Ecophysiological response and morphological adjustment of Argania spinosa L. Skeels under contrasting climates: case study of marginal populations

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Abstract
In this paper, we investigated the seasonal physiological performance and morphological adjustment of Argania spinosa growing under contrasting climatic and biogeographic conditions. Two marginal populations were selected in the main distribution area of the species, one at the North-west and the other one at the South-west. Trees from the North showed a Mediterranean pattern in Photosynthetic performances, exhibiting maximal carbon assimilation during spring and minimum in summer. In contrast, trees from the South showed a different pattern with maximum values recorded in winter and minimum in spring. Photochemical efficiency of PSII results evidenced the absence of damage to PSII in both sites, probably due to an efficient energy dissipation processed by carotenoid pigments. We recorded increased LMA values in the South, which improves drought resistance. Increasing stomatal length and decreasing stomatal density were registered during the drought season in both populations. A. Spinosa is sensitive to changes in the length of drought stress at its Southern limit. The scarcity of rainfall leading to persistent drought has limited the distribution of the species to the banks of dry Wadis and depressions, where it finds some water compensations during summer. In the North, the summer drought severely impacted the species carbon assimilation.

Introduction
For the twenty-first century, global climate observations show the existence of a warming trend, as well as higher frequency and magnitude of drought events. Increasing evidence show that climate extremes will be of larger intensity and frequency under expected climate change. Several climate forecast models amplify aridity, making North Africa a climate change hotspot with negative impacts on ecological systems. The challenge in the coming years will be to learn how carbon uptake and growth in species and communities will respond to these changes and how forest management strategies can be improved to alleviate the negative impacts of climate change on forests.

It is well known that vegetation structure, biogeochemical and physiological processes are sensitive to extreme droughts. The knowledge of the ecosystem’s response to periodic drought is essential to understanding their structure and function. Thus plant adaptations to drought in arid environments are essential for their survival. Under water-limiting conditions, leaves play a critical role in the adaptive mechanism, several strategies are adopted to reduce water losses under a water deficit environment.

The Argan [Argania spinosa (L.) Skeels] is a Moroccan endemic species with a tropical origin. Argan woodlands constitute a specific ecosystem of the South-west of Morocco at the edge of the Saharan Desert. This species plays essential ecological functions and indispensable socio-economic roles in arid and semiarid lands. As a multipurpose tree, A. spinosa sustains more than 2.5 million people, significantly alleviating poverty. In recognition of its local economic and ecological value, the Argan forest is protected by various United Nations entities and designated in 1998 as a biosphere reserve by UNESCO. In 2021, the 10 May was proclaimed the International Day of A. spinosa by the General Assembly of the United Nations.

It has been reported that a much larger area than today was covered by dense Argan woodlands, and a boundary contraction of 200 km has occurred at Northern and Southern limits. Although at the South limit of the species, precipitations decrease to less than 100 mm, the tree can grow following temporary watercourses (dry Wadis). The most vulnerable area is at the Southern limit, where A. spinosa ecosystem lost, during the last century, half of its surface area because of biotic and abiotic stress factors. Conditions become less favorable on the margins than the center of the distribution, owing to increased abiotic stress involving a reduction in population densities. Previous studies have presented useful information concerning the species response to drought, but no studies have been done on the performances of the species at its ecological South-western limit, where extreme environmental conditions are current. At its Southern ecological limit, an assessment of the Argan tree’s climate sensitivity is needed to understand and counteract its exposure to the future risks of climate warming. A. spinosa reaches its South-western ecological limit, of its main distribution area, in the Draa Basin. At this site, we assume that A. spinosa populations will probably be more vulnerable to the expected climate extremes than the Northern located populations. Adaptation to the south marginal habitat may imply some specific changes in species traits.

Here, we investigated the ecophysiological and morphological status of two populations growing under contrasting climatic
and biogeographic conditions. One of them is located in the North-western part of the species’ main distribution area. The other one growing in more arid conditions, at the South-western limit of the main distribution area of the species.

We measured several physiological and morphological parameters to assess both populations. This evaluation will allow us to estimate the consequences of climate change on the future performance of marginal *A. spinosa* populations, growing at the species limit of climatic tolerance.

Materials and methods

Study areas

Two experimental sites were chosen, one in the South limit of Argan tree range (29°06’24″N; 10°06’32″W), at Northern Guelmim hereafter South, where the trees are grouped along the banks bordering the ephemeral Oued Das. The climate is arid with mild winters due to the proximity of the Atlantic Ocean. Annual rainfall is particularly low, about 118mm. The other site located South of Smimou village (31°09’10″N; 9°42’10″W) called hereafter North, is characterized by a semi-arid climate with annual rainfall not exceeding 350 mm.

