A sand-fixing pioneer C₃ species in sandland displays characteristics of C₄ metabolism

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Abstract

A better understanding of the ecophysiology of sandy plants will facilitate the prediction of the community succession in sandy environment. The photosynthetic characteristics of Hedysarum fruticosum var. mongolicum, a sand-fixing leguminous C₃ species in Hunshandak Sandland (HS) of northern China, were monitored and compared with those of Salix gordejevii, a typical C₃ species co-existing with H. fruticosum in semi-fixed or fixed sand dunes. The maximal photosynthetic rate (A max ) and instantaneous water use efficiency (WUE i ) of H. fruticosum was approximately two times higher than those of S. gordejevii. Except Ribulose-1,5-bisphosphate carboxylase (RuBPcase), the activities of photosynthetic carbon assimilation enzymes such as phosphoenolpyruvate carboxylase (PEPcase), NAD-malic enzymes (NAD-ME), NADP-malic enzymes (NADP-ME), NAD-malate dehydrogenase (NAD-MDH), NADP-malate dehydrogenase (NADP-MDH) and pyruvate phosphate dikinase (PPDK), were at least five times greater in H. fruticosum than those in S. gordejevii, suggesting H. fruticosum might have a C₄ photosynthetic syndrome. However, stable carbon isotope analysis revealed that H. fruticosum had the δ¹³C value of −23‰, which was close to that of C₃ plants. Therefore, it is likely that H. fruticosum is either a C₃-C₄ intermediate species, or a C₃ species displaying C₄ metabolic characteristics in habitat of sand dune. The involvement of C₄ metabolism in H. fruticosum might account for its greater efficiencies for photosynthesis and water use, allowing H. fruticosum to colonize the shifting sand dune with high temperature, light intensities and water stress.

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1. Introduction

Alterations of photosynthetic pathways under environmental stress have been suggested to contribute to the adaptation of plants to the environmental stress (Ehleringer et al., 1997). For example, a switch from C₃ to Crassulacean acid metabolism (CAM) in Mesembryanthemum crystallinum is induced after salinity treatment (Winter and Smith, 1996). Hydrilla verticillata, a submerged aquatic plant, changes its photosynthetic pathway from C₁ to C₄ under conditions of CO₂-deficiency (Reskind et al., 1997). Therefore, environmental factors are of critical importance in the photosynthetic pathways change (Parcelo and Laueneroth, 1996; Ehleringer et al., 1997). Keeley (1999). One obvious instance is the selection of C₄ pathway under water stressed conditions. Under conditions of limiting CO₂ caused by water stress, C₄ plants display greater advantages over C₃ plants in terms of carbon gain (Edwards and Walker, 1983) because low CO₂ concentrations favor plants with C₄ photosynthetic pathway (Ehleringer et al., 1997). It has been reported that the expression and activity of key enzymes associated with C₄ metabolism, such as PEP-case, NAD-ME, NADP-ME, NAD-MDH, NADP-MDH and PPDK were also regulated by water conditions (Imazumi et al., 1990; Walker and Leegood, 1999; Ku et al., 2000).

Water availability is the most limiting factor for plant growth in arid and semi-arid areas. Shifting and semi-fixed sand dune in arid and semi-arid areas exert other environmental stress (for example, high temperature and low N availability) in addition to low soil water content for plant growth (Fryrear, 1995; Kaul, 1996). Some highly specific mechanisms (Escudero et al., 1999) including changes in
morphological (Yamada et al., 2000) and physiological characteristics (Tyree et al., 1998; Terwilliger et al., 2001) have been developed in plants under water stress. However, it is illusive whether and how plants in arid and semi-arid area develop specific photosynthetic metabolisms to colonize the sand dune environment.

