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Comparison of photosynthetic traits between two typical shrubs: legume and non-legume in Hunshandak Sandland

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Abstract

In Huanshandak Sandland, China, net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), water use efficiency (WUE), photochemical efficiency of photosystem 2 (F_v/F_m), and leaf nitrogen content were compared for *Hedysarum fruticosum* var. *mongolicum* (*H.f.m.*), a nitrogen fixing shrub, and *Salix gordejevii* (*S.g.*), a nitrogen non-fixing shrub. P_N, E, and g_s of the two shrubs were similar in trends, *i.e.* two peaks were observed in diurnal courses. However, except C_i, other parameters of *H.f.m.* were higher during the measured days than those of *S.g.* The midday depression of P_N was mainly due to decrease in stomata conductance and to reduction of F_v/F_m at midday. The higher P_N of *H.f.m.* was consistent with the higher leaf N content and there was a positive relation between them. In addition, several C_4 traits were found in *H.f.m.*, *i.e.* high saturation irradiance and WUE, low dark respiration rate, and C_i, which partly resulted in higher P_N. This seems to indicate that the C_3 plant *H.f.m.* may have C_4 photosynthesis pathway or C_4 enzymes.

Additional key words: adaptation to arid habitat; chlorophyll fluorescence; diurnal courses; *Hedysarum fruticosum* var. *mongolicum;* leaf N content; nitrogen use efficiency; photosynthesis; photosystem 2; *Salix gordejevii.*

Introduction

Nitrogen is a major element of the enzymes associated with photosynthesis (Evans 1989). Carbon fixing enzymes, such as ribulose-1,5-bisphosphate carboxylase (RuBPC), phosphoenolpyruvate carboxylase (PEPC), and pyruvate phosphate dikinase (PPDK) primarily accounted for above 50 % dissoluble protein in leaf. The content of these enzymes increased with the increasing N content (Yamazaki *et al.* 1986). In addition, the maximum rate of carboxylation and the potential rate of RuBP regeneration were positively correlated with N content in leaf (Wohlfahrt *et al.* 1999). Net photosynthetic rate (P_N) is positively related with leaf N content (Evans 1983, Rosati *et al.* 1999, Kazda *et al.* 2000). However, their extent differs in individual species, which reflects diverse nitrogen use efficiency (NUE) (Reich *et al.* 1998). In addition, the N validity and leaf longevity can also cause the different relationships between P_N and leaf N content.(Reich *et al.* 1994, 1995). When N content in soil is scarce or nitrogen validity is low, the plants that fix N_2 or have high NUE may have higher P_{max} than the N_2 non-fixing plants living in the same habitat. Higher P_N in leguminous plants than in the non-leguminous ones in natural ecosystems was reported by Jiang and He (1999) and Jiang and Zhu (2001). However, this is only reported as a phenomenon, the reason for it having not been discussed. Maeda *et al.* (1999) reported that the high P_{max} of *Myrica gale* var. *tomentosa*, an N_2 fixing shrub in Ozegahara moor, depended clearly on the leaf N content. However, the information on the comparison of photosynthesis in the N_2 fixing and non-fixing species is still limited. The above reports are insufficient to explain what processes contribute to higher P_N in a leguminous plant.

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Abbreviations: Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; F_v/F_m – photochemical efficiency of photosystem 2; g_s – stomatal conductance; *H.f.m.* – *Hedysarum fruticosum* var. *mongolicum*; NUE – nitrogen use efficiency; PEPC – phospho*enol*pyruvate carboxylase; PPDK – pyruvate phosphate dikinase; PPFD – photosynthetic photon flux density; P_{max} – the maximal photosynthetic rate; P_N – net photosynthetic rate; RuBPC – ribulose-1,5-bisphosphate carboxylase; *S.g.* – *Salix gordejevii*; T_{air} – air temperature; T_{leaf} – leaf temperature; WUE – water use efficiency.

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Is it really the higher N content in a leaf or the photosynthetic mechanism?

In this paper, the N content and the photosynthesis traits of two typical, N_2-fixing and non-N_2-fixing shrubs in Hunshandak Sandland, China were compared. Our aim was to explore the special photosynthetic mechanism of the leguminous plants grown in the same habitat, a semiarid sandland distributed in north China.

