‘波叶金桂’对干旱和高温胁迫的生理生态响应

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摘要 为了探讨植物对干旱、高温及协同胁迫的响应, 该研究以木犀(*Osmanthus fragrans*) ‘波叶金桂’为材料, 采用盆栽质量控水法模拟干旱胁迫(对照、轻度、中度和重度)和高温胁迫, 利用动态顶空气体循环吸附法和热脱附-气相色谱-质谱(TDS-GC-MS)联用技术对其挥发性有机化合物(VOCs)进行测定; 同时测定其非结构性碳(NSC)含量及次生代谢酶活性。结果表明: 干旱胁迫对‘波叶金桂’叶片NSC组分含量影响不显著; 可溶性糖和淀粉含量在高温胁迫下显著降低, 在协同胁迫后持续性下降, 重度协同胁迫下, 葡萄糖、果糖、蔗糖和淀粉分别比对照降低47.7%、46.4%、34.4%和38.2%。干旱胁迫和协同胁迫下3-羟基-3-甲基戊二酸单酰辅酶A还原酶(HMGR)、1-脱氧木酮糖-5-磷酸还原酶(DXR)活性表现出先上升后下降, 而脂氧合酶(LOX)活性表现出持续性上升趋势; 高温胁迫后, HMGR、DXR和LOX活性显著高于对照。干旱胁迫下萜烯类VOCs释放量表现出先上升后下降趋势, 中度干旱胁迫和高温胁迫下分别比对照高37.9%和32.3%; 协同胁迫下萜烯类释放量逐渐降低, 干旱、高温和协同胁迫诱导醛类释放量明显增加。上述结果表明: 干旱胁迫条件下, ‘波叶金桂’通过NSC进行自我渗透调节, 同时合成大量萜烯类化合物来提高抗旱性; ‘波叶金桂’调控萜烯类化合物合成以及绿叶挥发物(GLVs)的释放抵御高温胁迫; 协同胁迫下萜烯类化合物的合成途径受阻, ‘波叶金桂’提高GLVs合成与释放量抵御协同胁迫; 中度和重度协同胁迫导致‘波叶金桂’细胞膜严重受损, 自我调节能力降低。

关键词 木犀; 干旱高温胁迫; 非结构性碳; 挥发性有机化合物; 次生代谢酶

汪俊宇, 王小东, 马元丹, 傅卢成, 周欢欢, 王彬, 张汝民, 高岩 (2018). ‘波叶金桂’对干旱和高温胁迫的生理生态响应. 植物生态学报, 42, 681–691. DOI: 10.17521/cjpe.2018.0017

Physiological and ecological responses to drought and heat stresses in *Osmanthus fragrans* ‘Boyejingui’

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Abstract

Aims Drought and heat stresses are two critical environmental factors affecting the growth and development of plants; climate change has exacerbated the occurrence of these conditions in many parts of the world. To elucidate the mechanisms of responses to drought and heat stresses in *Osmanthus fragrans*, we studied changes in non-structural carbohydrate (NSC) and volatile organic compounds (VOCs) emissions using 3-year-old seedlings (cultivar ‘Boyejingui’) under conditions of low drought (LD), moderate drought (MD) and severe drought (SD), interactively with heat treatments (40 °C in daytime (12 h) and 30 °C at night (12 h)).

Methods The VOCs emissions were measured using the dynamic headspace air-circulation method, and the composition and content of VOCs were analyzed using the thermal desorption system/gas chromatography/mass spectrometer technique (TDS-GC-MS). We determined the content of NSC and activities of lipooxygenase (LOX), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) in seedlings under different drought and heat treatments.

Important findings The content of NSC in leaves was not significantly affected by drought stress. The contents of soluble sugar and starch were significantly reduced by heat stress as well as combination of drought and heat stresses. The combination of severe drought and heat treatment significantly reduced the contents of glucose, fructose, sucrose and starch by 47.7%, 46.4%, 34.4% and 38.2% ($p < 0.05$), respectively. There were differences

收稿日期Received: 2018-01-14 接受日期Accepted: 2018-05-12

基金项目: 国家自然科学基金(31470704和31270756)。Supported by the National Natural Science Foundation of China (31470704 and 31270756).

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among the activities of HMGR, DXR and LOX in response to drought and heat stresses. Under drought and combined stresses, the activities of HMGR and DXR increased first and then decreased, and the LOX activity increased. The activities of HMGR, DXR and LOX were significantly higher under heat stress than in controls. The release of terpenes increased first and then decreased with the level of drought treatments. MD and SD stress stimulated the release of terpenes by 37.9% and 32.3% ($p < 0.05$), respectively, but the combined stresses induced a reduction in the release of terpenes. The release of aldehydes increased significantly in response to drought, heat treatment, and their combination. The results indicated that ‘Boyejingui’ seedlings can enhance resistance to drought stress by adjusting contents of non-structural carbohydrate and releasing terpene compounds. They also adjusted the biosynthesis of terpene compounds and GLVs emission to resist against heat stress. However, the biosynthesis pathway of terpene compounds was blocked in the occurrence of both drought and heat stresses, when the enhanced release of GLVs facilitated the resistance. Under heat stress, moderate and severe drought caused severe damage to cell membranes, thus reducing the self-regulatory capacity.

