盐碱胁迫对2个葡萄品种光合及荧光特征的影响

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摘要: 土壤盐碱胁迫是限制作物生产力的世界性问题。很多研究关注盐分胁迫或者碱胁迫单因子对作物的影响,盐碱混合胁迫的研究较少。本研究按照研究区自然土壤盐碱主要成分和组成比例($NaCl$、Na_2SO_4、Na_2CO_3质量混合比例为1∶0.5∶0.5),设置了不同浓度的盐碱处理(0,100,200,400 mmol/L),模拟无胁迫、轻度胁迫、中度胁迫和重度胁迫。试验材料为2个葡萄品种克瑞生和摩尔多瓦的3年生实生苗。研究结果表明,轻度盐碱处理能在一定程度上改善2个品种的光合能力。高浓度盐碱胁迫条件下,两品种的净光合速率和气孔导度数值均比对照提高,光合-光响应曲线趋势表现一致。对于克瑞生,光合速率的提高得益于气孔导度和蒸腾速率的提高,但是较低的PSⅡ光化学效率降低了这种惠益。对于摩尔多瓦,较高的PSⅡ光化学效率帮助了其保持较为稳定的净光合速率。2个品种显示了对盐碱胁迫的不同适应性机制。

关键词: 盐碱胁迫;光合-光响应曲线;光合特征;荧光特征;葡萄

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Saline-alkaline Stress on Photosynthesis, Chlorophyll a Fluorescence of Two Cultivars of Grape

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Abstract: Soil salinization and alkalization is a worldwide problem limit productivity of horticultural crops. Many studies have focused on responses of crop to either salt or alkaline stress. However, seldom have studies focused on the effects of this mixed stress. In our experiment in Yinchuan, Northern China, the mixtures of three salts ($NaCl$, Na_2SO_4, Na_2CO_3 with proportion of 1∶0.5∶0.5) in various concentrations (0, 100, 200, 400 mmol/L) were used to simulate a range of soil natural salt-alkaline conditions. Three-year-old seedlings of two grape cultivars, Crimson (CRS) and Moldova (MDW), widely spread in the world, were planted in open field conditions under the treatments. The results indicate that light saline-alkaline treatments enhanced the photosynthetic capability in two grape cultivars. For the high concentration, net photosynthetic rate (Pn) and stomatal conductance (Gs) of CRS or MDW increased. In Pn light response curves, the same tendency was observed. For CRS, the increase of Pn benefited from high Gs and transpiration rate (Tr), however, a low Fv/Fm decreased such impacts. For MDW, high Fv/Fm helpfully maintain a relatively stable Pn. The two cultivars demonstrated different suitability regime to saline-alkaline stress.

Key words: Saline-alkaline stress; Photosynthesis; Chlorophyll a fluorescence; Photosynthetic light response curve; Grape cultivars

Saline-alkaline soils extend throughout the arid or semi-arid regions in the world[1] and limit the productivity of horticultural crops[2-3]. Many crop cultivars have been introduced into saline-alkaline area in order

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to select the most suitable ones and improve productivity. The ability to distinguish suitable cultivars in its early stage is very important since classical methods of screening for environmental tolerance are based on yield response and time consuming and expensive. As a result, the selection of ecophysiological parameters as useful criteria in early growth stages recently has become an active research topic for some crop species [4-5].

Ecophysiological characterization of gas exchange at the leaf level may represent useful criteria for breeding and cultivar selection and the prediction of potential plant behavior in response to environmental conditions [6]. Photosynthesis [4], water use efficiency [7] and chlorophyll fluorescence [5] are important parameters for detecting growth response of a cultivar to environments. For photosynthesis, there has been found a positive relationship between net photosynthetic rate and productivity [8]. Values of maximal photosystem (PS) II and photochemical efficiency as well as direction of change were found to have a close correlation with the growth rate for *Phalaenopsis* saplings [5]. Under conditions of saline-alkaline stress, such parameters behaved differently. Salt stress decreased photosynthesis through stomatal and nonstomatal factors [9]. However, there are conflicting reports on the relationship between the photosynthetic quantum yield (Fv/Fm) and salt treatments, whether Fv/Fm is significantly correlated with salt stress [21-22] or not [23-24]. High photosynthetic water use efficiency has always been regarded as tone of he best adaptive trait for water deficits [25] and this trait could be taken as a reference for selecting cultivars which grow well in region with pronounced water deficits.

