

# A COMPARISON OF THE PHYSIOLOGICAL RESPONSES OF THREE PINE SPECIES IN DIFFERENT BIOCLIMATIC ZONES IN TUNISIA

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(Received 12<sup>th</sup> Mar 2019; accepted 11<sup>th</sup> Jul 2019)

**Abstract.** Increasing aridity attributed to climate change is the main threat to the diversity and survival of Mediterranean forests including Tunisian pine species. The impact of this climatic condition in three pine species was examined in order to detect their responses to drought and guide their selection. Three Tunisian pine species: *Pinus halepensis*, *Pinus brutia* and *Pinus canariensis* growing in different climatic zones: humid, sub-humid, and semi-arid were studied in two months March and July 2016. Measurements carried out within this study are leaf gas exchange, twig water potential, and soil water content. The results showed a decrease in stomatal conductance and twig water potential in July in all pine species with increase in vapor pressure deficit and low soil water content. *Pinus halepensis*, *P. canariensis* and *P. brutia* had stronger stomatal control in semi-arid, sub-humid and humid climate, respectively (62, 95 and 63 mmol m<sup>-2</sup> s<sup>-1</sup>) and had higher stem water potential; for *P. halepensis* (-2.04 MPa) in semi-arid climate; for *P. canariensis* and *P. brutia* (-2.21, -2.39 MPa) in sub-humid and humid climate, respectively. *Pinus halepensis*, *P. canariensis* and *P. brutia* are better adapted to semi-arid, sub-humid and humid climates, respectively. Nevertheless, *P. halepensis* is considered the most resistant species as that could maintain higher photosynthesis, stomatal control and water use efficiency particularly in semi-arid climate. These findings help to assess the interaction between species and climate on the physiological response of pine species in mid-summer water deficit, and to select among these species the most resistant for future reforestation programs.

**Keywords:** drought, leaf gas exchange, water potential, Mediterranean forest, genetic variability

## Introduction

Mediterranean forest tree species are exposed to a range of pressures (Ramirez-Valiente et al., 2010) and are mainly vulnerable to drought (Allen et al., 2010; Choat et al., 2012). An increase in aridity is particularly expected in the Mediterranean regions and, is the main threat to the diversity and the survival of forest trees (Peñuelas et al., 2017). Pine forests are one of Mediterranean forest species that may be threatened by rising temperatures and reduced precipitations (Manzanera et al., 2016).

To resist to these hydric stress conditions (Rubio-Casal et al., 2010; Letts et al., 2011), *Pinus* species may develop mechanisms of tolerance or avoidance (David et al., 2007; Mittler, 2002). Resistance to high temperature and drought strongly differs among

plant species (Matías et al., 2012) being correlated to transpiration, hydraulic adaptations (Martínez-Vilalta et al., 2009) and water use efficiency (Warren et al., 2001). Plants protect themselves from excessive water losses (diffusion out of the leaf) under water-limited environments through a reduction of stomatal conductance (gs), which in turn leads to less carbon uptake (diffusion of CO<sub>2</sub> into the leaf) and possibly subsequent physiological stress (McDowell et al., 2008; Will et al., 2013), reduction of transpiration (Bréda et al., 1993; Granier et al., 2008), that has been recognized as the main environmental factor limiting plant photosynthesis on the global scale (Nemani et al., 2003).

This situation underlines the need to study leaf gas exchange as indicators of the ecophysiological tolerance of pine forests (Manzanera and Martinez-Chacon, 2007). Thus, pines species maintain rather constant leaf water potential in soils with low water status and/or under high evaporative demand (Martinez-Vilalta et al., 2004). Water use efficiency (WUE) is a critical metric parameter that quantifies the trade-off between photosynthetic carbon assimilation and transpiration at the leaf level (Farquhar et al., 1982). Leaf gas exchange and water potential variables were studied on three Mediterranean pine tree species located in three bioclimatic zones in Tunisia.

Aleppo pine (*Pinus halepensis* Mill.) is an important forest tree in the Mediterranean region (Klein et al., 2011). Its continental range extends from northern Africa and Middle East, up to southern Mediterranean Europe. It is native and the most abundant pine species in Tunisia (You et al., 2016). The majority of Aleppo pine forests are located in central and northwestern Tunisia, mainly in Kasserine, Kairouan, Kef, Seliana and Zaghouan (Ayari et al., 2012).

Calabrian pine (*Pinus brutia* Ten.), native to the eastern Mediterranean, can be found in many southern Mediterranean countries. It is the most pine specie that widely distributed in Turkey (Kucuk et al., 2012). Because of their drought tolerance, they are well adapted to dry summer conditions (Lopéz et al., 2016). In Tunisia, this species was introduced in 1960 in Souiniet, Tebaba, et Jebel Abderrahmane (Khouja et al., 2002).

