

Effects of Hydrogen Fluoride-stress on Physiological Characteristics of Theaceae Tree Seedlings

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Abstract. In this study, four Theaceae tree species were processed by setting up two hydrogen fluoride concentration gradient (including controls), the actual quantum yield (Y (II)) of PSII, the relative electron transport rate (ETR), non-regulatory energy dissipation quantum yield (Y(NO)), regulatory energy dissipation quantum yield (Y(NPQ)), non-photochemical quenching coefficient (NPQ) and the relative electrical conductivity and other physiological characteristic value were measured, and fuzzy membership function method was applied for comprehensive evaluation and stress resistance sequencing of these four species, which could help to explore the impact of HF-stress on physiological indicators of Theaceae tree seedlings, and effectively screen out tress species with high resistance to hydrogen fluoride; besides, it could provide the basis for selection of planting trees with the purpose of environmental protection, and also provide a reference for HF stress mechanisms study. This study showed that, under the circumstance of 500 ppm hydrogen fluoride stress, chlorophyll fluorescence indicator of Theaceae tree species presented a downward trend on the whole, which concretely embodied in varying degrees of decline of Y (II) of PSII, ETR, Y (NO), Y (NPQ), NPQ. And the cell membranes of trees species were damaged and the membrane permeability increased. Except for *Schima superba*, the relative conductivity value of other species showed an upward trend. The capacity of these four species in anti- hydrogen fluoride contamination was *Tutcheria championi* first, *Camellia oleifera* second, *Schima superba* third, *Gordonia axillaries* is the last.

1 Introduction

Hydrogen fluoride (HF) gas is one of the most toxic air pollutants [Leonard H.Weinstein,1977], which is discharged through brick, fertilizer, cement, smelting and other industrial processes into the atmosphere [Moyer D.Thomas&Ernest W.Alther,1966], and causes serious damage to the surrounding plants [Fornasiero R B, 2001] . And because HF can be accumulated in leaves of plants [AW Davison, et al., 1984], which could endanger the health of animals and humans through the food chain, and result in insect and virus mutation, human malnutrition and decreased physical function, and other problems [Mamta Baunthiyal et al., 2014]. HF mainly damages the plant by entering into its body in the form of gas and affects a variety of plant physiological processes [Gou Xiaohua et al., 2000], in which, PSII plays an important role in the formation of resisting stress in photosynthetic apparatus [Wei Xiaodong et al., 2012; Baker N R,1991]. And by the application of chlorophyll fluorescence dynamics, we did in-depth analysis of environmental stress impact on PSIIand other chlorophyll fluorescence parameters to screen out tree species with strong resilience.

At present, some studies have done discussing the mechanism how pollutants damage plants and also plant resistance comparison using its physiological characteristics, one example: how ozone affects some physiological indicators of plant [JIN Minghong et al., 2000], photosynthate, biomass [Grantz D A&Farrar J F, 1999; Dickson R E et al., 1998], chloroplast fluorescence properties and chloroplast fluorescence system II (PSII) [Guera A et al., 2005; Calatayud A et al., 2002]; another

example: how sulfur, fluoride and heavy metals affect chlorophyll fluorescence of the European thorn pine [Pawet M. Pukacki, 2000] and so on. This implies that we can tell the impact of HF on plants by measuring HF-stressed plant leaf green fluorescence, relative conductivity and other physiological indicators, process and evaluate the data to effectively filter out plant species with certain HF anti-stress, and then select plants with HF-resistance and better absorbing in the surrounding of industrial zone and establish artificial green ecological engineering system of different types. In addition, through the research of biological mechanism on plant anti-stress, we can plant and induce other plant species to provide their anti-pollution ability. Our research could tell how HF anti-stress plants effectively alleviate environmental pollution and improve the ecological environment, and has a great significance to the construction of ecological forest and the achievement of sustainable development of urban forestry.

2 Materials and Methods

2.1 Experimental Field

It was located on the campus of South China Agricultural University, Guangzhou City. (23 ° 09 'N, 113 ° 21' E). Test objects: The two-year container bag seedlings of *Schema superba*, *Gordonia axillaris*, *Tutcheria championi* and *Camellia oleifera* of Theacea.