Although the physiological impacts of salt have been studied widely, investigations of physiological effects of mixed saline-alkaline stress with different concentrations are scarce. The present study was conducted to quantify and evaluate photosynthesis and chlorophyll is a fluorescence under increasing saline-alkaline concentrations on the vegetation stage of two common grape cultivars.

1 Materials and Methods

1.1 Study area

The experiment was conducted in Helan Research Base (38°26′-38°48′, N; 105°53′-106°36′, E) of Minzu University of China, a temperate sandy land in Helan County, Ningxia Hui Autonomous Region, Northern China. The prevailing climate is of temperate arid type with annual mean temperature is 8.7℃. The study area receives an annual precipitation of 143-195 mm, 80%-90% of which falls between May and September. However, the potential annual transpiration is as large as 1 567-2 155 mm, ten times the annual precipitation. Since the research base was close to the Yellow River, the experimental sites can be irrigated during the growing season. The soil is a warping soil with a pH of 7.8-8.2, and soil organic matter content is 0.75%-4.00% in top 20 cm.

1.2 The crops and experimental design

Two grape cultivars, Crimson (CRS) and Moldova (MDW) were planted in the base research under open field conditions in the spring of 2009. The two cultivars are late-maturity varieties introduced from United States of America in 2000, with moderate tolerance to drought. Each treatment was arranged as row plots with three replicates, with each row 50 m long. Plants were arranged with 2 meter row spacing and 0.5 meter plant spacing. The field was previously planted to rice in 2007 and 2008 (irrigated). All plots were subjected to the same field management. Before sapling transplanting, soil was mixed with fertilizer at a rate of N 165 kg/ha, P 150 kg/ha, K 100 kg/ha and bedded up into a ridge with 1 meter width and 0.5 meter height. Both cultivars were planted on the ridges. In 2009, irrigations were applied five times, giving 20 mm before transplanting, during mid-June, mid-July, mid-August and September, respectively.

After growing for one and a half months, concordant saplings were selected for treatment. The mixtures of three salts ($NaCl$, Na_2SO_4, Na_2CO_3 with proportion of 1∶0.5∶0.5) in three concentrations (100, 200, 400 mmol/L) were used to simulate a range of soil natural salt-alkaline conditions: low (Sl), moderate (Sm) and high (Sh) concentrations. No salt utilization was regarded as a control treatment (CK). All ecophysiological parameters were measured starting after one week.

1.3 Ecophysiological parameters measurements

Photosynthetic parameters were measured in at least three intact, fully expanded sunlit leaves on three to four trees per treatment, on clear days in middle June, July, 2010. We measured P_N, Gs, and Tr with the Li-Cor LI-6400 gas exchange system (Lincoln, NE, USA). The photosynthetic light response curves were also measured. For each light response curve, leaves were equilibrated at saturating photosynthetic photon flux densities (PPFD) before initiation of the light response curve. Each light response curve consisted of measurements at six to twelve PPFDs from 1 to 2 000 μmol/(m^2·s). Leaves were allowed to equilibrate at each new PPFD (typically requiring 20 min or less) before measurement.

Chlorophyll fluorescence determinants were measured on the same leaves used for gas exchange measurements, as described by Dionisio-sese and Tobita [9]. Chlorophyll fluorescence in dark-adapted and light-adapted leaves was excited and measured using a portable chlorophyll fluorometer (PAM-2000, WALZ, Germany). Prior to measurements for each leaf was dark adapted for 30 min. Dark adapted measurements of minimal fluorescence (Fo), maximal fluorescence (Fm), and variable fluorescence (Fv) as well as the Fv/Fm ratio were obtained. Leaves were then allowed to equilibrate for 30 min at a photon flux density of 1 500 μmol/(m^2·s). Measurements of the minimal fluorescence of a light adapted leaf (F′o), maximal fluorescence during a saturating pulse (F′m), and steady-state fluorescence (Fs) were obtained. From these measurements both photochemical (qP) and non-photochemical (qN) quenching components were calculated. Using both light and dark fluorescence parameters, we calculated the following: maximum efficiency of PSII photochemistry in the dark-adapted state (Fv/Fm); the quantum yield of PSII electron transport, Φ_{PSII} = (F′m − Fs) / F′m; electron transport rate (ETR) was calculated as ETR = (F′m − Fs) / Fm × PPFD × 0.5, where PPFD is absorbed light in mmol photon/(m^2·s) and 0.5 is a factor that accounts for the partitioning of energy between PSII and PSI [10]; the nonphotochemical quenching coefficient, qN = 1 − (F′m − F′o) / (Fm − Fo).