H. fruticosum, a nodulated N₂-fixing leguminous C₃ species as a pioneer species in shifting sand dune, can tolerate environmental extremes in temperature, light and drought (Zhang et al., 2002, 2003). Net photosynthetic rate of this species is higher than that of other C₃ species found in the same area (Jiang and Zhu, 2001; Liu et al., 2003; Niu et al., 2003), while is similar to the C₄ species Agropyron squarrosum (Niu et al., 2003), Halerpestes salignosus, Calamagrostis epigeios and Cynodon aculeatae (Jiang et al., 1999). Moreover, H. fruticosum exhibits higher light saturated point and lower light compensation point (Niu et al., 2003). These features are reminiscent of a typical C₄ plant. However, previous study has suggested that H. fruticosum is a C₃ plant (Tang et al., 1999). Salix gordejevi, a typical C₃ species coexists with H. fruticosum in fixed or semi-fixed sand dune, cannot live in shifting sand dune. To understand the mechanisms responsible for the greater adaptive features of H. fruticosum to shifting sand dune, we studied photosynthetic properties of H. fruticosum, particularly its photosynthetic enzymes and resource use efficiency, in comparing with those of S. gordejevi. We hypothesized that there is the distinguished C₃ photosynthetic metabolism in H. fruticosum that underpins the marked tolerance of H. fruticosum to the sandy environments, thus making it as a pioneer species in the shifting sand dune.

2. Materials and methods

2.1. Study site

The investigation was conducted at an experiment site (42°23′N, 112°23′E) of Sandy Ecosystem Research Station of Chinese Academy of Sciences in Hunshandak Sandland, based in the middle of Xilingel league of inner Mongolia Autonomous Region of China. The prevailing climate is of the temperate arid and semi-arid type. The average annual temperature is about 1.7 °C, with the mean maximum in July and minimum in January values being 16.6 °C and −24.1 °C, respectively. The annual total radiation time is 3000–3200 h. The above 10 °C accumulated temperature varied from 2000 to 2600 °C. The frostless period is approximately 100 d. Annual precipitation is 250–350 mm, with uneven distribution throughout the year. The maximum values (30 mm month⁻¹) were observed between June and August and the minimum values (1 mm month⁻¹) between March and May. There is a great fluctuation in the rain-fall among years, ranging from 150 mm in drought year to 400 mm in wet year. The annual potential transpiration is 2000–2700 mm, which approximates seven times the total of annual precipitation. The main habitats in this area are shifting sand dune, semi-fixed dune and fixed dune, lowland and wetland. The soil nitrogen content in sand dune is very low (about 0.2 g/kg).

2.2. Plant species

H. fruticosum, a leguminous sub-shrub, is widely distributed in the sand dune in Hunshandak sandland. It is about 1.5–2.0 m in height with odd plumose compound leaves and axial root reaching the deepest of 4.6–6.6 m. It adapts to dry habitat and germinates easily. Seedlings of H. fruticosum show fast growth and spread stem up to 18 cm within the first year. It is a dominant species at the primary stage in the sand vegetation’s succession (Chen, 1986). S. gordejevi is a widely distributed perennial C₃ shrub in the studied area and co-excits with H. fruticosum in semi-fixed or fixed sand dune. S. gordejevi displays similar life span and growth pattern to those of H. fruticosum.

2.3. Measurements of gas exchange and leaf nitrogen contents

We selected the 4-year old H. fruticosum and S. gordejevi in semi-fixed sand dune. Three similar size plants per species were measured. Of each plant, three functional leaves were measured and their mean values as the final value of this plant.

Gas exchange parameters were measured using a LCA-4 Portable Photosynthetic System (ADC, Hoddesdon, UK). These parameters include light saturated (PPFD = 1500 μmol m⁻² s⁻¹) net photosynthetic rates (Amax), transpiration rate (E), intercellular CO₂ concentration (C𝑖), ambient CO₂ concentration (Cₐ) and water vapor pressure deficit. Measurements were conducted at around 10:00 a.m. when PPFD was above light saturated point on all clear days. Gas exchange and leaf nitrogen content (Nleaf) were measured respectively 40, 60, 80 and 100 d after pullulation in plants of H. fruticosum and S. gordejevi. Instantaneous water use efficiency (WUE) was calculated from ratios between A (net photosynthetic rate) and E, and photosynthetic nitrogen use efficiency (PNUE) was measured as A/Nleaf. Prior to each time’s measurement the CO₂ and H₂O analyzers were calibrated using CO₂ standards (460 μmol mol⁻¹) and WG-602 Water Vapor Generator (ADC, Hoddesdon, UK), respectively. Leaf areas were determined using an Area Meter (AM100, ADC, Hoddesdon, UK). During operation, air was collected from 6 m above the soil surface and dried (by passing through “drier”) to 20% relative humidity before being pumped into the analyzer. Flow rate of air through the leaf chamber was 375 ml min⁻¹. The central portion of leaves was approximately horizontal, and the leaf cuvette was clamped on this portion and kept in the horizontal so that the effect of leaf angle on incident photon flux was minimized. The full-expanded functional leaves in three plants per species were selected.
Immediately after photosynthesis measurements, the leaves used for the above measurements were harvested and then oven-dried at 70 °C and weighed. N concentration was measured colorimetrically by Kjeldahl acid-digestion method with a Technicon Auto Analyzer after extraction with sulfuric acid (Brenner and Mulvaney, 1982).