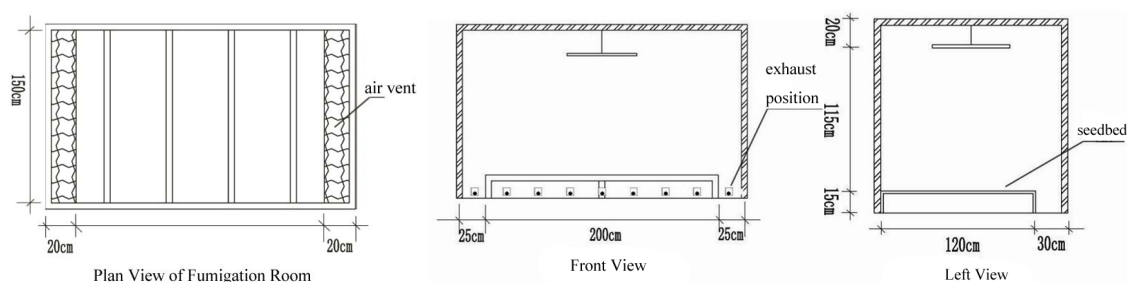


Fig.1 Schematic diagram of fumigation chamber

The experimental fumigation equipment was to simulate the natural dynamic smoke chamber (Fig. 1) with a volume of about 5.6 m³. The nursery bed for displaying seedlings was placed in the gas chamber with a height of about 15 cm, and a 40 V fan was equipped to stir gases in the fumigation chamber. Hydrogen fluoride gas was generated from the diffusion of hydrofluoric acid solution placed under the bottom of fumigation chamber, and then was mixed into the airflow blowing from axial flow fan. Then the airflow passed the gas chamber through the nursery bed from bottom to top, and was diffused by the fan to make the gas mass fraction to be equal. The gas was collected by a hand pump, and then the gas mass fraction was measured and monitored using HF detector tube produced by China Sciences Group, with the frequency of once every 2 hours.

We designed this experiment referring to the former research result [Chen Zhuomei et al., 2008], and set up a 500 ppm concentration and a control CK (HF concentration in the ambient atmosphere). During the test, gas of fumigation chamber was in a fluid state and was in a relatively stable controlled concentration. The experiment was conducted in October 2013, with fumigation of 12 h (7: 00-19: 00). At 9:00-11:30 of the second day after fumigation, we measured chlorophyll fluorescence indicators of leaves, and sheared the fresh leaves to measure the relative conductivity indicators with three replications.

2.2 Measurements of chlorophyll fluorescence characteristics

We selected three living leaves with robust growth from middle of the crown and measured them by application of OS1P-type portable chlorophyll fluorescence spectrometer produced by OPTI-SCIENCES Company. The measured indicators were: Y (II) of PSII, ETR, Y (NO), Y (NPQ), and NPQ.

2.3 Relative electrical conductivity measurements of leaves

We selected DDS-11AGA-type electrical conductivity meter. First, we clipped leaves of 0.3 g scrubbed with distilled water, then sucked dry the apparent moisture, and then put them into the conical flask, and then added 30 ml distilled water for standing 12 h at room temperature, during which we shook and stood it time to time. We measured relative electrical conductivity W1 with the

preheated conduct meter, then we put the conical flask stuffed with the plug into the boiling water for 20 min boiling, and then we took it out for cooling it to room temperature, and measured the total electrical conductivity W2. Membrane permeability was calculated according to the formula $W = (W1 / W2) \times 100\%$. Every time we used the electrical conductivity meter, we measured the liquid temperature, and adjusted the constant to set value 1.04.

