

储成才课题组主要从事农作物产量性状的分子基础及作物品种的分子设计改良研究,在水稻营养高效吸收利用、叶片衰老调控及源-库互作等分子机制和作物分子设计育种等研究领域发表了一系列原创性研究成果,为新一代“绿色超级稻”(少施化肥、少打农药、提高产量)及其他作物高产育种改良提供了全新的思路。近年来,已在 *Nature Genetics*, *Nature Plants*, *Genome Research*, *PNAS*, *Plant Cell* 等主流刊物发表论文 120 多篇,申请专利 40 多项,其中国际专利 8 项,与育种单位合作培育水稻新品种 3 个,累计推广面积达 1500 万亩。

## 水稻耐低温逆境研究：分子生理机制及育种展望

刘次桃<sup>1</sup>, 王威<sup>1</sup>, 毛毕刚<sup>2</sup>, 储成才<sup>1</sup>

1. 中国科学院遗传与发育生物学研究所, 植物基因组学国家重点实验室, 北京 100101

2. 湖南杂交水稻研究中心, 杂交水稻国家重点实验室, 长沙 410125

**摘要:** 低温严重影响水稻的地理分布、生长发育及产量。水稻在低温逆境下会产生一系列的生理及代谢变化, 如: 叶绿素荧光的改变, 电解质渗漏增加, 活性氧、丙二醛、蔗糖、脂质过氧化物、脯氨酸等代谢物含量升高, 植物内源激素 ABA 和 GA 的改变等。了解水稻在低温逆境下的生理代谢变化及低温应答分子机理对水稻耐低温性状的遗传改良具有重要的意义。本文系统总结了水稻在低温逆境下的生理代谢变化、已定位和克隆的耐低温基因/QTL, 以及水稻应答低温逆境信号转导机制的最新研究进展, 以期为水稻的耐低温育种提供参考。

**关键词:** 水稻; 低温胁迫; 耐低温相关基因; 数量性状; 信号传导

## Cold stress tolerance in rice: physiological changes, molecular mechanism, and future prospects

Citao Liu<sup>1</sup>, Wei Wang<sup>1</sup>, Bigang Mao<sup>2</sup>, Chengcai Chu<sup>1</sup>

1. State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

2. State Key Laboratory of Hybrid Rice, China National Hybrid Rice Research and Development Center, Changsha 410125, China

**Abstract:** Low temperature is a major factor affecting rice geographical distribution growth, development, and productivity. Cold stress mediates a series of physiological and metabolite changes, such as alterations in chlorophyll fluorescence,

收稿日期: 2018-01-08; 修回日期: 2018-02-13

基金项目: 国家自然科学基金青年基金项目(编号: 31501283)资助[Supported by the National Natural Science Foundation of China (No. 31501283)]

作者简介: 刘次桃, 博士, 研究方向: 水稻耐非生物逆境的基因克隆及功能分析。E-mail: liucitao2007@sina.com

通讯作者: 储成才, 博士, 研究员, 研究方向: 资源高效利用的分子基础和作物品种的分子设计。E-mail: ccchu@genetics.ac.cn

DOI: 10.16288/j.yczs.18-007

网络出版时间: 2018/2/26 10:54:00

URI: <http://kns.cnki.net/kcms/detail/11.1913.R.20180226.1053.002.html>

electrolyte leakage, reactive oxygen species (ROS), malondialdehyde (MDA), sucrose, lipid peroxides, proline, and other metabolites, plant endogenous hormones abscisic acid (ABA) and gibberellin (GA) also changes. In this review, we summarize the recent research progress on physiological and metabolic changes under low temperature, cold stress related loci and QTL reported by map-based cloning and genome-wide association analysis (GWAS), and some molecular mechanisms in response to low temperature in rice. We also discuss the future prospects on breeding cold tolerance varieties of rice.

**Keywords:** rice; cold stress; cold tolerance related genes; quantitative trait locus (QTL); signal transduction

水稻(*Oryza sativa* L.)是世界上最主要的粮食作物之一,世界上—半人口以水稻为主食<sup>[1,2]</sup>。水稻起源于热带、亚热带,相较于小麦(*Triticum aestivum* L.)、大麦(*Hordeum vulgare* L.)等作物,水稻对低温更加敏感。随着对粮食需求的日益增加,水稻种植区域不断由热带、亚热带地区向高海拔、高纬度地区扩张,水稻遭受冷害的机率也逐渐增加,世界上约有 24 个国家如中国、日本、朝鲜等都遇到过严重冷害问题<sup>[3]</sup>。我国稻区南北分布相差约 34 度(最北端漠河 53°27'N,最南端海南 18°90'N),海拔分布相差 2 700 米(从东南沿海到云贵高原),均有不同程度低温冷害情况。低温对水稻的影响主要发生在幼苗期和生殖生长期。水稻最适宜的生长温度是 25 ~ 30 。苗期,当环境温度低于 15 ,水稻的生理代谢就受到影响<sup>[2]</sup>;孕穗期特别是花粉母细胞减数分裂期,当温度低于 17°C 时结实率受影响<sup>[4]</sup>;开花授粉期,当环境温度持续 3 d 等于或者低于 20 时,将严重影响结实率<sup>[5,6]</sup>。例如在华南和长江中下游双季稻区,早稻经常受寒潮侵袭,常发生温度急剧降低或持续低温阴雨天气造成的不同程度春寒,引起烂秧、死苗,最终影响水稻产量<sup>[7-9]</sup>。生殖生长期,低温影响水稻的结实和灌浆<sup>[10]</sup>。在高纬度(如东北地区)和高海拔(如云贵高原)地区,水稻在生殖生长期受低温影响最为严重,平均每 3~4 年就会遭遇一次较大规模的冷害<sup>[11-13]</sup>。除此之外,在华南及长江中下游地区的晚稻也易遭受“寒露风”的危害。每年秋季“寒露”节气前后(9 月中下旬)是华南及长江中下游一带水稻抽穗扬花的关键时期,这时遇低温就会造成授粉和受精无法正常进行,水稻的瘪谷率大大增加,从而造成晚稻大幅减产<sup>[14]</sup>。尽管在全球气候变暖背景下,寒露风呈现减弱趋势,但近几年极端天气增多,寒露风等低温灾害又呈现增多趋势。低