2.4. Measurement of δ13 C

Fully expanded leaves from the selected species were collected at the anthesis stage, dried at 70 °C, grounded to pass 80 mesh and used for the measurement of carbon isotope composition (δ13 C). δ13 C was measured by Finnigan MAT 252 mass spectrometer in the Stable Isotope Laboratory, Institute of Geology and Geophysics, The Chinese Academy of Sciences. δ13 C (in ‰) reflects the molar abundance ratio of the plant leaf relative to that of a standard, the PDB carbonates (Ehleringer, 1991). Water use efficiency (WUE), PEPCase activity were determined as described by Zhang and Wu, 1986. PEPCase activity was determined as described by Sayre et al. (1979). Crude enzyme extract with leaves used for the above measurements were harvested were measured using the method of Tsuchida et al. (2001) and Sayre et al. (1979), respectively.

2.5. Enzyme assays

Leaf materials were taken from plants under full sun (PPFD of 1700 μmol m−2 s−1) at noon (11:00–12:30 h) and stored either in liquid N2 or at −80 °C for further use. Briefly, about 2 g fresh leaves that were cleaned and cut into small pieces placed in an ice-cold test tube with 4 mL of grinding media containing (mM): 100 Tris–HCl (pH 7.0), 10 MgCl2, 1.0 EDTA, 20 mercaptoethanol, 2% (w/v) PVP-10 and 10% (w/v) glycerol. Leaf extracts were centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was used for enzyme assay. Rubisco activity was assayed following the method of Tsuchida et al. (2001) and Sayre et al. (1979), respectively.

2.6. Statistical analysis

Analysis of variance (ANOVA) of leaf traits was carried out on each measurement and the results were analyzed by SPSS (10.0 for windows). The least significant differences (LSD) between the means were estimated at 95% confidence level. Calculations and linear regressions were performed using a SigmaPlot 8.0 program. Unless otherwise indicated, significant differences among different plants are given at P < 0.05.

3. Results

3.1. Gas exchange and leaf nitrogen concentrations

There was a growth-dependent increase in the maximal photosynthetic rates (Amax) for both H. fruticossum and S. gordejevii (Fig. 1a). For example, Amax in H. fruticossum increased from 24.2 μmol m−2 s−1 to 37.1 μmol m−2 s−1 between 40 and 80 d after pullulation (P < 0.05) (Fig. 1a). A similar growth-dependent increase in transpiration rate for the two species was observed (Fig. 1b). However, unlike Amax, transpiration rates of both species showed little difference during the period of measurement (P > 0.05) (Fig. 1b). Leaf N contents in H. fruticossum were about two times greater than in S. gordejevii (P < 0.05) (Fig. 1c). N contents in H. fruticossum and S. gordejevii measured at 40 d after pullulation were 1.57 and 0.8 g m−2, respectively. Leaf N contents in both species were relatively constant during the growth season (Fig. 1c). H. fruticossum had significantly lower C1/Ca

\[
\text{WUE} = \frac{\text{composition during carboxylation} (27\,‰)}{\text{composition during diffusion} (4.4\,‰)}
\]

where 

\[
\delta^{13} C = \frac{\text{composition of the ambient air (assumed to be } 8\,‰)}{\text{composition of } C_{\text{a}}}
\]

stands for the changes in isotopic composition favoring 13C discrimination are calculated from the following equation:

\[
\Delta^{13} C = \frac{\delta^{13} C_{\text{a}} - \delta^{13} C_{\text{p}}}{1 + \frac{1}{\epsilon}}
\]

where \( \delta^{13} C_{\text{a}} \) stands for the changes in isotopic composition favoring 13C, \( \delta^{13} C_{\text{p}} \) stands for the changes in isotopic composition favoring 13C during diffusion (4.4‰), \( \epsilon \) the change in isotopic composition during carboxylation (27‰), \( \delta^{13} C_{\text{a}} \) the isotopic composition of the ambient air (assumed to be ~8‰), \( C_{\text{a}} \) CO2 concentration in air; and \( C_{\text{p}} \) CO2 in plants, and \( \Delta W \) is the leaf-to-air vapor pressure deficit.

