

李菲菲,唐超兰,蒋红梅,等. 淹渍胁迫下枳砧温州蜜柑的形态特征和生理响应[J]. 华中农业大学学报, 2022, 41(5): 122-133.  
DOI: 10.13300/j.cnki.hnlkxb.2022.05.016

## 淹渍胁迫下枳砧温州蜜柑的形态特征和生理响应

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**摘要** 为探究淹渍胁迫后枳砧温州蜜柑(*Citrus unshiu* Marc.)植株的形态特征变化及其生理响应机制,以枳砧‘市文’温州蜜柑成年树为试材,进行盆栽土壤淹水胁迫处理,将植株根部分别淹渍处理2、5、10、15、20 d,以正常灌溉为对照(CK),观察其形态学及根系解剖结构特征的变化,并测定叶片的光合参数、叶片和根系的渗透调节物质、无氧呼吸酶活性、丙二醛含量及树体矿质营养元素等指标。结果显示:淹渍5 d时,根系开始出现异味且韧皮部细胞饱满膨胀;淹渍10 d时,新老叶片已呈现不同程度的黄化,根系开始腐烂、变色,淹渍20 d左右,树体干枯死亡。与对照相比,淹渍胁迫处理早期(5 d内),根系乙醇脱氢酶活性显著升高,随着淹渍胁迫时间的延长,根系中游离脯氨酸、可溶性糖和可溶性蛋白含量显著降低并维持在较低水平,丙二醛含量仅在淹渍10 d时显著高于对照。相同处理条件下,叶片中的叶绿素含量随淹渍时长持续下降,淹渍处理10 d时叶片的胞间CO<sub>2</sub>浓度显著升高,净光合速率、气孔导度和蒸腾速率显著下降。叶片中游离脯氨酸、可溶性蛋白、丙二醛和相对电导率自淹渍处理10~20 d均显著高于同期对照。淹渍胁迫5 d和20 d时,茎和叶片多数矿质营养积累未发生变化,根系中的P、K、Ca、Mg含量与对照相比显著减少。结果表明,枳砧温州蜜柑不同组织对淹渍胁迫后的生理响应存在明显差异,叶片相对电导率和根系乙醇脱氢酶对淹渍胁迫的响应敏感;长期淹渍胁迫影响植株的光合性能、渗透调节物质含量及乙醇脱氢酶活性的变化,使叶片和根系出现明显可见的胁迫症状,导致植株生长缓慢,甚至死亡。

**关键词** 温州蜜柑; 淹渍胁迫; 渗透调节物质; 植株形态; 生理特性

**中图分类号** S666.1 **文献标识码** A **文章编号** 1000-2421(2022)05-0122-12

柑橘是我国南方地区产量最大和分布最广的亚热带果树。由于多数柑橘产区春夏季节雨季较长,降雨量大,橘园常出现雨季积水浸根,甚至洪涝淹树不同程度的淹渍胁迫。因此,了解柑橘淹渍胁迫后的树体状况及淹渍响应生理机制对于正确评估柑橘淹渍灾害影响及灾后恢复性生产十分重要。

淹渍胁迫对植株的影响主要表现为植株形态和生理改变,引起植株根系腐烂,进而造成植株无氧呼吸加剧<sup>[1]</sup>、叶绿素降低、光合性能下降<sup>[2]</sup>、细胞膜伤害、代谢紊乱等,最终使得植株生长受到抑制、萎蔫、局部坏死或整株死亡<sup>[3]</sup>。研究<sup>[4]</sup>表明,柑橘砧木幼苗淹渍后表现叶脉黄化,最终导致叶片萎蔫干枯;淹渍条件下不同柑橘砧木品种的耐涝性差异明显,资阳

香橙、卡里佐枳橙和印度酸橘为耐涝砧木,枳为中度耐涝砧木,红橘、枸头橙和宜昌橙为不耐涝砧木<sup>[5]</sup>。枳砧脐橙1年生苗在淹渍后根系受损、生长量减少,叶片叶脉变黄、脉间相对保持绿色,根系和叶片的解剖结构也相应发生变化<sup>[6]</sup>。淹渍降低柑橘幼苗的光合作用,影响其生长和生物量分配,引起叶片气孔关闭,降低根系的导水率和pH值,但对叶片水势和相对含水量无影响<sup>[7-8]</sup>。柑橘在淹渍胁迫条件下积累活性氧造成细胞膜损伤,长时间淹渍胁迫使植株体内膜脂过氧化程度升高,并抑制某些蛋白质合成,造成可溶性蛋白含量下降、可溶性糖含量升高、抗氧化酶活性升高<sup>[6,9]</sup>。柑橘根系直接受到淹渍胁迫的伤害,导致根系活力降低,影响根系对养分的吸收和转运,

收稿日期: 2022-07-28

基金项目: 国家自然科学基金项目(32102313; 31872044); 国家柑橘产业技术体系建设专项(CARS-26)

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造成C/N失调<sup>[7]</sup>,也有研究报道淹渍胁迫影响柑橘对Fe的吸收<sup>[10]</sup>。

我国柑橘产区春夏季节连续阴雨易导致橘园淹渍频繁发生,淹渍胁迫对柑橘坐果及果实发育、产量品质形成、花芽分化均有较大影响,是当前柑橘生产亟需解决的问题之一<sup>[5,11]</sup>。国内外学者围绕柑橘砧木如枳橙、枳、资阳香橙、酸橙等以及部分柑橘类幼苗分别开展了淹渍胁迫的相关研究<sup>[4-10]</sup>,而温州蜜柑对淹渍胁迫响应机制的系统研究鲜有报道。因此,本研究以盆栽的枳砧‘市文’温州蜜柑为材料,从淹渍后温州蜜柑叶片和枳砧根系的形态学及解剖结构特征等表型变化着手,结合淹渍胁迫后植株的内在生理及生化指标变化,明确枳砧温州蜜柑在淹渍胁迫下的形态特征变化以及生理生化响应机制,以期为今后制定科学有效的栽培技术措施、降低胁迫灾害程度及柑橘耐涝良种选育提供理论指导。

