



## Performance assessment of three old pear cultivars (*Pyrus communis* L.) to cope drought caused by climate change

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### ABSTRACT

**Purpose:** The evaluation of the response of three old pear cultivars ('Meski Ardeb', 'Bougedma' and 'Alexandrine') to drought was performed through some physiological traits and leaf characteristics. This work aimed to highlight the behavior of these cultivars under water stress conditions. **Research method:** The experiment was carried out in a greenhouse under controlled conditions by the application of three water stress levels as a complete randomized block. Plants showed moderate water deficit after 10 days without watering and severe stress after 20 days without watering. Control plants were kept well watered. **Findings:** Data showed a similar cultivar response to water stress but with various degrees. The relative water content and the water potential of leaves decreased with the water stress severity in all cultivars. Stomatal conductance and photosynthetic assimilation followed the same path with water status parameters, and 'Alexandrine' showed the highest level of gas exchange parameters. Chlorophyll content and total soluble sugars concentration of leaves decreased in response to drought increase. However, the proline content and leaf characteristics were unaffected except for 'Bougedma' cultivar for which leaf area and succulence were reduced under severe stress. **Limitations:** No limitations were founded. **Originality/Value:** the three cultivars behaved differently against drought, these traits can be exploited for further breeding programs to face climate change impact.

## INTRODUCTION

Over the past years, the scenario of climate change predicted an increase in aridity. For Mediterranean type-environments, it has been recorded a decrease in precipitation with an increase in temperature. Vapor pressure deficits and high irradiance levels were also reported to take place (IPCC, 2007; Dai, 2011). As a consequence, water resource shortage is increasingly becoming a limiting factor for further development of fruit production. Therefore, crops are more and more subjected to a continuous and severe water deficit. That is why there is a need for information on the fruit cultivars behavior under drought conditions. In general, plant response to water deficit involves various adaptive mechanisms, leading to maintain several functions. These changes are manifested by the water status regulation through the ion homeostasis and osmotic adjustment. Under water stressed conditions, the stomata closure was the earliest response carried out in order to reduce water loss (Jones, 1973). In general, an important depression in photosynthetic activity associated with changes in water status occurred. The decrease of photosynthetic activity was attributed mainly to stomatal closure, as reducing its conductance was a major mean for controlling water loss from the leaves (Jones, 1992; Cornic, 2000). However, as the water stress continued, restrictions to the photosynthetic machinery were related more directly to metabolic impairments (Angelopoulos et al., 1996; Chartzoulakis et al., 1999; Dbara et al., 2016a). This could be related to the biochemical limitations of photosynthetic activity (Centritto et al., 2003; Aganchich et al., 2009, Evans et al., 2009). Moreover, the osmotic adjustment was well-known as an adaptive mechanism involved to escape stress by the accumulation of osmolytes such as, sugars, betaines and proline (Ben Ahmed et al., 2009; Sofu et al., 2004). Also, changes in pigments occurred to protect cellular compounds and the neutralization of reactive oxygen species took place to repair cellular damage. As a longer time response, plants regulate growth either by the reduced growth rate or by modification of organ anatomy. As has been mentioned, plant sensitivity to drought may be evaluated using different proxies of plant physiological status such as water relations, gas exchange characteristics and biochemical status (Centritto et al., 2003; Bacelar et al., 2007). On another hand, to avoid water deficit, plants showed acclimation ability (Bacelar et al., 2006). The leaf characteristics were changed with water stress such as reducing leaf area (Aganchich et al., 2009), stomata densities and leaf succulence to minimize the effect of abiotic stress (Mantovani, 1998). Plants showed a multiple adaptive response such as morphological, physiological, biochemical and molecular changes to avoid water stress.

Stressful conditions especially those due to lack of water which impacts varied among species and varieties. Therefore, researchers should develop reflections for the adoption of sustainable strategies to save water, especially for fruit trees as the most water-demanding crops. In addition to modifying production techniques, the choice of drought-resistant varieties is essential to overcome these difficulties. A deep knowledge of cultivars behavior face to abiotic stresses is necessary before fruit orchard establishment.

Recently, European pear (*Pyrus communis* L.) planting acreage has increased in Tunisia like many countries. The planting of new varieties is the new strategy adopted by farmers whereas local and old cultivars have been abandoned, and many of them are threatened by extinction. The new plantations limited the number of cultivars in spite of the genetic diversity in this species which is considered more than other pome fruit species (Lane, 1979). Its diversity is very important because the existence of gametophytic self-incompatibility process in flowering, fruiting and seed propagation in the past (Bell & Hough, 1986; Arzani, 2003). Morphological and physiological evaluation of cultivars under drought conditions

provide useful tool to clarify their adaptation to climate change in coming years and gives more information for plant breeders.

The present work aims the study of the morpho-physiological response to drought for three old pear cultivars. In this work, three pear cultivars were submitted to moderate and severe water stress with a morphological and physiological assessment.

