BRIEF COMMUNICATION

Chlorophyll *a* fluorescence responses of *Haloxylon ammodendron* seedlings subjected to progressive saline stress in the Tarim desert highway ecological shelterbelt

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Abstract

In order to assess the long-term impacts of saline groundwater irrigation to *Haloxylon ammodendron*, one of the main shrubs in the Tarim desert highway ecological shelterbelt, we irrigated the *H. ammodendron* seedlings with progressive saline groundwater ($3-30$ g L^{-1}, simulation environment in the Tarim desert highway ecological shelterbelt) and investigated the diurnal variations of chlorophyll *a* (Chl *a*) fluorescence parameters, such as maximal quantum yield of photosystem II (PSII) photochemistry (F_v/F_m), quantum yield of photochemical energy conversion in PSII (Y_{II}), the apparent rate of electron transport at the PSII level (ETR), photochemical quenching coefficient (q_P), non-photochemical quenching (NPQ), quantum yield of nonregulated non-photochemical energy loss in PSII (Y_{NO}) and quantum yield of regulated non-photochemical energy loss in PSII (Y_{II}), at approximately 2-h intervals. F_v/F_m with 5 g L^{-1} (S2) was lower than that with 2 g L^{-1} (S1) but a little higher than 20 g L^{-1} (S5), respectively. Under the low light [photosynthetically-active radiation (PAR) ≤ 250 μmol m^{-2} s^{-1}, at 08:00, 10:00 and 20:00 h of the local time], S1 kept the lowest Y_{II} and the highest Y_{NPQ}; while under the high light (PAR ≥ 1500 μmol m^{-2} s^{-1}), the Y_{II} performed S1>S2>S5, and the reverse Y_{NPQ}; under mild light (250 μmol m^{-2} s^{-1} $\leq$ PAR $\leq$ 1500 μmol m^{-2} s^{-1}), S1 remained the highest Y_{II}, no matter the light and the salinity, the similar Y_{NO} almost occurred basically. The results showed that the sand-binding plant *H. ammodendron* could regulate its energy-utilizing strategies. The S2 might be the most suitable salinity of the irrigation water for *H. ammodendron* in the Tarim desert highway ecological shelterbelt in the northwest of China.

Additional key words: energy dissipation; irrigation with salt groundwater; *PAM-2100*; saline stress.

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Plants adjust to changes in harsh environments, namely various stresses, in an attempt to optimize and preserve the function of the photosynthetic apparatus, which can be detected by the Chl *a* fluorescence changes of the plant to show its energy-utilizing proportion. Soil salinity stress, which can be induced by irrigation with salt water, is one of the abiotic stresses. The response of the plants to the saline water irrigation is one of the current focuses for plant stress physiology.

The Tarim desert highway ecological shelterbelt plants were irrigated by saline groundwater ($3-30$ g L^{-1}) bumped from the local wells to protect the security of the highway. The shelterbelt project, 436 km in length and 72–78 m in width, was fully lunched in 2003 and completed in 2006 and formed a green corridor in the Taklimakan desert. *H. ammodendron* (C. A. Mey) Bunge (Chenopodiaceae), dominant perennial in many areas of Asian deserts (Wu 1995), was used as one of the main

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shelterbelt shrubs, which is a valuable resource for the blown sand control (Dong *et al.* 2004). *H. ammodendron* plants also could be used to resist salinity, and were called xero-halophytes (Zhao and Li 1999). The growth status of the shelterbelt plants is an essential factor to sand controlling. The short photosynthetic performance of the shelterbelt plants indicates the plant status. Scientists have come to understand the biological and ecological characteristics and their physiological indices irrigated with salt water (Huang *et al.* 2003). The responses of *H. ammodendron* to salt stress have attracted a lot of attention. However, the course of the energy dissipation changes of *H. ammodendron* subjected to salt stress, especially the energy dissipation mechanisms, has not been understood well so far.

In recent years, noninvasive methods such as Chl *a* fluorescence were used to observe different types of plants and diseases affecting the photosynthetic machinery (Lichtenthaler 1996). Measurements of Chl fluorescence make it possible to evaluate the plant's photosynthetic energy-utilizing strategies and the extent of its tolerance to environmental stress (Maxwell and Johnson 2000).

