BEHAVIOUR OF *Cupressus dupreziana* A. Camus PLANTS UNDER WATER STRESS CONDITIONS

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Abstract. *Cupressus dupreziana* A. Camus is a critically endangered species endemic to Tassili n'Ajjer, where it survives despite extreme climatic conditions, with drought being a major limiting factor. The aim of this study is to examine the effect of water stress, induced by the cessation of irrigation, on seedlings of Tassili cypress. Although this species is adapted to aridity, its mechanisms for adaptation and tolerance to water stress have yet to be sufficiently explored. After a watering cessation of 100 days, water stress led to a significant reduction in relative water content (RWC), seedling growth, and the concentration of photosynthetic pigments. In contrast, the plants attempted to maintain their osmotic adjustment through the accumulation of proline and soluble sugars, with levels significantly increasing. This water stress also resulted in oxidative damage, as evidenced by the notable rise in hydrogen peroxide (H_2O_2) and malondialdehyde levels. However, despite these challenges, the plants have survived and been able to resume their growth thanks to a photo-protective system. This was facilitated by an increase in carotenoid concentration and the activation of antioxidant enzymatic defence mechanisms, as illustrated by the rise in activities of superoxide dismutase (SOD), catalase (Cat), ascorbate peroxidase (APX), and guaïacol peroxidase (GPOX). *Cupressus dupreziana* demonstrates significant capabilities for the rehabilitation of arid regions, thanks to its adaptation mechanisms and tolerance to water stress. This study highlights the importance of researching endemic species and their potential in combating desertification.

Keywords: antioxidant enzymes; *Cupressus dupreziana*; drought stress; endangered species; osmolytes; relative water content.

INTRODUCTION

Discovered by Captain Duprez in 1924, *Cupressus dupreziana* was described as a new species by Aimée Camus in 1926. It is a conifer endemic to Tassili n'Ajjer in the central Sahara of Algeria [81]. According to Abdoun and Beddiaf [1], the population of *Cupressus dupreziana* is now represented by 233 living trees. In a hyper-arid climate where rainfall does not exceed 30 mm, its resistance to aridity is of great importance. Notably, a radial growth rate ranging from 2.1 to 0.83 mm per year has been recorded in *Cupressus dupreziana* trees [24]. Whereas the *Cupressus atlantica* in Morocco records an average radial growth rate ranging from 1.1 to 2.11 mm per annum under a precipitation of 400 to 500 mm per annum [5]. The species not only tolerates drought but also exhibits good frost resistance [24, 81]. Currently, the IUCN classifies the Tassili cypress among the most endangered species of extinction [2]. This risk of disappearance is primarily linked to male apomictic reproduction, where embryos develop from pollen within female reproductive structures without fertilization [72]. This breeding strategy has an impact on the regeneration of the species, which is almost non-existent, as the majority (80%) of the seeds produced in situ by *Cupressus dupreziana* trees are entirely empty. Meanwhile, 10% consist of seeds with endosperm but without an embryo, and only 10% contain normal seeds with both an embryo and endosperm [71]. Drought and population isolation can limit sexual reproduction in *Myrtus nivellei, for example,* to cope with this, the species reproduces clonally and survives in a few humid refuges scattered across the Sahara[37]. The rate of natural regeneration currently observed in the Tassili cypress is insufficient to ensure the survival of its population. *Cupressus dupreziana* is facing various pressures due to pastoralism and the increasing demand for wood by the Tuaregs and tourists, which has caused a loss of 8% over the last 30 years [1]. These pressures are exacerbated by the region's vulnerability to climate change, characterized by rising temperatures and decreasing precipitation. Hence, as a complementary approach for conservation and propagation, *in vitro* culture was initially envisaged as a viable alternative strategy [13]. However, it appears that *ex situ* regeneration is possible with good success [39, 60, 69].

Vegetation distribution is strongly influenced by water availability, its unavailability is a major obstacle to species productivity [67]. Water stress alters the physiological processes of the plant at all levels of organization: morphology, physiology and metabolism [70]. Maintaining proper water levels in plant tissues ensures proper functioning of fundamental plant processes such as photosynthesis, where turgor plays a vital role in regulating osmotic pressure and water management. Water deficit leads to reduced photosynthetic capacity and decreased growth [9, 18]. In plants, a dynamic balance between the production of reactive oxygen species (ROS) and their detoxification systems is essential [15]. When maintained, this control enables ROS to play a crucial role as signaling molecules in the regulation of growth, development

and adaptive responses to abiotic stress conditions such as drought [30]. This balance can be disrupted, leading to oxidative damage in plants. At certain levels of stress this imbalance can lead to irreversible damage to the cell [74, 75, 78]. Indeed, ROS disturb enzymes activity and induce peroxidation of membrane lipids in plants. This last reaction leads to the formation of peroxidation products, such as malondialdehyde (MDA), which damage cell membranes and alter their integrity [20, 74]. Plants develop various defense systems to prevent ROS formation, or scavenging ROS. These antioxidant systems are enzymatic such as superoxide dismutase (SOD), ascorbate peroxidases (APX), glutathione reductase (GR), and catalases (Cat) [34, 68], and non-enzymatic antioxidants, such as ascorbic acid, alpha-tocopherol, polyphenols [69, 89], and also some saccahrides such as fructans and raffinose [87]. The plant also preserves cellular integrity via osmolytes and protective compounds [12, 31].

