Effects of water stress and high temperature on photosynthetic rates of two species of *Prosopis*

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A B S T R A C T

The main aim of this research was to compare the photosynthetic responses of two species of *Prosopis*, *Prosopis chilensis* (algarrobo) and *Prosopis tamarugo* (tamarugo) subjected to heat and water stress, to determine how heat shock or water deficit, either individually or combined, affect the photosynthesis of these two species. The photosynthetic rates expressed as a function of photon flow density (PFD) were determined by the O_2 liberated, in seedlings of tamarugo and algarrobo subjected to two water potentials: −0.3 MPa and −2.5 MPa and to three temperatures: 25 °C, 35 °C and 40 °C. Light response curves were constructed to obtain light compensation and light saturation points, maximum photosynthetic rates, quantum yields and dark respiration rates. The photochemical efficiency as the F_v/F_m ratio and the amount of RUBISCO were also determined under heat shock, water deficit, and under the combined action of both stress. Photosynthetic rates at a light intensity higher than 500 μmole photons m^{-2} s^{-1} were not significantly different ($P > 0.05$) between species when measured at 25 °C under the same water potential. The maximum photosynthetic rates decreased with temperature in both species and with water deficit in algarrobo. At 40 °C and −2.5 MPa, the photosynthetic rate of algarrobo fell to 72% of that of tamarugo. The quantum yield decreased in algarrobo with temperature and water deficit and it was reduced by 50% when the conditions were 40 °C and −2.5 MPa. Dark respiration increased by 62% respect to the control at 40 °C in tamarugo while remained unchanged in algarrobo. The photochemical efficiency decreased with both, high temperature and water deficit, without differences between species. RUBISCO content increased in algarrobo 35 °C. Water deficit reduced the amount of RUBISCO in both species. The results of this work support the conclusion that in both *Prosopis* species, the interaction between high temperature and water deficit affects photosynthesis responses greater than each individual stress, and that the interactive effect is more pronounce in algarrobo than in tamarugo.

1. Introduction

Prosopis chilensis (Chilean algarrobo) and *Prosopis tamarugo* (tamarugo) are leguminous trees of the semi-arid and hyper-arid regions of Northern Chile (20°17′–33° South Latitude). In these regions, these species are subjected to extreme environmental conditions such as high solar radiation [1] extreme temperatures [2,3], high salinity [4] and high water stress [5,6]. *P. tamarugo*, an endemic specie of the Atacama Desert (20°17′–20°50′ South Latitude) in the hyper-arid region of Northern Chile, has been characterized as a specie well adapted to water stress [5,6] with several physiological characteristics to avoid or to resist water deficit. Some of these characteristics include the presence of deep roots and the capacity of osmotic adjustments [5,7]. On the other hand, *P. chilensis* is a specie of the semi-arid Mediterranean region of Chile with pluviosity in winter months (from April to August).

With respect to temperature, both *Prosopis* species seem to have optimums for CO_2 assimilation at a higher temperature than the temperature reported for other species which have evolved in more Mediterranean climates. For instance, Pinto [8] observed that at 25 °C young plants of *P. chilensis* showed higher CO_2 assimilation rate than *P. tamarugo*, with an optimum at 30 °C and 32 °C. Similar results were found when the assimilation rate was measured in adult trees in morning hours of a spring day at Pampa del Tamarugal [1]. However, when the temperature was 31 °C in the afternoon of the same day, algarrobo photorespires while tamarugo continues to assimilate CO_2 [1].

The Chilean algarrobo has been characterized as a plant very tolerant to heat stress [2,3]. The heat thermotolerance of algarrobo is 6 °C higher than that of soybean, another leguminous plant considered to have good responses to heat shock [3]. The heat

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tolerance of algarrobo correlates with a high accumulation of heat shock proteins mainly HSP70 and ubiquitin [2,3,9].

Changes in cellular water balance and environmental temperatures cause altered growth in plants [10–12]. These two environmental conditions primarily cause a decrease in the photosynthetic activity which has been attributed, among other physiological changes, to the closure of stomata [11,13,14], to a high resistance to CO_2 flow from the mesophyll cells to the chloroplast stroma [15] and to alterations in the photochemical processes of the thylakoid membranes [17]. Thus, water deficit and heat shock can affect the light harvesting systems, the flow through the electron transport chain, NADPH and ATP synthesis, photosynthetic carbon reduction cycle in the chloroplast and the utilization of assimilates. On the other hand, the chlorophyll fluorescence of photosystem II increases under both water and temperature stresses, due to imperfect energy dissipation [14,18–20]. This, in turn, makes the photosynthetic efficiency decrease to a higher degree when both stress conditions are combined together.

Under water stress, not all physiological processes are equally affected. In plants, such as sugar beet and cotton, non-cyclic electron flow in the chloroplasts becomes affected only under severe water deficit when leaf water potential falls by more than 50% [21,22]. Both photosystems and the electron transport flow in the thylakoid membrane are less sensitive to changes in cellular water balance [23,24], as expected for physiological processes occurring in a non-aqueous medium, such as the lipid membranes. Therefore, water deficit may cause direct damage to the manganese–enzyme complex [15] affecting also biosynthesis of RUBISCO [25,26].

Under natural conditions, water deficit is associated with high temperature stress. Therefore, it is very difficult in plants to separate the effects induced by both environmental conditions [27,28]. As an example, water deficit causes stomatal closure which in turn, reduces the transpiration rate affecting heat dissipation. The consequence of this is an increase in the foliar temperature [22] leading to stomatal opening. Temperature also affects the stability of photosystem II, as has been previously reported [3].

Tamarugo and algarrobo are subjected in their natural environment to high temperature stress and water deficit during hours of high solar radiation and since they are well adapted to these conditions we wanted to test the hypothesis that: "the interaction between heat shock and water deficit has a more severe effect on the photosynthesis of these two species of *Prosopis*, than each of these stress conditions experienced individually". If this is the case, heat stress and water stress probably influence different processes of photosynthesis. Since tamarugo is better adapted to the hyper-arid conditions of the Atacama Dessert, we also examined whether the interaction of heat and water stress affects the photosynthesis of algarrobo more than tamarugo.

2. Materials and methods

2.1. Plant material

Chilean Algarrobo (*Prosopis chilensis*) seeds were collected from trees grown in Antumapu (Santiago–Chile). Tamarugo (*Prosopis tamarugo*) seeds were collected from trees grown in Canchones (Iquique–Chile). Seeds were germinated as described [3] and seedlings were watered with Hoagland II nutrient solution under conditions of greenhouses. After 7 months of growth, plants of similar size were selected for the experiments.