## **MATERIALS AND METHODS**

### **Plant material and growth conditions**

For the experiment we used three pear cultivars, two of which are Tunisian (Meski Arteb and Bouguedma) and a foreign one from France (Alexandrine). One-year-old pear plants were grown in plastic pots (capacity of 20 L) under normal daylight conditions in a greenhouse at the Regional Research Centre of Horticulture and Organic Agriculture (CRRHAB) at Chott-Mariem, centre-east Tunisia. Mean average day and night temperature in the greenhouse was respectively, 35 and 19°C, and day and night humidity was respectively 65 and 80%. All cultivars were grafted on quince BA29. Plants were homogenous having the same size.

Before starting the irrigation treatments, plants were transplanted in pots filled with substrate composed of sand and peat in a (1:2) volume ratio. Before two months of the beginning of the experiment, plants were abundantly irrigated with nutritive Hoagland's solution.

### **Treatments and experimental design**

Two drought stress levels were applied and compared to control, in which soil water potential remained constant by keeping plants well watered daily. Drought stressed plants showed moderate water deficit after 10 days without watering and severe stress after 20 days without watering. Treatments were arranged in a complete randomized design with five replications. A total of 45 plants were used (three varieties x three drought levels x five plants per treatments).

### **Measurements**

#### ***Leaf water status***

Leaf water status was measured by the relative water content and the leaf water potential. Samplings were realized at the end of drought period for each stressed treatment that is at 10 and 20 days. Leaf samples were harvested between 9:00 h and 10:00 h in the morning and immediately weighed. Values of leaf relative content (RWC) of fully expanded leaves from all plants taken from the same positions were determined (1) as:

$$\text{RWC} = ((\text{FW}-\text{DW})/(\text{TW}-\text{DW})) \times 100 \quad (1)$$

With FW, DW and TW are, respectively, fresh, dry and turgid weights. TW was obtained by immersing the petiole in distilled water for 24h at 2-4°C in dark. DW was determined after drying leaves at 80°C during 24h (Jones & Turner, 1978).

Midday leaf potential ( $\Psi_{\text{leaf}}$ ) was monitored at midday using a Scholander pressure chamber (PMS Instrument Company, USA). For each treatment, a number of six fully expanded leaves of the same age and position were measured.

### ***Gas exchange***

Leaf gas exchange of the central leaf section was measured using a portable gas exchange system LI-6400-40 (Li-Cor, Inc., Nebraska, and USA). Measurements were performed between 9 am and 11am on fully expanded leaf of at least three measurements for each plant. They were made at a saturating photon flux density (PPFD) of 1400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $[\text{CO}_2]$  of 400 ppm, leaf temperature of 25°C and relative humidity between 45 and 55%. Stomatal conductance (gs), photosynthetic assimilation (A) and transpiration rate (E) expressed respectively in  $\text{mmolm}^{-2}\text{s}^{-1}$ ,  $\mu\text{molm}^{-2}\text{s}^{-1}$  and  $\text{mmolm}^{-2}\text{s}^{-1}$ . Intrinsic water use efficiency (WUEi) and instantaneous water use efficiency (WUEins) were calculated as the ratio of A to gs and A to E, respectively (Dbara et al., 2016a).

### ***Leaf characteristics***

Leaf area ( $\text{mm}^2$ ) was measured using a digital planimeter (CID 203 Leaser). Stomata densities ( $\text{smm}^{-2}$ ) were also determined by preparing nail varnish ‘negatives’ of the abaxial leaf surface. These were placed on glass microscope slides and the number of stomata per unit leaf surface was counted using an Olympus (B07, BH-2, Olympus, Japan) microscope. Values were the mean of twelve samples per treatment.

Leaf succulence ( $\text{gmm}^{-2}$ ) was determined according to the following formula (2):

$$\text{SUC} = (\text{FW}-\text{DW})/\text{LA} \quad (2)$$

With: FW and DW are respectively fresh and dry weights, LA is the leaf area (Mantovani, 1998).

### ***Pigments determination***

Leaves were collected from the pear plants at the same time as the leaf gas exchange measurements. Total chlorophyll content was measured following the Arnon (1949) method. Fresh Leaves (1g) were homogenized in 20 mL 80% acetone (v/v), centrifuged and filtered. The chlorophyll extracted was adjusted to a final volume of 50 mL with 80% acetone. The supernatant was collected for reading with spectrophotometer (Biochrom Libra S32) at 663 and 645 nm, and the concentration was calculated using the following equation (3):

$$\text{Total chlorophyll (mg/100 g FW)} = (20.2 \times \text{OD663}) + (8.02 \times \text{OD645}) \quad (3)$$

### ***Proline determination***

Proline was determined spectrophotometrically following the ninhydrin method of Troll and Lindeslay (1955). 0.2g of fresh leaf sample was homogenized in 5ml 40% of methanol (w/v) and placed in water bath (Memmert) at 100°C during 30 min in glass tubes. A 1 mL aliquot of the supernatant was mixed with 2ml acidic acid, 2 mL of the reagent mixture (120 mL distilled water, 300 mL acetic acid and 80 mL orthophosphoric acid), 1 mL ninhydrin solution (25mg/ml) and incubated again in 100°C water bath for 1 h. After cooling, the mixture, 4 mL of toluene were added and mixed using vortex. The toluene fraction was collected for reading using a spectrophotometer (Biochrom Libra S32) at 520 nm, and toluene was a blank. Proline concentration was determined using calibration curve as  $\mu\text{mol proline g}^{-1} \text{FW}$ .

