

· 热点评述 ·

## 赤霉素和油菜素内酯信号通路双重调控助力小麦新一轮“绿色革命”

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**摘要** 自20世纪60年代以来, 半矮秆基因*Rht-B1b*和*Rht-D1b*的利用显著提高了小麦(*Triticum aestivum*)抗倒伏能力和收获指数, 使得全世界小麦产量翻了一番, 引发了农业第1次“绿色革命”。*Rht-B1b*和*Rht-D1b*编码植物生长抑制因子DELLA蛋白, 是赤霉素(GA)信号转导途径的负调控因子。DELLA蛋白积累抑制细胞分裂和细胞伸长, 导致矮化表型; 同时也抑制光合作用并降低氮素利用效率, 导致半矮化品种需要较高的化肥投入才能获得高产。如何“减肥增效”是实现低碳绿色农业所面临的重要问题。最近, 中国农业大学倪中福团队发现了具有育种应用价值的新型“半矮秆”基因模块, 证明通过对赤霉素和油菜素内酯(BR)信号通路的双重调控可实现矮秆高产小麦新品种培育。该团队鉴定并克隆了1个控制小麦株高和粒重的数量性状位点(QTL), 该QTL在衡597中存在1个约500 kb的*r-e-z*大片段缺失, 其中包括*Rht-B1b*基因和1个编码RING E3泛素连接酶的*ZnF-B*基因。研究发现, *ZnF-B*蛋白与油菜素内酯信号转导途径的抑制因子TaBKI1相互作用, 诱导TaBKI1降解, 从而促进BR信号转导。*ZnF-B*单敲除导致小麦株高和粒重降低, 影响小麦产量; *ZnF-B1*和*Rht-B1b*双敲除植株株高不变, 但小麦粒重和氮肥利用效率增高。该研究不仅揭示了BR信号转导调控的新机制, 而且提出了通过调控GA和BR双重信号转导机制实现农业可持续发展的育种新策略, 助力小麦新一轮“绿色革命”。

**关键词** 小麦, 绿色革命, 株高, 赤霉素, 油菜素内酯

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自20世纪60年代以来, “绿色革命”半矮秆农作物品种的育成和大面积推广有效解决了“高产与倒伏”之间的矛盾, 提高了农作物的收获指数和产量, 缓解了世界范围内因人口快速增长而引发的粮食危机(Khush, 1999; Pingali, 2012)。研究表明, “绿色革命”半矮化育种是通过调控植物激素赤霉素(gibberellin, GA)的生物合成和信号转导来实现(Liu et al., 2022)。水稻(*Oryza sativa*)的半矮秆基因*SD1*(*SEMI-DWARFING1*)编码GA合成酶GA-20ox2, 该基因突变导致GA合成受阻, 植物生长抑制因子DELLA蛋白积累, 抑制水稻生长, 从而产生半矮秆表型(Sasaki et al., 2002; Spielmeyer et al., 2002)。而小麦(*Triticum aestivum*)的半矮秆育种主要利用GA信号

转导的关键负调控位点*Rht-1*, 该位点的2个显性等位变异产生N端截短的*Rht-B1b*和*Rht-D1b*, 使得其编码的突变DELLA蛋白保持相对稳定, 不易被降解, 从而对GA不敏感, 产生半矮秆性状(Peng et al., 1999)。

水稻和小麦半矮秆高产品种株高降低、抗倒伏性增强, 但同时这些品种也表现出生长发育对氮肥响应减弱、根系吸收氮素能力下降以及氮肥利用效率低的弊端(Li et al., 2018; Wu et al., 2020; Liu et al., 2022)。为了增加水稻和小麦的产量, 农民不得不施加大量化肥。这不仅增加农业生产成本, 还导致环境污染, 引起土壤酸化和水体富营养化等一系列环境问题。近年来, 水稻株高与氮肥利用效率偶联和解偶联