温灾害导致我国每年粮食减产 3~5 亿吨<sup>[3,15]</sup>,因此培育耐低温水稻品种是水稻育种工作的重要方向。

植物耐低温胁迫是一个复杂的遗传性状,受多个基因/数量性状基因座控制。与其他农艺性状相比,水稻低温耐受性的遗传研究进展缓慢,目前只鉴定出少数耐低温基因<sup>[2,16,17]</sup>。本文系统总结了低温胁迫下水稻的生理代谢变化、已克隆的耐低温基因/QTL 以及水稻应答低温胁迫的信号转导途径,以期为水稻耐低温育种研究提供帮助。

## 1 水稻低温胁迫下生理代谢变化指标

低温不仅对水稻造成明显的外部损伤,如发芽率低、幼苗生长发育迟缓甚至死亡、结实率低等,还会引起一系列生理及代谢物的变化,如叶绿素荧光的改变,电解质渗漏增加,活性氧、丙二醛、蔗糖、脂质过氧化物、脯氨酸其他代谢物的含量升高<sup>[2]</sup>,植物激素脱落酸(abscisic acid, ABA)和赤霉素(gibberellins, GA)的改变等,这些生理水平的变化是衡量植物耐低温的生理指标。

### 1.1 叶绿素含量和叶绿素荧光

叶绿素含量高低和叶绿素荧光参数(最大荧光/可变荧光,  $F_v/F_m$ , 反映光系统 II 的最大光能转化效率)大小通常被作为判断植物耐受逆境胁迫的重要指标<sup>[18]</sup>。水稻在低温下会减少叶绿素合成以及叶绿体形成。因此,叶绿素含量以及  $F_v/F_m$  值变化是检验水稻对低温胁迫耐受能力的重要指标<sup>[18,19]</sup>。研究表明,在水稻和烟草(*Nicotiana tabacum* L.)中过表达 *OsiSAP8* 可以在低温条件下显著提高植物叶绿素含量和对低温的耐受能力<sup>[20]</sup>。此外,研究还发现 *OsAsr1* 过表达的转基因水稻  $F_v/F_m$  值显著升高,并在低温

条件下表现出明显的生长优势(表 1)<sup>[21]</sup>。

### 1.2 电解质渗漏(electrolyte leakages, EL)

细胞膜是水稻细胞最先感知低温冷害的部位，其在低温下的理化性质容易发生变化，从而导致细胞内电解质渗漏，因此电介质渗漏率常作为植物耐受低温的重要指标<sup>[22]</sup>。研究发现，在水稻中分别过表达 *OsOVPI* 和 *OsNAC5* 等基因导致植物的电解质渗漏率降低，从而表现出对低温的耐受性(表 1)<sup>[23, 24]</sup>。

### 1.3 活性氧(reactive oxygen species, ROS)与丙二醛(malondialdehyde, MDA)

水稻受低温等逆境胁迫时，细胞内氧代谢平衡

失调，产生 ROS(包括超氧阴离子  $O_2^-$ 、过氧化氢  $H_2O_2$  和氢氧根离子  $OH^-$ )，引发膜脂过氧化，从而造成细胞膜系统损伤<sup>[25]</sup>。ROS 还会促进多聚不饱和脂肪酸降解并产生 MDA，进而对植物组织和细胞进一步损害<sup>[26]</sup>。水稻对氧化胁迫的保护机制有两大系统，即酶系统和非酶系统。酶系统包括各种抗氧化酶，催化清除 ROS 反应的酶。其中超氧化物歧化酶(SOD)和过氧化氢酶(CAT)是两种最有效的抗氧化酶，这两种酶可以使超氧阴离子和过氧化氢变成水和氧分子，从而减轻 ROS 对细胞的伤害<sup>[27]</sup>。非酶系统包括各种抗氧化剂，其中还原型谷胱甘肽(GSH)和抗坏血酸(AsA)最为重要<sup>[28, 29]</sup>。如过量表达抗坏血酸过氧化物酶基因 *OsAPXa* 可以提高低温下抗坏血酸过氧化

表 1 水稻在低温下代谢和生理发生改变所涉及的基因

Table 1 Altered cold stress-related gene expression levels lead to metabolic and physiological changes in rice

生理代谢类型	基因	参考文献
光合作用		
叶绿素含量	<i>OsSAP8</i>	[20]
Fv/Fm	<i>CBF1/OsDREB1b</i> , <i>OsAsr1</i> , <i>OsASR3</i> , <i>OsCDPK7</i>	[56~58]
电介质渗漏	<i>CBF1/OsDREB1b</i> , <i>OsLti6</i> , <i>OsZFP245</i> , <i>OsOVPI</i> , <i>OsNAC5</i>	[23, 24, 47, 56, 59]
活性氧与丙二醛		
过氧化氢	<i>OsAPXa</i> , <i>OsMKK6</i> , <i>OsMPK3</i> , <i>OsNAC6</i> , <i>OsTrx23</i>	[29, 30, 60, 61]
超氧自由基	<i>OsAPXa</i>	[30]
羟基自由基	<i>OsAPXa</i>	[30]
丙二醛	<i>OsAPXa</i> , <i>OsMKK6</i> , <i>OsMPK3</i> , <i>OsNAC6</i>	[30, 60, 61]
可溶性糖		
蔗糖	<i>OsINV4</i> , <i>OsDREB1A</i>	[10, 62]
已糖	<i>OsINV4</i>	[10]
棉子糖	<i>OsDREB1A</i>	[62]
葡萄糖	<i>OsDREB1A</i>	[62]
果糖	<i>OsDREB1A</i>	[62]
海藻糖	<i>OsTPP1</i> , <i>OsTPP2</i> , <i>OsTPS1</i> , <i>OsNAC5</i>	[24, 35, 63]
低温相关氨基酸		
脯氨酸	<i>OsCOIN</i> , <i>OsDREB1A</i> , <i>OsMYB2</i> , <i>OsOVPI</i> , <i>OsNAC5</i> , <i>OsMYB4</i> , <i>OsPRP3</i> , <i>OsZFP245</i> , <i>OsMYB3R-2</i>	[23, 24, 42~47, 62, 64]
抗氧化剂		
抗坏血酸	<i>OsAPXa</i>	[30]
谷胱甘肽	<i>OsTrx23</i> , <i>OsCPK24</i>	[29, 65]
植物激素		
ABA	<i>OsPHS1</i> , <i>OsPHS 2</i> , <i>OsPHS 3-1</i> , <i>OsPHS 4</i> , <i>OsPDS</i> , <i>OsABA8ox1</i>	[51, 52]
GA	<i>SD1</i> , <i>D35</i>	[37]

