DOI: 10.1007/s11099-018-0794-8 PHOTOSYNTHETICA 56 (1): 468-477, 2018

Adaptation strategies of two leaf cohorts of *Prosopis juliflora* produced in spring and monsoon

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Abstract

Prosopis juliflora is an invasive leguminous tree species growing profusely under wide environmental conditions. Primary objective of this study was to investigate adaptation strategies evolved to deal with wide environmental conditions during different seasons. *P. juliflora* adapts through a production of leaves in two seasons, namely, the spring (the first cohort) and monsoon (the second cohort) with differing but optimal physiological characteristics for growth in respective seasons. Our studies show that the first cohort of leaves exhibit maximum carbon fixation under moderate temperatures and a wide range of PPFD. However, these leaves are sensitive to high leaf-to-air-vapor pressure deficit (VPD) occurring at high temperatures in summer resulting in senescence. While the second cohort of leaves produced during monsoon showed maximum carbon fixation at high irradiance and temperatures with low VPD, it is sensitive to low temperatures causing senescence in winter.

Additional key words: carbon assimilation; carboxylation efficiency; dark respiration rate; quantum efficiency; tree photosynthesis; water-use efficiency.

Introduction

Prosopis juliflora (Swartz), a leguminous tree, native of South America has been classified as an aggressive species and has invaded large areas in Africa, Australia, Sudan, and India (Pasiecznik 2001). It is a useful tree because it provides fuel-wood to village population as it grows profusely under varying seasonal conditions. In northern India, it grows in regions that have high temperatures and high light intensities for most of the year. However, during winter (December–January), the night temperatures fall to 2°C, while during summer (May–June), the day temperatures often exceed 45°C (Shirke and Pathre 2003, 2004a). Thus, trees of *P. juliflora* must adapt to these temperature extremes, especially, to the high temperatures in summer coupled with high leaf-to-air VPD. In our earlier and subsequent studies (Pathre *et al.* 1995, 1998), using diurnal gas-exchange and chlorophyll (Chl) fluorescence characteristics (Shirke 2001, Shirke and Pathre 2003), the physiological and biochemical changes related to sucrose-phosphate synthase and Rubisco (Sinha *et al.* 1998, Pathre *et al.* 2004, Shirke and Pathre 2004b) and the influence of VPD on the biochemistry and physiology of *P. juliflora* were determined (Shirke and Pathre 2004a).

Although *P. juliflora* has evergreen appearance, close observation showed that it produces two distinct cohorts of leaves, the first one in the spring (February–March), which declines gradually and senesces in summer (June–July), while the second cohort is produced during monsoon (June–July) and senesces in winter (December–January). We wondered if the two cohorts possess distinct characteristics related to photosynthesis suiting to the season, enabling the plant better competitive advantage over other trees for its survival. In the present study, we measured diurnal changes of several gas-exchange parameters, such as net photosynthesis (P_N), stomatal conductance (g_s), transpiration (E), water-use efficiency (WUE), dark respiration (R_D), and carboxylation efficiency (CE), in two distinct cohorts of *P. juliflora* produced during the optimal growth periods of spring and monsoon and during the period of their senescence. The study indicates peculiar adaptation and performance of *P. juliflora* throughout the year under widely differing environmental conditions.

Received 28 August 2017, *accepted* 19 February 2018, *published as online-first* 1 March 2018.

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Abbreviations: CE – carboxylation efficiency; Chl – chlorophyll; C_i – substomatal CO_2 concentration; E – transpiration; g_s – stomatal conductance to H_2O; P_N – net photosynthetic rate; P_{NLCP} – light-compensation point; P_{NLSP} – light-saturation point; P_{Nmax} – maximum photosynthetic rate; P_{NQE} – apparent quantum efficiency; R_D – rate of mitochondrial respiration in darkness; VPD – leaf-to-air-vapor pressure deficit; WUE – water-use efficiency; Γ – CO_2-compensation point.

Acknowledgements: The authors wish to thank the Director CSIR–NBRI for providing the necessary facility.

Materials and methods

Plant material and growth conditions: Six-to-eight-month-old, 20 cm tall seedlings of *Prosopis juliflora* (Swartz) DC. were transplanted to 10,000 cm^3 earthenware pots containing garden soil and organic manure (3:1) in early August and placed outdoors, at the terrace garden of the Institute at Lucknow, under natural light, temperature, and humidity conditions. During the experiments, plants were kept well-watered and fertilized fortnightly with Hoagland solution. Plants were grown for 18–24 months under these conditions and then were used for the present study. Each year a new set of plants 18–24 month old was used for the measurements. *P. juliflora* has a shallow root system and therefore the potted plants had comparable growth and photosynthesis as under field conditions. The new leaves of *P. juliflora* are produced with the onset of spring in February; it forms the first set of cohort, while the second cohort leaves are produced during monsoon, which begins in July. The leaves generally take about a month to attain their maturity.

Diurnal measurements of gas-exchange parameters: The gas-exchange measurements were made diurnally from pre-dawn to dusk in each month for three consecutive years. A total of more than 46 diurnal curves were obtained over a period of three years. However, the data obtained only on clear days were considered for analysis. For measurement, a mature leaf of 1.5–2 year old plant was enclosed in a 1,000-cm^3 leaf chamber of *LI-6200* portable photosynthesis systems (*Li-Cor Inc*., Lincoln, Nebraska, USA) under ambient conditions and three measurements on each leaf were recorded; 3–5 leaves were measured for each time point. The relative humidity during this period inside the chamber was maintained at 1–3% higher (to compensate boundary layer conductance) to that of outside environment by adjusting the air flow through the desiccant as described earlier (Shirke and Pathre 2004a). Dark respiration (R_D) was measured on a set of different leaves of the same plant by covering the chamber completely with black cloth after the measurements of photosynthetic parameters.

