

## 模拟酸雨对‘琯溪蜜柚’叶片抗氧化酶活性和光合作用的影响

张 琼, 陆銮眉, 戴清霞, 朱丽霞, 邹金美, 卞阿娜, 朱 眇, 李佳佳

(闽南师范大学生物科学与技术学院·园林植物生长发育与生态配置校级创新团队,福建漳州 363000)

**摘要:**【目的】探讨酸雨胁迫对‘琯溪蜜柚’叶片抗氧化酶活性和光合作用的影响。【方法】以 pH 值 5.6 为对照,采用 pH 2.5 和 pH 4.5 模拟酸雨对 1 a(年)生‘琯溪蜜柚’进行胁迫,研究酸雨对蜜柚叶片氧化伤害、抗氧化酶系统、气体交换参数和叶片结构的影响。【结果】pH 2.5 和 pH 4.5 模拟酸雨胁迫处理 3 个月,没有造成叶片可见伤害,叶片上表皮细胞排列整齐紧密,但 pH 2.5 模拟酸雨胁迫处理显著降低了叶肉中栅栏组织发育;pH 2.5 模拟酸雨胁迫处理 1 d 后,叶片 SOD 和 CAT 活性的升高减少 O<sub>2</sub><sup>-</sup> 和 H<sub>2</sub>O<sub>2</sub> 的积累,降低过氧化伤害,pH 2.5 模拟酸雨胁迫处理 3 d 之后引起叶片膜脂过氧化;pH 4.5 模拟酸雨胁迫处理 6 d 后虽然 3 种抗氧化系统酶活性显著高于对照,但不足以清除酸雨胁迫引起的活性氧,引起膜脂过氧化;模拟酸雨胁迫处理初期,2 种处理均显著抑制光合速率,酸雨处理 90 d 后,pH 2.5 酸雨显著抑制‘琯溪蜜柚’叶片光合速率,推测是由于氧化胁迫、栅栏组织发育等非气孔调节因素引起的,pH 4.5 酸雨处理下叶片具有较强自我修复能力,其光合速率没有受到显著抑制。【结论】‘琯溪蜜柚’具有较强的酸雨抗性,pH 4.5 中度酸雨胁迫对植株生长影响不显著,pH 2.5 重度酸雨胁迫显著抑制叶片光合作用。

**关键词:**‘琯溪蜜柚’;酸雨;抗氧化酶;光合作用;叶片解剖

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## Effects of acid rain on the leaves antioxidant activity and photosynthesis of *Citrus Grandis* (L.) Osbeck. ‘Guanximiyu’ seedlings

ZHANG Qiong , LU Luanmei , DAI Qingxia ,ZHU Lixia, ZOU Jinmei, BIAN Ana, ZHU Yang, LI Jiajia  
(School of Biological Sciences and Biotechnology, Minnan Normal University, Zhangzhou 363000, Fujian, China)

**Abstract:**【Objective】Acid rain has spread out from Europe beginning in the late 1950s. The contaminated regions have expanded from North America and Western Europe to developing countries, especially India and China. Acid rain may do harm to plants through changing their pH balance, increasing the leaching loss of nutrient elements, causing the destruction of leaf anatomy, and so on. *Citrus grandis* (L.) Osbeck. ‘Guanximiyu’ is a Rutaceae Citrus evergreen tree fruit whose pomelo is very delicious and nutritious and is an unusual species in Fujian Province. Fujian Province lies in the southeast of the China acid rain area and the pollution by acid rain is the main crisis of the pomelo industry. The majority of research on pomelo focused on the genetic quality, the fresh keeping techniques and the ecological planting mode, and so on. The study of the physiological ecology of the pomelo was given less emphasis. This study focuses on the foliar damage, antioxidant enzyme activity, gas exchange parameters and the leaves anatomical characteristics of *Citrus grandis* (L.) Osbeck. ‘Guanximiyu’ 1-year seedlings under acid rain treatment (pH 5.6, pH 4.5 and pH 2.5).【Methods】Healthy 1-year seedlings of *C. grandis* (L.) Osbeck. ‘Guanximiyu’ were planted in plastic pots with yellowish red soil in a greenhouse, with average day/night temperatures of 25 °C/20 °C and an average relative humidity of 75%. After two months of growing, three levels of acid rain (pH 5.6, pH 4.5 and pH 2.5) were developed. A completely

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作者简介:张琼,女,讲师,在读博士研究生,研究方向为植物生态学。Tel: 15006023059, E-mail: 349369029@qq.com