Materials and methods

Study area: The experiment was conducted in a warm desert of Hunshandak Sandland (42°30'~43°54' N; 112°45'~116°36' E), Inner Mongolia, China. The prevailing climate is of the temperate semiarid type with annual mean temperature of 1.7 °C, annual precipitation of 250-350 mm, and annual transpiration of 2 000-2 700 mm. The main habitats are shifting sand dune, semi-fixed sand dune, fixed sand dune, and lowland, among which lowland (grassland) is the dominant type. The coverage of vegetation in these habitats is <10, 10-40, >40, and 60 %, respectively. Recently, some fixed sand dunes have been reactivated and grassland degeneration has been accelerated due to the increasing human activity. Therefore, the shifting sand dune will account for higher proportion in the near future. *Hedysarum fruticosum* var. *mongolicum, Salix gordejevii, Caragara intermedia, Artemisia ordosia*, and other shrubs are the main species in both the semifixed and fixed sand dune. The growth period of these native species is from the middle of May to the end of September. Total N content in rhizosphere is very low (only 96.5 mg kg^{-1}).

Plants: *Hedysarum fruticosum* var. *mongolium* and *Salix gordejevii*, which grow on the sand dunes, are perennial shrubs, widely distributed in the study area. They survive in the shifting sand dunes as pioneer species because of their high aridity tolerance. *H.f.m.* can obtain a substantial amount of N from the atmosphere by N_2 fixation in root nodules (Tan and Li 1996). Both shrubs are crucial for the stabilisation of the shifting sand dunes.

Photosynthesis measurement: The experiment was conducted on 4 July and 2 August 2002 (clear days) throughout daytime at two-hour intervals. Photosynthetic photon flux density (PPFD), air temperature (T_{air}), leaf temperature (T_{leaf}), net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured using a portable gas exchange system (*LCA*-4, *ADC*, Hoddesdon, England). The leaf area was measured using an Area Meter (*AM100*, *ADC*, UK). Water use efficiency (WUE) was calculated as P_N/E. The central portion of most leaves was approximately horizontal, and the leaf cuvette was clamped on this portion of the leaf and kept in the horizontal position. In this way the effect of leaf angle on incident PPFD was minimised during the measurements. For measurements the fully expanded leaves in upper shoots were selected. Three replications were done for each species at each time.

Chlorophyll (Chl) fluorescence was measured using a portable plant efficiency analyser (*PEA*, *Hansatech*, King's Lynn, UK). F_0 (minimal fluorescence), F_m (maximal fluorescence), F_v (variable fluorescence), and F_v/F_m (maximal photochemical efficiency of PS2) were measured quickly after keeping the leaf for 30 min in dark. Red irradiance of 2 000 µmol m^{-2} s^{-1} was used for measurements. Three replications were made.

Irradiance response of P_N: The response of P_N to step changes in PPFD was examined in the field using the method introduced by Jiang and He (1999). A series of PPFD were produced by gradually altering the angle of leaf chamber. Each angle (*i.e.* the PPFD condition) was maintained for at least 5 min. P_N was recorded after steady date was reached. P_N-PPFD curves were plotted using the mean values of P_N measured at each PPFD Three replications were made.

Leaf N content: Leaves were collected from upper shoots on 4 July and on 2 August, respectively, then dried at 70 °C to a constant mass. Nitrogen content was measured using an automatic nitrogen analyser (*KJELTEC System, 1026* distilling unit, USA). Miller and Keoney (1982) introduced the method and calculation. Three replications were made.

Data analysis: The date sets of photosynthesis and Chl fluorescence were entered into an *EXCELL* spread sheet which included physiological variables, leaf areas, times, and plants. Analysis of variance of leaf traits was conducted on each measurement and the significance of plant mean square determined by testing against the error (species×replicate) mean square. The least significant differences (LSD) between the means were estimated at 95 % confidence level. Calculations and linear regressions were performed in a *Sigma-Plot 4.0* program. Significant differences among different plants are reported at $p < 0.05$, if not otherwise indicated.

Results

PPFD-P_N response curves: P_N of *H.f.m.* was saturated at 1 400 μmol m^{-2} s^{-1}, whereas that of *S.g.* at 1 100 μmol m^{-2} s^{-1}. The compensation irradiance for *H.f.m.* and *S.g.* was 22 and 35 μmol m^{-2} s^{-1}, respectively, and the dark respiration rate was 3.74 and 5.97 μmol m^{-2} s^{-1}, respectively. From 0 to 400 μmol m^{-2} s^{-1}, both shrubs responded rapidly. After a while, the curves were gradually at a plateau (Fig. 1). At each PPFD, P_N of *H.f.m.* was higher than that of *S.g.*, P_{max} of the former being 36 % higher than that of the latter. The different P_N response reflected the different mechanism of the two shrubs in radiation use efficiency.

