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Variation of biochemical content in the almonds of the endemic Argan tree (*Argania spinosa* (L.) Skeels) populations in Morocco

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Abstract: In this study, it was aimed to determine the variation of the biochemical characteristics of the argan [*Argania spinosa* (L.) Skeels] in natural distribution areas in Morocco. For this aim, it was used 13 populations, which are representative of the taxon on different sub-ecoregion in Morocco, to determine some biochemical compositions characters such as malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline, protein, flavonoid, phenol, glucose, sucrose, fructose, enzyme activities of superoxide dismutase (SOD), and peroxidase (POD) were analyzed. To determine variations among the population were analyzed using ANOVA. In addition, the phylogenetic relationship among the populations was revealed by Cluster Analysis. As a result of the research, significant differences were determined in terms of all parameters such as proline, protein, flavonoid, phenol, glucose, sucrose, fructose, MDA, H_2O_2, SOD, and POD of almond diversity among the populations. According to the Cluster Analysis, the results showed that the Tamanar population was relatively different from all other populations. The results obtained in the research confirm the high variety of different habitats in the natural distribution areas of argan in Morocco. The revealing of the diversity among the populations in the natural distribution area of this extremely degraded species is of primary importance for the conservation policies and sustainable use of the species.

Keywords: seed traits, almond, eco-region, provenances, Morocco, Argan

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Introduction

The argan tree (*Argania spinosa* (L.) Skeels) is a relic, and endemic species in Morocco (El Youbi et al., 2010; Díaz Barradasa et al., 2013). The argan tree is the most remarkable species in North Africa, due to its botanical and bioecological interest as well as its social-economical value of rural population. It is generally accepted that all argan trees disappeared from Northern Africa during the Quaternary glaciations, except in the Souss Valley, Morrocco where optimum conditions for the trees' survival remained (Kenny & Zborowski, 2007).

The argan forest covers about 8,280 km^2, mostly in the dry lowlands of the Souss Valley and on the sunny mountain spurs of the Anti-Atlas. The argan tree grows very slowly. It takes fifteen years to mature. The argan tree is extremely resistant to drought via a deep root system, which can live for 150 years, and sometimes more than 200 years. Therefore, the argan tree is often the ultimate warrior when the desert is encroaching on the Souss Valley. Because of these combined climatic and geologic factors, the valley and its surrounding mountains constitute an exceptional area, where the argan tree is exclusively endemic (Benzyane et al., 1999). For all these reasons, the argan forest has been declared a Biosphere Reserve by UNESCO in 1998. However, alarming signs have shown up in the argan forests; during the 20th century, its area has been reduced by half, and in some places, tree density is 66% lower than it was 50 years ago. Overgrazing by camels, goats, and excessive collection of wood are the main causes of this decline exacerbated by drought. Consequently, if no measures were taken, anticipated climate change would have accelerated this dramatic trend, and the argan tree would be severely endangered with subsequent irreversible desertification of the major part distribution area of the species (Morton & Voss, 1987; Kenny & Zborowski, 2007).

Argan has great medicinal and therapeutic benefits (Lizard et al., 2017; Idm'hand et al., 2020). In addition, it is highly sought after in cosmetics as a skin and hair-conditioning agent (El Abbassi et al., 2014). As the pulp of the argan leaves, and fruit is palatable to cattle and provides cheap feed for goats and other farm animals (Guillaume & Charrouf, 2013; Laaribya et al., 2017). Chemical analysis of argan fruit pulp has already shown the presence of lipids (Charrouf et al., 1991), polyisoprene (Pioch et al., 2011), saponins (Guillaume & Charrouf, 2011), and phenolics (Charrouf et al., 2007).

The argan is one of the most important floristic elements in Morocco, it is the only representative of the Sapotaceae family, which grows in the subtropical zone, and the *Argania* genus found in Morocco. This tree is fully exploited by the native populations for nutrition, medication, and cosmetics. The argan oil extracted from the seed is the main tree product for its large use (Kenny & Zborowski, 2007; Mechqoq et al., 2021). It is so important in the argan population for this kind of research in order to present the argan tree and define its specificities, and geographical conditions in both vertical and horizontal directions from sea level to altitudes of 1300–1500 m. (Ruas et al., 2011), and determine the variation of the biochemical characteristics of argan in different natural distribution areas in Morocco. It must be preserved these argan forests as a barrier to climate-induced desertification while helping the communities, that depend on these forests for a living, adapt and preserve their cultural heritage.

The argan tree grows in the study area in arid, and semi-arid bioclimate and is a multi-purpose tree that promotes the stability of local populations through income generation, increases resilience, and improves climate adaptation. It thus plays a very important role in achieving the three dimensions of sustainable development – economic, social, and environmental – at the local level. This research gives some orientation to protecting argan in different natural distribution areas in Morocco. Thanks to the biochemical properties determined according to the populations, it will be possible to determine the most suitable populations for restoration under the effects of climate change, and useful basic information for the nutrition, medicine, and cosmetic sector will be produced. In addition, revealing the diversity among the populations in the natural distribution area of this extremely degraded species is essential for the conservation policies and sustainable use of the species. Because, a good understanding of the variation within a species is necessary for its domestication, conservation, and sustainable management (El Kassaby, 2000). The aims of the present research are, to present the argan tree, define its specificities, report, and determine the variation of the biochemical characteristics of argan in natural distribution areas via different 13 populations in Morocco.