\[
\text{WUE} = C_{\text{a}} - \frac{\delta^{13} C_{\text{a}} - \delta^{13} C_{\text{p}}}{(b - a)} 
\]

\[
C_{\text{a}} = \frac{\delta^{13} C_{\text{a}} - \delta^{13} C_{\text{p}}}{b - a}
\]

\[
\Delta^{13} C = \frac{\delta^{13} C_{\text{a}} - \delta^{13} C_{\text{p}}}{1 + \frac{1}{\epsilon}}
\]

for further use. Briefly, about 2 g fresh leaves that were cleaned and cut into small pieces placed in an ice-cold test tube with 4 mL of grinding media containing (mM): 100 Tris–HCl (pH 7.0), 10 MgCl2, 1.0 EDTA, 20 mercaptoethanol, 2% (w/v) PVP-10 and 10% (w/v) glycerol. Leaf extracts were centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was used for enzyme assay. Rubisco activity was assayed following the method of Tsuchida et al. (2001) and Sayre et al. (1979), respectively.

NADP-MDH enzyme activity was assayed following the method of Sayre et al. (1979). Crude enzyme extract with a volume of 0.2 mL was mixed with 2.8 mL reaction solution containing (mM): 100 Tris–HCl (pH 7.0), 0.5 EDTA, 0.2 NADPH, 70 KCl), and 1 DTT. Oxaloacetic with final concentration of 2 mM was added to initiate the reaction. The reaction was performed at 30 °C and the change of absorbance at 340 nm was monitored.

PPDK reaction mixture contained (μM): 150 Tris–HCl buffer (pH 8.3), 18 MgSO4, 30 DTT, 0.45 NADH, 3 PEP, 3 AMP, 6 unit lactic dehydrogenase and diluted PPDK enzymes solutions. NAD-MDH activity was measured in reaction mixture of 0.5 μM NADH, 100 μM phosphoric acid buffer (pH 7.5). The reaction was initiated by adding 3 μM pyrophosphate natrium (1 μM oxaloacetate for NAD-MDH) and the absorption change at 340 nm was recorded.

Rubisco activities were expressed as μM CO2 carboxylase per min per soluble protein unit, while the other six enzymes activities were expressed as μM NADH oxidised per min per soluble protein unit. Total soluble proteins in the extracts were determined by the Coomassie Blue G 250 with bovine serum albumin as standard (Bradford, 1976).

3. Statistical analysis

Analysis of variance (ANOVA) of leaf traits was carried out on each measurement and the results were analyzed by SPSS (10.0 for windows). The least significant differences (LSD) between the means were estimated at 95% confidence level. Calculations and linear regressions were performed using a SigmaPlot 8.0 program. Unless otherwise indicated, significant differences among different plants are given at P < 0.05.
value than S. gordejevii (P < 0.05) (Fig. 1d). The C/Co values for both H. fruticosum and S. gordejevii were relatively independent of the growth (Fig. 1d).

3.2. Water and nitrogen use efficiency

There were significant (P < 0.05) differences in the instantaneous water use efficiency (WUEi) measured as A/E between H. fruticosum and S. gordejevii across the experimental period (Fig. 2a). WUEi value for H. fruticosum was approximately twice that of S. gordejevii throughout the period between 40 and 100 d after pullulation (P < 0.05) (Fig. 2a). There was a slight decline in WUEi for H. fruticosum, while relatively constant for S. gordejevii during the growing period (Fig. 2a).

Photosynthetic N use efficiency (PNUE) for H. fruticosum and S. gordejevii was comparable before 60 d after pullulation (Fig. 2b). However, there was a sharp increase in PNUE for H. fruticosum after 60 d pollution, but the PNUE for S. gordejevii declined slightly during the same period, leading to the PNUE for H. fruticosum being marginally (P < 0.10) higher than that for S. gordejevii at the time of 100 d after pullulation (Fig. 2b).