## 1 材料与方法

### 1.1 试验材料

试验于2019年5—6月在湖南农业大学温室大棚(28°17'99" N, 113°07'71" E)内进行。以栽植于黑色塑料桶(直径40 cm,高34 cm)中的9年生枳砧‘市文’温州蜜柑(*Citrus unshiu* Marc. ‘Ichifumi Wase’)为材料,栽培基质为 $V_{\text{锯木屑}}:V_{\text{河沙}}:V_{\text{草炭}}=2:1:0.5$ 。根据该地区春夏之交持续降雨规律性明显的特点,试验于5月16日开始,淹渍处理(waterlogging stress, WL)采用双套盆法,在打孔的桶外再套1个无孔的黑色塑料桶,控制淹水水面超过土壤表面1 cm。淹渍历时设2、5、10、15、20 d共5个处理;同期设置的对照(CK)进行正常水分管理,使基质相对含水量维持在(70±10)%。使用便携式土壤水分监测仪监测水分含量,确保相对含水量稳定。以上每个处理3次重复。大棚温湿度情况使用温湿度数据记录仪记录(图1)。

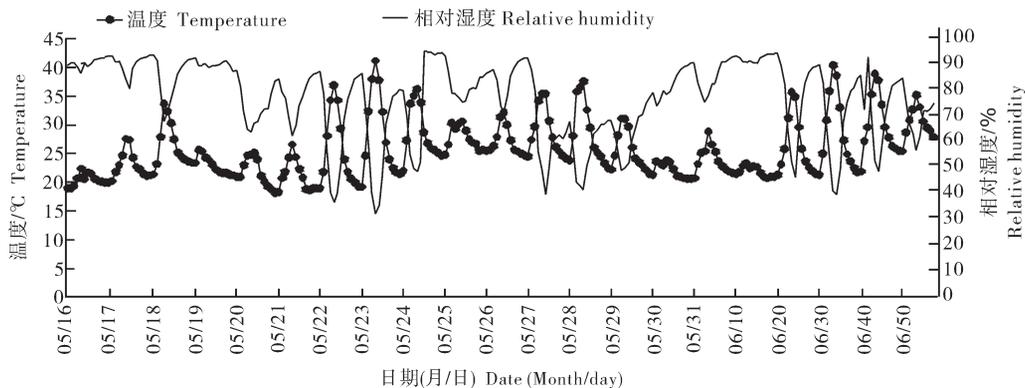


图1 试验期间大棚温湿度

Fig.1 Temperature and relative humidity in the greenhouse during the present experiment

### 1.2 主要仪器与设备

TDR 便携式土壤水分监测仪,美国 Spectrum technologies; DT-171 温湿度数据记录仪,中国 CEM; Panoramic MIDI 数字切片扫描仪,匈牙利 3DHISTECH; LI-6400 携式光合仪,美国 LI-COR Inc; Multiskan Sky 全波长酶标仪,美国赛默飞; DDS-11A 电导率仪,上海雷磁; KDN-103F 自动定氮仪,上海纤检; Thermo-902 超低温冰箱,美国赛默飞。

### 1.3 形态指标的测定

每个处理达到设定淹渍时间后,对叶片和根系拍照并观察其形态学变化;将试验材料整株拔出,先用自来水冲去土壤和杂质,再用蒸馏水冲洗干净,然

后用滤纸和吸水纸擦净材料上的水分,选取根尖粗度及成熟度相对一致的根段,放入乙醇-醋酸-福尔马林混合固定液(FAA 固定液)中保存,采用石蜡切片方法观察根系解剖结构<sup>[12]</sup>。

### 1.4 生理生化指标的测定

分别取 CK 和淹水处理 2、5、10、15、20 d 的植株细根和秋梢叶,其中叶绿素和相对电导率测定用新鲜叶片,其余均剪碎混匀、液氮速冻后放入-80 °C 超低温冰箱保存,矿质元素测定将试验材料的根、茎、叶分开,放入 105 °C 烘箱杀青 30 min 后,烘箱温度调至 70 °C 烘干至恒质量,样品粉碎后过孔径 0.15 mm 筛待测,所有测定指标均设 3 次重复。

叶绿素含量采用乙醇浸提法<sup>[13]</sup>;相对电导率采

用电导率仪法<sup>[13]</sup>;植物氮含量测定采用硫酸-过氧化氢消煮-蒸馏法,全磷(P)测定采用硫酸-过氧化氢消煮-钒钼黄比色法,全钾测定采用硫酸-过氧化氢消煮-火焰光度法,钙、镁、铁、锌测定采用微波消解仪进行消解,ICP法测定<sup>[14]</sup>。每处理组结束后,于当日09:00—12:00用便携式光合测定仪测定秋梢顶端第2~4片叶的净光合速率(net photosynthetic rate,  $P_n$ )、蒸腾速率(transpiration rate,  $T_r$ )、胞间二氧化碳浓度(intercellular  $CO_2$  concentration,  $C_i$ )及气孔导度(stomatal conductance,  $G_s$ )等光合参数<sup>[13]</sup>,设置光量子通量密度 $1\ 200\ \mu\text{mol}/(\text{m}^2\cdot\text{s})$ ,选取叶片中部测定,对照和处理每株5片叶,3次重复。

脯氨酸含量采用磺基水杨酸提取,酸性茚三酮法测定;可溶性蛋白含量采用BCA法测定<sup>[13]</sup>;可溶性糖含量采用蒽酮比色法测定<sup>[13]</sup>;丙二醛含量采用硫代巴比妥酸法测定<sup>[13]</sup>;脱氢酶采用TTC法<sup>[13]</sup>;乙醇脱氢酶活性测定参考Li等<sup>[15]</sup>的方法。所用试剂均

购自苏州科铭生物技术公司。

## 1.5 数据处理及分析

采用 Microsoft Excel 2016 软件和 Sigmaplot 10.0 软件进行数据处理和图表制作,采用 Photoshop CC 进行图片的整合。运用 SPSS 23.0 采用单因素(one-way ANOVA)和 Duncan's 法进行方差分析和显著性检验( $\alpha=0.05$ ),用 Pearson 法对叶片和根系间生理指标进行相关性分析。图表中数据为“平均值±标准误”。

## 2 结果与分析

### 2.1 淹渍胁迫对枳砧温州蜜柑外观形态和根系解剖结构的影响

春末夏初,大棚内日均温度 $25\ ^\circ\text{C}$ 左右,相对湿度73%左右,温州蜜柑外观形态和根系解剖结构受淹渍胁迫的影响随淹渍时间的延长而加剧。由图2可知,温州蜜柑淹渍2 d根系和地上部无明显变化;淹



