Medicinal Plant 2023, 14 (5): 42 -44 DOI: 10.19600/j.cnki.issn2152 -3924.2023.05.009

Chlorophyll Fluorescence Response of Persimmon Plants under Salt Stress

Xining GENG * , **Lihua XIE**, **Jingwen XU**, **Ruiyuan WANG**

Pingdingshan University, Henan Province Key Laboratory of Germplasm Innovation and Utilization of Eco-economic Wood Plant, Pingdingshang 467000, China

Abstract [**Objectives**] To study the photosynthetic response mechanism of persimmon seedlings to salt stress. [**Methods**] The chlorophyll fluorescence parameters of *Diospyros virginiana* and *Diospyros lotus* seedlings under 4% salt stress were studied by pot culture salt control method, including the minimal fluorescence (F_0), maximum fluorescence (F_m), potential activity of PS II (F_v/F_0), maximum photochemical efficiency of PS II (F_v/F_m), electron transport rate (ETR), actual photochemical efficiency of PS II (Y_{II}), and photochemical quenching coefficient (q_p). [**Results**] Under 4% salt stress, the maximum fluorescence (F_m), maximum photochemical efficiency of PS II (F_v/F_m), and photochemical quenching coefficient (q_p) of two persimmon plants decreased with time. The potential activity of PS II (F_v/F_0), actual photochemical efficiency of PS II (Y_{II}), and electron transport rate (ETR) decreased under salt stress. [**Conclusions**] This study indicates that the PS II reaction center in the persimmon leaves was damaged and the electron transport at the acceptor side was damaged under salt stress. It is expected to lay a foundation for the analysis of salt-tolerance mechanism of persimmon plants.

Key words Chlorophyll fluorescence, Salt stress, *Diospyros virginiana*, *Diospyros lotus*

1 Introduction

Diospyros is the largest genus in Ebenaceae that has more than 500 species with remarkable economical values, especially *Diospyros kaki* Thunb which has traditionally been used as an important food resource in China, Korea, and Japan[1]. *Diospyros* plants are pantropical and thrive in warm regions of the world such as China, Korea, Japan and the United States. In 2007, the global production of persimmon reached over 3.3 million t, with 70% coming from China[2]. *Diospyros* species have been ubiquitous to ethnic medication throughout the tropical regions. Leaves, barks, fruits, hard wood, and roots have been used as tonic, powder, and poultice to heal a wide range of illnesses such as asthma, dermatitis, hypertension, atherosclerosis, lumbago, hemorrhage, insomnia, biliousness, among others. Common usage includes febrifuge, carminative, astringent, sedative, anti-hypertentsive, vermifuge, constipation, and antidiuretic[3].

Some natural factors such as climate and unscientific agricultural operation will cause soil salinization. At present, the degree of soil salinization is becoming more and more serious, and the area is also expanding, which has become an ecological problem plaguing many countries[4]. With soil salinization becoming more and more serious, salt stress has gradually evolved into abiotic stress factors that seriously hinder plant growth and development. Salt stress is one of the main environmental factors that inhibit plant photosynthesis, which can lead to the damage of photosynthetic organs and inhibit plant photosynthesis. Chlorophyll fluorescence kinetics has been widely used in the research of photosynthetic physiology of plant stress resistance, which can quickly determine photosynthetic function without causing damage to plant leaves and reflect the changes of plant photosynthetic capacity under stress conditions[5]. Previous studies have been conducted on the growth, enzyme activity, photosynthesis and transcriptional response of persimmon plants under salt stress[6-9], but few studies have reported the chlorophyll fluorescence response of persimmon plants to salt stress. In this study, we explored the effects of salt stress at different time points on chlorophyll fluorescence parameters of persimmon leaves.

2 Materials and methods

2.1 Experimental materials The seeds of *Diospyros virginiana* and *D. lotus* were collected and sown for seedling cultivation. When the tested plants grew to 3 -4 leaves, the seedlings were planted in medium-size thickened plastic POTS with 3 plants per pot. The soil was mixed with matrix, sand and vermiculite (2 : 1 : 1). In May 2022, NaCl salt control test was carried out in the intelligent greenhouse of Pingdingshan University, Henan Province.