Field sampling and analyses

Ten individuals of each population were randomly marked for the study. Field measurements and sample collection were made on sunny days across two annual cycles from September 2016 to April 2018: summer (September 2016 and July 2017), winter (December 2016 and December 2017), and spring (April 17 and April 2018).

On fully expanded leaves exposed to full sunlight (three leaves per tree. n=30), the following parameters were measured each season: relative water content (RWC), gas exchange (A, g, Ci), maximal photochemical efficiency of photosystem II (Fv/Fm), chlorophyll a+b, chlorophyll a, chlorophyll b and carotenoid contents, free proline content, leaf mass area (LMA), stomatal density and stomatal length. For pigment and proline analysis, samples from each marked plant were kept refrigerated in individual hermetic plastic bags. Once in the laboratory, they were kept immediately at -24°C. For RWC, fresh weight was measured as soon as arriving at the laboratory within the same day.

RWC was determined according to the method described by Slatyer and Shmueli (1967).[^1] 0.5 g of fresh leaves were weighted (FW), then floated in cold distilled water for 24 hours to obtain turgid weight (TW). Then the leaves were oven-dried to give the dry weight (DW). Relative water content was calculated by the following formula:

\[
\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100.
\]

The net photosynthetic rate (A), stomatal conductance (g), and sub-stomatal CO2 concentration (Ci), were monitored by an infrared gas exchange analyzer (LCi-portable, ADC, UK) equipped with a light leaf chamber. The measurements were taken in-situ on three leaves per tree, during a clear sunny day, between 8:00 and 11:00h solar time to avoid midday depression of photosynthesis. The results must be expressed on a projected leaf area basis. Hence, leaves used for gas exchange activity were scanned, and leaf surface was measured using the MSurim Pro software.

Chlorophyll a fluorescence measurements were carried out at midday on three leaves per plant previously dark-adapted for 20 minutes with a clip holder. The pulse-amplitude modulation technique was used via a portable fluorimeter (OSp5+, ADC, UK).[^15] A brief saturating pulse was applied, and F0 (initial minimal fluorescence) and Fm (maximal fluorescence) were recorded. The maximum photochemical efficiency of PSII of dark-adapted leaves was automatically calculated as: \(\text{Fv}/\text{Fm} = (\text{Fm} - \text{F0})/\text{Fm}\).

Photosynthetic pigments were extracted from each sample with 80% acetone and determined spectrophotometrically following Lichtenthaler (1987).[^16] The sample absorbance at 470, 646.8, and 663.2 nm was measured, and total chlorophyll, chlorophyll a and b, and carotenoid contents were computed.

Free proline content in leaves was determined by a spectrophotometer according to the Ninhydrin acid method of Bates et al., (1973).[^17] Proline concentration was estimated using L-proline for the standard curve.

Stomatal density was determined as described by Camargo and Marenco (2011), using leaf imprints.[^18] The number of stomata and guard cell length was measured using an ocular micrometer in a light microscope system, 40x objective (Olympus, UK). Imprints were taken from three fully expanded leaves per tree and three samples per leaf.

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Figure 1 Average maximum (Tmax) and minimum (Tmin) monthly air temperature, monthly rainfall and relative humidity % (RH) during 2016/17/18 annual cycles in the two study sites.
Leaf mass area was determined according to le Maire et al. (2008). One or two leaves were scanned then measured with the “Mesurim Pro” software. Scanned leaves were oven-dried for 24 hours at 100 °C before being weighted. Leaf mass area was calculated as the dry leaf mass to leaf surface area ratio (g m⁻²).

STATISTICA 10 software was used to perform Two-way ANOVAs to assess the effect of season and site (independent variables), on ecophysiological variables (dependent variables). Post-hoc Bonferroni test was used for multiple comparisons of means. The statistical test was considered significant at the P<0.05 level. We contrasted the values of each parameter to detect seasonal differences between sites using Student’s t-tests.

**Results**

Over the study period, annual precipitation in the North was 27.57 mm during the 2016/2017 annual cycle and 100.16 mm during 2017/2018 (Figure 1). In the South, about 15.98 mm during 2016/2017, and 38.78 mm during 2017/2018. Both hydrological cycles were exceptionally dry, the first one being the driest (96% lower than the annual mean in the North, and 86% lower in the South). Summer maximal temperature was respectively 45.54°C and 44.63°C in the North, and 44.56°C and 43.34°C in the South.