### ***Total sugars determination***

Total sugars were quantified following the phenol-sulfuric acid method (Robyt & White, 1987). For that, 0.4 g of fresh leaves was homogenized in 5ml 80% of methanol (w/v) and

placed in water bath (Memmert) at 70°C during 30 min in glass tubes. Then, 1 ml of the extract was mixed with 1 ml of phenol (5%) and 5 ml sulfuric acid. The mixture was cooled and the absorbance was read using a spectrophotometer (Biochrom Libra S32) at 640 nm. Total sugars concentration was determined using calibration curve made with different concentration of glucose (from 0.05 to 0.3 mg/ml).

### **Statistical analysis**

All data were subjected to a two-way analysis of variance (ANOVA) to test the effect of watering regime and cultivar using R 3.5.1 for Windows. Means were compared using the Duncan's multiple range tests at the 5% significance level.

## **RESULTS AND DISCUSSION**

### **Leaf water status**

Relative water content (RWC) of pear leaves was affected by water regimes (Fig. 1). After 10 days of water stress, the three showed a reduced RWC, without significant differences as compared to the control. Later, in more stressed conditions (severe stress) a large decrease was recorded for three cultivars. Differences were statistically significant comparatively to control and non-significant between cultivars. In control plants for all cultivars RWC was maintained above 90% however, it was reduced to about 60%. This parameter was suggested as a sound index of water status in tissue culture plants (Diaz-Perez et al., 1994). It was strongly affected by water deficit for different species such as apple (Bolat et al., 2014). By the contrary, olive leaves showed a small non-significant effect following different water regimes (wahbi et al., 2005). In overall the RWC is negatively correlated with the increase of water deficit and cultivars showed the same behavior.

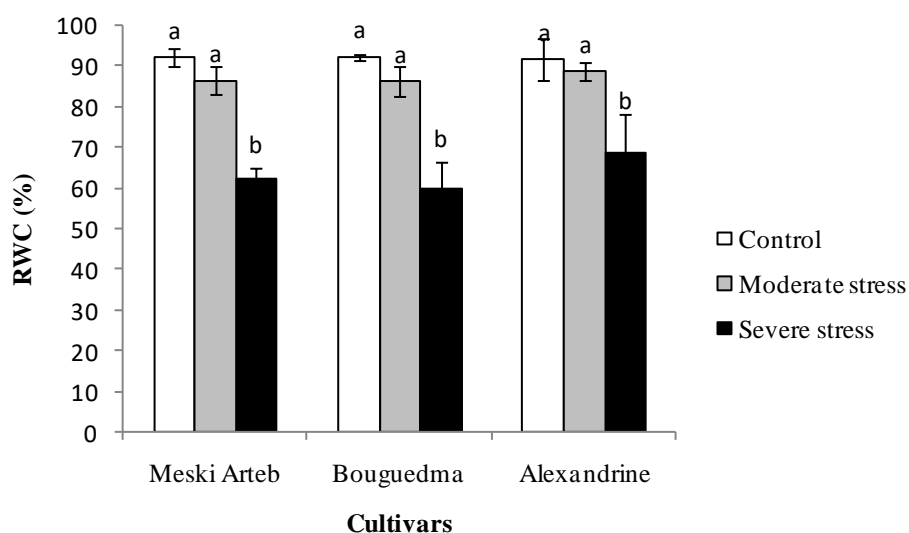
Leaf water potential ( $\psi$ ) measurements revealed differences between treatments and cultivars (Fig. 2). In fact, at moderate stress 'Meski Arteb' and 'Alexandrine' maintained the same level of  $\psi$  compared to control whereas the 'Bouguedma' cultivar showed a small insignificant decrease. Values varied between -2.5 MPa and -1.25 MPa. By continuing the water restriction for 20 days all cultivars showed a low values especially 'Bouguedma' and 'Meski Arteb'. Only 'Alexandrine' even under more water deficit maintained constant leaf water potential. Results are partially in accordance with previous which affirmed that in condition of water stress leaves tended to reduce leaf water potential (Marsal et al., 2008). Also, Bueckert (2013) affirmed that all water potential values become increasingly negative when a plant becomes stressed. Thus, the degree of reduction of RWC and  $\psi$  was correlated to cultivars and to the severity of stress. In this experiment, 'Bouguedma' cultivar trends to decrease these parameters even though at moderate conditions. 'Meski Arteb' cultivar was affected only at severe stress. 'Alexandrine' showed a slight change in water status only at level of RWC.

In general, drought led to the reduction of both RWC and  $\psi$ . According to previous investigations, it was affirmed that RWC was a more significant indicator of plant stress than leaf water potential (Cornic & Massacci, 1996; Ben Rouina et al., 2007).