Materials and methods

Material

This study was conducted on the almonds of 13 different argan populations from Morocco (Table 1). The seeds obtained from Morocco were sampled to represent the inland and coastal parts of the country. When the fruits ripen, the seeds were collected during the usual harvesting period, between May and June 2018. The localization distributions of 13 argan populations are shown in Figure 1.

Table 1. Geographical and habitat characteristics of the sampled argan tree populations

Population number	Population name	Altitude (m)	Latitude	Longitude	Climate
1	Aoujdad-Azıar	200	−9.69458	30.5563	Semi-arid
2	Argana	780	−9.12385	30.7847	Semi-arid
3	Tamanar	227	−9.80856	30.7423	Semi-arid
4	Aoulouz	736	−8.15603	30.6849	Arid
5	Admine	90	−9.36146	30.3330	Semi-arid
6	Anzi	400	−9.35903	29.6615	Arid
7	Ain Asmama	1480	−9.25822	30.7527	Subhumid
8	Lakhsass	900	−9.75488	29.3627	Arid
9	Arghen	520	−8.60863	30.5033	Semi-arid
10	Sidi Ifni	300	−10.16550	29.3802	Arid
11	Ait Baha	560	−9.15263	30.0745	Arid
12	Taznakht	1244	−9.49133	30.6691	Semi-arid
13	Tasgaou Drar	525	−9.58931	30.7565	Semi-arid

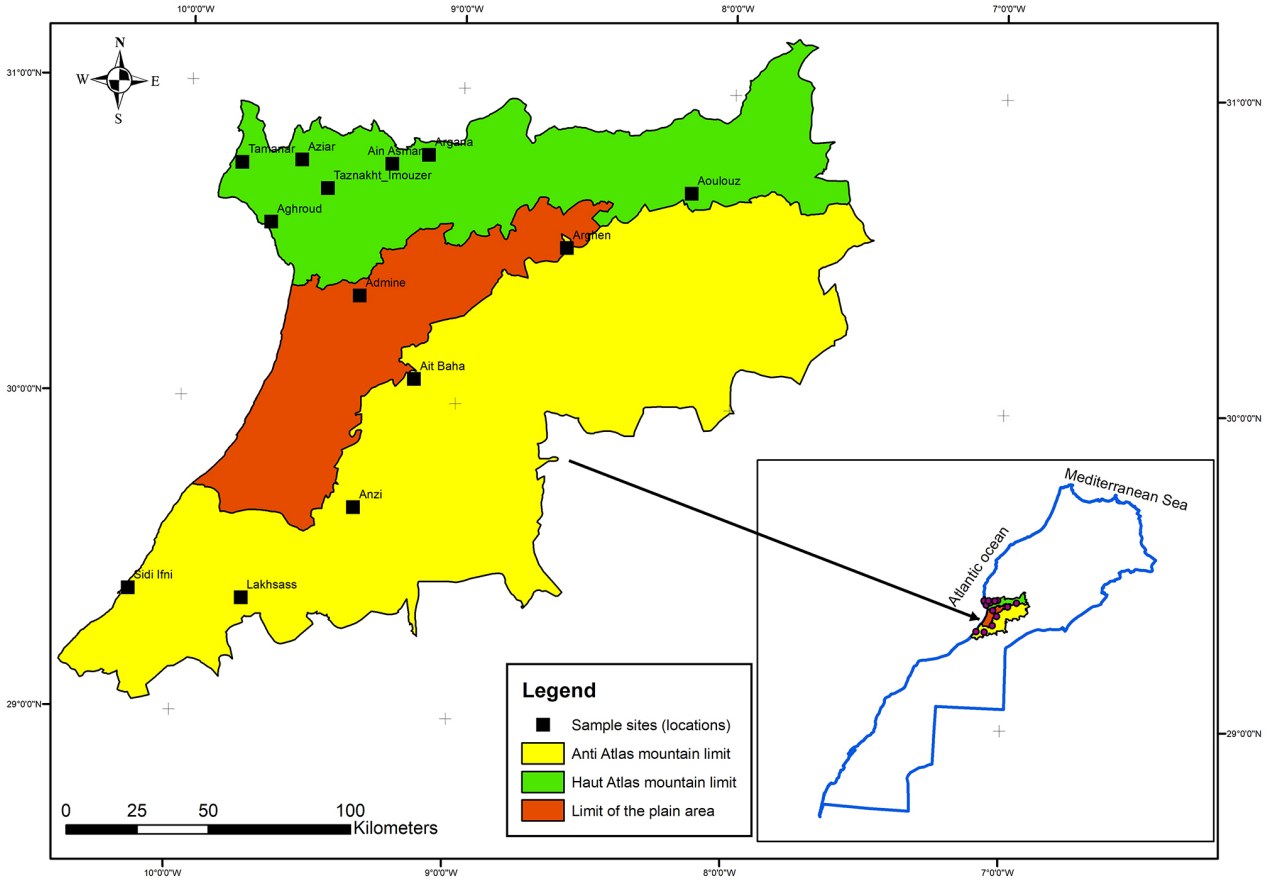

Fig. 1. Locations of 13 different populations in the investigation area (southwestern Morocco)

