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·技术方法·

# 水稻水杨酸代谢突变体高通量筛选方法的建立与应用

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**摘要** 水杨酸(SA)是植物免疫的关键防御信号分子。植物SA的定量分析对于SA代谢途径及其生物学功能研究至关重要。利用高效液相色谱仪(HPLC)和液相-质谱联用仪(LC-MS)测定SA含量是目前常用方法，但难以实现高通量测定。水稻(*Oryza sativa*)中SA合成代谢途径目前尚未完全解析，高效筛选水稻SA相关突变体对于阐明其代谢途径具有重要意义。该文对已有基于SA生物传感菌株*Acinetobacter* sp. ADPWH_*lux*估算SA的分析方法进行了改良，建立了水稻SA高通量估算方法，简化了样品采集和提取过程，省去样品称重、组织研磨及离心等耗时步骤，整个操作流程便捷且高效。同时，利用已报道的水稻SA代谢相关遗传材料验证了该方法的可行性，并使用该方法筛选了钴-60诱变的水稻突变体库，获得一批水稻SA含量发生显著变化的突变体，采用HPLC法对突变体内源SA进行了验证。该方法可用于SA代谢突变体的遗传筛选及其代谢相关酶鉴定，对水稻等作物的SA代谢及生物学功能研究具有重要的应用价值。

**关键词** 水杨酸测定，生物传感器，高通量方法，水杨酸代谢突变体，水稻

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水杨酸(salicylic acid, SA)又称邻羟基苯甲酸，自1828年，德国药理学家Johann Andreas Buchner从垂柳(*Salix babylonica*)皮中分离出水杨苷(一种具有水杨基的醇β-葡萄糖苷)以来(Ding and Ding, 2020)，至1859年，Hermann Kolbe首次通过人工方法合成了SA。SA作为一种重要的植物激素，可调节植物的生长和胁迫应答(丁秀英，2001；Rivas-San Vicente and Plasencia, 2011; Ahmad and Prasad, 2012；曹栋栋等，2020; Koo et al., 2020; van Butselaar and Van den Ackerveken, 2020; Saleem et al., 2021; Waadt et al., 2022; Shields et al., 2022)。SA处理的烟草(*Nicotiana tabacum*)植株对烟草花叶病毒(TMV)的抗性增强(White, 1979)；同时，在病原菌侵染后，烟草和黄瓜(*Cucumis sativus*)植株中的SA水平急剧升高(Malamy et al., 1990; Métraux et al., 1990; Rasmussen et al., 1991)，表明SA也参与植物的免疫应答。将细菌中编码的水杨酸羟化酶NahG转入烟草后获得的转基因植株中，SA积累减少，未产生系统获得性抗性(systemic acquired resistance, SAR)，表明SA是SAR产生必需的信号分子(Gaffney et al., 1993)。对SA生物合成或感知缺陷的拟南芥(*Arabidopsis thaliana*)突变体的深入研究表明，SA对局部组织中的模式触发免疫(pattern-triggered immunity, PTI)和效应因子触发免疫(effector-triggered immunity, ETI)也很重要(Zhang and Li, 2019)。

目前，植物中已知SA合成途径主要有2条：异分支酸合成酶(isochorismate synthase, ICS)途径和苯丙氨酸解氨酶(phenylalanine ammonia-lyase, PAL)途径(Dempsey et al., 2011)。在第1条途径中，异分支酸合成酶(ICS)催化质体中的分支酸转化为异分支酸，后者通过氨基转移酶PBS3 (AvrPphB Susceptible 3)催化产生异分支酸-谷氨酸加合物(ICS-*9*-Glu)，然后其自发分解为SA (Rekhter et al., 2019)。在第2条途径中，PAL将苯丙氨酸(Phe)转化为反式肉桂酸

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(*trans*-cinnamic acid, *t*-CA), 之后由肉桂酰辅酶A (cinnamoyl-CoA)连接酶(cinnamoyl-CoA Ligase, CNL)催化生成肉桂酰辅酶A (Wang et al., 2024), 后者被异常花序分生组织1 (abnormal inflorescence meristem 1, AIM1)氧化形成苯甲酸(benzoic acid, BA) (Xu et al., 2023), 然后通过假设的苯甲酸-2-羟化酶(benzoic acid 2-hydroxylase, BA2H)对BA羟基化产生SA (Peng et al., 2021)。目前对SA生物合成的认识大部分基于拟南芥中ICS途径的全面解析, 水稻(*Oryza sativa*)以及小麦(*Triticum aestivum*)等作物的SA生物合成途径尚待阐明。

植物激素的定性和定量在研究植物激素的作用机理、植物代谢途径和遗传改良等方面起重要作用。近年来, 植物激素检测方法发展迅速(提取后直接用于检测或经固相萃取(solid-phase extraction, SPE)小柱前处理后检测), 检测方法多样, 主要包括酶联免疫测定法(enzyme-linked immunosorbent assay, ELISA)、高效液相色谱(high performance liquid chromatography, HPLC)、气相色谱(gas chromatography, GC)、液相色谱-质谱联用(HPLC-mass spectrometry, HPLC-MS)、气相色谱-质谱联用(GC-MS)以及毛细管电泳(capillary electrophoresis, CE)等(Liu et al., 2017)。从只能检测1种植物激素(Sanders et al., 1989)发展到能检测上千种植物代谢物(Yang et al., 2022)。目前, 植物内源SA含量的测定主要依赖HPLC或HPLC-MS法(Verberne et al., 2002; Aboul-Soud et al., 2004)。但这些方法样品提取过程复杂且对测定样品纯度要求高, 仪器成本高昂, 并需专业的实验技术人员操作, 耗时较长, 无法满足大批量样品检测的需求。

Huang等(2005)开发了一种SA生物传感器, 他们将*lux*基因插入不动杆菌菌株*Acinetobacter* ADP1基因组中, 命名为*Acinetobacter* sp. ADPWH_*lux*。操纵子*lux*存在5个结构基因, 分别编码2条多肽链(luxA和luxB)、组成荧光脂酶, 还原酶luxC、转移酶luxD和合成酶luxE聚合组成的复合物脂肪酸还原酶。当反应底物存在时, *lux*基因编码的荧光脂酶催化脂肪醛产生脂肪酸以及波长约为490 nm的蓝绿光。*luxCDE*编码的脂肪酸还原酶复合物则将脂肪酸又还原成脂肪醛, 从而构成循环的反应体系(Meighen, 1993)。该生物传感器对SA、甲基SA和合成的SA衍生物乙酰水杨酸具有高度特异性(Huang et al., 2005), 且发光强度与SA的浓度在很大范围内相关(李超等, 2009), 因此适用于植物粗提物中SA的定量分析。