2.4 Fuzzy membership function method

Fuzzy membership function method is a good method for comprehensive evaluation of resistance. The larger the mean of comprehensive evaluation was, the stronger the resistance became [Su Yonghua,2007]. Membership function calculation formula is: $U(X_i) = (X_i - X_{min}) / (X_{max} - X_{min})$. $U(X_i)$ is the subordinative function value; X_i is the stress resistance coefficient of a certain indicator in the same tree species ($X_i = \text{treatment group indicator value} / \text{control group indicator value}$); maximum X_{max} and X_{min} are respectively the maximum value and the minimum value of stress resistance coefficient of a certain indicator in the same tree species. If the measured indicator showed a negative correlation with the comprehensive evaluation result, we should use anti-membership function to realize quantitative transformation. Here, the anti membership function calculation formula is: $U(X_i) = 1 - (X_i - X_{min}) / (X_{max} - X_{min})$.

3. Results

3.1 Comparisons of the actual quantum yield Y(II) of PSII in each tree species

In the condition of light, the Y(II) of PS II could reflect the actual photosynthetic efficiency of plants at present. In the stress environment, organisms in plant were injured by externality, and their photosynthetic capacity was weakened accordingly. As shown in figure 2-1, in regard to the Y(II) of PSII which reflected the actual plant photosynthetic efficiency, four tree species all decreased, in which, the decreasing amplitude of *Gordonia axillaris* was as much as 65.0%, and decreasing amplitude of *Schima superba*, *Tutcheria championi* and *Camellia oleifera* was within 50%, as respectively 40.2%, 28.8%, 42.40%.

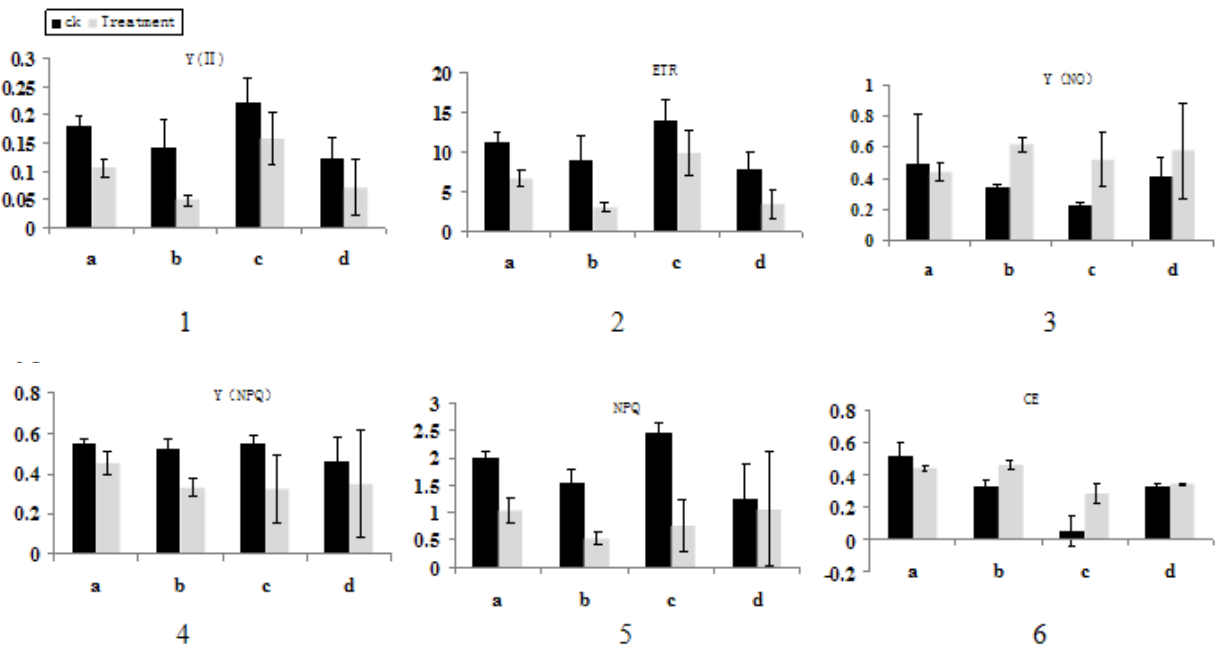


Figure. 2 Two hydrogen fluoride concentration gradient (including controls) in four Theaceae tree species .

a, *Schima superba*; b, *Gordonia axillaris*; c, *Tutcheria championi* ;d, *Camellia oleifera*.

