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Gas exchange and chlorophyll fluorescence response to simulated rainfall in *Hedysarum fruticosum* var. *mongolicum*

S.L. NIU, L.H. LI*, G.M. JIANG*, L.M. GAO, Y.G. LI, Y. PENG, and M.Z. LIU

*Laboratory of Quantitative Vegetation Ecology, Institute of Botany, Chinese Academy of Sciences,
20 Nanxincun, 100093, Beijing, P.R. China*

Abstract

The response of gas exchange and chlorophyll fluorescence along with changes in simulated rainfall were studied in water stressed plants *Hedysarum fruticosum* var. *mongolicum (H.f.m.)*. Net photosynthetic rate (P_N), stomatal conductance (g_s), leaf water potential (Ψ_{leaf}), and apparent carboxylation efficiency (P_N/C_i) were significantly increased with the increase of rainfall. However, they did not change synchronously. The complete recovery of both P_N and P_N/C_i appeared 3 d after watering while g_s and Ψ_{leaf} were recovered 1 d after treatment. Gas exchange characters increased sharply from 5 to 15 mm rainfall and then maintained steady state with increasing rainfall. During the initial phase of water recovery, photosystem 2 (PS2) activity was not affected and its complete recovery occurred also 3 d after rainfall. Hence the recovery of P_N was attributed to both opening of stomata and increase in carboxylation efficiency. Furthermore, PS2 activity was really impaired by water stress and could recover to the normal status when the water stress disappeared.

Additional key words: apparent carboxylation efficiency; gas exchange; leaf water potential; net photosynthetic rate; soil water content; stomatal conductance.

Introduction

Water availability affects plant growth, especially in arid and semi-arid regions, where plants are often subjected to periods of drought. Plants can adapt to arid environment by eco-physiological adjustments (Sala and Tenhunen 1994) such as stomata regulation (Brestic *et al.* 1995, Lawlor 1995). At least under mild drought, stomata dominantly control the decline of photosynthetic rate (Cornic and Fresneau 2002). Another important factor contributing to the depression of photosynthesis is the non-stomatal factor, which was attributed to reduced carboxylation efficiency (Wise *et al.* 1991), decreased RuBP regeneration (Tezara and Lawlor 1995), or to a reduced amount of functional ribulose-1,5-bisphosphate carboxylase/oxygenase (Kanechi *et al.* 1995). Changes of net photosynthetic rate (P_N) with the progress of water stress were studied in-depth by Souza *et al.* (2003). However, most of the water-control experiments were performed in greenhouse and the living conditions have been changed greatly. Few field studies were reported as yet.

As to the studies of chlorophyll (Chl) *a* fluorescence, most investigations have shown that PS2 is either unaffected (Lu and Zhang 1998, Shangguan *et al.* 2000) or affected little only under very severe drought (Saccardy *et al.* 1998). Thus the photosynthetic apparatus, and particularly photosystem 2 (PS2), is more sensitive to heat stress than to water stress (Bilger *et al.* 1987, Yordanov 1992). Nevertheless, opposite results have also been reported (Balaguer *et al.* 2002, Souza *et al.* 2003). Therefore this controversial topic needs further studies.

Hedysarum fruticosum var. *mongolicum* (*H.f.m.*) is a pioneer species largely distributed in shifting sand dunes. Due to its sand-fixation ability it is often regarded well adapted to drought and shifting sand conditions compared with other sand species (Chen 1986). The study of its response to water stress and re-watering may contribute to understanding the eco-physiological adjustment of xerocoles to drought.

The aim of this study was to evaluate the responses

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*Corresponding author; fax: (+8610) 62595380, e-mail: jgm@ht.rol.cn.net; llinghao@ns.ibcas.ac.cn

Abbreviations: Chl – chlorophyll; F_0 – initial fluorescence; F_m – maximal fluorescence; F_v/F_m – photochemical efficiency of photosystem 2; g_s – stomatal conductance; *H.f.m. – Hedysarum fruticosum* var. *mongolicum*; P_N – net photosynthetic rate; P_N/C_i – apparent carboxylation efficiency; SWC – soil water content; Ψ_{leaf} – leaf water potential.