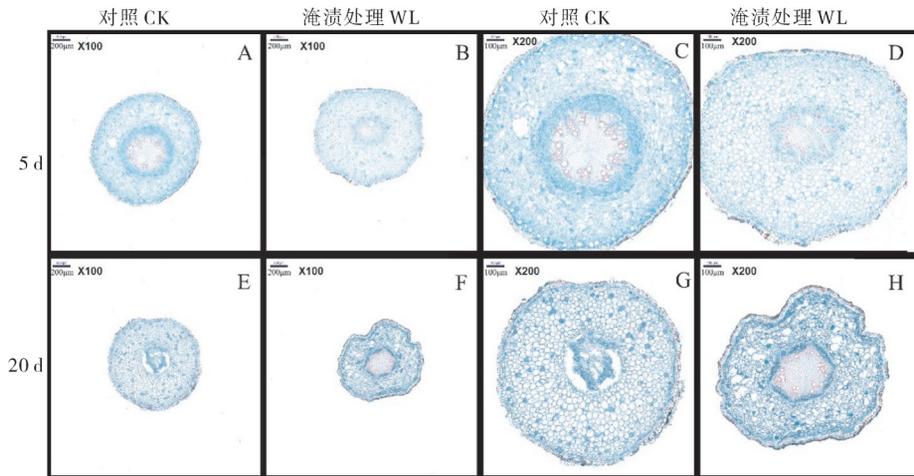
CK:对照;WL:淹渍处理。下同。CK:Control;WL:Waterlogging treatment.The same as follows.

图2 淹渍胁迫后枳砧温州蜜柑地上部(A)和根系(B)形态变化

Fig.2 Morphological changes of the shoots(A) and root(B) of satsuma mandarin with trifoliate orange rootstocks after waterlogging stress

渍5 d根系有轻微异味,地上部变化不明显;淹渍10 d,根系轻微腐烂、变臭,地上部老叶出现不同程度的黄化、褶皱且叶脉变黄褐色,新叶失水、脉间黄化、叶尖焦枯,并伴随轻微落叶;淹渍15 d根系腐烂加剧、部分韧皮部脱落、臭味加重,地上部枝条顶端出现焦枯,大量叶片边缘卷曲、干枯,树体近1/2的叶片

自翼叶处产生离层、脱落;淹渍20 d根系腐烂,韧皮部脱落严重,根系和近基部茎有酒味,整株叶片干枯褐黄,落叶数超过1/2,果实黄褐色形成僵果,部分自行掉落。温州蜜柑淹渍5 d其根系解剖结构排列整齐,与CK相比细胞更加饱满膨胀,淹渍20 d其韧皮部与木质部分离(图3)。



A和C分别代表CK在处理5 d时根系横截面;B和D分别代表淹渍处理5 d时根系横截面;E和G分别代表CK在处理20 d时根系横截面;F和H分别代表淹渍20 d时根系横截面。A and C respectively represents roots cross section of CK 5 d, B and D respectively represents roots cross section of flooded 5 d, E and G respectively represents roots cross section of CK 20 d, F and H respectively represents roots cross section of flooded 20 d.

图3 淹渍胁迫下枳砧温州蜜柑根系解剖结构变化

Fig.3 Changes on root anatomical structure of trifoliate orange grafted with satsuma mandarin after waterlogging stress

## 2.2 淹渍胁迫对枳砧温州蜜柑叶绿素含量和光合性能的影响

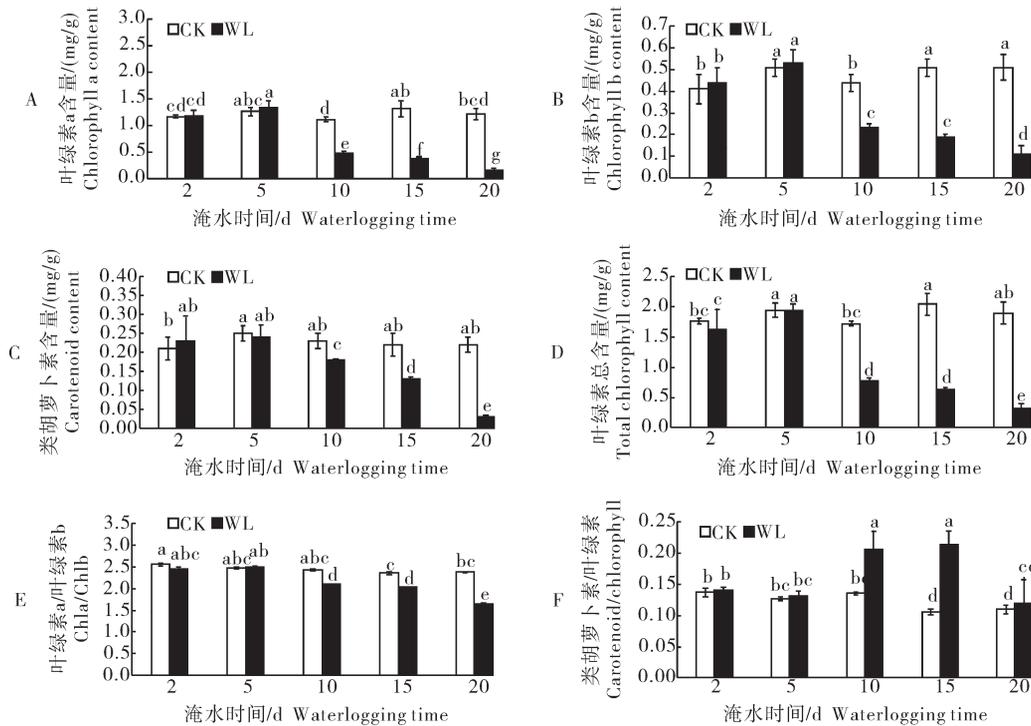
淹渍胁迫导致温州蜜柑叶片中叶绿素及类胡萝卜素的含量显著降低。其中,温州蜜柑淹渍处理叶片中的叶绿素含量从第10天始显著降低,且随胁迫时间的延长持续下降。温州蜜柑叶片中叶绿素a (chlorophyll a, Chl a)、叶绿素b (chlorophyll b, Chl b)和类胡萝卜素(carotenoid, Car)的含量在淹渍胁迫5~10 d变化最为剧烈,特别是淹渍10 d时各色素组分分别较同期CK显著降低57.14%、47.73%、21.74%,且随淹渍时间的延长而持续下降,淹渍20 d降至最低值,此时温州蜜柑叶片中Chl a、Chl b、Car的含量分别较同期CK降低86.07%、78.43%、86.36%,Chl总含量仅是CK的11.97%(图4)。淹渍胁迫对温州蜜柑叶片总叶绿素及各色素含量的影响趋势相同;此外,比较叶绿素a/叶绿素b(Chl a/Chl b)的变化可见,Chl a/Chl b在淹渍胁迫后逐渐降低且Chl a比Chl b下降更快(图4E)。Car/Chl在淹渍胁迫