The fluorescence yields F_o or F_o' can be measured with dark-adapted or illuminated samples, respectively (Kooten and Snel 1990). Cailly *et al.* (1996) first presented expressions, based on basic fluorescence parameters that describe the partition of absorbed excitation energy in PSII between three fundamental pathways. The expressions showed three parts in terms of the complementary quantum yields of PSII: (*1*) Φ_{II} (Y_{II}), photochemical conversion, (*2*) Φ_{NPQ} (Y_{NPQ}), regulated thermal energy dissipation related to NPQ to protect the photosynthetic apparatus initiatively, and (*3*) Φ_{NO} (Y_{NO}), primarily constitutive losses, corresponding to the sum of nonregulated heat dissipation plus some of the fluorescence emission. $\Phi_{II} + \Phi_{NPQ} + \Phi_{NO} = 1$, $\Phi_{II} = (F_m' - F)/F_m' = \Delta F/F_m'$,$\Phi_{NPQ} = F/F_m' - F/F_m$, $\Phi_{NO} = F/F_m$ (Cailly *et al.* 1996).

The experiments were performed in the Taklimakan Desert Research Station/Tazhong Botanical Garden, Chinese Academy of Sciences, in the hinterland of the Taklimakan Desert in Xingjiang Uygur Autonomous Religion (39°01' N, 83°36' E, 1100 m a.s.l.), where the annual average precipitation is 24.6 mm, annual mean evaporation capacity 3,638.6 mm, mean air temperature 12ºC, average July temperature 75.3ºC, average December temperature –8.1ºC, maximum air temperature 45.6ºC, minimum air temperature –22.2ºC, and active accumulated temperature (≥ 10ºC) 4,618.6ºC, which show the extremely arid condition. The serious blown sand disasters are characterized also by an annual average wind speed of 2.5 m s^{-1}, a maximum instantaneous wind speed of 20.0 m s^{-1}, and total annual sand-shifting windy days of more than 130 d.

60 yearling seedlings, about 30–40 cm high, healthy and free of diseases and pest damages, irrigated sufficiently every 10 days from early April to the end of September, 2009, with different salt groundwater 2 g L^{-1} (S1), 5 g L^{-1} (S2), 10 g L^{-1} (S3), 15 g L^{-1} (S4), 20 g L^{-1} (S5), and 28 g L^{-1} (S6), respectively were potted in pots outside. The soil salt concentration was assessed, with the mass of soil:water = 1:5, after 3 min dissolved shaked by a shock-bed, by soil filtrate electric conductivities measured by the conductivity thermometer *SY-3* (*Nanjing Institute of Soil Science, Chinese Academy of Sciences*, Nanjing, China).

In situ Chl *a* fluorescence was measured on 15, 20, and 25 August 2009, which were all clear days. The highest PAR was 1,800 µmol m^{-2} s^{-1}, air temperature was about 33–37ºC. Chl *a* fluorescence of every plant per treatment was measured at predawn and at approximately 2-h intervals for the diurnal variation from 08:00 to 20:00 following the method (Nogués *et al.* 1998), with a portable Chl *a* fluorescence fluorometry (*PAM-2100, Walz GmbH*, Effeltrich, Germany), equipped with a Leaf-Clip Holder (*Walz, model 2030-B*), which can monitor PAR and leaf temperature simultaneously. Dark-adapted green leaves, which had been placed into darkness for 30 min or longer, were given an irradiance (0.5 µmol m^{-2} s^{-1}), then F_o was measured, and F_m after SP (PAR > 6,000 µmol m^{-2} s^{-1}, for 100–200 ms). Actinic light for F_m' measurement was about 1,500 µmol m^{-2} s^{-1} (3–5 min). At the end of the measurement, F_o' was estimated after a far-red irradiance (10–20 s). Measurements of $\Delta F/F_m'$ and other parameters were performed in 6 repetitions for each treatment. The derived Chl *a* fluorescence parameters $\Delta F/F_m'$, F_v/F_m, q_P, NPQ, Y(II), Y_{NO}, and Y_{NPQ} were arcsine-transformed prior to statistical analysis (Sokal and Rohlf 1981). One-way and two-way *ANOVA* (salt × light: $p<0.05$ or $p<0.01$) were used to test the effect of salinity and PAR on Chl *a* fluorescence parameters using *SPSS 11.0 for Windows*, (Chicago. IL, USA), $p=0.05$. The least significant differences (LSD) between the means were estimated at 95% confidence level. Results were presented as means ± SE ($n = 6$). The graphs were processed by *SigmaPlot 8.0*.