For the analysis of total CO_2 fixed, water transpired, water-use efficiency, and carbon lost by respiration per day in each month in response to the seasonal change in environment, data were obtained from the integrated area of the diurnal curves averaged from the data monitored over three-year period in the corresponding months.

Effect of light, temperature and CO_2: In order to analyze the photosynthesis response in the leaves of different cohorts to environmental factors, such as light and temperature under natural conditions, the light- and temperature-response curves were generated using diurnal data measured in months of March and May (the first cohort) and that in July and January (the second cohort). P_N were plotted against PPFD (400–700 nm), the nonrectangular hyperbola was fitted, and the apparent quantum-use efficiency (P_{NQE}), the light-saturation point (P_{NLSP}), light-compensation point (P_{NLCP}), and the maximum photosynthetic rate (P_{Nmax}) were calculated from the light-saturation curve using the "*AC_i and AQ Response Curve Analysis Software* (*version 1.0, Li-COR, 2/2008*)". Similarly the temperature response to photosynthesis was analyzed from the measurements made between 8.00–12.00 h, and only those readings were considered where the PPFD was between 1,000–1,400 μmol(photon) m^{-2} s^{-1}.

In order to determine the carboxylation efficiency in leaves of the two cohorts, P_N/C_i response curves were generated. The P_N/C_i response studies were carried out according to McDermitt *et al.* (1989). A fully expanded leaf was enclosed in a 250-cm^3 leaf chamber of *Li-6200* system in open mode (Shirke and Pathre 2004a) and was allowed to reach a steady-state. The system was then closed and the draw-down rate of CO_2 was recorded until the CO_2-compensation point (Γ) was reached. The data for P_N and substomatal CO_2 concentration (C_i) were computed for every 5 μmol mol^{-1} draw-down of CO_2. Each measurement of P_N/C_i required approximately 30–45 min.

Statistical analysis: All values reported are mean of at least three–five independent experiments. The means ± SD are shown in the figure or the table unless mentioned otherwise. The levels of significance were compared between leaves of the first cohort *vs*. leaves of the second cohort of *P. juliflora* by analysis of variance (*ANOVA*), performed with *MS Excel* and multivariate analysis of variance (*MANOVA*) using *SAS 9.1* (*SAS institute Inc. Cary*, NC, USA). Standard polynomial quadratic functions were fitted to observed responses of P_N to increasing PPFD, leaf temperature, and C_i using *SigmaPlot* (*ver. 9.0.1*).

Results

Annual course of photosynthetic parameters in response to environmental parameters: *P. juliflora* remains productive throughout the year because of the presence of functional leaves related to two cohorts that overlap. Summer is the most adverse season with high temperature and high VPD. It is followed by a favourable monsoon season when the temperatures are moderately high but VPD is low (Fig. 1*A*,*B*) and light intensity is high and of a longer duration. The appearance of young leaves of the second cohort during this season produces P_{Nmax} reaching as high as 475 and 410 mmol(CO_2) m^{-2} d^{-1} in August and September, respectively. As expected, *E* was