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of photosynthesis in water stressed *H.f.m.* plants to water irrigation under field conditions, both in terms of CO_2 assimilation measured by leaf gas exchange and of the state of photosynthetic apparatus as assessed by Chl *a* fluorescence measurements. The hidden hypothesis is that photosynthesis in water irrigated *H.f.m.* plants is elevated by stomatal and non-stomatal factors, and Chl *a* fluorescence parameters are really influenced by water condition.

Materials and methods

Study area: The investigation was conducted at Hunshandak Sandy Ecosystem Research Station of the Chinese Academy of Sciences (42º23´N, 112º23´E) lying in the middle of Xilingel League of Inner Mongolia Autonomous Region of China. For details about the study area see Niu *et al.* (2003).

Plants: *H.f.m.* is a perennial sub-shrub that distributes in the fixed sand dune or semi-fixed dune in Hunshandak Sandland. It is about 1.0–1.5 m in height with odd plumose compound leaves and axial root reaching the deepest of 4.6–6.0 m. It is tolerant to dry habitat and easily germinates (Chen 1986). The seedlings grow fast and spread stem up to 18 cm at the first year of development. They grow from May to September.

Experimental design: In the early summer (May) of 2002, 18 circular plots with 100 cm diameter were put on three-years seedlings of *H.f.m.* with an aluminous frame (50×50×50 cm, length×width×depth). Each plot was centred on a single plant and contained no other plants except the target species. The treated plants had similar height and width, and kept distance of at least 100 cm from plot to plot. All the treatments were assigned randomly with three replications. A series of simulated rainfall, *i.e.* 0 (control), 5, 10, 15, 20, and 25 mm were designed to irrigate the plants, 18 individuals were tested. Water was supplied uniquely in the afternoon of 15 August using a handheld sprinkler. It had not been raining since 15 d before the experiment, with the soil water status being severe dry. The days following the simulated precipitation were all sunny with temperature changing from 26 to 37 °C.

Gas exchange was measured 1 and 3 d after watering, *i.e.* on 16 and 18 August 2002. P_N, stomatal conductance (g_s), and internal CO_2 concentration (C_i) were measured using an *LCA*-4 Portable Photosynthetic System (*ADC*, Hoddesdon, England). The measurements started from 10:00 when photosynthetic photon flux density (PPFD) was above saturating irradiance and stopped at 12:00 (to avoid high irradiation stress). Conditions for measurements were ambient CO_2 concentration (C_a) of 350 µmol mol^{-1}, vapour pressure deficit (VPD) of 2.0±0.4 kPa, leaf temperature of 35±0.26 °C, and PPFD of 1 800±42 µmol m^{-2} s^{-1}. The apparent carboxylation efficiency was evaluated as P_N/C_i (Flexas *et al.* 2001). During operation, the leaf cuvette was kept horizontally so that the effect of leaf angle on incident photon flux was minimised during the measurements. For measurements fully expanded functional leaves in upper shoots were selected. Leaf areas for calculation of gas exchange were measured by an Area Meter (*AM100, ADC*, Hoddesdon, England). Three leaves of each treated plant were used.

Chl fluorescence of full-expanded leaves near those for gas exchange measurement 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 immediately after keeping the leaves for 30 min in dark. A red irradiance of 2 000 µmol m^{-2} s^{-1} was used for measurements.

Leaf water potential (Ψ_{leaf}) was measured with a *WP4* Dewpoint Potential Meter (*Decagon Devices*, Pullman, WA, USA). Full-expanded leaves with their twig were taken near to the tops of plants and then kept in a sealed plastic bag and stored in an icebox after being collected. Measurements were done immediately after bringing leaves into laboratory. Calibration was checked hourly. Five to eight maturing leaves were sampled for each determination. Small linear leaves or leaf sections were put into chamber for measurements. Mature leaf tissue was used to minimise growth effects on Ψ_{leaf}.