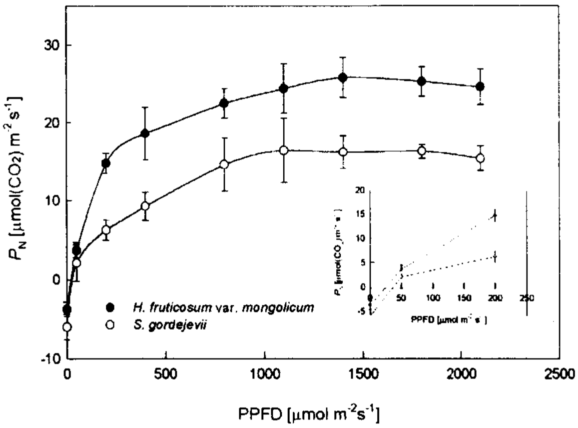

Fig. 1. Irradiance (PPFD) response curves of net photosynthetic rate (P_N) of *H. fruticosum* var. *mongolicum* and *S. gordejevii*. Error bars are ±S.E.

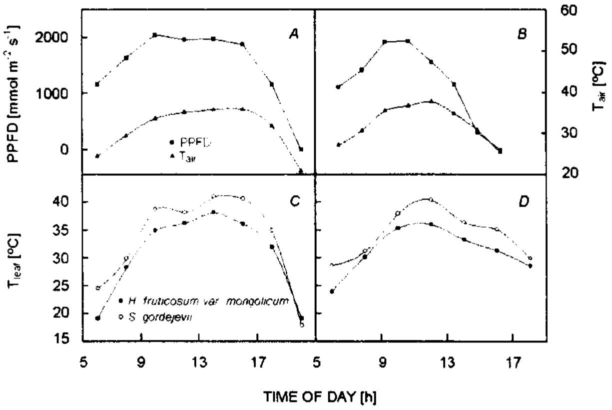

Fig. 2. Diurnal changes in photosynthetic photon flux density (PPFD), air temperature (T_{air}), and leaf temperature (T_{leaf}) on 4 July (*A, C*) and 2 August (*B, D*).

Diurnal course of photosynthesis: The time with PPFD above 1 000 μmol m^{-2} s^{-1} and T_{air} higher than 25 °C was about twelve hours during the experimental days. T_{leaf} of *H.f.m.* was 2.7 and 2.6 °C lower than that of *S.g.* on 4 July and 2 August, respectively. On average, T_{leaf} of the two shrubs was 2-4 °C higher than T_{air} under high PPFD (Fig. 2).

Under such conditions, the P_N of the shrubs showed a two-peak pattern on both days (Fig. 3*A,B*). The first peak appeared at 08:00, while the other, which was much smaller than the first one, appeared during 16:00-18:00. Compared with *S.g.*, *H.f.m.* exerted a significantly sharper second peak. The diurnal patterns of *E*, g_s, and WUE were similar with that of P_N (Fig. 3). At midday, g_s of *H.f.m* was 52 and 47 % lower than the maximal one on July 4 and August 2, respectively, whereas that of *S.g.* was reduced by 82 and 83 % (Fig. 3*E,F*). During the daytime, *H.f.m* showed greater P_N, *E*, g_s, and WUE than *S.g.* For example, P_{max} of *H.f.m.* was 25 and 65 % greater than that of *S.g*, respectively, on 4 July and 2 August, while on average, P_N of the former was 48 and 63 % greater than the latter (Fig. 3*A,B*).

C_i showed different patterns from those of P_N (Fig. 3*G,H*). The values decreased from the morning until 16:00 and then increased. C_i was the largest at 20:00 because of the enhanced respiration rate. Of the two days, C_i of *H.f.m.* was lower than that of *S.g.* though both showed similar trends.

Changes in Chl fluorescence: Under the experimental conditions, the values of F_v/F_m for the two shrubs were similar (Fig. 4). Generally, F_v/F_m decreased from the morning and increased in the afternoon. However, that of *S.g.* decreased more rapidly than that of *H.f.m.*, especially after 10:00. It reached the lowest value at 18:00, when F_v/F_m of *S.g.* was 13 and 16 % lower than that of *H.f.m.* on 4 July and 2 August, respectively. After 18:00, F_v/F_m began to increase. The minimum of F_v/F_m of *H.f.m.* in a day was about 11 % lower than the maximum on both 4 July and 2 August, whereas those of *S.g.* were 56 and 18 % lower on July 4 and August 2, respectively.