后显著升高,从而引起淹渍植株叶片的颜色更多地呈现黄色(图2A、图4F)。

由表1可知,温州蜜柑淹渍处理10 d时,光合作用也受到了强烈抑制, $P_n$ 、 $G_s$ 、 $T_r$ 分别较CK显著下降76.01%、68.62%、59.36%, $C_i$ 浓度显著升高。

## 2.3 枳砧温州蜜柑渗透调节物质对淹渍胁迫的响应

温州蜜柑根系中的渗透调节物质从淹渍2 d开始持续下降,叶片中的渗透调节物质在淹渍10 d后升高。淹渍树体根系中的脯氨酸(proline, Pro)含量随淹渍时间的延长呈下降趋势,淹渍20 d时较CK显著下降91.44%(图5A);叶片中的Pro含量显著升高,淹渍10 d时较同期CK上升60.90%,差异显著(图5B)。与同期对照相比,淹渍组根系中的可溶性蛋白(soluble protein, SP)含量自5 d开始显著下降,淹渍10 d时,较CK显著降低79.61%,随后变化趋缓直至试验结束(图5C);而淹渍组叶片SP含量表现为先降低后增加的趋势,在淹渍5 d内较同期CK显著



不同字母表示在 $\alpha=0.05$ 水平差异显著。下同。Different letters indicate significant differences at  $\alpha=0.05$  level. The same as below.

图4 淹渍胁迫下枳砧温州蜜柑叶片叶绿素变化

Fig.4 Changes of chlorophyll content of satsuma mandarin leaves with trifoliolate orange rootstocks after waterlogging stress

表1 淹渍胁迫10 d后枳砧温州蜜柑光合参数变化

Table 1 Changes of photosynthetic parameters of satsuma mandarin leaves with trifoliolate orange rootstocks after 10 d of waterlogging stress

处理 Treatment	净光合速率/ $(\mu\text{mol}/(\text{m}^2\cdot\text{s}))$ Net photosynthetic rate	气孔导度/ $(\text{nmol}/(\text{m}^2\cdot\text{s}))$ Stomatal conductance	胞间 $\text{CO}_2$ 浓度/ $(\mu\text{mol}/\text{mol})$ Intercellular $\text{CO}_2$ concentration	蒸腾速率/ $(\text{mmol}/(\text{m}^2\cdot\text{s}))$ Transpiration rate
CK10 d	$6.67\pm 0.69^*$	$0.110\ 9\pm 0.022\ 4^*$	$268.4\pm 9.49$	$4.38\pm 0.91^*$
WL10 d	$1.60\pm 0.28$	$0.034\ 8\pm 0.008\ 5$	$292.6\pm 14.33^*$	$1.78\pm 0.38$

注:\*表示同一指标处理间在 $\alpha=0.05$ 水平差异显著。Note:\* indicates significant difference at  $\alpha=0.05$  level between treatments.

下降,自淹渍10 d开始显著上升,淹渍20 d较CK增加了1.69倍,差异显著(图5D)。处理组根系中的可溶性糖(souble sugar, SS)含量随淹渍时间延长而呈下降趋势,淹渍5 d和20 d分别较同期CK显著降低44.96%、36.08%(图5E);处理组叶片SS含量至淹渍15 d仍与CK无显著性差异,淹渍20 d才开始大量积累,并较同期CK显著上升75.55%(图5F)。根系中的渗透物质先对对淹水胁迫作出响应,温州蜜柑根系中的渗透物质均在淹渍2~5 d时表现出显著下降的趋势,而叶片中的渗透物质随淹渍时间的延长呈现显著上升趋势。

## 2.4 枳砧温州蜜柑根/叶膜脂过氧化代谢对淹渍胁迫的响应

淹渍逆境下温州蜜柑根/叶的丙二醛(malondialdehyde, MDA)及相对电导率(relative electrical conductivity, EL)均呈现动态变化。温州蜜柑淹渍处理后,叶片MDA含量表现为随处理时间延长呈逐渐增加的趋势(图5H)。自淹渍10 d起,叶片的MDA含量开始显著增加,达到CK的1.66倍;到淹渍15、20 d时,MDA含量分别是CK的2.25、2.53倍,差异显著;根系中的MDA含量表现出下降-上升-下降的变化趋势(图5G)。温州蜜柑叶片的EL淹渍后10 d开始显著升高且随处理时间的延长表现稳定,在淹渍15 d

最高,为同期CK的3.35倍(图5I)。

### 2.5 枳砧温州蜜柑根系无氧呼吸酶系统对淹渍胁迫的响应

淹渍胁迫下,温州蜜柑根系活力明显降低,无氧呼吸酶活性在淹渍早期急剧升高而后随淹渍时间的延长呈下降趋势。脱氢酶(dehydrogenase, DHA)活性是反映根系活力的重要指标。淹渍后温州蜜柑根系的DHA活性总体呈下降趋势,且从淹渍10 d开始显著低于CK,直至淹渍处理结束(图6A)。淹渍胁迫

后温州蜜柑根系的ADH活性呈先升高后降低的趋势。乙醇脱氢酶(alcohol dehydrogenase, ADH)活性在淹渍2、5 d分别是CK的1.54倍、1.21倍,自淹渍10 d开始较CK显著下降78.57%,而后随淹渍时间的增加而持续下降(图6B)。