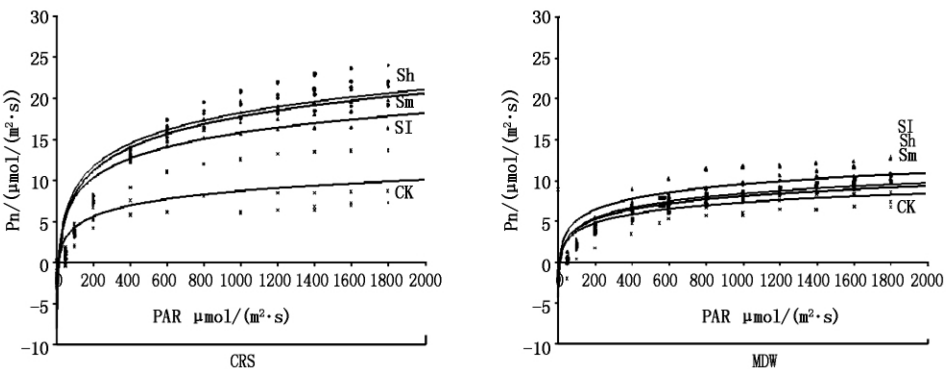
1.4 Data analysis

Differences in physiological variables among four treatments were tested using one-way ANOVA at each data measurement. Means were compared using Duncan′s multiple range tests, at a Significantly level of $P < 0.05$. Correlate coefficients among physiological variability were calculated. The statistical analysis was performed employing SPSS 16.0 (SPSS Inc, Chicago, IL, USA)

2 Results and Analysis

2.1 Photosynthetic light response curve

The light response curves (Fig. 1) indicated that P_N of CK were lowest for both cultivars. For CRS, P_N of high concentration treatment was the highest, followed by Sm, Sl. For MDW, light treatment was the highest followed by Sh.



(Sl, low stress; Sm, moderate stress; Sh, high stress) The photosynthetic light response curves were fit for an exponential function.

Fig. 1 Photosynthetic light response curves for treatment of saline-alkaline stress and control (CK) seedlings of grape cultivar CRS and MDW in July 2010

2.2 Gas exchange

Figure (Fig. 2) of gas exchange of the two cultivard indicates that saline-alkaline treatment improves the photosynthetic capability of the two grape cultivars to varying degrees... For cultivar CRS, P_N and Gs in the high concentration treatment increased by 89.5% and 143%, respectively, compared to CK (no treatment); two values of Moldova (MDW) increased by

35% and 10%. The results also indicated that moderate concentration treatment of CRS has the highest P_N and Gs, for MDW, both belonged to treatment Sh. Also, the results indicated that saline-alkaline treatment improved the water use efficiency, from 1.5 to 2.8 – 3.3.

2.3 Chlorophyll fluorescence

From application of saline-alkaline stress, ETR has increased significantly for both cultivars (Fig. 3). However, higher saline-alkaline concentration slowed such effects. Such trends also were found for φPS Ⅱ. As for qN, there existed an inverse trend. Saline-alkaline treatment enlarged the value of CRS, and reduced those of MDW. However, the highest saline-alkaline concentration treatment had a reduced Fv/Fm for both cultivars. Finally, CRS had a higher Fv/Fm than MDW under same saline-alkaline treatment.

2.4 Diurnal light intensity

The light intensity in the experimental site showed a high value (more than 1 500 μmol/(m^2·s)) during 11:00 – 15:00. At 17:00 in the afternoon (Fig. 4), there still existed a comparatively high value (1 200 μmol /(m^2·s)), and then quickly declined to 600 μmol/(m^2·s).