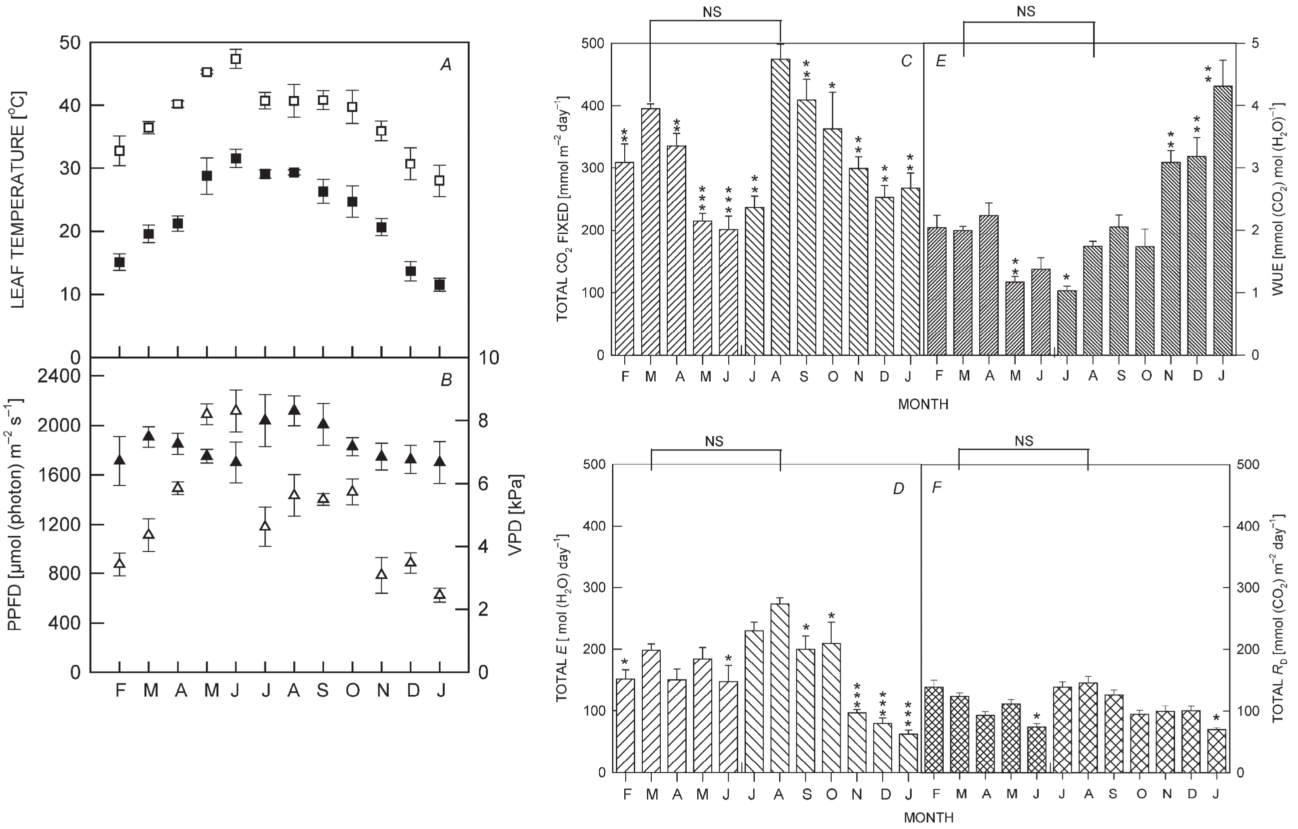

Fig. 1. Annual course of the daily minimum and maximum of photosynthesis related to environmental parameters. (*A*) maximal (□) and minimal (■) leaf temperature, (*B*) maximal photosynthetic photon flux density (PPFD) (▲), maximal leaf-to-air vapour pressure deficit (VPD) (△). Annual course of (*C*) the total CO_2 fixed per day, (*D*) total daily water loss by transpiration, (*E*) the total water-use efficiency (WUE), and (*F*) total CO_2 respired, calculated by integrating the diurnal net photosynthesis, transpiration, and the dark respiration (R_D) curves. SD is calculated from data of leaves of at least twelve independent plants over a period of three years for panels *A*, *B*, *C*, *D,* and *E*. SD is from data of leaves of four independent plants over a period of one year for panel *F*. *ANOVA* was performed for pairs of corresponding values of March with values in different months within the leaves of the first cohort and between August and other months within the leaves of the second cohort and between values of March and values of August of leaves of *Prosopis juliflora*. Significantly different values are indicated as (*P<0.05), (**P<0.001), and (***P<0.0001), while NS signifies insignificant differences at the P>0.05.

also high (Fig. 1*D*) but the WUE (Fig. 1*E*) during August to October was around 2 mmol(CO_2) mol(H_2O)$^{-1}$. The photosynthesis significantly decreased gradually in subsequent months in October–November (autumn) giving around 300 mmol(CO_2) m^{-2} d^{-1} and reached minimum in December [253 mmol(CO_2) m^{-2} d^{-1}] due to a decrease in temperatures (Fig. 1*A*) that lead to senescence of the leaves. The *E* was low due to lower temperatures and consequently the WUE was rather high reaching above 4 mmol(CO_2) mol(H_2O)$^{-1}$ in January.

R_D, which contributes to loss of carbon, did not differ in different months although in general it was high in the younger leaves in the spring and monsoon and low in the older leaves (Fig. 2*F*).

Diurnal changes in photosynthetic parameters in two cohorts of *P. juliflora*: The diurnal changes in the gas-exchange and environmental parameters for new leaves (March and August) and senescing leaves (June and January) of the first and second cohort are shown for data obtained from one year in Fig. 2. The diurnal PPFD pattern indicates that PPFD was more than saturating for most of the day throughout the year (Fig. 2*A*,*D*,*G*,*J*). In monsoon, the highest PPFD of around 2,000 μmol(photon) m^{-2} s^{-1} was observed, while in other seasons it was around 1,600 μmol(photon) m^{-2} s^{-1}. Unlike PPFD diurnal pattern, temperature showed substantial variation in monsoon and winter leaves (Fig. 2*H*,*K*). The leaf temperatures of the second cohort of leaves in monsoon varied from 30 to 41°C, while in winter it varied from 10 to 26°C. The leaf temperature of the first cohort of leaves varied from 20 to 37°C in the spring (Fig. 2*B*) and 26 to 43°C in summer (Fig. 2*E*). The diurnal pattern of VPD was similar in the spring and monsoon (1–4.5 kPa). It was higher in summer (1.5–7.0 kPa) and the lowest (0.5–2.5 kPa) in winter (Fig. 2*C*,*F*,*I*,*L*).