randomized design was applied. Each treatment had fifteen replicates, and a 200 mL acid rain solution was watered every two days. The leaves were harvested at 1, 3, 6 and 12 days after acid rain treatment. The malondialdehyde (MDA), superoxide dismutase (SOD), catalase(CAT) and peroxidase(POD) of the leaves were measured with kits. The photosynthesis rate ( $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), stomata conductance ( $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), intercellular  $\text{CO}_2$  concentration ( $\mu\text{mol CO}_2 \cdot \text{mol}^{-1}$ ) and transpiration rate ( $\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) were measured by using a photosynthetic Monitor (Li—6400XT) at 1, 3, 6, 12 and 90 day after acid rain treatment. The leaf anatomical structures were observed by using the paraffin section method. After 90 days treatment, 5-10 matured leaves in each treatment were selected for a paraffin section. These leaves were made by permanent slicing through fixing, dehydrating, embedding and so on. The leaves anatomical structures were measured by Image-Pro Plus 6.0. The experimental data were sorted out by Excel 2007 and analyzed with one-way ANOVA by SPSS19.0.【Results】The leaves treated by pH 2.5 and 4.5 acid rain did not show any visible injury and the surface layer of the cells were arranged in neat rows. And the pH 2.5 acid rain significantly decreased the thickness of the palisade cells and significantly increased the thickness of the sponge tissues compared with the pH 5.6 treatment. pH 4.5 acid rain did not have any significant effect on the thickness of the palisade cells and sponge tissues compared with the pH 5.6 treatment. The leaves did not show any oxidative damage due to the increase in SOD and CAT until after the third day being treated by pH 2.5 acid rain. pH 4.5 acid rain caused oxidative damage after six days of being treated by acid rain and the activities of SOD, CAT and POD were higher than the control treatment (pH 5.6). The photosynthesis rates were decreased by pH 2.5 and 4.5 acid rain during the initial period of acid rain stress. After three months of acid rain stress, the photosynthesis rate with the pH 2.5 treatment was lower than the control treatment and it was speculated this was because of non-stomata factors (oxidative damage, the decrease of palisade cell and so on). The photosynthesis rates in the pH 4.5 acid rain treatment were not affected after three months. The stomata conductance and transpiration rates in the pH 2.5 treatment were always significantly lower than the control treatment throughout the treatment process. The results showed that pH 2.5 treatment significantly restrained stomatal conduction and affected water absorption and transport. Stomata conductance in the pH 4.5 treatment was always significantly lower than the control treatment on the first day and the 12th day. And stomata conductance in the pH 4.5 treatment was not significantly different from the control treatment on the third day, the ninth day and the 90th day. The transpiration rate in the pH 4.5 treatment was significantly lower than the control treatment most of the time.【Conclusion】During the initial phases of the experimentation, the acid rain treatment did not cause any oxygen issues due to the increasing of the antioxidants activity. With a prolonged treatment time, acid rain treatment induced lipid peroxidation damage. pH 2.5 acid rain treatment did not cause any visible blade damage, but significantly decreased the thickness of the palisade tissues compared with the pH 5.6 treatment. pH 2.5 acid rain treatment significantly decreased the net photosynthetic rate compared with the pH 5.6 treatment. Under the pH 4.5 acid rain treatment, the net photosynthetic rate was not significantly decreased due to the ability to repair itself.

**Key words:** *Citrus grandis* (L.) Osbeck. ‘Guanximiyou’; Acid rain; Antioxidant enzyme; Photosynthesis; Leaf anatomy

酸雨自20世纪50年代后期在欧洲爆发,污染范围不断扩大,现已扩展到发展中国家,特别是印度和中国,危害最为严重<sup>[1-3]</sup>。近年来酸雨对植物影响已有大量报道,包括叶片结构<sup>[4]</sup>、种子发芽<sup>[5]</sup>、植物生长<sup>[6]</sup>、矿质营养吸收<sup>[7]</sup>和光合作用<sup>[8-10]</sup>等方面。酸雨对

植株伤害首先反映于叶片,酸雨破坏植物叶表面角质层,损害叶片表皮结构,使得酸性成分通过气孔或表皮扩散进入细胞<sup>[7,9,11]</sup>。叶片是光合器官,而光合作用是植物最为重要的生理活动,酸雨对光合速率的影响随酸雨pH、植物种类和发育阶段等因素不同

而不同<sup>[8]</sup>。不同植物对酸雨耐性阈值不同,乃至同一种植物不同品种不同无性系耐酸性也不同<sup>[12]</sup>,耐性较高植物可通过提高活性氧代谢相关基因、锌指蛋白类转录因子、钙相关基因和NAC转录因子基因表达等方面提高抗酸性<sup>[8,13-15]</sup>。

‘琯溪蜜柚’[*Citrus grandis* (L.) Osbeck. ‘Guanximiyou’]为芸香科柑橘属常绿乔木果树,其果实汁多、酸甜可口、营养丰富,具有较高经济价值,是福建省特有的柚类品种<sup>[16-17]</sup>。‘琯溪蜜柚’经过几十年芽变选育,已有多个品种(系),其中‘三红’品种市场竞争力和经济效益最高,目前是平和县推广最多的品种<sup>[18]</sup>。福建省位于华东酸雨区,酸雨频度较高,漳州被划为国家酸雨区,降水年平均pH≤4.5,土壤严重酸化,酸雨污染是该地区柚类产业面临的主要危机<sup>[19-20]</sup>。对蜜柚的研究多侧重其遗传品质<sup>[17]</sup>、生长性状质量评价<sup>[20]</sup>、采后保鲜<sup>[21]</sup>、鲜果深加工<sup>[22]</sup>和生态种植<sup>[23]</sup>等方面,很少对其个体生理生态特性,尤其是污染环境胁迫下的响应与调节机制进行研究。笔者以‘琯溪蜜柚’为材料,人工模拟酸雨,测定‘琯溪蜜柚’叶片抗氧化酶活性和光合参数,以期为酸雨区‘琯溪蜜柚’种植提供理论依据,为确定南亚热带果园生态系统耐酸雨生态安全阈值提供科学依据。

## 1 材料和方法

### 1.1 材料

取长势均匀的‘琯溪蜜柚’幼苗(1 a生嫁接苗,砧木为酸柚,‘三红’品种,株高30~40 cm)栽培于塑料花盆(端部直径20 cm,底部直径12 cm,高度16 cm)。花盆填入果园黄红壤2 cm,土壤pH为5.1,全N含量( $\omega$ ,后同)1.2 g·kg<sup>-1</sup>,黏粒含量50.1%。幼苗盆栽适应2个月后进行酸雨胁迫处理。