Huang等(2006)利用水杨酸生物传感器分析了抗病过程中烟草叶片的水杨酸含量。Marek等(2010)利用*Acinetobacter* sp. ADPWH_*lux*对拟南芥SA响应病菌进行了高通量快速测定, 证实了该方法能检测拟南芥不同基因型间SA水平的差异, 可用于SA水平改变突变体的遗传筛选。目前对水稻进行SA突变体筛选的报道较少。水稻叶片较大且相较于拟南芥叶片更为坚硬, 打孔器取样法及使用96孔PCR板处理并不适用, 要实现水稻的水杨酸相关突变体库筛选需要一种简便的大规模筛选方法。本研究建立了水稻的SA检测流程, 利用已知SA遗传材料进行了验证, 并利用该方法从钴-60射线辐射诱变水稻突变体库筛选到SA含量变化的突变体, 用于后续研究。

## 1 材料与方法

### 1.1 材料

#### 1.1.1 实验材料

粳稻品种日本晴(*Oryza sativa* L. subsp. *japonica* 'Nipponbare') (NIP)及抗病品种IRBB7, 2 500份日本晴(NIP)和抗病品种IRBB7经钴-60射线辐射诱变的M_2代株系, 日本晴背景的基因敲除突变体*oss5h*DM/NIP和过表达材料*S5H1*-OE, 不动杆菌*Acinetobacter* sp. ADPWH_*lux*。

#### 1.1.2 仪器和试剂

NaCl和甲醇(色谱醇)购自国药集团化学试剂有限公司。SA标准品购自西格玛奥德里奇(上海)贸易有限公司。酵母提取物和胰蛋白胨购自赛默飞世尔科技(中国)有限公司。

其它仪器和试剂包括: 高速冷冻离心机和移液器(艾本德(上海)实验室科技有限公司)、高效液相色谱仪1260 (安捷伦科技(中国)有限公司)、全自动样品快速研磨仪(上海净信实业发展有限公司)、多功能涡旋振荡器(上海达姆实业有限公司)、高压蒸汽灭菌锅(上海博科公司)、隔水式培养箱(上海一恒仪器有限公司)、酶联免疫检测仪INFINITE 200 PRO (帝肯(上海)

贸易有限公司)、微量紫外分光光度计NanoDrop 2000c (赛默飞世尔科技(中国)有限公司)、多用途旋转摇床QB-208 (海门市其林贝尔仪器制造有限公司)和氮吹仪(毕克气体仪器贸易(上海)有限公司)。

### 1.2 方法

#### 1.2.1 细菌培养

将*Acinetobacter* sp. ADPWH_*lux*菌种在无抗LB平板上划线，在37°C培养箱中过夜培养。挑取单菌落，接入加有5 mL无抗LB的试管中。37°C下220 r·min$^{-1}$摇床培养约12小时。然后按1:20 (v/v)的比例接菌，将1 mL上述菌液接入加有20 mL无抗LB的50 mL锥形瓶中, 37°C下摇床培养2–4小时，直至OD=0.4。

#### 1.2.2 样品处理方法

水稻在96孔PCR板(管底经切割处理)上水培约3周(每板加入3株野生型，1株*oss5h*DM/NIP和*S5H1*-OE作为对照)。每株水稻苗剪取2枚叶片(首个样品称重约0.015 g，其余样品无需称重，参照第1个样品叶片长度剪取)，揉成团塞入对应的已加600 μL LB的96孔2 mL板中, 96孔PCR板与96孔2 mL板位置一一对应。将96孔2 mL板在95°C条件下水浴30分钟，每隔5分钟将96孔板盖子重新盖好(高温使盖子顶开)。待96孔2 mL板中的叶片提取液冷却至室温后，吸取50 μL叶片提取液至对应的黑色酶标板中，酶标板与96孔2 mL板位置一一对应(每次实验每板水稻苗做2板重复)。然后，吸取50 μL制备好的菌液加入酶标板中。在37°C恒温培养箱中黑暗静置培养1.5小时。最后用酶联免疫检测仪测定相对发光值(每一酶标板需测2次)。数据处理：去除96孔板96个数据中无样的数据，按照标准曲线粗略换算成SA浓度，选取高于或低于NIP叶片SA含量约40%的作为候选样品进行第2次筛选。

#### 1.2.3 酶联免疫检测仪的操作方法

打开电脑中酶联免疫检测仪关联软件Tecan i-control (版本: 1.11.10)。在软件主界面“Measurements”选项中双击“Luminescence”2次。将酶标板放入仪器中。单击“start”开始检测。

#### 1.2.4 高效液相色谱法测定SA含量

取水稻叶片用液氮研磨，称取约0.1 g磨成粉末的样品，放入2 mL离心管中，加入1 mL 80%甲醇(色谱纯), 4°C下使用多用途旋转摇床旋转混匀4小时。4°C下18 000 ×*g*离心10分钟，吸取上清液于新的2 mL离心管中，剩余沉淀重新加入0.6 mL 100%甲醇(色谱纯), 4°C下使用多用途旋转摇床旋转混匀4小时。4°C下18 000 ×*g*离心10分钟，吸取上清，并入第1次的上清液中，用氮吹仪吹干后加入300 μL 30%甲醇(色谱纯)，用多功能涡旋振荡器重悬，4°C下使用多用途旋转摇床旋转混匀4小时, 4°C下18 000 ×*g*离心10分钟，离心后用1 mL一次性无菌注射器吸取上清，再用0.22 μm滤膜过滤，将所得提取液放入相应标记的样品瓶中，待测(Zhao et al., 2019)。

### 1.3 数据统计和分析

实验数据取3次生物学重复或3次技术重复的平均值。使用GraphPad Prism 9 (https://www.graphpad.com/)软件对数据进行统计分析。用*t*检验对实验数据进行差异显著性分析，$P$<0.05表示差异显著，$P$<0.01表示差异极显著，$P$<0.001也表示差异极显著。

