Physiological and biochemical responses of drought-acclimated *Pistacia atlantica* Desf. to short-term irrigation

Respuestas fisiológicas y bioquímicas de *Pistacia atlantica* Desf. aclimatada a la sequía frente al riego a corto plazo

Respostas fisiológicas e bioquímicas de *Pistacia atlantica* Desf. aclimatada à seca à irrigação de curto prazo

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Abstract

Pistacia atlantica Desf. is a key drought-tolerant tree in hyper-arid North African ecosystems, yet little is known about how its osmolytes and phenolic-based antioxidant system respond when water availability is transiently improved. This study evaluated the short-term effects of irrigation on osmolyte levels, phenolic composition, and antioxidant activity in drought-acclimated *P. atlantica* trees. Ten adult trees in Igli region (southwestern Algeria) were assigned to non-irrigated and irrigated groups. Leaves were sampled before and after 90 days of irrigation. The relatively small sample size reflects the limited number of trees available and suitable for the study criteria in the area. Relative water content, proline, glycine betaine, total phenolics, and individual phenolics (HPLC-DAD) were determined. Results showed that irrigation increased leaf water content by about 12 % and significantly reduced proline (−25 %), glycine betaine (−22 %), and total phenolics (−30 %), while IC50 increased by 25 %. All quantified phenolics decreased after irrigation, with maximum reductions of −24 to −27 % were observed for chlorogenic, ferulic, and *p*-coumaric acids and quercetin, whereas gallic, syringic, ellagic acids and naringenin showed smaller declines (−8 to −16 %). The close coupling between osmolytes, key hydroxycinnamic acids and flavonols, and antioxidant activity indicates an integrated defence network that is highly sensitive to water availability and central to drought resistance and recovery in this species.

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Resumen

Pistacia atlantica Desf. es una especie arbórea clave tolerante a la sequía en los ecosistemas hiperáridos del norte de África; sin embargo, se conoce poco sobre cómo sus osmólitos y su sistema antioxidante basado en compuestos fenólicos responden cuando la disponibilidad de agua mejora de manera transitoria. Este estudio evaluó los efectos a corto plazo del riego sobre los niveles de osmólitos, la composición fenólica y la actividad antioxidante en árboles de *P. atlantica* aclimatados a la sequía. Diez árboles adultos en la región de Igli (suroeste de Argelia) se asignaron a grupos no irrigados e irrigados. Se recolectaron hojas antes y después de 90 días de riego. El tamaño de muestra relativamente reducido refleja el número limitado de árboles disponibles y adecuados según los criterios del estudio en la zona. Se determinaron el contenido relativo de agua, la prolina, la glicina betaína, los fenoles totales y los compuestos fenólicos individuales (HPLC-DAD). Los resultados mostraron que el riego incrementó el contenido hídrico foliar en aproximadamente un 12 % y redujo significativamente la prolina (−25 %), la glicina betaína (−22 %) y los fenoles totales (−30 %), mientras que el IC_{50} aumentó en un 25 %. Todos los compuestos fenólicos cuantificados disminuyeron tras el riego, con reducciones máximas de −24 a −27 % observadas en los ácidos clorogénico, ferúlico y *p*-cumárico, así como en la quercetina, mientras que los ácidos gálico, siríngico y elágico, junto con la naringenina, mostraron descensos más moderados (−8 a −16 %). La estrecha relación entre osmólitos, ácidos hidroxicinámicos clave y flavonoles, y la actividad antioxidante indica la existencia de una red de defensa integrada altamente sensible a la disponibilidad hídrica y fundamental para la resistencia a la sequía y la recuperación en esta especie.

Palabras clave: *Pistacia atlantica*, estrés por sequía, marcadores bioquímicos, osmolitos, compuestos fenólicos.