### 1.2 试验设计

根据闽南地区多年自然降水的化学组成,调配SO<sub>4</sub><sup>2-</sup>与NO<sub>3</sub><sup>-</sup>摩尔比5:1的酸雨母液<sup>[24-26]</sup>。一般将pH小于5.6的大气降水称为酸雨,本研究以pH 5.6模拟酸雨为对照<sup>[24,27]</sup>。在母液中加入自来水配制出pH值为5.6、4.5、2.5的模拟酸雨溶液,每个处理重复15盆。用模拟酸雨对‘琯溪蜜柚’盆栽进行浇灌,2 d 1次,每次浇灌200 mL 模拟酸雨,并对叶子喷淋酸雨,以叶面滴水为止。至试验完成90 d后,共浇灌酸雨45次,试验期间如果植株缺水,补充浇灌去离

子水。

### 1.3 抗氧化系统指标测定

酸雨胁迫1、3、6和12 d,选取成熟叶片(自上而下第2次分枝的第4~6枚叶片)分别采用苏州科铭生物技术有限公司生产的丙二醛测试盒、超氧化物歧化酶测试盒、过氧化物酶测试盒和过氧化氢酶测试盒测定丙二醛(MDA),超氧化物歧化酶(SOD),过氧化氢酶(CAT)和过氧化物酶(POD)活性。

### 1.4 光和参数测定

酸雨胁迫1、3、6、12和90 d利用美国Li-6400XT光合测定仪测定‘琯溪蜜柚’叶片净光合速率 $P_n$ ( $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )、气孔导度 $G_s$ ( $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )、胞间CO<sub>2</sub>浓度 $C_i$ ( $\mu\text{mol} \cdot \text{mol}^{-1}$ )、蒸腾速率 $T_r$ ( $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )。每个处理选取成熟叶片20~25枚测定。

### 1.5 叶片切片制作

酸雨处理90 d后,每组处理中选取成熟叶片5~10枚(自上而下第2次分枝的第4~6枚叶片),每一叶剪取中脉两侧5 mm×5 mm样块制作石蜡切片;将切好的叶片浸入FAA固定液中,经过脱水、浸蜡、包埋、切片、染色、透明、封片等常规制作石蜡切片步骤制成永久装片。在连接数码相机(Olympus DP-50, Japan)的奥林巴斯显微镜(OYLMPUS IX81, Japan)下镜检拍照,应用Image-Pro Plus 6.0软件测量叶片解剖数量特征。

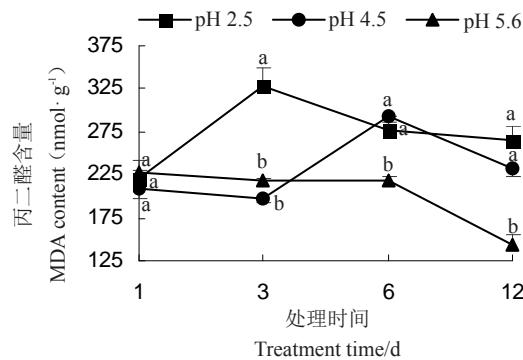
### 1.6 数据处理

应用Excel 2007整理试验数据,运用SPSS 19.0软件对数据进行单因素方差分析,并进行多重比较(SSR法),显著性水平为 $p < 0.05$ 。数据作图采用Origin 8.0软件。

## 2 结果与分析

### 2.1 酸雨胁迫对‘琯溪蜜柚’叶片抗氧化系统的影响

2.1.1 酸雨胁迫对MDA含量的影响 酸雨胁迫对‘琯溪蜜柚’叶片MDA含量的影响见图1。随着酸雨胁迫时间的延长,pH 2.5处理下MDA含量先增加后降低,pH 4.5处理下MDA含量缓慢增加后降低。实施酸雨处理1 d后,与对照相比,pH 2.5和pH 4.5处理下叶片MDA含量与对照差异不显著。酸雨处理3 d后,pH 2.5处理下叶片MDA含量显著高于对照,pH 4.5处理下MDA含量与对照差异不显著,处理6 d和12 d,2种酸雨胁迫下叶片MDA含量均显



不同小写字母表示差异达显著水平( $p < 0.05$ )。下同。

Different small letters indicate significant difference ( $p < 0.05$ ) according to Duncan's test among acid rain treatments in the same treatment. The same below.

图1 模拟酸雨对琯溪蜜柚叶片MDA含量的影响

Fig. 1 Effect of simulated acid rain on MDA content in leaves of the pomelo

著高于对照,但2种酸雨胁迫间MDA含量差异不显著。

**2.1.2 酸雨胁迫对抗氧化酶活性的影响** 酸雨胁迫对‘琯溪蜜柚’叶片SOD含量的影响见图2。与对照处理相比,pH 2.5处理1 d‘琯溪蜜柚’叶片SOD含量

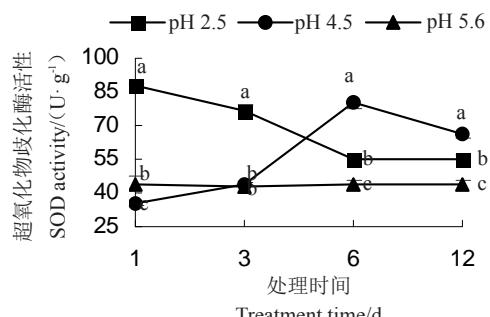


图2 模拟酸雨对‘琯溪蜜柚’叶片SOD活性的影响

Fig. 2 Effect of simulated acid rain on SOD activity in leaves of the pomelo

显著升高,处理6和12 d SOD含量下降,但也显著高于对照。pH 4.5处理1 d SOD含量显著低于对照,而后增加再降低,处理12 d SOD含量显著高于对照。

由图3可知pH 2.5和4.5处理1 d CAT活性均显著高于对照处理,处理3 d CAT活性下降,处理至12 d,pH 2.5处理下叶片CAT活性低于处理第1天,但显著高于对照处理,pH 4.5处理与对照处理差异不显著。