## 2 结果与讨论

### 2.1 绘制*Acinetobacter* sp. ADPWH_*lux*菌株的发光强度标准曲线

首先建立*Acinetobacter* sp. ADPWH_*lux*的发光强度标准曲线。将SA标准品加入600 μL LB溶液中，使其终浓度分别为0.1、0.2、0.4、0.8、1、1.5、2、2.5、3、3.5、4、4.5和5 nmol·mL$^{-1}$。将配制好的SA标准品混合液置于96孔深孔板中, 95°C下加热30分钟，冷却至室温后，吸取50 μL混合液(总量)至黑色酶标板中，最后加入提前制备好的50 μL菌液，37°C下静置黑暗培养1.5小时。用酶联免疫检测仪检测发光值。获得该菌株在不同SA浓度下的发光强度。根据发光强度结果绘制该菌株的发光强度标准曲线，曲线显示菌株的发光强度与SA浓度成正相关，可用于SA的粗略测定。

### 2.2 高通量测定水稻SA含量的操作流程

根据得到的标准曲线(图1)，拟利用*Acinetobacter* sp. ADPWH_*lux*建立一个大批量快速测定水稻SA含量的方法。图2显示改良后的对水稻样品处理以及

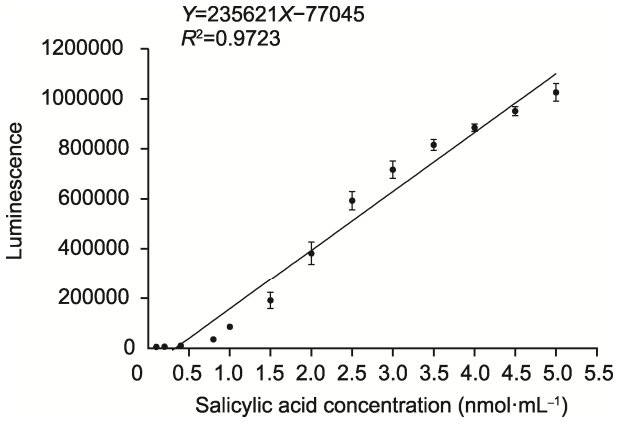


**图1** *Acinetobacter* sp. ADPWH_*lux*菌株的发光强度标准曲线标准曲线方程是$Y$=235621$X$–77045，决定系数$R^2$ =0.9723。数据为平均值±标准差($n$=3)。

**Figure 1** Standard curve of luminescence of *Acinetobacter* sp. ADPWH_*lux* strain
Equation: $Y$=235621$X$–77045, Coefficient of determination: $R^2$=0.9723. Data are means ± SD ($n$=3).

测定的操作流程。首先，利用水培进行大规模种植，避免土培的费工费时，并在发芽后对水稻种子进行初步筛选，选取发芽一致的种子放入96孔PCR板中，确保水稻幼苗长势一致。由于水稻幼苗叶片较大，且一般呈细长形，故采用固定长度对质量进行标准化取样(图2)。将同一批水培苗0.015 g样品所对应的叶片长度进行衡量，约为5 cm的叶片2枚，后续直接用尺子进行测量后快速取样，既加快了取样速度，也避免了由于取样过程耗时而导致的植物激素含量变化。使用96孔深孔板(U型底，容量2 mL)作为取样板，可使样品更好地浸泡在LB溶液中，SA释放更彻底，且取样后的96孔深孔板直接放入水浴锅中，操作更简便。筛选整个96孔PCR板的水稻样品需2.5小时，在95°C下水浴0.5小时过程中进行下一板水稻样品的取样，这样1天可以筛选18个96孔PCR板的水稻样品，将近1 700个单株材料。传统的HPLC和LC-MS法测定有前处理步骤(需对样品进行提取和浓缩等)，测定1个样品的SA含量至少需15分钟，1天最多能完成96个单株材料的测定。因此，本方法大幅提高了筛选效率。