Resumo

Pistacia atlantica Desf. é uma espécie arbórea-chave tolerante à seca em ecossistemas hiperáridos do Norte da África; no entanto, pouco se sabe sobre como seus osmólitos e seu sistema antioxidante baseado em compostos fenólicos respondem quando a disponibilidade hídrica é transitoriamente melhorada. Este estudo avaliou os efeitos de curto prazo da irrigação sobre os níveis de osmólitos, a composição fenólica e a atividade antioxidante em árvores de *P. atlantica* aclimatadas à seca. Dez árvores adultas na região de Igli (sudoeste da Argélia) foram distribuídas em grupos não irrigados e irrigados. Folhas foram coletadas antes e após 90 dias de irrigação. O tamanho amostral relativamente reduzido reflete o número limitado de árvores disponíveis e adequadas aos critérios do estudo na área. Foram determinados o conteúdo relativo de água, prolina, glicina betaína, fenólicos totais e compostos fenólicos individuais (HPLC-DAD). Os resultados mostraram que a irrigação aumentou o conteúdo hídrico foliar em cerca de 12 % e reduziu significativamente a prolina (−25 %), a glicina betaína (−22 %) e os fenólicos totais (−30 %), enquanto o IC_{50} aumentou em 25 %. Todos os compostos fenólicos quantificados diminuíram após a irrigação, com reduções máximas de −24 a −27 % observadas para os ácidos clorogênico, ferúlico e *p*-cumárico e a quercetina, enquanto os ácidos gálico, siríngico e elágico, bem como a naringenina, apresentaram reduções mais moderadas (−8 a −16 %). A estreita associação entre osmólitos, ácidos hidroxicinâmicos-chave e flavonóis, e a atividade antioxidante indica uma rede de defesa integrada altamente sensível à disponibilidade hídrica e central para a resistência à seca e a recuperação nesta espécie.

Palavras-chave: *Pistacia atlantica*, stress hídrico, marcadores bioquímicos, osmólitos, compostos fenólicos.

Introduction

Algeria's climate ranges from Mediterranean in the north to hyper-arid in the south, with a sharp decline in rainfall and rise in temperature extremes along this gradient. Climate change has intensified these contrasts. *Pistacia atlantica* Desf., a drought-tolerant tree of the Anacardiaceae family, is well adapted to this climatic variability and is widely distributed across Algeria's ecological zones. Its resilience to water scarcity, heat, and poor soils highlights its ecological plasticity. Additionally, the species holds ethnobotanical and economic importance, with its resin, fruits, and wood used in traditional practices (Belaid *et al*., 2024; Hamitouche *et al*., 2024; Ifticene-Habani *et al*., 2025)particularly those found in its seeds and leaves. To promote its use, an ethnobotanical survey was conducted among herbalists and other knowledgeable individuals in the Naâma region, utilizing 100 questionnaires divided among 25 municipalities. The findings revealed that leaves (42 %. Plants employ diverse survival and tolerance strategies to manage drought stress, primarily through leaf-based adaptations that regulate transpiration. A critical physiological reaction involves closing stomata to prevent dehydration; however, this action inadvertently limits carbon dioxide absorption and can lead to the toxic buildup of reactive oxygen species (ROS) (Doghbage *et al*., 2024). To counteract oxidative damage, they often increase the synthesis of antioxidant secondary metabolites, notably phenolic compounds and flavonoids, which help protect cellular components and sustain metabolic function (Mohammadi *et al.*, 2023). The intensity of this biochemical response varies depending on species genotype (Doghbage *et al*., 2024). Yet, it is not clear to what extent the phenolic profile and antioxidant potential of mature *P. atlantica* trees grown under hyper-arid field conditions adjust when water availability is transiently improved, nor how these changes are coordinated with proline, glycine betaine, and leaf water status. Addressing this gap is important both for understanding the plasticity of this keystone species and for assessing how short irrigation pulses influence its defensive metabolism. This study aims to characterize the osmolyte content and phenolic status of *P. atlantica* under persistent drought stress, to determine how and to what extent these traits shift during short-term irrigation, and to identify the phenolic compounds most responsive to changes in water availability.

Materials and methods

Study zone

Igli is a commune in the Beni Abbes province of southwestern Algeria, located between 2°10'–2°20' W longitude and 30°20'–30°35' N latitude. It lies approximately 160 km south of Bechar and 67 km south of Taghit (Figure 1). The region has a hyperarid desert climate, with annual rainfall rarely exceeding 50 mm (Figure 2). Summers are extremely hot, with temperatures often exceeding 45 °C and daytime humidity dropping below 10%. Winters are milder, ranging from 4 to 18 °C, with pronounced diurnal temperature fluctuations (Figure 3) (Abdelbaki *et al.*, 2022; Djoudi *et al.*, 2024). Weather data were acquired using Power Data Access Viewer (DAV) (power.Iarc.nasa.gov) from (Nasa Power, 2025).