3.2 Comparisons of relative electron transport rate (ETR) in each species

ETR, also known as the relative photosynthetic electron transport rate or relative photosynthetic electron transfer rate of PSII, is a parameter of measuring apparent electron transfer conditions of carbon fixation caused by photochemical reaction in the photosynthetic chain under the actual light

intensity. Because the plants' body were injured in the stress environments, so ETR and Y (II) of PSII in each species had the same trends, and ETR of each species all showed downtrend (Figure 2-2), in which, *Gordonia axillaris* and *Camellia oleifera* declined more than 50%, with respectively, 65.4%, 55.8%; *Schima superba* and *Tutcheria championi* declined within 50%, as respectively 40.4%, 29.0%.

3.3 Comparisons of non-regulatory energy dissipation quantum yield Y (NO) in each species

Y (NO) refers to the condition that the incident light intensity exceeds the acceptable degree of plants, which is an important indicator of photo damage. The larger the value Y (NO) increases, the more serious the plant body damages. As shown in Figure 2-3, with the exception of 9.8% decline of Y (NO) in *Schima superba*, Y (NO) in the other species all showed an increased trend, in which *Gordonia axillaris* and *Camellia oleifera* respectively increased by 83.7% and 39.3%, while *Tutcheria championi* increased by up to 130.7%.

3.4 Comparisons of regulatory energy dissipation quantum yield Y (NPQ) in each species

Y (NPQ) is an auto-regulation indicator which shows the plant requires light protection, and when a plant body is damaged, the ability of regulation will be reduced. As shown in Figure 2-4, Y (NPQ) in the four species declined in varying degrees, in which, *Tutcheria championi* declined the most, 41.7%, *Gordonia axillaris* followed, 36.4%, and *Schima superba* and *Camellia oleifera* left behind, 18.7% and 24.0% respectively.

3.5 Comparisons of non-photochemical quenching coefficient (NPQ) in each species.

When it is in photosynthesis under illumination, NPQ reflects the ability of the plant dissipating excess energy into heat energy, i.e. light protection. As shown in Figure 2-5, NPQ in each species was in a decreasing trend, which reflected that the ability of the plant dissipating excess energy into heat energy reduced. For the four species tested, *Gordonia axillaris* and *Tutcheria championi* declined by more than 50%, specifically 65.4% and 69.2% respectively, and *Schima superba* and *Camellia oleifera* declined by less than 50%, specifically 48% and 16.2% respectively.

3.6 Comparisons of relative electrical conductivity values in each species

Relative electrical conductivity is an important physiological and biochemical indicator which reflects the status of membrane system. When the plant is under the condition of stress or other damage, its membrane proteins will be damaged, which later could cause cytoplasm extravasation to increase the relative electrical conductivity and could directly reflect the degree of injury to the plant. The smaller the value of the relative electrical conductivity changes, the smaller the damage will be, on the contrary, the greater the damage of the body, the larger the value of the relative electrical conductivity. As shown in Figure 2-6, except for the decreased relative electrical conductivity value of *Schima superba*, the other three species showed a rise condition. The relative electrical conductivity value of *Schima superba* declined by 15.4%, while *Gordonia axillaris* and *Camellia oleifera* increased by 39.4% and 3.0% respectively, and *Tutcheria championi* rise increased as high as 460%.

3.7 Comprehensive evaluation of anti-HF contamination characteristics in each species

Different plants resist or withstand stress of the external environment in different ways, and the mechanism of anti-fluoride contamination is also very complex, which is affected by many complex interaction of the external morphology, internal structure, physiological and biochemical characteristics, etc.. Many scholars believe that a single anti-fluoride indicator could not fully and accurately reflect the overall ability of plants to adapt to hydrogen fluoride contamination. Therefore, this study used many indicators for comprehensive evaluation.