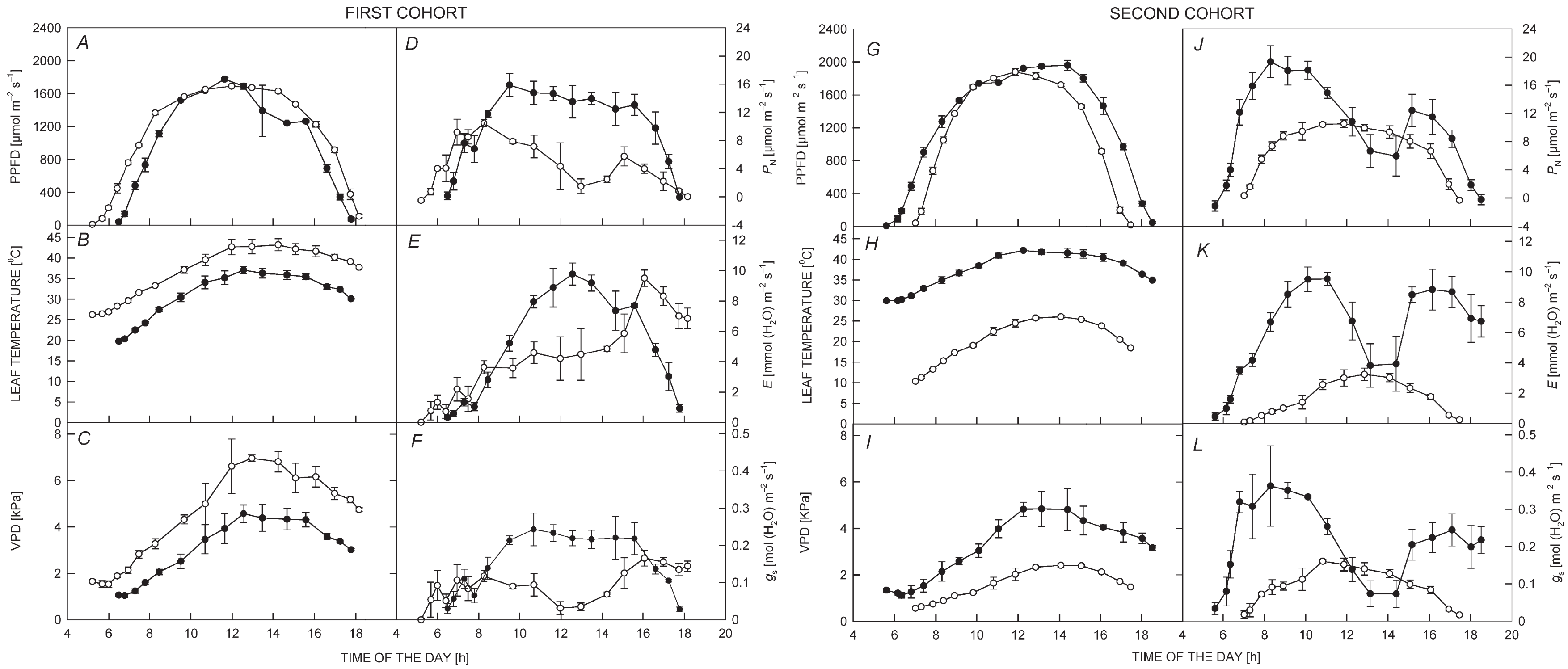

Fig. 2. Diurnal variations in photosynthetic parameters in *Prosopis juliflora* leaves of the (*A–F*) first cohort in the months of March, spring (*filled symbol*), May, summer (*hollow symbol*), and (*G–L*) second cohort in August, monsoon (*filled symbol*) and January, winter (*hollow symbol*). (*A*,*G*) Irradiance (PPFD), (*B*,*H*) leaf temperature, (*C*,*I*) leaf-to-air vapour pressure deficit (VPD), (*D*,*J*) net photosynthetic rate (P_N), (*E*,*K*) transpiration (*E*), and (*F*,*L*) stomatal conductance (g_s) ± SD is from data of leaves of four independent plants obtained from one year.

Leaves of the first cohort: During the spring, as the temperature and VPD increases after the winter, new leaves of the first cohort start appearing in February and simultaneously the old leaves start shedding and by March the plants are full of mature new leaves. In March, the leaf temperatures reach 33°C and the rates of photosynthesis reach above 16 μmol(CO_2) m^{-2} s^{-1} (Fig. 2*A*,*J*). The diurnal photosynthesis curve was dome-shaped during the spring month and thus P_N was high for a longer period of the day (Fig. 2*D*). The g_s also increased in the spring, it was around 0.26 mol m^{-2} s^{-1} in March while 0.16 mol m^{-2} s^{-1} in January (Fig. 2*F*,*L*). The *E* was higher at around 9.8 mmol m^{-2} s^{-1} (Fig. 2*E*). For these leaves, the situation in summer months is different. The leaf temperature and VPD are high and the photosynthesis is high at 8.00 h and then goes down showing a typical depression and a moderate rise around 16.00 h. When the leaf temperature rose to around 45°C, the P_N decreased to its minimum and the stomata were almost closed as indicated by very low values of g_s (Fig. 2*F*). Maximum *E* in May was 9.7 mmol(H_2O) m^{-2} s^{-1} at around 16.00 h, while throughout the day, it was around 5.0 mmol m^{-2} s^{-1} (Fig. 2*E*).

Leaves of the second cohort: After the summer, with the onset of monsoon by the end of June, the leaves of the second cohort start emerging as the temperatures and VPD decrease. The gas-exchange parameters were monitored in the monsoon month of August (Fig. 2*G*–*L*). On clear days, the P_N reached a maximum of over 20 μmol(CO_2) m^{-2} s^{-1} (Fig. 2*J*) associated with high g_s (Fig. 2*L*) and high *E* (Fig. 2*K*). The VPD increased during the day (Fig. 2*I*) and the P_N was inversely proportional to the VPD values. The first peak, which occurred at around 9:00 h, showed a value of 20 μmol(CO_2) m^{-2} s^{-1} while the second peak, which occurred at 16:00 h, showed a value of 15 μmol (CO_2) m^{-2} s^{-1} (nearly 77% of the morning rate). A distinct mid-day depression was seen at around 14:00 h showing only 23% of the morning P_N. The g_s and the *E* were parallel to the P_N. The maximum g_s observed in August was more than 0.35 mol(H_2O) m^{-2} s^{-1}, during the midday depression (Fig. 2*L*).