## 2.3 样品处理和筛选方法可行性验证

之前，本实验室报道了水稻中能将SA羟基化转变为2,5-DHBA的羟基化酶*OsS5H1*和*OsS5H2*基因，并创

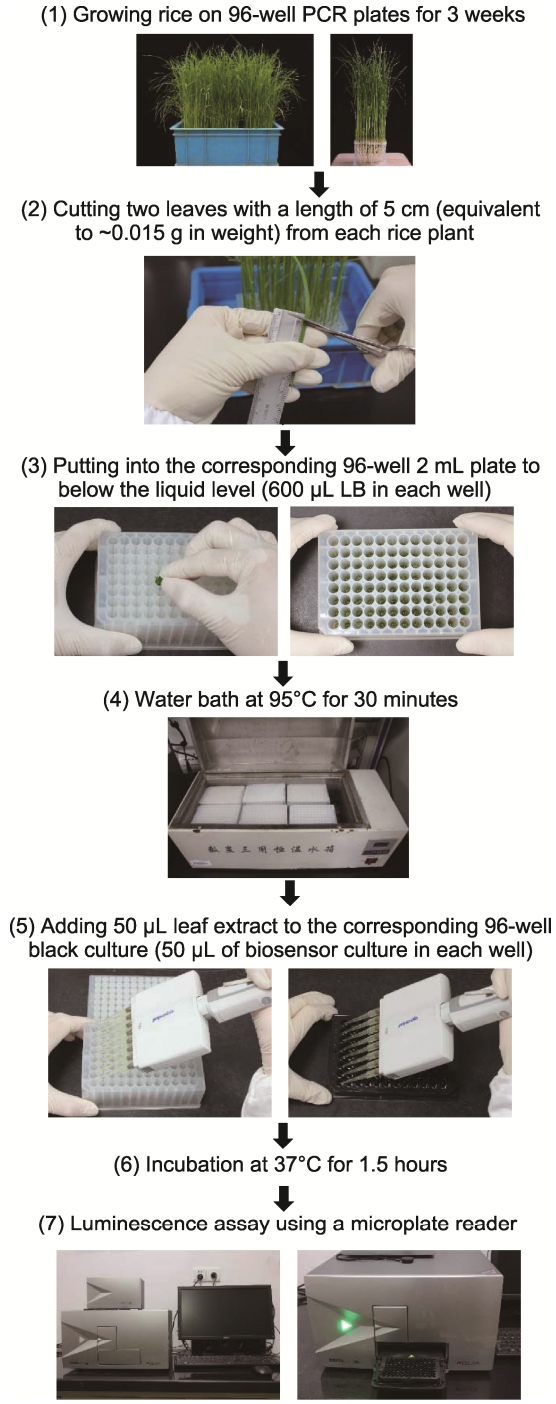


**图2** 利用水杨酸(SA)生物传感器*Acinetobacter* sp. ADPWH_*lux*菌株高通量测定水稻SA含量的改良操作流程(改自Marek et al., 2010)
(1) 水稻在96孔PCR板(管底经切割处理)水培约3周(每板加入3株野生型，1株*oss5h*DM/NIP和*S5H1*-OE作为对照); (2) 剪取每株水稻苗5 cm的叶片2枚(约0.015 g); (3) 揉成团塞入对应的已加600 μL LB的96孔2 mL板中, 96孔PCR板与96孔2 mL板位置一一对应; (4) 将96孔2 mL板在95°C下水浴30分钟，每隔5分钟将96孔板盖子重新盖好(高温会使盖子顶开); (5) 待96孔

2 mL板中的叶片提取液冷却至室温后，吸取50 μL叶片提取液至已提前加入50 μL生物传感器的黑色酶标板中，酶标板与96孔2 mL板位置一一对应(每次实验每板水稻苗做2板重复)；(6) 37°C下恒温黑暗静置培养1.5小时；(7) 用酶联免疫检测仪测定相对发光值(每一酶标板需测2次)。

**Figure 2** Modified manipulating process for high-throughput determination of salicylic acid (SA) content using SA biosensor *Acinetobacter* sp. ADPWH_*lux* strain in rice (adapted from Marek et al., 2010)
(1) Rice was hydroponic for about 3 weeks in 96-well PCR plates (The bottom of tube was cut) (As controls, 3 wild type,1 *oss5h*DM/NIP and *S5H1*-OE plants were added to each plate); (2) Took two leaves of 5 cm (about 0.015 g) from each rice seedling; (3) Kneaded the leaf into a 2 mL 96-well plate with 600 μL LB, and the positions of the 96-well PCR plate and the 96-well 2 mL plate were one-to-one corresponding; (4) The 2 mL 96-well plate was bathed in water at 95°C for 30 minutes (the lid of the 96-well plate should be re-closed every 5 minutes, because high temperature would make the lid open); (5) After the leaf extract liquid from the 2 mL 96-well plate was cooled to room temperature, 50 μL of the extract liquid was pipetted into the black enzyme-labeled plate that had been added with 50 μL biosensor in advance, and the position of the enzyme-labeled plate was one-to-one corresponding to the 96-well 2 mL plate (two plates were repeated for each rice seedling in each experiment); (6) Incubation at 37°C in a incubator for 1.5 hours in the dark; (7) The relative luminescence value was measured with an enzyme-linked immunodetector (each enzyme plate needs to be measured twice).

制了水稻*OsS5H1*和*OsS5H2*基因敲除突变体*oss5h*-DM/NIP及*OsS5H1*过表达材料*S5H1*-OE (Zhang et al., 2022)。*oss5h*DM/NIP表现出内源性SA含量增加，*S5H1*-OE则表现出内源性SA含量降低。为验证高通量测定水稻SA操作流程的可行性，利用该方法检测了野生型日本晴(NIP)、*oss5h*DM/NIP以及*S5H1*-OE的SA发光强度，结果分别为281 009.00、450 300.00和136 309.67 (图3A)。估算0.015 g NIP、*oss5h*DM/NIP和*S5H1*-OE幼苗叶片样品中的水杨酸含量分别为60.78、89.52和36.21 nmol·g$^{-1}$，相较于NIP，*oss5h*-DM/NIP的发光强度升高47.28%，*S5H1*-OE的发光强度降低40.41%。

为进一步验证发光强度检测结果的准确性，对同一批NIP、*oss5h*DM/NIP和*S5H1*-OE水稻幼苗叶片进行了HPLC检测，结果显示其内源性SA含量分别为73.50、119.14和25.14 nmol·g$^{-1}$，与其SA发光强度测定结果相吻合(图3B)，说明该初筛方法可以用于后续

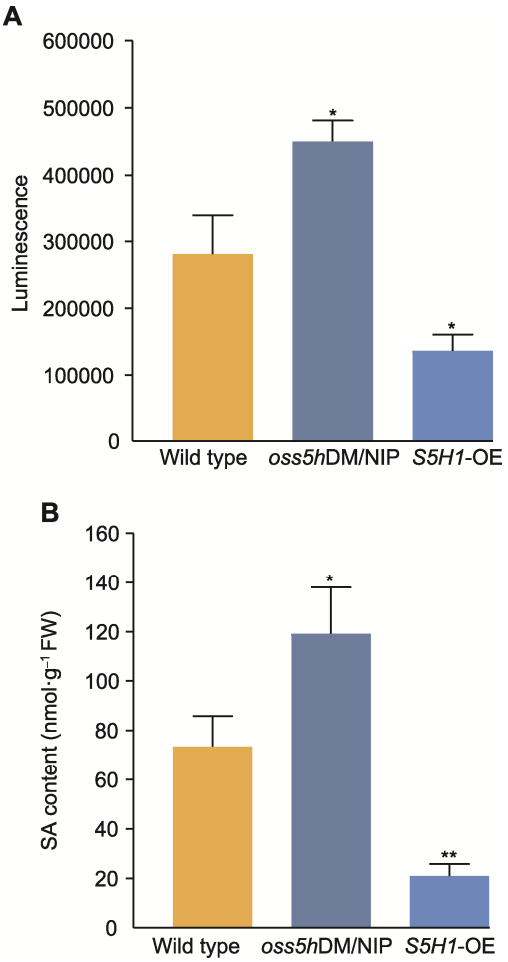


**图3** NIP、*oss5h*DM/NIP和*S5H1*-OE的水杨酸(SA)发光强度**(A)**及内源性SA含量**(B)**
水稻在温室条件下培养，培养条件完全相同。数据为平均值±标准差($n$=3)。使用$t$检验进行统计学分析，*$P$<0.05; **$P$<0.01