2.2. Water potential determination of plants under water stress

To determine the water potential under water stress, a group of 24 plants of both species was subjected to water restrictions for 90 days. The water potentials were determined by Scholander's chamber, using the methodology described [29]. Plants of *Prosopis* grown at 25 °C began to show symptoms of withering when the leaf water potential reached −2.5 MPa after 49 days of water deficit (Fig. 1). After 90 days of water restrictions, algarrobo showed permanent withering with a potential of −5 MPa, while tamarugo reached a potential of −4.2 MPa with loss of leaves, although on rewatering the plants recovered.

2.3. Growth conditions of tamarugo and algarrobo plants

Plants of seven months old were transferred to a controlled environment growth chamber and acclimated for 4 days at 25 °C. The conditions of the acclimation chambers were: light intensity 130 μmole photons m^{-2} s^{-1}, relative humidity 70% ± 5% and photoperiod of 12 h.

2.4. Water and temperature treatments

The experimental design used corresponded to a factorial of $3 \times 2 \times 2$. Each treatment had five replicates of three plants each. Algarrobo and tamarugo plants were subjected to the followings treatments: (1) forty five plants were water-restricted until the water potential reached −2.5 MPa. The plants were separated into three groups of 15 plants each and treated at 25 °C, 35 °C and 40 °C in growth chambers. Three groups of 15 plants each were also used as control. The temperature treatments were performed for a period of 2 h.

2.5. Determination of oxygen evolution to define the photosynthetic parameters

The oxygen evolution measurements were performed in folioles of equal area using a Clark's electrode as described previously [30]. The folioles were placed in the oxygraph chamber in the last 30 min of the temperature treatment. The oxygraph temperature was maintained by immersion of the oxygraph chamber in a circulating water bath kept at the desired temperature. The tubing system of the bath for water circulation was insulated to avoid small variations in the temperature (of about 1 °C). A thermocouple was included in the interior of the water tubing outlet which was connected to a digital tester model HC-81 to record the water temperature.

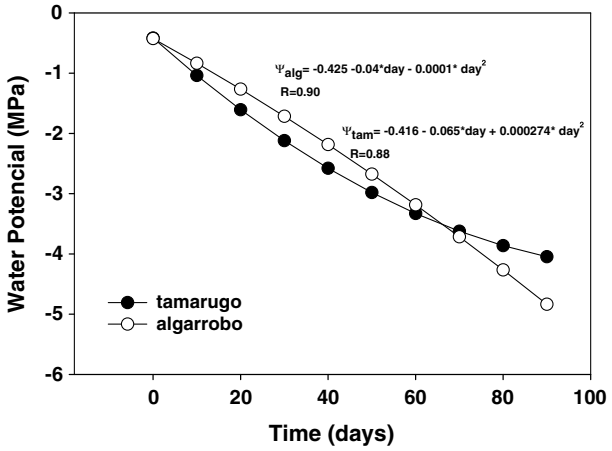

Fig. 1. Determination of water potentials in leaves of tamarugo and algarrobo during water restriction. Plants were maintained under water restriction until the plants showed symptoms of withering. After 90 days of water restrictions, algarrobo showed irreversible withering and tamarugo had loss of leaves. The regressions were calculated using a quadratic equation of the second degree.

Five folioles of different plants were subjected to each temperature treatment. Foliar area was determined by a correlation between area and fresh weight of the sample by linear regression (Eqs. (1) and (2)). These regressions were calculated using the values obtained from 50 samples of each species.

2.5.1. Algarrobo

$$\text{Leaf area} = -0.278 + 74.055 * \text{foliole fresh weight} \quad (1)$$

$r = 0.9920$.

2.5.2. Tamarugo

$$\text{Leaf area} = -0.250 + 38.865 * \text{foliole fresh weight} \quad (2)$$

$r = 0.9938$.

2.6. Light intensity treatments

Different light intensities were applied to the folioles placed in the oxygraph chamber for the temperature and water treatment experiments. The light intensities were generated by voltage changes using a control box (Hansatech model L.S 3), which automatically regulated the light intensity each minute. The applied intensities were: 0, 50, 75, 100, 200, 500, 600, 650, 700, 900 and 1075 μmole photons $m^{-2}\,s^{-1}$. Exceptionally, the intensities of 0 and 1075 μmole photons $m^{-2}\,s^{-1}$ were applied for 3 min in order to stabilize the response of the plant to these conditions.

2.7. Determination of photosynthetic parameters

All photosynthetic parameters were determined by O_2 evolution. Quantum yield, maximum photosynthetic rate, dark respiration and the light compensation point were obtained at saturating CO_2 concentration using the light response curves. All the photosynthetic responses obtained were automatically stored in a computer and processed using the program LD. The Photon Flux Density (PFD) (μmole photons $m^{-2}\,s^{-1}$) was calculated as a function of the oxygen evolution rate (μmole $O_2\,m^{-2}\,s^{-1}$). To obtain the maximum photosynthetic rates, the data was adjusted to a rectangular hyperbola given by Eq. (3). The quantum yield was estimated from the initial slope by applying linear regression to low-photon flux data of the light response curve. The projection of the straight line to the *X-axis corresponds to the light compensation point*

$$A = \frac{\Phi * Q + A_{\max}\sqrt{(\Phi * Q + A_{\max})^2 - 4\Phi * Q * \Theta * + A_{\max}}}{2\Theta} - R_d \quad (3)$$

where A is the gross photosynthetic rate, Φ is the apparent quantum efficiency, Q is the photosynthetic active radiation, $A_{\max}$ is the light-saturated rate of evolved O_2, Θ is the convexity or curvature factor, and R_d is the dark respiration.

2.8. Determination of stomatal conductance

The stomatal conductance was determined using a porometer (CIRAS, UK). For this, five plants of each species were used per temperature and water treatment. The folioles remained attached to the plant during the measurements. For heat shock experiments, the chamber of the porometer was set at the same temperature of the treatment using a temperature controlled water bath (25 °C, 35 °C and 40 °C). The measurements were performed under a light intensity of 900 μmole photons $m^{-2}\,s^{-1}$. The foliar area was corrected by Eqs. (1) and (2).

2.9. Fluorescence experiments

Chlorophyll fluorescence was measured in a Photosynthetic Fluorescence Emission Analyzer (PEA, Hansatech, UK), according to the methodology described by Walker [31]. The measurements were performed in both, growth chambers and greenhouse.

2.10. Determination of RUBISCO content

The content of RUBISCO was estimated by the amount of protein present in the small and large RUBISCO subunit bands resolved by SDS–PAGE. The SDS–PAGE analysis was performed according to Laemli [32]. The gels were stained with Coommasie Blue. The small and large subunits of RUBISCO were electroeluted from the gel in 25 μM TRIS [33] and the protein content of each band was determined by Bradford's test [34].

