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Effects of parasitic plant *Cistanche deserticola* on chlorophyll *a* fluorescence and nutrient accumulation of host plant *Haloxylon ammodendron* in the Taklimakan Desert

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Abstract: The parasitic plant *Cistanche deserticola* attaches to *Haloxylon ammodendron*, a perennial shrub with high tolerance to salinity and drought. However, little was known about the parasite-host relation between the two species. Effects of the parasite on chlorophyll *a* fluorescence and nutrient accumulation in the host plant (*H. ammodendron*) were investigated in the Taklimakan Desert. Some photosynthetic parameters of both host and non-host *H. ammodendron* plants were measured by *in vivo* chlorophyll *a* fluorescence technology in the field. The assimilating branches of host and non-host plants were collected and nutrient and inorganic ion contents were analyzed. The results from field experiments showed that the infection of *C. deserticola* reduced the non-photochemical quenching of the variable chlorophyll fluorescence (*NPQ*) and the potential maximum quantum yield for primary photochemistry (F_v/F_m) of the host. Compared with non-host plants, the host *H. ammodendron* had low nutrient, low inorganic ion contents (Na^+ and K^+) and low K^+/Na^+ ratios in the assimilating branches. It suggested that *C. deserticola* infection reduced the nutrient acquisition and caused damage to the photoprotection through thermal dissipation of the energy of the photosystem II in the host, resulting in a decrease in the tolerance to salinity and high radiation. It was concluded that the attachment of the parasite plant (*C. deserticola*) had negative effects on the growth of its host.

Keywords: parasite-host relation; nutrient acquisition; inorganic ion content; non-photochemical quenching; the Taklimakan Desert

Haloxylon ammodendron, a perennial shrub of family Chenopodiaceae, is mainly distributed in the deserts and semi-deserts of the Northern Hemisphere (Xu *et al.*, 2010). Due to its high tolerance to drought and salinity, it was often planted as shelter belts in the northwest of China. This perennial plant is known as the host of *Cistanche deserticola*, a perennial parasitic herb of family Orobanchaceae. Because of its own inability to photosynthesis, *C. deserticola* attaches underground directly to the roots of the dicotyledonous plant *H. ammodendron* (Naran *et al.*, 1995). Growth of *C. deserticola* depends completely on its

host, which provides carbohydrate, minerals, and even water. Recent studies paid more attention to the interaction between *C. deserticola* and its host (Zheng *et al.*, 2006; Tan *et al.*, 2007). It was reported that this parasitic plant decreased relative water content and water retention capability in the assimilating branches of *H. ammodendron* (Li *et al.*, 2009a), damaged the protective enzyme system and osmotic adjustment system, caused a decrease in the host's drought resistance (Li *et al.*, 2009b), and reduced the biomass of

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the host plant (Tan *et al.*, 2004; Huang *et al.*, 2009). However, effects of the parasitic plant *C. deserticola* on the photosystem activity of *H. ammodendron* were little known.

The Taklimakan Desert, the biggest desert in China, is known for extreme high temperature and drought, and especially for wind blown sand (Wang, 2003). *Haloxylon ammodendron* was planted as shelter belts to protect the desert highway from sand encroachment (Li *et al.*, 2008). In the mean time, *C. deserticola* was often artificially attached to the roots of *H. ammodendron* to produce an economic crop. Pharmacological research has shown that extracts from *Cistanche* plants possess a wide spectrum of health benefits, such as enhancing immunity, anti-aging and anti-fatigue (Jiang and Tu, 2009). *Cistanche deserticola* is known as Rou Cong Rong (Renshen in Chinese) due to its medicinal properties (Naran *et al.*, 1995). Its English common name is Ginseng.

Photosynthesis is one of the most important metabolic activities in plants. Photosystem II (PSII) is thought to be the most sensitive site of inhibition in plants to environmental stress (Perales-Vela *et al.*, 2007; Rapacz, 2007). The study described in this paper aimed to investigate the effect of *C. deserticola* infection on photosystem II activity in the host plant *H. ammodendron* by *in vivo* chlorophyll *a* fluorescence technology. Further, due to the practice of irrigation with salty water, the status of inorganic ions and nutrient accumulation between the host and non-host plants were also investigated.