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的机理解析取得系列重大进展。水稻生长调节因子GRF4 (GROWTH-REGULATING FACTOR 4)与GIF1 (GRF-INTERACTING FACTOR 1)相互结合, 激活下游氮素吸收同化和光合固碳相关基因的表达, 增强光合作用和氮代谢, 进而促进植物的生长发育。DELLA蛋白抑制GRF4与GIF1的结合, 减弱GRF4转录激活能力, 进而降低氮肥的利用效率(Li et al., 2018)。氮响应调控因子NGR5 (NITROGEN-MEDIATED TILLER GROWTH RESPONSE 5)可招募PRC2蛋白复合体, 调控下游分蘖控制基因的组蛋白修饰抑制基因表达, 进而促进水稻分蘖。GA通过其受体GID1促进NGR5蛋白降解, 导致表观遗传修饰水平降低, 进而增强靶基因的转录激活活性。而DELLA蛋白与NGR5互作, 竞争性结合GID1, 减弱NGR5降解(Wu et al., 2020)。这些研究表明, 在水稻中维持半矮化优良性状不变的情况下, 通过筛选GRF4和NGR5的优异等位基因, 可在减少氮肥投入的同时提高水稻和小麦的产量, 实现“少投入、多产出、保护环境”的育种目标。但目前小麦株高和氮肥利用效率之间的偶联机制尚不明确, 缺乏调控氮高效利用的矮秆小麦关键基因及其优异单倍体型。因此, 鉴定新的“绿色革命”关键基因及其优异单倍体型, 对小麦高产和氮高效利用协同改良的分子设计育种具有重要意义。

油菜素内酯(brassinosteroids, BRs)是调控作物株高的另一重要植物激素, 其合成缺陷或信号转导异常均导致作物矮化(Tong and Chu, 2018)。研究表明, GA信号转导途径关键元件DELLA蛋白可直接与BR信号通路转录因子BZR1相互作用, 抑制BZR1的转录活性; 同时, BR通过BZR1促进GA合成基因的表达, 诱导DELLA蛋白降解, 释放对BZR1的抑制作用, 从而促进植物生长(Bai et al., 2012; Gallego-Bartolome et al., 2012; Li et al., 2012; Tong et al., 2014)。尽管GA的合成与信号转导相关基因已广泛应用于培育半矮秆高产作物品种, 但BR合成和信号转导相关的矮秆基因是否具有育种应用价值鲜有报道, 亟需开展系统深入的研究。

中国农业大学倪中福团队长期致力于小麦杂种优势利用和高产抗逆关键基因挖掘, 在小麦遗传育种与新品种培育方面取得了一系列重要进展。最近, 该团队在小麦“绿色革命”新基因挖掘和机制解析方

面取得了重要进展(Song et al., 2023)。他们以石4185和衡597为亲本材料构建了遗传群体, 将1个控制株高和千粒重的主效QTL定位在小麦4B染色体上, 通过图位克隆和序列比对, 确定在衡597中存在1个约500 kb的大片段缺失, 并将其命名为r-e-z片段。为分析r-e-z单倍体型对小麦农艺性状的影响, 研究人员通过杂交和连续回交构建了2种单倍体型的近等基因系, 即含有r-e-z片段的NIL-Shi和不含有该片段的NIL-Heng。研究发现, 2个近等基因系的株高相近, 但NIL-Heng表现出更为优异的农艺性状, 包括株型紧凑、茎秆粗壮、旗叶宽厚、麦穗硕大、千粒重增加, 并具有较高的氮肥利用效率。田间测产显示, 无论高密度还是低密度种植, NIL-Heng均表现出更高的氮肥利用效率和收获指数, 平均产量较NIL-Shi增加约12%。此外, NIL-Shi在大田中会出现部分倒伏的现象, 而NIL-Heng即使在高密度种植的情况下也未发现倒伏。上述结果表明, r-e-z片段缺失在维持半矮秆的同时显著提高了小麦的氮肥利用效率, 增加了小麦产量。