During periods of drought, plants engage in different adaptive responses. Thus, the maintenance of turgor in plant cells is the most important factor for plant biosynthesis under a lower water potential [58]. Maintaining optimal cell turgor depends on the type, intensity and duration of stress, which indirectly determine the level required to ensure physiological processes such as photosynthesis and enzymatic activity [67, 73].

Adaptation to drought has often been associated with the climate of natural habitats, species inhabiting arid areas have developed more effective adaptation strategies to survive drought compared to their congeners living in sub humid or humid regions [44, 56]. Algeria is facing challenges related to water deficit, particularly in arid and semi-arid areas, these areas are increasingly threatened by increased desertification. Indeed, Algeria has shifted from a semi-arid country to a practically arid country due to the increase in temperatures and the decrease in precipitation [11, 54].

The critically endangered *Cupressus dupreziana* has been extensively studied in demography, genetics and ecology. However, despite its precarious status, no research has yet explored its behavior in the face of water stress. Acquiring this knowledge is of paramount importance for the preservation of this unique and threatened species. Our work aims to be a preliminary and essential step to fill this gap by studying the impact of water stress on this species naturally adapted to an arid climate. This original research aims to provide fundamental information on the resilience of *cupressus dupreziana* to drought conditions. Indeed, understanding how this plant responds to water stress situations is crucial for better stress management wich is essential to improve the survival of plants in arid environments. Our study is a scientific contribution to developing integrated strategies for long-term conservation.

MATERIALS AND METHODS

Plant material

The plants under study were obtained from the germination of cypress of Tassili seeds that were gathered in the Tassili n'Ajjer natural park in the far southeast of Algeria (latitude 26°20′N to 24°00′N, longitude 5°00′E to 10°00′E) under hyper-arid climate. The seeds were stored for 10 months and used without any treatment prior to germination, Their germination rate was approximately 3.4%

At the University of Blida1's Laboratory of Biotechnologies of Plant Production, one-year-old *Cupressus dupreziana* plants were kept in a naturally lit greenhouse in a semi-controlled setting. After a month of regular watering (two times per week), the plants were subjected to water stress by ceasing watering for 100 days, while the control group continued to receive regular watering for the same period. The samples were collected only in the morning at the same hour and quickly processed in order to avoid variations due to the plant's diurnal rhythm. After collection, the samples were immediately placed on dry ice to slow down metabolic activity, and then stored at -80°C. With the exception of the growth rate, which was recorded every 20 days, all parameters were measured at the conclusion of the experiment.

Growth rate

The shoot growth was measured before, every 20 days and after stopping watering using a graduated tape, counting 5 measurements. The growth rate was calculated by subtracting the height of the plant from the height measured after 20 days.

Relative water content (RWC)

The water content of the seedlings was determined through the fresh weight (FW), dry weight (DW) and turgid weight (TW) (after imbibition in distilled water) of each sample according to the following formula:

$$RWC\ (\%) = (FW - DW) / (TW - DW) \times 100.$$

Photosynthetic pigments

The extraction of chlorophyll and carotenoids was carried out from 100 mg of fresh leaf material in 10 mL of 80% acetone. The absorbance of the samples was read at wavelengths of 470, 663 and 647 nm [47].

Proline

According to the method of Troll and Lindsley [86], simplified and developed by Dreier and Goering [23], modified by Magné and Larher [50]. A sample of fresh plants was ground in 40% methanol. The reaction medium contained 1 mL of the plant extract to which are added 1 mL of glacial acetic acid, 1 mL of ninhydrine reagent (1%) and 5 mL of toluene. The optical density is measured at a wavelength of 520 nm. The values obtained are converted into proline concentration using a standard curve.

Soluble sugar

The determination of total soluble sugars was carried out according to the protocol of DuBois *et al.* [25]. The extraction was carried out in 80% ethanol and recovered with distilled water then mixed with 5%

phenol and concentrated sulfuric acid. The optical density was measured at 490 nm and converted into concentration of soluble sugars using a standard curve.