图4显示酸雨处理1 d后,各酸雨处理下‘琯溪

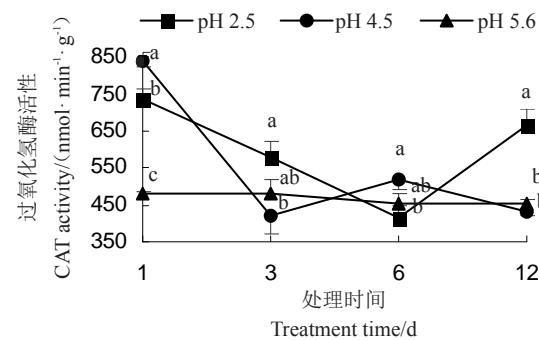


图3 模拟酸雨对‘琯溪蜜柚’叶片CAT活性的影响

Fig. 3 Effect of simulated acid rain on CAT activity in leaves of the pomelo

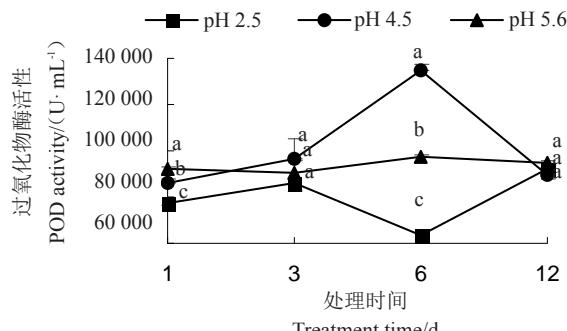


图4 模拟酸雨对‘琯溪蜜柚’叶片POD活性的影响

Fig. 4 Effect of simulated acid rain on POD activity in leaves of the pomelo

蜜’柚叶片POD活性均低于对照,酸雨胁迫越大,POD活性越小。随着处理时间的延长,各处理下‘琯溪蜜柚’叶片POD活性呈现增加趋势,至处理12 d时,各处理下‘琯溪蜜柚’叶片POD活性与对照无显著差异。

## 2.2 酸雨胁迫对‘琯溪蜜柚’叶片气体交换参数的影响

由表1可知pH 2.5和pH 4.5处理1 d和3 d净光合速率均显著低于对照处理;处理6 d和12 d,pH 2.5处理净光合速率显著低于对照,而pH 4.5处理6 d后与对照处理不显著,但处理至12 d后,净光合速率显著低于对照处理;酸雨胁迫90 d后,pH 2.5处理净光合速率显著低于对照,pH 4.5处理下净光合速率与对照差异不显著。由此表明‘琯溪蜜柚’叶片对酸雨的光合响应依据胁迫程度和时间的不同而不同,长期酸雨胁迫下pH 4.5处理没有显著抑制净光合速率,pH 2.5处理降低植株叶片碳同化速率,显著抑制叶片光合作用。

在pH 2.5处理下,气孔导度和蒸腾速率在各检

表 1 模拟酸雨对‘琯溪蜜柚’叶片气体交换参数的影响

Table 1 Effect of simulated acid rain on gas exchange parameters in leaves of the pomelo

指标 Index	处理 Treatment	时间 Time/d				
		1	3	6	12	90
净光合速率 Photosynthesis rate/ ( $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	pH 2.5	4.734 $\pm$ 0.200 b	8.052 $\pm$ 0.242 c	6.985 $\pm$ 0.194 b	5.607 $\pm$ 0.181 b	7.004 $\pm$ 0.143 b
	pH 4.5	4.661 $\pm$ 0.178 b	11.730 $\pm$ 0.157 b	8.367 $\pm$ 0.308 a	5.569 $\pm$ 0.260 b	8.773 $\pm$ 0.195 a
	pH 5.6	5.907 $\pm$ 0.305 a	12.657 $\pm$ 0.335 a	8.038 $\pm$ 0.306 a	8.978 $\pm$ 0.205 a	8.871 $\pm$ 0.174 a
气孔导度 Stomata conductance/ ( $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	pH 2.5	0.167 $\pm$ 0.007 b	0.089 $\pm$ 0.005 b	0.075 $\pm$ 0.011 a	0.050 $\pm$ 0.003 b	0.109 $\pm$ 0.007 b
	pH 4.5	0.140 $\pm$ 0.006 c	0.217 $\pm$ 0.012 a	0.104 $\pm$ 0.009 a	0.064 $\pm$ 0.006 b	0.225 $\pm$ 0.005 a
	pH 5.6	0.203 $\pm$ 0.010 a	0.233 $\pm$ 0.017 a	0.100 $\pm$ 0.008 a	0.151 $\pm$ 0.009 a	0.238 $\pm$ 0.011 a
胞间 CO <sub>2</sub> 浓度 Intercellular CO <sub>2</sub> concentration/ ( $\mu\text{mol} \cdot \text{mol}^{-1}$ )	pH 2.5	302.467 $\pm$ 1.734 a	214.733 $\pm$ 4.067 b	194.714 $\pm$ 13.325 a	220.500 $\pm$ 10.406 c	294.750 $\pm$ 6.556 a
	pH 4.5	294.810 $\pm$ 0.801 b	254.889 $\pm$ 2.340 a	213.917 $\pm$ 8.323 a	243.815 $\pm$ 5.923 b	302.185 $\pm$ 1.626 a
	pH 5.6	292.722 $\pm$ 1.410 b	248.857 $\pm$ 4.279 a	215.556 $\pm$ 8.677 a	291.500 $\pm$ 3.241 a	298.571 $\pm$ 2.024 a
蒸腾速率 Transpiration rate/( $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	pH 2.5	2.593 $\pm$ 0.070 b	1.956 $\pm$ 0.088 c	1.938 $\pm$ 0.235 b	0.951 $\pm$ 0.066 c	1.385 $\pm$ 0.095 c
	pH 4.5	2.392 $\pm$ 0.072 b	4.038 $\pm$ 0.097 b	2.738 $\pm$ 0.194 a	1.315 $\pm$ 0.101 b	3.290 $\pm$ 0.059 b
	pH 5.6	3.254 $\pm$ 0.118 a	4.672 $\pm$ 0.265 a	2.657 $\pm$ 0.184 a	2.539 $\pm$ 0.114 a	3.790 $\pm$ 0.119 a