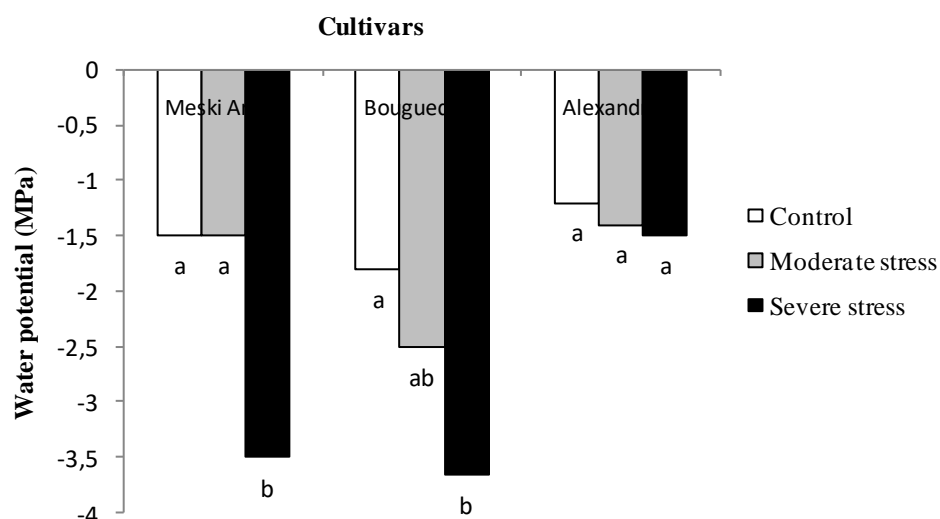
### **Leaf gas exchange**

Recorded results showed that the stomatal control appeared the first line of defense against water stress. Even under moderate water stress, all cultivars decreased the stomatal conductance (gs) of at least of 30% (Table 1). By continuing the water deficit, the stomatal conductance decreased as well. 'Alexandrine' reached a highest level under severe water

stress. Likewise, it was largely affirmed that the stomatal control was the first response of gas exchange in water stress conditions (Jones, 1992). For different species and at different level of water deficit, stomata tended to close. For pear and apple species it was shown that  $g_s$  decreased when water potential was suddenly reduced (Marsal et al., 2000, Jackson, 2003). Also, olive plants submitted to water stress under controlled conditions presented lower  $g_s$  comparatively to control (Dbara et al., 2016 a). The comparison of four olive cultivars  $g_s$  reduction showed significant differences (Petridis et al., 2012).



**Fig. 1.** Relative leaf water content (RWC) at different levels of water stress for Meski Arteb, Bouguedma and Alexandrine cultivars. Vertical bars represent standard error of the mean of 3 replications. For each cultivar, different letters indicate significant differences at  $p \leq 0.05$ .



**Fig. 2.** Midday leaf water potential ( $\psi$ ) at different levels of water stress for Meski Arteb, Bouguedma and Alexandrine cultivars. Different letters within cultivars are significantly different at  $p \leq 0.05$ .

As  $g_s$ , the photosynthetic assimilation (A) showed significant effect on water stress (Table.1). In fact, values of A decreased with moderate and severe water stress for all cultivars. At more stressing conditions, A was reduced reaching values less than  $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ . However, the Alexandrine cultivar presented the highest value compared to others. This result confirmed those of Marsal et al. (2000) which indicated that drought limited the  $g_s$  and A of pear trees. Some previous studies illustrated the same response where it was affirmed that the carbon assimilation of olive tree under water stress was reduced but the degree of reduction differ with cultivars (Pertidis et al., 2012). Also it was reported that  $g_s$  was more affected by water regime than A. In another way, Centritto et al. (2003) found that the patterns of leaf photosynthesis were similar to those of  $g_s$ . Although these values of A and  $g_s$  were rather low, irrespective of water supply.

So, all cultivars decreased their gas exchange under water stress and Alexandrine slightly showed a small difference as compared to the two other cultivars. By studying the characteristics of the pear cultivars, Dong et al. (2015) indicated that the photosynthetic rate had significant exponential correlation with the intercellular  $\text{CO}_2$  concentration, transpiration rate, and stomatal conductance. Also, the photosynthetic rate was mainly affected by stomatal limitation and some selected cultivars were classified as high photosynthetic genetic resources. Stomatal closure is the first plant response to water limitation that influenced gas exchange process. It limited the photosynthesis without other changes in the energetic of photosynthesis process (Flexas & Medrano, 2002). Furthermore, it has been reported that there are both stomatal and non-stomatal limitations to photosynthesis but a hydraulic limitation exists within the stem to leaf pathway (Smit et al., 2020). As it has been affirmed by Abboud et al. (2021) generally the difference between cultivar responses can be largely attributed to stomatal behaviour. A coordinated adjustment in stomatal responses may represent an adaptive advantage in conditions of water deficit.