3. Results

The photosynthetic light response curves obtained at different temperatures and water potentials showed that the photosynthetic rates measured at the light saturation point decreased when temperature and water deficit increase (Fig. 2). Light saturation points

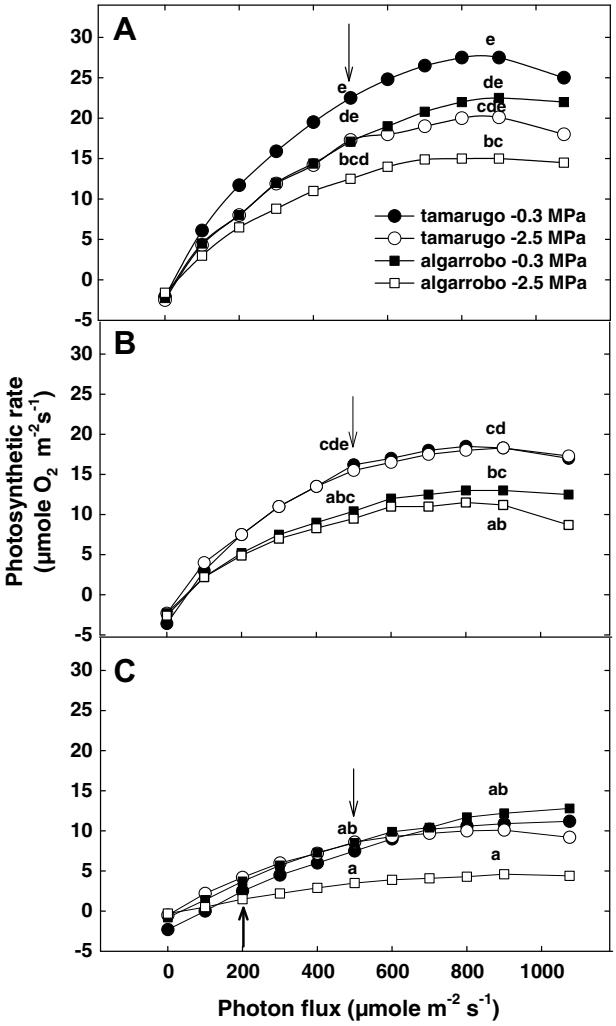

Fig. 2. Light response curves of tamarugo and algarrobo, subjected to different temperatures and different water conditions. (A) at 25 °C, (B) at 35 °C, (C) at 40 °C. Thin arrows indicate the light saturation points for all treatments except for algarrobo at −2.5 MPa and 40 °C (thick arrow).

were at 500 μmole photons m^{-2} s^{-1} in all conditions in both species except for algarrobo at 40 °C and −2.5 MPa (Fig. 2). Under these conditions the light saturation point fell to 200 μmole photons m^{-2} s^{-1}.

The maximum photosynthetic rate seen in the photosynthetic light response curves corresponds to the maximum absolute value of photosynthesis measured at a determined light intensity [31]. In our experiments this maximum photosynthetic rate was produced at 500 μmole photons m^{-2} s^{-1} for both species under all temperature and water potential conditions (Fig. 2, Table 1). However, the statistical analyses showed that no significant differences ($P > 0.05$) were found between maximum photosynthetic rates of plants subjected to the same water potentials when the light intensities exceeded 500 μmole photons m^{-2} s^{-1}, (Fig. 2). At a high temperature (40 °C) and a low water potential (−2.5 MPa) the photosynthetic rates, measured at the light saturation point, greatly decreased in both species and *P. chilensis* was most affected.

With the aim of separating the effect of each factor (temperature and water deficit) on the maximum photosynthetic rate, an analysis was performed for each factor without considering the effect of the other factor. Taking in account only the temperature, it was found that tamarugo at 25 °C has a maximum photosynthetic rate (24.3 μmole m^{-2} s^{-1}) greater than that of algarrobo (19.8 μmole m^{-2} s^{-1}, $P \leqslant 0.05$; Table 2). A similar situation occurred when plants were exposed to 35 °C. In both species the maximal photosynthetic rates were greatly affected at 40 °C, with an almost 50% reduction ($P \leqslant 0.05$) with respect to the control treatment (25 °C).

When the analysis was performed considering only the water potentials (Table 2), both species under normal water availability had similar maximum photosynthetic rates ($P > 0.05$). However, when the two *Prosopis* species were subjected to a water deficit (−2.5 MPa) the maximum photosynthetic rate of algarrobo decreased to a value significantly lower than that of tamarugo (71%, $P \leqslant 0.05$; Table 2).

The combined factors (temperature and water potential) produced an increase in the light compensation point (LCP). In tamarugo, the light compensation point increased from 24.8 at 25 °C to 72.3 μmole photon at 40 °C with a water potential of −0.3 MPa (Table 1), figures substantially greater than those observed in algarrobo (31.9 at 25 °C to 41.9 μmole photon at 40 °C) with a water potential of −0.3 MPa. The combined action of water deficit (−2.5 MPa) and high temperatures (35 °C and 40 °C), produced an increase in the LCP of algarrobo, which increased from 24.9 at 25 °C to 59.9 μmole photon at 40 °C, while in tamarugo the LCP did not change. The maximum photosynthetic rates, measured at the light compensation point decreased with temperature and water deficit respect to control treatments ($P \leqslant 0.05$; Table 1). As in the case of the photosynthetic rates measured at the light saturation point, algarrobo subjected to 40 °C and −2.5 MPa has the lowest photosynthetic rate (Table 1).

Table 2
Effects induced by temperature and by water deficit on maximum photosynthetic rates of tamarugo (TAM) and algarrobo (ALG) measured at light saturation point (LSP)

Treatments	Maximum photosynthesis rate at LSP (μmole O_2 m^{-2} s^{-1})	Homogeneous group ($P \leqslant 0.05$)	Rates relative to control (%)
TAM 25 °C	24.3	c	100.0
TAM 35 °C	18.9	b	77.8
TAM 40 °C	12.5	a	51.4
ALG 25 °C	19.8	b	100.0
ALG 35 °C	13.2	a	66.7
ALG 40 °C	9.1	a	48.0
TAM −0.3 MPa	20.2	b	100.0
TAM −2.5 MPa	16.9	b	83.7
ALG −0.3 MPa	16.3	ab	100.0
ALG −2.5 MPa	12.0	a	73.6

Different letters denote significance of differences.