**Figure 3** The salicylic acid (SA) luminescence **(A)** and SA content **(B)** of wild type (NIP), *oss5h*DM/NIP and *S5H1*-OE
Rice plants were cultured in greenhouse, and the culture conditions were the same. Data are means ± SD ($n$=3). The statistical significances were calculated by $t$-test, * $P$<0.05; **$P$<0.01

大批量筛选。

## 2.4 利用高通量方法对水稻突变体进行筛选

为验证大批量筛选的准确性，以*oss5h*DM/NIP和*S5H1*-OE为对照，进行以下实验：将3粒NIP (或IRBB7)，*oss5h*DM/NIP和*S5H1*-OE各1粒种子随机播种在96孔PCR板上并做好标记，其余位置播种M_2代株系的种子。培养3周后用该方法检测96孔PCR板上所有水稻的SA发光强度(图4A)。测定结果能直观且快速地

区分出*oss5h*DM/NIP、*S5H1*-OE和M_2代突变体。至此，确定了本研究建立的水稻样品快速制备及SA测定方法的可行性。

为探索该方法在水稻SA代谢突变体遗传筛选中的应用，对2 500份钴-60射线辐射诱变的NIP和IRBB7的M_2代株系进行筛选。在96孔PCR板的第1竖列种3粒NIP (或IRBB7)、2粒*oss5h*DM/NIP和2粒*S5H1*-OE水稻种子，其余每一竖列种1份M_2代株系水稻的8粒种子(随机挑选)，每次播种27个96孔PCR板，10天播种1次，共计播种228板，分批次筛选。初步得到1个高SA水平的突变体和4个低SA水平的突变体，分别为*41-4A* (high level SA)、*133-3H* (low level SA)、*134-6D* (low level SA)、*144-5B* (low level SA)和*144-5F* (low level SA) (图4B)。这些突变体名称中的第1个数字代表96孔板编号，后1个符号代表在96孔板上的位置。将每一板的突变体荧光值与NIP (或IRBB7)、*oss5h*DM/NIP和*S5H1*-OE进行比较，同时根据标准曲线计算出突变体SA发光强度对应的SA浓度(图4B; 表1)，结果依次为1.91、0.36、0.42、0.52和0.64 nmol·mL$^{-1}$，由约0.015 g样品估算SA在水稻幼苗叶片中的含量分别为76.42、13.21、16.77、20.64和25.44 nmol·g$^{-1}$ (表1)。其中, *41-4A* (high level SA)

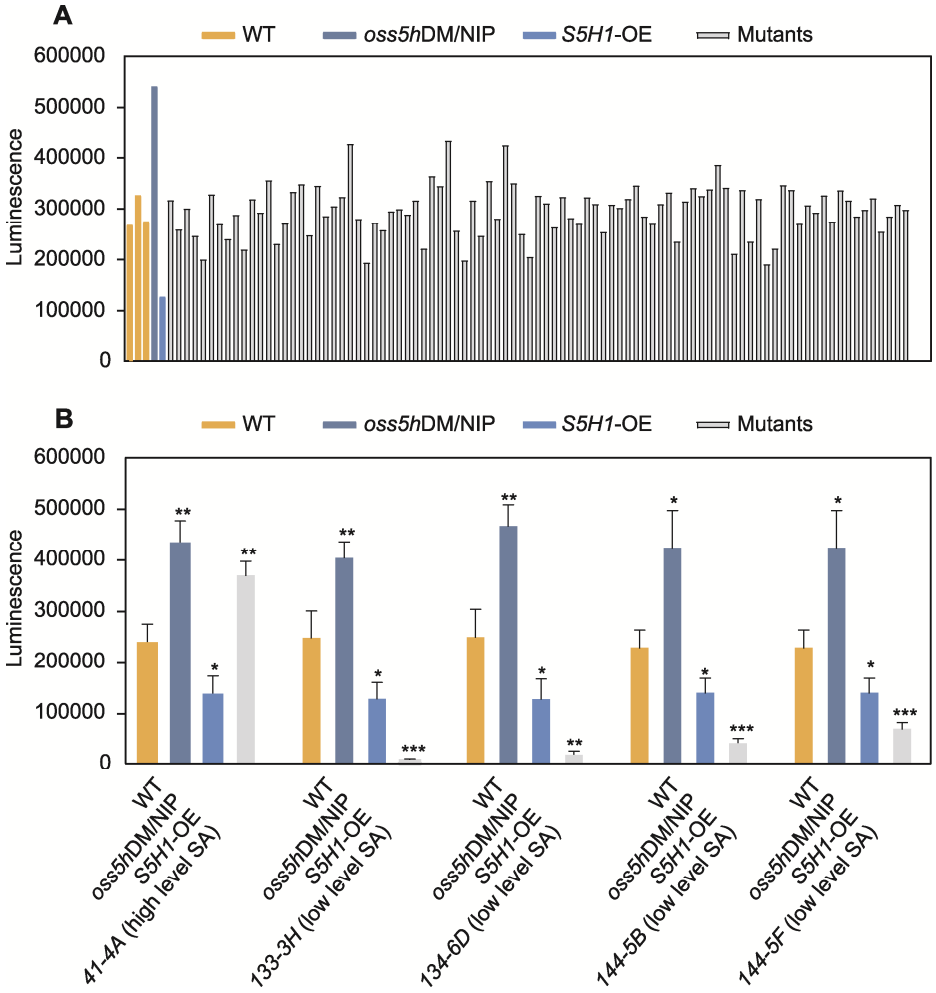


**图4** 96孔PCR板上所有水稻的水杨酸(SA)发光强度**(A)**及筛选候选突变体的SA发光强度**(B)**
数据为平均值±标准差(*n*=3)。使用*t*检验进行统计学分析, * *P*<0.05; ***P*<0.01; ****P*<0.001

**Figure 4** The salicylic acid (SA) luminescence of all rice on the 96-well PCR plate **(A)** and SA luminescence in the selected candidate mutants **(B)**
Data are means ± SD (*n*=3). Statistical significance was calculated by *t*-test, * *P*<0.05; ** *P*<0.01; *** *P*<0.001