测时间段均显著低于对照处理,表明酸雨在胁迫初期抑制气孔开度,影响水分吸收和转运。胞间二氧化碳浓度处理 1 d 时,显著高于对照,而后降低,处理至 12 d,低于对照 14.7%。在 pH 4.5 处理下,‘琯溪蜜柚’叶片气孔导度在处理 1 和 12 d 时,显著低于对照,其他时间与对照差异不显著,蒸腾速率在多数时

间点均显著低于对照处理。

### 2.3 酸雨胁迫对‘琯溪蜜柚’叶片解剖结构的影响

由图 5 可见蜜柚叶片上表皮为单表皮,各处理上表皮细胞均排列紧密。为更好地呈现酸雨对叶片解剖结构的影响,采用相对比例表示叶片各结构发育状况(表 2)。单因素方差分析显示与对照相

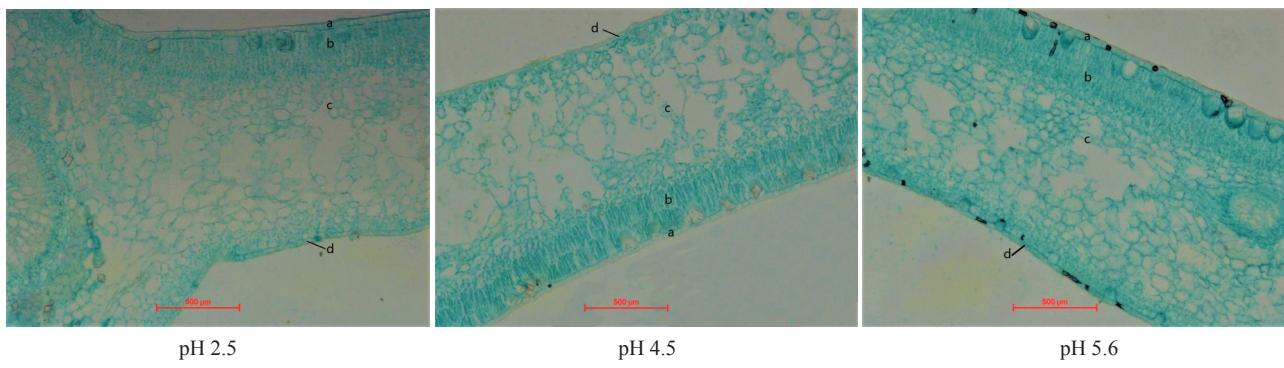


图 5 酸雨处理下‘琯溪蜜柚’叶片横切解剖结构

Fig. 5 Leaves anatomical characteristics of pomelo under acid rain treatment

表 2 模拟酸雨对‘琯溪蜜柚’叶片解剖结构的影响

Table 2 Effect of simulated acid rain on leaves anatomical characteristics of the pomelo

处理 Treatment	上表皮比例 Ratio of upper epidermis/%	下表皮比例 Ratio of low epidermis/%	栅栏组织比例 Ratio of palisade tissue/%	海绵组织比例 Ratio of spongy tissue/%
pH 2.5	2.73 $\pm$ 0.37 a	1.79 $\pm$ 0.15 b	18.95 $\pm$ 1.71 b	76.53 $\pm$ 2.07 a
pH 4.5	2.64 $\pm$ 0.11 a	2.13 $\pm$ 0.13 ab	22.87 $\pm$ 0.59 a	72.35 $\pm$ 0.58 b
pH 5.6	3.32 $\pm$ 0.24 a	2.31 $\pm$ 0.18 a	23.30 $\pm$ 0.79 a	71.07 $\pm$ 0.88 b

比 2 种模拟酸雨处理没有显著影响上表皮细胞厚度比例,pH 2.5 酸雨胁迫显著降低下表皮比例,pH 4.5 酸雨胁迫没有显著影响下表皮比例。栅栏薄壁组织是进行光合作用的主要位点,其发育程度影响

植物体光合效率。多重比较结果显示 pH 2.5 酸雨处理显著降低叶肉中栅栏组织厚度比例,而显著增加海绵组织比例,pH 4.5 酸雨胁迫对叶肉组织影响不显著。

### 3 讨 论

植物抗逆性与体内抗氧化系统有关,酸雨胁迫下随着胁迫强度和时间增加,抗氧化酶活性也随之变化,需要SOD、CAT和POD等抗氧化酶和抗氧化物质相互配合,才能提高植物体清除活性氧的能力,而超过植物耐受阈的高强度酸雨会导致植物体活性氧积累超过抗氧化系统的自净极限,引起氧化伤害<sup>[27]</sup>。本研究酸雨胁迫初期,由于琯溪蜜柚叶片SOD和CAT活性升高,减少了O<sub>2</sub><sup>·</sup>和H<sub>2</sub>O<sub>2</sub>积累,降低了氧化伤害,随着酸雨胁迫时间的延长,植物体内活性氧生产能力大于清除能力,引起细胞膜脂过氧化。

酸雨直接淋洗叶片,易对叶片造成即时伤害,如龙眼叶片在pH 4.5酸雨胁迫6 h后就检测出生理损伤(K<sup>+</sup>大量渗漏)<sup>[24]</sup>,本研究琯溪蜜柚在pH 2.5和4.5处理1 d后,叶片气孔导度和净光合速率均被显著抑制,但随着处理时间的延长,pH 4.5酸雨胁迫对光合的抑制效果降低,而pH 2.5重度酸雨胁迫对光合作用的抑制效果没有缓解。许多研究也表明随着酸雨强度增加,光合作用被抑制程度也增加,如加拿大一枝黄花和木芙蓉随着模拟酸雨酸度的增加,光合速率也持续降低<sup>[28-29]</sup>。