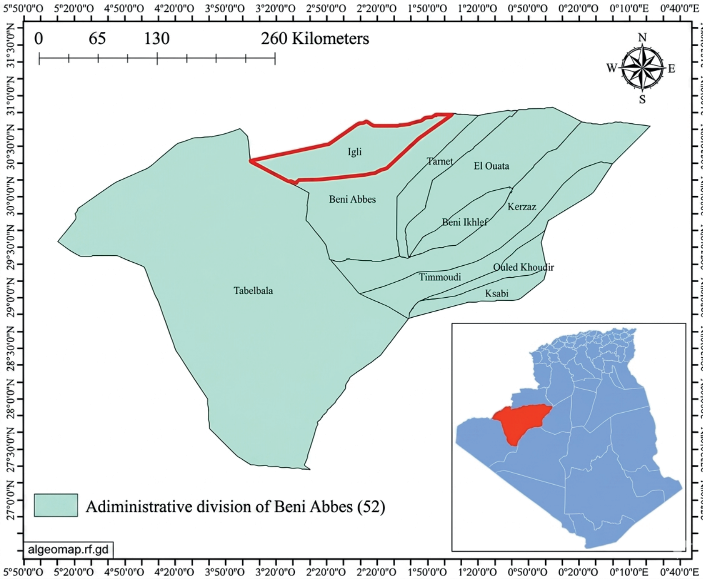

Figure 1. Geographic localization of Igli - Beni Abbes (*Algeomap*, 2025).

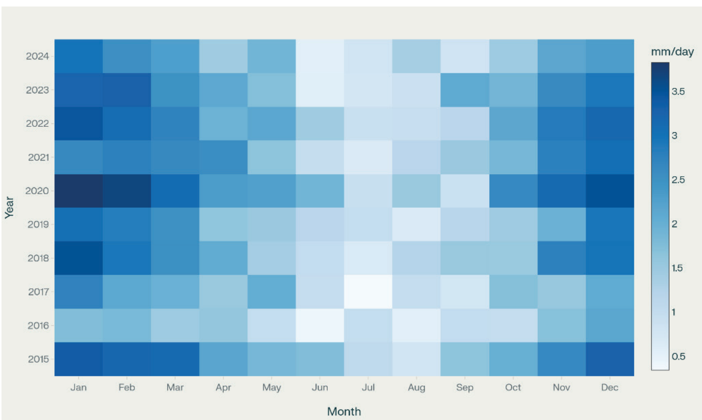

Figure 2. Monthly accumulated precipitation heatmap (2015-2024).

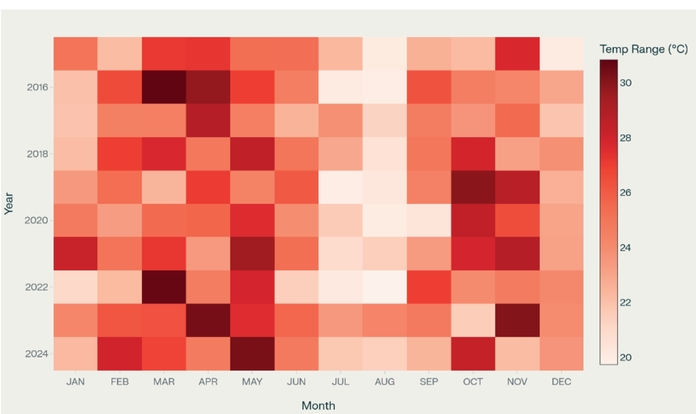

Figure 3. Monthly temperature range heatmap (2015-2024).