In the determination of populations, care has been taken to sample from different habitats to represent coastal and inland ecosystems from the northernmost to the southernmost of the distribution area and from high mountain to flat areas. The argan tree in this study area is a thermophilic and xerophilic tree, with an arid to semi-arid warm and temperate bioclimate. The locations of the populations have an annual precipitation amount between 250 and 400 mm according to the average of the data of the last 30 years (Table 1).

Methods of biochemical analysis

Malondialdehyde (MDA)

The amount of malondialdehyde (MDA) was performed based on the method of Lutts et al. (1996). Fresh weight of seeds (500 mg) was extracted with 10 mL of trichloroacetic acid (1% w/v). The extract was centrifuged at 10,000 rpm for 10 min, and the supernatant (2.0 mL) was added to 4.0 mL of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA). The solution was heated at 95 °C for 60 min

and then immediately cooled in an ice bath and centrifuged at 10000 g for 10 min. The absorbance was recorded at 532 and 600 nm by spectrophotometer. By subtracting the absorption value at 600 nm, the MDA content was assessed using its absorption coefficient of 155 nmol cm-1 and expressed as μmol g^{-1} fresh weight.

Hydrogen Peroxide (H_2O_2)

The amount of Hydrogen Peroxide (H_2O_2) was determined following Velikova et al. (2000). First, seeds were frozen with liquid nitrogen and ground with 10 mL 0.1% (w/v) trichloroacetic acid. The mixture was centrifuged at 15.000 × g for 15 min at 4 °C. 0.5 mL supernatant aliquoted and 1 mL 1 M KI and 0.5 mL 10 mM K_2HPO_4 buffer (pH 7.0) was added. The reaction mixture was subjected to dark conditions for 60 min. The absorbance values of the samples were read at 390 nm. The H_2O_2 amount was calculated with a standard curve.

Proline

The amount of proline was made using the method of Bates et al. (1973). A 500 mg sample was crushed in a mortar with 10 mL of 3% sulfosalicylic acid solution. The mixture was filtered with cheesecloth, 2 mL was taken from the filtrate and transferred to 25 mL glass tubes. 2 mL of ninhydrin and 2 mL of glacial acetic acid (acidic ninhydrin) were added to 2 mL of sample in a glass tube. The mouth of the tubes was closed with parafilm, and the top is pierced several times with a pin. It was boiled at 95 °C for 1 hour and then cooled in an iced environment. Toluene, which was cooled in the refrigerator the night before or cooled by keeping it in an iced environment 1 hour before, was added to the cooled samples. When toluene was added, two separate phases were formed. It was taken from the upper phase and transferred to quartz tubes. Absorbance was read at 520 nm. Toluene was used to stabilize the spectrophotometer. Proline standards were prepared. With the equation obtained from these, the amount of proline was calculated (X = Y-0.086/0.1522).

Soluble protein

The soluble protein content of almonds was determined according to the modified Bradford (1976) method. About 500 mg of almonds were extracted with 5 mL of 50 mM KH_2PO_4 (pH 7) buffer. 1000 μL was taken from samples centrifuged in Eppendorf tubes at +4°C at 15 000 rpm for 20 minutes and 2.5 mL of Coomassie Brillant Blue G-250 (CBB) was added to it. After 10 minutes of incubating samples, their absorbance was measured with the help of a standard BSA graph at 595 nm on a UV-vis spectrophotometer, and almond protein content was determined as mg g^{-1} fresh weight (FW).

Superoxide dismutase (SOD) and Peroxidase (POD)

For the preparation of enzyme extracts; 0.5 g of seeds were taken and homogenized with 50 mM (pH 7.6 phosphate buffer solution (5 mL) containing 0.1mM Na-EDTA (ethylenediamine tetraacetic acid disodium salt)) in the sample. Subsequently, homogenized samples were centrifuged for 15 min at 15000 g and +4 °C the enzyme activities in the resulting supernatant were measured as superoxide dismutase (SOD) and peroxidase (POD). The SOD activity of seed samples was measured according to the method applied by Cakmak and Marschner (1992). The SOD reaction mixture (3 mL) contained 13 mM methionine, 10 μM EDTA, 50 mM sodium phosphate buffer (pH 7.8), 2 μM riboflavin, 75 μM NBT and 0.1 mL of the enzyme extract. The reaction mixtures were lightened under light (60μ mol m^{-2} s^{-1}) for 10 min and the absorbance was recorded by recording at 560 nm using a spectrophotometer. Blank solutions were held in the dark. Enzyme activity was estimated as the quantity of enzyme causing a 50% reduction in absorbance. The activity of SOD was expressed as U mg^{-1} protein. The activity of POD was performed following Zhang et al. (2006) method. The POD reaction mixture contained 2 mL buffer substrate (8 mM guaiacol, 100 mM sodium phosphate pH 6.4), 1 mL of 24 mM H_2O_2, and 0.5 mL of enzyme extract. Absorbance values were recorded twice at 30-sec intervals at 470 nm. POD activity was expressed as ΔA470 min^{-1} mg^{-1} protein.