物酶的活性,减少细胞内脂类物质的过氧化反应和 MDA 含量,从而提高水稻在低温下的结实率(表 1)<sup>[30]</sup>。

#### 1.4 可溶性糖与脯氨酸

水稻在低温逆境下会积累大量的可溶性糖(包括蔗糖、已糖、棉子糖、葡萄糖、果糖和海藻糖)。可溶性糖在低温逆境中可以作为细胞的渗透调节物质,稳定细胞膜和原生质胶体;同时还为其他有机物的合成提供碳骨架和能量<sup>[31-33]</sup>。过量表达海藻糖合成的关键基因 *OsTPP1*、*OsTPP2* 和 *OsTPS1* 均能显著提高水稻对低温的耐受性(表 1)<sup>[34,35]</sup>。

水稻在孕穗期,特别是花粉母细胞减数分裂期遇低温时,糖(包括蔗糖、葡萄糖、果糖)在花药中积累,同时伴随着蔗糖分解酶活性降低,单糖转运蛋白表达量下调,从而导致供应到绒毡层和花粉粒的蔗糖不足。而蔗糖是合成淀粉的主要成分,这就造成了花粉不育<sup>[10, 36]</sup>。Sakata 等<sup>[37]</sup>研究发现,外施蔗糖能极大地提高低温下水稻花粉的育性并提高结实率。这可能的解释是:在低温下,虽然流(从花药到花粉)受阻,但是增加外源的蔗糖,最终仍能增加库(花粉)的蔗糖含量,进而合成淀粉增多,可育花粉增多,结实率提高。

在低温下水稻还会积累大量的脯氨酸。脯氨酸广泛参与渗透调节、碳氮代谢、保护多数酶类物质,避免其变性失活<sup>[38]</sup>。同时,脯氨酸还具有稳定多聚核糖体、维持蛋白合成的作用<sup>[39]</sup>。在逆境条件下,脯氨酸还能够清除逆境反应产生的过量氢离子( $H^+$ ),维持细胞质中有氧呼吸的最佳 pH<sup>[40]</sup>。另外,脯氨酸通过其疏水基团与蛋白质结合来提高蛋白的亲水性<sup>[41]</sup>。研究发现,*OsCOIN*、*OsMYB2*、*OsMYB4*、*OsMYB3R-2* 和 *OsZFP245* 等基因的过表达植株均表现出脯氨酸含量显著提高和对低温耐受力的增强(表 1)<sup>[42-47]</sup>。

#### 1.5 ABA 与 GA 含量的改变

ABA 在低温逆境中扮演着重要角色<sup>[48]</sup>。一般而言,低温下增加 ABA 含量可能放大 ABA 信号,启动下游 ABA 应答耐低温相关基因表达量改变,从而提高植物对低温的耐受性。然而植物适应逆境与生

长发育是两个相互矛盾的过程:适应逆境就会牺牲生长发育,反之亦然<sup>[49]</sup>。在持续性低温逆境下,植物需要保持正常生长与适应逆境之间的平衡,以避免逆境信号过度放大<sup>[49, 50]</sup>。因此在低温逆境下,维持相对低的 ABA 水平有利于提高水稻对低温的耐受力。Du 等<sup>[51]</sup>研究发现,类胡萝卜素(ABA 合成的前体)的突变体 *phs1*、*phs2*、*phs3-1*、*phs4* 与 *PDS-RNAi* 干涉表达的转基因水稻在苗期和生殖生长期水稻内源 ABA 水平降低,但对低温的耐受性有所提高。过量表达水稻 ABA 代谢基因 *OsABA8ox1* 降低水稻幼苗内源 ABA 水平,在 15℃ 低温处理下,提高了转基因株系对低温的耐受力;但在 4℃ 条件下,转基因株系 27-5 提高了对低温的耐受力;株系 27-3 的 *OsABA8ox1* 表达量远比株系 27-5 的 *OsABA8ox1* 表达量高,相应的 ABA 含量 27-3 比 27-5 低很多,但是却表现出对低温敏感<sup>[52]</sup>。用水稻绒毡层特异性表达启动子 *OsG6B* 融合小麦 ABA 代谢基因 *TaABA8OH1* 的转基因水稻减少了花药中 ABA 含量,提高了花粉的育性和结实率<sup>[53]</sup>。过量表达 *OsNAC095* 提高水稻幼苗内源 ABA 水平,却使水稻对低温敏感<sup>[54]</sup>。Oliver 等<sup>[36]</sup>报道水稻敏感品种 *Doongara* 与耐低温品种 *R31* 在孕穗期经低温处理,内源 ABA 含量均升高,但是耐低温品种 *R31* 内源 ABA 升高幅度要低得多,加之本身 ABA 含量低,表现出 *R31* 更耐低温。这些结果表明,无论是苗期还是抽穗期,在一定的阈值范围内,维持相对低的 ABA 水平,有利于提高水稻对低温的耐受性;但是极低内源 ABA 水平对植物适应低温逆境是极不利的。

外施 ABA 能提高苗期水稻低温耐受力<sup>[55]</sup>。这可能是在苗期外施 ABA 能促使 ABA 信号放大,促进低温相关基因表达,进而提高水稻对低温的耐受性。但是在孕穗期,外施 ABA,导致 ABA 积累,而大量 ABA 抑制蔗糖转化酶和单糖转运蛋白基因表达,这就导致大量糖包括蔗糖在花药中积累,不能运输到花粉中,从而导致水稻花粉不育,结实率降低<sup>[10, 36, 53]</sup>。

孕穗期遇低温,水稻内源的赤霉素(gibberellins, GA)含量降低,GA 合成突变体 *sd1* 和 *d35* 对低温敏感;外施 GA 处理,能使 GA 合成突变体在低温下花粉可育性得到恢复,进而结实率得到恢复<sup>[37]</sup>。