Plant materials

The plant material consisted of *Pistacia atlantica* Desf. trees selected from natural populations in the Igli region. Trees were chosen based on similar morphological characteristics, including approximate age, visually assessed from trunk diameter, canopy development, and bark texture, as well as size and health status, to ensure homogeneity. To avoid anthropogenic influence, only individuals located far from agricultural fields, urban or rural infrastructure, water supply networks, sewage systems, and roads were selected. This ensured that all sampled trees were growing under comparable, natural arid conditions without exposure to artificial water sources. The botanical identification was conducted by Dr. Guenaia Abdelkader at the Department of Biology, University of Bechar. A voucher specimen has been deposited in the herbarium under the number gr:1/2002.

Experiment design

Ten *Pistacia atlantica* trees were selected and divided into two groups of five. For the first group, a circular watering basin approximately 1 meter in radius and 10 cm deep was carefully dug around each tree. The depth was intentionally chosen to avoid cutting or damaging the surface root system during excavation. These five trees were irrigated twice weekly for 90 days, from March to May, with approximately 90 L of water applied per irrigation. The remaining five trees served as a control group and were left under natural conditions without any supplemental watering.

Sampling and preparation of extracts

Leaf samples were randomly collected from both irrigated and non-irrigated *Pistacia atlantica* trees at two time points: prior to the initiation of irrigation and after 90 days of treatment. Only fully expanded, mature leaves were randomly collected, regardless of canopy position. Relative water content (RWC) of fresh leaves was assessed as described by Smart & Bingham (1974). The four batches of leaves were then shade-dried at ambient temperature until they became brittle and easily breakable. Hydroethanolic extracts were prepared by macerating 20 g of powdered leaves in 100 mL of a 50:50 (v/v) ethanol–water solution for 24 hours. The mixtures were filtered and concentrated using a rotary evaporator.

Proline and glycine betaine content

Proline content was measured as described by Bates *et al*. (1973). Leaf powder was homogenized in 3 % sulfosalicylic acid, centrifuged, and the supernatant was reacted with acid ninhydrin and glacial acetic acid at 98°C for 30 minutes. After cooling, proline was extracted with toluene, and the absorbance was recorded at 520 nm.

Glycine betaine was quantified as described by Grieve & Grattan (1983). Leaf powder (0.5 g) was extracted with a toluene-water mixture, shaken at 25°C for 24 h, filtered, and sequentially reacted with 2 N HCl and potassium triiodide in an ice bath, then combined with ice-cooled water and dichloroethane. After phase separation, the absorbance of the organic layer was measured at 365 nm for quantification.

Total phenolics and antioxidant activity

The content of total phenolic compounds was estimated using Folin-Ciocalteu assay as described by Singleton & Rossi (1965). The results were expressed in milligram equivalents of gallic acid per gram of dry matter (EGA.g^{-1} DW). IC50 was calculated from the graph plotting inhibition percentages against extract concentrations. Trolox and gallic acid were used as positive controls (Toul *et al.,* 2022).

HPLC

A validated HPLC method utilizing a Waters 2695 Alliance HPLC system (Waters Inc., Milford, CT, USA), equipped with a UV-Vis DAD. The separation was performed on a C18 reverse-phase column (4.6 × 250 mm, 5 µm). Gradient elution consisted of 2.5 % acetic acid in acetonitrile (solvent A) and water (solvent B), delivered at 1.2 mL.min^{-1}. Detection was performed with a diode array covering 270–320 nm. The gradient profile was as follows: 0-3 min (10 % A), 3-10 min (10-25 % A), 15-20 min (25-40 % A), 20-25 min (40-50 % A), 25-28 min (50-10 % A), 28-30 min (10 % A) (Zhao *et al*., 2015).

The validation method was assessed in accordance with recent multi-analyte HPLC standards, focusing on linearity, recovery, precision, and selectivity (Walfish, 2006).

Statistical analysis

The study used a two-group field experimental design comparing irrigated and non-irrigated trees. All data were analyzed using *IBM SPSS Statistics*, 2020 (Version 27.0), with results presented as mean ± SD from three independent replicates. Differences among treatment groups were evaluated using one-way ANOVA followed by Tukey's HSD post hoc test ($\alpha = 0.05$). Pearson correlation coefficients were calculated to investigate relationships among phenolic compounds, biochemical markers, and antioxidant activity ($p < 0.05$ considered significant). HPLC method validation included linearity assessment ($R^2 > 0.998$), LOD/LOQ determination, recovery percentages, and % RSD repeatability values.