The soil filtrate conductivities at different depths were shown (Table 1). The up-trend with the salinity increasing can clearly be seen. Salt stress elicited a significant decline of F_v/F_m during progressing salt stress. (2 g L^{-1}: $F_v/F_m = 0.745 \pm 0.11$ a; 5 g L^{-1}: $F_v/F_m = 0.649 \pm 0.10$ b; 20 g L^{-1}: $F_v/F_m = 0.620 \pm 0.15$ c, $p<0.01$, $n = 6$). Diurnal variations of some Chl *a* fluorescence parameters, such as PAR, leaf temperature, q_P, NPQ, and ETR, were shown in Fig 1.

q_Ps of the three salt degrees in middle were all lower than the other. q_Ps of the S2 seedlings were significantly lower ($p<0.05$) than those of S1 plants (Fig. 1*C*). At 08:00 and 10:00, q_P of S2 was lower than the other two, but there were no significant differences among the three salt degrees in 12:00 and 20:00. ETR showed the similar changing principles as q_P. The maximum ETR of S1 occurred around 16:00 (PAR was about 1,500 µmol

Table 1. The soil filtrate conductivities in depth, irrigated with differently saline water. The measurements were performed under room temperature, 25ºC, the mass ratio of soil and water was 1:5 (the means ± SE, n = 6. Significant difference was shown at α=0.05).

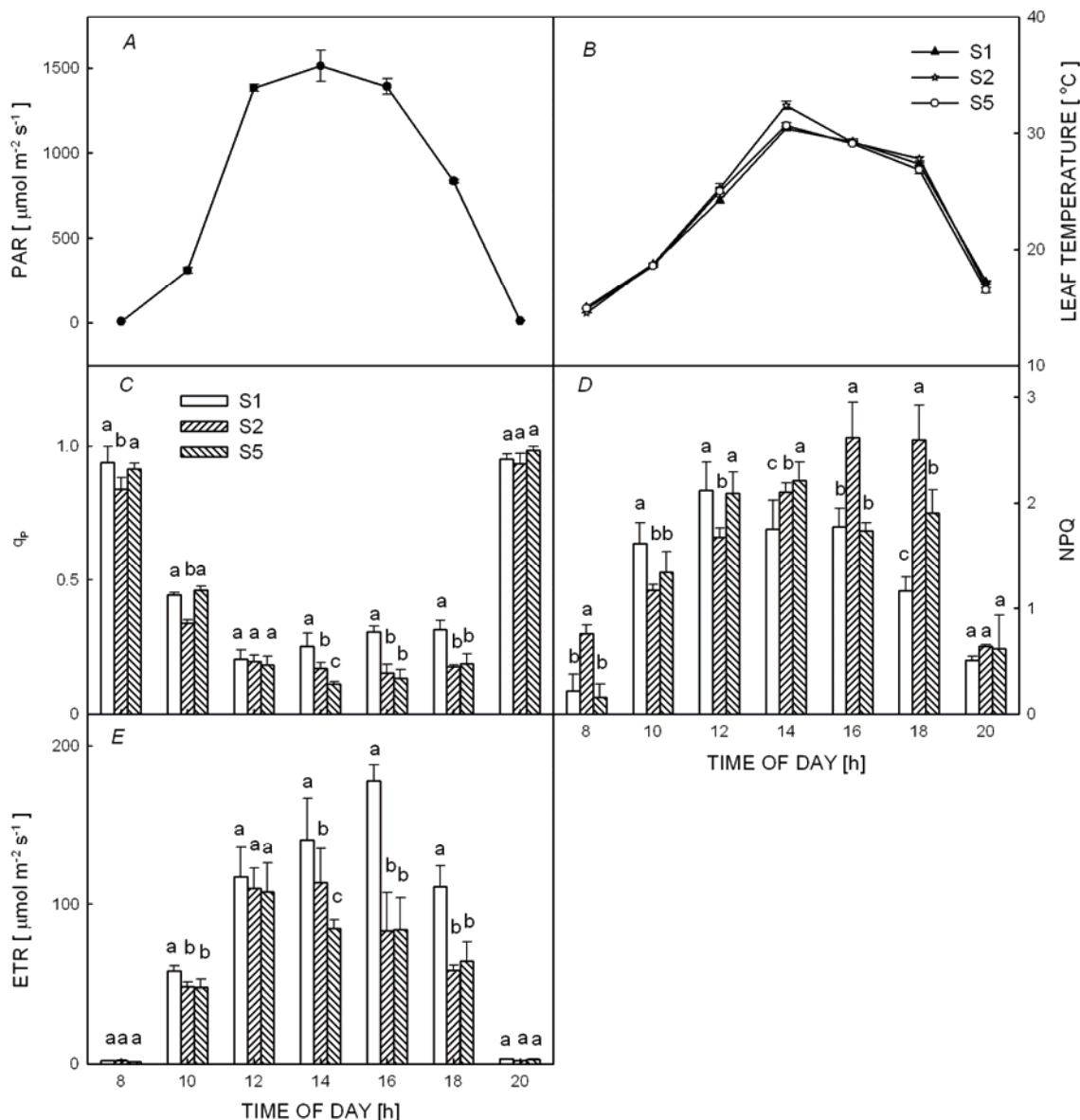| | Depth [cm] | Salinities [g L^{-1}] | | | | | |
		2	5	10	15	20	28
Conductivity [mS cm^{-1}]	5	0.118 ± 0.009[e]	0.712 ± 0.010[d]	0.982 ± 0.020[c]	1.37 ± 0.080[b]	1.071 ± 0.032[c]	4.47 ± 0.119[a]
	15	0.212 ± 0.011[f]	1.485 ± 0.020[d]	1.196 ± 0.030[e]	1.98 ± 0.068[c]	2.33 ± 0.055[b]	3.91 ± 0.112[a]
	30	0.46 ± 0.015[f]	0.906 ± 0.010[d]	0.721 ± 0.012[e]	2.79 ± 0.104[b]	1.534 ± 0.028[c]	3.29 ± 0.097[a]