For RWC, ANOVA test results show significant differences between sites, seasons, and site x season, respectively, p<0.05, p<0.001, and p<0.001. The population from the North showed a seasonal pattern with maximum values recorded during the wet season and the minimum during summer (Table 1). A similar pattern was recorded in the South, but the decrease in the spring season was more pronounced than in the Northern population. The recorded values reached 69.62±2.9% in April 2018.

The net photosynthesis rate (A_N) showed a significant seasonal pattern in both sites (Figure 2a). In the North, the highest values were recorded during spring 2018 (13.58±1.92 μmol m⁻² s⁻¹), and the lowest during summer 2017 (2.73±1.43 μmol m⁻² s⁻¹). In the South, the highest values were recorded during winter 2017 (9.22±2.88 μmol m⁻² s⁻¹), and the lowest in spring 2017 (4.52±2.14 μmol m⁻² s⁻¹). A similar pattern was recorded for the stomatal conductance rate (g_s) in both sites (Figure 2b). Concerning sub-stomatal CO₂ concentration (Ci), in the South it increased in the summer of both cycles and spring and winter 2017. However, in the North, it increased in winter 2017 (Figure 2c).

Maximum photochemical efficiency (Fv/Fm) shows no significant differences between sites during the study period (Figure 3). The two populations presented Fv/Fm values ranging between 0.7 and 0.77, hence close to the optimum.

In the North, the minimum total chlorophyll content was recorded in September, after the summer stress, and the maximum values were recorded in April (Table 1). There were significant differences in the seasonal pattern of all photosynthetic pigment parameters (p<0.01). The carotenoids to total chlorophyll content ratio (Car/Chl) increased in summer for both populations. Chlorophyll a to chlorophyll b ratio (Chl a/b) and Car/Chl ratio show a significant difference between seasons (p<0.001), but no difference between stations. Maximum values of Chl a/b ratio were registered during winter 2016 in both sites (Table 1).

Concerning the seasonal variation of...
free proline content in leaves (Table 1), the maximum values were recorded during winter in both sites. The ANOVA test shows significant differences between sites (p<0.05), seasons (p<0.01), and site x season (p<0.01).

Trees from the North show the highest values of stomatal length during the annual cycle 2016 and 2017 (Figure 4a), and significant differences were registered between the two populations. However, there were no significant differences between the two sites in December 2017 and April 2018. In both populations, the highest values of stomatal length were recorded during summer. Concerning stomatal density, no significant differences were recorded between sites except in April 2017 (p<0.05). A seasonal pattern was registered with decreasing values in summer and increasing in the favorable period (Figure 4b).

Over the annual two cycles, the lowest LMA values occurred in the trees from the North population (Table 1). In both sites, the highest values were recorded during September 2016. There was a significant difference between sites (p<0.001), seasons (p<0.001), and site x season (p<0.05).

**Discussion**

Throughout the study period, all the studied parameters exhibited a significant seasonal variation in both sites. Indeed, several studies have shown adjustments of many physiological and morphological parameters, in response to climatic fluctuations.

Leaf water relations data provide a valuable indication of the capacity of woody species to maintain gas exchange, water use, and growth during the drought of the summer period. For RWC, the results of the two-way ANOVA analysis showed significant differences between sites, seasons, and site x season (p<0.05, p<0.001, p<0.001), respectively. The Northern population presented high values of RWC during winter and spring, which is common in Mediterranean species. The lowest values were recorded in summer. However, the population from the South exhibited a different pattern with low values of RWC recorded during spring and summer, suggesting that drought conditions begin in the spring.

The photosynthetic performances of *A. spinosa* depend on the season and the station’s geographical situation. In the North, maximum rates of ASn were recorded during spring and minimum values in summer. The main drops in carbon assimilation were due to stomatal closure to avoid water losses. This behavior has been described in most Mediterranean species and is in accordance with previous work on *A. spinosa*. The population from the South exhibited a different pattern. High values of Ci and low values of ASn were recorded during summer 2017. Otherwise, it has been shown that

Table 1. Relative water content RWC, Total chlorophyll content Chl a+b (µg mg⁻¹ FM), chlorophyll a/b ratio Chl a/b, Total carotenoids to total chlorophyll content ratio (Car/Chl), Free proline content (mol g⁻¹ FM), Leaf mass area LMA (g m⁻²) in randomly selected leaves of *A. spinosa* in the North and the South over the 2016-2017-2018 annual cycles. Values represent mean ± SE, n=30. Asterisks indicate significance levels for the comparison between the two sites within each season by student’s t-tests (*P<0.001; *P<0.05).