Results and discussion

Table 1 reports the quantitative changes in water content, proline, glycine betaine, total phenolics, and antioxidant capacity in leaf extracts as a function of irrigation treatment.

Table 1: Biochemical Markers and Antioxidant Activity.

Parameter	Units	Control (T0)	Non-irrigated	Irrigated
Water content	% FW	75.2 ± 2.1^{a}	74.8 ± 2.3^{a}	84.5 ± 1.8^{b}
Proline	µmol.g^{-1} FW	350.0 ± 15.0^{a}	367.5 ± 15.8^{a}	262.5 ± 11.2^{b}
Glycine betaine	µmol.g^{-1} FW	40.0 ± 2.0^{a}	40.8 ± 2.0^{a}	31.2 ± 1.6^{b}
Total phenolics	mg GAE.g^{-1} DW	65.0 ± 3.0^{a}	67.0 ± 3.1^{a}	45.5 ± 2.1^{b}
DPPH IC50	µg.mL^{-1}	15.0 ± 0.8^{a}	13.3 ± 0.1^{a}	18.8 ± 1.0^{b}
Trolox IC50	µg.mL^{-1}	6.1 ± 0.1^{a}		
Gallic acid IC50	µg.mL^{-1}	2.3 ± 0.1^{a}		

Notes: Superscript letters indicate statistically significant differences (ANOVA Tukey HSD, $p < 0.05$). FW = fresh weight; DW = dry weight.

Non-irrigated *Pistacia atlantica* trees maintained stable physiological profiles over 90 days, with consistent low water content (75 % FW), high osmolyte accumulation (proline, glycine betaine), and elevated phenolic content and antioxidant capacity ($p > 0.05$ vs. T0). Irrigation significantly increased hydration (+12 %), reduced osmolytes (-25 %), phenolics (-30 %), and weakened antioxidant capacity (IC50: +25 %, $p < 0.05$).

The parallel behavior of proline, glycine betaine, total phenolics, and antioxidant activity clearly indicates a tightly integrated drought-defense system. Under non-irrigated conditions, proline and glycine betaine remain high and statistically unchanged between the initial and 90-day samplings, while total phenolic content and DPPH IC50 also show no significant variation, indicating that trees maintain a stable, drought-adapted biochemical status over time. When irrigation is restored for 90 days, leaf water content increases and this is accompanied by a coordinated quantitative decrease of approximately one quarter for proline, around one fifth for glycine betaine, and roughly 30 % for total phenolics, together with an increase of about 25 % in DPPH IC50. This coupling shows that osmolyte accumulation is not an isolated response: high proline and glycine betaine levels are associated with high phenolic content and strong antioxidant potential, whereas their decline signals a relaxation of the overall defense network once water becomes non-limiting.

Table 2 shows that all phenolic compounds remain statistically unchanged between the two non-irrigated groups ("a"; $p > 0.05$), indicating a stable phenolic profile under sustained drought. In contrast, irrigation for 90 days significantly reduces the concentration of most phenolics ("b"; oneway ANOVA, Tukey's HSD, $p < 0.05$), with percentage decreases ranging from about -8 % (gallic acid) to around -27 % for the most drought-responsive markers (chlorogenic, ferulic, *p*coumaric acids, and quercetin). Intermediate reductions (-15 to -20 %) are observed for caffeic acid, rutin, kaempferol, naringenin, syringic, and ellagic acids.

Table 2. Phenolic compounds concentrations (mg.g^{-1} DW).