Hydrogen peroxide (H_2O_2)

A sample of 100 mg of fresh plant material was ground in 0.1% trichloroacetic acid and then centrifuged at 12,000 rpm for 15 min at 4°C; the supernatant was collected in 10 mM phosphate buffer at pH 7 and then mixed with 1 M potassium iodide KI. The extract was incubated in the dark for an hour [88]. The optical density was measured at 390 nm and converted into concentration of H_2O_2 using a standard curve.

Lipid peroxidation

Lipid peroxidation was determined by the malondialdehyde content. Samples of fresh plant material (100 mg) were ground in 1% TCA, centrifuged at 12,000 rpm for 20 min at 4°C. A volume of 1 mL of 0.5% thiobarbituric acid in 20% TCA was added to 500 mL supernatant and placed in a water bath at 95°C for 30 min. After cooling, the mixture was centrifuged and the optical density was measured at 532 and 600 nm. The absorbance reading at 600 nm was used to deduce the absorption due to other substances other than the MDA-TBA2 complex [36]. The MDA content was calculated using the molar extinction coefficient of MDA ($\varepsilon = 155\ mM^{-1}cm^{-1}$).

Total proteins

Protein extraction was performed in 0.1 M Tris-HCl extraction buffer (pH=8.1) in which fresh plant material was ground and centrifuged at 12,000 rpm for 20 min at 4°C. A volume of 3 mL of Bradford reagent was added to the extract and homogenized by vortexing. Optical density was measured at 595 nm and converted into concentration of proteins using a standard curve.

Superoxide dismutase (SOD)

The measurement of SOD activity is carried out according to the method of Marklund and Marklund [52], modified by Boucelha *et al.* [15]. The reaction medium contains 50 mM Tris-HCl buffer pH 8.2, 1 mM EDTA and 100 µM of the plant extract, the reaction is initiated by the addition of 1 mM pyrogallol, its autoxidation results in increased absorption at 420 nm. The activity of SOD was expressed in units per minute and per mg of protein.

Catalase (Cat)

The principle of determining the activity of Cat is based on the power of this enzyme to eliminate H_2O_2. In order to measure the activity of catalase, the reaction medium is composed of 100 µL of plant extract and 2 mL of 100 mM potassium phosphate buffer, pH 7.5, the reaction begins with the addition of 15 µL of 10 mM H_2O_2 and the variation of the optical density is measured at 240 nm [6]. Activity is expressed in nmoles of H_2O_2 degraded per minute and per mg of proteins, using the molar extinction coefficient of H_2O_2 ($\varepsilon = 36\ mM^{-1}cm^{-1}$).

Ascorbate peroxidase (APX)

This enzyme was measured according to the method described by Nakano and Asada [60]. The reaction medium (2 mL) consists of 100 mM potassium phosphate buffer (KH_2PO_4/K_2HPO_4) pH 7, 1 mM Na_4EDTA, 50 mM ascorbate, 10 mM H_2O_2 and 100 µL of enzymatic extract. The reaction was initiated by adding ascorbate and monitored for 30s. The activity was expressed in µmoles of oxidized ascorbate per min and per mg of protein. Molecular extinction coefficient of ascorbate at 290 nm and 25°C is ($\varepsilon = 2.88\ M^{-1}\cdot cm^{-1}$).

GPOX

According to MacAdam *et al.* [49] modified by Boucelha *et al.* [15], the reaction mixture was composed of 0.1 M potassium phosphate buffer pH 6.5 and 10 mM H_2O_2, the reaction was initiated by the addition of 100 µL of guaïacol (36 mM). The activity was monitored as a function of time. The variation of the absorbance is measured at 470 nm. The enzymatic activity was expressed in µmoles of guaïacol oxidized per minute and per mg of proteins. The GPOX activity was calculated using the molar extinction coefficient of guaïacol ($\varepsilon = 26.6\ mM^{-1}cm^{-1}$).

Statistical analysis

Statistical analyses were performed using SPSS software version 21.0.0 for Windows. Analyses were repeated 5 times and means followed the same trend according to the Shapiro Wilk normality test. When data did not meet the conditions of normality and homoscedasticity, non-parametric tests were performed (Mann-Whitney test). Means were compared by Student's t test and differences were considered significant at the $P < 0.05$ level.

RESULTS

The independent samples student's t-test verifies that all the parameters measured in the Tassili cypress plants exposed to water stress are significantly different (Table 1).

Growth rate

Stem growth of control plants is progressive throughout the experimental period as it records a growth increase of more than 67% on the 100th day of the experiment, the growth progression is similar in plants subjected to a watering stop during the first 20 days (Fig. 1B). Then until the 40^{th} day the growth slows down by about 13% compared to the growth percentage in control plants, when the stress intensifies between the 40^{th} and 80^{th} day of the experiment, the growth speed stabilizes (Fig. 1A) Stressed plants completely stop their growth during the last 20 days of the experiment. The decrease in growth is significantly different.