The analysis performed to determine the individual effects of temperatures or water potentials on the light compensation point allowed us to detect that water potential did not significantly affect the LCP in either species (Table 3). However, light compensation point increased considerably at 40 °C ($P \leqslant 0.05$) in both species (Table 3).

At 25 °C without water restrictions, algarrobo showed a higher water conductance rate than tamarugo (300 vs. 130 μmole water m^{-2} s^{-1}; Fig. 3). This is in agreement with the wider stomata and with the higher number of stomata per mm^2 of *P. chilensis* compared to *P. tamarugo* [35]. As the temperature increased (35 °C), the transpiration rate fell dramatically in algarrobo due to stomatal closure with a reduction in water loss of almost 94%. The conductance rate of tamarugo slightly increased with temperatures up to 30 °C. When the temperature increased to 35 °C the water conductance of this specie was reduced to 67%. At 40 °C both species had very similar and low conductance rates.

The quantum yield (QY) of tamarugo with normal water supply (−0.3 MPa) was not affected by temperature ($P > 0.05$) (Table 4). Algarrobo, however, under the same conditions of water availability, displayed a significant decrease in the QY at 40 °C ($P \leqslant 0.05$). When water stress was applied (−2.5 MPa) tamarugo was able to maintain its QY even at 40 °C (0.076 μmole O_2 [μmole photon]$^{-1}$),

Table 1
Combined effects induced by temperature and by water deficit on light saturation points (LSP), on light compensation points (LCP) and on photosynthetic rates measured at light saturation points and at light compensation points of tamarugo (TAM) and algarrobo (ALG) plants

Treatments	LSP (μmole O_2 μmole photon^{-1})	Maximum photosynthesis rates at LSP (μmole O_2 m^{-2} s^{-1})	Homogeneous groups ($P \leqslant 0.05$)	Photosynthesis rate relative to control	LCP (μmole photon)	Maximum photosynthesis rates at LCP (μmole O_2 m^{-2} s^{-1})	Homogeneous groups ($P \leqslant 0.05$)	Photosynthesis rate relative to control (%)
TAM 25 °C −0.3 MPa	500	23.5	e	100.0	24.8	27.9	f	100.0
TAM 35 °C −0.3 MPa	500	17.1	cde	72.8	39.7	19.0	de	68.1
TAM 40 °C −0.3 MPa	500	8.4	ab	35.7	72.3	14.5	cde	52.0
TAM 25 °C −2.5 MPa	500	18.0	de	76.6	30.9	22.1	ef	79.3
TAM 35 °C −2.5 MPa	500	16.8	cd	71.5	27.7	16.1	cde	57.7
TAM 40 °C −2.5 MPa	500	9.4	ab	40.0	36.3	13.1	ab	47.0
ALG 25 °C −0.3 MPa	500	17.9	de	100.0	31.9	22.7	ef	100.0
ALG 35 °C −0.3 MPa	500	10.4	abc	58.7	31.3	15.4	bcd	67.8
ALG 40 °C −0.3 MPa	500	9.8	ab	54.7	41.9	11.7	abc	51.5
ALG 25 °C −2.5 MPa	500	13.4	bcd	74.9	24.9	17.4	bcde	76.8
ALG 35 °C −2.5 MPa	500	11.0	abc	61.5	31.0	11.1	abcd	48.9
ALG 40 °C −2.5 MPa	200	2.6	f	14.5	59.9	7.9	a	34.8

Different letters denote significance of differences.

Table 3
Effects induced by temperature and by water deficit on light compensation points (LCP) of tamarugo (TAM) and algarrobo (ALG)

Treatments	LCP (μmole photon)	Homogeneous group ($P \leqslant 0.05$)	LCP relative to control (%)
TAM 25 °C	27.8	a	100.0
TAM 35 °C	33.7	a	121.2
TAM 40 °C	54.3	b	195.3
ALG 25 °C	28.4	a	100.0
ALG 35 °C	31.2	a	110.0
ALG 40 °C	50.9	b	177.0
TAM −0.3 MPa	45.6	a	100.0
TAM −2.5 MPa	31.6	a	69.0
ALG −0.3 MPa	35.0	a	100.0
ALG −2.5 MPa	38.6	a	110.0

Different letters denote significance of differences.

Table 5
Effects induced by temperature and by water deficit on quantum yield of tamarugo (TAM) and algarrobo (ALG)

Treatments	Quantum yield (μmole O_2 [μmole photon]$^{-1}$)	Homogeneous group ($P \leqslant 0.05$)	QY relative to control (%)
TAM 25 °C	0.081	bc	100.0
TAM 35 °C	0.088	c	108.6
TAM 40 °C	0.070	b	86.4
ALG 25 °C	0.069	b	100.0
ALG 35 °C	0.075	bc	108.7
ALG 40 °C	0.050	a	72.5
TAM −0.3 MPa	0.079	b	100.0
TAM −2.5 MPa	0.080	b	98.8
ALG −0.3 MPa	0.071	ab	100.0
ALG −2.5 MPa	0.059	b	83.0

Different letters denote significance of differences.

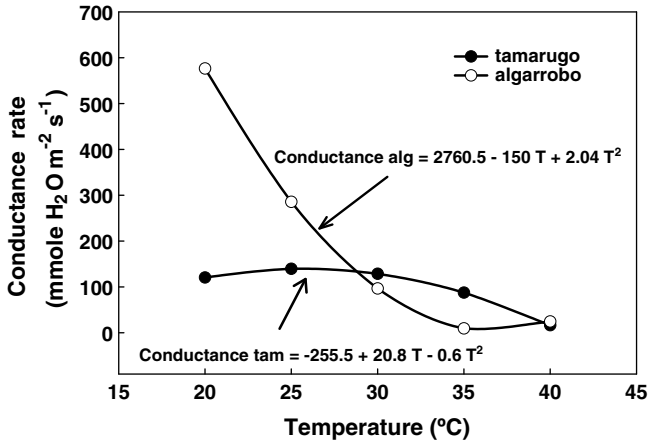

Fig. 3. Effects induced by temperature on the leaf conductance rates of tamarugo and algarrobo. The experiments were conducted at a water potential of −0.3 MPa. Each point was obtained by a regression curve calculated according to the equations shown in the figure.

while the QY of algarrobo decreased to 0.038 (μmole O_2 [μmole photon]$^{-1}$) at this temperature.

No significant differences in the QY were found between tamarugo and algarrobo at 25 °C and 35 °C when temperature without water deficit was considered in the analysis (Table 5). However, at 40 °C algarrobo had a lower QY than tamarugo ($P \leqslant 0.05$). This is in agreement with the photosynthetic rate determined at 40 °C. The water potential itself did not affect the QY in tamarugo (0.79 at −0.3 MPa to 0.80 at −2.5 MPa) whereas, in algarrobo QY fell from 0.71 at −0.3 MPa to 0.59 at −2.5 MPa decreasing to a 74% of the QY of the control (Table 5).