Phenolic Compound	RT (min)	Control (T0)	Nonirrigated (90 days)	Irrigated (90 days)
Gallic acid	4.2	2.45 ± 0.08^{a}	2.48 ± 0.09^{a}	2.26 ± 0.08^{b}
Chlorogenic acid	6.5	1.88 ± 0.07^{a}	1.92 ± 0.08^{a}	1.38 ± 0.06^{b}
Caffeic acid	7.8	0.85 ± 0.04^{a}	0.83 ± 0.04^{a}	0.68 ± 0.03^{b}
Syringic acid	9.2	0.70 ± 0.03^{a}	0.72 ± 0.03^{a}	0.61 ± 0.02^{b}
p-Coumaric acid	11.3	0.60 ± 0.03^{a}	0.61 ± 0.03^{a}	0.45 ± 0.02^{b}
Rutin	14.1	0.92 ± 0.05^{a}	0.95 ± 0.05^{a}	0.75 ± 0.03^{b}
Quercetin	16.8	3.22 ± 0.10^{a}	3.28 ± 0.11^{a}	2.43 ± 0.09^{b}
Kaempferol	19.3	0.55 ± 0.03^{a}	0.57 ± 0.03^{a}	0.44 ± 0.02^{b}
Naringenin	21.5	0.40 ± 0.02^{a}	0.39 ± 0.02^{a}	0.33 ± 0.01^{b}
Ferulic acid	23.7	0.72 ± 0.03^{a}	0.74 ± 0.03^{a}	0.53 ± 0.02^{b}
Ellagic acid	26.1	0.37 ± 0.02^{a}	0.38 ± 0.02^{a}	0.31 ± 0.01^{b}

Notes: Superscript letters indicate statistically significant differences (ANOVA Tukey HSD, $p < 0.05$).

Figure 4 presents the Pearson correlation matrix between proline, glycine betaine, total phenolics, and individual phenolic compounds in *Pistacia atlantica* leaves. The heatmap shows strong positive correlations (r up to ~0.98) among osmolytes, total phenolics, and all measured phenolic compounds, indicating coordinated accumulation of biochemical markers under drought stress.

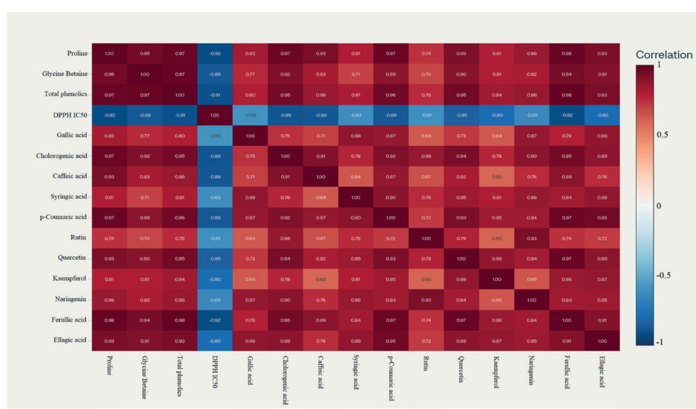

Figure 4. Pearson correlation heatmap of osmolytes, phenolic compounds, and antioxidant activity.

The strong positive correlations among these variables support this interpretation. Proline, glycine betaine, and total phenolics are very tightly correlated ($r \geq 0.94$), and they also show high positive correlations with most individual phenolic compounds, notably quercetin, chlorogenic acid, ferulic acid and *p*-coumaric acid, with correlation coefficients frequently above 0.93. In contrast, DPPH IC50 is inversely related to this entire cluster, so samples with higher osmolytes and phenolics consistently have lower IC50 and thus stronger radical-scavenging efficiency. Quantitatively, the decreases of 20 to 30 % in proline, glycine betaine and total phenolics after irrigation are mirrored by a comparable proportional loss in antioxidant efficiency, indicating that osmotic adjustment and phenolic accumulation contribute together to the antioxidant status of the leaves. A similar co-regulation of osmolytes and phenolics under drought has been described in several crops and woody species: Ashraf & Foolad (2007) reported that proline and glycine betaine accumulation improve osmotic adjustment and protect membranes while also supporting antioxidant systems under abiotic stress. It has been reported that abscisic acid and ROS signalling simultaneously drive proline/glycine betaine biosynthesis and activation of the phenylpropanoid pathway, leading to higher total phenolics and stronger radical scavenging under water deficit, followed by down-regulation upon re-watering (Uzilday *et al.,* 2024)Plant roots exert hydrotropism in response to moisture gradients to avoid drought stress. The regulatory mechanism underlying hydrotropism involves novel regulators such as MIZ1 and GNOM/MIZ2 as well as abscisic acid (ABA.