Leaf N content: On 4 July, N contents in leaves were 1.66±0.10 and 0.79±0.03 g kg^{-1}, respectively, for *H.f.m.* and *S.g.*, while on 2 August they were 1.68±0.05 and 0.91±0.06 g kg^{-1}, respectively. Leaf N content of *H.f.m* was 52 and 46 % higher than that of *S.g.* on 4 July and 2 August, respectively.

Discussion

Hunshandak Sandland is about 1 330 m above sea level. Under such condition, irradiance and T_{air} are normally high by the influence of the continental arid climate, especially in July and August. Local plants have special photosynthesis mechanism to adapt to the living conditions through the long history of adaptation. For instance, the decrease in g_s, by which the plants can reduce water evaporation, could result in midday depression of net P_N (Raschke and Resemann 1986, Knapp and Smith 1989). In addition, the depression of photo-chemical efficiency of PS2 could also account for the P_N decrease (Faria *et al.* 1996, Jiang and Zhu 2001). In this study, we found similar responses (Fig. 3*A,B*) for both *H.f.m.* and *S.g.* The closing of stomata, together with the depression of PS2 photochemical efficiency, resulted in P_N decrease at midday (Figs. 3*E,F* and 4). However, the photosystems of both shrubs were not destroyed even under the severe conditions, at irradiance >2 200 μmol m^{-2} s^{-1} and T_{leaf} >38 °C, as indicated by the quick recovery of F_v/F_m when irradiance became moderate (Fig. 4). This indicates that both species can dissipate excessive energy by a special mechanism to protect the photosystems from damage (Krause and Weis 1991). Our results suggested the high adaptability of *H.f.m.* and *S.g.* to arid habitats and both species are the ideal pioneer species when restoring the degraded sand dunes in Hunshandak Sandland.

During the two periods of measuring, P_N of *H.f.m.* was always remarkably higher than that of *S.g.*, which can be explained as follows:

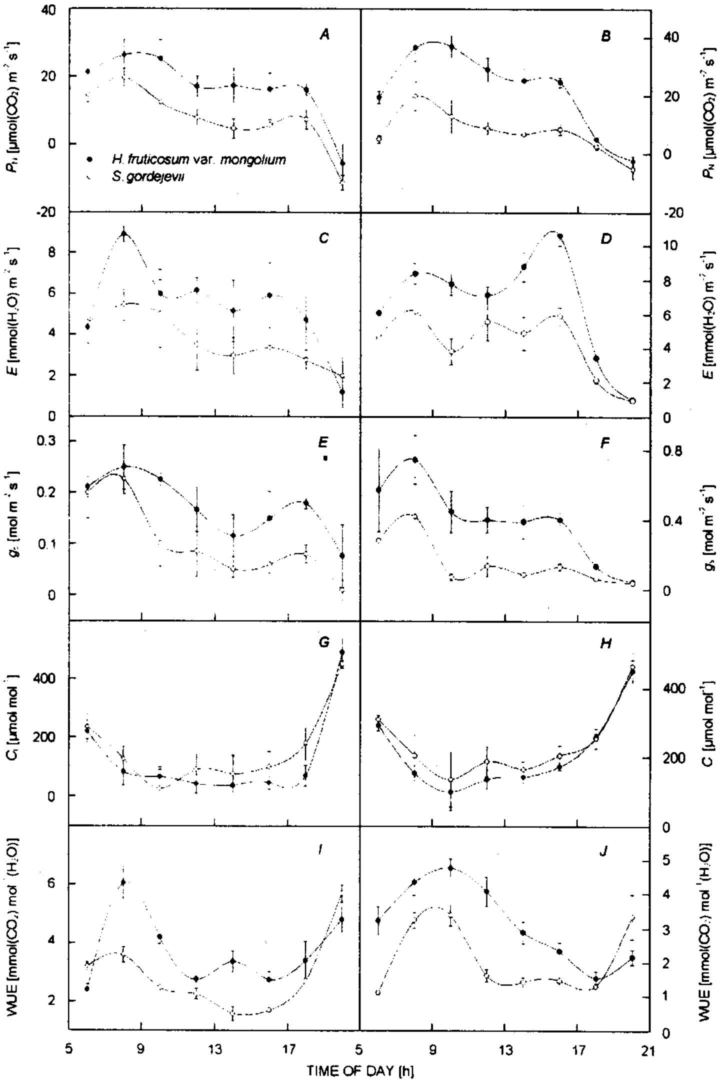

Fig. 3. Diurnal changes in net photosynthetic rate (P_N), transpiration rate (*E*), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and water use efficiency (WUE) on 4 July (*left panels*) and 2 August (*right panels*). Error bars are ±S.E.