1 Materials and methods

1.1 Study area

The study was carried out at the Taklimakan Station for Desert Research, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences (39°06'N, 83°40'E). The station is located in the centre of the Taklimakan Desert with typical characteristics of continental climate. Annual precipitation is only 10.7 mm and evaporation exceeds 3,800 mm. Due to extremely high evaporation and little precipitation, the area is hyper-arid and natural conditions are very poor.

1.2 Materials and samples

Host plants (with *C. deserticola* attached) and non-

host plants were selected for this study in field. Host plants were selected after the parasite was observed to be attached to the roots. Non-host plants were selected as the control after at least 3 years without parasite infection. *H. ammodendron* plants of similar ages were chosen so that variability could be reduced.

Four individual host and non-host plants of *H. ammodendron* were selected, and five assimilating branches from each plant were tagged for the measurement of chlorophyll *a* fluorescence. At the same time, assimilating branches from each plant were collected and air-dried for 48 h at 60°C for nutrient and ion analysis.

1.3 Methods

1.3.1 Chlorophyll *a* fluorescence measurement

Chlorophyll *a* fluorescence parameters were measured by a portable fluorometer (PAM-2100, Walz, Germany). The measurements were carried out in the morning following the leaves adapting to dark conditions for the whole night. Photosynthetic available radiation (*PAR*) was controlled at a level lower than 10 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ to ensure full dark-adaptation. The minimum fluorescence (F_o) was obtained by measuring modulated light that was sufficiently low ($<100 \mu\text{mol}/(\text{m}^2\cdot\text{s})$) not to induce any significant variable fluorescence and the maximal fluorescence (F_m) was determined by a 0.8 second saturating pulse at 8,000 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ in dark-adapted leaves. The variable fluorescence F_v is calculated as $F_m - F_o$, and the potential maximum quantum yield for primary photochemistry (F_v/F_m) was obtained according to the function $(F_m - F_o)/F_m$ (Genty *et al.*, 1989). In natural radiation, following adaptation to full light, leaves were measured once each 2 h from 6:00 to 18:00 to obtain the steady-state fluorescence (F_s) and the maximal fluorescence level during natural illumination (F_m'). The non-photochemical quenching (*NPQ*) of the variable chlorophyll fluorescence was calculated as $(F_m - F_m')/F_m'$ (Krause and Weis, 1991). The relative electron transport rate (*rETR*) was calculated as $PAR \times (F_m' - F_s)/F_m' \times 0.84 \times 0.5$, where, *PAR* is photosynthetic available radiation in leaf; 0.5 is a factor that assumes equal distribution of energy between the two photosystems; and 0.84 is assumed leaf absorbance (Genty *et al.*, 1989; Ralph and Gademann, 2005).

1.3.2 Determination of nutrient status

Samples of dry leaf were digested with sulfuric-perchloric acid ($\text{H}_2\text{SO}_4/\text{HClO}_4$) using a block heater. Nitrogen was analyzed using the Kjeldahl method with an auto sample analyzer (Tecator Digestion system 6, Höganäs, Sweden). Potassium concentrations of samples were measured using an atomic absorption spectrometer (Thermo Solaar M, Thermo Electron, USA) and phosphorus was determined spectrophotometrically after molybdenum antimony impedance colorimetry (Cresser and Parsons, 1979).

1.3.3 Determination of inorganic solutes

The finely ground dry leaf samples were placed in test tubes containing distilled water. The tubes were incubated in boiling water for 1 h. N and K concentrations of the solutions were analyzed using an atomic absorption spectrometer (Thermo Solaar M, Thermo Electron, USA; Warwick and Halloran, 1992).

1.4 Statistical analysis

Except where referred to in the text, all of the treatments were repeated three times. A one-way analysis of variance (ANOVA) was performed using SPSS software (SPSS 13.0). Tests of difference between two values used Student's *T* test at the 5% level of significance. Duncan's analysis was used in Post Hoc Multiple Comparisons also at the 5% level of significance.