The HPLC data further clarify which phenolic compounds are most involved in this response. Although all quantified phenolics decrease significantly after irrigation, their sensitivity differs: chlorogenic acid, ferulic acid, p-coumaric acid and quercetin show the largest relative declines, on the order of 24–27 %, while caffeic acid and kaempferol drop by about 20 %, rutin and naringenin by roughly 18–17 %, and syringic, ellagic and gallic acids by about 8–16 %. This gradient suggests that hydroxycinnamic acids and quercetin are the most drought-responsive phenolics in *P. atlantica* leaves. Similar quantitative hierarchies have been reported in other species subject to water deficit: in drought-stressed amaranth, for example, chlorogenic, ferulic and p-coumaric acids and flavonols such as quercetin, rutin and kaempferol increased markedly under stress and declined after re-watering, in parallel with changes in total antioxidant capacity (La *et al.,* 2023). Reviews of phenylpropanoid responses to drought stress likewise highlight these hydroxycinnamates and flavonols as the most plastic and functionally important constituents of the phenolic response (Wani *et al.,* 2024).

This pattern is consistent with the known structure–activity relationships of these molecules. Phenolic acids such as caffeic and chlorogenic acids, with ortho-dihydroxyl substitution, and ferulic acid, with a methoxy and hydroxyl group on a conjugated side chain, are recognized as particularly effective radical scavengers and can stabilize phenoxyl radicals efficiently, while flavonols such as quercetin and rutin, with multiple hydroxyl groups and a conjugated C2=C3 double bond, rank among the most potent natural antioxidants (Kadoma & Fujisawa, 2008; Razzaghi-Asl *et al.,* 2013). By contrast, mono-hydroxylated phenolic acids like p-coumaric or syringic acid, although still active, usually contribute less per mole and rely more on their concentration to impact total antioxidant capacity (Skroza *et al.,* 2022). In this light, the 25 % irrigation-induced decline in chlorogenic, ferulic, p-coumaric acids and quercetin in *P. atlantica* leaves can be expected to have a disproportionate effect on DPPH IC50, consistent with the observed 25 % increase in IC50 in irrigated trees.

Integrating these findings into the broader *Pistacia* literature elucidates the synergistic role of osmolytes and phenolic compounds in mediating drought resistance. Previous research established that *P. atlantica* naturally possesses elevated phenolic and flavonoid concentrations, characterized by low IC50 values (Benmahieddine *et al*., 2023; Toul *et al*., 2017, 2022). Seasonal and regional surveys have further demonstrated that phenolic content and antioxidant capacity in *P. atlantica* leaves are higher in more arid or stressful environments and decline in milder conditions, indicating environmental control over these traits (Bendeddouche *et al*., 2025; Chelghoum *et al*., 2021) we investigate the effect of different abiotic environmental factors on the chemical constituents of Pistacia atlantica Desf and their bioactive potential. Altitude, temperature, precipitation, and harvest season were the key factors. Forty-three samples of P. atlantica, including leaves and galls, were collected from two different bioclimatic areas (Tilghemt and Aflou. These results provide a mechanistic framework demonstrating that water availability, for a given population and phenological stage, independently modulates the coordinated synthesis of osmolytes and individual phenolics. Specifically, under chronic drought, *P. atlantica* maintains an enriched pool of key phenolics (chlorogenic, ferulic, *p*-coumaric acids and quercetin) alongside elevated osmolytes, a state that is quantitatively reversed upon prolonged irrigation, revealing the interconnected roles of osmotic adjustment and antioxidant defense as plastic survival strategies in response to environmental stressors in this species.

Conclusion

Short-term irrigation of drought-acclimated *P. atlantica* trees results in a clear reduction of osmotic and antioxidant defences, with proline and glycine betaine decreasing by about 20–25 %, total phenolics by roughly 30 %, and the main individual phenolic compounds by approximately 8–27 %. The close quantitative association among osmolytes, key hydroxycinnamic acids and flavonols, and antioxidant activity indicates a coordinated defence network that is highly responsive to changes in water availability and supports drought resistance. These results highlight the central contribution of osmolytes and specific phenolic compounds to the resilience and recovery of *P. atlantica* under hyper-arid conditions.

The scope of this study was influenced by the limited number of accessible trees meeting the selection criteria, which may limit the extent to which the conclusions can be extrapolated. Future research should therefore target a study area with larger and more representative tree populations.

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