Fig. 1. Diurnal patterns in *A*: photosynthetically active radiation (PAR); *B*: leaf temperature; *C*: photochemical quenching (q_P); *D*: non-photochemical quenching (NPQ); *E*: electron transport rate (ETR). S1 (2 g L^{-1}), S2 (5 g L^{-1}), and S5 (20 g L^{-1}). Symbols shown were the means ± SE (n = 6). Significant difference was shown at α=0.05 level.

m^{-2} s^{-1}, and leaf temperature was about 27ºC), and decreased afterwards (Fig. 1*E*). However, for S2 ETR$_{max}$ peaked at 14:00 (PAR was around 1,625 µmol m^{-2} s^{-1}, and leaf temperature was about 31ºC), and de-

creased thereafter. For S5, the peaked time was 12:00, the peaked PAR and leaf temperature was 1,400 µmol m^{-2} s^{-1} and 25ºC, respectively (Fig. 1*A*, *B*, *E*). The results showed the stress time and PAR under S1, S2, and S5.
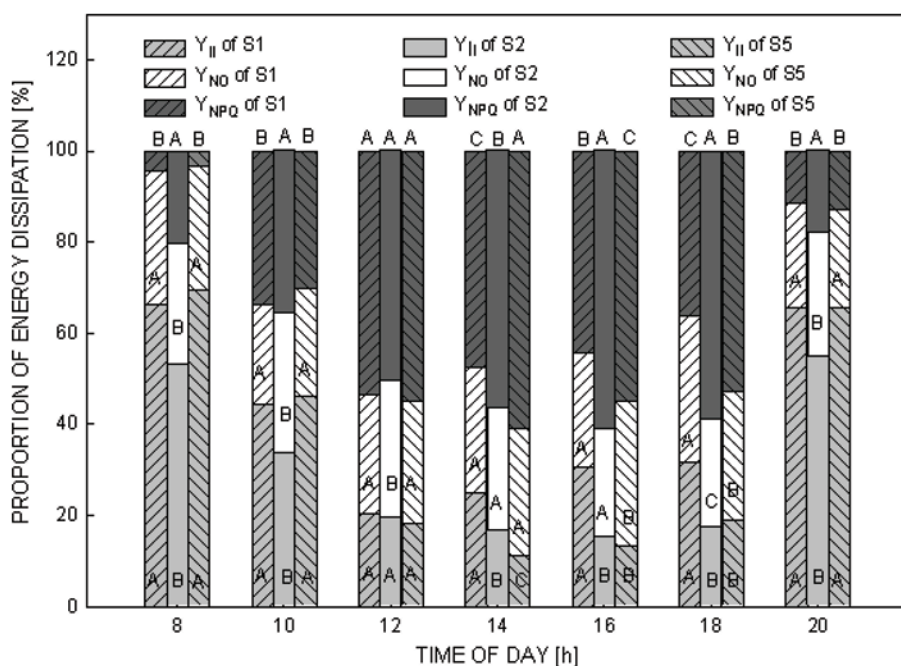
Fig. 2. Three parts in terms of the complementary quantum yields of PSII: (*1*) quantum yield of photochemical energy conversion in PSII (Y_{II}), (*2*) quantum yield of regulated non-photochemical energy loss in PSII (Y_{NPQ}) and (*3*) quantum yield of nonregulated non-photochemical energy loss in PSII (Y_{NO}) with salt stress were shown. Y_{II} (■) – effective quantum yield of PSII photochemistry; Y_{NO} (□) – quantum yield of non-regulated energy dissipation of PSII; Y_{NPQ} (■) – quantum yield of regulated energy dissipation of PSII. *Bars* represent least mean values (*n* = 6) for treatments. Values of the same quantity designated with different letters are statistically different at *p*<0.01. *Pattern bars* represent S1 (2 g L^{-1}, ▨), S2 (5 g L^{-1}, no slash), and S5 (20 g L^{-1}, ◩) respectively.