2 Results

2.1 Diurnal variation in leaf temperature and PAR

Diurnal variation in leaf temperature and PAR showed a single peak at noon. There was a small difference between these two parameters. The PAR rose from 6:00 in the morning, and reached a peak at about 12:00 noon. At 18:00 in the afternoon, it almost fell back to the same level as it was at 6:00. The curve of diurnal variation in leaf temperature showed a delay behind the PAR curve. The peak was observed at 14:00, after which the leaf temperature had a slow decline. The value was still about 30°C at 18:00 in the afternoon, which was the same as it was at 10:00 in the morning (Fig. 1).

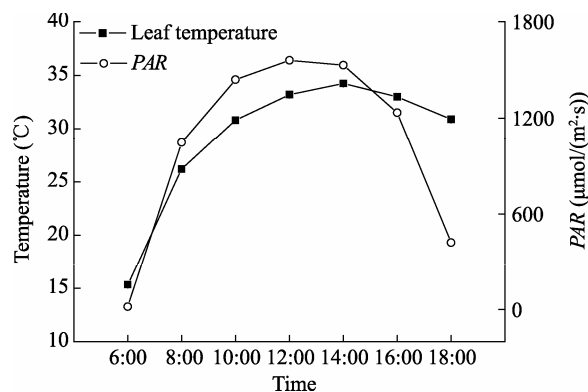


Fig. 1 Curves of diurnal variation in leaf temperature of *H. ammodendron* and photosynthetic available radiation (PAR) during the experimental period in the Taklimakan Desert. $n=20$.

2.2 Chlorophyll *a* fluorescence

The maximum quantum yield for photochemistry (F_v/F_m) was significantly different between non-host and host plants ($P<0.05$). The values of F_v/F_m in the non-host and the host plants were 0.775 ± 0.002 and 0.759 ± 0.003 , respectively. F_v/F_m was higher in the former than in the latter (Fig. 2). The diurnal variation curve of the relative electron transport rate ($rETR$) in non-host plants showed a single peak, which was similar to the curve for photosynthetic available radiation (PAR). In host plants, however, no clear peak was observed. At almost all times, there was no significant difference between host and non-host plants, except at 12:00 noon (Fig. 3).

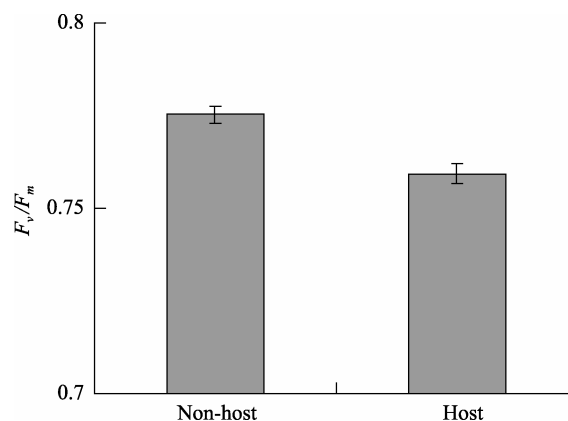


Fig. 2 The maximum quantum yield for primary photochemistry (F_v/F_m) in host and non-host *H. ammodendron*. Little bars indicate S.E., $n=20$. The figure shows a significant difference between non-host and host plants using Student's *T* test ($P<0.05$).

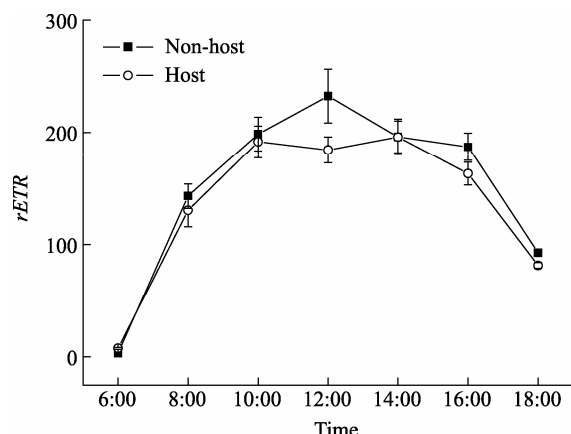


Fig. 3 Diurnal variation curves of relative electron transport rate ($rETR$) in host and non-host *H. ammodendron*. Little bars indicate S.E., $n=20$. No significant difference between the mean values for host and non-host plants was observed at any time of the day, except at 12:00 noon ($P<0.05$). Differences within treatments at different times of the day were not compared.