The quantum requirement (1/QY) is related to the energy needed to produce one mole of O_2 [31]. Water potential did not significantly affect the 1/QY at any temperature in the case of tamarugo. In algarrobo the 1/QY did not change with a water potential of −2.5 MPa and temperatures of 25 °C or 35 °C. In algarrobo, the 1/QY increased twice under a water deficit of −2.5 MP at 40 °C (Table 4). As with the QY, a water deficit with increasing temperature produced a decrease in the efficiency of energy storage in both species. In conclusion, the energy efficiency of tamarugo is less affected by water deficit compared with the energy efficiency of algarrobo.

Table 6 shows the combined effect of temperature and water potential on dark respiration. At −0.3 MPa the dark respiration of tamarugo increased with temperature. At 35 °C the respiration rate was almost 73% higher than that of the control at 25 °C. At 40 °C ($P \leqslant 0.05$) the dark respiration rate of tamarugo became twice the rate of the control. With a water potential of −2.5 MPa, the dark respiration rate of tamarugo did not change at all with temperature and had values similar to that of the control. In algarrobo the dark respiration rate did not significantly change with temperature at any water potential. When the analyses of dark respiration was performed for each stress factor individually (Table 7), the dark respiration significantly increased in tamarugo at 35 °C and 40 °C while in algarrobo the respiration rate remained unchanged.

Table 4
Combined effects induced by temperature and by water deficit on quantum yields (QY) and on quantum requirements (1/QY) of tamarugo (TAM) and algarrobo (ALG)

Treatments	QY (μmole O_2 [μmole photon]$^{-1}$)	Homogeneous group ($P \leqslant 0.05$)	1/QY (μmole photon [μmole O_2]$^{-1}$)	Homogeneous group ($P \leqslant 0.05$)	Energy by photons (kJ)	Theoretical minimal * Energy efficiency (%)
Theoretical minima					1.408	34.0
TAM 25 °C −0.3 MPa	0.084	b	12.0	ab	2.094	22.9
TAM 35 °C −0.3 MPa	0.090	b	11.6	a	1.954	24.5
TAM 40 °C −0.3 MPa	0.064	ab	16.3	abc	2.746	17.4
TAM 25 °C −2.5 MPa	0.079	b	13.0	abc	2.235	21.4
TAM 35 °C −2.5 MPa	0.085	b	12.1	ab	2.077	23.1
TAM 40 °C −2.5 MPa	0.076	b	14.4	abc	2.323	20.6
ALG 25 °C −0.3 MPa	0.076	b	13.4	abc	2.323	20.6
ALG 35 °C −0.3 MPa	0.074	b	15.6	abc	2.376	20.2
ALG 40 °C −0.3 MPa	0.057	c	17.4	c	3.080	15.6
ALG 25 °C −2.5 MPa	0.063	c	16.0	abc	2.798	17.1
ALG 35 °C −2.5 MPa	0.077	b	14.1	abc	2.288	20.9
ALG 40 °C −2.5 MPa	0.038	a	26.8	d	4.629	10.3

Theoretical minimal* = Walker [31].
Respect to storage energy by evolved O_2 = 479 kJ.
Different letters denote significance of differences.

Table 6
Combined effects induced by temperature and by water deficit on dark respiration rate of tamarugo (TAM) and algarrobo (ALG)

Treatments	Dark respiration rate (μmole of evolved O_2 m^{-2} s^{-1})	Homogeneous groups ($P \leqslant 0.05$)	Rate relative to control (%)
TAM 25 °C −0.3 MPa	2.2	a	100.0
TAM 35 °C −0.3 MPa	3.8	b	172.7
TAM 40 °C −0.3 MPa	4.7	c	213.6
TAM 25 °C −2.5 MPa	2.7	ab	122.7
TAM 35 °C −2.5 MPa	2.4	ab	109.1
TAM 40 °C −2.5 MPa	2.8	ab	127.3
ALG 25 °C −0.3 MPa	2.5	ab	100.0
ALG 35 °C −0.3 MPa	2.2	ab	88.0
ALG 40 °C −0.3 MPa	2.7	ab	108.0
ALG 25 °C −2.5 MPa	1.6	a	64.0
ALG 35 °C −2.5 MPa	2.4	ab	96.0
ALG 40 °C −2.5 MPa	2.3	ab	92.0

Different letters denote significance of differences.

Table 7
Effects induced by temperature and by water deficit on dark respiration rates of tamarugo (TAM) and algarrobo (ALG)

Treatments	Dark respiration rates (μmole of evolved O_2 m^{-2} s^{-1})	Homogeneous group ($P \leqslant 0.05$)	Rates relative to control (%)
TAM 25 °C	2.3	c	100.0
TAM 35 °C	3.0	ab	130.4
TAM 40 °C	3.7	ab	161.0
ALG 25 °C	2.0	c	100.0
ALG 35 °C	2.2	c	110.0
ALG 40 °C	2.5	c	125.0
TAM −0.3 MPa	3.5	a	100.0
TAM −2.5 MPa	2.5	b	71.0
ALG −0.3 MPa	2.4	b	100.0
ALG −2.5 MPa	2.1	b	87.5

Different letters denote significance of differences.

Table 8
Combined effects induced by temperature and by water deficit on photochemical efficiency (F_v/F_m) of tamarugo (TAM) and algarrobo (ALG)

Treatments	F_v/F_m	Homogeneous groups ($P \leqslant 0.05$)	F_v/F_m relative to control%
TAM 25 °C −0.3 MPa	0.86	cd	100.0
TAM 35 °C −0.3 MPa	0.84	bcd	97.6
TAM 40 °C −0.3 MPa	0.82	b	95.3
TAM 25 °C −2.5 MPa	0.85	bcd	98.9
TAM 35 °C −2.5 MPa	0.83	b	96.5
TAM 40 °C −2.5 MPa	0.76	a	88.4
ALG 25 °C −0.3 MPa	0.86	d	0.0
ALG 35 °C −0.3 MPa	0.84	bcd	97.6
ALG 40 °C −0.3 MPa	0.83	bc	96.5
ALG 25 °C −2.5 MPa	0.83	bc	96.5
ALG 35 °C −2.5 MPa	0.82	b	95.3
ALG 40 °C −2.5 MPa	0.77	a	89.5

Different letters denote significance of differences.

The water potential reduced the dark respiration rate in the case of tamarugo to 71% of the control ($P \leqslant 0.05$).