Glucose, Fructose, and Sucrose

The calculations for the quantities of glucose, fructose, and sucrose were done in accordance with the protocols of Pearson et al. (1976). Approximately 1 g sample was homogenized in 50 mL ethanol (80%) at +4 °C for 24 hours. After incubation, the suspensions were filtered through Whatman No. 4 filter paper and this filtrate was used for glucose determination. To measure fructose, the remaining sample residue was incubated again in 30 ml of distilled water at 4°C for 24 h. Then, the solution was filtered through Whatman No. 4 filter paper and the filtrate was used for fructose determination. The residue from the sample used for fructose determination was incubated in 30 ml of 52% perchloric acid at 4°C for 24 h. The resulting suspension was filtered through Whatman No. 4 filter paper and the filtrate obtained was used to determine sucrose. The contents of glucose, fructose, and sucrose were determined by using standard calibration curves of glucose, fructose, and sucrose and were expressed as mg g^{-1} fresh weight.

Total polyphenols

The total polyphenol contents were determined according to the method of Folin and Denis (1915).

Approximately 1 g sample was homogenized in 15 mL acetone (80%) and filtered through Buckner's funnel. The residue was washed several times with 80% acetone and the final volume was adjusted to 50 mL with 80% acetone. The reaction mixture in Nessler's tubes consisted of 1 mL of plant extract, 10 mL of 20% Na_2CO_3, and 2 mL of Folin-Denis reagent. The mixture was kept at room temperature for 2–3 hours and the final volume was adjusted to 1000 mL with distilled water. The reaction mixture was adjusted to a final volume of 50 mL with distilled water. The absorbance of the blue color developed after 20 min was measured at 660 nm in a spectrophotometer.

Total flavonoid

The determination of total flavonoid was carried out according to the method of Luximon-Ramma et al. (2002). The reaction mixture was prepared by adding 1.5 mL of the extract to 1.5 mL of methanolic $AlCl_3$ (2%), and the samples were then kept at room temperature for 10 min. The absorbance was then measured at 420 nm. Samples were prepared in triplicate, and the average value was calculated. A similar procedure was used for the reference compound quercetin, and the standard curve was plotted (10 µg/mL to 100µg/mL, $R^2 = 0.920$). The data obtained were expressed as milligrams of quercetin equivalents (QE)/g of sample.

Statistical Analysis

Nine repeated samples were prepared for each parameter in order to obtain data, and analyses were performed in the laboratory. One-Way ANOVA was performed to determine whether each parameter of the chemical compounds detected on the almonds differed significantly by population. These findings were presented using the IBM SPSS statistic 23.0 program. The difference that existed among each of the processes, according to the ANOVA results and the significant differences among main values of processes was determined by Duncan's Multiple Test (D'sMT). As a result of the D'sMT, the groups were given in alphabetical order as letters along with the arithmetic mean and standard error. In addition, the phylogenetic relationship among the populations was revealed by Cluster analysis.

Results

The almonds belonging to 13 different populations of argan were analyzed and compared for 11 different biochemical characteristics. All the biochemical variables analyzed showed significant differences based on the populations (Tables 2, 3).

As can be seen in Table 2, the highest H_2O_2 value (36.05±0.1 µmol/g**)** was determined in the population "Arghen", and the lowest value in the population "Tasgou Drar". According to the results of the D'sMT, H_2O_2 values were clustered in seven homogeneous groups. The highest MDA values were determined in the populations of "Taznakht" (45.05±0.1 µmol/g), and "Argana" (44.06±0.48 µmol/g). "Taznakht" and "Argana" populations were included in the first homogeneous group. The lowest is the population "Ain Asmama" (31.47±0.4 µmol/g). The populations with the lowest and highest values in terms of MDA were all located in the High Atlas Mountain Zone in the north (Fig. 1). According to the results of the D'sMT, MDA values were clustered in eight homogeneous groups (Table 2).