A significant difference between the host and the non-host plants was observed in non-photochemical quenching of the variable chlorophyll fluorescence. The values of NPQ in the host plants were obviously lower than those in the non-host plants at any time of the day ($P<0.05$). But no significance difference in values was observed within the host or the non-host plants between readings taken at different times in the day (Fig. 4).

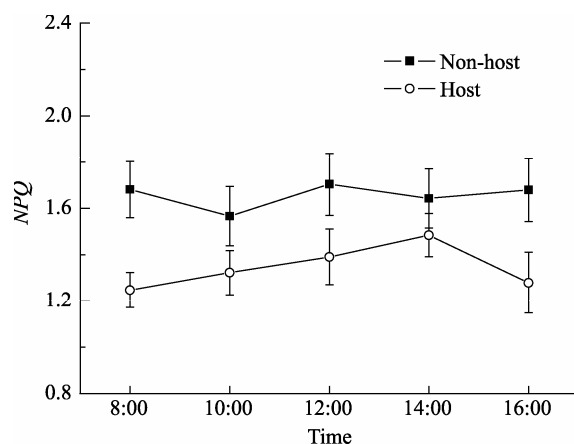


Fig. 4 The diurnal variation curves of the non-photochemical quenching (NPQ) of the variable chlorophyll fluorescence in host and non-host *H. ammodendron*. $n=20$. Little bars indicate S.E. The results within treatments are not shown because the differences at different times in non-host or host plants were not significant (Duncan test at $P<0.05$).

2.3 Nutrient and inorganic ion contents

The host plants showed a lower nutrient content than non-host plants. The contents of N and K in the as-

similating branches of *H. ammodendron* host plants were respectively only 59% and 52% of the contents found in non-host plants. The former values were obviously lower than the latter ones. The content of P showed no difference between the host and non-host plants (Table 1).

The assimilating branches of infected *H. ammodendron* had lower contents of Na^+ and K^+ . The contents of Na^+ and K^+ in the non-hosts were 1.15 and 2.07 times the contents of the host plants, respectively. The contents of K^+ had a more significant drop in the host plants. So, K^+/Na^+ ratio was 0.74 ± 0.57 and 0.41 ± 0.67 in the non-host and the host plants, respectively. The former was significantly higher than the latter ($P<0.05$) (Table 1).

3 Discussion and conclusion

The field experiment carried out in the Taklimakan Desert showed that *C. deserticola* infection reduced the relative electron transport ratio (at 12:00 noon), the non-photochemical quenching of PSII, and the maximum quantum yield for primary photochemistry of PSII (F_v/F_m) in host plants of *H. ammodendron*, resulting in decreased accumulation of nutrients and inorganic ions in the host plants.

The maximum quantum yield for primary photochemistry (F_v/F_m), which is the most frequently used fluorescence parameter, is often regarded as an indicator for understanding the responses of plants under environmental stress (Roháček, 2002). F_v/F_m can represent the amount of energy trapped in the reaction centers (RCs) of photosystem II in relation to energy absorbed (Strasser *et al.*, 2000). Lower F_v/F_m values may indicate inactivity in parts of PSII RCs (Li *et al.*, 2004). In the present study, the value of F_v/F_m was higher in non-host plants than in hosts. Therefore it is concluded that the infection of *C. deserticola* caused damage to the photosynthetic apparatus of *H. ammodendron* plants, especially to PSII RCs.

In chlorophyll *a* fluorescence studies, NPQ is another frequently used parameter. The parameter is linearly related to the excess radiation and the extent of development in leaves, and so it is often used as an indicator of the excess radiation energy dissipation to heat in the PSII antenna complexes (Demmig-Adams

Table 1 Nutrient and inorganic ion contents in host and non-host *H. ammodendron*.