In the winter, which covers the months of November to January, these leaves experience somewhat lower PPFD but more importantly the photoperiod was only 6–7 h as compared to over 8 h in other seasons (Fig. 2*G*). There was also a drastic drop in the leaf temperatures (Fig. 2*H*) reaching as low as 10°C in the morning hours in January, while the maximum leaf temperatures were around 25°C during the afternoon hours. The VPD values were less than 3.0 kPa (Fig. 2*I*). The diurnal photosynthesis curve in these winter months was dome-shaped, but the P_N was just around 10.5 μmol(CO_2) m^{-2} s^{-1} (Fig. 2*J*) with low leaf temperature accompanied by a substantial drop in the g_s which was 0.16 mol(H_2O) m^{-2} s^{-1} (Fig. 2*L*). The maximum *E* in the winter months was around 3.25 mmol(H_2O) m^{-2} s^{-1} as compared to 9.5 mmol(H_2O) m^{-2} s^{-1} in August (Fig. 2*K*).

Diurnal changes in dark respiration (R_D) in leaves of two cohorts of *P. juliflora*

The first cohort: In the spring (Fig. 3*A*), the R_D showed a peak around noon and then decreased gradually in the evening. In March, the maximum rate observed was above 4.5 μmol(CO_2) m^{-2} s^{-1}.

During the summer months of May (Fig. 3*B*), R_D increased with the leaf temperature, which increased subsequently with the increase in PPFD. The leaf temperatures reached maximum at noon and the R_D was around 2.6 μmol(CO_2) m^{-2} s^{-1} at this time. These high rates remained more or less steady throughout the day and decreased in the evening, at sunset, when the PPFD and temperatures decreased to 0.93 μmol(CO_2) m^{-2} s^{-1} in May.

The second cohort: In the monsoon months of August (Fig. 3*C*), the R_D increased rather sharply to its maximum of 4.22 μmol(CO_2) m^{-2} s^{-1} and remained above 4.0 μmol m^{-2} s^{-1} throughout the day and decreased sharply in the evening. The rate at pre-dawn and sunset were 1.45 and 1.63 μmol(CO_2) m^{-2} s^{-1}, respectively.

In the winter, in January (Fig. 3*D*), the maximum R_D were quite low as compared to the monsoon month with a maximum of 2.14 μmol(CO_2) m^{-2} s^{-1}. In the winter, the R_D at dawn and in the evening were also low, 0.71 and 0.64 μmol(CO_2) m^{-2} s^{-1}, respectively.

Effect of light, temperature and CO_2 on two cohorts of *P. juliflora*

Effect of light

Light-saturated rates of CO_2 assimilation (P_{Nmax}) and quantum efficiency: In order to analyze the P_{Nmax}, the rates were plotted as a function of PPFD for the leaves of two cohorts, spring and monsoon. The P_N measured from predawn till 11:00–11:30 h were used for this purpose as these points provided light intensity from low to more than saturating one. Data from three years were used (Fig. 4*A*).

Net photosynthesis responded hyperbolically to PPFD in the leaves of the two cohorts. In the leaves of the first cohort in March (spring), the P_{Nmax} was around 15.34 μmol(CO_2) m^{-2} s^{-1} and the saturating PPFD was around 1,397 μmol(photon) m^{-2} s^{-1} (Fig. 4*A*). The photosynthesis was more or less constant beyond the saturating light. In the second cohort leaves in August, PPFD was saturating at substantially lower intensity, *i.e.*, 654 μmol(photon) m^{-2} s^{-1}, yet the P_{Nmax} was 20.19 μmol(CO_2) m^{-2} s^{-1} (Fig. 4*A*). These rates were reflected in the quantum efficiency that was calculated from the initial slope of the light-response curve of photosynthesis (Table 1). The quantum yield was low in the spring, with a value of 0.019 mol(CO_2) mol^{-1}(photon) (Table1), while in the leaves of the second cohort, this was over 0.041mol(CO_2) mol^{-1} (photon) in monsoon.

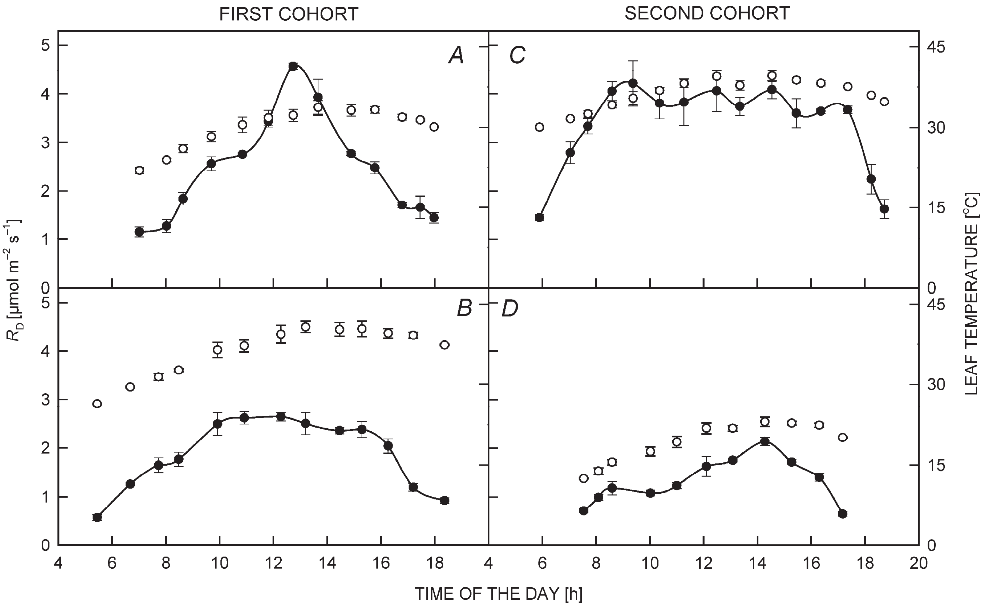

Fig. 3. Diurnal variations in dark respiration in the leaves of the two cohorts of *Prosopis juliflora* (*filled symbol*) and the respective leaf temperatures (*hollow symbol*). In (*A*) spring, (*B*) summer, (*C*) monsoon, and (*D*) winter. ± SD is from average of four readings on four separate plants obtained from one year data. R_D – dark respiration rate.