Canary pine (*Pinus canariensis*) is an endemic species of the Canary Islands (de Nascimento et al., 2009). Despite its small distribution area (western islands, occupying much of La Palma, Tenerife, El Hierro and Gran Canaria, the species grows across a wide climate (from xeric conditions to mixed forest). In Tunisia, this species was introduced since 1965 in Souiniet, Henchir Naam, Jebel Abderrahmane (Khouja, 2001).

The purpose of this study was: (i) to assess drought tolerance of three pine species from the gas exchange data and water potential, (ii) to detect if climate has an effect on the degree of tolerance of species to drought, and (iii) to select the most droughts tolerant pine species. Therefore, our objective was to compare the physiological responses to drought in three pine species, Calabrian pine (*Pinus brutia*), Canary pine (*Pinus canariensis*) and Aleppo pine (*Pinus halepensis*), in different bioclimatic zones of Tunisia.

## Materials and methods

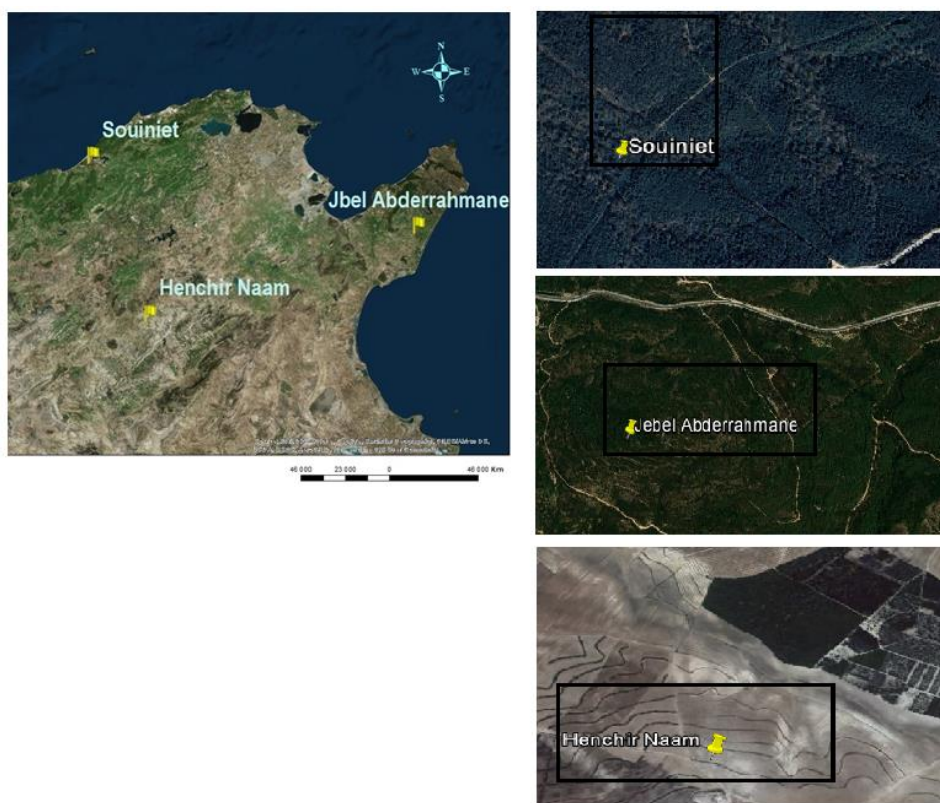
### Study sites

The study was carried out in three different arboretums (*Fig. 1*) on three pine species (*Pinus halepensis*, *P. brutia* and *P. canariensis*). The first, Souiniet “SNT”, is located in northwest Tunisia (*Table 1*). The shrub layer is composed of trees of *Arbutus unedo*,

*Erica scoparia*, *Erica arborea*, *Myrtus communis*, *Phillyrea media*, *Halimium halimofolium*, *Cistus salvifolius* and trees of *Quercus suber*

The second, Jebel Abderrahmane “JAB”, is located in northeast Tunisia. Vegetation consists of Mediterranean maquis with *Quercus coccifera*, *Erica arborea*, *Calycotome intermedia*, *Halimium halimofolium*, *Pistacia lentiscus*, and *Phillyrea media*.