在r-e-z片段中包含3个高度保守的基因Rht-B1、EamA-B和ZnF-B。为分析其中哪个基因对调控r-e-z单倍体型小麦株型和千粒重有主要贡献, 研究人员利用基因编辑技术在小麦Fielder品种中分别构建了这3个基因的敲除材料(Song et al., 2023)。Fielder具有与NIL-Shi一样的单倍体型, 在4B染色体上含有Rht-B1、EamA-B和ZnF-B三个基因。与Fielder对照相比, 敲除Rht-B1 (rht1-bb)导致小麦株高增加14.22 cm, 千粒重增加5.59 g; 敲除ZnF-B (znf-bb)使得小麦株高降低8.4 cm, 千粒重减少1.74 g; 而敲除EamA-B (eama-bb)对小麦的株高和千粒重无明显影响。这些结果说明, 在r-e-z片段缺失小麦中, ZnF-B缺失可导致株高和千粒重降低的表型, 但对穗长无明显影响; 而Rht-B1b敲除显著增加小麦株高、穗长和千粒重。研究还发现, 同时敲除Rht-B1和ZnF-B表现出与Fielder类似的株高, 但穗长、籽粒长度和千粒重显著增加。因此, 缺失ZnF-B具有与Rht-1相似的矮秆性状, 但对穗部发育和千粒重的负效应小于Rht-B1b和Rht-D1b, 具有在半矮秆育种中替代“绿色革命”基因的巨大潜力。

为解析ZnF调控小麦株高和千粒重的分子机理, 研究人员同时敲除了ZnF在基因组A、B、D中的同源

基因，构建了 $znf-aabbdd$ 突变体。该突变体的株高、穗长、籽粒长度、籽粒宽度和千粒重均较Fielder显著降低。转录组学分析显示，BR合成基因 $TaDWF4$ 和 $TaBRD2$ 以及BR信号转导基因 $TaBRI1$ 、 $TaTUD1$ 、 $TaRAVL1$ 、 $TaDLT$ 和 $TaBZR1$ 在 $znf-aabbdd$ 突变体中的表达量均上升。BR含量测定显示，Fielder与 $znf-aabbdd$ 突变体中内源的BR含量并无显著差异。

此外， $znf-aabbdd$ 突变体的胚芽鞘变短，并表现为对BR不敏感。这些结果表明，ZnF是一个BR信号转导的正调控因子(Song et al., 2023)。

近年来，BR信号转导研究进展迅速。在植物体BR含量低时，BR受体BRI1被负调控因子BKI1结合，抑制BRI1活性，阻断BR信号转导。当植物体内BR含量升高时，BR被位于质膜上的受体BRI1和共受体BAK1