2 耐低温相关基因/QTL 鉴定和克隆

2.1 图位克隆且精细定位的 QTL

近 20 年来, 以 Silewah、Koshihikari、M202、Norin-PL8、东乡野生稻、昆明小白谷、丽江新团黑谷等高海拔或高纬度的耐低温材料作为供体亲本, 构建了大量重组自交系(recombinant inbred lines, RIL)、双单倍体系(double haploid, DH)、回交自交系(backcross inbred lines, BIL)和近等基因系(nearly isogenic lines, NIL)等群体, 在水稻的 12 条染色体上定位到 250 多个与低温相关的 QTL<sup>[66~72]</sup>, 但大多数只局限在 QTL 的初步定位。目前, 水稻中精细定位

的仅有 5 个孕穗期耐低温 QTL(*qLTB3*<sup>[73]</sup>、*qCTB7*<sup>[11]</sup>、*qCTB8*<sup>[12]</sup>、*qCT-3-2*<sup>[15]</sup>和 *qCTB10-2*<sup>[74]</sup>, 表 3), 5 个苗期耐低温 QTL(*qCTS4*<sup>[75]</sup>、*qCtss11*<sup>[76]</sup>、*qSCT1*<sup>[77]</sup>、*qSCT11*<sup>[77]</sup>和 *qLOP2/qPSR2-1*<sup>[78]</sup>, 表 3), 1 个苗期和成熟期耐低温 QTL(*qRC10-2*<sup>[79]</sup>)以及 1 个萌发期耐低温 QTL(*qLTG-9*<sup>[80]</sup>, 表 3)。克隆且有功能鉴定的耐冷基因仅有 7 个, 包括 *qLTG3-1*、*COLD1*、*qCTS-9*、*GSTZ2*、*LTG1*、*Ctb1* 和 *CTB4a*(表 2)。*qLTG3-1* 编码一个富含甘氨酸(GRP)的保守结构域, 第 17 位氨基酸的差异(L17H)决定在水稻在低温条件下种子萌发的强与弱<sup>[81]</sup>。*COLD1* 编码一个 G 蛋白信号调节因子, 它能与 G 蛋白  $\alpha$  亚基 RGA1 互作, 促进  $\text{Ca}^{2+}$  内流并增强 G 蛋白的 GTP 酶活性, 进而增强水稻的耐寒

表 2 基于图位克隆的耐低温基因

Table 2 Cold tolerance genes based on map-based cloning technique

基因	LOC 号	是否有遗传功能验证	时期	表型鉴定	参考文献
<i>qLTG3-1</i>	LOC_Os03g01320	是	萌发期	萌发率	[81]
<i>COLD1</i>	LOC_Os04g51180	是	苗期	成活率	[82]
<i>qCTS-9</i>	LOC_Os09g24440	是	苗期	成活率	[83]
<i>GSTZ2</i>	LOC_Os12g10730	是	苗期	成活率	[84]
<i>LTG1</i>	LOC_Os02g40860	是	营养生长期	株高、分蘖数、产量	[85]
<i>Ctb1</i>	LOC_Os04g52830	是	孕穗期	结实率	[86]
<i>CTB4a</i>	LOC_Os04g04330	是	孕穗期	结实率	[3]

表 3 精细定位的耐低温 QTL

Table 3 Fine mapped cold tolerance QTL

QTL	候选基因 LOC 号或区间	是否有遗传功能验证	时期	表型鉴定	参考文献
<i>qLTB3</i>	LOC_Os03g57680 LOC_Os03g59200	否	孕穗期	结实率	[73]
<i>qCTB7</i>	RI02905–RM21862	否	孕穗期	结实率	[11]
<i>qCTB8</i>	RM5647–PLA61	否	孕穗期	结实率	[12]
<i>qCT-3-2</i>	Chr.3:1770855–2199078	否	孕穗期	结实率	[15]
<i>qCTB10-2</i>	LOC_Os10g11820 LOC_Os10g11730 LOC_Os10g11770 LOC_Os10g11810	否	孕穗期	结实率	[74]
<i>qCTS4</i>	CT245–CT236	否	苗期	成活率	[75]
<i>qCtss11</i>	LOC_Os11g40130 LOC_Os11g40160	否	苗期	成活率	[76]
<i>qSCT1</i>	LOC_Os01g69910	否	苗期	成活率	[77]
<i>qSCT11</i>	LOC_Os11g37720	否	苗期	成活率	[77]
<i>qLOP2/ qPSR2-1</i>	LOC_Os02g45450	否	苗期	成活率	[78]
<i>qRC10-2</i>	LOC_Os10g34790 LOC_Os10g34840	否	苗期和成熟期	成活率	[79]
<i>qLTG-9</i>	L9-25D–ID-1	否	萌发期	萌发率	[80]

性<sup>[82]</sup>。*qCTS-9* 在亲本丽江新团黑谷、黄山占 2 号及其重组自交系 RIL 群体的启动子区有一个 InDel 标记与低温的耐受性显著相关,过表达 *qCTS-9* 提高水稻对低温的耐受性<sup>[83]</sup>。*OsGSTZ2* 第 99 位的苯丙氨酸(Ile)对提高水稻苗期耐低温起重要作用<sup>[84]</sup>。*LTG1* 编码酪蛋白激酶 I,其编码区 357 位的氨基酸替换(I357K)对于低温环境下水稻的生长速率、抽穗期和产量都有重要的影响<sup>[85]</sup>。*Ctb1* 编码一个含有 F-box 结构域的蛋白,它与一个 E3 泛素连接酶亚基 Skp1 互作,参与泛素-蛋白酶体途径的低温信号传导<sup>[86]</sup>。*CTB4a* 编码一个保守的富亮氨酸受体样激酶 LRR-RLK(leucine-rich repeat receptor-like kinase),其与 ATP 合成酶的  $\beta$  亚基 AtpB 互作,影响 ATP 合成酶的活性,为低温下水稻灌浆提供能量供应。Zhang 等<sup>[3]</sup>分析了 119 个水稻品种的单倍型,结果显示 *CTB4a* 启动子区域的 3 个 SNP(分别是 ATG 上游 -2536、-2511 与 -1930)的差异决定不同水稻品种对低温耐受能力的强弱。