RWC

The relative water content in stressed plants at the end of the experiment is approximately 47.04 ± 3.2% (Fig. 2) which represents a drop of approximately 38.5% compared to control plants (76.49 ± 3.86%) this drop in RWC is highly significant.

Table 1. Morphological, physiological and biochemical responses to water stress of *Cupressus dupreziana* each value represents the mean ± standard error. Different letters indicates significant differences at $P < 0.05$.

Parameters studied	Control		Stressed	
Growth rate ($cm \times day^{-1}$)	7.08	$\pm 0.640^a$	2.61	$\pm 0.508^b$
RWC (%)	76.49	$\pm 3.856^a$	47.04	$\pm 3.203^b$
Chlorophyll a+b ($mg \times g^{-1}fw$)	0.81	$\pm 0.030^a$	0.51	$\pm 0.035^b$
Chlorophyll a/b	4.48	$\pm 0.702^a$	1.56	$\pm 0.202^b$
Carotenoids ($mg \times g^{-1}fw$)	0.11	$\pm 0.020^a$	0.29	$\pm 0.020^b$
MDA ($\mu g \times g^{-1}$)	1.63	$\pm 1.632^a$	2.88	$\pm 0.068^b$
Proline ($mg \times g^{-1}fw$)	0.56	$\pm 0.16^a$	15.64	$\pm 0.17^b$
Soluble sugars ($mg \times g^{-1}fw$)	6.48	$\pm 0.14^a$	19.28	$\pm 0.45^b$
H_2O_2 ($nmoles \times g^{-1}fw$)	0.37	$\pm 0.374^a$	1.70	$\pm 0.018^b$
SOD ($U.min^{-1} \times mg^{-1}$ prot)	73.94	$\pm 2.230^a$	144.38	$\pm 1.901^b$
CAT ($\mu moles \times min^{-1} \times mg^{-1}$ prot)	0.24	$\pm 0.017^a$	0.92	$\pm 0.027^b$
APX ($\mu moles \times min^{-1} \times mg^{-1}$prot)	0.10	$\pm 0.004^a$	0.31	$\pm 0.019^b$
GPOX ($\mu moles \times min^{-1} \times mg^{-1}$prot)	0.11	$\pm 0.035^a$	0.64	$\pm 0.117^b$

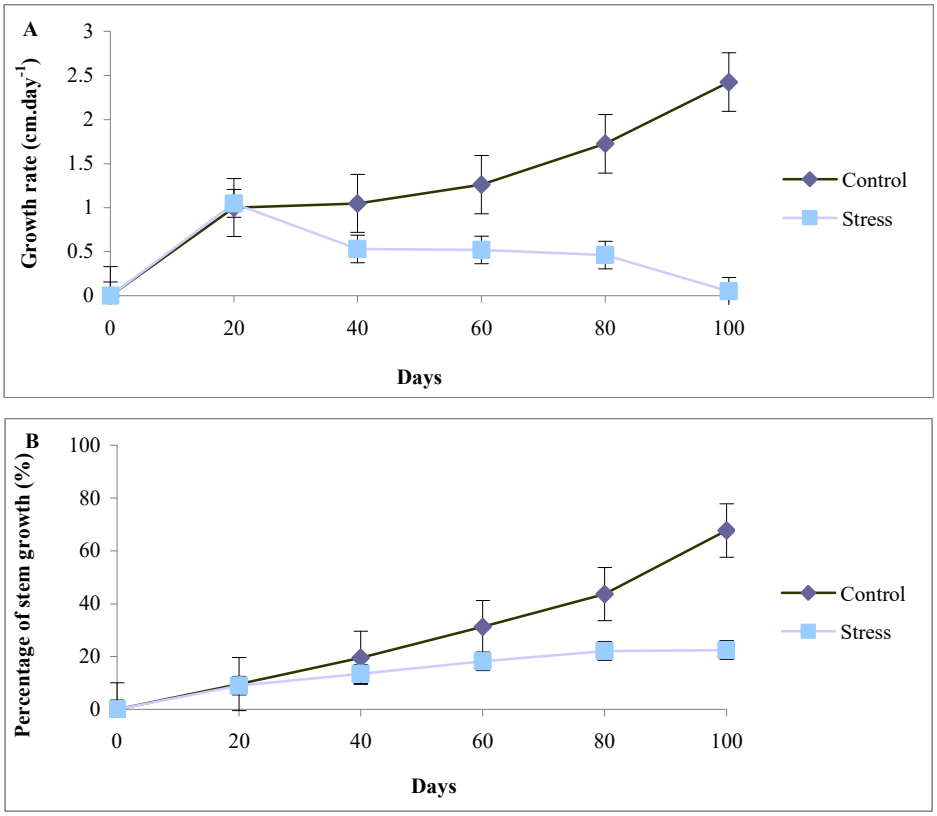

Figure 1. Effect of water stress on growth rate ($cm \times day^{-1}$) (A) and percentage of stem growth compared to initial height (%) (B) of *C. dupreziana* plants. Error bar represents standard error.