Soil water content (SWC) in each treatment was investigated with a *Delta-T* Device Moisture Meter (Profile Probe, type *PR1/6*). Four probes were buried around each target plant in four separate directions. Measurements were taken at 20-cm depth intervals, beginning from 0 cm, and stopping at 80 cm of the soil layer.

Data analysis: Analysis of variance (*ANOVA*) of leaf traits was conducted on each measurement and the significance of plant mean square was determined by testing against the error mean square. The least significant differences (LSD) between the means were estimated at 95 % confidence level. The ultimate date of each characteristic for comparison was the mean of three replications. All the statistical analyses were performed using the *SPSS 10.0* package (*SPSS*, Chicago, USA).

Results

SWC: Simulated precipitation increased SWC of each layer of the soils where the target plants were distributed. For example, when 25 mm 'rainfall' was applied, SWC of soils at the layers of 20, 40, 60, and 80 cm beneath the target plants were, respectively, 5, 5, 3, and 3 fold of those where no rainfall was applied. Vertically, SWC was elevated from 20 to 60 cm at any water amount treatment, but kept steady when soil layer was deeper than 60 cm (Fig. 1).

Gas exchange and Ψ_{leaf} response to simulated rainfall: Water treatment significantly improved P_N, g_s, Ψ_{leaf}, and P_N/C_i since the first day after simulated rainfall (Fig. 2). However, P_N and P_N/C_i reached their maximal values only 3 d after treatment while g_s and Ψ_{leaf} fully recovered 1 d after treatment. All the four measured characters raised rapidly from 5 to 15 mm, and then were steady or increased slowly with the increasing rainfall. The maximum values of P_N, g_s, Ψ_{leaf}, and P_N/C_i were 76, 285, 79, and 88 % higher, respectively, than those of the control plants.

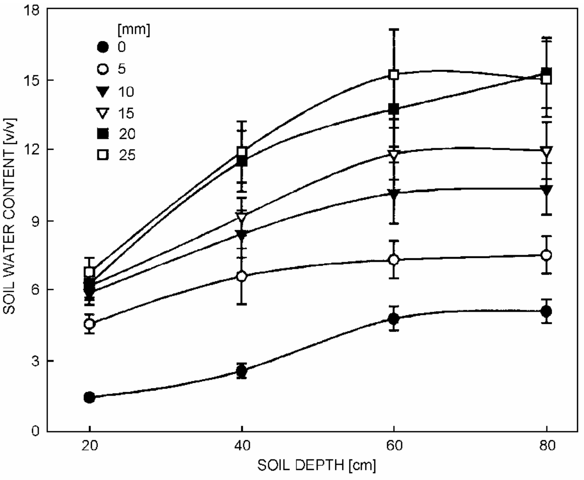

Fig. 1. Changes of soil water content in 20, 40, 60, and 80 cm layers below sand surface among different simulated rainfall events (0 to 25 mm) in Hunshandake Sandland. *Error bars* are ±S.E.