Table 1 Stress resistance coefficient values of comprehensive evaluation indicators in four Theaceae species

Species	$X_{Y(II)}$	X_{ETR}	$X_{Y(NO)}$	$X_{Y(NPQ)}$	X_{NPQ}	X_K
<i>Schima superba</i>	0.598	0.596	0.902	0.813	0.520	0.846
<i>Gordonia axillaris</i>	0.350	0.346	1.837	0.636	0.346	1.394
<i>Tutcheria championi</i>	0.712	0.710	2.307	0.583	0.308	5.600
<i>Camellia oleifera</i>	0.576	0.442	1.393	0.760	0.838	1.030

In the comprehensive evaluation method of this study, stress resistance coefficient value of each indicator of the four Theaceae species is shown in Table 1, and each membership function value of each indicator obtained by calculation is shown in Table 2.

Table2 Membership function of each indicator in Theacea species under hydrogen fluoride environment

Species	U(XY(II))	U(XETR)	U(XY(NO))	U(XY(NPQ))	U(XNPQ)	U(X _K)	Comprehensive Evaluation	Stress Resistance Ranking
<i>Schima superba</i>	0.685	0.686	0.000	1.000	0.401	0.000	0.462	3
<i>Gordonia axillaris</i>	0.000	0.000	0.665	0.228	0.071	0.115	0.180	4
<i>Tutcheria championi</i>	1.000	1.000	1.000	0.000	0.000	1.000	0.667	1
<i>Camellia oleifera</i>	0.625	0.264	0.349	0.769	1.000	0.039	0.508	2

4 Discussions

In summary, there are individual differences in the anti-HF contamination test of these four Theaceae species. After fumigation, each indicator showed significant fluctuation which implies that each individual has different response speed and mechanism to the external environment and different plant has its own resistance performance to the external hydrogen fluoride contamination, and has its own critical concentration and time. [Gou Xiaohua, 2000].

As for restrictions of the experimental conditions, the experimental material was only seedlings. And the differences of their resistance between big trees are still needed to be explored.

In addition, there are many indicators for measuring resistance of the plant. This study only selected relative electrical conductivity and chlorophyll fluorescence indicators for evaluation. How to choose objective indicators more systematically and to conduct a comprehensive study of resistance is still a problem needing to be further explored.

5 Conclusions

This study showed that, under the 500 ppm HF environmental stress, physiological indicators of the four species presented the following changes compared with the control CK:

(1) Y(II) of PSII(z) showed a downward trend, and decrease amplitude was respectively as follows: 65.0% of *Gordonia axillaris* > 42.40% of *Camellia oleifera* > 40.2% of *Schima superba* > 28.8% of *Tutcheria championi*;

(2) ETR showed a downward trend, and decrease amplitude was respectively as follows: 65.4% of *Gordonia axillaris* > 55.8% of *Camellia oleifera* > 40.4% of *Schima superba* > 29.0% of *Tutcheria championi*;

(3) Y (NO) showed an increased trend except for *Schima superba* (a decline of 9.8%), and amplitude was respectively as follows: 130.7% of *Tutcheria championi* > 83.7% of *Gordonia axillaris* > 39.3% of *Camellia oleifera*;

(4) Y (NPQ) showed a downward trend, and decrease amplitude was respectively as follows:

41.7% of *Tutcheria championi* > 36.4% of *Gordonia axillaris* > 24.0% of *Camellia oleifera* > 18.7% of *Schima superba*;

(5) NPQ showed a downward trend respectively: 69.2% of *Tutcheria championi* > 65.4% of *Gordonia axillaris* > 48% of *Schima superba* > 16.2% of *Camellia oleifera*;

(6) In addition to the relative electrical conductivity value of *Schima superba* (a decline of 15.4%), the rest showed an increase trend respectively: 460% of *Tutcheria championi* > 39.4% of *Gordonia axillaris* > 3.0% of *Camellia oleifera*.

The comprehensive evaluation showed that, the anti-HF contamination characteristics of the four Theaceae species in this experiment ranked: *Tutcheria championi* > *Camellia oleifera* > *Schima superba* > *Gordonia axillaris*.

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