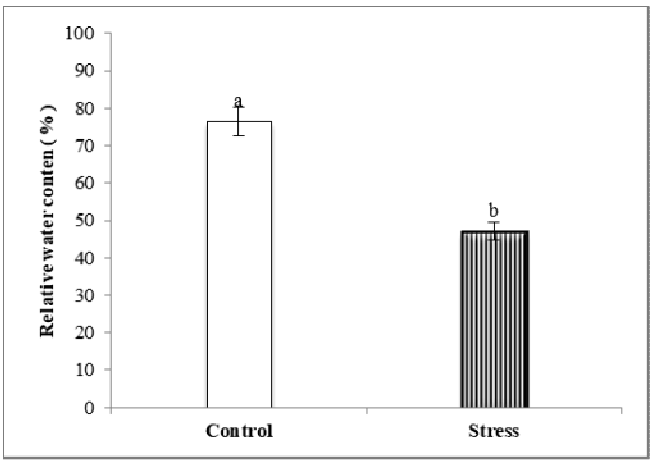

Figure 2. Effect of water stress on the relative water content (RWC) of seedlings of *Cupressus dupreziana* after 100 days of water stress. Error bar represents standard error. Different alphabetical letters indicate a significant difference ($P < 0.05$) between means.

Photosynthetic pigments

Water stress caused a significant decrease in total chlorophyll content in stressed plants (37.04%) compared to control plants and similarly a very significant decrease (65.18%) in the Chla/Chlb ratio. The carotenoid content in stressed plants was about 2.6 times greater than that in control plants (Fig. 3).

Proline

Proline content in stressed plants increased significantly to approximately 27.9 times the proline content in control plants (Fig. 4).

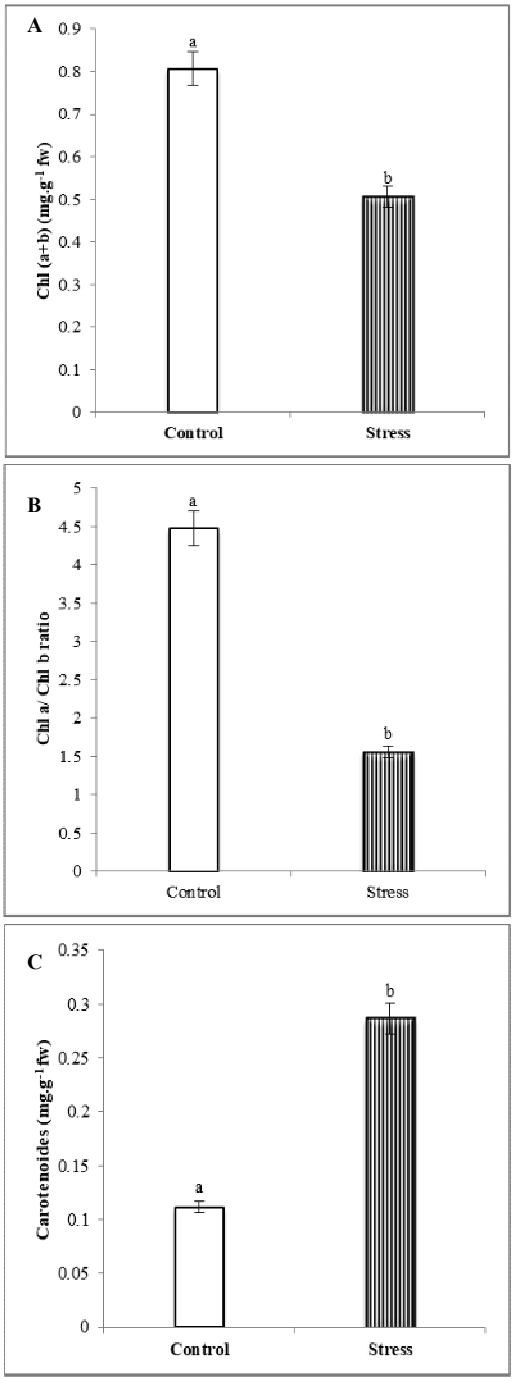

Figure 3. Effect of water stress on total chlorophyll pigment contents (A), Chl a/Chl b ratio (B) and carotenoid content (C) of *C. dupreziana* plants. Error bar represents standard error. Different alphabetical letters indicate a significant difference ($P < 0.05$) between means.

Soluble sugars

Watering cessation caused a significant increase (197.5%) in soluble sugar content in stressed *C. dupreziana* plants compared to controls (Fig. 5).