3.3. Stable carbon isotope

To determine the photosynthetic types of the two species, we measured the stable carbon isotope (δ13C). δ13C in H. fruticosum was significantly higher than that in S. gordejevii at anthesis stage, i.e., −23.6‰ ± 1.4 and −28.5‰ ± 1.1 for H. fruticosum and S. gordejevii, respectively (P < 0.05) (Fig. 3a). The higher value of stable carbon isotope led to the lower value of the carbon isotope discrimination (Δ13C) for H. fruticosum than for S. gordejevii (Fig. 3b). Water use efficiency, calculated from the measured δ13C values, was 47% higher for H. fruticosum than for S. gordejevii (Fig. 3c). The ratio between intercellular and atmospheric partial pressures...
of CO₂ based on the δ1³C values (Cᵢ/Cₐ) was 43% lower for *H. fruticosum* than for *S. gordejevi* (Fig. 3d).

### 3.4. Photosynthetic enzymes

RuBP activities showed similar values in *H. fruticosum* and *S. gordejevi* along with the growing period. Activities of all the C₄ enzymes assayed, including PEP, NAD-ME, NADP-ME, NAD-MDH, NADP-MDH and PPDK, were at least five times higher for *H. fruticosum* than for *S. gordejevi* (Fig. 4). There were growth-dependent increases in the enzyme activities for *H. fruticosum* (Fig. 4) whereas those for *S. gordejevi* remained relatively constant throughout the growing period (Fig. 4). All enzymes' activities in *H. fruticosum* reached peak values at approximately 80 d after pullulation. There was a linear increase in the ratio between PEPcase and RuBPCase for *H. fruticosum* between 40 and 100 d after pullulation (Fig. 4a–g). After that the enzymes' activities exhibited a sharp decline, leading to the differences in the activities for the two species being smaller at 100 d after pullulation. There was a linear increase in the ratio between PEPCase and RuBPCase for *H. fruticosum* got peak value of 0.8 at 40 d after pullulation and then declined to 0.24 at 80 d after pullulation (Fig. 4h).

### 4. Discussion

The greater capacity for *H. fruticosum* to adapt to the sandy habitats has drawn much attention in recent years (Roels et al., 2001). Previous studies have attributed the adaptation of *H. fruticosum* to sand environment to its clonal propagations (Zhang et al., 2001, 2003) and/or to its superior survival ability under sand burial (Zhang et al., 2002). We suggest that the presence of C₄ photosynthetic metabolism is likely to account for the higher efficiencies for photosynthesis and water use in *H. fruticosum*, thus enabling it to better adapt to the high temperatures, high light intensities and low water availability that occur in the shifting sand dune than *S. gordejevi*. This might be the reason why *H. fruticosum* can grow in shifting sand dune as a pioneer species while *S. gordejevi* cannot.

*H. fruticosum* was distinguished by its higher *A*ₘₐₓ (Fig. 1a) and similar transpiration rate (Fig. 1b) compared to *S. gordejevi*, which resulted in the greater WUE i in *H. fruticosum* relative to *S. gordejevi* (Fig. 2a). It has been reported that there was trade-off relationship between WUE i and PNUE (Field et al., 1983; Mulkey et al., 1991; Wang et al., 1998; Chen et al., 2004). This is because diffusion of CO₂ into leaves is regulated by stomata that are governed by the compromise of increasing CO₂ fixation over excessive water loss. In order to decrease water losses from stomata, plants displaying conservative water use usually limit rates of photosynthesis per unit of nitrogen. However, *H. fruticosum* exhibited higher values in both WUE i and PNUE (Fig. 3). This might be due to its great photosynthetic rate and thus made a high water and nitrogen use efficiency.

Carbon isotope discrimination (Δ¹³C), an integrated, long-term indicator of gas exchange performance (Farquhar et al., 1989), has long been used as an important, convenient and available parameter for estimating long terms WUE and Cᵢ/Cₐ (Wright et al., 1988). The higher calculated WUE in *H. fruticosum* than in *S. gordejevi* (Fig. 3c) was consistent with WUE i (Fig. 2a), suggesting that *H. fruticosum* may belong to conservative water use types at both short and long times of growing. It is well known that sufficient supply of CO₂ (high Δ¹³C) can improve the rate of assimilation (Le Roux-Swarthout et al., 2001). However, the lower
Fig. 4. Ribulose-1,5-biphosphate carboxylase (a) phosphoenolpyruvate carboxylase (b), NAD-malate dehydrogenase (c), NADP-malate dehydrogenase (d), NAD-malate enzymes (e), NADP-malic enzyme (f), pyruvate phosphate dikinase (g) and PEPcase/RuBPcase ratio (h) in *Hedysarum fruticosum* and *Salix gordjevii* during growing season (mean ± S.E.).