	N*	P	K*	Na**	K**	K ⁺ /Na ⁺ **
	(mg/g DW)					
Non-host	21.08±1.14	4.65±0.18	42.67±0.48	55.53±0.90	41.15±0.51	0.74±0.57
Host	12.47±0.27	3.97±0.12	22.13±0.07	48.14±1.28	19.86±0.84	0.41±0.67

Note: Each value is shown as mean±SE ($n=3$). * shows significant differences between non-host and host plants ($P<0.05$).

et al., 1996). *NPQ* is thought to be due to down regulation of PSII antenna efficiency and light-induced photoprotection through thermal dissipation of energy (Kramer *et al.*, 2004). In this study, a value of *NPQ* in the host lower than that in the non-host was observed. *C. deserticola* infection resulted in decreasing photoprotection through thermal dissipation of energy in the host. Lu *et al.* (2003) reported that high light induced higher *NPQ* and the increase of photoprotection in the halophyte *Artimisia anethifolia*. The decrease in photoprotection seemed to associate with the damage to antenna complexes in PSII.

Nitrogen (N), phosphorus (P) and potassium (K) are the most essential nutrients to plants and are often the limiting factors in plant growth in natural habitats (Bucher, 2007). The hemi-parasite *Santalum album* reduced the accumulation of nitrogen (N) in legume and non-legume hosts (Radomiljac *et al.*, 1999). Matthies and Egli (1999) showed that host biomass is significantly reduced under low nutrient conditions, suggesting that limiting resources (such as nutrients) play the major competitive role between host and parasite. In the Taklimakan Desert, available nutrients in the soil are very scarce. Contents of nitrogen and phosphorus are especially low (Gu *et al.*, 2002). Our results showed that nitrogen and phosphorus contents were significantly lower in host plants than in non-host plants (Table 1). This suggested that *C. deserticola* infection deprived host plants of nutrients.

Haloxylon ammodendron, which is distributed across dry deserts and salt pans, has high tolerance to osmotic and salt stress (Tobe *et al.*, 2000). Tobe *et al.* (2000) showed that this species accumulated some inorganic ions, such as Na⁺ and Cl⁻, to enhance the tolerance to salinity. Further, Wang *et al.* (2004) and Song *et al.* (2006) proved that inorganic ions, especially Na⁺, were important in osmoregulation for *H. ammodendron* to help it adapt to saline and arid environments. In this study, *C. deserticola* infection led to a decrease in the Na⁺ content in host plants com-

pared to non-host plants. Intracellular K⁺ homeostasis is critical for plant salt tolerance (Chen *et al.*, 2007). Higher external Na⁺ disrupts K⁺ homeostasis in the cell by competing with K⁺ for the same uptake sites, so K⁺/Na⁺ ratios are often indications of the capacity of plants to counteract salinity stress (Chen *et al.*, 2005). Ghars *et al.* (2008) concluded, in comparison of the salt tolerance of the two plant species of *Thellungiella halophila* and *Arabidopsis thaliana*, that the high NaCl tolerance in *Thellungiella halophila* was associated with a good K⁺ supply, resulting in high K⁺/Na⁺ ratios. Our study showed that, compared with non-host plants, host plant accumulated less K⁺ and achieved lower K⁺/Na⁺ ratios in assimilating branches. This suggests that *C. deserticola* infection decreases the tolerance to salinity of *H. ammodendron*.

Parasitic plants have profound effects on the ecosystems in which they occur. They produce major impacts on host growth, allometry and reproduction, resulting in changes in competitive balances between host and non-host species (Press and Phoenix, 2005). Some parasite infections had no effect on the crop yield of host plants (Rambakudzibga *et al.*, 2002). In our study, the parasitic plant *C. deserticola* had negative effects on the growth of its host. *C. deserticola* infection reduced nutrient acquisition, inorganic ion accumulation and the photoprotection through thermal dissipation of energy in the host by PSII, resulting in the decrease of tolerance to high radiation and salinity stress. *C. deserticola* infection produced a negative effect on nutrient acquisition and the PSII activity of *H. ammodendron*. Therefore, providing sufficient nutrients and water to the host plant *H. ammodendron* during its growth period can achieve a high productivity of the parasite *C. deserticola*.

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