### 2.6 叶片和根系生理生化指标的相关性

淹渍胁迫后,叶片与根系的抗逆理化指标多数呈极显著负相关。根系Pro和SP与叶片Pro、SP、SS、MDA和EL呈负相关,根系的SS与叶片的Pro、

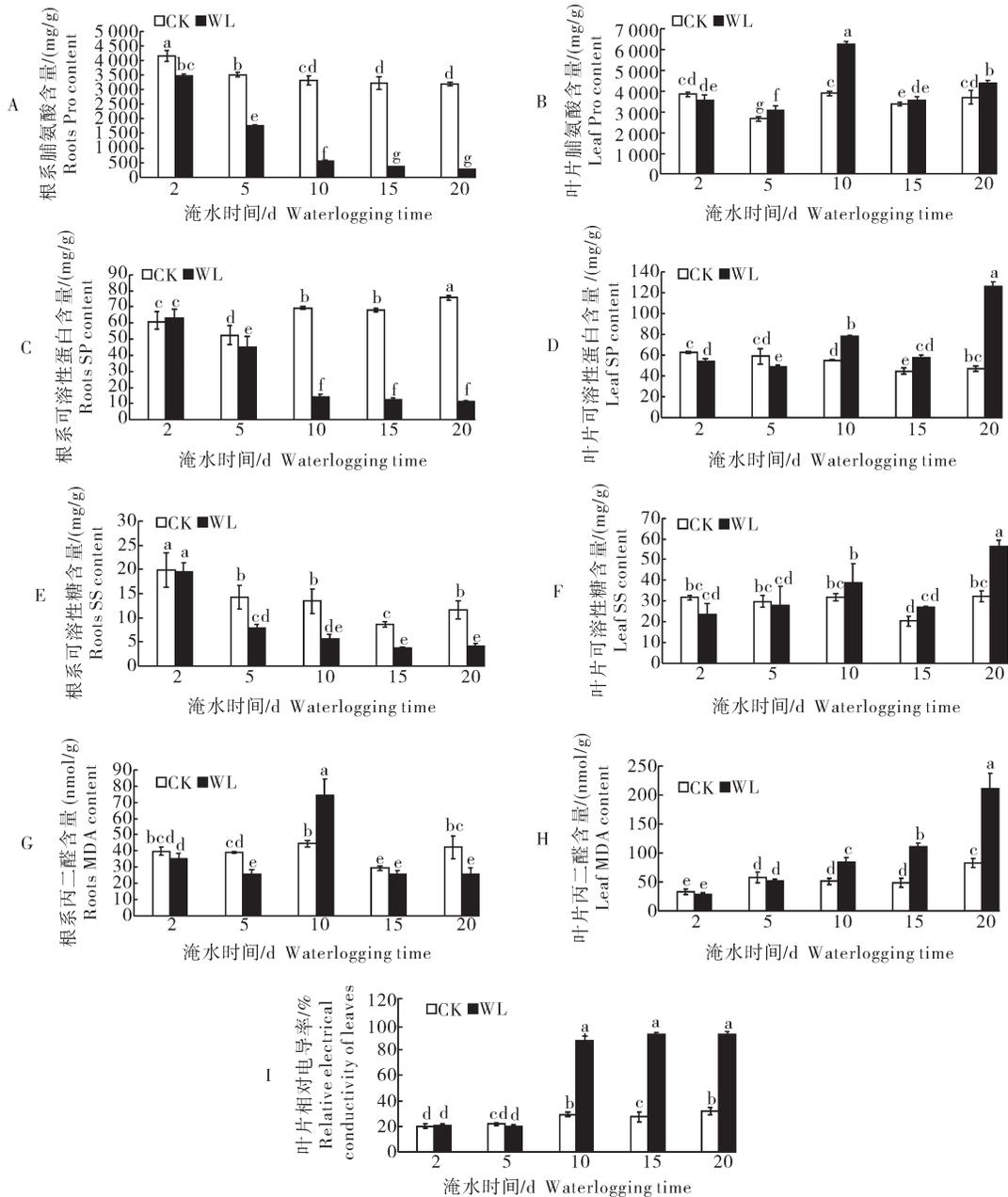


图5 淹渍胁迫下枳砧温州蜜柑根系(A、C、E、G)和叶片(B、D、F、H、I)的渗透调节物质和膜脂过氧化程度变化  
 Fig.5 Changes on osmotic regulation substance and membrane lipid peroxidation in satsuma mandarin with trifoliate orange rootstocks roots(A,C,E,G) and leaves(B,D,F,H,I) after waterlogging stress

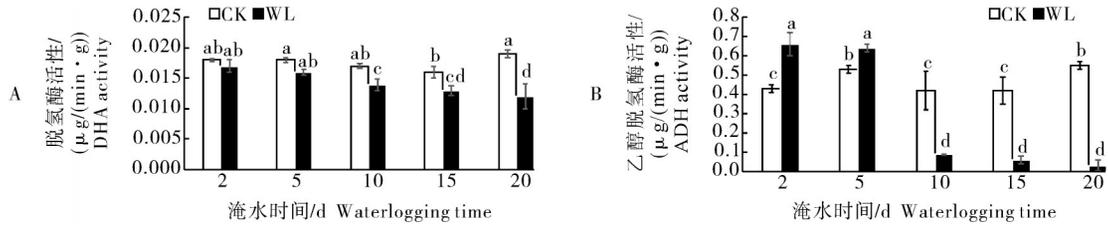


图6 淹渍胁迫下枳砧温州蜜柑根系活力DHA(A)和乙醇脱氢酶ADH(B)活性变化

Fig.6 Changes of trifoliate orange grafted with satsuma mandarin root DHA(A) and anaerobic respiration enzyme activity(B) after waterlogging stress

MDA和EL也呈负相关;根系的MDA和叶片Pro呈极显著正相关( $r=0.669$ )(表2)。

2.7 淹渍胁迫对枳砧温州蜜柑树体矿质营养积累的影响

淹渍胁迫导致枳砧温州蜜柑根系中P、K、Ca、Mg含量减少,茎、叶中的各矿质元素含量无显著变化。根系中P含量在淹渍5d和20d时均显著降低,20d时较CK降低40%(图7D);根系中K含量在淹渍5d时无显著变化,20d时较CK显著降低(图7G);与对照相比,淹渍植株茎、叶中的P、K含量无显著性变化(图7E、F、H、I)。根系中的Ca、Mg含量在淹渍20d时均显著下降,分别较CK下降35.84%和65.7%;淹渍处理下茎、叶中的Ca、Mg含量无显著变化(图8)。温州蜜柑淹渍后,根、茎、叶中的N、Fe、Zn