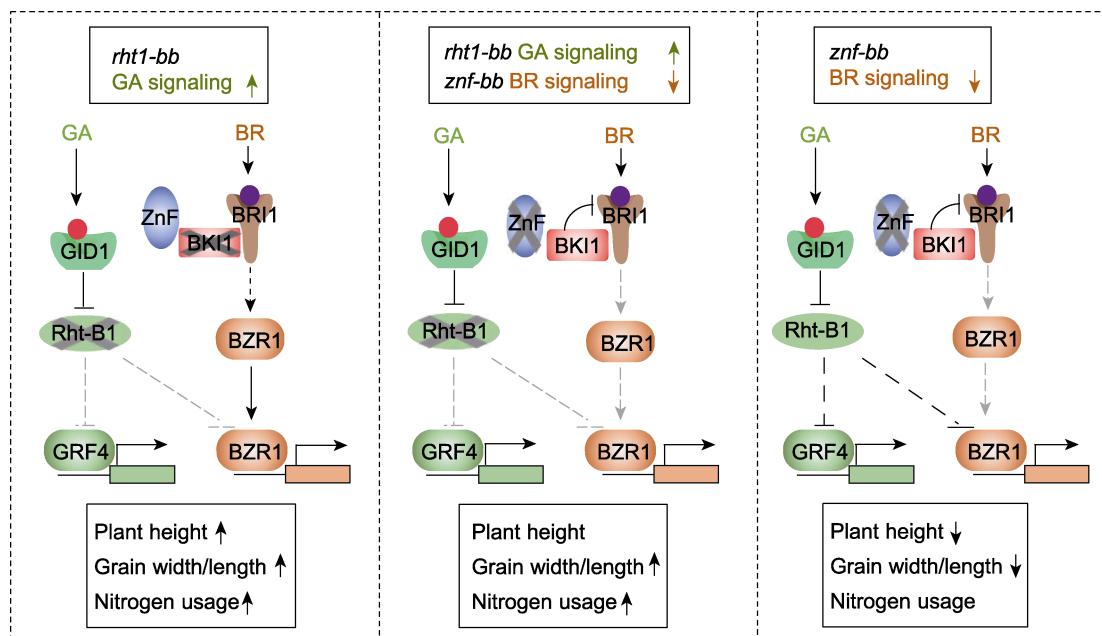


图1 “绿色革命”基因模块Rht-B1-ZnF调控小麦重要农艺性状示意图

Rht-B1是赤霉素(GA)信号通路负调控因子，GA结合受体GID1后诱导Rht-B1蛋白降解，解除Rht-B1对转录因子GRF4和BZR1的抑制作用，从而增加株高及提高千粒重和氮肥利用效率。ZnF是油菜素内酯(BR)信号通路的正调控因子，BR结合受体BRI1后促进ZnF与BR信号转导负调控因子BKI1结合，泛素化并促进BKI1降解，解除BKI1对BRI1的抑制作用，触发信号级联传递，进而激活转录因子BZR1，从而增加株高，提高千粒重。单独敲除Rht-B1 (*rht1-bb*)激活GA信号，导致小麦株高、千粒重和氮肥利用效率提高；单独敲除ZnF (*znf-bb*)抑制BR信号，导致株高和千粒重降低；同时敲除Rht-B1和ZnF，小麦株高不变，但千粒重和氮肥利用效率提高。Rht-B1-ZnF基因模块为新一轮“绿色革命”高产高效育种提供了具有育种利用价值的基因资源。实线箭头表示激活目的基因；虚线箭头表示以未知机制激活目的基因；T型实线表示抑制目的基因活性；T型虚线表示以未知机制抑制基因活性。

**Figure 1** Schematic diagram of the “Green Revolution” Rht-B1-ZnF gene module regulating wheat agronomic traits. Rht-B1, a negative regulator of gibberellin (GA) signaling pathway, physically interacts with transcription factors GRF4 and BZR1 to inhibit their DNA binding ability. GA binding to the soluble receptor GID1 induces the degradation of Rht-B1, and releases the repression of Rht-B1 on GRF4 and BZR1, thereby promoting wheat height, 1000-grain weight and nitrogen use efficiency. ZnF is a positive regulator of brassinosteroid (BR) signaling pathway. BR binding to BRI1 promotes the binding ability of ZnF to BKI1, which directly associates with BRI1 to inhibit BRI1 activity. ZnF promotes BKI1 degradation to activate BRI1 and BR signaling, thereby promoting plant height and 1000-grain weight. *Rht-B1* deletion (*rht1-bb*) activated GA signaling, and resulted in the dramatically increased in plant height, 1000-grain weight and nitrogen use efficiency, whereas *ZnF-B* deletion (*znf-bb*) inhibited BR signaling, and led to the reduced plant height and 1000-grain weight. The *rht1-bb/znf-bb* double mutant did not change the plant height but increased grain yield and nitrogen use efficiency. The “Green Revolution” Rht-B1-ZnF gene module can be used to next generation breeding which aims to crops with high yield and high nitrogen use efficiency. The solid-line arrow indicates activation of the target gene; the dashed-line arrow represents activation of the target gene by an unknown mechanism; the T-shaped solid line indicates inhibition of target gene activity; the T-shaped dashed line represents inhibition of gene activity by an unknown mechanism.

所感知, 触发级联信号, 抑制糖原合成酶激酶BIN2, 促进转录因子BZR1和BES1调控众多下游靶基因表达, 从而调控植物的生长发育(Han et al., 2023)。*ZnF*编码1个具有7次跨膜结构域的RING E3泛素连接酶。为解析*ZnF*在质膜上如何调控BR信号转导, 研究人员分析了*ZnF*与小麦BR信号转导途径关键元件TaBRI1、TaBAK1以及TaBKI1相互作用。结果显示, *ZnF*特异地与TaBRI1和TaBKI1相互作用, 但不与TaBAK1相互作用。BR处理能增强*ZnF*与TaBKI1的结合, 但减弱*ZnF*与TaBRI1的结合。在TaBRI1存在时, *ZnF*与TaBKI1的结合能力增强。进一步研究发现, *ZnF*在细胞质膜上特异地泛素化TaBKI1, 并促进TaBKI1降解。这些结果表明, BR通过TaBRI1增强*ZnF*与TaBKI1的结合, 诱导TaBKI1被泛素化降解, 解除TaBKI1对TaBRI1的抑制作用, 进而促进BR信号转导(Song et al., 2023)。