The third arboretum, Henchir Naam “HNM”, is located in northwest Tunisia and is characterized by a semi-arid climate with moderate winters and hot dry summers. Pine trees: *P. halepensis*, *P. brutia* and *P. canariensis* are found in forest mosaics along with other tree species, including *Picris echioides*, *Phalaris truncata*, *Brassica amplexicaulis*, *Euscari comosum* and *Centaurea nicaensis*.

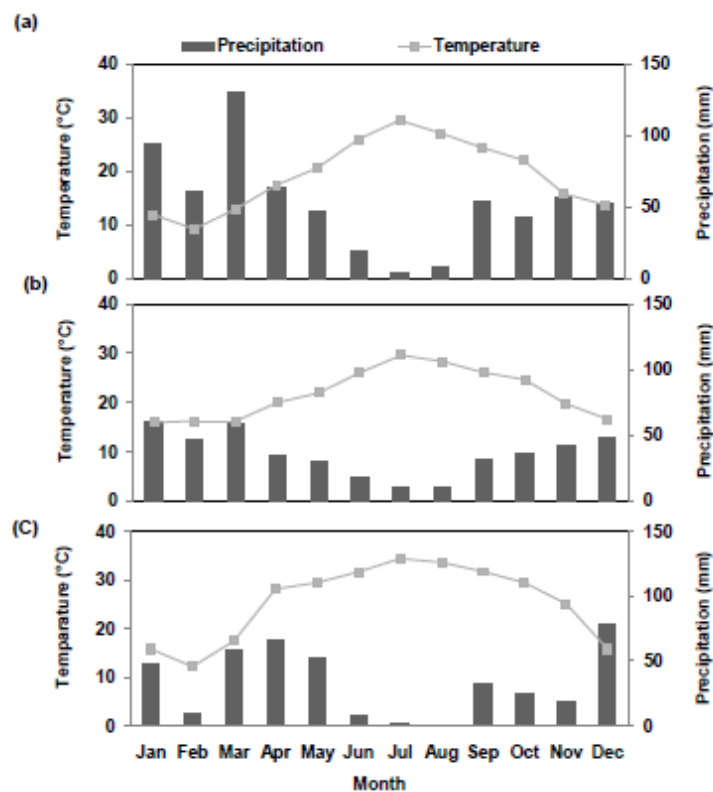


**Figure 1.** Localisation map of three experimental stations Souiniet, Jebel Abderrahmane and Henchir Naam Tunisia forest

**Table 1.** Geographical and climatic characteristics of three sites in which pine species grow

Sites	Climate	Latitude (N)	Longitude (E)	Altitude (m a.s.l)	Mean annual precipitation (mm) (1997-2016)
Souiniet	Humid	36°47'920"	8° 48'495"	492	1749.885
Jebel Abderrahmane	Sub-humid	36°40'086"	10°40'582"	255	611.751
Henchir Naam	Semi-arid	36°13'258"	9°10'374"	450	479.513

Figure 2 shows the monthly distribution of precipitation and temperature in 2016 at the three sites Souiniet, Jbel Abderrahmane and Henchir Naam.



**Figure 2.** Climatograph of study site: (a) Souiniet site (humid climate), (b) Jbel Abderrahmane (sub-humid) and (c) Henchir Naam (semi-arid). The climatograph illustrates the monthly distribution of precipitation (P) and temperature (T) in 2016

### Sampling

Three experiments were carried out in this study: leaf gas exchange, twig water potential, and soil water content on 11th in the month of March and July 2016 in SNT, JAB, and HNM.

Three different trees of each pine species were considered of a high of  $13.5 \pm 2.5$  m and mean diameter at breast height (DBH) of  $16 \pm 0.3$  cm. From each tree, three branches were collected. Thus, a total nine branches of each pine species were taken for leaf gas exchange (Santiago and Mulkey, 2003). The measurements of leaf gas exchange and twig water potential were taken immediately after the collection of branches.

### Leaf gas exchange measurements

Leaf gas exchange were measured using a Licor 6400® (Li-Cor, Lincoln, NE, USA) on two-year old needles leaves. At the top of each tree, three lateral branches 90 cm in length were cut with pruning shears from three different trees of each pine species ( $n = 27$ ) in the morning between 06:00 and 08:00 a.m, and immediately measured in the field. Branches placed in a bucket containing cold water during the measurements to maintain leaf gas exchange at a constant level. Photosynthetic rate of the leaves was measured at the ambient CO<sub>2</sub> concentration (400 ppm) with an open infrared gas analysis system (Li-Cor 6400-40 equipped with a red-blue LED source; Li-Cor Inc., Lincoln, NE, USA). Measurements for attached leaves were operated at 25 °C leaf