MDA

Water stress significantly increases the malondialdehyde level in stressed plants. The malondialdehyde content is 1.8 times higher compared to the MDA content recorded in control plants (Fig. 6).

Hydrogen peroxide (H_2O_2)

The application of water stress caused a significant increase in the H_2O_2 content of stressed plants up to 4.6 times the content of control plants (Fig. 7).

Enzymes (SOD, CAT, APX et GPOX)

Tassili cypress plants show a significant increase in catalase, SOD, APX and GPOX (Fig. 8). These increases in the activity of enzymes in stressed plants are 283.3%, 95.3%, 210% and 481.8% respectively compared to control.

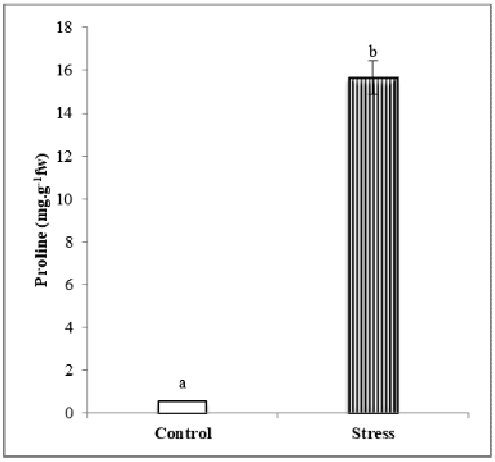

Figure 4. Effect of water stress on the proline content in plants *C. dupreziana*. Error bar represents standard error. Different alphabetical letters indicate a significant difference ($P < 0.05$) between means.

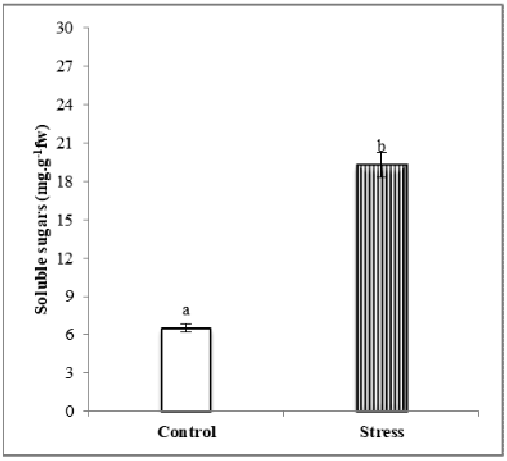

Figure 5. Effect of water stress on the soluble sugars content in plants *C. dupreziana*. Error bar represents standard error. Different alphabetical letters indicate a significant difference ($P < 0.05$) between means.

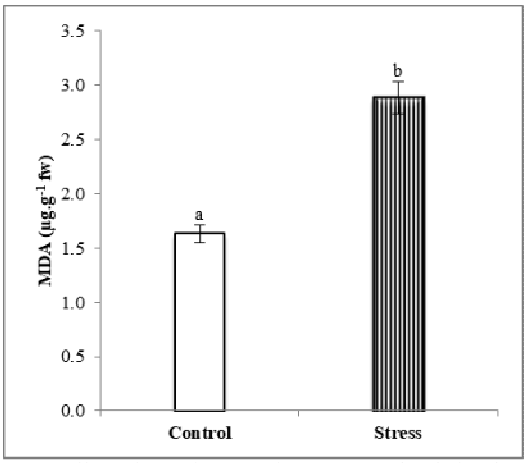

Figure 6. Effect of stopping watering on MDA levels in plants *C. dupreziana*. Error bar represents standard error. Different alphabetical letters indicate a significant difference ($P < 0.05$) between means.

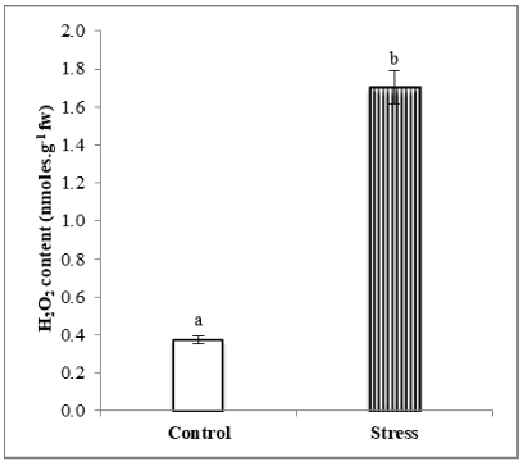

Figure 7. Effect of stopping watering on H_2O_2 content in plants *C. dupreziana*. Error bar represents standard error. Different alphabetical letters indicate a significant difference ($P < 0.05$) between means.