Key words *Osmanthus fragrans*; drought and heat stress; non-structural carbohydrate; volatile organic compounds; secondary metabolic enzymes

Wang JY, Wang XD, Ma YD, Fu LC, Zhou HH, Wang B, Zhang RM, Gao Y (2018). Physiological and ecological responses to drought and heat stresses in *Osmanthus fragrans* ‘Boyejingui’. *Chinese Journal of Plant Ecology*, 42, 681–691. DOI: 10.17521/cjpe.2018.0017

非结构性碳水化合物(NSC)作为植物体内的可溶性碳库, 不仅为植物生长发育提供碳源与能源, 还能调控植物生理活动(Hoch *et al.*, 2003); 同时, 植物体内NSC的积累是一种重要的渗透调节保护机制(Lin *et al.*, 2014; Murcia *et al.*, 2015)。干旱胁迫导致欧洲云杉(*Picea abies*)树根NSC含量减少, 在地上和地下组织之间的碳分配、使用和运输方面有显著差异(Hartmann *et al.*, 2013), 随干旱胁迫时间的延长, 刺槐(*Robinia pseudoacacia*)叶片NSC含量降低(Zhang *et al.*, 2015)。Marias等(2017)研究发现热处理能够诱导西黄松(*Pinus ponderosa*)和花旗松(*Pseudotsuga menziesii*)淀粉含量减少, 果糖和葡萄糖含量增加, 总NSC和蔗糖含量并没有受到影响。随着高温干旱协同胁迫加剧, 新疆梨(*Pyrus sinkiangensis*)可溶性糖含量呈上升趋势, 淀粉含量逐渐降低(穆蓁蓁, 2015)。受到环境胁迫时, 植物初级代谢发生紊乱, 可溶性糖的积累受到严重影响。蔗糖裂解为果糖和葡萄糖, 不仅作为代谢物质和能量来源, 还充当信号分子, 通过信号转导途径识别, 协调合成代谢和分解代谢过程, 促进植物生长发育(Li & Sheen, 2016)。

植物释放的挥发性有机物(VOCs)能够维持类囊体膜的完整性, 起到抗氧化作用(Velikova *et al.*, 2011; Arab *et al.*, 2016); 在抵御逆境胁迫方面发挥重要的信号作用(Loreto & Schnitzler, 2010; Bourtsoukidis *et al.*, 2014)。Arab等(2016)研究海枣(*Phoenix dactylifera*)发现, 高温诱导使异戊二烯排放大幅增加; 同时, 在干旱胁迫下脂肪酸增加。Staudt等(2017)

发现温度升高能够提高*Quercus ilex*和*Quercus pubescens*类异戊二烯的排放量; 干旱胁迫提高单萜排放量, 同时减少类异戊二烯的排放。高温胁迫后花旗松倍半萜、水杨酸甲酯、芳樟醇比单萜排放释放量增加更快(Joó *et al.*, 2011)。刘芳等(2013)研究发现, 迷迭香(*Rosmarinus officinalis*) VOCs中萜类和醛类化合物受到干旱胁迫诱导表现出显著增加。VOCs释放有助于诱导植物非生物应激性抵抗, 增强植物抵御非生物胁迫能力(周帅等, 2012)。

植物VOCs释放受到相关酶的调控。甲羟戊酸和甘油醛磷酸/丙酮酸两条途径是合成萜烯类化合物的主要途径(Dudareva *et al.*, 2013), 3-羟基-3-甲基戊二酸单酰辅酶A还原酶(3-hydroxy-3-methylglutaryl CoA reductase, HMGR)和1-脱氧木酮糖-5-磷酸还原酶(1-deoxy-D-xylulose 5-phosphate reductoisomerase, DXR)分别是两个途径的关键限速酶(Ramak *et al.*, 2013), 直接调控萜烯类化合物的生物合成(Moses *et al.*, 2013)。植物释放绿叶挥发物(GLVs)是由环境胁迫诱发的一种防御反应, 植物通过调控其中关键酶——脂氧合酶(lipoxygenase, LOX)——催化不饱和脂肪酸形成GLVs (Hassan *et al.*, 2015)。因而HMGR、DXR和LOX活性的提高诱导VOCs的形成是植物增强抗胁迫能力的重要前提。

木犀(*Osmanthus fragrans*)为木犀科常绿乔木或灌木, 中国十大名花之一, 树形优美, 芳香袭人, 是重要的园林芳香植物。木犀品种资源丰富, ‘波叶金桂’作为金桂种群中观赏价值很高的优异品种, 具有很高的研究价值。在全球气候变暖的大环境背

景下, 杭州夏季持续高温干旱, 导致大量植物受到伤害, 成为制约当地农林业可持续发展的关键限制因子(陈柯辰, 2013; Hijioka *et al.*, 2014)。为了研究植物非结构性碳、VOCs及相关酶代谢对干旱高温交互作用的响应, 本研究以‘波叶金桂’为对象, 通过控制水分和温度模拟干旱高温胁迫对非结构性碳含量、VOCs组分以及次生代谢酶活性的影响, 揭示‘波叶金桂’在防御干旱高温胁迫反应中可溶性碳库的变化和VOCs含量与次生代谢酶活性的生理响应调节机制, 以及‘波叶金桂’在抵抗干旱、高温及协同胁迫方面的应对对策; 同时为植物多样性保护、园林植物的合理利用以及可持续发展提供参考。

1 材料和方法

1.1 供试材料

试验材料为三年生‘波叶金桂’(浙江杭州画境种业有限公司提供)。2016年4月选取长势良好的苗木栽植于盛有培养土(泥炭:沙土:蛭石体积比1:2:1, 干质量1.5 kg)的花盆中(直径25 cm, 高30 cm), 苗高40 cm左右, 每盆1株, 共计40盆。苗木培植于温室(昼/夜30 °C/25 °C, 相对湿度40%)进行正常的栽培管理。充分缓苗1个月, 再实施控水胁迫。

1.2 试验处理

选取盆栽苗40盆, 随机分为8组, 其中4组用作干旱处理, 剩余4组干旱处理后再进行高温处理。

干旱胁迫: 试验根据土壤含水量设置对照(CK, 75%田间持水量(FC))、轻度干旱(LD, 60%FC)、中度干旱(MD, 40%FC)、重度干旱(SD, 20%FC) 4个水分梯度。采用称质量法控制土壤水分含量, 每天18:00称质量并补充消耗的水分, 维持土壤含水量。每个处理5盆, 每盆作为1个独立重复实验, 每个梯度土壤含水量达标后维持处理3天, 第4天10:00测定各项指标。