temperature and at an airflow rate of 300 cm<sup>3</sup> min<sup>-1</sup>. The vapor pressure deficit was kept at 1.2 ± 0.2 kPa. During the experiment, leaf temperature was maintained at 25 °C, photon flux density at 1500 μmol m<sup>-2</sup> s<sup>-1</sup>, ambient CO<sub>2</sub> molar ratio (Ca) at 400 ppm and humidity of the incoming air were kept at 60%. The variables measured were: leaf stomatal conductance (gs, in mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), net carbon assimilation (An, in μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), and transpiration (Tr, in mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>). The instantaneous water use efficiency (WUE, in μmol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O) was determined according to *Equation 1*:

$$WUE = \frac{An}{Tr} \quad (\text{Eq.1})$$

### ***Twig water potential (TWP)***

Water potential was measured at mid-day between 12:00 and 14:00 on three small twigs (5-7 cm long) from the considered trees using a Scholander pressure chamber (SKPM 1400®, Skye Instruments Ltd., Powys, UK) (Scholander et al., 1965).

### ***Soil water content***

Soil water content (SWC) was monitored using a time domain refractometry (TDR, Trase system I, Soil Moisture Equipment Corp., and USA) for the three sites.

### ***Statistical analysis***

Data analysis were done using Analysis of Variance ANOVA (SAS version 9.0) by GLM procedure and the mean values of species were compared using SNK test (Student-Newman-Keuls) tests at 95% confidence interval (P < 0.05). Pearson correlation coefficient was used to evaluate the correlations between physiological parameters studied.

## **Results**

### ***Gas exchange measurements at the leaf level***

Drier conditions during the summer had a strong effect on gas exchange activity. Leaf gas exchange revealed significant differences in all variables (carbon net assimilation, transpiration and water use efficiency) between species (p < 0.001) and sites (p < 0.001), except for stomatal conductance where there was a significant difference between species (p < 0.05) but no difference between sites (p = 0.145). For any given species, there were differences among sites (p < 0.001) except for stomatal conductance in *Pinus canariensis* (p = 0.812). For any given site, there were differences among species (p < 0.001). The interaction between species x sites was significantly (p < 0.001) (*Table 2*).

At the leaf level, An was lower for all pine species in July compared to March, except for *P. halepensis*, which registered the highest values. Moreover, *P. halepensis* showed a sharp decrease (52%) in An from March to July in semi-arid climate (p < 0.001) than the other two species. For *P. canariensis*, there was a slight difference in reduction of An between humid, sub-humid and semi arid climates (44.07%, 41.77%

and 48.7%). While for *P. brutia*, the reduction of An was (51%, 47.56% and 46.23%) in humid, sub-humid and semi arid climates (Table 2).

Transpiration rate, (Tr) was significantly lower in July than in March ( $p < 0.001$ ) and between sites ( $p = 0.006$ ). *Pinus canariensis*, *P. halepensis* and *P. brutia* showed a sharp decrease from March to July ( $p < 0.001$ ) in sub- humid, humid and in semi-arid climates. It was (77.72%, 77.5% and 82.30%, respectively) (Table 2).

Water use efficiency rate, (WUE) was significantly different between months and species ( $p < 0.001$ ). Higher WUE values were measured in July in semi-arid climate. The overall means were (17.44 and 15.52  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ), in *P. halepensis*, and *P. brutia*, respectively except for *P. canariensis* the highest value was measured in sub-humid climate (15.03  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ).

There was also a difference in water use efficiency between climates for the same species however, in *P. canariensis* the difference was more pronounced between humid and semi-arid (7.71  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ , 8.5  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) and JAB (15.03  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ).

**Table 2.** Net assimilation rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), stomatal conductance ( $\text{mmol m}^{-2} \text{ s}^{-1}$ ), and water use efficiency ( $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) in March and July of *Pinus canariensis*, *Pinus halepensis*, *P. brutia* in Souiniet, Jebel Abderrahmane and Henchir Naam. Mean  $\pm$  SE with distinct letters are significantly different at 5% (SNK test)