## 2.2 全基因组关联分析(genome-wide association study, GWAS)

虽然图位克隆是挖掘新基因的重要手段,但是图位克隆所得基因的遗传背景仅局限于构建遗传群体的双亲及它们的重组后代。利用图位克隆所进行的研究没有考虑到除亲本之外水稻种质资源的遗传多样性,并且局限于东亚的几个代表性的籼稻×粳稻亚种定位群体。GWAS 是以自然群体的同一染色体或不同染色体上不同座位的等位基因之间的遗传连锁不平衡为基础,从而对目标性状与遗传标记(或候选基因)的相关性进行分析的方法。利用全基因组范围单核苷酸多态性(single nucleotide polymorphism, SNP)为遗传标记,进行全基因组水平的相关性分析,能够有效发掘影响复杂性状的基因变异和优异等位变异<sup>[87-89]</sup>,从而为分子设计育种提供更为广泛的基因资源。

Fujino 等<sup>[90]</sup>收集了日本北海道 63 个水稻品种,并利用 GWAS 分析鉴定了 6 个抽穗期相关的 QTL 和 13 个低温萌发相关 QTL。Pan 等<sup>[14]</sup>利用 174 个来源于中国的水稻核心种质进行低温下萌发实验,获得 22 个低温萌发相关的 QTL(图 1,表 5)。Shakiba

等<sup>[91]</sup>利用水稻种质资源库 RDP1 中 400 多个水稻核心种质进行萌发期和生殖生长期耐低温筛选,分析并鉴定到 42 个苗期耐低温 QTL 和 29 个生殖生长期耐低温 QTL(图 1,表 5)。Wang 等<sup>[7]</sup>采用 RDP1 中 295 份水稻品种,对三叶期的水稻进行低温处理 3 天,以成活率为耐低温指标,利用 GWAS 分析获得了 67 个分布在水稻 11 条染色体上的苗期耐低温 QTL(图 1,表 4,表 5)。此外,该研究还发现一个候选基因 *Osryh1* 在耐低温品种与敏感品种间有一个 InDel 标记跟低温耐受性相关(图 1,表 3)。Lv<sup>[92]</sup>等对 529 份水稻核心种质在四叶期时进行自然条件下的耐低温处理(5 ~12 °C),以电解质渗漏为耐低温指标,获得了 132 个苗期低温关联位点。此外,该研究中的单倍型关联分析显示, *OsMYB2* 具有明显的籼粳基因型分化,粳稻型 *OsMYB2* 的水稻品种具有更强的耐低温能力(图 1,表 4)。Schläppi 等<sup>[8]</sup>利用来源于美国农业部(USDA)的 202 份水稻微核心种质进行苗期低温处理,通过 5 种苗期耐低温指标进行 GWAS 分析,获得 48 个耐低温相关 QTL(图 1,表 5)。但是目前通过 GWAS 获得的多数耐低温的 QTL 区间仍然很大,候选基因过多,耐低温主效基因仍不明确,还需要结合遗传学及转录组、蛋白组和代谢组等其他手段以获得更精确的定位和候选基因,并验证其遗传功能,为水稻分子育种提供优异的耐低温等位基因。

## 3 耐低温胁迫信号传导途径

植物作为不可移动的生物,在长期进化过程中产生了一系列适应逆境(如低温)的机制。植物适应低温的信号转导途径主要包括依赖 ABA 的信号转导途径和不依赖 ABA 的信号转导途径。低温信号转导途径主要组分包括核心转录因子和蛋白激酶。核心转录因子如 CBF(C-repeat-binding factor),蛋白激酶如 SNF1(sucrose nonfermenting 1 protein kinase)和 MAPK(mitogen-activated protein kinase)等。低温信号促进植物体离子平衡、水分运输以及代谢和基因表达的重调,进而维持低温环境下离子平衡和细胞稳定<sup>[98]</sup>。