表2 淹渍胁迫下枳砧温州蜜柑叶片和根系生理生化指标的相关系数

Table 2 Correlation coefficients of the physiological and biochemical indexes between leaves and roots of satsuma mandarin with trifoliate orange rootstocks under waterlogging stress

项目 Item	Pro	SP	SS	MDA	EL
Pro	-0.506**	-0.580**	-0.507**	-0.726**	-0.903**
SP	-0.530**	-0.657**	-0.524**	-0.663**	-0.914**
SS	-0.347**	-0.359	-0.357	-0.647**	-0.741**
MDA	0.669**	0.071	0.113	-0.149	0.184

注:\*\*表示相关系数达到极显著水平(0.01)。Note:\*\* represent significantly different 0.01 level.

含量波动较小(图7、图8)。

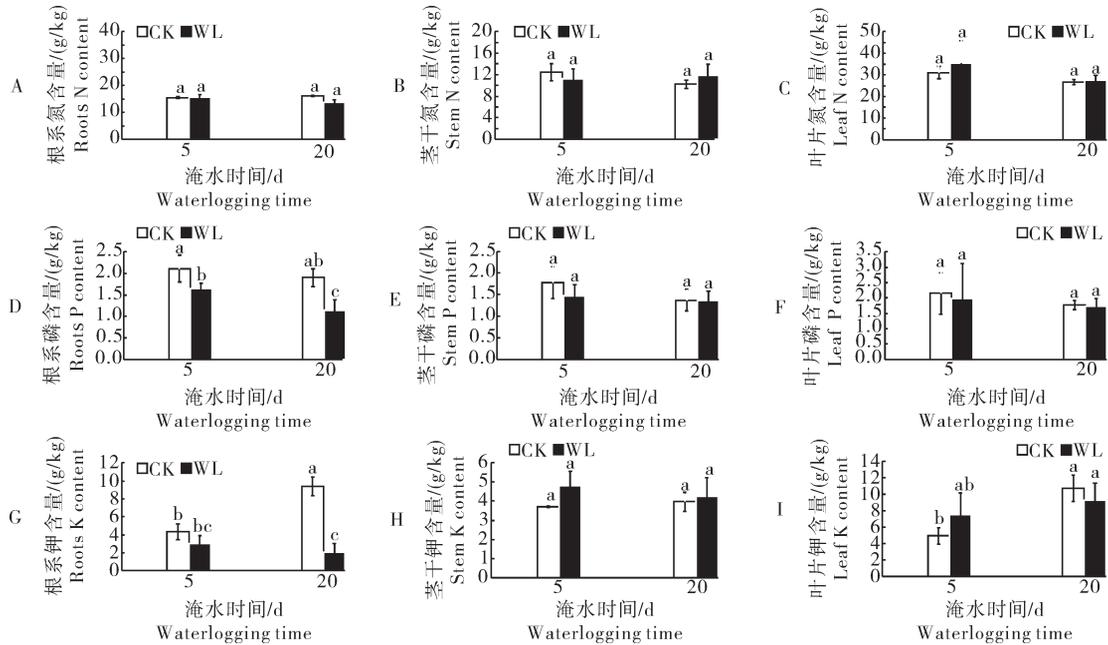


图7 淹渍胁迫对枳砧温州蜜柑树体内N(A、B、C)、P(D、E、F)、K(G、H、I)含量的影响

Fig.7 Effects of flooding stress on contents of N(A,B,C),P(D,E,F) and K(G,H,I) in satsuma mandarin trees with trifoliate orange rootstocks

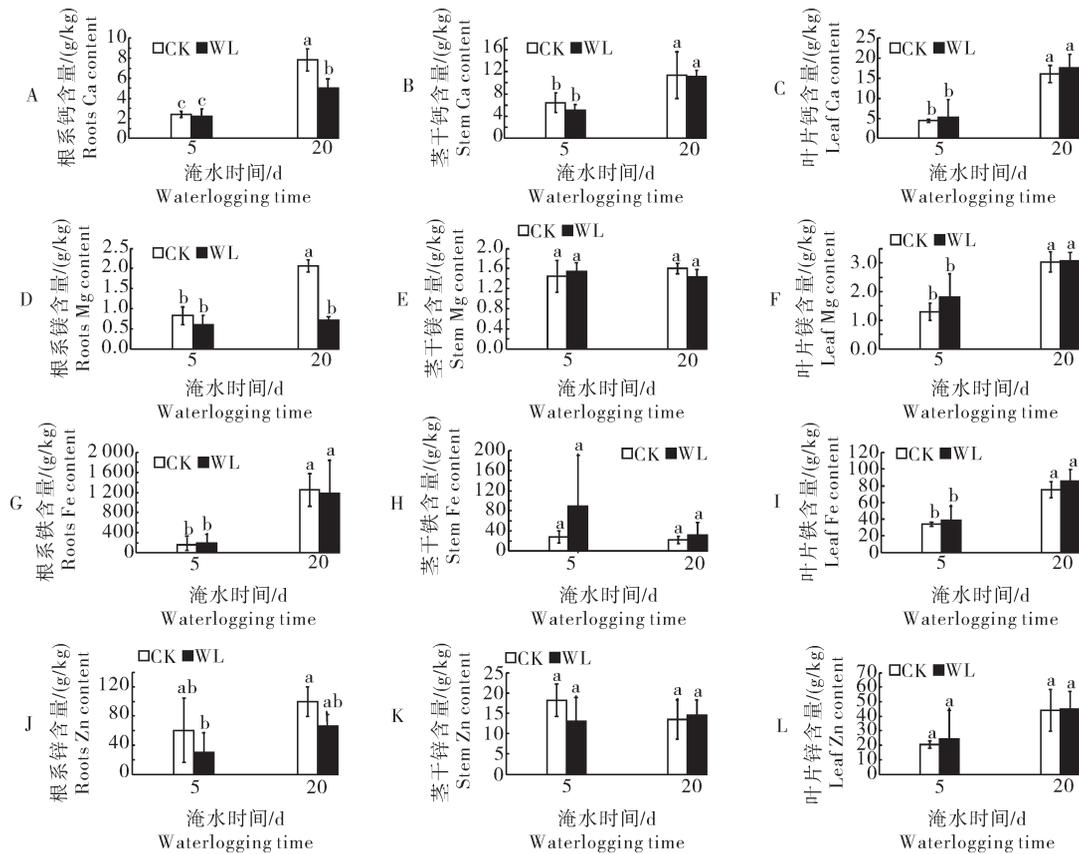


图8 淹渍胁迫对枳砧温州蜜柑树体Ca(A、B、C)、Mg(D、E、F)、Fe(G、H、I)、Zn(J、K、L)含量的影响  
Fig.8 Effects of flooding stress on contents of Ca(A,B,C),Mg(D,E,F),Fe(G,H,I) and Zn(J,K,L) in satsuma mandarin trees with trifoliate orange rootstocks

