


**Figure 3: Changes in starch contents of *Pistacia atlantica* plants grown in Balagray and Derna regions.**

### 3.4. Changes in soluble protein contents.

The studied plants showed a significant increase in the contents of proteins, in the *Pistacia atlantica* plant from the Derna , the values were (3.47µg/g), respectively (Fig. 4). On the other hand, Balagrae plant samples recorded relative decrease in soluble protein (2 .68µg/g).

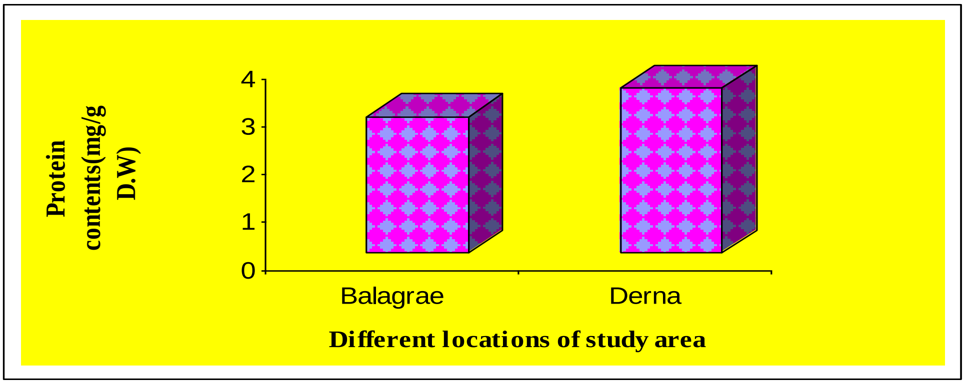


**Figure 4: Changes in protein contents of *Pistacia atlantica* plants grown in Balagray and Derna regions.**

### 3.5. Changes in minerals contents.

The results in given in (Table, 1) showed the mineral contents in soil and leaves of *Pistacia atlantica* plants cultivated in Balagrae and Derna areas. The results showed that plants grown in Derna areas resulted in significant increases in levels of ions, phosphorus, nitrogen, potassium, and calcium, compared to plants grown in Balagrae. These results were related to the soil content of nutrients, where the stock of Derna area was higher compared to Balagrae.

**Table1. Certain minerals contents as P, N, $K^+$ and $Ca^{+2-}$ contents of soil and leaves of *Pistacia atlantica* developing in two different regions of Al-Jabal Al-Akhdar area (Libya)**

| Samples | | Mineral contents (%) | | | |
|---|---|---|---|---|---|
| | | P | N | $K^+$ | $Ca^{+2}$ |
| Balagrae | **Leaf** | 2.8 | 1.55 | 0.27 | 0.17 |
| | **Soil** | 4.37 | 0.87 | 019 | 0.18 |
| Derna | **Leaf** | 4.28 | 2.66 | 0.28 | 0.29 |
| | **Soil** | 4.67 | 1.97 | 0.23 | 0.23 |

## 4. Discussion

According to the results obtained in this study, which was conducted on the *Pistacia atlantica* trees in the regions of Derna and Belgra, the study showed that there were differences in some physiological properties for all the studied measurements, where all concentrations were high in the leaves of the growing trees in Derna, and slight differences were recorded in the contents of minerals between the leaves in the two selected areas and also in soil content. The influence of climatic changes between regions is considered one of the factors that most affect the contents of the studied descriptions.

On the other hand, changes in pigments contents were found to be affected by exposure climate change and soil compositionas well as plant species or genotypes (Doganlar ZB *et al.*, 2010). The concentrations of Chl (Chl a, and Chl b, ) are indicators of light-use efficiency and, in turn, production efficiency. In addition, Chl a and Chl b are the main components of the

reaction centre and light-harvesting complexes (Sharma PK *et al.,* 2001) . Hence, the composition of (Chl a/b) mainly reflects the allocation of plants to different photosynthetic apparatus protein complexes as well as the efficiency balance between light -harvesting and electron transport
(Li M, Zhang *et al.,* 2008).These high-carotene levels with seasonality could be attributable to the high incidence of thesun's UV index ange of the sun's spectrum intodeeper of the tissue. Carotenoids are necessary for photo protection of photosynthesis and they play an important role as a precursor in signaling during the plant development under a biotic/biotic stress. Decrease in carotenoids lead to degradation of B-carotene and formation of Zeaxanthins, which are apparently involved in protection against photo-inhibition(Murakeozy *et al.,* 2003) .Nowadays, enhanced carotenoids contents in plants are of considerable attention for breeding as well as genetic engineering in different plants (Parida *et al.,* 2005). Soluble carbohydrates accumulation in plants has been widely reported as a response to climate change, salinity or drought, despite a significant decrease in net $CO_2$ assimilation rate (Parida *et al.,* 2002).