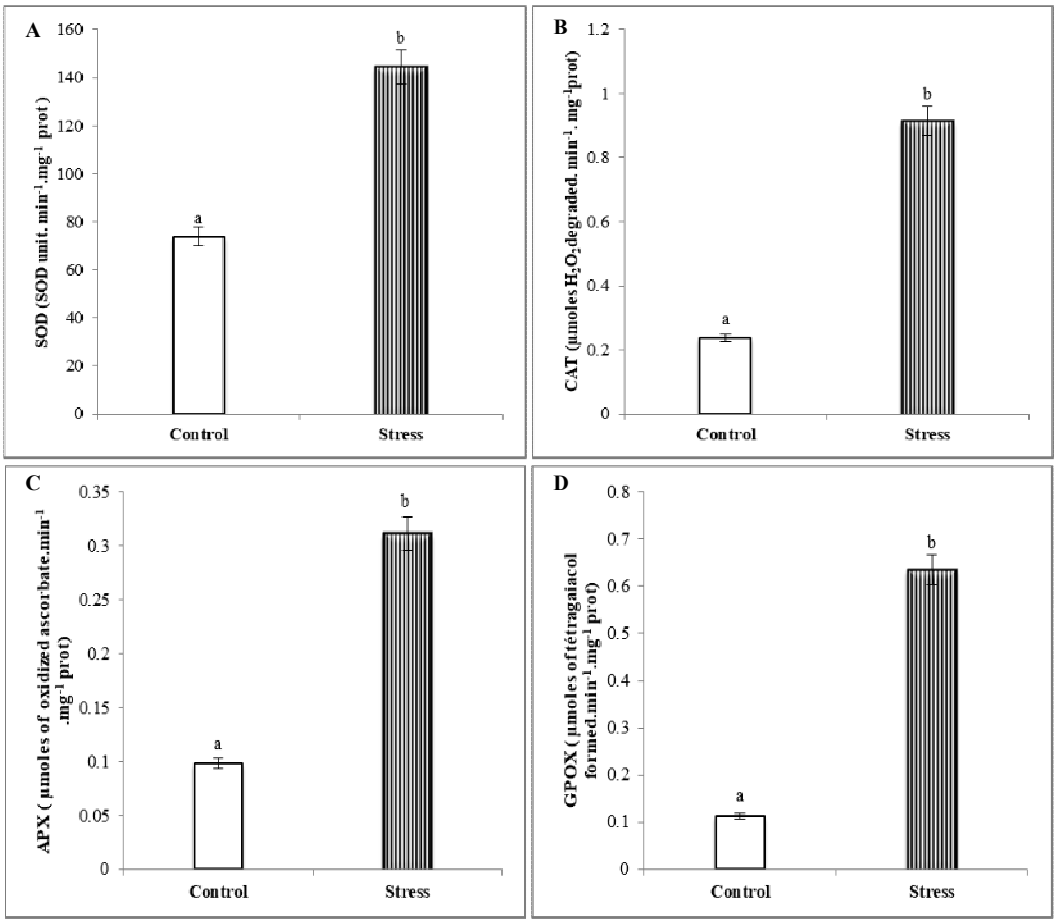

Figure 8. Effect of stopping watering on the activity of SOD (A), on the activity of CAT (B), on the activity of APX (C) and on the activity of GPOX (D) in plants of *C. dupreziana*. Error bar represents standard error. Different alphabetical letters indicate a significant difference ($P < 0.05$) between means.

DISCUSSION

Knowledge about the behavior of *Cupressus dupreziana*, an endangered species, under environmental stress is scarce, making this study particularly important. The results obtained will allow us to better understand the response mechanisms of this threatened species to drought conditions.

Growth is one of the physiological processes most sensitive to drought [90]. It is accomplished by cell division, enlargement and differentiation and involves genetic, physiological and biochemical factors and their interactions that determine the quality and quantity of plant growth and is highly influenced by water deficit [15, 43]. The slowdown in the growth of Tassili cypress plants is only affected from the 20th day