The highest proline value (2.4±0.1 µmol/g) was detected in the population "Tamanar", while the lowest values in populations "Ain Asmama" (1.42±0.03 µmol/g) and "Admine"

Table 2. The values of biochemical characteristics according to the populations (Abdaloğlu, 2022)

Population name	H_2O_2 (µmol/g)	MDA (µmol/g)	Proline (µmol/g)	Protein (mg/g)	SOD (EU/mg protein)	POD (EU/mg protein)
Admine	33.85±0.06b	35.81±0.17f	1.42±0.01g	35.86±0.23e	2.22±0.04h	0.28±0.00f
Ain Asmama	33.09±0.09b	31.47±0.40h	1.42±0.03g	49.26±0.03c	6.48±0.06a	0.22±0.00g
Ait Baha	14.72±0.07d	37.75±0.51e	1.89±0.01d	33.10±0.02h	0.35±0.01m	0.16±0.00h
Anzi	16.95±2.31c	43.09±0.37bc	1.75±0.04e	35.25±0.08f	4.04±0.02d	0.76±0.01c
Aoujdad Aziar	15.54±0.19cd	41.61±0.39cd	2.08±0.01c	36.42±0.31d	2.04±0.01i	0.57±0.01d
Aoulouz	4.70±0.7g	41.80±0.15cd	1.53±0.03fg	35.69±0.07e	2.49±0.04g	0.89±0.02b
Argana	32.78±0.20b	44.06±0.48ab	2.23±0.04b	56.38±0.09b	3.64±0.02e	0.08±0.00i
Arghen	36.05±0.10a	33.26±0.29g	1.57±0.00f	33.59±0.10g	1.60±0.01k	0.38±0.00e
Lakhsass	7.44±0.23f	33.36±0.21g	1.50±0.02fg	35.78±0.03e	3.31±0.01f	0.77±0.02c
Sıdı Ifni	12.79±0.08e	42.84±0.75bc	1.68±0.01e	33.47±0.07g	4.41±0.01c	0.91±0.02b
Tamanar	14.17±0.08de	38.12±1.30e	2.40±0.10a	65.81±0.05a	0.75±0.01l	0.52±0.01d
Tasgou Drar	4.60±0.05g	41.20±0.52d	2.22±0.06b	33.35±0.07gh	1.78±0.00j	0.35±0.02e
Taznakht	7.26±0.03f	45.05±0.10a	1.90±0.02d	36.56±0.09d	5.97±0.02b	1.16±0.05a
F value & P level	326.82***	72.81***	73.88***	6 925.99***	5 382.94***	343.23***

Note: Each letter represents the homogeneous group formed by multiple test analysis.

Table 3. Values of sucrose, glucose, fructose, flavanoid, and phenol according to the populations (Abdaloğlu, 2022)

Population	Sucrose (µg/gr)	Glucose (µg/gr)	Fructose(µg/gr)	Flavanoid (mg/g)	Phenol (mg/g)
Admine	11.86±0.07e	23.83±0.17f	1.81±0.02b	2.12±0.00 de	1.84±0.03e
Ain Asmama	12.16±0.03cd	24.24±0.16ef	0.73±0.01e	2.14±0.01de	2.36±0.02d
Ait Baha	12.25±0.05bc	25.96±0.32c	2.86±0.05a	2.11±0.00 e	1.53±0.04fg
Anzi	12.37±0.02ab	24.06±0.15f	0.42±0.00h	2.12±0.01de	3.40±0.01b
Aoujdad Aziar	12.40±0.02a	24.96±0.18d	0.28±0.02j	2.12±0.00de	4.22±0.06a
Aoulouz	11.82±0.05e	25.31±0.21d	0.31±0.01ij	2.19±0.00a	1.73±0.01ef
Argana	12.07±0.02d	26.26±0.23c	0.95±0.02d	2.18±0.01ab	1.88±0.02e
Arghen	12.15±0.05cd	23.99±0.26f	0.64±0.01f	2.12±0.00de	2.92±0.04c
Lakhsass	12.35±0.03ab	24.81±0.16de	1.71±0.06c	2.08±0.00f	1.57±0.25fg
Sıdı Ifni	12.26±0.03bc	24.82±0.22de	0.53±0.01g	2.14±0.02cde	1.25±0.01hi
Tamanar	12.14±0.05cd	25.02±0.15d	0.36±0.01hi	2.18±0.00ab	3.54±0.11b
Tasgou Drar	12.30±0.02ab	30.64±0.14a	0.39±0.00hi	2.17±0.02abc	1.35±0.00gh
Taznakht	12.14±0.06cd	27.48±0.28b	0.31±0.01ij	2.15±0.02bcd	1.03±0.07i
F value & P level	18.37***	78.29***	972.69***	9.77***	155.28***

Note: Each letter represents the homogeneous group formed by multiple test analysis.