高温胁迫: 将对照组和每个干旱梯度胁迫后的苗木, 放入人工气候箱(昼/夜40 °C/28 °C, 12 h/12 h, 相对湿度40%, 光照强度400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)进行高温胁迫处理2天。将对照组进行高温胁迫作为高温组, 其他作为协同组。每个处理5盆, 每盆作为1个独立重复实验。

1.3 试验方法

可溶性糖含量测定: 称取0.5 g植物叶片, 研磨至匀浆, 加蒸馏水9 mL, 沸水浴提取10 min, 不断搅

拌。冷却, 离心(3000 $\text{r}\cdot\text{min}^{-1}$, 5 min)后取上清液。蔗糖、果糖和葡萄糖含量分别采用果糖和蔗糖试剂盒(南京建成科技有限公司)、葡萄糖试剂盒(上海荣盛生物药业有限公司)测定, 具体方法按说明书操作。

淀粉含量测定: 将上述提取可溶性糖后的沉淀用80%乙醇冲洗1遍, 加蒸馏水3 mL, 搅拌均匀, 沸水浴15 min。冷却后, 加入冷的高氯酸(9.2 $\text{mmol}\cdot\text{L}^{-1}$) 4 mL, 搅拌提取20 min, 蒸馏水定容至25 mL, 混匀, 离心(3 000 $\text{r}\cdot\text{min}^{-1}$, 10 min)。取上清液, 采用蒽酮硫酸法进行测定。

HMGR和DXR酶液提取: 称取0.5 g冰冻叶片, 加5 mL Tris-HCl缓冲液(50 $\text{mmol}\cdot\text{L}^{-1}$, pH值7.5), 冰浴条件下研磨至匀浆, 10 000 $\text{r}\cdot\text{min}^{-1}$ 离心30 min (4 °C), 取上清液, 用于HMGR和DXR活性的测定以及蛋白质含量测定。

HMGR活性测定: 测定方法参照Toroser和Huber (1998)的方法并略作修改。反应总体积为1 mL。取980 μL Tris-HCl缓冲液(150 $\text{mmol}\cdot\text{L}^{-1}$, pH值7.5), 加入20 μL 酶液。立即计时, 用紫外分光光度计在340 nm处检测还原型辅酶II (NADPH)的氧化情况, 每隔10 s检测一次, 连续测定5 min, 测得无底物 β -羟[基]- β -甲戊二酸单酰辅酶A (HMG-CoA)的情况下NADPH的氧化速率(*a*)。反应体系同上, 取940 μL 反应液, 加20 μL 酶液, 再加40 μL HMG-CoA母液。操作步骤同上, 测得有HMG-CoA情况下的NADPH的氧化速率(*b*)。HMGR活力单位: 一个活力单位为1 min氧化1 nmol NADPH所需酶量(1 U)。

DXR活性测定: 参照Hasunuma等(2008)的方法并略作修改。反应总体积1 mL。取980 μL Tris-HCl缓冲液(150 $\text{mmol}\cdot\text{L}^{-1}$, pH值7.5), 加入20 μL 酶液。立即计时, 用紫外分光光度计在340 nm处检测NADPH的氧化情况, 每隔10 s检测一次, 连续测定5 min, 测得无底物DXP的情况下NADPH的氧化速率(*a*)。反应体系同上, 取955 μL 反应液, 加20 μL 酶液, 再加25 μL DXP母液。操作步骤同上, 测得有DXP情况下的NADPH的氧化速率(*b*)。DXR活力单位: 一个活力单位为1 min氧化1 nmol NADPH所需酶量(1 U)。

蛋白质含量测定: 参照Bradford (1976)的方法进行测定。

LOX酶液提取: 称取0.5 g叶片剪碎, 用9 mL

Tris-HCl缓冲液(100 mmol·L⁻¹, pH值7.8)冰浴研磨至匀浆, 10000 r·min⁻¹离心10 min (4 °C), 得到上清液。

LOX活性的测定参照Hatanaka (1993)的方法并做修改。3.25 mL反应体系中含有3.0 mL醋酸缓冲液(100 mmol·L⁻¹, pH值5.0)、0.2 mL底物和0.05 mL酶液, 测定在3 min内234 nm波长处吸光度的变化。LOX活力单位: 234 nm波长处, 以亚油酸为底物, 1 min反应体系吸光度增加0.1为1个活力单位(1 U)。

1.4 VOCs采集及分析方法

在9:00–11:00, 采用动态顶空气体循环采集法采集‘波叶金桂’VOCs, 气体流量设置为0.1 m³·min⁻¹, 采样30 min。VOCs成分分析采用TDS-GC-MS法。仪器正常工作条件参数设置参照Gao等(2005)的方法。TDS (TD3, GERSTEL, Mulheim, Germany)工作条件: 载气压力20 kPa; 进样口温度250 °C; 热脱附温度250 °C, 保持10 min; 冷阱温度-100 °C, 保持3 min; 冷阱进样时温度骤然升至260 °C。GC (7890A, Agilent, Delaware, USA)工作条件: 色谱柱为30 m × 250 μm × 0.25 μm的HP-5MS柱; 升温程序: 初始温度40 °C, 保持4 min后, 以6 °C·min⁻¹速率升至250 °C, 保持3 min后, 以10 °C·min⁻¹的速率升至270 °C, 保持5 min。MS (5975C, Agilent, Delaware, USA)工作条件: 电离方式为EI; 电子能量为70 eV, 原子质量范围: 28–450; 接口温度280 °C; 离子源温度为230 °C; 四级杆温度150 °C。