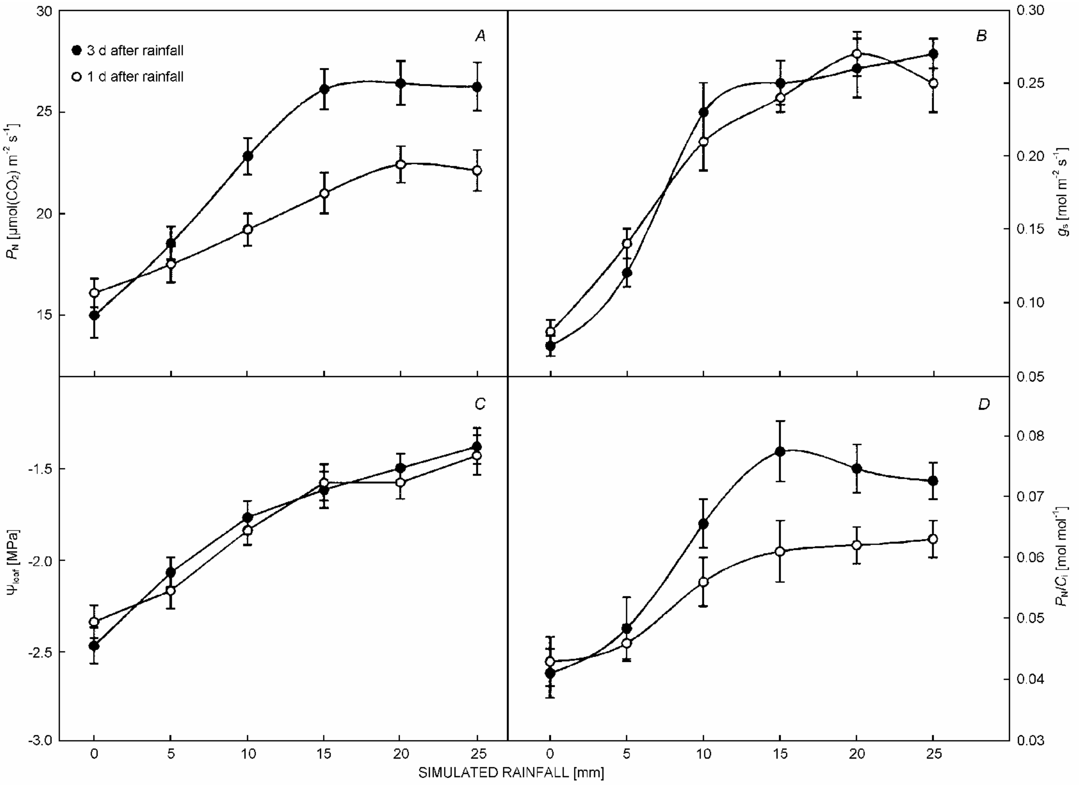

Fig. 2. Net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), leaf water potential, Ψ_{leaf} (*C*), and apparent carboxylation efficiency, P_N/C_i (*D*) of *Hedysarum fruticosum* var. *mongolicum* under simulated rainfall treatments. *Error bars* are ±S.E.

Chl *a* fluorescence response to water treatments: Under water treatments, F_0 was ever decreased (Fig. 3*A*), and F_m (Fig. 3*B*) and F_v/F_m (Fig. 3*C*) were ever improved with the increasing rainfall, 3 d after the treatment. But there were no significant differences between the control plants and treated plants within 1 d. The maximal values of F_m and F_v/F_m occurred when 20 mm rainfall was supplied, which were 49 and 25 % higher, respectively, than in the control plants.

Relationships between P_N, g_s, F_v/F_m, and Ψ_{leaf}: P_N, g_s, and F_v/F_m showed significant linear correlations with Ψ_{leaf} both 1 and 3 d after treatment (Fig. 4). Nevertheless, the slopes of P_N *vs.* Ψ_{leaf} and F_v/F_m *vs.* Ψ_{leaf} 3 d after treatment were higher than 1 d after treatment. Nevertheless, the slope of g_s *vs.* Ψ_{leaf} was similar for the two measuring times.

Discussion

Gas exchange and PS2 response to simulated rainfall: The recovery of P_N after simulated rainfall indicated that water stress already affected photosynthetic activity in *H.f.m.* P_N recovered with the opening of stomata on the first day after irrigation, which indicates that the decreases in P_N under water stress are mainly the result of stomatal closure (Campos *et al.* 1999, Cornic 2000). However, P_N did not recover to the maximum 1 d after