光合速率下降主要是由于气孔和非气孔因素影响,当酸雨胁迫下随着气孔的关闭而C<sub>i</sub>也相应降低,表明光合作用降低主要由气孔关闭造成;如果气孔关闭而C<sub>i</sub>不变,甚至还有所提高,则表明光合作用的下降主要由于非气孔因素引起的<sup>[29-30]</sup>。有些研究表明,酸雨破坏植物叶片结构,在可见损伤叶片上导致植物气孔不同程度永久性开放,使得污染物容易侵入,水分散失增加,在无可见损伤叶片上气孔与正常叶片的气孔一样是可调节的<sup>[31]</sup>。本研究中‘琯溪蜜柚’对酸雨有较强抗性,即使在pH 2.5重度酸雨长期胁迫下‘琯溪蜜柚’叶片也无可见损伤,‘琯溪蜜柚’叶片通过调节气孔开度,减少吸入污染物,以适应酸雨胁迫,但也显著抑制蒸腾作用,从而影响水分和营养元素的运输,由胞间二氧化碳浓度推测气孔调节不是长期酸雨胁迫对光合作用抑制的主要影响因素。酸雨胁迫下叶片叶绿体易遭受破坏,使得叶绿体光还原活性降低,也使细胞代谢受阻<sup>[32-33]</sup>,本研究中‘琯溪蜜柚’叶片在酸雨胁迫下遭受氧化伤害,膜脂过氧化,影响光合结构的完整性,推测是光合抑制的主要因素。pH 4.5处理初期光合速率的下降主要

是由非气孔因素影响,处理3个月后叶片表现出较强的自我修复能力,其光合速率没有受到显著抑制。

植物形态特点和生物学特性对酸雨抗逆性起到决定性作用,在重度酸雨胁迫下,酸雨能够破坏许多植物叶片表面的蜡质和角质层,损害植物表皮结构,如王伟等<sup>[34]</sup>研究显示,pH 3.0酸雨处理下青菜叶片近轴表皮细胞和栅栏细胞发生瓦解。而有些植物如山茶花叶片革质,表皮覆盖较厚蜡质层和角质层,对酸雨液滴的滞留时间较短,对酸雨中有害离子侵入有结构形态上的排外作用,重度酸雨胁迫后,没有造成明显的伤斑<sup>[27]</sup>。本研究中蜜柚叶片质地革质,酸雨抗性亦较强,叶片横切面解剖图显示近轴表皮细胞在pH 2.5重度酸雨胁迫3个月后排列整齐,重度酸雨没有显著影响上表皮厚度,栅栏组织亦规则排列,但与对照处理相比,酸雨显著降低栅栏组织厚度,在一定程度上抑制光合作用。

### 参考文献 References:

- [1] ABBASI T, POOMIMA P, KANNADASAN T, KANNADASAN S A. Acid rain: past, present, and future[J]. International Journal of Environmental Engineering, 2013, 5(3): 229-272.
- [2] LIU T W, JIANG X W, SHI W L, CHEN J, PEI Z, ZHENG H. Comparative proteomic analysis of differentially expressed proteins in beta-aminobutyric acid enhancing *Arabidopsis thaliana* tolerance to simulated acid rain[J]. Proteomics, 2011, 11: 2079-2094.
- [3] PRIHATIN J, COREBIMA A D, ARIFFIN, GOFUR A. The effect of exposure of mulberry to acid rain on the defects cocoon of *Bombyxmori* L.[J]. Procedia Environmental Sciences, 2015, 23: 186-191.
- [4] 付晓萍,田大伦,黄智勇.模拟酸雨对植物形态学效应的影响[J].浙江林学院学报,2006(5): 521-526.  
FU Xiaoping, TIAN Dalun, HUANG Zhiyong. Effects of simulated acid rain on phytomorphology[J]. Journal of Zhejiang Forestry College, 2006(5): 521-526.
- [5] LIU T W, WU F H, WANG W H, CHEN J, LI Z J, DONG X J, PATTON J, PEI Z M, ZHENG H L. Effects of calcium on seed germination, seedling growth and photosynthesis of six forest tree species under simulated acid rain[J]. Tree Physiology, 2011, 31(4): 402-413.
- [6] 廖广社,许建新,许涵,庄雪影.模拟酸雨对黄槐幼苗生长的影响[J].广东园林,2005, 31(5): 37-41.  
LIAO Guanshe, XUE Jianxin, XU Han, ZHUANG Xueying. Effects of acid rain treatments on growth in *Cassia surattensis* seedlings[J]. Guangdong Landscape Architecture, 2005, 31 (5): 37-41.