1.5 数据分析

所有数据均为5次重复的平均值±标准误差。利用Origin 9.0软件进行数据处理、图表制作和统计分析, 统计方法采用单因素方差分析, 进行Turkey多重比较($p < 0.05$)。采用双因素方差分析法分析高温×干旱处理的交互作用。通过TDS-GC-MS技术分析, 获得VOCs总离子流色谱图, 采用NIST2008谱库并进行定性分析组分, 以色谱峰的峰面积表示成分的相对含量。

2 结果和分析

2.1 干旱、高温及协同胁迫对葡萄糖含量的影响

图1显示了干旱、高温及协同胁迫对‘波叶金桂’叶片葡萄糖含量的影响。干旱胁迫下葡萄糖含量呈现持续上升趋势, 在重度干旱胁迫下达到最高值, 比对照增加了13.8% ($p < 0.05$); 高温胁迫下葡萄糖含量减少, 比对照降低了15.5% ($p < 0.05$); 协同胁迫

图1 干旱高温处理对波叶‘波叶金桂’叶片葡萄糖含量的影响(平均值±标准误差, $n = 5$)。不同大写字母表示干旱处理的差异显著, 不同小写字母表示高温处理的差异显著($p < 0.05$, 根据LSD测验)。**, $p < 0.01$ 。CK, 对照; LD, 轻度干旱; MD, 中度干旱; SD, 重度干旱。

Fig. 1 Effects of drought and heat stress on glucose content in leaves of *Osmanthus fragrans* cv. ‘Boyejingui’ (mean ± SE, $n = 5$). Different uppercase letters indicate significant differences of drought and different lowercase letters denote statistically significant differences of heat stress treatment ($p < 0.05$, according to LSD test). **, $p < 0.01$. CK, control; LD, light drought; MD, moderate drought; SD, severe drought.

胁迫下比高温胁迫下降幅度大, 与对照相比, 轻度、中度、重度协同胁迫分别减少了25.6%、33.9%和47.7% ($p < 0.05$), 表明高温和协同胁迫导致‘波叶金桂’叶片葡萄糖含量减少。

2.2 干旱、高温及协同胁迫对果糖含量的影响

干旱、高温及协同胁迫对‘波叶金桂’叶片果糖含量的影响见图2。在干旱胁迫下果糖含量变化不明显; 高温胁迫下果糖含量比对照降低了15.4% ($p < 0.05$); 果糖含量在协同胁迫下持续性下降, 与对照相比, 轻度、中度和重度协同胁迫下分别降低了19.3%、27.5%和46.4% ($p < 0.05$), 下降幅度显著大于单一高温胁迫, 显著影响果糖的合成与分解。

2.3 干旱、高温及协同胁迫对蔗糖含量的影响

图3显示了干旱、高温及协同胁迫对‘波叶金桂’叶片蔗糖含量的影响。蔗糖含量随着干旱胁迫强度的增强呈现先升高后降低的趋势, 分别在轻度和重度干旱胁迫达到最高值和最低值, 与对照相比分别增加了12.3% ($p < 0.05$)和减少了15.3% ($p < 0.05$); 高温胁迫下蔗糖含量比对照减少了9.8% ($p < 0.05$); 轻度、中度、重度协同胁迫下蔗糖含量与对照相比分别减少了13.6%、26.0%和34.4% ($p < 0.05$)。轻度干旱促进蔗糖的积累, 高温、协同胁迫促进蔗糖的分解, 为‘波叶金桂’抵御胁迫提供能量。

图2 干旱高温处理对‘波叶金桂’叶片果糖含量的影响(平均值±标准误差, $n = 5$)。不同大写字母表示干旱的差异显著, 不同小写字母表示高温的差异显著($p < 0.05$, 根据LSD测验)。**, $p < 0.01$ 。CK, 对照; LD, 轻度干旱; MD, 中度干旱; SD, 重度干旱。

Fig. 2 Effects of drought and heat stress on fructose content in leaves of *Osmanthus fragrans* cv. ‘Boyejingui’ (mean $\pm$ SE, $n = 5$). Different uppercase letters indicate significant differences of drought and different lowercase letters denote statistically significant differences of heat stress treatment ($p < 0.05$, according to LSD test). **, $p < 0.01$. CK, control; LD, light processing; MD, moderate drought; SD, severe drought.

图3 干旱高温处理对‘波叶金桂’叶片蔗糖含量的影响(平均值±标准误差, $n=5$)。不同大写字母表示干旱的差异显著, 不同小写字母表示高温的差异显著($p < 0.05$, 根据LSD测验)。**, $p < 0.01$ 。CK, 对照; LD, 轻度干旱; MD, 中度干旱; SD, 重度干旱。

Fig. 3 Effects of drought and heat stress on sucrose content in leaves of *Osmanthus fragrans* cv. ‘Boyejingui’ (mean $\pm$ SE, $n = 5$). Different uppercase letters indicate significant differences of drought and different lowercase letters denote statistically significant differences of heat stress treatment ($p < 0.05$, according to LSD test). $p < 0.01$. CK, control; LD, light drought; MD, moderate drought; SD, severe drought.