The photochemical efficiency of photosystem II (PSII) was estimated according to Havaux [36], based on the F_v/F_m ratio. The combine effect of heat stress and water deficit affects the photochemical efficiency (Table 8) in both species. In tamarugo the F_v/F_m fell from 0.86 at 25 °C and −0.3 MPa to 0.76 at 40 °C and −2.5 MPa, translating into an 11.6% reduction in the photochemical efficiency of this species. Similarly, in algarrobo the ratio fell from 0.86 at 25 °C and −0.3 MPa to 0.77 at 40 °C and −2.5 MPa with a 10.5% reduction in the photochemical efficiency which was not significantly different to tamarugo. Together, heat shock and water deficit, had a more severe impact on the PSII than each stress separately.

Table 9
Effects induced by temperature and by water deficit on photochemical efficiency (F_v/F_m) of tamarugo (TAM) and algarrobo (ALG)

Treatments	F_v/F_m	Homogeneous group ($P \leqslant 0.05$)	F_v/F_mrelative to control (%)
TAM 25 °C	0.85	b	100.0
TAM 35 °C	0.83	b	97.6
TAM 40 °C	0.80	a	94.1
ALG 25 °C	0.84	b	100.0
ALG 35 °C	0.83	b	98.8
ALG 40 °C	0.80	a	95.2
TAM −0.3 MPa	0.84	bc	100.0
TAM −2.5 MPa	0.82	ab	97.6
ALG −0.3 MPa	0.84	c	100.0
ALG −2.5 MPa	0.80	a	95.2

Different letters denote significance of differences.

The analysis performed considering only the temperature (Table 9) showed that there was no effect on the F_v/F_m at 35 °C. At 40 °C there was a significant decrease in the F_v/F_m in both species ($P \leqslant 0.05$) indicating a negative effect of heat shock on PSII. In tamarugo, the ratio decreased from 0.85 to 0.80 and, in algarrobo, from 0.84 to 0.80. Water deficit also decreased the F_v/F_m from 0.84 to 0.82 in tamarugo while in algarrobo the decrease of F_v/F_m was from 0.84 to 0.80 (Table 9).

The effects of temperature and water deficit on the RUBISCO content were determined as a mean to detect the effects of these two stress conditions on the principal enzyme participating in CO_2 assimilation. For this, the large and small subunits of RUBISCO were purified preparatively from leaves of plants subjected to different temperatures and to two water potentials. Fig. 4A shows the combined effects of temperature and water deficit on the RUBISCO content. Under normal irrigation the amount of the enzyme does not change in tamarugo with increasing temperature. In algarrobo the amount of RUBISCO increased by 153% at 35 °C returning to normal levels at 40 °C (Fig. 4A and B). Under water deficit, the amount of RUBISCO decreased in both species at 35 °C or 40 °C becoming 37% of the control at 40 °C and −2.5 MPa in tamarugo and 53% of the control at 40 °C and −2.5 MPA in algarrobo (Fig. 4A and C). This decrease was similar to that observed if only a water deficit was applied (Fig. 4C).

4. Discussion

From the analysis of the light saturation curves it is possible to conclude that tamarugo plants grown at 25 °C and −0.3 MPa have the highest photosynthetic rates under the different photon flows applied. The maximum photosynthetic rates decreased with increasing temperature in both species. Superimposing a water deficit reduces the photosynthetic rates in both species, affecting algarrobo more severely. If a water deficit and an increase in temperature were applied simultaneously, the photosynthetic rates fell in tamarugo and algarrobo beyond those obtained at 35 °C and −0.3 MPa. At 40 °C and −2.5 MPa the photosynthetic rate of tamarugo was reduced by 60% compared to that of 35 °C without water deficit. However, the photosynthetic rate of algarrobo fell by 80% under the same conditions, showing a greater susceptibility to extreme temperatures if a water deficit is also applied. These findings demonstrate that the photosynthetic processes are differently affected by these stresses. As Fufezan et al. [37] have indicated the photosynthetic rates at light saturation are greatly influenced by temperature. Thus, low and high temperatures

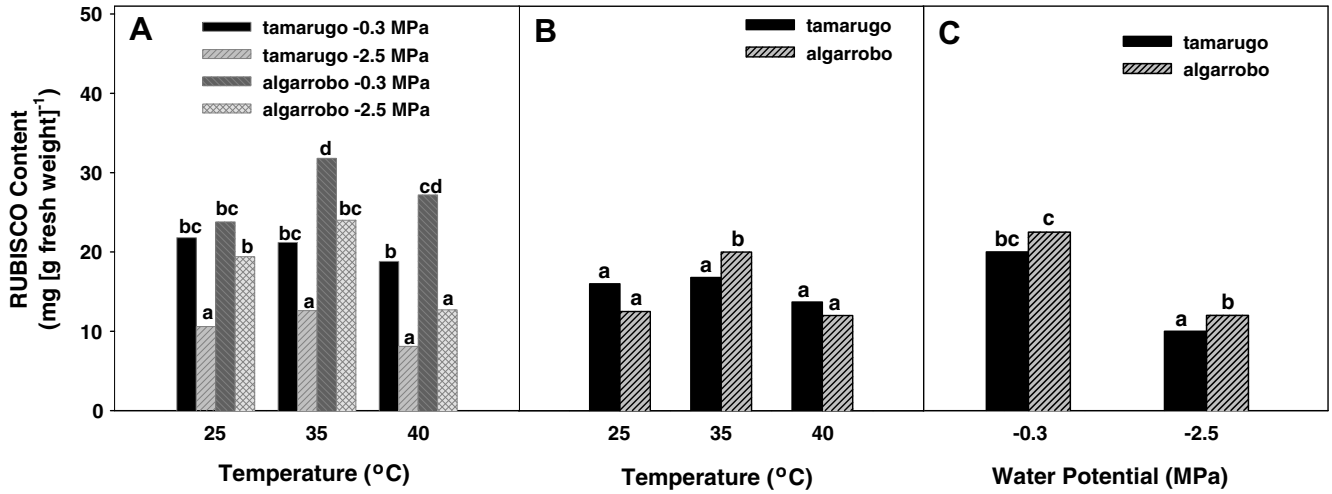

Fig. 4. Effects induced by temperature and water potential on the content of RUBISCO of tamarugo and algarrobo. (A) The combined effect of temperature and water potential on the content of RUBISCO. (B) The effect of temperature on the content of RUBISCO. (C) The effect of the water potentials on the content of RUBISCO. The bars are the average of three independent experiments. The significance of differences are denoted by different letters.

reduce the photosynthetic rates while intermediate temperatures maintain the photosynthesis rate at maximum. Consistent with our results, the temperature effect also depend on the plant species [38]. Temperature seems to affect similarly the enzymatic processes of photosynthesis since the amount of RUBISCO became maximal in algarrobo at the intermediate temperature of 35 °C. Others Calvin Cycle enzymes are probably affected in a similar manner by temperature and high temperature could also influence the enzyme complex involved in the photolysis of water and the D1 and D2 proteins of PSII. It has been reported [3,37] that D1 and D2 proteins are separated from the light harvesting complex when the temperature increases, resulting in interrupted in electron transport of PSII.