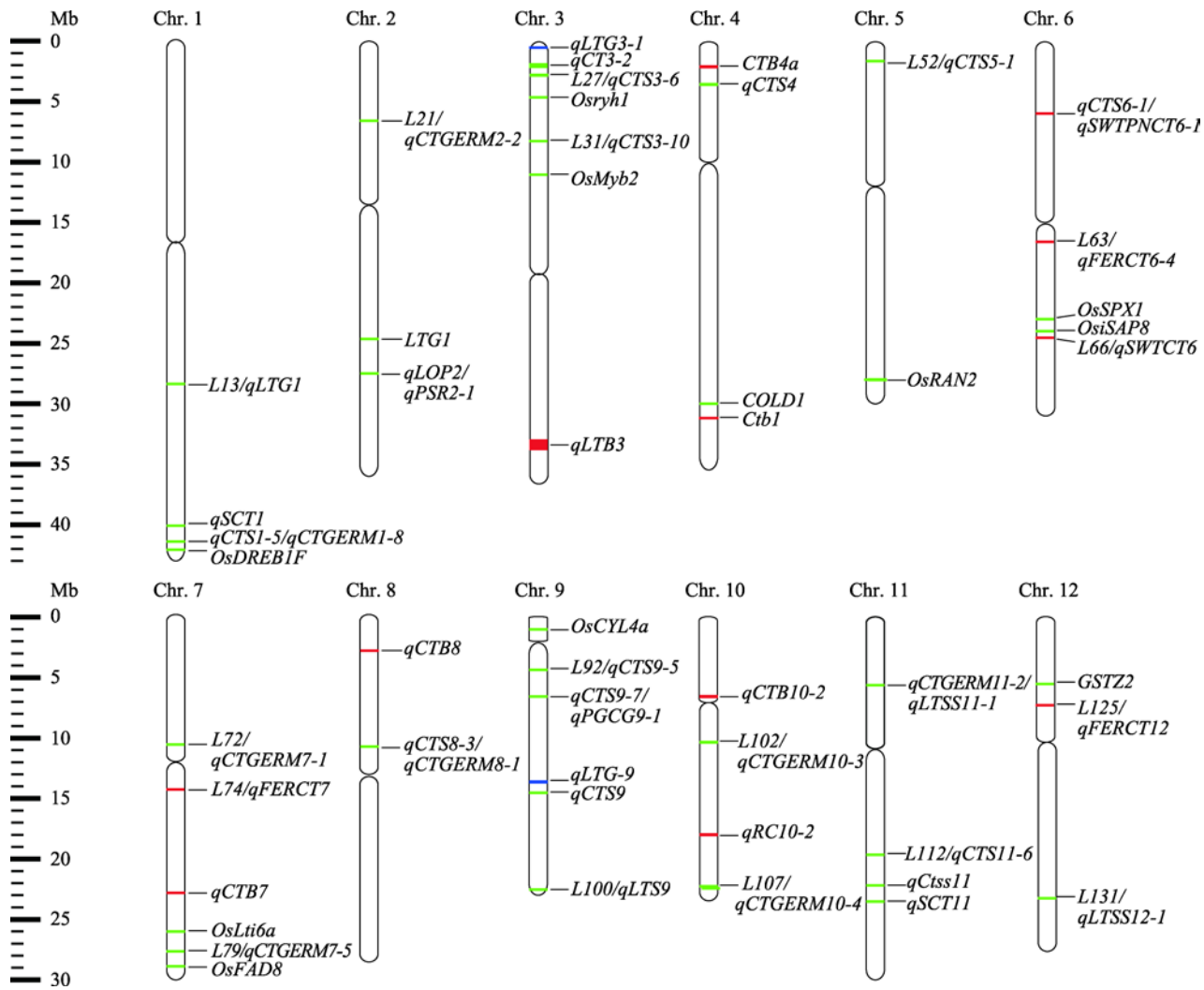


图 1 经图位克隆和 GWAS 定位的耐低温 QTL

Fig. 1 Fine-mapped and GWAS-mapped cold tolerance QTL

绿色代表苗期耐低温位点，红色代表孕穗期耐低温位点，蓝色代表萌发期耐低温位点。

表 4 GWAS 预测的候选耐低温基因

Table 4 Cold tolerance candidate genes predicted by GWAS

QTL	候选基因	LOC 号	是否有遗传功能验证	时期	表型鉴定	参考文献
<i>qCTS1-6</i>	<i>OsDREB1F</i>	LOC_Os01g73770	是	苗期	成活率	[7, 93]
<i>qCTS3-1</i>	<i>qLTG3-1</i>	LOC_Os03g01320	是	苗期	成活率	[7, 81]
<i>qCTS3-9</i>	<i>Osryh1</i>	LOC_Os03g09140	否	苗期	成活率	[7]
<i>qCTS6-5</i>	<i>OsSPX1</i>	LOC_Os06g40120	是	苗期	成活率	[7, 94]
<i>qCTS7-5</i>	<i>OsFAD8</i>	LOC_Os07g49310	是	苗期	成活率	[7, 95]
<i>qCTS9-1</i>	<i>OsCYL4a</i>	LOC_Os09g02270	是	苗期	成活率	[7, 96]
<i>L33</i>	<i>OsMyb2</i>	LOC_Os03g20090	是	苗期	电解质渗漏	[43, 92]
<i>L48</i>	<i>Ctb1</i>	LOC_Os04g52830	是	苗期	电解质渗漏	[6, 86, 92]
<i>L56</i>	<i>OsRAN2</i>	LOC_Os05g49890	是	苗期	电解质渗漏	[92, 97]
<i>L66</i>	<i>OsSAP8</i>	LOC_Os06g41010	是	苗期	电解质渗漏	[20, 92]
<i>L77</i>	<i>OsLti6a</i>	LOC_Os07g44180	是	苗期	电解质渗漏	[59, 92]

L：locus(位点)；qCTS：QTL for cold tolerance at seedling stage(苗期耐低温 QTL)。

表 5 通过 GWAS 获得的耐低温可重复定位的 QTL 区段

Table 5 Cold tolerance QTL co-localized by at least two independent GWAS

QTL	染色体	物理位置(Mb)	SNP (Mb)	是否有遗传功能验证	时期	参考文献
<i>L13/qLTG1</i>	1	28.260~28.458	28.359	否	苗期、萌发期	[8, 92]
<i>qCTS1-5/qCTGERM1-8</i>	1	41.518~41.786	41.676	否	苗期、萌发期	[6, 7]
<i>L21/qCTGERM2-2</i>	2	6.557~6.624	6.557	否	苗期、萌发期	[6, 7]
<i>L27/qCTS3-6</i>	3	2.851~3.173	2.680	否	苗期	[7, 92]
<i>L31/qCTS3-10</i>	3	8.060~8.248	8.192	否	苗期	[7, 92]
<i>L48/qCTGERM4-5</i>	4	31.247~31.428	31.436	否	苗期	[6, 92]
<i>L52/qCTS5-1</i>	5	1.865~2.058	1.970	否	苗期	[7, 92]
<i>qCTS6-1/qSWTPNCT6-1</i>	6	5.927~6.229	6.194	否	苗期、孕穗期	[6, 7]
<i>L63/qFERCT6-4</i>	6	16.588~16.770	16.659	否	苗期、孕穗期	[6, 92]
<i>L66/qSWTCT6</i>	6	24.708~24.901	24.812	否	苗期、孕穗期	[6, 92]
<i>L79/qCTGERM7-5</i>	7	27.651~27.844	27.749	否	苗期、萌发期	[6, 92]
<i>L72/qCTGERM7-1</i>	7	10.448~10.627	10.525	否	苗期、萌发期	[6, 92]
<i>L74/qFERCT7</i>	7	13.981~14.471	14.316	否	苗期、孕穗期	[6, 92]
<i>qCTS8-3/qCTGERM8-1</i>	8	10.438~10.903	10.506	否	苗期、萌发期	[6, 7]
<i>qCTS9-7/qPGCG9-1</i>	9	6.303~6.503	6.403	否	苗期、萌发期	[7, 8]
<i>L100/qLTS9</i>	9	22.701~22.887	22.701	否	苗期	[8, 92]
<i>L92/qCTS9-5</i>	9	4.304~4.488	4.350	否	苗期	[7, 92]
<i>L102/qCTGERM10-3</i>	10	10.165~10.335	10.257	否	苗期、萌发期	[6, 92]
<i>L107/qCTGERM10-4</i>	10	22.258~22.298	22.298	否	苗期、萌发期	[6, 92]
<i>qCTGERM11-2/qLTSS11-1</i>	11	5.500~5.710	5.590	否	苗期、萌发期	[6, 8]
<i>L112/qCTS11-6</i>	11	19.811~20.004	19.898	否	苗期	[7, 92]
<i>L125/qFERCT12</i>	12	7.118~7.312	7.243	否	苗期、孕穗期	[6, 92]
<i>L131/qLTSS12-1</i>	12	23.082~23.272	23.173	否	苗期	[8, 92]