2.4 干旱、高温及协同胁迫对淀粉含量的影响

从图4可以看出: 干旱、高温及协同胁迫下‘波

叶金桂’叶片淀粉含量发生了不同程度的变化。随着干旱胁迫强度的增加, 淀粉含量表现出先上升后下降趋势, 在重度干旱胁迫下达到最低值, 比对照减少了20.6% ($p < 0.05$); 高温胁迫下淀粉含量比对照减少了14.0% ($p < 0.05$); 与对照相比, 轻度、中度和重度协同胁迫下淀粉含量分别降低了20.5%、29.9%和38.2% ($p < 0.05$), 协同胁迫下明显降低了‘波叶金桂’叶片中的淀粉含量。

2.5 干旱、高温及协同胁迫对HMGR活性的影响

由图5可见, 随着干旱胁迫强度的增强, ‘波叶金桂’叶片HMGR活性先上升后下降, 在轻度干旱胁迫下达到最大值, 与对照相比增加了57.1% ($p < 0.05$), 重度干旱胁迫下减少了22.1% ($p < 0.05$); 高温胁迫下HMGR活性比对照增加了43.2% ($p < 0.05$); 干旱高温协同胁迫后, 与对照相比, HMGR活性在轻度协同胁迫下增加了52.8% ($p < 0.05$), 在重度协同胁迫下减少了37.7% ($p < 0.05$)。

2.6 干旱、高温及协同胁迫对DXR活性的影响

如图6所示, 在干旱胁迫下, 随着胁迫强度的增强, ‘波叶金桂’叶片DXR活性呈现先上升后下降的趋势, 与对照相比, 中度干旱胁迫下增加了56.7% ($p < 0.05$), 重度干旱胁迫下略有减少; 高温胁迫下DXR活性比对照增加了41.1% ($p < 0.05$); 协

图4 干旱高温处理对‘波叶金桂’叶片淀粉含量的影响(平均值±标准误差, $n = 5$)。不同大写字母表示干旱的差异显著, 不同小写字母表示高温的差异显著($p < 0.05$, 根据LSD测验)。**, $p < 0.01$ 。CK, 对照; LD, 轻度干旱; MD, 中度干旱; SD, 重度干旱。

Fig. 4 Effects of drought and heat stress on starch content in leaves of *Osmanthus fragrans* cv. ‘Boyejingui’ (mean $\pm$ SE, $n = 5$). Different uppercase letters indicate significant differences of drought and different lowercase letters denote statistically significant differences of heat stress treatment ($p < 0.05$, according to LSD test). **, $p < 0.01$. CK, control; LD, light drought; MD, moderate drought; SD, severe drought.

图5 干旱高温处理对‘波叶金桂’叶片3-羟基-3-甲基戊二酰辅酶A还原酶活性的影响(平均值±标准误差, $n = 5$)。不同大写字母表示干旱的差异显著, 不同小写字母表示高温的差异显著($p < 0.05$, 根据LSD测验)。**, $p < 0.01$ 。CK, 对照; LD, 轻度干旱; MD, 中度干旱; SD, 重度干旱。

Fig. 5 Effects of drought and heat stress on 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) activity in leaves of *Osmanthus fragrans* cv. ‘Boyejingui’ (mean $\pm$ SE, $n = 5$). Different uppercase letters indicate significant differences of drought and different lowercase letters denote statistically significant differences of heat stress treatment ($p < 0.05$, according to LSD test). **, $p < 0.01$. CK, control; LD, light drought; MD, moderate drought; SD, severe drought.

图6 干旱高温处理对‘波叶金桂’叶片1-脱氧木酮糖-5-磷酸还原酶活性的影响(平均值±标准误差, $n = 5$)。不同大写字母表示干旱的差异显著, 不同小写字母表示高温的差异显著($p < 0.05$, 根据LSD测验)。**, $p < 0.01$ 。CK, 对照; LD, 轻度干旱; MD, 中度干旱; SD, 重度干旱。

Fig. 6 Effects of drought and heat stress on 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) activity in leaves of *Osmanthus fragrans* cv. ‘Boyejingui’ (mean $\pm$ SE, $n = 5$). Different uppercase letters indicate significant differences of drought and different lowercase letters denote statistically significant differences of heat stress treatment ($p < 0.05$, according to LSD test). **, $p < 0.01$. CK, control; LD, light drought; MD, moderate drought; SD, severe drought.

同胁迫下DXR活性呈现先升高后下降的趋势, 轻度协同胁迫下与对照相比增加了45.6% ($p < 0.05$), 重度协同胁迫下降低了36.9% ($p < 0.05$)。

2.7 干旱、高温及协同胁迫对LOX活性的影响

从图7可以看出, 干旱、高温及协同胁迫后, ‘波叶金桂’叶片LOX活性发生了不同程度的变化。随着干旱胁迫强度的增强, LOX活性呈上升趋势, 重度干旱胁迫下比对照增加了55.4% ($p < 0.05$); 高温胁迫下LOX活性比对照增加了39.7% ($p < 0.05$); 轻度、中度和重度协同胁迫下与对照相比分别增加了60.9%、67.7%和39.8% ($p < 0.05$)。

2.8 干旱、高温及协同胁迫对挥发物的影响

干旱、高温和协同胁迫, ‘波叶金桂’叶片VOCs种类和含量发生显著变化, VOCs共鉴定出23种化合物(表1)。随着干旱胁迫强度的增加, VOCs释放量呈上升趋势, 中度和重度干旱胁迫下VOCs释放量分别比对照增加了24.1%和22.8% ($p < 0.05$)。干旱胁迫下萜烯类化合物释放量呈现先增加后减少的趋势, 轻度和中度干旱胁迫下萜烯类化合物释放量分别比对照增加了16.8%和37.9% ($p < 0.05$), 重度干旱胁迫下减少了12.1% ($p < 0.05$); 干旱胁迫下醛类化合物释放量分别比对照增加了35.3%、46.8%和124.5% ($p < 0.05$), 诱导释放新增苯基丁醛和辛烯醛。高温胁迫醛类、萜烯类和酯类化合物分别比对照增加了83.6%、32.3%和70.3% ($p < 0.05$)。同时诱

图7 干旱高温处理对‘波叶金桂’叶片脂氧合酶活性的影响(平均值±标准误差, $n = 5$)。不同大写字母表示干旱的差异显著, 不同小写字母表示高温的差异显著($p < 0.05$, 根据LSD测验)。*, $p < 0.05$; **, $p < 0.01$ 。CK, 对照; LD, 轻度干旱; MD, 中度干旱; SD, 重度干旱。

Fig. 7 Effects of drought and heat stress on lipooxygenase (LOX) activity in leaves of *Osmanthus fragrans* cv. ‘Boyejingui’ (mean $\pm$ SE, $n = 5$). Different uppercase letters indicate significant differences of drought and different lowercase letters denote statistically significant differences of heat stress treatment ($p < 0.05$, according to LSD test). **, $p < 0.01$. CK, control; LD, light drought; MD, moderate drought; SD, severe drought.