Moreover, the calculation of  $\text{WUE}_i$  and  $\text{WUE}_{ins}$  showed differences between treatments and cultivars (Table.1). The  $\text{WUE}_i$  increased in stressed plants with no significant difference between moderate and severe stress. However, the  $\text{WUE}_{ins}$  was high in moderate stress in all cultivars. On the other hand, it has been shown that WUE was 1.5 – 2 times that of more arid regions (Fong et al., 2020).

In addition, the establishment of correlation between RWC and in different cultivars, results presented differences (Figure.3). In fact, Alexandrine and Bouguedma cultivars showed a high correlation (0.86 and 0.89 respectively) compared to Meski Ardeb (0.64). The significant relationship between A and RWC that previously found illustrating the decrease of A resulted from water scarcity. Decreasing RWC induced a decrease. Lawlor (2002) affirmed that at a small value of RWC,  $g_s$  reach a minimum but A may continue to decrease. Also, Boussadia et al. (2008) found a relationship between A and RWC for two olive cultivars but with different  $R^2$ .

### **Leaf characteristics**

The determination of leaf area, stomata density and leaf succulence for different cultivars under various water stress level showed some differences (Table 2). In fact, water stress slightly reduced the leaf area of ‘Meski Ardeb’ and ‘Alexandrine’ cultivars, especially with severe stress. These two cultivars were characterized by an important leaf area comparatively to ‘Bouguedma’ which had small leaves, and was affected differently. Results were in accordance with previous observations which illustrated the effect of water stress on the reduction of leaf area for various species such as olive (Aganchich et al., 2009) and strawberry (Caulet et al., 2014). Morphological adaptations in response to water stress

involved reduction of leaf area for minimization of water loss (Singer et al., 2003). The smaller leaf area of plants cultivated under drought conditions was considered a drought avoidance mechanism, which leads to a better control of water loss by transpiration (Aranda et al., 2005).

In general, it was affirmed that plant growth directly depended on cell volume and thus on cell turgor pressure (Bueckert, 2013). Consequently, when subject to drought, their growth was inhibited (Jackson, 2003). Also it was noted that drought can reduce plant productivity by inhibiting growth and photosynthesis (Taiz & Zieger, 1998).

Concerning the stomata density, it was noted that the abundance of stomata was similar for all treatments and cultivars. This characteristic was more related to the species and sometimes to cultivars (Dbara et al., 2016a). Nevertheless, some papers indicated that stomatal characteristics such as frequency and size were strongly affected by the species but occasionally by environmental factors (Dong & Zhang, 2000; Munir et al., 2011). For pistachio, drought tolerant cultivars had lower SD compared to sensitive cultivars (Sun et al., 2013, Esmailpour et al., 2016).

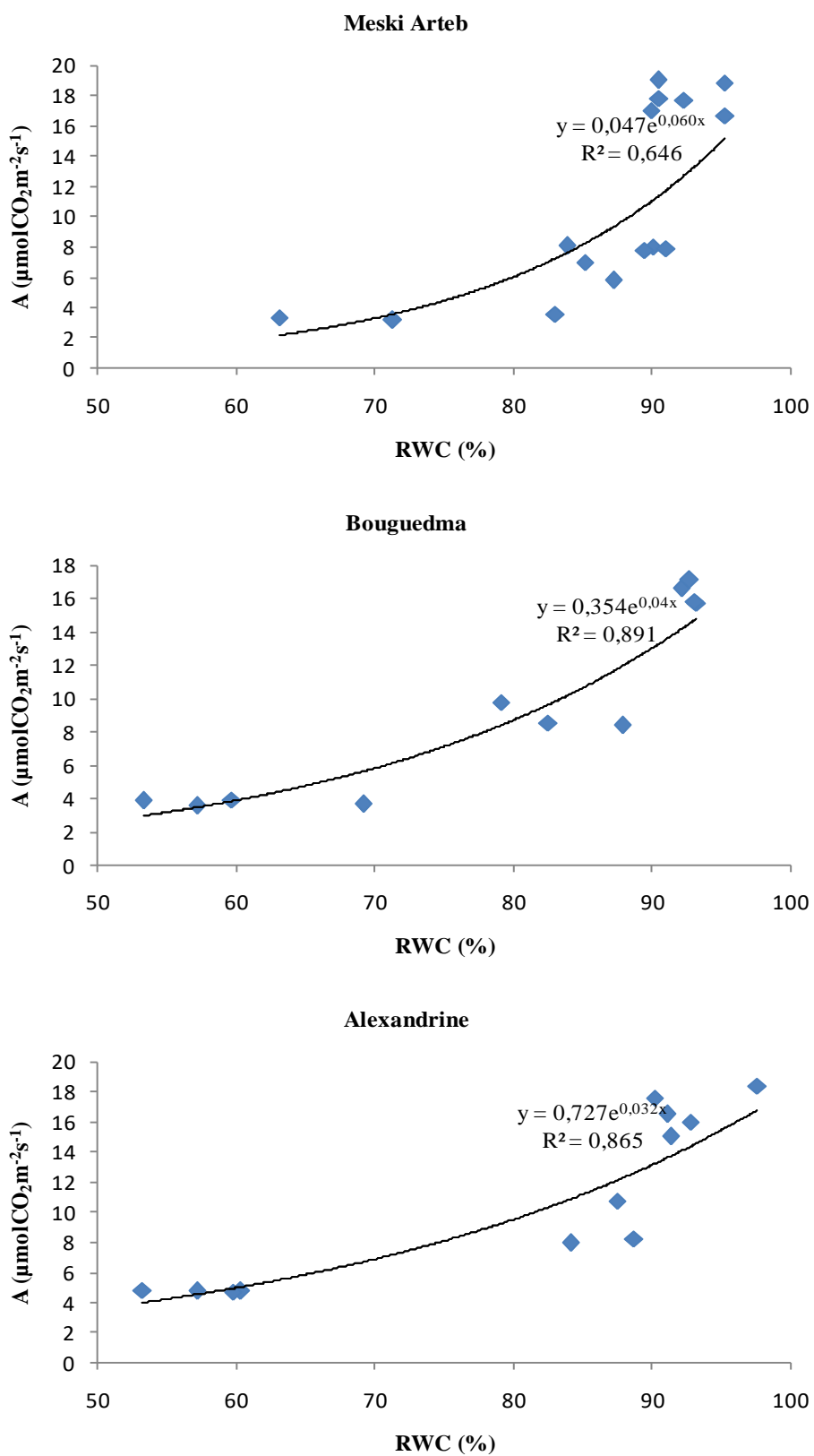
The estimation of leaf succulence proved that it decreased with the severity of water stress for all cultivars. Differences were highly significant particularly under severe stress. Likewise previous studies on olive trees submitted to drought, SUC was greatly reduced (Roussos et al., 2010). SUC of leaves was significantly higher in control due mainly to the supply of sufficient quantity of water sustaining leaf tissue turgor.