**表1** 突变体水杨酸(SA)发光强度在标准曲线中对应的SA浓度及估算的水稻叶片SA含量

**Table 1** The salicylic acid (SA) concentration corresponding to the SA luminescence of mutant in the standard curve and estimated SA content in rice leaves

| No. | Sample name | Luminescence | SA concentration in extracting solution (nmol·mL$^{-1}$) | Estimated SA content in rice leaves (nmol·g$^{-1}$) |
|---|---|---|---|---|
| 1 | NIP | 240906.67±33164.52 | 1.35±0.14 | 53.98±5.63 |
| | *41-4A* | 373098.67±23587.55** | 1.91±0.10* | 76.42±4.00* |
| 2 | NIP | 251577.33±48090.31 | 1.39±0.20 | 55.79±8.16 |
| | *133-3H* | 7778.33±1563.11*** | 0.36±0.01*** | 13.21±0.27*** |
| 3 | NIP | 253330.67±50076.11 | 1.40±0.21 | 56.09±8.50 |
| | *134-6D* | 21748.67±2827.30** | 0.42±0.01** | 16.77±0.48** |
| 4 | NIP | 232799.67±29917.16 | 1.32±0.13 | 52.60±5.08 |
| | *144-5B* | 44528±4907.68*** | 0.52±0.02*** | 20.64±0.83*** |
| 5 | NIP | 232799.67±29917.16 | 1.32±0.13 | 52.60±5.08 |
| | *144-5F* | 72798.67±7982.44*** | 0.64±0.03*** | 25.44±1.36*** |

根据标准曲线计算提取液中的SA浓度，根据0.015 g水稻叶片样品计算叶片SA含量。发光强度数据为平均值±标准差($n$=3)。使用$t$检验进行统计学分析, *$P$<0.05; **$P$<0.01; ***$P$<0.001

SA concentration was calculated from the standard curve and SA content of rice leaves was calculated according to leaf mass 0.015 g. Luminescence are means ± SD ($n$=3). Statistical significances were calculated by $t$-test, *$P$<0.05; **$P$<0.01; ***$P$<0.001

相较于NIP升高41.57%，*133-3H* (low level SA)、*134-6D* (low level SA)、*144-5B* (low level SA)和*144-5F* (low level SA)相较于NIP分别降低76.32%、70.10%、60.76%和51.63%。

## 2.5 利用HPLC检测水稻突变体内源性SA含量

为进一步确认筛选结果，使用HPLC检测法对日本晴(NIP)以及筛选获得的突变体*41-4A* (high level SA)、*133-3H* (low level SA)、*134-6D* (low level SA)、*144-5B* (low level SA)和*144-5F* (low level SA)进行了SA含量检测，结果显示其内源性游离SA含量分别为85.74、140.71、2.13、26.34、23.51和32.98 nmol·g$^{-1}$，与其发光强度趋势一致，说明筛选获得的突变体SA代谢水平发生了变化(图5)。

## 2.6 讨论

本研究建立了使用SA生物传感器*Acinetobacter* sp. ADPWH_*lux*快速测定水稻组织中SA含量的方法。此方法取样快速且测定过程所需成本低，样品制备组织量收集、研磨和离心所用时间少，适用于对温室水培植物进行大批量快速筛选。但测得的SA浓度的精准度不高，需在每板中加入野生型(对照)、*oss5h*DM/NIP

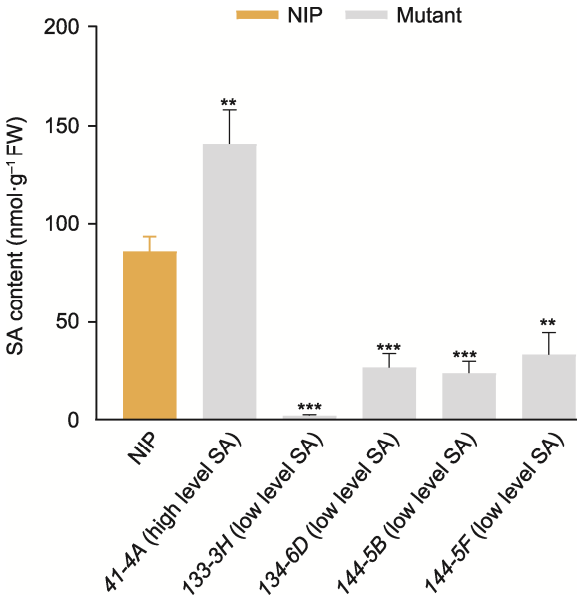


**图5** 野生型(NIP)和5个水杨酸(SA)水稻突变体的SA含量柱状图

FW: 鲜重。数据为平均值±标准差($n$=3)。使用$t$检验进行统计学分析, **$P$<0.01; ***$P$<0.001

**Figure 5** Histogram of salicylic acid (SA) content in wild type (NIP) and 5 SA rice mutant plants

FW: Fresh weight. Data are means ± SD ($n$=3). The statistical significance was calculated by $t$-test, **$P$<0.01; ***$P$<0.001

和*S5H1*-OE样品以保证测定数据的可靠性。尽管测定的数值不能代表精确的SA浓度, 但可利用HPLC或LC/MS方法进一步验证获得的突变体材料及测定准确的SA浓度。该方法可用于水稻SA代谢途径及其调控相关突变体的遗传筛选和后续的图位克隆, 对其它作物的SA含量测定也具有较强的借鉴意义。

**致谢** 英国生态与水文中心(UKCEH)和英国研究与创新机构(UKRI)提供*Acinetobacter* sp. ADPWH_*lux*菌株(菌株使用需通过MTA授权), 特此一并致谢。

## 作者贡献声明

叶灿: 完成实验、分析数据和撰写论文; 姚林波、金莹、高蓉、谭琪和李旭映: 完成实验并分析数据; 张艳军: 提供技术支持; 陈析丰和马伯军: 提供遗传学材料; 章薇和张可伟: 设计实验、分析数据并撰写论文。

## 参考文献

**Aboul-Soud MAM, Cook K, Loake GJ** (2004). Measurement of salicylic acid by a high-performance liquid chromatography procedure based on ion-exchange. *Chromatographia* **59**, 129–133.

**Ahmad P, Prasad MNV** (2012). Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability. New York: Springer Press. pp. 312–466.