(*1*) P_N was positively correlated with leaf N content (Mooney *et al.* 1981, Evans 1989, Anten *et al.* 1996, Mohammad *et al.* 1997). In the semi-fixed or fixed sand dunes, N content is probably the limiting factors of P_N because of its scarcity. The N content fixed by white clover per year is about 26.9 g(N) m^{-2} (Ledgard 1991). From this, we can suspect that *H.f.m.* can compensate for the scarcity of N in soil by N_2 fixing ability so as to guarantee photosynthesis. After the calculation of NUE (P_N/N) introduced by Hirose and Werger (1987), the NUE of *S.g.* was similar to that of *H.f.m.*, which indicated that the different leaf N content resulted in the difference of P_N. The higher P_N of *H.f.m.* just depended on the nodule N-fixing ability.

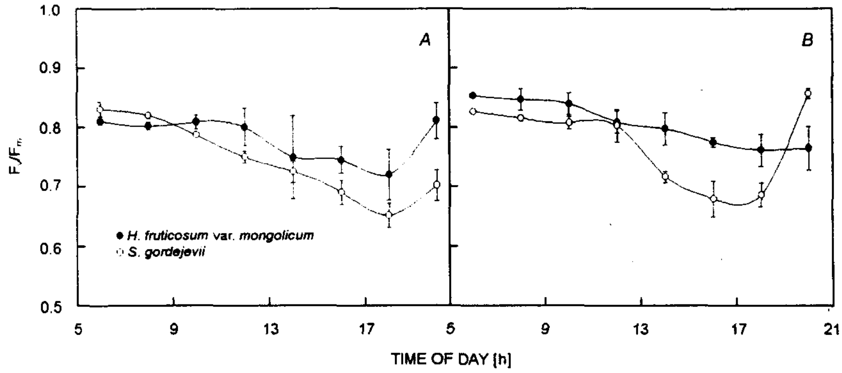

Fig. 4. Diurnal changes of photochemical efficiency of photosystem 2 (F_v/F_m) of *H. fruticosum* var. *mongolicum* and *S. gordejevii* on 4 July (*A*) and 2 August (*B*). Error bars are ±S.E.

(*2*) The differences could also be analysed by the relationship between sources and sink. P_N would be enhanced by an increased sink activity (Bagnall *et al.* 1988). Compared with that of the non-leguminous plant, about one third of photosynthesis production of the leguminous plant will be used for N_2 fixing and synthesis, carbon skeleton, and reductant (Minchin and Summerfield 1981). So *H.f.m.* was likely to have a larger sink capacity than *S.g.*, which could result in the higher P_N under the condition of smooth "stream".

(*3*) The difference in carbon fixing between *H.f.m.* and *S.g.* might also account for the diverse P_N. Higher saturation irradiance and WUE, lower dark respiration rate and C_i of *H.f.m.* (Figs. 1 and 3) suggest that *H.f.m.* can keep a higher photosynthetic potential even under high irradiance, high temperature, and low C_i, which are the typical traits of C_4 plants (Jenkins and Hatch 1985, Hatch 1992, Iglesias *et al.* 1997, Orsenigo *et al.* 1997). However, there are no C_4 leguminous plants in Inner Mongolia (Tang *et al.* 1999). So we speculate that C_3 plant *H.f.m.* might have C_4 photosynthesis pathways or C_4 enzymes. The viewpoint that C_3 plants have the C_4 pathway has already been reported (Duffus and Rosie 1973, Moore *et al.* 1989, Agarie *et al.* 1997). Similarly, *H.f.m.* may have C_4 pathway or C_4 enzymes under the special habitats, such as high temperature, drought, and high irradiance in Hunshandak Sandland, just like *Flaveria* and *Eleocharis vivipara* (Ueno *et al.* 1988, Moore *et al.* 1989, Reiskind *et al.* 1989, 1997).

In addition, the higher P_N of *H.f.m.* may also be due to its higher resistance to photoinhibition. Lesser decrease of *H.f.m.* than *S.g.* in g_s and F_v/F_m at midday (Figs. 3*E,F* and 4) suggests that the photoinhibition of *H.f.m.* was at a lower degree than that of *S.g.* which attributes to a much higher P_N of *H.f.m.* than *S.g.* during the whole day.

From the above results, we conclude that *H.f.m.* has a greater photosynthesis capability than *S.g.*, though they have similar diurnal trends. The N fixing ability of *H.f.m.* probably maintains higher leaf N content and thus higher P_N. The presence of C_4 photosynthetic pathway or C_4 enzymes in *H.f.m.* cannot be excluded, which needs further studies.

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