Light-compensation point (P_{NCP}): P_{NCP} in leaves of the first cohort in the spring was 27 µmol(photon) m^{-2} s^{-1}, while in the second cohort leaves during monsoon it was around 45 µmol(photon) m^{-2} s^{-1} (Table1). The *E* calculated from the light-curves in the respective leaves of the two cohorts were 0.5 and 1.9 µmol(CO$_2$) m^{-2} s^{-1} in the spring and monsoon, respectively (Table 1).

Effect of temperature: In order to analyze the potential effects of leaf temperature on the diurnal pattern, the P_N were plotted against the leaf temperature using the data from diurnal study of three years (Fig. 4*B*) as mentioned in the Materials and methods. Interestingly, the temperature optima for photosynthesis showed a shift, the first cohort functioning during the spring demonstrated the optimum temperature of 30°C, while during monsoon in the second cohort, it showed a temperature optima at a higher temperature of 35°C (Fig. 4*B*). However, the shift in the temperature optima was not significant.

Effect of CO$_2$: The P_N *vs.* the leaf internal CO$_2$ concentration (C_i) curves were monitored in the leaves of the two cohorts in the spring and monsoon (Fig. 4*C*) and the carboxylation efficiency (CE) and the CO$_2$-compensation point (Γ) were calculated (Table 1).

In March (1st cohort), the photosynthetic apparatus seemed to function at its maximum efficiency as the CE was maximum (0.172 mol m^{-2} s^{-1}) and Γ was low at 44 µmol(CO$_2$) mol^{-1} (Fig. 4*C*, Table 1). The leaf temperature was around 30°C for the spring cohort, while it was around

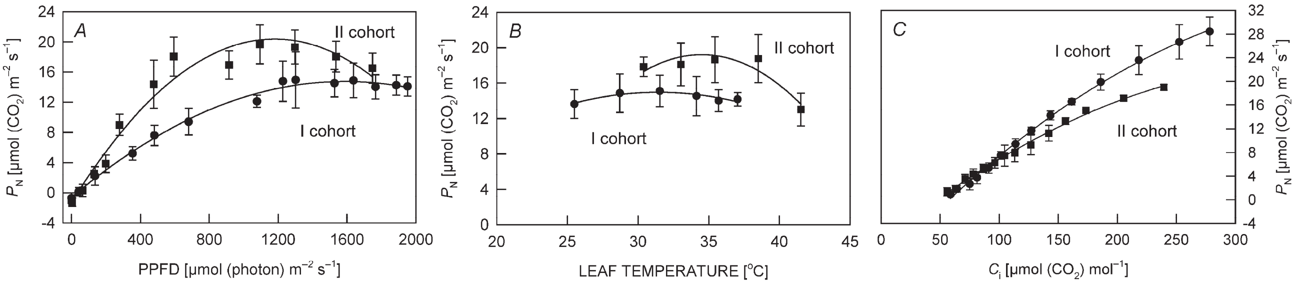

Fig.4. Responses of net photosynthesis (P_N) in the in the leaves of the first cohort (spring, ●) and the second cohort (monsoon, ■) (*A*) as a function of incident photosynthetic photon flux density (PPFD), (*B*) to leaf temperature, and (*C*) to substomatal CO$_2$ concentration (C_i). SD is from data of leaves of at least twelve independent plants over a period of three years for panels *A* and *B*, while for panel *C*, ± SD is from set of four independent experiments. The linear and quadratic terms for the polynomial regressions for P_N under increasing PPFD or increasing internal CO$_2$ concentration, C_i was significant at $P<0.0001$, while P_N under increasing leaf temperature was not significant at $P>0.05$.

Table 1. Comparison of parameters derived from the light and P_N/C_i curves in the leaves of the two cohorts of *Prosopis juliflora*. ± SD is from data of leaves of at least twelve independent plants over a period of three years for light curve and from set of four independent experiments for P_N/C_i curve. Significantly different values are indicated (*P<0.05), (**P<0.001), and (***P <0.0001). P_{NLSP} – light-saturation point; P_{NCP} – light-compensation point; P_{NQE} – apparent quantum efficiency; P_{Nmax} – maximal photosynthesis at saturating PPFD; R_D – respiration rate; CE – carboxylation efficiency; Γ – CO_2-compensation point.

Parameter	First cohort Spring	Second cohort Monsoon
P_{NLSP} [μmol(photon) m^{-2} s^{-1}]	1,397.20 ± 188.62	653.65 ± 77.78*
P_{NCP} [μmol(photon) m^{-2} s^{-1}]	27.32 ± 4.07	45.44 ± 9.18*
P_{NQE} [mol(CO_2) mol^{-1}(photon)]	0.019 ± 0.0027	0.041 ± 0.0067*
P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]	15.34 ± 1.93	20.19 ± 2.34*
R_D [μmol(CO_2) m^{-2} s^{-1}]	0.5 ± 0.073	1.9 ± 0.26**
CE [mol m^{-2} s^{-1}]	0.172 ± 0.002	0.164 ± 0.018***
Γ [μmol(CO_2) mol^{-1}(CO_2)]	44 ± 2.12	60.75 ± 5.68***

35°C in the monsoon cohort. During monsoon (2nd cohort) on clear day, the CE was high at 0.164 mol m^{-2} s^{-1} and Γ was around 60 μmol mol^{-1} (Fig. 4*C*, Table 1). Thus, the CE was maximum in the spring and monsoon, the Γ also was low in these two seasons. The results indicate that the photosynthesis efficiency was at its maximum in these two seasons.