- [7] 张宇飞,方向民,陈伏生,宗莹莹,顾菡娇,胡小飞.模拟酸雨对红壤区茶树器官氮磷含量及其化学计量比的影响[J].应用生态学报,2017,28(4): 1309-1316.  
ZHANG Yufei, FANG Xianmin, CHEN Fusheng, ZONG Yingying, GU Hanjiao, HU Xiaofei. Influence of simulated acid rain on nitrogen and phosphorus contents and their stoichiometric ratios of tea organs in a red soil region, China[J]. Chinese Journal of Applied Ecology, 2017, 28(4): 1309-1316.
- [8] 牛力.模拟酸雨对拟南芥某些生理特性和基因表达谱的影响[D].厦门:厦门大学,2009.  
NIU Li. Effects of simulated acid rain on some physiological characteristics and gene expression profiles of *Arabidopsis thaliana*[D]. Xiamen: Xiamen University, 2009.
- [9] 宋晓梅,曹向阳.模拟酸雨对不同园林植物叶片生理生态特性的影响[J].水土保持学报,2017,24(2): 365-370.  
SONG Xiaomei, CAO Xiangyang. Effect of simulated acid rain on the physiological and ecological characteristics of different garden plants[J]. Research of Soil and Water Conservation, 2017, 24(2): 365-370.
- [10] LI Y H, WANG D, XU X T, GAO X X, SUN X, XU N J. Physiological responses of a green algae (*Ulva prolifera*) exposed to simulated acid rain and decreased salinity[J]. Photosynthetica, 2017, 55(4): 623-629.
- [11] 童永忠,刘超,周志勤,傅柳松,吴杰民.酸雨对土壤—植物系统的环境效应研究[J].浙江农业大学学报,1994,20(1): 89-91.  
TONG Yongzhong, LIU Chao, ZHOU Zhiqin, FU Liusong, WU Jiemin. Studies on the environmental influence of acid rain against soil- plant system[J]. Journal of Zhejiang Agricultural University, 1994, 20(1): 86-91.
- [12] 黄俊文,黄真池,曾彩萍,谢观燕,庞恒声,章武.酸雨胁迫对两种桉树光合生理及保护酶活性的影响[J].分子植物育种,2017,15(8):3272-3277.  
HUANG Junwen, HUANG Zhenchi, ZENG Caiping, XIE Guanyan, PANG Hengsheng, ZHANG Wu. Effects of acid rain stress on photosynthetic physiology and protective enzyme activities of two *Eucalyptus* species[J]. Molecular Plant Breeding, 2017, 15(8): 3272-3277.
- [13] 乔芳.拟南芥对三种类型模拟酸雨不同响应机制研究[D].厦门:厦门大学,2014.  
QIAO Fang. Studies on differential mechanisms of *Arabidopsis thaliana* in response to three types of simulated acid rain[D]. Xiamen: Xiamen University, 2014.
- [14] 高世超,钟凤林,林义章,赵瑞丽.青花菜NAC转录因子克隆及在酸雨胁迫下的表达分析[J].分子植物育种,2014,12(4): 740-747.  
GAO Shichao, ZHONG Fenglin, LIN Yizhang, ZHAO Ruili. Cloning of NAC transcription factor (*Brassica oleracea* var. *italica*) and its expression in broccoli under acid rain[J]. Molecular Plant Breeding, 2014, 12(4): 740-747.
- [15] 王丽红,孙静雯,王雯,周青.酸雨对植物光合作用影响的研究进展[J].安全与环境学报,2017,17(2): 775-780.  
WANG Lihong, SUN Jingwen, WANG Wen, ZHOU Qing. Research advances in effects of acid rain on plant photosynthesis[J]. Journal of Safety and Environment, 2017, 17(2): 775-780.
- [16] 林燕金,林旗华,姜翠翠,卢新坤.福建省柚类产业发展现状及对策[J].东南园艺,2014(5): 39-42.  
LIN Yanjin, LIN Qihua, JIANG Cuicui, LU Xinkun. Industry status and countermeasures of pomelo industry in Fujian province[J]. Southeast Horticulture, 2014(5): 39-42.
- [17] 王梨媚,潘永娟,杨莉,蔡盛华,黄新忠.‘琯溪蜜柚’荧光定量PCR内参基因的筛选[J].果树学报,2013,30(1): 48-54.  
WANG Lihuan, PAN Yongjuan, YANG Li, CAI Shenghua, HUANG Xinzhong. Validation of internal reference genes for qRT-PCR normalization in ‘Guanxi sweet pummelo’ (*Citrus grandis*)[J]. Journal of Fruit Science, 2013, 30(1): 48-54.
- [18] 吴培衍,张金文,张荣标,张志鸿.琯溪蜜柚大树高接三红蜜柚技术试验[J].林业调查规划,2017,42(4):127-129.  
WU Peiyang, ZHANG Jinwen, ZHANG Rongbiao, ZHANG Zhishong. Experiment on top grafting with three red honey pomelo on Guanxi honey pomelo tree[J]. Forest Inventory and Planning, 2017, 42(4): 127-129.
- [19] 赵卫红.福建省主要城市降水离子特征及沉降量现状分析[J].亚热带资源与环境学报,2008,3(3): 19-24.  
ZHAO Weihong. On characteristics of precipitation ion and current sedimentation situation in main cities of Fujian province[J]. Journal of Subtropical Resources and Environment, 2008, 3(3): 19-24.
- [20] 钱笑杰,林晓兰,肖靖,李发林.福建果园土壤pH值、养分关系与土壤肥力质量评价研究——以福建省漳州市平和县琯溪蜜柚园地为例[J].福建热作科技,2017,42(1):9-15.  
QIAN Xiaojie, LIN Xiaolan, XIAO Jing, LI Falin. Evaluation research between soil pH, nutrition and soil fertility in Fujian orchard[J]. Fujian Science & Technology of Tropical Crops, 2017, 42(1): 9-15.
- [21] 张小红,赵依杰,潘东明,林航.琯溪蜜柚果实采后有机酸代谢[J].果树学报,2010,27(2): 193-197.  
ZHANG Xiaohong, ZHAO Yijie, PAN Dongming, LIN Hang. Study on the post-harvest metabolism of organic acids in fruit of Guanximiyu pomelo cultivar[J]. Journal of Fruit Science, 2010, 27(2): 193-197.
- [22] 张素芳,肖安风,杨远帆,胡阳,姜泽东,陈峰,倪辉.柚苷酶处理对琯溪蜜柚果汁抗氧化活性的影响[J].中国食品学报,2016,16(9): 60-67.  
ZHANG Sufang, XIAO Anfeng, YANG Yuanfan, HU Yang, JIANG Zedong, CHEN Feng, NI Hui. Effects of naringinase treatment on the antioxidant activity of Guanxi pomelo juice[J]. Journal of Chinese Institute of Food Science and Technology, 2016, 16(9): 60-67.
- [23] 李发林,曾瑞琴,危天进,李延,林晓兰,武英,钱笑杰,杨忠义,