**Table 1.** Stomatal conductance (gs), photosynthetic assimilation (A), Transpiration (E), intrinsic water use efficiency (WUEi) and instantaneous water use efficiency (WUEins) measured at different levels of water stress (control, moderate and severe stress) for Meski Ardeb, Bouguedma and Alexandrine cultivars

Cultivars	Treatments	Stomatal conductance (gs)	Photosynthesis (A)	Transpiration (E)	WUEi ( $\mu\text{molmol}^{-1}$ )	WUEins
Meski Ardeb	Control	0.32±0.04a	18.12±0.94 a	5.28±0.07 a	55.89b	3.42 b
	Moderate stress	0.07±0.005b	7.92±0.11 b	1.63±0.25 b	105.99ab	5.89a
	Severe stress	0.02±0.003c	3.34±0.22 c	1.34±0.24 b	139.63a	2.04 b
Bouguedma	Control	0.28±0.002 a	16.30±0.66 a	5.97±0.64 a	57.44 b	2.72 b
	Moderate stress	0.10±0.018 b	8.90±0.76 b	4.18±0.95a b	84.05 a	4.42 a
	Severe stress	0.04±0.00 c	3.60±0.26 c	2.01±0.03 b	89.60 a	0.86c
Alexandrine	Control	0.32±0.011 a	16.56±1.23 a	6.28±0.12 a	51.42 b	2.63 b
	Moderate stress	0.11±0.004 b	9.16±0.004 b	2.94±0.08ab	83.33 a	3.04 a
	Severe stress	0.06±0.005 c	4.76±0.10 c	3.01±0.24 b	72.34 a	1.61 c
ANOVA	C	<0.001	<0.001	<0.001	<0.001	<0.001
	T	<0.001	<0.001	<0.001	<0.001	<0.001
	C×T	*	*	*	*	*
		<0.001	<0.001	<0.001	<0.001	<0.001

Values are means ± SE of three replicates. Means followed by different letters within a column indicate significant differences between treatments for each cultivar separately at p<0.05, based on Duncan's means test.





**Fig. 3.** The relationship between A and RWC for Meski Arteb, Bouguedma and Alexandrine. Regression lines are fitted to the data.

**Table 2.** Leaf characteristics of different pear cultivars under various water regimes

Cultivar	Treatment	LA (cm <sup>2</sup> )	SD (s/mm <sup>2</sup> )	SUC (g/cm <sup>2</sup> )
Meski Arteb	Control	21.84 ±3.06a	170±18.87a	0.014 ±0.002a
	Moderate stress	20.50 ±3.24a	158.33±3.81a	0.014 ±0.002ab
	Severe stress	15.31 ±1.30b	184.16±17.01a	0.012 ±0.001b
Bouguedma	Control	14.79 ±2.87a	154.16±3.81a	0.020 ±0.008a
	Moderate stress	13.10 ±3.83a	119.16±7.21a	0.015 ±0.004b
	Severe stress	13.90 ±2.19a	110.83±5.20a	0.014 ±0.001b
Alexandrine	Control	18.14 ±4.93a	152.5±14.14a	0.017 ±0.003a
	Moderate stress	11.92 ±1.60b	136.66±18.08a	0.016 ±0.003ab
	Severe stress	11.87 ±2.92b	187.5±11.45a	0.014 ±0.003b

Leaf area (LA), Stomata density (SD) and succulence (SUC). Values represent mean ± Standard error (n=9). Means should be compared vertically. Those followed by different letters indicate significant differences (P < 0.05) using a one-way ANOVA with LSD post-hoc test.