Table 2. The average heights of *H. ammodendron* seedlings irrigated with differently saline water. The test plots, in which the seedlings of *H. ammodendron* were simultaneously planted, were sufficiently irrigated every 10 days from early April to the end of September 2009. The heights of *H. ammodendron* seedlings were accordingly recorded at the end of September (the means ± SE, *n* = 6). Significant difference was shown at α=0.05 level.

Salinity [g L^{-1}]	2	5	10	15	20	27.9
Height [cm]	142.8 ± 15.3[d]	186.2 ± 9.2[a]	170.5 ± 11.2[b]	156.3 ± 5.5[c]	156.5 ± 6.8[c]	129.6 ± 5.1[e]

NPQ increased progressively as salt stress intensified, particularly at 16:00 and 18:00 (Fig. 1*D*). For S1, the NPQ $_{max}$ time and PAR were 12:00 and 1,400 μmol m^{-2} s^{-1}; but for S2, the same occurred at 16:00 and 18:00 (PAR = 1,500, and 800 μmol m^{-2} s^{-1}), while the S5 peak appeared 14:00 (PAR = 1,610 μmol m^{-2} s^{-1}).

A significant decrease of Y_{II} was observed between S1 and S2 (Fig. 2) during daytime, but there were obvious enhancements at 08:00, 10:00, and 20:00 when salinity elevated from S2 to S5. Moreover, there was a continuous decrease in ETR among the three salinities over the course of the day (Fig. 1*E*). No obvious effect on Y_{NO} occurred with elevated salinities, but a few changes of the diurnal still could be found at 16:00, 18:00 and 20:00 during the salinity increase, yet unaffected at other times (Fig. 2). The Y_{NPQ} values at S1 and S2 obtained at 12:00 increased by 1,156% and 149% than in the morning (08:00), respectively. Interestingly, at S5, Y_{NPQ} at noon was 1,522% higher than that at 08:00, the diurnal

changes of Y_{NPQ} and NPQ showed similar patterns (Figs. 1, 2).

Irrigation with salt groundwater increased the soil salt concentration (Table 1), which subsequently injured the growth of plants directly. The declines in growth were observed along with the increasing groundwater salinities at the end of September, 2009. Salt stress resulted in a growth stimulation which was optimal at S2 (5 g L^{-1}) salt groundwater. The decreased heights of seedlings subjected to elevated salinity (Table 2) were often associated with drops in their absorbed energy.

Reduction of photosynthetic rates under salt stress, one of the environmental stresses, has been noted by Bongi and Loreto 1989, Everard *et al.* 1994, and Redondo-Gómez *et al.* 2007. We found that all of the ETRs in *H. ammodendron* did not decrease along with increasing of salinity stress. Although the ETR of S2 and S5 plants sharply decreased compared with the S1 seedlings, S5 performed similarly to S2 in daily course

(Fig. 1). This suggested that *H. ammodendron* could maintain photochemical activities as salinity stress increased from mild to severe level under mild light. The significant decrease of ETR accompanied with the significant climbs of quantum yield of regulated energy dissipation (Y_{NPQ}) was observed under high light, but there were almost no changes of nonregulated energy dissipation (Y_{NO}). We detected that the coefficient of photochemical quenching (q_P) decreased, and non-photochemical quenching (NPQ) increased significantly under severe salt stress plus high light (Fig. 1).