- 郑涛,张世祺.平和县琯溪蜜柚山地果园径流氮磷含量变化[J].中国农学通报,2017,33(27): 117-123.
- LI Falin, ZENG Ruiqin, WEI Tianjin, LI Yan, LIN Xiaolan, WU Ying, QIAN Xiaojie, YANG Zhongyi, ZHENG Tao, ZHANG Shiqi. Change of runoff nitrogen and phosphorus content: mountain orchard of Guanxi pomelo in Pinghe[J]. Chinese Agricultural Science Bulletin, 2017, 33(27): 117-123.
- [24] 邱栋梁,刘星辉.模拟酸雨伤害龙眼的酸度阈值研究[J].热带作物学报,2003,24(1): 31-35.
- QIU Dongliang, LIU Xinghui. Study on the pH threshold of acid rain injury to longan[J]. Chinese Journal of Tropical Crops, 2003, 24(1): 31-35.
- [25] 汤大纲,王玮,庞燕波,刘红杰,王山珊,王榕海.氮氧化物在闽南地区酸雨中的贡献[J].环境科学研究,1996,9(5): 38-40.
- TANG Dagang, WANG Wei, PANG Yanbo, LIU Hongjie, WANG Shanshan, WANG Ronghai. Contribution of NO<sub>x</sub> to acid rain in Minnan area[J]. Research of Environmental Sciences, 1996, 9(5): 38-40.
- [26] 林万生.福建省漳州市降雨化学组成综合分析[J].广州化工,2014,42(7):119-121.
- LIN Wansheng. Analysis on the characteristics of chemical composition of precipitation in Zhangzhou city, Fujian province[J]. Guangzhou Chemical Industry, 2014, 42(7): 119-121.
- [27] 赵栋,潘远智,邓仕槐,尚鹤,王芳,陈睿.模拟酸雨对山茶生物特性的影响[J].南京林业大学学报(自然科学版),2010,34(5): 39-42.
- ZHAO Dong, PAN Yuanzhi, DENG Shihuai, SHANG He, WANG Fang, CHEN Rui. Effects of simulated acid rain on physiological characteristics of *Camellia japonica*[J]. Journal of Nanjing Forestry University (Natural Sciences Edition), 2010, 34(5): 39-42.
- [28] 王子健,朱欣,高松.酸雨、遮阴胁迫对加拿大一枝黄花光合生理特征的影响[J].河南师范大学学报(自然科学版),2015,43(2): 113-118.
- WANG Zijian, ZHU Xin, GAO Song. Effects on photosynthetic characteristics of *Solidago canadensis* under acid rain or shading stress[J]. Journal of Henan Normal University (Natural Science Edition), 2015, 43(2): 113-118.
- [29] 王应军,邓仕槐,姜静,尚鹤,林波,孙亚琴,胡晓梅.酸雨对木芙蓉幼苗光合作用及抗氧化酶活性的影响[J].核农学报,2011,25(3): 588-593.
- WANG Yingjun, DENG Shihuai, JIANG Jing, SHANG He, LIN Bo, SUN Yaqin, HU Xiaomei. Effects of acid rain on photosynthesis and antioxidant enzyme activity of *H. mutabilis* L. seedlings[J]. Journal of Nuclear Agricultural Sciences, 2011, 25(3): 588-593.
- [30] 郑飞翔,温达志,旷远文.模拟酸雨对柚木幼苗生长、光合和水分利用的影响[J].热带亚热带植物学报,2006,14(2): 93-99.
- ZHENG Feixiang, WEN Dazhi, KUANG Yuanwen. Effects of simulated acid rain on the growth, photosynthesis and water use efficiency in *Tectona grandis*[J]. Journal of Tropical Andsubtropical Botany, 2006, 14(2): 93-99.
- [31] 付晓萍,田大伦.酸雨对植物的影响研究进展[J].西北林学院学报,2006,21(4): 23-27.
- FU Xiaoping, TIAN Dalun. Research progress of the effect of acid rain on plant[J]. Journal of Northwest Forestry University, 2006, 21(4): 23-27.
- [32] 宋莉英,柯展鸿,孙兰兰,彭长连.模拟酸雨对3种菊科入侵植物光合特性的影响[J].植物学报,2013,48(2):160-167.
- SONG Liying, KE Zhanhong, SUN Lanlan, PENG Changlian. Effect of simulated acid rain on gas exchanges of three composite invasive plants[J]. Chinese Bulletin of Botany, 2013, 48(2): 160-167.
- [33] 黄卓辉,魏家绵.光合磷酸化偶联机制研究—VIII、6-苄氨基嘌呤对光合磷酸化的促进作用[J].植物生理学报,1984,10(2): 161-167.
- HUANG Zhuohui, WEI Jiamian. Studies on the coupling mechanism of photophosphorylation--VIII, the stimulatory effect of 6-benzylaminopurine on photophosphorylation in chloroplasts isolated from spinach[J]. Plant Physiology Journal, 1984, 10 (2): 161-167.
- [34] 王伟.模拟酸雨处理的青菜显微和亚显微结构观察及部分生理指标测定[J].环境科学,1988,9(3): 12-17.
- WANG Wei. Effects of simulated acid rain on microstructure and ultrastructure as well as physiological index of green vegetable[J]. Environmental Science, 1988, 9(3): 12-17.