### Pigments and Biochemical status

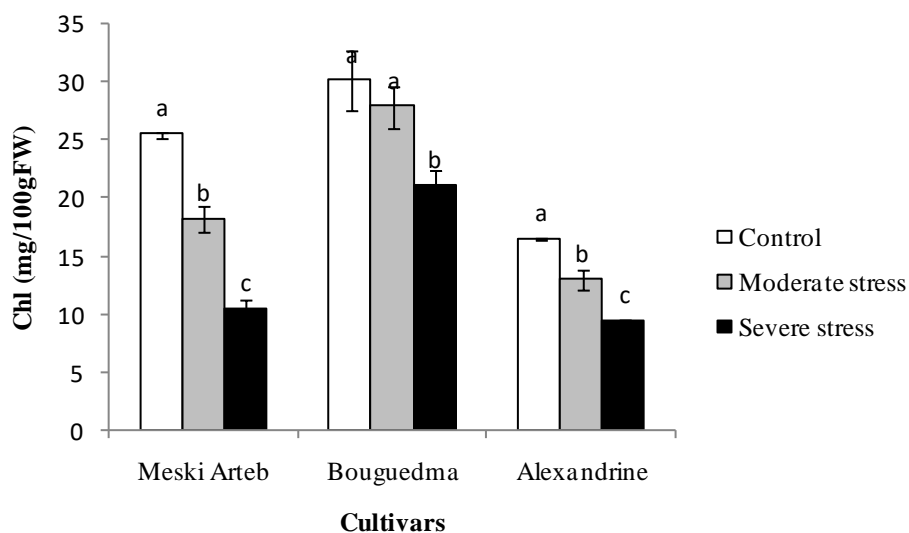
Total chlorophyll content decreased with water stress (Fig. 4). The most concentrated leaf was observed in 'Bouguedma' cultivar. Results were in accordance with others which affirmed that drought reduced chlorophyll content of leaves (Gholami et al., 2012; Bolat et al., 2014, Jin et al., 2015). Also, it was noted that drought induced a significant reduction of Chlorophyll a, Chlorophyll b and total Chlorophyll in different strawberry cultivars (Caulet et al., 2014). Egert and Tevini (2002) indicated that the decrease in the chlorophyll or protein concentration would be a typical symptom of oxidative stress. Comparing the chlorophyll concentration of different cultivars, it was noted significant differences under the same conditions of water stress (Gholami et al., 2012) as shown in our results. The highest values were observed in 'Bouguedma' followed by 'Meski Arteb' then 'Alexandrine'.

Proline concentration in leaves was unaffected by water regimes for all studied cultivars (Fig. 5). Values ranged around 0.085 µg/g FM for the three cultivars under different levels of water stress. Proline content of leaves stayed constant despite it was indicated in many studies of the effect of water stress that it increased with the level of stress (Zahran & Razia, 2009; Jin et al., 2015; Dbara et al., 2016a). Our results were in accordance with Bolat et al. (2014) which affirmed that proline contents of apple leaves increased with the impact of stress but this was not statistically significant. Also, it was affirmed that the accumulation of proline in leaves started at a severe water stress where  $\psi$  decreased from -0.8 to -1.7 MPa (Bueckert, 2013). In a previous study, it was proved that the water stress applied on pear orchard increased the proline content of leaves only for 'Jules Guyot' cultivar but it was unaffected for 'Meski Arteb' (Dbara et al., 2016b).

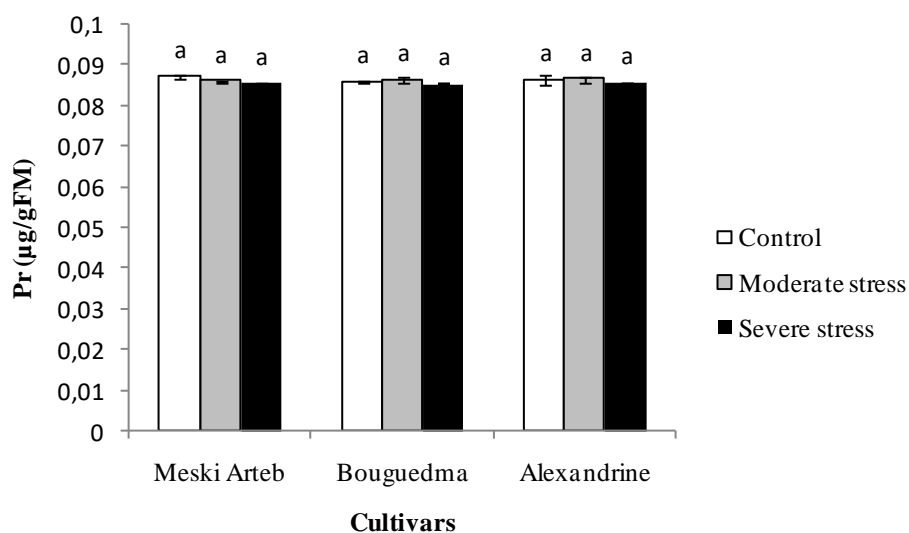
The determination of soluble sugars in leaves demonstrated important levels for three cultivars under severe water stress (Fig. 6). This result illustrated the osmotic adjustment involved for escaping the water stress. In general, many plants adapted to a steadily increasing drought conditions by increasing the solute concentration in the cell cytosol. Sugars, organic acids, sugar alcohols, and free amino acids were all solutes that come from suspended carbon and protein metabolism, and ions (K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>) were pumped from the vacuole aiming turgor maintaining (Bueckert, 2013). According to this author, the sugar accumulation occurred at very low water potential (-1.0 to -1.8 MPa), after or at the same time with proline accumulation. Also, Abboud et al. (2021) presented that metabolic changes, including the accumulation of soluble sugars and proline have been also triggered by water stress.