(1.42±0.01 µmol/g). The "Admine" population with the lowest proline value is located in the plain area, while the "Ain Asmama" population was located in the High Atlas Mountains zone. In addition, the highest protein value (65.81±0.05 mg/g) was also detected in the population "Tamanar", while the lowest value (33.1±0.02 mg/g) was in the population "Ait Baha". The "Tamanar" population with the highest value in terms of protein was located in the High Atlas, and the "Ait Baha" population with the lowest value was located in the Anti Atlas Mountains Zone (Fig. 1). According to the results of D'sMT, seven homogeneous groups were formed in terms of proline value and eight homogeneous groups in terms of protein value (Table 2)

The highest SOD value (6.48±0.06 EU/mg protein) was detected in the population "Ain Asmama", and the lowest value (0.35±0.01 EU/mg protein) in the population "Ait Baha". It is noteworthy that there is a more than 18-times difference between the highest and lowest populations in terms of SOD value. Of these two populations, which have a remarkable difference between them, "Ain Asmama" High Atlas Mountains with the highest SOD value and "Ait Baha" population with the lowest SOD value are located in the Anti Atlas Mountains Zone (Fig. 1). In addition, a difference in SOD value as well as POD value in terms of the highest and lowest population values was detected more than 14 times. The highest POD value was detected in the population "Taznakht" (1.16±0.05 EU/mg protein), and the lowest value was detected in the population "Argana" (0.08±0.00 EU/mg protein). The populations with the lowest and highest values in terms of POD were all located in the same zone, High Atlas Mountain in the North (Fig. 1). According to the results of D'sMT, 13 homogeneous groups were formed in terms of SOD value and nine homogeneous groups in terms of POD value (Table 2).

As can be seen in Table 3, the highest sucrose values were detected in populations "Aoujdad Aziar" (12.4±0.02 µg/gr), "Anzi", "Lakhsass", and "Tasgou Drar", respectively; the lowest values were in populations of "Admine" (11.86±0.07 µg/gr and "Aoulouz" (11.82±0.05 µg/gr). From the results obtained; It is understood that the lowest or the highest sucrose values are not only related to the location of the populations but also that both the highest and the lowest sucrose values were determined in populations in different zones.

It has been determined that the population "Tasgou Drar" has the highest value in terms of glucose (30.64±0.14 µg/gr) and sucrose (12.30±0.02 µg/gr) content and population "Ait Baha" has the highest value (2.86±0.05 µg/gr) in terms of fructose content. More than 10 times differences were found among the populations with the highest "Ait Baha" and lowest "Aziar" fructose values. According to the results of D'sMT, 13 populations formed 5 homogeneous groups in terms of sucrose, 6 in terms of glucose, and 10 in terms of fructose content (Table 3). The highest flavonoid contents were found in the populations of "Aoulouz" (2.19±0.00 mg/g), "Argana" and "Tamanar" (2.18±0.01mg/g), and "Tasgou Drar" (2.17±0.02 mg/g). The lowest is the population "Lakhsass" (2.08±0.00 mg/g). The populations with the highest value in terms of flavonoids were located in the High Atlas Mountains zone, while the population with the lowest value was located in the Anti Atlas Mountains Zone. In addition, the lowest phenol values were found in "Taznakht" (1.03±0.07 mg/g) and "Sıdı Ifni" (1.25±0.01 mg/g) populations, while the highest value in "Aoujdad Aziar" (4.22±0.06 mg/g) population. According to the results of D'sMT, 6 homogeneous groups for flavonoid values and 9 homogeneous groups were formed according to phenol content.

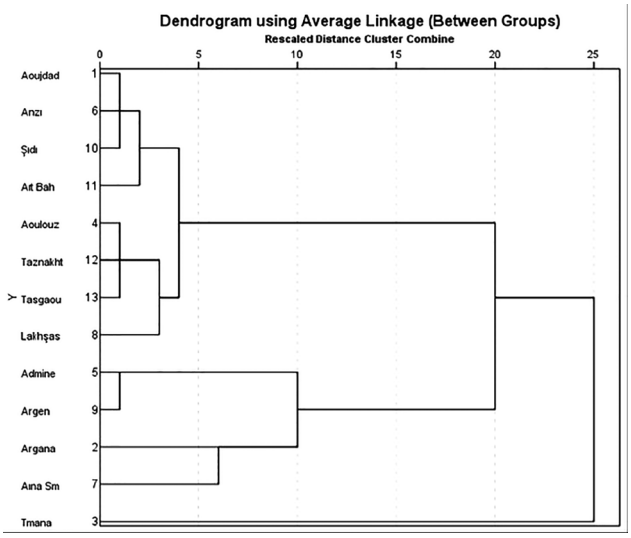

Fig. 2. Cluster analysis based on the populations

According to the Cluster Analysis conducted for 11 biochemical variables related to almonds of the 13 argan populations, the differences among populations were given as dendrogram (Fig. 2). The results showed that the Tamanar population was relatively different from all other populations. The remaining 12 populations were clustered into two main subgroups. Aoujdad Aziar, Anzi, Şidi Ifni, Ait Baha, Aoulouz, Taznakht, Tasgaou Drarand Lakhşass populations are located in the first group, while Admine, Argen, Argana, and Ain Asmama populations were in the second group. The distribution pattern of sampling of diverse populations in clusters indicates that the genetic diversity was not according to geographical origin. The results of cluster analysis support the results of variance analysis.