L : locus (位点) ; qCTS : QTL for cold tolerance at seedling stage (苗期耐低温 QTL) ; LTG : low temperature tolerance at germination stage (萌发期耐低温) ;CTGERM :cold tolerance at germination stage (萌发期耐低温) ;FERCT :cold tolerance associated with spikelet fertility(以结实率为评价标准的耐低温) ; SWTPNCT : cold tolerance associated with seed weight per panicle(以单穗重量为评价标准的耐低温) ; SWTCT : cold tolerance associated with seed weight per plant(以单株产量为评价标准的耐低温) ; PGCG : plumule growth rate after cold germination(低温萌发后胚芽生长速率) ; LTS : low temperature survival (低温处理后存活率) ; LTSS : low temperature seedling survivability(苗期低温处理后存活率)。

3.1 ABA 依赖途径

植物激素 ABA 不仅在种子萌发、休眠、气孔开闭等生理过程中起重要作用，而且还对植物应对逆境条件起重要的调节作用，主要表现为 ABA 能在逆境下促进多数逆境应答基因的表达<sup>[99]</sup>。ABA 信号传导通路由 ABA 受体 PYR/PYL/RCAR (pyrabactin resistance/pyrabactin resistance-like/regulatory component of abscisic acid receptor)、负调控因子 2C 类蛋白磷酸酶(type 2C protein phosphatase, PP2C)、正调控因子 SNF1 相关的蛋白激酶 2(SNF1 related protein kinase 2, SnRK2)和转录因子 AREB/ABF 等 4 个核心组分共同组成一个双重负调控系统(PYR/

PYL/RCAR| PP2C| SnRK2-ABF/AREB)，调控 ABA 应答反应<sup>[100~103]</sup>。低温条件下，水稻内源的 ABA 升高，PYR/PYL/RCAR 与 ABA 结合，并与 PP2C 互作，抑制 PP2C 与 SnRK2 结合，SnRK2 便能磷酸化转录因子如 AREB、ABF 等，磷酸化的转录因子激活 ABA 响应基因表达，从而提高植物对低温的耐受性<sup>[104]</sup>。然而在正常条件下，内源 ABA 含量不变，PP2C 与 SnRK2 互作阻止 SnRK2 磷酸化下游的底物，从而遏 ABA 应答基因的表达(图 1)<sup>[104]</sup>。水稻中 ABA 受体 *OsPYL3* 和 *OsPYL9* 正向调节 ABA 信号，过表达 *OsPYL3* 和 *OsPYL9* 均能显著提高水稻耐冷性<sup>[105]</sup>。除了核心组分 PYL-PP2C-SnRK2-ABF 之外，ABA 信号通路的组分还包括 Ca<sup>2+</sup>、ROS、一氧化氮(NO)、