In drought resistance the interaction between rootstock and cultivar has been previously studied (Tatari et al., 2019). In fact, in this research all the cultivars are grafted onto BA29, the defined behavior of which was essentially linked to the variety. Moreover, it has been

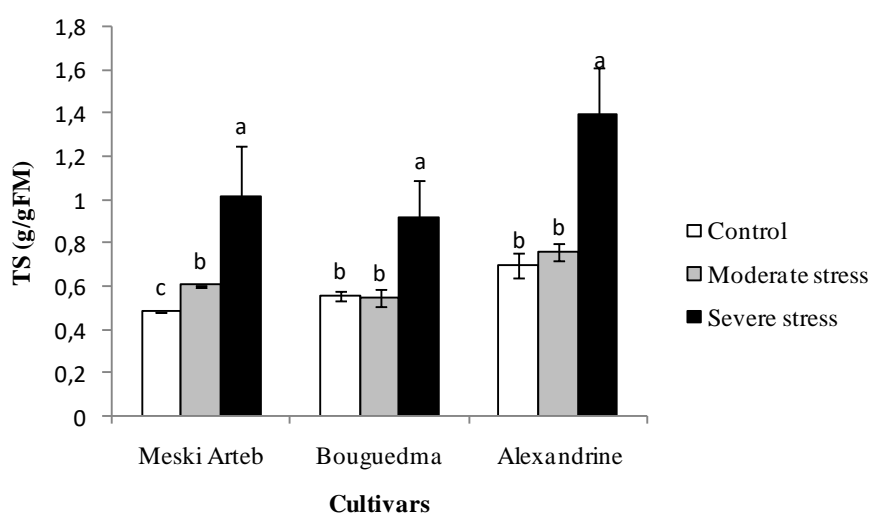
recorded that BA 29 rootstocks were less affected under water stress than those on other rootstocks (Sharma & Sharma, 2008). This shows that the effects found are related to the interaction with the variety.



**Fig. 4.** Leaf chlorophyll content (Chl in mg per 100g of fresh matter) at different levels of water stress for Meski Arteb, Bouguedma and Alexandrine cultivars. Vertical bars represent standard error of the mean of 3 replications. Different letters within cultivars are significantly different at  $p \leq 0.05$ .



**Fig. 5.** Leaf proline content (Pr in µg per g of fresh matter) at different levels of water stress for Meski Arteb, Bouguedma and Alexandrine cultivars. Vertical bars represent standard error of the mean of 3 replications. Different letters within cultivars are significantly different at  $p \leq 0.05$ .



**Fig. 6.** Leaf total sugar content (TS g per g of fresh matter) at different levels of water stress for Meski Arteb, Bouguedma and Alexandrine cultivars. Vertical bars represent standard error of the mean of 3 replications. Different letters within cultivars are significantly different at  $p \leq 0.05$ .

## CONCLUSION

From this work we conclude the importance of cultivars diversity because it behaved differently at different water stress degree. In fact, relative water content, leaf water potential showed a similar trend with the stomatal conductance and carbon assimilation. Alexandrine had slight differences with the other cultivars (Meski Arteb and Bouguedma). It appeared the relatively most resistant cultivar to water stress which presented a small reduction of leaf water potential leading to partial stomatal closure and partial carbon assimilation despite the smaller chlorophyll concentration contained in leaves.

As it was indicated previously the three pears cultivars reduced the carbon assimilation in response to stomatal control. This is due to the perturbation of the water status involving the osmotic adjustment (increase of sugars concentration). The Alexandrine cultivar showed the highest photosynthesis, 'Meski Arteb' slightly reduced leaf area and 'Bouguedma' preserved the chlorophyll concentration. Therefore, all cultivars behaved similarly but with different degree. A further detailed research is also needed to elucidate the underlying biochemical processes, anatomical and genetic parameters which are responsible for differential responses of pear cultivar.

### Conflict of interest

The authors have no conflict of interest.

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