Discussion

The research results on the biochemical contents of almonds belonging to different populations of the species confirm the high variety of different habitats of the species in the natural distribution area. Abdelaziz et. al. (2014) emphasized that there are greater variations within the population than among the populations. Studies of genetic diversity based on molecular markers confirmed the existence of high genetic diversity within and among populations of Argan in Morocco (Mouhaddab et. al., 2017).

Phytochemical composition of argan fruits reveals different classes of bioactive compounds, including essential oils, fatty acids, triacylglycerols, flavonoids and their acylglycosyl derivatives, monophenols, phenolic acids, cinnamic acids, saponins, triterpenes, phytosterols, ubiquinone, melatonin, new aminophenols, and vitamin E (Khallouki et al., 2017). It contains high levels of antioxidant compounds (Khallouki et al., 2017). The high antioxidant effect of argan oil has been emphasized by many researchers, which is associated with its phenolic compounds (Khallouki et al., 2003; Cherki et al., 2005; Valavanidis et al., 2004). However, because of the extract's complexity in measuring antioxidant activity, all the methods did not give the same results (Mansour et al., 2019).

Abiotic and biotic stress factors, senescence, and silvicultural interventions may increase the severity of oxidative stress by disturbing such balance (Zimmermann et al., 2005). Drought, which is one of the abiotic stress factors, can cause an increase in reactive oxygen species (ROS) such as superoxide ion (O^{-2}), hydroxyl radical (OH), and H_2O_2. In particular, enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) can eliminate the negative effects of reactive oxygen species in plant tissues with their scavenging roles. The activity of these metabolites and antioxidant enzymes causes a high level of resistance to oxidative damage and minimizes damage to cells (Alscher, 1989; Tabatabaei, 2015). Within the scope of this research, it was determined that "Ain Asmama" and "Taznakht" populations localized in different ecological regions with "Sidi Ifni" and "Anzi" had the highest values in terms of SOD. This result may indicate that SOD values are related not only to the influence of environmental conditions but also to the genetics of populations.

Within the scope of this research; It is believed that cellular endurance increases in populations where SOD and POD enzyme activities increase. It has been found that enzyme activities play a protective role, especially in the populations remaining in the High Atlas Mountain Zone. However, in populations with low SOD and POD enzyme activities, an increase in the amount of protein can provide this effect. Because the activation of enzymes responsible for the hydrolysis of proteins can be suppressed. When the first three highest values of proline and protein amounts were taken into account, higher values were found in the populations located in the High Atlas Mountain Zone in the north. According to Chakhchar et al. (2016) in a study conducted on the physiological and antioxidant properties of argan trees belonging to coastal and inland ecotypes; In a study using populations such as "Admine", "Rabia", "Aoulouz", and "Lakhssas", it was concluded that the protective mechanisms of inland ecotypes are higher compared to coastal ecotypes. In this study, Chakhchar et al. (2016) the highest SOD value, which supports the research result, was detected in the "Ain Asmama", and "Taznakht" populations located in the inner ecotypes of the Atlas Mountains. However, Chakhchar et al. (2016) conducted research on two contrasting coastal ecotypes (Admin and Rabia) and two contrasting inland ecotypes (Aoulouz and Lakhssas) to reveal

whether SOD activation promotes the activation of argan tree isoenzymes to edaphic drought tolerance and recovery under rehydration. As a result of the research, a significant amount of carbonyl group, H_2O_2, and superoxide radical accumulation was recorded in the leaves of plants under stress, reflecting oxidative stress in terms of biochemical reactions. In parallel, it was observed that total SOD and their isoenzymes protect the cell against inducing oxidative stress. All adaptive features can make inland ecotypes an elite source of drought tolerance in drought and semi-arid environments and become the new focus of argan tree breeding and improvement.

Morphology, functions, and biochemistry of cells, tissues, and organs of the plants change during their developmental stages such as seedlings, juvenile, adult, and senile stages (Hackett, 1985). An increase in tissue and organ deformation along with aging stimulates the accumulation of lipid peroxidation products such as MDA, and ketonic compounds, and the ROS derivatives such as H_2O_2 and SOD anions in plant cells and tissues (Koussevitzky et. al, 2007). However, the plants can destroy these compounds, which reduce cellular activity and stimulate oxidative stress in plants, using APX, CAT, and POD as enzymatic compounds, and non-enzymatic compounds such as carotenoids, phenolic, flavonoids, proline, and sucrose (Szabados & Savoure, 2009). Within the scope of this study, the highest and the lowest MDA values were determined in the populations of "Taznakht", "Argana, and "Ain Asmama". The populations with the lowest and highest values in terms of MDA were all located in the High Atlas Mountain Zone in the North. This result can be related to the genetic characteristics of the populations and the development stages of the argan individuals constituting the population.