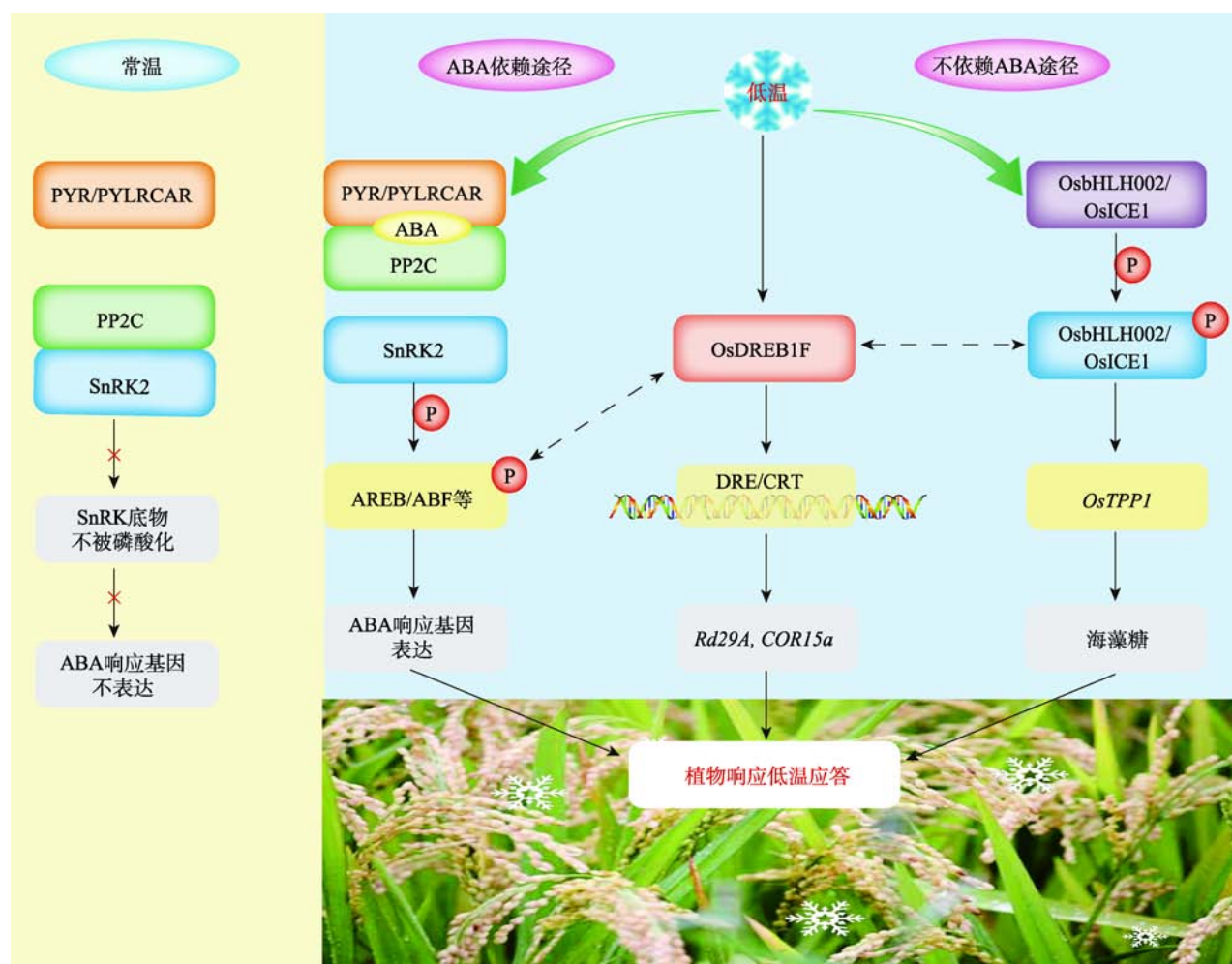


图2 水稻耐低温胁迫的信号传导途径

Fig. 2 Rice cold signal transduction pathway

水稻应答低温逆境信号分为 ABA 依赖途径和不依赖 ABA 途径。低温下水稻内源的 ABA 升高, 与 ABA 受体 PYR/PYL/RCAR 和 2C 类蛋白磷酸酶(PP2C)相结合, SNF1 相关蛋白激酶 2(SnRK2)磷酸化下游转录因子如 AREB/ABF 等, 进一步激活下游 ABA 响应低温应答基因表达, 从而提高水稻对低温的耐受性<sup>[104]</sup>; 然而在常温下, 由于 PP2C 与 SnRK2 相结合, SnRK2 就丧失了磷酸激酶活性, 下游底物也就不能被磷酸化, 也就没有低温应答反应<sup>[104]</sup>。OsbHLH002/OsICE1 受丝裂原活化蛋白激酶 OsMAPK3 磷酸化, 磷酸化的 OsbHLH002/OsICE1 激活下游海藻糖磷酸酶基因 *OsTPP1* 的表达, 水稻中海藻糖含量升高, 进而提高水稻对低温的耐受性<sup>[110]</sup>。转录因子 OsDREB1F 可能被上游 OsbHLH002/OsICE1 激活, 再激活下游含 DRE/CRT 低温响应基因 (*COR*) 的表达, 参与不依赖 ABA 的耐低温调控途径; 也可能直接参与不依赖 ABA 的耐低温调控途径; 还可能激活下游含 AREB/ABF 这类转录因子, 参与 ABA 依赖的耐低温信号传导途径<sup>[93]</sup>。

磷脂分子以及其他的激酶如 MAPK 等<sup>[98]</sup>。

### 3.2 不依赖 ABA 途径

CBF/DREB1(C-repeat-binding factor/dehydration-responsive element binding factor)是一类非 ABA 依赖的低温应答信号途径中重要的转录因子。CBF 属于 AP2/ERF (APETALA 2/ethylene responsive transcription factors)转录因子家族的一个亚家族, AP2/

ERF 家族主要分为 AP2、ERF、DREB 和 RAV(related to VP1/ABI3)4 个亚家族<sup>[106, 107]</sup>。CBF 含有 AP2 保守结构域, 在低温条件下, 其能结合含有核心元件为 CCGAC(又称 CRT, C-repeat)的低温响应基因(*CORs*)启动区, 并在转录水平上激活 *CORs*<sup>[108]</sup>。CBF 基因通常受 bHLH 类转录因子 ICE1(inducer of CBF expression 1)调控<sup>[108]</sup>。因此, 该低温调控途径又称为 ICE-CBF-COR 途径。现有研究证明, 在水稻中过量

表达 DREB1 亚家族的 *OsDREB1D*、*OsDREB1F* 均能提高水稻对低温的耐受性<sup>[93,109]</sup>。丝裂原活化蛋白激酶 OsMAPK3 磷酸化 OsbHLH002/OsICE1 降低 OsbHLH002 的泛素化水平, 进而促进活性 OsbHLH002 的积累; OsbHLH002 能激活下游基因 *OsTPP1* 的表达, 提高水稻海藻糖含量, 进而提高水稻对低温的耐受性(图 2)<sup>[110]</sup>。此外, 还有一些 CBF 基因受 ABA 诱导, 如 *OsDREB1F* 等。*OsDREB1F* 既参与不依赖 ABA 信号传导途径, 又参与 ABA 依赖信号传导途径<sup>[93]</sup>(图 1), 这就暗示着 ABA 依赖的信号传导途径与 ICE-CBF-COR 途径有重叠<sup>[111]</sup>。

## 4 结语与展望

未来水稻耐低温育种可以主要集中在以下几个方向: (1) 利用基因工程技术过表达耐低温基因或是敲除低温敏感基因。如过量表达 *OsMyb4*<sup>[44]</sup>、*OsNAC5*<sup>[24]</sup> 等; 敲除低温敏感基因, 如 *OsbZIP52*<sup>[112]</sup>; (2) 基于分子标记和连锁图谱获得了大量的耐低温相关 QTL 或基因, 而这些 QTL 遗传标记或基因是基于籼粳之间或栽培稻与野生稻之间差异而获得的, 因此, 可以利用分子标记辅助选择, 选择携带耐低温相关 QTL 或等位基因的供体亲本, 农艺性状优良主栽品种为轮回亲本, 经多代回交后将供体耐低温 QTL 或等位基因转育到受体材料中, 在育种群体世代繁殖过程中逐步开展表现型和基因型鉴定, 选择耐低温且农艺性状优良的品系; (3) 在改良多基因控制的复杂性状时, 分子标记辅助选择和分子标记辅助选择育种存在两个方面的缺陷: 一是基于双亲的 QTL 定位结果有时不具有普遍性, 遗传群体中的 QTL 定位结果不能很好地应用于育种群体中; 二是耐低温多由多个微效基因控制, 不能将这些数量基因位点有效地应用于数量性状改良。全基因组选择 (genomic or genome-wide selection, GS) 是在高密度分子标记基因型鉴定的情况下, 利用覆盖全基因组的 SNP 标记或等位基因型, 以及群体中个体的表型数据, 估计每个标记的遗传效应, 从而得到基因组估计的育种值 (genomic estimated breeding value, GEBV)。在后续的育种群体中, 利用每一个标记的估计效应和个体的基因型鉴定数据, 预测个体的表型或育种值, 然后根据预测表型选择优良后代<sup>[113~116]</sup>; (4) 利用基因组编辑技术改良水稻品种, 如将籼稻品

种中的 *COLD1*<sup>Ind</sup> 基因型替换为粳稻型的 *COLD1*<sup>Jap</sup>。

总之, 随着对水稻低温胁迫应答机制的深入理解, 水稻耐低温育种改良也逐渐成为可能。

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