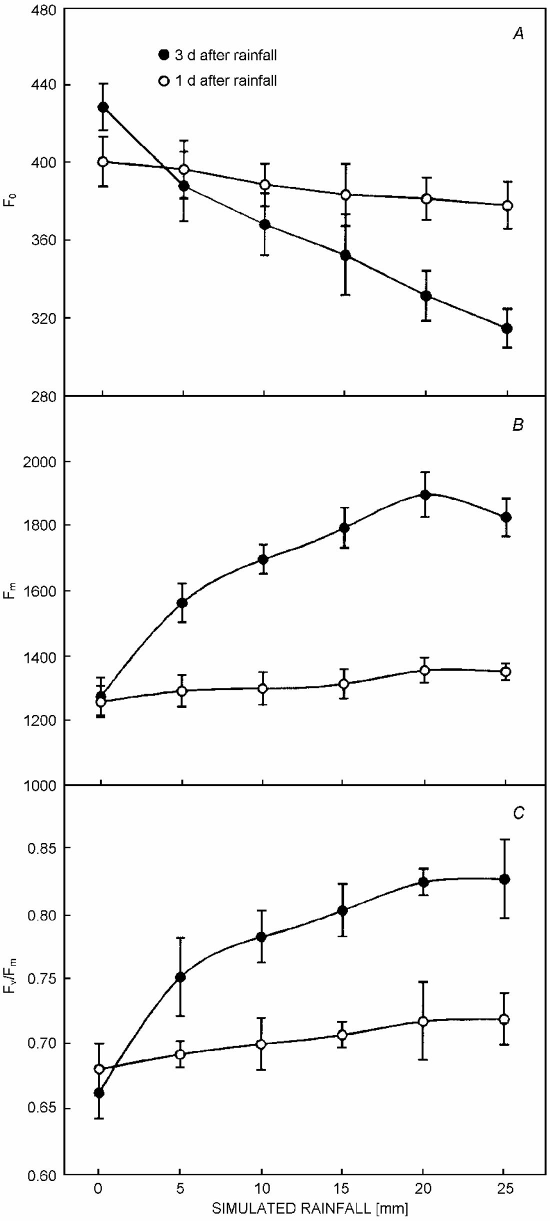

Fig. 3. Initial fluorescence, F_0 (*A*), maximal fluorescence, F_m (*B*), and photochemical efficiency of photosystem 2, F_v/F_m (*C*) of *Hedysarum fruticosum* var. *mongolicum* under simulated rainfall treatments. *Error bars* are ±S.E.

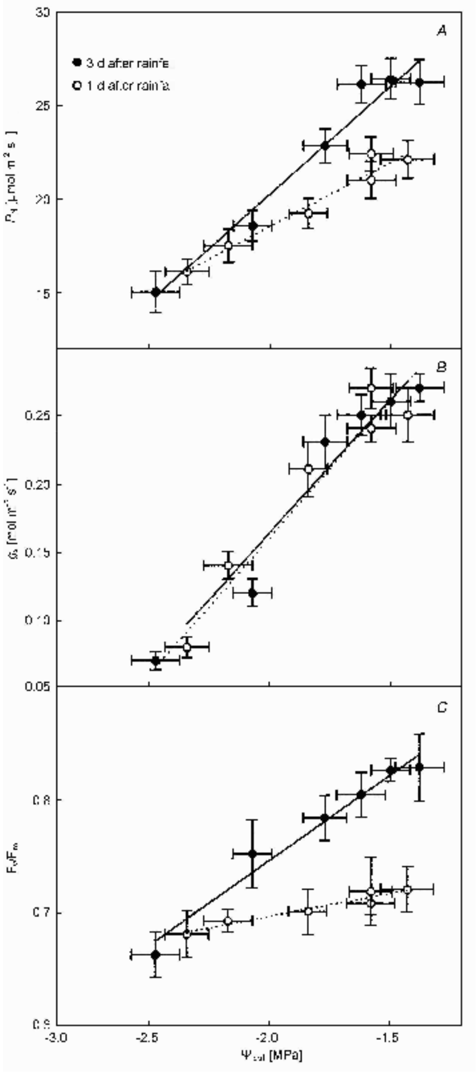

Fig. 4. Relationship between leaf water potential, Ψ_{leaf} and net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), and photochemical efficiency of photosystem 2, F_v/F_m (*C*). *Error bars* are ±S.E. Correlation and *r*-values: For P_N, 3 d after rainfall: $P_N = 11.551\ \Psi_{leaf} + 43.334$, $r^2 = 0.9598$; 1 d after rainfall: $P_N = 7.291\ \Psi_{leaf} + 33.194$, $r^2 = 0.9589$. For g_s, 3 d after rainfall: $g_s = 0.2021\ \Psi_{leaf} + 0.5641$, $r^2 = 0.953$; 1 d after rainfall: $g_s = 0.1954\ \Psi_{leaf} + 0.5546$, $r^2 = 0.9812$. For F_v/F_m, 3 d after rainfall: $F_v/F_m = 0.1511\ \Psi_{leaf} + 1.0479$, $r^2 = 0.9699$; 1 d after rainfall: $F_v/F_m = 0.0405\ \Psi_{leaf} + 0.7769$, $r^2 = 0.9366$.