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<th>Jul 17</th>
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<tr>
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<td>72.67±5.25</td>
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<td>Car/Chl</td>
<td>0.28±0.03</td>
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<tr>
<td>Proline</td>
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<td>LMA</td>
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<td>104±11</td>
<td>106±8</td>
<td>107±17</td>
<td>107±13</td>
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Figure 3. Maximum photochemical efficiency of PSII (Fv/Fm) in randomly selected leaves of *A. spinosa* in the North and the South over the 2016-2017-2018 annual cycles. Values represent mean ± SE, n=30. Asterisks indicate significance levels for the comparison between the two sites within each season by student’s t-tests (*P<0.001; *P<0.05).
increasing Ci indicate that non-stomatal effects mainly cause A<sub>N</sub> reduction. An increase in proline concentration has been registered during winter in both sites. This is in accordance with Díaz Barradas et al. (2010) that associated the increase of proline concentration to the cold temperatures, as the species is described as a thermophile. Furthermore, Hare et al. (1998) reported that proline, acting as an electron acceptor, may assist in counteracting photo-inhibitory damage under adverse conditions, even those that do not cause cellular dehydration. Indeed, in our study, despite the water availability in winter in both sites, cold temperature combined with high radiations may have increased the risk of photo-inhibition, that have been avoided by proline accumulation.

Studying drought-adaptation to arid conditions, stomatal density, and aperture and their regulation are of key interest. Stomata range in size from about 10 to 80 μm in length, depending on the species and the environmental conditions. In our study, both populations exhibited small stomatal size, about 16 to 22 μm in length. Hence, this allows them to regulate opening-closure faster and more finely, enabling

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<td>Stomatal length (μm)</td>
<td>25</td>
<td>20</td>
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<tr>
<td>Stomatal density</td>
<td>10</td>
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Figure 4. Stomatal length (a) and stomatal density (b) in randomly selected leaves of A. spinosa in the North and the South over the 2016-2017-2018 annual cycles. Values represent mean ± SE, n=30. Asterisks indicate significance levels for the comparison between the two sites within each season by student’s t-tests (*P<0.05).
them to respond faster to microclimatic fluctuations during drought, improving leaf water use efficiency. On the other hand, trees from both sites showed seasonal fluctuations, increasing stomatal length, and decreasing stomatal density during the drought season. A lower stomatal density can reduce stomatal conductance and transpiration rate and be an important structural trait of water conservation. A significant negative relationship was found between stomatal length and StoD in both sites (Data not shown). Suggesting a high regulation by changing stomatal size and density induced by drought conditions.

It is well known that water availability plays a primordial role in modifying leaf structure. The highest LMA values of *A. spinosa* were recorded in the South during all the studied periods. Increasing LMA is interpreted by several authors as an adaptation to dryness; this increases the plant’s resistance to physical stresses. Moreover, higher LMA results in higher leaf compactness which improves drought resistance. When the water potential of the leaf is modified, less elastic leaves lose less water. Therefore, these leaves can extract water from drier soil than more mesophytic leaves. The maximum LMA values coincided with the exceptional drought of summer 2016, which was extremely dry in both sites.

**Conclusions**

In conclusion, *A. spinosa* from both sites showed a conservative water use strategy to cope with the drought. A rigorous stomatal control, with the concurrence of other morpho-physiological adaptations, including an effective photoprotection, allowed the species to balance carbon acquisition and water loss by transpiration and minimizing the exposure of its tissues to stress.

Our results show that the species is sensitive to changes in the length of drought stress at its South limit. We registered a limitation in the carbon assimilation rates starting from the spring season. The scarcity of rainfall leading to persistent water deficit from spring to late summer has limited the distribution of the species in these areas. Indeed, the species is confined along the banks of dry wadis and depressions, where it finds some water compensations during summer stress. In the North, the species maintained a high photosynthetic rate during growing period. However, the summer drought severely impacted carbon assimilation rates.

Given the increasing drought imposed by expected global climate change, the distribution of marginal populations of *A. spinosa* may be constricted. Thus, long-term monitoring of more functional traits of the species is needed to evaluate the ability of the species to persist in marginal ecosystems.

**References**