**Cao DD, Chen SY, Qin YB, Wu HP, Ruan GH, Huang YT** (2020). Regulatory mechanism of salicylic acid on seed germination under salt stress in kale. *Chin Bull Bot* **55**, 49–61. (in Chinese)
**曹栋栋, 陈珊宇, 秦叶波, 吴华平, 阮关海, 黄玉韬** (2020). 水杨酸调控盐胁迫下羽衣甘蓝种子萌发的机理. 植物学报 **55**, 49–61.

**Dempsey DA, Vlot AC, Wildermuth MC, Klessig DF** (2011). Salicylic acid biosynthesis and metabolism. *Arabidopsis Book* **9**, e0156.

**Ding PT, Ding YL** (2020). Stories of salicylic acid: a plant defense hormone. *Trends Plant Sci* **25**, 549–565.

**Ding XY, Zhang J, Su BL, Xu HF** (2001). Role of salicylic acid in plant disease resistance. *Chin Bull Bot* **18**, 163–168. (in Chinese)
**丁秀英, 张军, 苏宝林, 徐惠风** (2001). 水杨酸在植物抗病中的作用. 植物学通报 **18**, 163–168.

**Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H, Ryals J** (1993). Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* **261**, 754–756.

**Huang WE, Huang LF, Preston GM, Naylor M, Carr JP, Li YH, Singer AC, Whiteley AS, Wang H** (2006). Quantitative *in situ* assay of salicylic acid in tobacco leaves using a genetically modified biosensor strain of *Acinetobacter* sp. ADP1. *Plant J* **46**, 1073–1083.

**Huang WE, Wang H, Zheng HJ, Huang LF, Singer AC, Thompson I, Whiteley AS** (2005). Chromosomally located gene fusions constructed in *Acinetobacter* sp. ADP1 for the detection of salicylate. *Environ Microbiol* **7**, 1339–1348.

**Koo YM, Heo AY, Choi HW** (2020). Salicylic acid as a safe plant protector and growth regulator. *Plant Pathol J* **36**, 1–10.

**Li C, Zhou L, Zhang YJ, Song FP, Zhang J** (2009). Influence of ions conditions on *salR* gene in *Acinetobacter* sp. ADP1. *Biotechnol Bull* (9), 57–63. (in Chinese)
**李超, 周琳, 张永军, 宋福平, 张杰** (2009). 离子环境对*Acinetobacter* sp. ADP1的*salR*基因活性的影响. 生物技术通报 (9), 57–63.

**Liu SJ, Wu YN, Fang CG, Cui Y, Jiang N, Wang H** (2017). Simultaneous determination of 19 plant growth regulator residues in plant-originated foods by QuEChERS and stable isotope dilution-ultra performance liquid chromatography-mass spectrometry. *Anal Sci* **33**, 1047–1052.

**Malamy J, Carr JP, Klessig DF, Raskin I** (1990). Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science* **250**, 1002–1004.

**Marek G, Carver R, Ding YZ, Sathyanarayan D, Zhang XD, Mou ZL** (2010). A high-throughput method for isolation of salicylic acid metabolic mutants. *Plant Methods* **6**, 21.

**Meighen EA** (1993). Bacterial bioluminescence: organization, regulation, and application of the *lux* genes. *FASEB J* **7**, 1016–1022.

**Métraux JP, Signer H, Ryals J, Ward E, Wyss-Benz M, Gaudin J, Raschdorf K, Schmid E, Blum W, Inverardi B** (1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* **250**, 1004–1006.

**Peng YJ, Yang JF, Li X, Zhang YL** (2021). Salicylic acid: biosynthesis and signaling. *Annu Rev Plant Biol* **72**, 761–791.

**Rasmussen JB, Hammerschmidt R, Zook MN** (1991).

Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*. *Plant Physiol* **97**, 1342–1347.

**Rekhter D, Lüdke D, Ding YL, Feussner K, Zienkiewicz K, Lipka V, Wiermer M, Zhang YL, Feussner I** (2019). Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid. *Science* **365**, 498–502.

**Rivas-San Vicente M, Plasencia J** (2011). Salicylic acid beyond defence: its role in plant growth and development. *J Exp Bot* **62**, 3321–3338.

**Saleem M, Fariduddin Q, Castroverde CDM** (2021). Salicylic acid: a key regulator of redox signaling and plant immunity. *Plant Physiol Biochem* **168**, 381–397.

**Sanders IO, Smith AR, Hall MA** (1989). The measurement of ethylene binding and metabolism in plant tissue. *Planta* **179**, 97–103.

**Shields A, Shivnauth V, Castroverde CDM** (2022). Salicylic acid and *N*-hydroxypipecolic acid at the fulcrum of the plant immunity-growth equilibrium. *Front Plant Sci* **13**, 841688.

**van Butselaar T, Van den Ackerveken G** (2020). Salicylic acid steers the growth-immunity tradeoff. *Trends Plant Sci* **25**, 566–576.

**Verberne MC, Brouwer N, Delbianco F, Linthorst HJM, Bol JF, Verpoorte R** (2002). Method for the extraction of the volatile compound salicylic acid from tobacco leaf material. *Phytochem Anal* **13**, 45–50.

**Waadt R, Seller CA, Hsu PK, Takahashi Y, Munemasa S, Schroeder JI** (2022). Plant hormone regulation of abiotic stress responses. *Nat Rev Mol Cell Biol* **23**, 680–694.

**Wang YK, Jin GC, Song SY, Jin YJ, Wang XW, Yang SQ, Shen XX, Gan YB, Wang YX, Li R, Liu JX, Hu JP, Pan RH** (2024). A peroxisomal cinnamate: CoA ligase-dependent phytohormone metabolic cascade in submerged rice germination. *Dev Cell* **59**, 1363–1378.

**White RF** (1979). Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology* **99**, 410–412.

**Xu L, Zhao HY, Wang JB, Wang XM, Jia XQ, Wang L, Xu Z, Li RL, Jiang K, Chen ZX, Luo J, Xie XD, Yi KK** (2023). AIM1-dependent high basal salicylic acid accumulation modulates stomatal aperture in rice. *New Phytol* **238**, 1420–1430.

**Yang CK, Shen SQ, Zhou S, Li YF, Mao YY, Zhou JJ, Shi YH, An LX, Zhou QQ, Peng WJ, Lyu Y, Liu XM, Chen W, Wang SC, Qu LH, Liu XQ, Fernie AR, Luo J** (2022). Rice metabolic regulatory network spanning the entire life cycle. *Mol Plant* **15**, 258–275.