Variable	Species	Site/month					
		SNT		JAB		HNM	
		March	July	March	July	March	July
An	PC	5.65 $\pm$ 0.06 <sup>c</sup>	3.16 $\pm$ 0.08 <sup>c</sup>	7.23 $\pm$ 0.01 <sup>b</sup>	4.21 $\pm$ 0.045 <sup>b</sup>	5.96 $\pm$ 0.11 <sup>c</sup>	3.06 $\pm$ 0.22 <sup>c</sup>
	PH	6.24 $\pm$ 0.22 <sup>b</sup>	3.43 $\pm$ 0.05 <sup>b</sup>	7.1 $\pm$ 0.11 <sup>b</sup>	4.12 $\pm$ 0.07 <sup>a</sup>	8.99 $\pm$ 0.06 <sup>a</sup>	4.36 $\pm$ 0.06 <sup>a</sup>
	PB	8.38 $\pm$ 0.02 <sup>a</sup>	4.12 $\pm$ 0.25 <sup>a</sup>	8.2 $\pm$ 0.09 <sup>a</sup>	4.3 $\pm$ 0.44 <sup>a</sup>	6.64 $\pm$ 0.13 <sup>b</sup>	3.57 $\pm$ 0.08 <sup>b</sup>
Tr	PC	1.42 $\pm$ 0.03 <sup>a</sup>	0.41 $\pm$ 0.02 <sup>a</sup>	1.23 $\pm$ 0.12 <sup>b</sup>	0.28 $\pm$ 0.01 <sup>c</sup>	1.53 $\pm$ 0.05 <sup>a</sup>	0.36 $\pm$ 0.03 <sup>a</sup>
	PH	1.2 $\pm$ 0.11 <sup>b</sup>	0.27 $\pm$ 0.01 <sup>c</sup>	1.18 $\pm$ 0.12 <sup>b</sup>	0.33 $\pm$ 0.01 <sup>a</sup>	0.82 $\pm$ 0.01 <sup>c</sup>	0.25 $\pm$ 0.01 <sup>b</sup>
	PB	1.45 $\pm$ 0.04 <sup>a</sup>	0.32 $\pm$ 0.05 <sup>b</sup>	1.4 $\pm$ 0.21 <sup>a</sup>	0.31 $\pm$ 0.01 <sup>b</sup>	1.3 $\pm$ 0.21 <sup>b</sup>	0.23 $\pm$ 0.02 <sup>b</sup>
gs	PC	450 $\pm$ 0.04 <sup>b</sup>	106 $\pm$ 0.00 <sup>a</sup>	440 $\pm$ 0.03 <sup>c</sup>	95 $\pm$ 0.00 <sup>b</sup>	510 $\pm$ 0.02 <sup>b</sup>	116 $\pm$ 0.07 <sup>a</sup>
	PH	410 $\pm$ 0.01 <sup>b</sup>	82 $\pm$ 0.00 <sup>b</sup>	620 $\pm$ 0.04 <sup>b</sup>	96.6 $\pm$ 0.00 <sup>b</sup>	410 $\pm$ 0.01 <sup>c</sup>	62 $\pm$ 0.00 <sup>a</sup>
	PB	550 $\pm$ 0.08 <sup>a</sup>	63 $\pm$ 0.00 <sup>c</sup>	720 $\pm$ 0.06 <sup>a</sup>	102 $\pm$ 0.00 <sup>a</sup>	630 $\pm$ 0.06 <sup>a</sup>	110 $\pm$ 0.04 <sup>a</sup>
WUE	PC	3.97 $\pm$ 0.10 <sup>c</sup>	7.71 $\pm$ 0.5 <sup>b</sup>	5.87 $\pm$ 0.65 <sup>a</sup>	15.03 $\pm$ 0.24 <sup>c</sup>	3.89 $\pm$ 0.14 <sup>c</sup>	8.5 $\pm$ 1.21 <sup>c</sup>
	PH	5.2 $\pm$ 0.57 <sup>b</sup>	12.70 $\pm$ 0.81 <sup>a</sup>	6.02 $\pm$ 0.57 <sup>a</sup>	12.48 $\pm$ 0.49 <sup>a</sup>	11 $\pm$ 0.21 <sup>a</sup>	17.44 $\pm$ 0.69 <sup>a</sup>
	PB	5.91 $\pm$ 0.27 <sup>a</sup>	12.87 $\pm$ 2.87 <sup>a</sup>	5.86 $\pm$ 0.94 <sup>a</sup>	13.87 $\pm$ 1.7 <sup>a</sup>	5.10 $\pm$ 0.81 <sup>b</sup>	15.52 $\pm$ 1.6 <sup>b</sup>

An: Net assimilation rate Tr: transpiration rate gs: stomatal conductance WUE: water use efficiency, Sp: species, PC: *Pinus canariensis*, PH: *P. halepensis* and PB: *P. brutia*

In July, there was a high positive correlation between An and gs:  $r^2$  coefficient. The correlation was more important in *P. halepensis*, *P. brutia*, and *P. canariensis* in semi-arid climate than others climates. The coefficients were ( $r^2 = 0.623$ ,  $r^2 = 0.58$ ,  $r^2 = 0.95$ ), respectively (Table 3).