表1 波叶金桂叶片挥发性有机化合物的主要成分(峰面积, $A \times 10^5 \cdot 10 \text{ g}^{-1}$)(平均值 $\pm$ 标准误差)
Table 1 The main components of the volatile organic compounds (VOCs) from the leaf of *Osmanthus fragrans* ‘Boyejingui’ (peak area, $A \times 10^5 \cdot 10 \text{ g}^{-1}$) (mean $\pm$ SE)

序号 No.	挥发性有机物 Volatile organic compounds	分子式 Chemical formula	28 ℃				40 ℃			
			对照 Control	轻度干旱 Light drought	中度干旱 Moderate drought	重度干旱 Severe drought	对照 Control	轻度干旱 Light drought	中度干旱 Moderate drought	重度干旱 Severe drought
1	己烯醛 3-Hexenal	C ₆ H ₁₀ O	3.33 $\pm$ 0.23	0.58 $\pm$ 0.65	2.27 $\pm$ 0.12	—	3.61 $\pm$ 0.26	—	—	1.15 $\pm$ 0.05
2	2-甲基-4-戊烯醛 2-Methyl-4-pentenal	C ₆ H ₁₀ O	0.99 $\pm$ 0.11	2.36 $\pm$ 0.23	—	5.97 $\pm$ 0.35	—	2.88 $\pm$ 0.12	3.14 $\pm$ 0.16	1.69 $\pm$ 0.13
3	2-己烯醛 (E)-2-Hexenal	C ₆ H ₁₀ O	2.48 $\pm$ 0.18	2.14 $\pm$ 0.23	2.54 $\pm$ 0.21	8.67 $\pm$ 1.14	4.53 $\pm$ 0.54	4.35 $\pm$ 0.08	8.01 $\pm$ 0.24	7.03 $\pm$ 0.19
4	苯基丁醛 4-Phenylbutanal	C ₁₀ H ₁₂ O	—	1.85 $\pm$ 0.11	—	—	—	5.48 $\pm$ 0.13	2.58 $\pm$ 0.06	2.74 $\pm$ 0.06
5	山梨醛 Sorbaldehyde	C ₆ H ₈ O	1.08 $\pm$ 0.06	2.82 $\pm$ 0.08	1.90 $\pm$ 0.04	3.05 $\pm$ 0.64	1.80 $\pm$ 0.08	—	2.61 $\pm$ 0.11	2.33 $\pm$ 0.08
6	辛烯醛 2-Ethylhexenal	C ₈ H ₁₄ O	—	1.91 $\pm$ 0.04	4.86 $\pm$ 0.81	—	3.77 $\pm$ 0.12	1.37 $\pm$ 0.06	0.67 $\pm$ 0.02	—
7	壬醛 n-Nonanal	C ₉ H ₁₈ O	—	—	—	—	0.76 $\pm$ 0.02	1.21 $\pm$ 0.04	0.90 $\pm$ 0.05	0.53 $\pm$ 0.02
8	反-3-己烯醇 trans-3-Hexenol	C ₆ H ₁₂ O	6.80 $\pm$ 0.45	8.07 $\pm$ 0.62	7.85 $\pm$ 0.67	4.12 $\pm$ 0.35	9.26 $\pm$ 1.61	2.50 $\pm$ 0.27	5.38 $\pm$ 1.01	3.20 $\pm$ 0.32
9	(3E)-3-壬烯醇 (3E)-3-Nonen-1-ol	C ₉ H ₁₈ O	—	—	—	—	—	—	1.24 $\pm$ 0.07	1.17 $\pm$ 0.11
10	2-乙基己醇 2-Ethylhexan-1-ol	C ₈ H ₁₈ O	4.28 $\pm$ 0.14	1.25 $\pm$ 0.08	—	1.93 $\pm$ 0.07	—	8.29 $\pm$ 0.61	2.08 $\pm$ 0.10	2.15 $\pm$ 0.09
11	反罗勒烯 Ocimene	C ₁₀ H ₁₆	1.65 $\pm$ 0.06	1.53 $\pm$ 0.11	2.02 $\pm$ 0.08	1.85 $\pm$ 0.05	1.55 $\pm$ 0.08	—	0.80 $\pm$ 0.02	—
12	薄荷烯 cis-p-Menthane	C ₁₀ H ₂₀	3.38 $\pm$ 0.04	—	2.03 $\pm$ 0.06	—	1.50 $\pm$ 0.12	2.48 $\pm$ 0.21	—	1.08 $\pm$ 0.05
13	柠檬烯 Limonene	C ₁₀ H ₁₆	3.83 $\pm$ 0.07	1.42 $\pm$ 0.12	1.61 $\pm$ 0.12	1.74 $\pm$ 0.10	3.29 $\pm$ 0.29	1.65 $\pm$ 0.15	1.67 $\pm$ 0.03	0.94 $\pm$ 0.04
14	罗勒烯Ocimene	C ₁₀ H ₁₆	0.18 $\pm$ 0.01	3.25 $\pm$ 0.24	2.58 $\pm$ 0.22	1.44 $\pm$ 0.14	1.40 $\pm$ 0.10	—	0.94 $\pm$ 0.05	—
15	α -蒎烯alpha-Pinene	C ₁₀ H ₁₆	0.07 $\pm$ 0.01	2.68 $\pm$ 0.16	3.13 $\pm$ 0.02	2.52 $\pm$ 0.09	5.24 $\pm$ 0.34	4.83 $\pm$ 0.37	3.60 $\pm$ 0.34	2.69 $\pm$ 0.27
16	紫苏烯 Perillen	C ₁₀ H ₁₄ O	0.34 $\pm$ 0.04	0.53 $\pm$ 0.05	1.05 $\pm$ 0.11	1.60 $\pm$ 0.06	1.03 $\pm$ 0.02	0.63 $\pm$ 0.01	0.54 $\pm$ 0.02	—
17	长叶烯 Longifolene	C ₁₅ H ₂₄	1.77 $\pm$ 0.13	3.69 $\pm$ 0.05	2.05 $\pm$ 0.23	0.71 $\pm$ 0.03	0.83 $\pm$ 0.01	1.46 $\pm$ 0.04	1.03 $\pm$ 0.02	0.88 $\pm$ 0.04
18	丁香烯 π Caryophyllen	C ₁₅ H ₂₄	—	—	—	—	—	1.07 $\pm$ 0.05	—	—
19	顺-3-乙酸叶醇酯 cis-3-Hexenyl acetate	C ₈ H ₁₄ O ₂	1.55 $\pm$ 0.06	1.61 $\pm$ 0.86	2.25 $\pm$ 0.61	4.73 $\pm$ 1.37	6.11 $\pm$ 1.01	2.63 $\pm$ 0.31	2.20 $\pm$ 0.92	4.09 $\pm$ 0.56
20	丙烯酸正己酯 Hexyl acrylate	C ₉ H ₁₆ O ₂	1.90 $\pm$ 0.09	—	—	—	—	2.58 $\pm$ 0.18	1.55 $\pm$ 0.02	1.08 $\pm$ 0.04
21	异丁酸庚酯 Heptyl isobutyrate	C ₁₁ H ₂₂ O ₂	—	—	0.96 $\pm$ 0.03	5.66 $\pm$ 0.63	2.45 $\pm$ 0.23	6.00 $\pm$ 0.93	3.10 $\pm$ 0.43	2.47 $\pm$ 0.41
22	丁酸庚酯 Heptyl butyrate	C ₁₁ H ₂₂ O ₂	1.39 $\pm$ 0.05	1.31 $\pm$ 0.03	4.22 $\pm$ 0.08	2.05 $\pm$ 0.39	5.02 $\pm$ 0.71	4.40 $\pm$ 1.21	2.79 $\pm$ 0.94	2.18 $\pm$ 0.52
23	丁酸丁酯 Butyl butyrate	C ₈ H ₁₆ O ₂	3.87 $\pm$ 0.14	3.07 $\pm$ 0.54	5.93 $\pm$ 0.35	1.71 $\pm$ 0.48	1.25 $\pm$ 0.08	—	—	—
合计			38.89	40.07	48.25	47.75	53.4	52.81	44.83	37.37