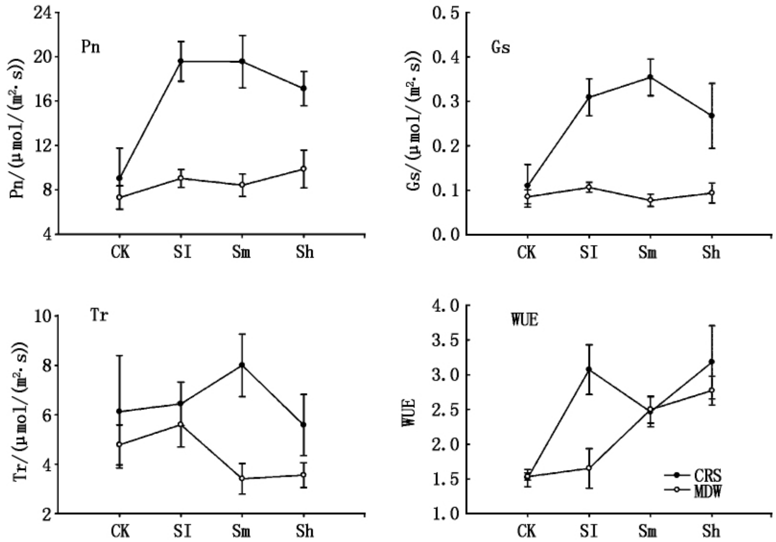


Fig. 2 Variation in mean (and standard error) leaf net photosynthetic rate (P_N), stomatal conductance (Gs), transpiration rate (Tr) and photosynthetic water use efficiency (WUE) under saline-alkaline stress and control (CK) of seedlings of grape cultivar CRS and MDW over the course of the experiment. Each point represents either 10 – 12 samples measured on each sampling date.

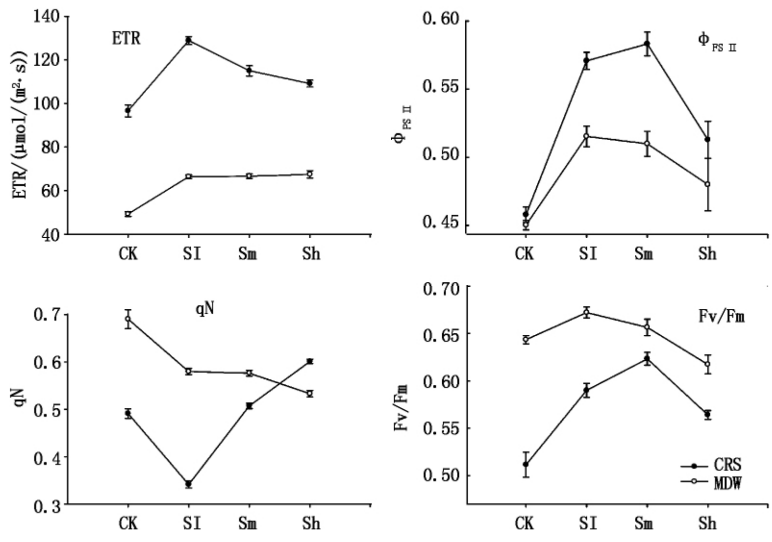


Fig. 3 Variation in mean (and standard error) leaf ETR, $\Phi_{PS II}$, qN and Fv/Fm of saline-alkaline stress and control (CK) seedlings of grape cultivar CRS and MDW over the course of the experiment. Each point represents either 10 – 12 samples measured on each sampling date.

2.5 Relationship

For both cultivars, P_N had a significant positive correlation (Tab. 1) with Gs or Tr, and a significant negative correlation with Ci or qN. Gs had a significant positive correlation with Tr, and a significant negative correlation with Ci or qN. The correlation between Ci and Tr was significantly positive for MDW, but significantly negative for CRS.

Tab. 1 Correlation among physiological parameters in all treatments for two grape cultivars

CRS	Pn	Gs	Ci	Tr	ETR	φPSⅡ	qN	Fv/Fm
Pn	1							
Gs	0.665**	1						
Ci	-0.981**	-0.626**	1					
Tr	0.502**	0.921**	-0.483**	1				
ETR	0.206	0.222	0.172	0.188	1			
φPSⅡ	0.121	0.139	-0.141	-0.160	0.454**	1		
qN	-0.116	-0.089	-0.025	-0.051	0.179	-0.344*	1	
Fv/Fm	0.042	0.142	0.177	0.151	-0.135	0.166	-0.323*	1
MDW	**Pn**	**Gs**	**Ci**	**Tr**	**ETR**	**φPSⅡ**	**qN**	**Fv/Fm**
Pn	1							
Gs	0.770**	1						
Ci	-0.524**	-0.860**	1					
Tr	0.217**	0.742**	0.633**	1				
ETR	0.105	0.133	0.055	0.078	1			
φPSⅡ	0.111	0.093	-0.070	0.190	0.330**	1		
qN	-0.063	0.279*	0.323**	0.351**	0.239*	0.184	1	
Fv/Fm	0.176	-0.170	-0.217	-0.337*	-0.035	-0.137	-0.260	1

Note: **. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

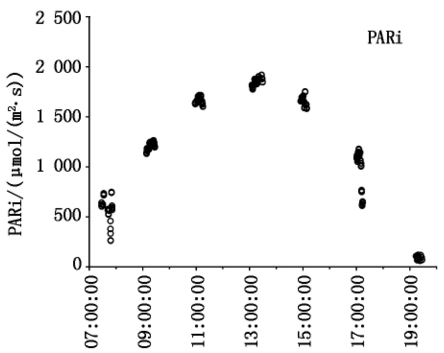


Fig. 4 The light intensity of experimental site in Yinchuan for a typical day in July, 2010.