### Stomatal control and twig water potentials

Stomatal conductance was significantly lower in July than in March ( $p < 0.001$ ) for all pine species. The highest rate of decline of stomatal conductance between the two months was recorded for *P. halepensis*, *P. canariensis* and *P. brutia* in semi-arid, sub-

humid and humid climates, respectively. The rate of loss was (84.87, 78.41 and 88.54%), respectively (Table 2).

Twig water potentials revealed a significant difference between species ( $p < 0.001$ ), sites ( $p < 0.001$ ) and months ( $p < 0.001$ ). For any given species, there were differences among sites ( $p < 0.001$ ) and for any given site; there were differences among species ( $p < 0.001$ ). The interaction between species and sites was significantly ( $p < 0.001$ ).

**Table 3.** Correlation coefficient  $r^2$  of An and gs in July of *P. halepensis*, *P. brutia* and *P. canariensis* in Souiniet, Jebel Abderrahmane and Henchir Naam

Sites	<i>P. halepensis</i>	<i>P. brutia</i>	<i>P. canariensis</i>
Souiniet	0.49	0.46	0.42
Jebel Abderrahmane	0.53	0.56	0.65
Henchir Naam	0.62	0.58	0.95

An: Net assimilation rate, gs: stomatal conductance

The water potential decreases significantly from March to July in all pine species in different climates (sites). The lowest rates of decline of water potential between the two months were 17.91%, 31.40% for *P. halepensis* and *P. canariensis* in semi-arid climate. For *P. brutia* the lowest rate was 47.53% detected in humid climate (Table 4).

**Table 4.** Water potential ( $\Psi$ : MPa) of *P. canariensis*, *P. halepensis*, and *P. brutia* in two months March and July in Souiniet, Jebel Abderrahmane, and Henchir Naam. Mean  $\pm$  SE with distinct letters are significantly different at 5% (SNK test)

Pine species	Souiniet		Jebel Abderrahmane		Henchir Naam	
	March	July	March	July	March	July
PC	-1.54 $\pm$ 0.01 <sup>a</sup>	-2.62 $\pm$ 0.01 <sup>c</sup>	-1.68 $\pm$ 0.01 <sup>a</sup>	-2.21 $\pm$ 0.01 <sup>a</sup>	-2.42 $\pm$ 0.01 <sup>c</sup>	-3.18 $\pm$ 0.01 <sup>c</sup>
PH	-1.7 $\pm$ 0.02 <sup>c</sup>	-2.58 $\pm$ 0.01 <sup>b</sup>	-2.56 $\pm$ 0.01 <sup>c</sup>	-3.47 $\pm$ 0.02 <sup>c</sup>	-1.73 $\pm$ 0.02 <sup>b</sup>	-2.04 $\pm$ 0.00 <sup>a</sup>
PB	-1.62 $\pm$ 0.01 <sup>b</sup>	-2.39 $\pm$ 0.01 <sup>a</sup>	-1.92 $\pm$ 0.00 <sup>b</sup>	-2.99 $\pm$ 0.05 <sup>b</sup>	-1.57 $\pm$ 0.00 <sup>a</sup>	-2.46 $\pm$ 0.01 <sup>b</sup>

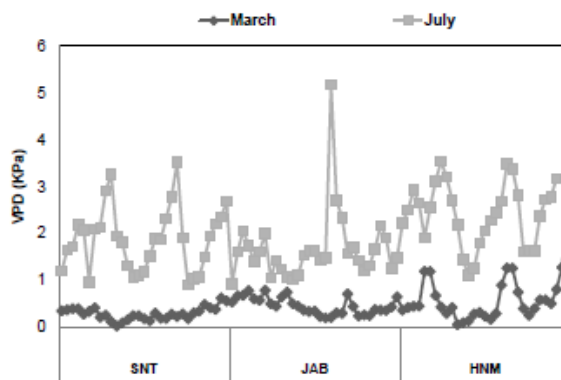
PC: Pinus canariensis, PH: P. halepensis and PB: P. brutia

Stomatal conductance (gs) decreased significantly as VPD increased in July in three sites (Fig. 3). In July and under semi-arid climate with (VPD = 2.4 kPa), the lowest minimum rate in gs was recorded in *Pinus halepensis* (62 mmol m<sup>-2</sup> s<sup>-1</sup>). While in a sub-humid climate and with (VPD = 1.68 kPa), stomatal conductance was lower in *P. canariensis* (95 mmol m<sup>-2</sup> s<sup>-1</sup>). In a humid climate and with (VPD = 1.9 kPa), *P. brutia* species recorded the lowest stomatal conductance value (63 mmol m<sup>-2</sup> s<sup>-1</sup>).