—, 未检测到化合物。
—, the compound was not detected

导新增辛烯醛、壬醛和异丁酸庚酯3种新组分。协同胁迫萜烯类化合物释放量减少, 醛类化合物释放量增加, 轻度、中度和重度协同胁迫下醛类化合物释放量分别比对照增加了94.0%、127.3%和96.3% ($p < 0.05$), 新诱导出丁香烯、壬醛、3-壬烯醇、辛烯醛、苯基丁醛和异丁酸庚酯。

3 讨论

NSC是参与植物生命代谢的重要物质, 作为“可移动碳库”为植物体生长发育提供碳源和能源, 其碳库大小和组分含量可反映出NSC在植物体的

碳代谢和碳平衡(潘庆民等, 2002; 李蟠等, 2008), 在逆境条件下维持碳平衡是树木抵抗胁迫的应对机制(McDowell *et al.*, 2008; Choat *et al.*, 2012)。相关研究表明, 一定程度干旱胁迫下植物积累可溶性糖进行渗透调节, 但也有研究表明, 干旱胁迫能够导致植物叶片气孔关闭, 减小光合速率, 可能迫使植物耗竭NSC储备, 导致因碳饥饿而死亡(Maguire & Kobe, 2015)。温度升高能够加强细胞呼吸作用, 消耗体内大量的可溶性糖和淀粉, 为抵御高温胁迫提供能量, 最终出现负碳平衡, 导致NSC含量减少。Trifilò等(2017)研究发现月桂(*Laurus nobilis*)长期轻

度缺水使植株生长速度减慢,可溶性NSC含量降低,淀粉含量增加;热处理导致西黄松和花旗松幼苗葡萄糖和果糖含量增加,淀粉含量减少,总NSC和蔗糖含量没有受到热处理的影响(Marias *et al.*, 2017);赵超等(2017)研究发现,木薯(*Manihot esculenta*) NSC含量在轻度干旱胁迫下升高,而在严重干旱胁迫下大幅度降低。本研究结果显示,随着干旱胁迫强度的增强,葡萄糖含量增加,淀粉含量降低,与Trifilò等(2017)的研究结果不一致,可能是因为淀粉降解为可溶性糖进行渗透调节。说明‘波叶金桂’通过积累葡萄糖来调节体内细胞渗透势,保持一定含水量,缓解干旱胁迫造成的不利影响,从而保障‘波叶金桂’正常生理活动来适应和抵御干旱胁迫(Klein *et al.*, 2014)。葡萄糖含量的提高一方面来源于蔗糖和淀粉的降解(图2, 图3);另一方面是通过光合作途径合成。高温胁迫后,‘波叶金桂’体内可溶性糖和淀粉含量减少,并在协同胁迫后持续性下降,协同胁迫后的研究结果与穆蓁蓁(2015)研究结果不一致,原因可能是实验设计不一致,穆蓁蓁(2015)的对照组是灌水和喷水处理。高温和干旱高温协同胁迫,一方面导致呼吸速率加强,消耗大量可溶性糖,同时还形成应激防御物质(表1),导致NSC所有组分含量显著下降;另一方面协同胁迫可能导致光合器官遭到破坏,使光合作用受到抑制,可溶性糖合成降低(Silva *et al.*, 2010)。