2.2 Salt treatment of persimmon plants The method of multiple salt application was adopted, and 1.00% NaCl solution was prepared for each concentration under salt treatment for the first time, and the concentration was increased by 1.00% step by step every 24 h. When the salt concentration reaches the set concentration, water is then watered according to the evaporation situation to balance the evaporation amount, and at the same time, the plastic tray under the plastic pot is thickened, so that the solution flowing out of the water can be returned to the basin in time to prevent the loss of salt. The salt concentration was set at 4.00% and 6 strains were repeated. After 8 d of salt solution treatment, the basic chlorophyll fluorescence parameters of the leaves of Ameri-

Received: June 20, 2023 Accepted: September 4, 2023
Supported by Science and Technology Research Project of Henan Provincial Science and Technology Department (222102110444); Introduction of Talent of Pingdingshan University (PXY-BSQD-202109).
* Corresponding author. E-mail: geng_xn@yeah.net

can persimmon seedlings were determined by portable chlorophyll fluorescence analyzer PAM-2500 (WALZ, Germany), and the leaves at the same position of each plant were selected for continuous measurement for 4 days.

2.3 Determination of chlorophyll fluorescence parameters

After dark adaptation treatment for 15 min, the minimal fluorescence (F_0) and maximum fluorescence (F_m) were determined, and the variable fluorescence F_v ($F_v = F_m - F_0$) and the maximum photochemical efficiency of PS II (F_v/F_m) were calculated. After light adaptation, the minimum fluorescence (F_0), maximum fluorescence (F_m), electron transport rate (*ETR*), actual photochemical efficiency of PS II (Y_{II}), photochemical quenching coefficient (q_p) are automatically given.

3 Results and analysis

3.1 Effects of salt stress on chlorophyll a fluorescence parameters of persimmon leaves On the third day after chlorophyll fluorescence parameters measurement, the leaves of *D. virginiana* in the treatment group were normal, while the leaves of *D. lotus* in the treatment group were shed. The results (Table 1) showed that with the increase in time after 4% salt solution treatment, the minimal fluorescence (F_0) of the leaves of *D. virginiana* and *D. lotus* first increased and then gradually decreased, indicating that the PS II reaction center was damaged or reversibly deactivated. F_m is the maximum fluorescence in the dark, which refers to the fluorescence intensity when all PS II reaction centers are closed after complete dark adaptation, and all non-photochemical processes are minimized, which can reflect the electron transfer situation after PS II.

With the increase in time after treatment with 4% salt solution, the F_m of persimmon leaves first increased and then gradually decreased, the F_m of persimmon leaves gradually decreased by 32.5% compared with the first day on the 4th day, the F_m of *D. virginiana* leaves gradually decreased, and the F_m of *D. lotus* leaves on the 3rd day decreased by 22.1% compared with the first day, indicating that the electron transfer ability of plant PS II reaction center was weakened.

F_v/F_0 is often used to measure the potential activity of PS II, F_v/F_m refers to the maximum photochemical efficiency of PS II. They are two important parameters to indicate the photochemical reaction status. With the increase in time after treatment with 4% salt solution, F_v/F_0 and F_v/F_m of the two persimmon genera showed a slow increase at first and then a sharp decrease (Table 1). The decrease in *D. virginiana* on the 4th day was 53.3% compared with the 1st day, and the decrease of *D. lotus* on the 3rd day was 24.0% compared with the 1st day, indicating that the salt stress caused damage to PS II. The potential activity and primary light energy conversion efficiency of PS II were weakened, and photoinhibition of photosynthesis occurred in plants.

Table 1 Effects of salt stress on F_0, F_m, F_v/F_m, F_v/F_0

Chlorophyll fluorescence parameters	Group	1	2	3	4
F_0	CK_v	0.722 ±0.104	0.728 ±0.071	0.794 ±0.107	0.770 ±0.047
	T_v	0.680 ±0.097	0.762 ±0.052	0.617 ±0.059	0.579 ±0.223
	CK_l	0.842 ±0.051	0.893 ±0.027	0.791 ±0.075	0.820 ±0.051
	T_l	0.869 ±0.048	0.847 ±0.057	0.768 ±0.081	
F_m	CK_v	1.609 ±0.303	1.575 ±0.223	1.738 ±0.216	1.613 ±0.279
	T_v	1.270 ±0.177	1.511 ±0.303	1.063 ±0.127	0.857 ±0.431
	CK_l	1.668 ±0.134	1.676 ±0.128	1.467 ±0.147	1.495 ±0.016
	T_l	1.694 ±0.164	1.666 ±0.157	1.320 ±0.126	
F_v/F_m	CK_v	0.547 ±0.033	0.533 ±0.052	0.543 ±0.020	0.515 ±0.054
	T_v	0.462 ±0.063	0.482 ±0.090	0.417 ±0.039	0.270 ±0.146
	CK_l	0.491 ±0.059	0.466 ±0.029	0.457 ±0.066	0.452 ±0.029
	T_l	0.484 ±0.042	0.489 ±0.037	0.418 ±0.025	
F_v/F_0	CK_v	1.218 ±0.151	1.169 ±0.279	1.194 ±0.096	1.083 ±0.235
	T_v	0.881 ±0.233	0.981 ±0.358	0.722 ±0.116	0.411 ±0.259
	CK_l	0.989 ±0.235	0.876 ±0.103	0.868 ±0.255	0.828 ±0.097
	T_l	0.950 ±0.155	0.967 ±0.142	0.722 ±0.070	