Discussion

Trees growing in the north Indian plains experience high temperature (>30°C) for nine months during the year and therefore the trees have evolved strategies not only to tolerate but to perform at high temperatures. However, unlike temperate tree species, low temperatures are detrimental for growth of tropical species and high VPD leads to stomatal closure limiting photosynthesis drastically. Therefore, the optimum seasons for growth of the trees in this region are the spring and monsoon, while the summer and winter exert considerable stress during the year (Shirke and Pathre 2003, 2004a). In monsoon, the photosynthetic rate showed a tendency of two-peak response, a typical midday depression phenomena. The major factor for this phenomenon could be the high VPD values (Shirke and Pathre 2003, 2004a).

As the season progresses from the spring to summer, there is a cumulative stress of increasing light intensity and its duration, temperature, and VPD. This environmental stress results in decreased g_s for a longer period (Fig. 2*F*,*L*). This depression in gas exchange results in a decrease of daily carbon assimilation but prevents water loss (Figs. 1*A*; 2*D*,*J*) when the evaporative demand is the highest leading to near optimization of carbon assimilation to the water supply (Cowan 1982). A dependency of stomatal responsiveness to atmospheric humidity has been shown earlier in *P. juliflora* (Shirke and Pathre 2004a) and other species (Franks *et al.* 1997).

In this study, we observed that the maximum CO_2 was fixed in the spring and monsoon when the leaves of the two different cohorts matured and the total CO_2 fixed was 395 and 475 mmol(CO_2) d^{-1}, respectively (Fig. 1*C*). Under natural habitat in a semiarid ecosystem in Venezuela, the total CO_2 fixed by *Prosopis juliflora* throughout the day was in the range of 280 mmol(CO_2) d^{-1} (Tezara *et al.* 1998). However, authors have not indicated the season, when the measurements were performed. Weber *et al.* (1985) observed that *Acer saccharum* leaves fixed approximately 355 mmol(CO_2) m^{-2} d^{-1} during September on a typical day without any apparent environmental stress. These values are comparable with *P. juliflora* values, obtained in our study. The rates observed in the present study are higher than those obtained for tree species in the Mediterranean region (Tenhunen *et al.* 1985). This was expected keeping in view the environmental conditions.

In the Mediterranean species, the total *E* was very low as compared to *P. juliflora*. Water transpired by *Q. coccifera* was approximately 70 mol(H_2O) m^{-2} d^{-1}, *Q. suber* and *A. unedo* transpired approximately 30 mol(H_2O) m^{-2} d^{-1} (Tenhunen *et al.* 1987). The WUE in *Q. coccifera* was 2.86 mmol(CO_2) mol^{-1}(H_2O) d^{-1}, while in *Q. suber* and *A. unedo* it was 4.0 and 3.33 mmol(CO_2) mol^{-1}(H_2O) d^{-1}, respectively (Tenhunen *et al.* 1985). In *P. juliflora*, the WUE during the year ranged between 1.0 mmol(CO_2) mol^{-1}(H_2O) d^{-1} in summer and 4.0 mmol(CO_2) mol^{-1}(H_2O) d^{-1} in winter. These values are comparable with values reported by other workers for different species belonging to temperate and Mediterranean regions. Even the diverse species, such as Scots pine, possess similar WUE (Troeng and Linder 1982). Cernusak *et al.* (2007, 2011) compared the whole plant WUE in tropical leguminous and non-leguminous tree species using isotopic analyses namely $\delta^{13}C$, $\delta^{18}O$, and $\delta^{15}N$. They observed that leguminous species had a higher value of WUE as compared to the non-leguminous species. Apparently nodule forming nitrogen fixers like *P. juliflora* use water more efficiently than non-nitrogen fixers.