植物VOCs是体内重要的次生代谢产物,在抵御环境胁迫中发挥着重要的抗氧化作用(Loreto & Schnitzler, 2010; Silva *et al.*, 2010; Jud *et al.*, 2016)。刘芳等(2013)研究迷迭香(*Rosmarinus officinalis*)发现干旱胁迫导致VOCs释放总量减少,但种类增加,萜烯类化合物和绿叶挥发物相对含量显著增加;Hartikainen等(2009)研究欧洲山杨(*Populus tremula*)发现,温度升高显著增加单萜和绿叶挥发物释放量,但在罗勒(*Ocimum basilicum*)中萜烯类化合物呈现降低趋势(程璐璐等, 2015)。HMGR和DXR活性调控着萜烯类化合物的合成量,在防御反应中发挥重要应激防御作用。Morshedloo等(2017)研究发现,轻度和中度水分胁迫下牛至(*Origanum vulgare*)精油含量显著增加,同时HMGR转录上调。本研究中,萜烯类化合物释放量与HMGR、DXR活性变化趋势基本一致(表1),说明HMGR和DXR活性能够调控萜烯类化合物释放量。在干旱胁迫下‘波叶金桂’通过

释放萜烯类化合物防止活性氧大量累积,降低氧化胁迫的伤害,发挥应激保护作用(Vickers *et al.*, 2009)。同时高温能诱导提高HMGR和DXR活性促使萜烯类化合物合成并释放,有助于膜的稳定性(Arab *et al.*, 2016)。在重度干旱胁迫下萜烯类化合物减少, HMGR和DXR活性受到抑制,也可能是严重干旱导致气孔关闭,萜烯类化合物的释放通道受到阻碍。协同胁迫处理萜烯类化合物释放量降低,一方面是HMGR和DXR活性受到抑制;另一方面可能是协同胁迫导致光合产物NSC含量减少,供给合成挥发物途径的碳源减少,影响到萜烯类化合物的生物合成。

GLVs是由脂氧合酶(LOX)催化不饱和脂肪酸形成的含氧VOCs,是植物应激反应过程中产生的一类具有抵御能力的物质(Santino *et al.*, 2010),在参与植物防御机制和应激适应中发挥着重要作用(Hatanaka, 1993; Loreto & Schnitzler, 2010)。Nahar等(2015)研究发现,高温胁迫显著增加了绿豆(*Vigna radiata*)的LOX活性;干旱胁迫下小麦(*Triticum aestivum*)各器官LOX活性显著升高(马超等, 2017); Vitale等(2008)发现,干旱胁迫明显诱导冬青栎(*Quercus ilex*) LOX活性增加,同时(E)-2-己烯醇和(Z)-3-己烯醛释放量也相应增加。本实验结果显示,重度干旱胁迫下‘波叶金桂’ LOX活性显著增加,诱导GLVs释放量相应增加,可能是‘波叶金桂’细胞膜受到损伤,活性氧积累触发和刺激LOX途径,促进GLVs释放以清除活性氧,发挥抗氧化作用(Agrawal *et al.*, 2009)。高温有利于提高LOX活性,增加GLV释放量;协同胁迫严重破坏细胞膜结构,诱导细胞膜脂质过氧化,醛类化合物释放量显著增加(表1)。

植物体内的初生代谢和次生代谢途径处于动态平衡, NSC是合成次生代谢产物的主要来源, VOCs的排放可能会大幅减少植物的固碳量(Dicke & Loreto, 2010; Peñuelas & Staudt, 2010; Copolovici *et al.*, 2014)。本研究中,干旱胁迫下‘波叶金桂’通过蔗糖和淀粉降解积累葡萄糖维持正常膨压进行渗透调节;同时,还给次生代谢途径供给碳源合成萜烯类化合物(表1),以此降低活性氧对‘波叶金桂’细胞膜的伤害。高温胁迫加强呼吸作用,消耗可溶性糖和淀粉为萜烯类化合物的合成提供能量与碳源,萜烯类化合物释放量显著增加;协同胁迫导致细胞

结构被破坏, 碳摄取和利用受到限制, 供应到次生代谢途径的碳源和能源减少, 最终萜烯类化合物的合成减少, ‘波叶金桂’体内活性氧大量积累以及细胞膜脂质过氧化, 在中度和重度协同胁迫下对‘波叶金桂’细胞膜造成严重伤害, 从而导致GLVs释放量增加。

综上所述, NSC既为植物自我调节提供能源和碳源, 同时又作为渗透调节物质进行渗透调节。干旱胁迫下‘波叶金桂’通过降解淀粉和蔗糖积累葡萄糖, 进行渗透调节维持正常膨压, 同时提高次生代谢酶活性调控萜烯类化合物大量合成, 从而抵御干旱胁迫。重度干旱胁迫导致GLVs释放量增加, ‘波叶金桂’细胞膜已经受到伤害; 高温胁迫下‘波叶金桂’呼吸作用加快, 可溶性糖和淀粉降解, 为抵御胁迫提供能量和碳源, 同时提高次生代谢酶活性, 促进萜烯类化合物合成, 从而抵御高温胁迫; 协同胁迫后, 可溶性糖含量明显下降, 萜烯类化合物的生物合成受阻, ‘波叶金桂’通过提高LOX活性合成大量GLVs; 中度和重度协同胁迫对‘波叶金桂’的伤害已经超过‘波叶金桂’自身生理生化调节的阈值, 细胞膜已经严重受损并导致组织损伤, 无法正常发挥保护机能。

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