Note: CK_v indicates control group in *D. virginiana*; T_v indicates treatment group in *D. virginiana*; CK_l indicates control group in *D. lotus*; T_l treatment group in *D. lotus*. The same below.

3.2 Effects of salt stress on kinetic parameters of chlorophyll a fluorescence induction in persimmon leaves Y_{II} is the actual photochemical quantum yield of PS II, which reflects the proportion of excitation energy used in photochemical pathways to excitation energy entering PS II, and is an important index of plant photosynthetic capacity. Y(*II*) of persimmon leaf decreased under salt stress, which decreased by 55.1% and 23.9%, respectively, on the 4th and 3rd day of treatment (Table 2). The photochemical quenching coefficient (q_p) is related to the photochemical reaction of PS II, reflecting the share of light energy absorbed by the pigment of PS II antenna for the electron transfer of photochemical reaction, and reflecting the openness of the PS II reaction center to a certain extent.

With the increase in time after 4% salt solution treatment, the photochemical quenching coefficient q_p showed a slow increase at first and then a sharp decline, and the two species of persimmon decreased by 37.1% and 10.1% on the 4^{th} and 3^{rd} day of treatment, respectively (Table 2), indicating that the reoxidation capacity of Q_A was weakened and the electron transfer on the PS II accepter side was damaged by salt stress. *ETR* is the apparent photosynthetic electron transport rate.

With the increase in time after salt solution treatment, *ETR* showed a downward trend in general, and the *ETR* decreased by 55.1% and 23.9% for two persimmon species, respectively (Table 2).

Table 2 Effects of salt stress on $Y(II)$, q_p, ETR

Chlorophyll fluorescence parameters	Group	1	2	3	4
$Y(II)$	CK_v	0.436 ±0.066	0.406 ±0.067	0.426 ±0.022	0.380 ±0.037
	T_v	0.312 ±0.070	0.380 ±0.118	0.263 ±0.029	0.140 ±0.102
	CK_l	0.324 ±0.084	0.287 ±0.040	0.337 ±0.036	0.315 ±0.035
	T_l	0.306 ±0.076	0.330 ±0.070	0.233 ±0.029	
q_p	CK_v	0.794 ±0.087	0.757 ±0.066	0.783 ±0.029	0.737 ±0.008
	T_v	0.674 ±0.106	0.774 ±0.133	0.630 ±0.051	0.424 ±0.250
	CK_l	0.649 ±0.091	0.617 ±0.075	0.741 ±0.041	0.695 ±0.043
	T_l	0.625 ±0.114	0.669 ±0.107	0.559 ±0.078	
ETR	CK_v	36.850 ±5.523	34.233 ±5.665	35.917 ±1.883	32.040 ±3.149
	T_v	26.367 ±5.943	32.067 ±9.961	22.150 ±2.483	11.840 ±8.600
	CK_l	27.283 ±7.090	24.267 ±3.379	28.467 ±3.078	26.533 ±2.930
	T_l	25.833 ±6.488	27.833 ±5.916	19.667 ±2.434	