Dark respiration is a major pathway through which the carbon is lost. During the photosynthesis, photorespiration in C_3 plants, including tree species, accounts for considerable loss of fixed carbon. During the dark period, a further loss occurs particularly if the night temperatures are high. Respiration was high during the months of March and August that corresponded to months of high photosynthesis. Apparently, recently fixed carbon through photosynthesis is probably a substrate for respiration. Further, these are the months when new leaves appear and their demand for carbon for their growth is higher. This probably is the reason why respiration during these periods needs to be higher to provide required carbon skeletons and the energy to the growing leaves. Irrespective of the season, the rates come down considerably in the evening and remain low also at the dawn. Interestingly, the respiration rates are lower during the stress periods of winter and hot summer. The estimates from this study indicated that maximum R_D was approximately 20% of the maximum P_N values in all the months, except in the peak growth months of August/September, and March, when these values were approximately 30%. However, when the percentage of total R_D during the photoperiod as compared to the total CO_2 fixed during the day was calculated, it showed variation ranging from 26 to 59%. The annual average of the total R_D was 36.6% of annual average CO_2 fixed during the day. Amthor (1991) suggested that about half of the carbon fixed in photosynthesis (excluding photorespiration) is lost *via* respiratory processes. If one was to account for root, stem, and other tissue respiration, the respiration values would also probably approach 50% of the rates of photosynthesis in *P. juliflora*. In most studies of the carbon balance in plants or plant organs, it is assumed that dark respiration in the light continues at the same rate as in darkness. However, there is evidence that light inhibits dark respiration in photosynthetic tissues (Kirschbaum and Farquhar 1987, Villar *et al.* 1994, Atkin *et al.* 2000). The inhibition of R_D in light may occur due to metabolites from photosynthesis (ATP, NADPH) acting on the respiratory enzymes as respiratory regulators (Atkin and Tjoelker 2003). However, the mechanism of inhibition is not clear and appears to be complex (Villar *et al.* 1995, Foyer and Noctor 2000, Atkin and Macherel 2009). The acclimation of respiration and its degree to long-term changes in temperature is highly variable within and among plant species (Atkin and Tjoelker 2003). Several authors have shown that the extent of inhibition of dark respiration by light ranges between 17 and 66% in different species of agricultural or silvicultural interest (Villar *et al.* 1994, Weerasinghe *et al.* 2014). A comparison of leaf temperature and its dark respiration showed that temperature alone does not govern dark respiration. Pearcy (1977) observed in *Atriplex lentiformis* from desert habitats that increased growth temperatures (43°C day/30°C night) resulted in decreased dark respiration at all the temperatures, these plants also showed steady photosynthetic rates at high temperatures. Thus the improved net photosynthetic performance in the high temperature-acclimated desert plants appeared to be due to a combination of decreased respiration rates, decreased temperature dependence of respiration, and an apparent increased thermal stability of photosynthetic CO_2 exchange. In a similar way, the low dark respiration rates, even at very high temperatures, indicate the adaptability of *P. juliflora* to extreme temperatures as also observed by Pearcy (1977).

For many species, the temperature optimum for photosynthesis varies with the thermal environment in which the plants are growing, such that the thermal optima remain in tune with mean daytime air temperatures (Hikosaka *et al.* 2006, Smith and Dukes 2013). Although many desert species exhibit a capacity for thermal acclimation of their photosynthetic apparatus, a number of species, such as *Encelia farinosa*, do not show changes in temperature optima when grown under different thermal regimes (Ehleringer and Björkman 1978). Heat stress inhibits net photosynthesis primarily by decreasing the activation state of Rubisco *via* inhibition of Rubisco activase (Crafts-Brandner and Salvucci 2000, Salvucci and Crafts-Brandner 2004).

It appears that the rates of various energy dissipating mechanisms also alter with seasons. Such mechanisms include changes in the CO_2-compensation concentration, the carboxylation efficiency, and lipid composition in such a way as to increase their tolerance to increased temperature (Huner *et al.* 1998). *P juliflora* showed a shift in temperature optimum to suit the seasonal variation in temperature. Earlier studies from our laboratory (Shirke and Pathre 2003) had shown that this species uses energy dissipation pathways to dissipate excess energy and manages to prevent photoinhibition/photodestruction. We suggest that the fluctuations in temperature optima and energy-dissipating pathways are important seasonal adaptations of *P. juliflora*.

Bauer and Martha (1981) re-examined the CO_2-compensation point (Γ) in 32 morphologically or ecologically different C_3 plants. They determined the Γ at 20°C under normal O_2 content and light saturation. They observed that Γ lies between 31 and 40 ppm. In *P. juliflora*, in the winter month of January at 20°C and saturating light, Γ was approximately 30 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$, which was within the range, as shown by Bauer and Martha (1981). This observation according to them indicates a close relationship between the photosynthetic and photorespiratory rate in a leaf and leads to the supposition that the actual minimum CO_2 concentration inside the leaves is almost the same for most of the C_3 plants at a given favourable temperature.

While the general photosynthetic parameters observed in this study for *P. juliflora* are comparable to other tree species growing in different conditions as observed by other authors, a comparison of the leaves of the two cohorts has not been reported yet. Our study brought out distinct differences in the light saturation, shift in optimum temperature, compensation point, carboxylation efficiency, quantum efficiency, respiration, water-use efficiency, *etc*. in the two types of leaves produced by the tree. These two types seem to enable the tree to maximize the productivity under contrasting environmental conditions through the year and may give a competitive advantage *vis-à-vis* compare to other deciduous trees that remain leafless in the winter season. These results suggest that *P. juliflora* adapted to the environmental conditions by producing distinctly different leaves suiting to harsh summer and somewhat cold winter seasons. The underlying differences resulting in two types of leaves with different characteristics is not known and need to be studied further.

Conclusions: *P. juliflora* trees must adjust to diurnal and seasonal changes in temperature, vapor pressure deficit, and light intensities and their duration. *P. juliflora* seems to adapt to these changes by developing two distinct cohorts which appear in two different seasons of monsoon and spring. The leaves of the spring cohort are subjected in summer to extremes of high temperatures and high light along with high VPD, while the leaves of monsoon cohort face extremely low temperatures in the winter. The leaves regulate their stomata to optimize photosynthesis and balance their *E* losses.

The first cohort of *P. juliflora* leaves fixed about 39% of the total annual CO_2, which comprises the period of February to June during which the leaves face the harsh summers for about three months. On the other hand, the second cohort of leaves fixed the rest 61% of the total CO_2 under relatively cooler environmental conditions covering the period from July to January. The WUE was 2.0 mmol(CO_2) mol^{-1}(H_2O) in the first cohort of leaves (March) and was marginally lowered to 1.75 mmol(CO_2) mol^{-1}(H_2O) in the second cohort leaves (August). *P. juliflora* trees thus remain productive throughout the year by forming two different sets of leaves during a year as compared to the deciduous tropical trees where the trees have no leaves during the winter.

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