$C_4/C_3$ (Figs. 1d and 3d) in *H. fruticosum* does not seem to inhibit photosynthetic rate yet. This might ascribe to the higher carboxylation ability of CO$_2$ in *H. fruticosum* (Fig. 4a). In addition, the higher PEPcase activities in *H. fruticosum* (Fig. 4b) could also function as a CO$_2$-pump to condense CO$_2$ in the chloroplasts. $A_{\text{max}}$ of *H. fruticosum* during the growing period occurred at 80 d after pullulation (Fig. 1a) when photosynthetic enzymes displayed peak activities (Fig. 4). These findings indicate that the higher $A_{\text{max}}$ in *H. fruticosum* was independent of the $C_i/C_a$ (Fig. 1d), but dependent on the photosynthetic enzymes’ activities.

The photosynthesis performance in our present study, together with the expression of $C_4$ photosynthetic enzymes (Fig. 4) and higher resource use efficiency, indicating that *H. fruticosum* is likely to be a $C_4$ species. However, the $\delta^{13}C$ value of $-23\%$ in this species (Fig. 2) is comparable to a typical $C_3$ plants so far characterized (Farquhar et al., 1989). Therefore, *H. fruticosum* is unlikely to be a $C_4$ plant. Rather, it could be $C_3$-$C_4$ intermediate species or a $C_3$ species with $C_4$ photosynthetic pathways. The $C_3$-$C_4$ intermediate species represent a stage in the evolutionary transition from the $C_3$ to the $C_4$ photosynthetic mechanism (Fresco et al., 1999). $C_4$ photosynthetic pathways in $C_3$ plants have been reported in other species such as *Flaveria browii* (Cheng et al., 1988), *Eleocharis vivipara* (Ueno, 2001) and *Bienertia cycloptera* (Voznesenskaya et al., 2002). In the previous studies, the ratio between PEPcase and RuBPcase (Li et al., 2001) and/or the presence of higher activity of $C_4$ enzymes have been widely used as indicators for the existence of $C_4$ pathways (Hatch and Burnell, 1990). Our results showed that both the absolutely activity of $C_4$ enzymes (Fig. 4a–g) and ratio between PEPcase and RuBPcase were higher (Fig. 4b), suggesting that a $C_4$ photosynthetic metabolism predominates in *H. fruticosum*.

In the shifting sand dune of Hunshandak, the environment is often associated with high light intensities, high temper-
atures and dryness. It was reported that water content in shifting sand dune was only 3–4% in the similar area (Guo et al., 2000). To minimize water losses by transpiration, *H. fruticosum* may have to reduce the stomatal conductance, thus leading to a reduction of CO₂ diffusion into leaves. This would account for the observed lower C₄/C₃ in *H. fruticosum* than in *S. gordonii* (cf. Figs. 1d and 3d). Increase in the rate of the C₄ photosynthetic enzymes (Fig. 4) to enhance the carboxylation ability might be used by *H. fruticosum* as a mechanism to adapt to the lower intercellular CO₂ regime because C₄ metabolism has an efficient CO₂ pump (Van Caemmerer and Furbank, 2003). Therefore, it is likely that the C₄ metabolisms are induced in the C₃ plant of *H. fruticosum* under the conditions of HS. As to its real photosynthetic types in *H. fruticosum*, some anatomical features and the initial carboxylation conducts measured by ¹⁴C₀₂ need to be further studied.

It is of ecological importance for *H. fruticosum* to have C₄ photosynthetic metabolism and N₂-fixing ability to colonize and survive in the shifting sand dune where phosphorus, nitrogen and water contents are very low. The higher carboxylation efficiency and greater resource use efficiency due to its markedly higher C₄ photosynthetic enzyme activities, allowing it to compensate for the lower intercellular CO₂ concentration caused by the closing of stomata in Hunshandak Sandland. The establishment of *H. fruticosum* in bare sand dune would improve the soil nitrogen status, facilitating the later succession plants in sand dune. These characteristics provide an ecophysiological advantage for *H. fruticosum* to adapt to the conditions of shifting sand dune.

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