The greater photosynthetic rate reduction of algarrobo compared with that of tamarugo seems to be corroborated by the data of the light compensation points. In both species the light compensation point showed an increase in value when temperature increased to 40 °C. However, at 40 °C and −2.5 MPa the light compensation point of algarrobo was considerably greater than that of tamarugo under the same conditions. This increase in the light compensation point of algarrobo at high temperature and water deficit requires a greater of photon flow to reach the equilibrium between the O_2 liberated in photosynthesis and the O_2 absorbed by dark respiration. Since the rate of dark respiration does not significantly change in algarrobo under these conditions, we conclude that photosynthesis provided more energy for algarrobo than dark respiration, whereas the reverse is true for tamarugo.

A similar situation to tamarugo has been reported [39] for *Prosopis juniflora* under field conditions at a temperature of 40 °C. One possible explanation for this could be that high temperature reduces the capture of energy by the light harvesting complex, or that the electron flux is partially interrupted at the PSII reaction centre and, therefore, this species requires more photons to achieve the photolysis of the water.

The higher respiration rate of tamarugo could be also the consequence of a higher metabolism induced by the increasing temperature, as has been reported by Farrar [40] and by Luyssaert et al. [38].

Water deficit, in turn, seems not to affect the LCP beyond the temperature effect, except in algarrobo at 40 °C. Acevedo et al. [5,6] reported that *P. tamarugo* plants were able to carry out osmotic adjustment when treated at −3.00 MPa for 210 days. This is in agreement with the results of the present work, since tamarugo seems to tolerate the water deficit well and better than algarrobo. Extreme conditions of 40 °C and −2.5 MPa are present in the natural environment where tamarugo is found (Pampa del Tamarugal). Measurements of the water potentials of small branches in tamarugo under natural conditions showed that these became as low as −5.00 MPa at hours of the day (12.00AM–4.00PM) when the temperature is as high as 36–37 °C in the afternoon of spring days [1,6]. Under these conditions, *P. tamarugo* has a CO_2 assimilation rate higher than *P. chilensis* which seems to be photorespiring at this time [1]. The photorespiration rate of algarrobo at 35–37 °C during afternoon hours of spring days in the Atacama Desert agrees with the higher conductance rates of algarrobo stomata at moderate temperatures and normal water availability. Algarrobo has wider and more stomata per mm^2 of leaf than tamarugo [35]. Other mesquites and C3 plants grown in desert conditions such as *P. glandulosa* [41] and olive trees [42] show similar relationships between photosynthetic rates and stomata conductance.

The results of photochemical efficiency suggest that PSII is affected more under water deficit and high temperature than under one of these two stresses. This suggests that water deficit and high temperature might be influencing different processes or different levels of the same process of PSII or activating different signaling pathways causing reduction in photochemical efficiency. Osmotic stress produced inactivation of PSI and PSII decreasing the cellular volume in the cyanobacteria *Synnechoccocus*. Such effects were reversible when the osmolyte sorbitol, was removed from the experiment [43]. Since osmotic stress causes a water deficit, we speculate that under water stress PSI and PSII might be partially inactivated in *Prosopis* sp. Since tamarugo tolerate the water deficit more effectively, the inactivation of PSI and PSII by lack of water might be reduced in this species as compared with algarrobo.

Quantum yield significantly decreases with temperature and with water potential for algarrobo ($P \leqslant 0.05$). At 40 °C and −2.5 MPa the QY decreased dramatically in algarrobo to a value lower than those recorded when each factor is considered separately, indicating that the effect of these two stresses are additive on algarrobo. This also suggests that each stress condition is activating different signaling transduction pathways. It is known that heat shock disrupt the membrane bound complexes of mitochondria decreasing the electron transport [44]. Similar to mitochondria the membrane bound complexes of chloroplasts might be disrupted decreasing the electron transport between both photosystems.

This, in turn will increase the triplet state of chlorophyll a [37]. As a consequence there is an induction of oxidative stress with the formation of reactive oxygen species (ROS) since the electrons from water are not reducing the chlorophylls a of the Reaction Center. This may explain the great decay to 50% in the QY of algarrobo at 40 °C and −2.5 MPa with respect to control plants. Temperature will also affect membrane fluidity and probably affects the Chloroplast enzymes, such as the enzyme complex which performs the photolysis of water and those participating in the Calvin cycle.

Under both stress conditions, the quantum requirement of algarrobo is in the order of 26.8 μmole photons [μmole O_2]$^{-1}$. The minimum thermal requirement is 8 photons per fixed CO_2 or evolved O_2[45,47]. If red light (680 nm) is used as a light source with an energy equivalent of 176 kJ mole^{-1}, then 8 mole of photons correspond to 1.408 kJ of energy. If 479 kJ is estimated to be the storage energy per mole of evolved O_2, then the theoretical efficiency of the energy conversion will be equal to 34% under conditions of 25 °C, pH7, and one atmosphere of pressure (479 kJ/1408 kJ × 100%) [33]. This is the theoretical efficiency under which the photosynthetic system could work.

In the present work, the photosynthetic rates correlated with the energy requirement or quantum requirement (1/QY) which in tamarugo increases from 12 μmole photons m^{-2} s^{-1} at 25 °C and −0.3 MPa to 14.4 μmole photons m^{-2} s^{-1}, when the conditions were 40 °C and −2.5 MPa ($P \leqslant 0.05$). In algarrobo the 1/QY doubled from 13.4 μmole photons m^{-2} s^{-1} at 25 °C and −0.3 MPa to 26.8 μmole photons m^{-2} s^{-1} at 40 °C and −2.5 MPa ($P \leqslant 0.05$). These results are also in agreement with a decrease in the light saturation point of algarrobo (200 μmole photons m^{-2} s^{-1}) under these extreme conditions.

As has been reported [8], diverse factors can affect the quantum yield, which in turn alter the photosynthetic efficiency for energy conversion. Ehleringer and Bjorkman [48] and Ehleringer et al. [49] showed that the QY and therefore the 1/QY of C3 plants, are affected by high temperature due to a change in gas solubility, thus changing the CO_2 and O_2 concentrations in the cell. If so, this would explain the increase in the dark respiration rate of tamarugo a 40 °C and −0.3 MPa. The QY can also be changed by the radiation levels under which the plants are grown; such changes are similar in all C3 plants [50].