watering in spite of the complete recovery of stomata (Fig. 2), which reflects the non-synchronous recovery of g_s and P_N. Therefore the non-stomatal limitation of photosynthesis might have functioned during stress and the initial stage of recovery. The depression of F_v/F_m (Fig. 3*C*) and carboxylation efficiency (Fig. 2*D*) also proved this. With the progress of recovery, P_N was completely recovered when the inhibition was relieved 3 d after irrigation. So, in water-stressed *H.f.m.* plants, where stomata closure played a major role in limiting photosynthesis, a non-stomatal limitation was probably also present, which confirms our hypothesis. This result was in accordance with that for pot experiments (Souza *et al.* 2003).

Contrary to previous studies (Saccardy *et al.* 1998, Shangguan *et al.* 2000), Chl *a* fluorescence parameters in our study were sensitive to water conditions. Re-watering led to an increase of 25 % in F_v/F_m values (Fig. 3*C*), indicating the occurrence of possible damages of photochemical activity under water stress. However, the damages of photochemical activity in *H.f.m.* seemed to be not very important, since P_N was recovered 1 d after irrigation, though F_v/F_m lacked alternation at that time (Fig. 3*C*). The slow recovery of fluorescence parameters revealed a long lasting down-regulation of PS2 activity (Long *et al.* 1994). Different to the pot experiment, the F_v/F_m value changed much more in our study and it stopped decreasing as soon as re-watering was applied, whereas the continued stress appeared 1 d after watering in the study of Souza *et al.* (2003).

Water use strategy of *H.f.m.*: *H.f.m.* might be categorised as conservative water user. Because small amount of rainfall (15 mm) could satisfy its need for photosynthesis (Fig. 2*A*), it did not open stomata further with the increase of 'rainfall' for the sake of stopping water losses from transpiration. The redundant rainfall might be conserved in deeper soils for potential extending growth in later drought period, just like in *Hilaria jamesii* (Schwinning *et al.* 2002). Such species could have stronger drought resistance, which is reflected by lower Ψ_{leaf} (Fig. 2*C*) that represents the higher ability of plant to absorb deep soil water (Boyer 1982, Tangpremsri *et al.* 1991).

Ψ_{leaf} regulates gas exchange and F_v/F_m: The studies on changes of Ψ_{leaf} and their relationships with other ecophysiological characters reflect plant tolerance to water deficit (Saliendra *et al.* 1995, Alder *et al.* 1996). In our study, g_s increased as Ψ_{leaf} increased along with the water treatment (Fig. 4*B*), as previously observed in other plant species (Prior *et al.* 1997, Oren *et al.* 1999). g_s sensitive to the changes of Ψ_{leaf} can be regarded as a protective mechanism against water loss under water stress (Saliendra *et al.* 1995, Alder *et al.* 1996). P_N and F_v/F_m were also closely correlated with Ψ_{leaf} in our study (Fig. 4*A,C*). This might be owing to the strong dependence of these parameters on stomatal conductance (Chaves 1991). The strong dependence of gas exchange and F_v/F_m on Ψ_{leaf} indicated higher drought tolerance of *H.f.m.* in sand area.

In conclusion, the photosynthesis recovery of *H.f.m.* after stress relief was attributed to both stomatal and non-stomatal factors, and PS2 activity was sensitive to water conditions. *H.f.m.* needs small amount of rainfall on account of its response to water treatments. The conservative water use strategy allows it successfully survive in drought shifting sand dunes.

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