### 3 讨论

#### 3.1 淹渍胁迫下枳砧温州蜜柑表型特征和叶片光合指标的变化

淹渍条件下植物气体扩散受阻、叶绿素降解、光合速率下降、产生无氧呼吸,干扰植物正常的生理代谢及生长发育,为了适应淹渍条件,保障正常的生理代谢功能,植株地上部和地下部形态通常会发生改变<sup>[16-17]</sup>。前人的研究表明,柑橘砧木及脐橙幼苗的叶片在淹渍胁迫时先是叶脉失绿黄化而脉间保持绿色,之后逐步出现黄化、萎蔫等症<sup>[4,6]</sup>;其中,红橘和枸头橙幼苗分别在淹渍处理的第13和14天开始出现萎蔫症<sup>[5]</sup>,枳砧‘大分四号’温州蜜柑幼树在淹渍4d开始出现可见症<sup>[18]</sup>,而枳砧脐橙幼苗在淹渍40d才出现淹水胁迫症<sup>[6]</sup>。本研究中枳砧‘市文’温州蜜柑在淹渍处理5d时叶片无明显可见症状,淹渍10d时出现不同程度的失绿黄化、萎蔫及轻微落叶等症,推测不同柑橘类型淹渍症状出现的时间可能与其耐涝性强弱有关<sup>[4-6]</sup>。枳砧脐橙幼苗在淹渍

胁迫15d时叶片外观及微观结构均无明显变化,但在淹渍10d时,根皮层细胞连接松散,并出现了便于输送氧气和运输根系中积累的乙醇、二氧化碳等物质的裂生性气腔<sup>[6]</sup>。本研究中,枳砧温州蜜柑根系也早于叶片(10d)于淹渍处理5d时表现出异常,且多数生理指标此时也发生了显著变化,推测根系是受淹渍逆境直接伤害的器官,淹水后植株根系先发生了一系列形态结构变化和生理生化方面的适应性调节,以减缓伤害。

已有研究表明,水分胁迫下,果树叶片的叶绿素降解加剧<sup>[19]</sup>,叶片衰老,光合性能降低<sup>[20]</sup>。叶绿素是植物在光合作用中吸收、传递和转换光能的载体<sup>[21]</sup>,类胡萝卜素不仅能辅助植物的光吸收,还能作为抗氧化物清除光呼吸中产生的自由基<sup>[22]</sup>。本研究中,叶绿素含量的显著下降表明淹渍胁迫降低了枳砧温州蜜柑叶片对光能的同化能力,这与黄翼<sup>[5]</sup>在枳砧脐橙幼苗、刘超颖等<sup>[6]</sup>在枳砧脐橙幼苗上的研究结果一致。逆境胁迫条件下叶片净光合速率降低的原因可分为气孔和非气孔限制因素<sup>[5]</sup>。本研究中,淹渍胁迫

下枳砧温州蜜柑叶片的净光合速率、气孔导度和蒸腾速率与同期对照相比显著降低,胞间CO<sub>2</sub>浓度显著升高,这与钟娟等<sup>[23]</sup>在金桔上的研究结果一致且符合Farquhar等<sup>[24]</sup>提出的光合作用的非气孔限制判断标准。表明淹渍胁迫抑制了温州蜜柑的光合特性,而非气孔限制因素是导致本研究中的砧木温州蜜柑光合作用受抑制的主要因素。

### 3.2 枳砧温州蜜柑渗透调节物质和膜脂过氧化代谢对淹渍胁迫的响应

植物在淹渍逆境中启动的细胞膜脂过氧化系统可导致生物膜严重损伤<sup>[25]</sup>,为适应淹渍逆境,植物会通过调节相应的代谢机制从而缓解伤害,渗透调节就是其中非常重要的调节机制之一<sup>[26]</sup>。前人的研究表明,植物在逆境条件下会通过调节体内的有机渗透物质如脯氨酸、可溶性糖和可溶性蛋白等以维持细胞的渗透势和水分平衡<sup>[27-28]</sup>。根系生理是评价抗逆性能的有效指标<sup>[29]</sup>,脐橙<sup>[6,8]</sup>淹渍胁迫后根系SP和SS含量在淹渍早期就出现了变化。本研究结果表明,温州蜜柑叶片和根系的膜脂过氧化程度、渗透调节物质等多数植物逆境响应指标在叶片和根系中呈显著负相关的趋势,表明温州蜜柑地上、地下部响应淹渍胁迫的模式不同;此外,多数逆境响应指标在根系淹渍胁迫5 d后即显著低于对照,而叶片胁迫10 d后显著高于对照,淹渍胁迫持续5 d左右枳砧温州蜜柑根系即发生伤害,但此时地上部分未表现出异常,而进一步的淹渍胁迫导致地上部呈现明显症状时,树体的淹渍伤害已加重。上述结果表明,温州蜜柑叶片和根系组织对淹渍胁迫的响应机制不一致。淹渍逆境造成柑橘叶片和根系组织的形态、生理和代谢发生不同程度的变化,柑橘根系先受到胁迫,通过改变生长形态以期在短期内适应水淹环境。