**Zhang YJ, Yu QL, Gao SL, Yu NN, Zhao L, Wang JB, Zhao JZ, Huang P, Yao LB, Wang M, Zhang KW** (2022). Disruption of the primary salicylic acid hydroxylases in rice enhances broad-spectrum resistance against pathogens. *Plant Cell Environ* **45**, 2211–2225.

**Zhang YL, Li X** (2019). Salicylic acid: biosynthesis, perception, and contributions to plant immunity. *Curr Opin Plant Biol* **50**, 29–36.

**Zhao JZ, Yu NN, Ju M, Fan B, Zhang YJ, Zhu EG, Zhang MY, Zhang KW** (2019). ABC transporter OsABCG18 controls the shootward transport of cytokinins and grain yield in rice. *J Exp Bot* **70**, 6277–6291.

# Establishment and Application of a High-throughput Screening Method for Salicylic Acid Metabolic Mutants in Rice

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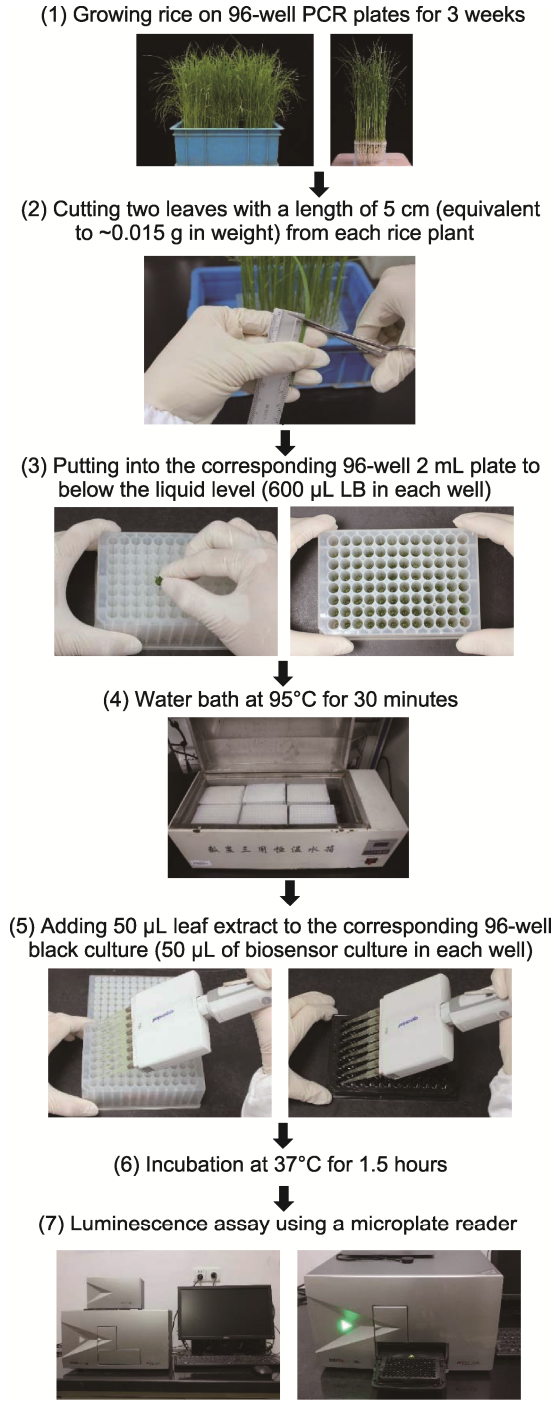
**INTRODUCTION:** Salicylic acid (SA) plays an important role in the plant immune system. The quantitative analysis of SA in plants is fundamental to studying SA metabolism and its biological functions. Although high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC/MS) are widely used for SA determination, their low throughput limits their suitability for large-scale analysis. However, the SA biosynthetic pathway in rice is not well understood, highlighting the need for efficient methods to screen SA-related mutants and elucidate SA metabolic pathways.

**RATIONALE:** Current methods for measuring endogenous SA levels, such as HPLC and LC/MS, involve labor-intensive sample preparation, making them unsuitable for high-throughput analysis. While a *lux* gene-based SA biosensor has been successfully used in tobacco and *Arabidopsis*, a reliable and efficient method for SA detection in rice remains unavailable. To address this problem, we optimized sample processing and operational workflows to enable high-throughput SA quantification in rice plants.

**RESULTS:** We developed a streamlined, high-throughput method for SA quantification in rice, eliminating time-consuming steps such as sample weighing, tissue grinding, and centrifugation. This approach significantly simplifies the process while maintaining efficiency and accuracy. We validated the method's feasibility using published rice SA metabolic mutants. We then applied it to screen a Cobalt-60 induced rice mutant library, identifying mutants with altered SA metabolism. Endogenous SA levels in these mutants were confirmed using HPLC. The results demonstrate the method's effectiveness in screening SA-related metabolic mutants, providing a valuable tool for studying SA metabolism and its roles in rice and other crops. The method was validated using known SA genetic materials. SA content-altered mutants were successfully isolated for further research.

**CONCLUSION:** This study establishes a rapid and cost-effective method for measuring SA content in rice tissues using the SA biosensor *Acinetobacter* sp. ADPWH_*lux*. Given the pivotal role of SA in plant defense, our method adopts streamlined sampling process, requiring only leaf clipping and boiling in LB medium, and dramatically reduces the time and effort associated with tissue collection and processing. This high-throughput approach is well-suited for large-scale screening of greenhouse-grown or hydroponic plants, providing a powerful platform for advancing research on SA metabolism and its biological functions in crops.




**Modified manipulating process for high-throughput determination of salicylic acid (SA) content using SA biosensor *Acinetobacter* sp. ADPWH_*lux* strain in rice**

**Key words** salicylic acid assay, biosensor, high throughput method, salicylic acid metabolism mutant, rice

**Ye C, Yao LB, Jin Y, Gao R, Tan Q, Li XY, Zhang YJ, Chen XF, Ma BJ, Zhang W, Zhang KW** (2025). Establishment and application of a high-throughput screening method for salicylic acid metabolic mutants in rice. *Chin Bull Bot* **60**, 586–596.

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