研究人员对全球范围内的556份小麦品种进行分析, 发现仅有12份中国小麦品种具有*r-e-z*片段缺失的单倍体型, 且这些品种大部分表现为麦穗增大、千粒重增加而株高变化不明显的优良性状。研究人员将含有*r-e-z*片段缺失单倍体型的小麦品种Erwa与含“绿色革命”矮秆基因的农大4803杂交, 并利用分子标记技术选育出含有*r-e-z*片段缺失单倍体型的4个品系。田间测产显示, 这4个品系比目前主栽的高产品种良星99增产6.84%–15.25%。上述研究结果表明, *r-e-z*缺失是一个新的“绿色革命”优异单倍体型, 不仅能有效降低株高还具有进一步提高小麦产量的潜力(Song et al., 2023)。

该项研究是小麦功能基因组研究的重大进展之一, 不仅鉴定到BR信号转导途径1个新的关键元件*ZnF*, 而且进一步完善了BR信号转导调控机制; 揭示了通过敲除*Rht-B1*激活GA信号和敲除*ZnF*抑制BR信号来协同调控小麦株高和籽粒发育的分子机制; 进一步提出了通过双重调控GA和BR信号转导机制来设计新型半矮秆高产小麦品种的育种策略, 助力新一轮“绿色革命”, 为低碳绿色农业发展奠定了新的理论基础(图1)。

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## Coordinated Regulation of Gibberellin and Brassinosteroid Signalings Drives Toward a Sustainable “Green Revolution” by Breeding the New Generation of High-yield Wheat

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**Abstract** Since the 1960s, the utilization of semi-dwarfing genes *Rht-B1b* and *Rht-D1b* has significantly improved the lodging resistance and harvest index of wheat (*Triticum aestivum*), leading to a doubling of global wheat production and triggering the “Green Revolution” in agriculture. *Rht-B1b* and *Rht-D1b* encode plant growth-inhibiting factors, DELLA proteins, which are negative regulatory factors in the gibberellin (GA) signaling pathway. Accumulation of DELLA proteins not only inhibits cell division and elongation, leading to a dwarf phenotype, but also suppresses photosynthesis and nitrogen use efficiency, resulting in semi-dwarf varieties requiring higher fertilizer inputs to achieve high yields. Addressing the challenge of “reducing fertilizer inputs while increasing efficiency” is a crucial issue for achieving green and low-carbon agriculture. Recently, Zhongfu Ni and his colleagues from China Agricultural University identified a novel “semi-dwarfing” regulatory module with potential breeding applications and demonstrated that reducing brassinosteroid (BR) signaling could enhance grain yield of wheat “Green Revolution” varieties (GRVs). They isolated and characterized a major QTL responsible for plant height and 1000-grain weight in wheat. Positional cloning and functional analysis revealed that this QTL was associated with a ~500 kb fragment deletion in the Heng597 genome, designated as *r-e-z*, which contains *Rht-B1* and *ZnF-B* (encoding a RING E3 ligase). *ZnF-B* was found to positively regulate BR signaling by triggering the degradation of BR signaling repressor BRI1 Kinase Inhibitor (TaBKI1). Further experiments showed that deletion of *ZnF-B* not only caused the semi-dwarf phenotypes in the absence of *Rht-B1b* and *Rht-D1b* alleles, but also enhanced grain yield at low nitrogen fertilization levels. Thus, manipulation of GA and BR signaling provides a new breeding strategy to improve grain yield and nitrogen use efficiency of wheat GRVs without affecting beneficial semi-dwarfism, which will drive toward a new “Green Revolution” in wheat.

**Key words** wheat, Green Revolution, plant height, gibberellin, brassinosteroid

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