### Soil water content

Soil water content (SWC) showed significant differences between months ( $p < 0.001$ ) and sites ( $p = 0.017$ ). The interaction term was also significant ( $p = 0.02$ ), being higher in March than in July (Table 5). In July, the correlation between soil water content and gs was high and positive for *P. brutia*, *P. canariensis*, and *P. halepensis*  $r^2 = 0.916$ ,  $r^2 = 0.798$  and  $r^2 = 0.931$ , respectively in all sites.

Soil water content was negatively correlated with WUE for all pine species under these climates: for *P. halepensis*, *P. brutia* and *P. canariensis* ( $r = -0.84$ ;  $r = -0.79$ ;  $r = -0.78$ ), in a semi-arid, humid and sub-humid climate, respectively.



**Figure 3.** Vapor pressure deficit in kPa (VPD) in March and July 2016 in SNT (Souiniet), JAB (Jbel Abderrahmane), and HNM (Henchir Naam)

**Table 5.** Soil water content (%) for *P. canariensis*, *P. halepensis*, and *P. brutia* in three sites Souiniet, Jbel Abderrahmane, and Henchir Naam between March and July. Mean  $\pm$  SE with distinct letters are significantly different at 5% (SNK test)

Pine species	Souiniet		Jebel Abderrahmane		Henchir Naam	
	March	July	March	July	March	July
PC	34.63 $\pm$ 0.02 <sup>c</sup>	17.43 $\pm$ 0.01 <sup>b</sup>	47.80 $\pm$ 0.01 <sup>a</sup>	19.23 $\pm$ 0.01 <sup>ab</sup>	35 $\pm$ 0.01 <sup>b</sup>	21.13 $\pm$ 0.01 <sup>a</sup>
PH	58.43 $\pm$ 0.01 <sup>a</sup>	21.93 $\pm$ 0.03 <sup>a</sup>	45.90 $\pm$ 0.02 <sup>ab</sup>	17.72 $\pm$ 0.02 <sup>b</sup>	48.57 $\pm$ 0.01 <sup>b</sup>	19.82 $\pm$ 0.00 <sup>a</sup>
PB	48.43 $\pm$ 0.01 <sup>b</sup>	19.46 $\pm$ 0.01 <sup>b</sup>	44.67 $\pm$ 0.02 <sup>ab</sup>	20.80 $\pm$ 0.05 <sup>a</sup>	43.93 $\pm$ 0.00 <sup>a</sup>	18.60 $\pm$ 0.03 <sup>a</sup>

PC: *P. canariensis*; PH: *P. halepensis*, and PB: *P. brutia*

## Discussion

### *Ecophysiological measurements and stomatal control*

Drought may reduce leaf net carbon assimilation by both stomatal and metabolic limitations (Farquhar and Sharkey, 1982). In addition, the decrease in stomatal conductance is the main factor of photosynthesis inhibition during dehydration as previously reported (Cornic and Fresneau, 2002; Flexas and Medrano, 2002).

In this study, a significant decrease in “ $g_s$ ” and “ $A_n$ ” was found in July in *P. halepensis*, *P. canariensis* and *P. brutia* under different climates compared to results of March. Both parameters ( $g_s$  and  $A_n$ ) plummeted concomitantly as aridity increased. In Spain, Manzanera et al. (2016) found the same results on *Pinus sylvestris*, *Pinus pinea* and *Pinus halepensis*.

In our study, *P. halepensis*, *P. canariensis* and *P. brutia* suggest a higher stomatal control and decreased their water potential in semi-arid, sub-humid and humid climate, respectively to a lesser in the other climates. Also a strong reduction of stomatal conductance under drought conditions slows transpiration allowing plants to keep high water potentials (Table 2).

In France, Lebourgeois et al. (1998) found that pine species were tolerant to drought and a significant decrease of stomatal conductance of *Pinus nigra* was not correlated



with obvious variation in water potential. They usually exhibit drought avoidance strategy with efficient stomatal control of transpiration loss, a decrease of stomatal conductance (up to 30%) and no change in water potential.

In July, our results (*Table 2*) revealed that the net carbon assimilation of *Pinus halepensis* was lower in humid climate ( $3.43 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than sub-humid climate ( $4.12 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). However, Aleppo pine showed a higher assimilation rate than the other pine species in semi-arid climate ( $4.36 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Similarly, in Spain, Salazar-Tortosa et al. (2018) found that net carbon assimilation in *P. halepensis* was ( $3.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in the same season July 2014. It can maintain  $\text{CO}_2$  uptake and photosynthesis by mean lower stomatal control.