H_2O_2 is an important compound that acts as a signaling molecule in cellular tissues and cells. It has a toxic effect on the limited value of this compound. Oxidative stress in cellular components stimulates lipid peroxidation. A decrease in H_2O_2 level is also effective in reducing antioxidant enzyme activities (Gill & Tuteja, 2010; Foyer et. al., 2017). Drought stress has significantly increased H_2O_2 and lipid peroxidation. In addition, moderate and severe drought stress increased CAT, SOD, POD, polyphenoloxidase, and lipoxygenase activities depending on time. According to the research, it has been determined that inland ecotypes are more tolerant than coastal ecotypes. According to the canonical discriminant analysis, inland ecotypes are basically separated from coastal ecotypes by the biochemical characteristics of polyphenol oxidase, SOD, and MDA (Chakhchar et al., 2016). The SOD enzyme activity inhibits the H_2O_2 toxic effect (Del Rio et al., 2018). In this research, the highest H_2O_2 value was determined in the population "Arghen" in Anti Atlas Mountain Zone, and the lowest value in the population "Tasgou Drar" in High Atlas Mountain Zone. However, this difference cannot be explained by regional differences alone.

Sucrose, fructose, and glucose are reducing sugars produced by photosynthesis, which are important roles in regulating intracellular pressure, plant-water relations, transportation photoassimilates as well as protecting cellular membranes (Stitt et al., 2007). Zhar et al. (2016) stated that the total sugar content of argan fruits with different morphotypes at different ripening stages in semi-continental and littoral regions shows significant variation. Generally, the ripe stage for all morphotypes is characterized by the greatest total sugar content values. In addition, emphasized that this result can be due to the environmental and genetic factors effect that could have an impact on the sugar content. In the north and the High Atlas Mountain zone, the lowest fructose value was observed in the "Aoujdad Aziar" population, while the sucrose value was found to be the highest in the same population. This may indicate that glucose, fructose, and sucrose monomers are involved in starch synthesis in the cell and are key compounds for metabolic reactions (Kuruger & Volin, 2006).

Generally, the maturation stage of argan fruits influenced significantly all biochemical compounds, and the maturation degree was positively and strongly correlated with all phytochemical compounds. In addition, the region had a significant effect on total protein (Zhar et al., 2016). The total proteins of all morphotypic argan fruit increased significantly and gradually from the green to the ripe stage (Zhar et al., 2016). Other authors established that the protein content of plants could vary with soil, climatic conditions, and cultivars' origin (Amira et al., 2011; Tlili et al., 2011). Zhar et al. (2016) emphasized in their study that a gradual increase and significant difference was found in the phenolic content at different stages of maturity according to the four morphotypes of argan fruits. It was found that argan fruits with spherical morphotypes of populations in semi-continental regions have the highest phenolic content.

Among the non-enzymatic compounds contributing to the elimination of ROS accumulation and lipid peroxidation damages, proline and total soluble proteins are particularly important (Mattioli et al., 2009). In addition, phenolic compounds and flavonoids are also important non-enzymatic compounds affecting the growth and development of plants (Harborne, 1980). Besides, they play an important role in increasing tolerance to environmental changes through their functions such as cell wall activities, distribution of assimilates and osmotic regulation (Bernhardt & Tierney, 2000). Flavonoids, which are the most common phenolic compounds, are

important because they are free radical scavengers and they have the task of regulating enzyme activities (Mammadov, 2014). Phenols, which are organic, crystalline, and colorless substances, show a red color when they come into contact with air. It has been determined that the populations of the north Atlas zone are rich in phenolic substances. This may have ensured the preservation of oxidative degradation. Phenolic compounds can be considered an energy source that allows plants to maintain their life. Under adverse climatic conditions, plants synthesize a large amount of phenolic compounds, increasing their resistance to climatic conditions. At the same time, these compounds are involved in the formation of supporting substances of cells and tissues (Mammadov, 2014). In this study, it is thought that the biosynthesis of phenolic compounds is higher in the populations located in the north Atlas zone, which may increase the endurance of the plant by protecting the lipid layer of the membranes from dispersal.

Conclusions

Significant differences were found among the populations in terms of all biochemical variables measured. So, the results confirm the high variety of different habitats in the natural distribution areas of the species. However, the distribution pattern of sampling of diverse populations in clusters indicates that the genetic diversity was not according to the geographical origin.

With the extensive use of genetic diversity based on the chemical and biochemical properties of argan seeds, ecological protection, and sustainable use opportunities can be improved. The use of a large gene pool, both the number of individuals representing the population and the interpopulation variation in genetic diversity, should not be ignored for breeding programs.

These results indicate the urgency of integrated preparation and effective implementation of both in-situ and ex-situ conservation strategies for argan species and populations that have been subjected to degradation for many years. Home to the only biosphere reserve of argan in the world, Morocco is facing a unique challenge. The results may give indications for genetic selection programs and the preservation of the trees that constitute the main source of argan oil, which must be preserved in order to maintain its quality and potency.

Conflicts of interest statement

The paper is produced from Gülbahar Abdaloğlu's master's thesis, which was conducted under the supervision of Prof. Dr. Sezgin Ayan. The authors declare that there is no conflict of interest regarding the publication of this paper.

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