Under high light, three parts of energy utilizing did not perform the same types along with the progressive salinities. Y_{NPQ} represents the part of the absorbed irradiance that is dissipated as heat (Demmig-Adams *et al.* 1996). Usually, plants show NPQ values varying from 0 to 3.5 under saturating irradiance; these values may vary according to species (Maxwell and Johnson 2000). In the results, we found the huge changes of Y_{NPQ} under high light and severe salt stress, but the recovery of the Y_{II} and stable Y_{NO} could be seen to suggest the upper limits of the salt and light. Since $Y_{II} + Y_{NPQ} + Y_{NO} = 100\%$, according to the previous demonstration of Kramer *et al.* (2004), both Y_{NPQ} and Y_{NO} factors contributed to the effects of salinity stress on the reduction of Y_{II}. When subjected to salinity stress, the plant dissipated the excess energy in form of heat *via* the regulated photoprotective NPQ mechanism firstly (Bilger *et al.* 1995, Demmig-Adams *et al.* 1996). Therefore, the Y_{II} reduction of the *H. ammodendron* seedlings irrigated with salt groundwater could be due to the reasons mentioned above. In this study, Y_{NPQ} mainly increased with the increase of water salinities under high light. Therefore, it is reasonable that Y_{II} of S1 was notably higher than that in S2 and S5 plants under high light, indicating distinct stresses along with the increase of water salinities. The different change trends of q_P with different salinity groundwater could give farther evidence (Fig. 1*C*).

Under lower light, S2 had higher Y_{NPQ} than S1 and S5, which suggested that the proper salt stress could enhance the Y_{NPQ} to protect the damage to the photosynthetic apparatus, but too high salt stress might inhibit PSII. Allakhverdiev *et al.* (2002) reported the different effects of light and salt stress on PSII in cyanobacterium *Synecchocystis* sp. PCC 6803: strong light induced photodamage to PSII, whereas salt stress inhibited the repair of photodamaged PSII and does not accelerate damage to PSII directly. Baker and Rosenqvist (2004) showed that high salt treatment at high irradiance elicited acute photoinhibition and increased thermal energy dissipation *via* the xanthophyll cycle at midday. Salt stress inhibited PSII activity, which had been identified as an early event of plant responses to salt stress under field condition, leading to a limitation of carbon uptake by leaves and slow growth (Bilger *et al.* 1995). Plants of the high salt treatment 5 g L^{-1} and 20 g L^{-1} were in different phases of the salt stress therefore they exhibited differences in NPQ as compared to the low salt treatment, the 5 g L^{-1} treatment had higher NPQ (at midday) compared to the 2 g L^{-1} treatment, which meant it had higher induction of the photoprotective process (Fig. 1). However, in the 20 g L^{-1} treatment NPQ was lower (during most hours of the day) compared to the 5 g L^{-1} treatment, which might suggest a possible impairment of the photoprotective processes under higher salinity. We could get this point from the fact that both 5 g L^{-1} and 20 g L^{-1} treatments had relatively close F_v/F_m values. We could see this point confirmed in Table 2. However, as salt stress continued, especially when the groundwater salinity elevated from 2 g L^{-1} to 5 g L^{-1}, we found that the seedlings´ height did not decrease but increased. This may mean the feasible groundwater salinity for *H. ammodendron* is about 5 g L^{-1}. The maximum light which could not stress *H. ammodendron* might be of 1,400 µmol m^{-2} s^{-1} whatever the salinities of groundwater are in the hinterland of the Taklimakan desert.

In summary, we found that the upper limit of light was 1,400 µmol m^{-2} s^{-1} plus irrigation with 5 g L^{-1} saline groundwater, under which almost no stress of light and salt occured to *H. ammodendron*. We also found that, under high light, the dominant factor inhibiting photosynthesis in *H. ammodendron* was the increase of Y_{NPQ}, which corresponded to the fraction of energy dissipated in form of heat *via* the regulated photoprotective NPQ in progressing of salt stress; under mild- and low light, dilute salt groundwater could make a higher Y_{NPQ}. Y_{NO}, which reflects the fraction of energy that is passively dissipated in form of heat and fluorescence, kept almost stable during the increase of the saline groundwater.

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