In this study, temperature and water deficit did not affect the energy efficiency of tamarugo. However, the energy efficiency of algarrobo fell by 50% of the control at 40 °C and −2.5 MPa. The decay of the photosynthetic efficiency of algarrobo under these conditions affects the energy conversion and, therefore, 1/QY increased because the evolution of one mole of O_2 requires more energy.

The dark respiration rate does not change with the water potential in either species when the temperature was 25 °C, although it was affected at 40 °C. In tamarugo, the dark respiration at 40 °C is almost twice that achieved at 25 °C (3.7 vs. 2.3 mmole O_2 m^{-2} s^{-1}). It is possible that under these conditions tamarugo requires extra energy for metabolic processes since higher temperatures accelerate metabolism. These results are coincident with the higher energy requirement of tamarugo at 40 °C (2.869 kJ) compared to that at 25 °C (2.112 kJ).

A respiratory rate maintained by plants under water stress could be related to the results reported [51,52] where it was found that water deficit makes plants more tolerant to high temperatures; in other words, water deficit seems to provide cross protection to plants to extreme temperatures [52,53], suggesting that this cross protection may be given by proteins of PSII and by changes in the composition of chloroplast lipids. If water stress changes the lipid composition of thylakoids, the susceptibility of chloroplasts to respond to temperature fluctuations will be altered, affecting the physiology of photosynthesis since lipid composition is related to membrane fluidity. Similar changes may also affect the mitochondrial membranes and, therefore, the dark respiration rates will be altered.

The cross protection provided by water deficit could also be related to heat shock proteins whose levels increase in the cell and in chloroplasts and mitochondria under various stress conditions [1,3,53]. If this is the case, tamarugo will be more tolerant to desert conditions than algarrobo. However, algarrobo seems to be a tree highly tolerant to temperature stress [2] with significant accumulation of the heat shock proteins HSP70 and ubiquitin. Our results, however, suggest that algarrobo is more susceptible to water stress than tamarugo, and therefore, the cross protection, if there is any, is more efficient in tamarugo than in algarrobo. The greater susceptibility of algarrobo to water stress might also be related to the higher conductance rates of algarrobo stomata and the fact that under mild field conditions algarrobo photorespires.

The increase of respiratory activity in tamarugo under normal water availability and higher temperature is probably due to the energy requirement demanded by a higher metabolic activity. Such metabolic demand occurs because many enzymes are more active at intermediate temperatures and because there is synthesis of protective proteins under stress such as the heat shock proteins [53]. At the same time that synthesis of protective proteins occurs, in most organisms, the synthesis of house-keeping proteins stops [44]. However, in a study of the pattern of synthesized proteins with ^{35}S-methionine, it was found that in algarrobo the synthesis of heat shock proteins occurs without decreasing the synthesis of house-keeping proteins present at normal conditions [2].

An increase in metabolic activity during heat shock also occurs because the composition of membranes lipids changes. Fatty acids of the bipolar lipids will change from unsaturated to saturated fatty acids to stabilize membrane fluidity. It is important to note that both species at 25 °C did not differ in their respiration rates. The difference between tamarugo and algarrobo was first detected at 35 °C, and was maximal at 40 °C ($P \leqslant 0.05$). At this temperature and under illumination *P. chilensis* photorespires while *P. tamarugo* does not, [1]. It might well be that less photorespiratory activity provides tamarugo with natural tolerance to water deficit but makes it more susceptible to heat shock. This susceptibility to heat shock may induce a higher dark respiration rate. Recently López and Cardemil (2008) [54] have shown that *P. tamarugo* has less HSP70 and ubiquitin expression than *P. chilensis*, indicating that algarrobo is better protected than tamarugo to heat shock, at least by the protection conferred by heat shock proteins.

The photochemical efficiency of PSII was estimated according to Havaux [36] based on the F_v/F_m ratio. The ratio was not affected at 35 °C when plants from either species were well irrigated. This is also in agreement with the notion that plant photosynthesis is not affected up to 38 °C if the water status is normal [36]. At 40 °C both species showed a significant decrease ($P \leqslant 0.05$) in the F_v/F_m ratio indicating that heat shock affects PSII. The combined temperature-water stress produced a more severe effect on PSII decreasing the F_v/F_m ratio by 11.6% and 10.5% for tamarugo and algarrobo respectively. From this, it is possible to conclude that both stress conditions are affecting different components of PSII or affecting PSII through different signalling pathways.

High temperatures seem not to affect the biochemical fixation of CO_2 in algarrobo. At 35 °C there is a positive effect on the amount of RUBISCO in algarrobo although at 40 °C the amount of RUBISCO is back to normal levels. However, water deficit decreases the amount of RUBISCO in both species suggesting that this stress affects either the turnover or the synthesis of the enzyme. It remains to be determined whether water deficit also affects other enzymes of the Calvin cycle in algarrobo and tamarugo. These

results are in agreement with Lawlor et al. who demonstrated that water deficit decreases RUBISCO biosynthesis.

From our results, it is possible conclude that:

1. Most of the photosynthetic parameters studied in this research were affected by temperature and water deficit.
2. The effects on photosynthesis processes, such as phosynthesis rates, quantum yield, quantum requirements, photochemical efficiency, and the amount of RUBISCO by water deficit or by temperature, when separated, were milder than those induced by both stress conditions combined.
3. A water deficit applied at 40 °C severely affected algarrobo photosynthesis. However, under normal water conditions algarrobo photosynthesis is at the level of tamarugo photosynthesis at all temperatures applied.
4. Dark respiration rates do not change in algarrobo either under water deficit, under high temperature or under both stress conditions.
5. Dark respiration significantly increases in tamarugo with temperature at any water status. This increase in dark respiration is probably related to a higher energy requirement of this species to maintain metabolic processes. This is coincident with a mayor quantum requirement of tamarugo at 40 °C (2.869 kJ) as compared with the quantum requirement at 25 °C (2.112 kJ).
6. The photochemical efficiency of PSII is affected equally in both species with temperature and water deficit, with a greater effect when both stress conditions are combined.
7. The amount of RUBISCO is more greatly affected in tamarugo than in algarrobo under water deficit conditions. This suggests that in tamarugo the biochemical processes are more severely influenced by water deficit, while in algarrobo the photochemical processes are more severely influenced by this stress.

5. Abbreviations

QY	quantum yield
1/QY	quantum requirement
PSII	photosystem II
PFD	photon flux density

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