4 Discussion

Under normal circumstances, light energy absorbed by chlorophyll is mainly consumed through three ways: photosynthetic electron transfer, chlorophyll fluorescence emission and heat dissipation[10]. There is a relationship between these three pathways, and the change in photosynthesis and heat dissipation will cause the corresponding change of fluorescence emission. Therefore, photosynthesis and heat dissipation can be explored through the observation of fluorescence. The results of this study showed that the maximum fluorescence (F_m), the maximum photochemical efficiency of PS II (F_v/F_m) and photochemical quenching coefficient (q_p) of the leaves of two persimmon plants decreased with the increase of time after salt stress, and the potential activity (F_v/F_0), the actual photochemical efficiency (Y_{II}) and the electron transport rate (*ETR*) of the leaves decreased under salt stress. The results indicated that salt stress had caused photoinhibition, resulting in damage to the potential active center of PS II, inhibition of photochemical activity of PS II, and affected the transfer of photosynthetic electrons from PS II reaction center to Q_A, Q_B and P_Q library, which was not conducive to the transfer of excitation energy from light-trapping pigment protein complex (LHC) to PS II. These results were similar to those of rice[11], tomato[12], lettuce[13], and Amur grape[14] when exposed to salt stress.

References

[1] GUO DL, LUO ZR. Genetic relationships of the Japanese persimmon *Diospyros kaki* (Ebenaceae) and related species revealed by SSR analysis [J]. Genetics and Molecular Research, 2011, 10(2): 1060 –1068.

[2] BUTT MS, SULTAN MT, AZIZ M, *et al.* Persimmon (*Diospyros kaki*) fruit: Hidden phytochemicals and health claims [J]. EXCLI Journal, 2015(14): 542 –561.

[3] XIE CY, XIE ZS, XU XJ, *et al.* Persimmon (*Diospyros kaki* L.) leaves: A review on traditional uses, phytochemistry and pharmacological properties[J]. Journal of Ethnopharmacology, 2015(163): 229 –240.

[4] LIN S, SUN M. Analysis of physiological response and salt tolerance mechanism of *Crossostephium chinense* and four species of chrysanthemum under salt stress[J]. Acta Botanica Boreali-Occidentalia Sinica, 2017, 37(6): 1137 –1144. (in Chinese).

[5] ZHANG A, QI M, ZHANG Y. A discussion on chlorophyll fluorescence induction parameters and their measurement[J] Journal of Nuclear Agricultural Sciences, 2008, 22(6): 909 –912. (in Chinese).

[6] GIL-MUNOZ F, PEREZ-PEREZ J, QUINONES A, *et al.* Intra and interspecific variability of salt tolerance mechanisms in *Diospyros* genus[J]. Frontiers in Plant Science, 2020(11): 1132.

[7] SUN F, ZHOU B, SUN M. The physiological and biochemical response of *Diospyros lotus* L. seedlings to cross-stress of water and salt[J]. Journal of Shandong Forestry Science and Technology, 2009, 39(5): 1 –4. (in Chinese).

[8] WEI P, YANG Y, FANG M, *et al.* Physiological response of young seedlings from five accessions of *Diospyros* L. under salinity stress[J]. Korean Journal of Horticultural Science & Technology, 2016, 34(4): 564 –577.

[9] INCESU M, CIMEN B, YESILOGLU T, *et al.* Growth and photosynthetic response of two persimmon rootstocks (*Diospyros kaki* and *D. virginiana*) under different salinity levels[J]. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 2014, 42(2): 386 –391.

[10] WU CA, MENG QW, ZHOU Q, *et al.* Comparative study on the photooxidative response in different wheat cultivar leaves[J]. Acta Agronomica Sinica, 2003(3): 339 –344. (in Chinese).

[11] TSAI YC, CHEN KC, CHENG TS, *et al.* Chlorophyll fluorescence analysis in diverse rice varieties reveals the positive correlation between the seedlings salt tolerance and photosynthetic efficiency[J]. BMC Plant Biology, 2019, 19(1): 403.

[12] SHIN YK, BHANDARI SR, CHO MC, *et al.* Evaluation of chlorophyll fluorescence parameters and proline content in tomato seedlings grown under different salt stress conditions[J]. Horticulture, Environment, and Biotechnology, 2020, 61(3): 433 –443.

[13] SHIN YK, BHANDARI SR, JO JS, *et al.* Response to salt stress in lettuce: Changes in chlorophyll fluorescence parameters, phytochemical contents, and antioxidant activities[J]. Agronomy, 2020, 10(11): 1627.

[14] QIN HY, AI J, XU PL, *et al.* Chlorophyll fluorescence parameters and ultrastructure in Amur grape (*Vitis amurensis* Rupr.) under salt stress [J]. Acta Botanica Boreali-Occidentalia Sinica, 2013, 33(6): 1159 –1164. (in Chinese).