3 Discussion and conclusion

Saline-alkaline stress induced diurnal changes in photosynthesis, as well as in stomatal conductance. For most plant species studied, eg omato [11-12], castor bean [13], citrus plant [14], olive [15], salinity causes a decline of net photosynthetic rate, with greater reduction for salt sensitive plants. Different salt concentrations has different effects on Pn. At moderate salt concentration (100-200 mmol/L NaCl), salt didn´t significantly lower the Pn capacity of *Populus alba* L. [16], and even stimulated the Pn rate and thus improved whole plant growth for halophyte [17]. However, these effects were inhibited under high salt concentration [12,17]. Although salt stress induced a decrease in the Pn of most plant, in our study, under mixture saline-alkaline stress, the Pn of grape cultivars was enhanced to different degrees.

Pn was determined by many environmental factors, such as PPFD, intercellular CO_2 concentration, leaf conductance and leaf temperature, etc. In this study, there existed a significant positive correlation between Pn and Gs, and a significant negative correlation between Pn and Ci for both cultivars. An increase of stomatal conductance always causes an inrease of Tr [11,18-19], which might be the reason for the increase of Pn in the treatments other than CK. However, for cultivar MDW, the Tr value was low even with high stomata conductance. Stomatal conductance was regarded as one of parameters to assess leaf activity. High salt concentration may impaired leaf stomata in extreme conditions, make stomata open or insensitive to environmental changes [20], and reduce transpiration activity. In our experiment, high salt treatment produced a high Gs in the daytime and even at 19:00, the transpiration rate was also higher than other treatments with a significant level, Pn was the highest than other treatments. High saline-alkaline concentration (400 mmol/L) perhaps enhanced stomata activity by Na^+, and this might be the reason for the higher Pn in treatment Sh. The decrease of Tr might cause the obvious increase in the water use efficiency under all stress conditions in this study.

Higher salinity not only affected gas exchange, but also changed PSII functional characteristics, associated with increased non-photochemical quenching [17]. Leaf chlorophyll is a fluorescence response to salt stress for most plant species has been reported. Tomato plant, growth was significantly decreased with increasing salinity, and photochemical quenching (qP), the non photochemical quenching (qN), and the linear electron transport rate (ETR) can be used as criteria parameters for selection of salt tolerance in this species [12]. For tomato, NaCl significantly decreased photochemical quenching coefficient, efficiency of the excitation energy capture by open PSII reaction centers (Fv/Fm) and actual quantum yield of photosynthesis (Φ_{PSII}) [11]. As for castor bean, all salt stress induced a continuous reduction in Fm, Fv/Fm, Φ_{PSII} with highest at low salt level and lowest at high salt level, qN in stressed plants were all significantly higher than control [13]. For citrus, salt stress induced rapid reductions of performance of PSII and photosynthetic efficiency. In sorghum, Netondo et al reported that maximum quantum yield of photosystem II (PSII; Fv/Fm), photochemical quenching coefficient (qP) and electron transport rate (ETR) significantly decreased, but non-photochemical quenching (qN) increased substantially under saline conditions [1]. However, for rice cultivars contrasting in salinity tolerance, Fv/Fm was only slightly affected by salt stress, whereas qN increased in sensitive cultivars with increasing salt stress [9]. It is suggested that sensitivity to salt stress in cereals might thus be associated with both reduction in PSII photochemical efficiency and enhanced qN to dissipate excess energy. In this study, the photochemical quenching (qP) and the efficiency of open PSII centers (Φ_{PSII}) insignificantly decreased with increasing salinity level. Compared with significant decrease in other plant, such as tomato [11], olive [15], and castor bean [13], the impacts of salt stress on both parameters (qP and Φ_{PSII}) were not significant for both grape cultivars. Still, such impacts were reduced in midday conditions. This reduction was caused perhaps by strong light intensity (1 500 – 2 000 $\mu mol/(m^2 \cdot s)$) and high leaf temperature (40 – 42℃) in the midday. Comparing all parameters, Φ_{PSII} and qN can be used as criteria for selecting of salt tolerance of grape cultivar.

In conclusion, for CRS, the increase of Pn benefitted from high Gs and Tr, however, low Fv/Fm decreased this response. For MDW, high Fv/Fm helpful maintain a relative stable Pn, but Tr had a significant negative influence. Two cultivars demonstrated different suitable regime to saline-alkaline stress.

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