### 3.3 枳砧温州蜜柑根系无氧呼吸酶系统对淹渍胁迫的响应

根系是植物从土壤中吸收、运输水分及养分的重要器官,其活力的高低是衡量植物抗逆性的重要指标之一。随着淹水胁迫时间的延长,猕猴桃的根系活力逐渐下降,其下降程度受基因型的影响<sup>[29]</sup>。桃<sup>[30]</sup>、火龙果<sup>[31]</sup>在淹渍胁迫后根系活力均下降,本研究中的枳砧温州蜜柑在淹渍胁迫下根系的DHA活性随着淹渍时间的延长也呈显著下降趋势。灌溉、雨水过多或洪水等易造成植物根系缺氧,而低氧可诱导植物产生多种参与无氧呼吸的酶,其中ADH

酶是无氧呼吸时糖酵解途径最后一步的关键酶<sup>[32]</sup>,可将乙醛转化为乙醇,以降低对细胞自身的毒害作用,并维持能量平衡<sup>[7]</sup>。耐涝性强的中山杉<sup>[33]</sup>在长期水淹条件下其根系的ADH酶活性一直保持较高水平,而本研究中砧木温州蜜柑根系的ADH酶活性在淹渍胁迫下呈先升后降趋势,这与其他经济类果树如甜樱桃<sup>[32]</sup>、桃<sup>[34]</sup>的研究结果一致。砧木温州蜜柑根系的ADH酶活性在淹渍2~5 d显著升高,表明根系已开始响应淹渍缺氧逆境,在淹渍10 d后,伴随着ADH酶活性的迅速下降至较低水平,DHA活性也显著下降,表明根系活力已经显著减弱,长期持续淹渍胁迫可能对树体已经造成了不可逆的伤害。

ADH活性及叶片EL作为耐涝性的重要生理指标,已广泛应用于观赏植物<sup>[35]</sup>、无花果<sup>[36]</sup>和乌桕<sup>[37]</sup>的耐涝性鉴定评价,本研究中的枳砧温州蜜柑根系ADH活性在胁迫2 d时显著升高,叶片EL在淹渍10 d显著升高,其变化与淹渍胁迫后对应组织中多数抗逆指标和树体表观症状变化趋势密切相关,推测根系ADH活性和叶片EL可作为早期判断枳砧温州蜜柑根系和叶片受到淹渍伤害程度的参考指标。

### 3.4 淹渍胁迫对枳砧温州蜜柑树体矿质营养积累的影响

植物主要依靠根系吸收矿质元素,淹渍胁迫直接对根系造成伤害,减少根系对矿质元素的吸收量。研究表明,玉米<sup>[38]</sup>、棉花<sup>[39]</sup>在淹渍胁迫后N、P、K含量显著降低。柑橘在淹渍胁迫后根系对养分的吸收和转运受到影响,淹渍胁迫会导致卡列里佐枝橙幼苗中的总N含量和浓度逐渐降低,碳水化合物如淀粉和蔗糖从叶片向根系的转运显著减少,造成C/N失调<sup>[7]</sup>。也有研究表明,长期淹水会影响柑橘幼苗对Fe的吸收和分布<sup>[10]</sup>。本研究中淹渍胁迫下枳砧温州蜜柑根系中的N含量变化不显著,但P、K、Ca、Mg含量显著降低,可能与不同柑橘类型对矿质营养的吸收存在差异有关<sup>[40]</sup>。温州蜜柑茎、叶中的矿质养分含量均未受淹渍胁迫的显著影响,淹渍胁迫下柑橘不同组织器官中矿质营养的积累机制可能存在差异,尚需进一步验证。

本研究结果表明:枳砧温州蜜柑的地上部和地下部组织响应淹渍胁迫的敏感程度及响应存在显著差异。根系是直接受到淹渍胁迫伤害的器官,较叶片更早响应淹渍胁迫,根系ADH活性变化与根系受

伤害程度紧密相关,叶片EL与受淹渍胁迫程度密切相关。

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## Morphological characteristics and physiological responses of satsuma mandarin (*Citrus unshiu* Marc.) with trifoliolate orange rootstock (*Poncirus trifoliolate* (L.) Raf.) under waterlogging stress

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**Abstract** The potted adult satsuma mandarin trees were treated under waterlogging stress for 2, 5, 10, 15, and 20 d and normal irrigation was used as the control to observe the changes of its morphological and root anatomical characteristics to study the changes of morphological characteristics and physiological response mechanism of satsuma mandarin (*Citrus unshiu* Marc. 'Ichifumi Wase') under waterlogging stress. The photosynthetic parameters of leaves, the osmotic adjustment substances of leaves and roots, the activity of anaerobic respiratory enzymes, the content of malondialdehyde and the mineral nutrients of tree were determined. The results showed that the root system began to have an odor and the phloem cells were full and swollen after 5 days of waterlogging. The new and old leaves had appeared different degrees of yellowing, and the root system began to rot and discolored after 10 days of waterlogging. The tree body withered and died after being submerged for about 20 days. Compared with the control, the activity of alcohol dehydrogenase in the root system was significantly increased at the early stage (within 5 days) of waterlogged stress. The content of free proline, soluble sugar and soluble protein was significantly decreased and maintained at low levels, and the content of malondialdehyde was significantly higher than that of the control only after 10 days of waterlogging. Under the same treatment conditions, the content of chlorophyll in leaves continued to decrease with the waterlogging time. The net photosynthetic rate, stomatal conductance, and transpiration rate of leaves decreased significantly after 10 days of waterlogging, and the concentration of intercellular CO<sub>2</sub> was significantly increased. The content of free proline, soluble protein, malondialdehyde and the relative electrical conductivity in leaves under waterlogging was significantly higher than that of the control at the same period for 10-20 d after waterlogging. Under waterlogging stress for 5 and 20 days, the accumulation of most mineral nutrients in stems and leaves did not change, and the content of P, K, Ca and Mg in root decreased significantly compared with the CK. It is indicated that there are significant differences in the physiological responses of different tissues and organs to waterlogged stress in satsuma aurantium grafted in trifoliolate orange rootstock, and the relative electrical conductivity of leaves and the alcohol dehydrogenase in root are sensitive to waterlogged stress. Long-term waterlogging stress affects the photosynthetic performance, the content of osmotic regulation substance and the activity of alcohol dehydrogenase, causing obvious stress symptoms in leaves and roots, resulting in the slow growth and even death of plants.

**Keywords** satsuma mandarin (*Citrus unshiu* Marc.); waterlogging stress; osmotic regulation substances; plant morphology; physiological characteristics

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