Soluble sugar accumulation may be due to further transformation of starch to sugars or less consumption of carbohydrates by the tissues in saline conditions (Marschner, 2001).Carbohydrates accumulations under Unsuitable conditions for growth (stress) play a leading role in osmoprotection, osmotic adjustment, carbon storage, and radical scavenging (Ashraf ,2004).Carbohydrates are supplied mainly through the process of photosynthesis and photosynthesis rates is usually lower in plants exposed to salinity and especially to NaCl (Russell, 1999). The decrease in protein content may be caused by enhanced protein degradation process because of increased protease activity that is found to increase under stress conditions. Another explanation for protein degradation under saline environment has been reported due to decrease in the availability of amino acids and denaturation of enzymes involved in protein synthesis (Russell, 1999). Nitrogen, in one form or another, accounts for about 80% of the total mineral nutrients absorbed by plants (Sharpley et *al.,* 1992). Decreased nitrogen uptake under unsuitable conditions for growth (climate change ) occurs due

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to interaction between $Na^+$ and $NH_4^+$ and/or between $Cl^-$ and $NO_3^-$ that ultimately reduce the growth and yield of the crop (Page *et al.*, 2006). This reduction in $NO_3^-$ uptake is associated with $Cl^-$ antagonism or reduced water uptake under unsuitable conditions for growth conditions (Page *et al.*, 2006).Phosphorous is the second major nutrient for plant growth as it is an integral part of different biochemical like nucleic acids, nucleotides, phospholipids, and phosphoproteins. Phosphate compounds act as "energy currency" within plants (Russell, 1999).The interaction between salinity and phosphorus nutrition of plants is equally as complex as that between salinity and nitrogen. In most cases, salinity decreases the concentration of phosphorous in plant tissue (Page *et al.*, 2006) but the results of some studies indicate salinity either increased or had no effect on phosphorous uptake. Phosphate availability is reduced in saline soils not only because of ionic strength effects that reduce the activity of phosphate but also because phosphate concentrations in soil solution are tightly controlled by sorption processes and by the low-solubility of phosphorous. Calcium plays an essential role in processes that preserve the structural and functional integrity of plant membranes, stabilize cell wall structures, regulate ion transport and selectivity, and control ion-exchange behavior as well as cell wall enzyme activities. Because calcium appears to be readily displaced from its membrane binding sites by other cation, these functions may become seriously impaired by reduced calcium availability. Increasing the external concentration of calcium largely counteracted this displacement (Blaha *et al.*, 2000). Potassium is the most prominent inorganic plant solute, and as such makes a major contribution to the low osmotic potential in the stele of the roots that is a prerequisite for turgor-pressure-driven solute transport in the xylem and the water balance of plants (Page *et al.*, 2006). Potassium ion is the major cation within plants, which counterbalances the negative charge of anions. The potassium ion stabilizes the pH, osmotic potential, and turgor pressure within cells. It also plays a crucial role in the activation of the enzymes involved in the metabolism and synthesis of proteins and carbohydrates as it plays a major role in binding tRNA to ribosomes (Blaha *et al.*, 2000).Moreover, potassium ions contribute to the osmotic adjustment of cells plants under environmental stresses (Shahbaz *et al.*, 2007).. Different plant studies indicated that high level of external $Na^+$ caused a decrease in both potassium and calcium, concentrations in plant tissues of many plant species (Blaha *et al.*, 2000).. This reduction in potassium concentration in plant tissue might be due to the antagonism of $Na^+$ and potassium at uptake sites in the roots, the influence of $Na^+$ on the potassium transport into xylem or the inhibition of uptake processes (Shahbaz *et al.*, 2007). Most woody plant genera show considerable inter-specific variation in leaf osmotic parameters (Ashraf, 2004).that reflects, at least partly, their distribution in relation to climatic and edaphic conditions. The genus *Pistacia atlantica* forms the dominant canopy in many forest and land ecosystems in Libya, and is endowed with its tolerance to a wide range of environmental conditions from arid conditions and the ability to cope with very low water potential (Roberts *et al.*, 1977). indicated that in Mediterranean forests, topography led to changes in vegetation and thus leading to changes in absorbed solar energy, moisture and the temperature of forest environment (Amrutha *et al.*, 2019).

## Conclusion

Through the results of our study, which included an evaluation of some of the physiological characteristics of the plant growing in two different regions in Al Jabal Al Akhdar, which have large numbers of diverse plants, we found that there are differences in all the studied characteristics between the two regions, mainly due to the topography of the place and the difference in climate, which in turn affected the composition of the soil. This has been confirmed by many studies that focused on climatic changes and the differences they cause in the content of plants growing in different regions. These results would contribute to a more comprehensive understanding of the potential geographical distribution pattern and the distribution of suitable habitats of these target species. This could provide useful information and reasonable reference for us to make recommendations for implementing long-term conservation, regional management, and reintroduction for these species.

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