The net carbon assimilation of *P. canariensis* was lower in July in semi-arid than humid climate but it maintain the high value in sub-humid climate. While, for *P. brutia* the net carbon assimilation was lower in semi arid climate but it can keep a high net assimilation in humid and sub-humid climates (*Table 2*).

### ***Ecophysiological mechanisms driving WUE enhancement***

In March and July, physiological activity and phenological development of the three pines species were critical. In March water availability in the soil was important and the radiation was not excessive, which leads to having a maximum value of photosynthesis, and the development of shoots and needles began. Whereas, in July water availability in the soil was limited; the radiation was excessive and lead to having minimal photosynthesis. The development of the shoots was complete and that of the needles was in progress.

For all studied pine species, the large fluctuations in WUE between March and July appear to be mainly related to changes in environmental conditions (soil water content, precipitation and temperature). Thus, soil water content was negatively correlated with WUE for all pine species under semi-arid, humid and sub-humid climate. These results revealed the degree of adaptation and resistance of three pine species particularly in these climates under drought conditions. The significant interaction observed between site (climate) and species clarify the behaviour of each pine species: *Pinus canariensis* have better water use efficiency in sub-humid than in humid and semi-arid. *Pinus brutia* have a high water use efficiency in the three sites with pronounced value in semi-arid. However, Aleppo pine showed higher water-use efficiency in different climates, supporting the notion that assimilation was less limited by climatic conditions (*Table 1*). As far as Klein et al. (2012) found that Aleppo pine has the ability to survive and grow in various environments indicates thus is a highly tolerant species. In contrary to previous study realized by Sardans and Peñuelas (2007) and Vila et al. (2008) confirmed that Aleppo pine is expected to suffer from changes in timing and duration of drought, particularly in spring and summer.

### ***The effect of VPD and soil water content on stomatal response of pine species***

Oren et al. (1999) emphasized that high VPD may be one of the signals that lead to stomatal closure. In our study, stomatal conductance decreased in July for all pine species in different climates with the increase of the VPD. Both high VPD and low SWC explained the reductions in stomatal conductance, transpiration and photosynthesis in all pine species under different climates (*Table 2*). The results were also in agreement with those reported in USA by Sulman et al. (2016) that worked on

*Acer saccharum*, *Liriodendron tulipifera*, *Sassafras albidum*, *Quercus alba*, *Quercus velutina*, and *Quercus rubra*. Under drought conditions and with low SWC or high VPD, these 5 plant species close stomata reduce transpiration and photosynthesis.

*P. brutia* was more resistant to drought in two climates humid and semi arid. Awada et al. (2003) showed that *P. brutia* was a drought-resistant species occupying the driest sites around the Mediterranean Basin. Furthermore, *P. canariensis* was resistant to drought in the three climates in Tunisia with less tolerance to humid climate. Jiménez et al. (2005) showed that *P. canariensis* was able to modulate its physiology (with a good stomatal control), depending of the site where it grows with. However, the performance of *P. halepensis* was much higher as much as this species exhibited higher stomatal control, higher photosynthesis and water use efficiency in a dry month (July) and under semi-arid climates. Salazar-Tortosa et al. (2018) showed that Aleppo pine would require less water in the carbon capture process when it is planted with limited water availability. This strategy allowed *P. halepensis* to be one of the more conservative species.

## Conclusion

The findings showed significant differences in leaf water potential and gas exchange measurements among pine species. The responses are depending not only on species but also on the climate in which they grow.

Under dry conditions, *P. halepensis*, *P. canariensis* and *P. brutia* are better adapted to a semi-arid, sub-humid and humid climate, respectively. All pine species avoid drought by reducing stomatal conductance to water through conservation of soil water content at high VPD and thus avoiding future drought. While this strategy would result in a net increase in WUE, it would be at the expense of photosynthesis during periods of high VPD. The findings of these experiments may help to quantify the impact of mid-summer water deficit on Mediterranean pines and evaluate their potential responses to future climate regimes. Monitoring of these three species is required to reveal how ecological restoration is progressing and where management interventions are required.

Therefore, in Tunisia, it is highly recommended to plant *P. halepensis* in semi-arid climate, *P. brutia* was recommended to be planted in humid and semi-arid climates. While, it is recommended to plant *P. canariensis* in sub-humid and semi-arid climates.

**Acknowledgements.** This work was supported and funded by the INCREDIBLE project (funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 774632). Thanks to Mokhtar Baraket for his considerable comments for manuscript and thanks to National Institute of Meteorology of Tunis for providing meteorological data from different regions.

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