of stopping watering. This slowdown is due to the reduction in cell growth which is due to the reduction in RWC, due to water stress, which also causes a reduction in turgor pressure [51, 53, 82]. These results are similar to those observed in one-year-old Atlas cypress, Atlas cedar and maritime pine saplings in which the reduction in stem growth appeared after the 20th day of drying linked to a lowering of the water potential [93]. The stem growth of cypress plants started to stop around the 80th day after water cessation, where a zero growth rate was recorded on the 100th day. This growth arrest can be explained by the significant drop in RWC at the end of the experiment, which is reflected by the interruption of water flow that causes the inhibition of cell elongation of higher plants due to severe water deficiency [66]. Our results are consistent with those observed in Japanese cypress and Japanese cedar where stopping watering inhibited stem growth [59]. Severe water stress can lead to a significant decrease in the relative water content of plants [4, 84, 92]. Our results also show that water stress applied to Tassili cypress plants and which is considered as severe stress led to a significant drop in relative water content, this can be explained according to Anderson *et al*. [6] by the fact that the lack of water in the soil can lead to an increase in the water potential in root cells, which makes plants more resistant to water absorption, consequently a drop in water content in the plant. The RWC observed in stressed plants is lower than that observed in *Casuarina glauca* during severe water stress whose RWC is approximately 63% [4], but higher than that observed in *Acacia tortilis* where the RWC decreases to 38.05% [41], and that mentioned by Nogués and Baker [65], where it decreases to 20% in *Lavandula stoechas*. The lack of water resources is measured through photosynthetic activity by the measurement of chlorophyll. The results of our study clearly show a decrease in total chlorophyll in plants subjected to water stress compared to control plants; these results are close to those observed in maritime pine where water stress causes a significant drop in chlorophyll of 30% [26]. Under water stress, plants experience a decrease in water content, which affects the structure and functionality of chlorophyll [17, 28]. Largely, chloroplast damage by ROS can lead to lipid peroxidation and, consequently, chlorophyll destruction. This degradation reduces the photosynthetic capacity of plants, leading to a decrease in metabolite production [19, 38, 55, 85]. In addition to the degradation of existing chlorophyll, the synthesis of new chlorophyll molecules is also reduced under water stress. This means that plants are unable to compensate for the loss of chlorophyll by producing new molecules, thus exacerbating the decrease in photosynthesis [10, 80].

The survival and adaptation of plants to drought largely depend on their ability to maintain their cellular turgor and adjust their osmotic balance [21]. The maintenance of cellular turgor is ensured by the synthesis and accumulation of osmolytes that prevent water loss from the cells [48]. As is the case for other species, when the Tassili cypress was under severe water stress or the RWC was low (47%), the proline and soluble sugars content increased considerably in order to avoid a further decrease in relative water content. The very high proline content at the time of water stress also provides protection of plant membranes and proteins [42], which has been observed in *Pistacia atlantica* [68] and *Atriplex halimus* [16]. While the accumulation of soluble sugars, which could be linked to starch degradation or inhibition of starch synthesis, [14, 32], in addition to their role in osmo-adjustment also proceeds as local antioxydants by scavenging ROS near sensitive membranes and complement the action of other antioxidants molecules mentioned above [69, 87, 89]. Similar results have been reported in *Pinus pinea* [83], *Eucalyptus microtheca* [46] and *Juglans regia* [61]. Among the consequences of water stress is the generation of oxidative stress that causes the formation of free radicals such as H_2O_2 [29, 45]. The H_2O_2 content increased significantly when plants underwent water stress, our results are supported by those observed in *Acacia raddiana* where the H_2O_2 content reaches 200% under a RWC lower than 60% [40] and those observed in *Populus cathayana* [91]. Simultaneously, malondialdehyde (MDA) level is often used as an indicator of oxidative damage due to stress, our study showed that *Cupressus dupreziana* plants subjected to water stress present a significant increase in MDA level this is due to the presence of reactive oxygen species, which have a high reactivity and disrupt plant metabolism by causing lipid peroxidation, which causes membrane alteration [8, 33, 79]. In order to protect against the harmful effects of oxidative stress, plants have established defense mechanisms, such as the production of protective pigments such as carotenoids [3, 27], under the drought conditions a very significant accumulation of carotenoids was observed in stressed plants, in parallel with the significant increase of the production of ROS, in this case, carotenoids can act as effective antioxidants by neutralizing these ROS and protecting photochemical processes [35]. The accumulation of antioxidant enzymes such as superoxide dismutases (SOD), ascorbate peroxidase (APX), guaïacol peroxidases (GPOX) and catalases (Cat) is considered to have a protective role against oxidative stress [29]. Our results show a huge increase in SOD, GPOX, and APX in stressed plants. These enzymes constitute the line of defense against the toxic effects of high levels of ROS [33, 57, 76], and acting as a main mechanism for neutralizing H_2O_2 [22, 30, 64]. Thus contributing to the maintenance of cellular redox balance under water stress [7, 63, 77].

In conclusion, the cypress of Tassili managed to survive during a drought period. In our study, it maintained, even weakly, its growth for 100 days, proof that *Cupressus dupreziana* had the ability to

maintain relatively high tissue water potential even though its growth totally ceased at 100th day under a low relative water content (47%) which is considered a critical value. The maintaining of turgor is possible due to solutes accumulation as proline and soluble sugars. Moreover, the cell's structure integrity is preserved by dint of the activation of antioxidant machinery. This study contributes to advancing the understanding of the physiological and metabolic responses of the Tassili cypress under water stress conditions. It forms part of a broader holistic investigation of this species, which is essential both for its conservation and for its potential application in the reforestation of arid ecosystems.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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