

Can heterotrimeric G proteins improve sustainable crop production and promote a more sustainable Green Revolution?

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The Green Revolution of the 1960s dramatically boosted cereal yields, as semi-dwarfism brought lodging resistance and improved harvest index. However, the worldwide spread of Green Revolution variations (GRVs) has resulted in high-input, resource-intensive agriculture that cannot be sustained over the long term. The challenges to agricultural sustainability caused by the combination of environmental degradation, global climate change, and population growth, include: (i) how to maintain stable grain yield

and quality in the face of climate extremes and warmer average temperatures, (ii) how to reduce dependence on fertilizer resources needed to grow high-yielding GRVs, (iii) how to break the trade-off between grain yield and quality, and (iv) how to improve utilization of marginal lands. To cope with these challenges and ensure food security, new GRVs that can better cope with environmental fluctuations are urgently needed to sustainably enhance crop yields.

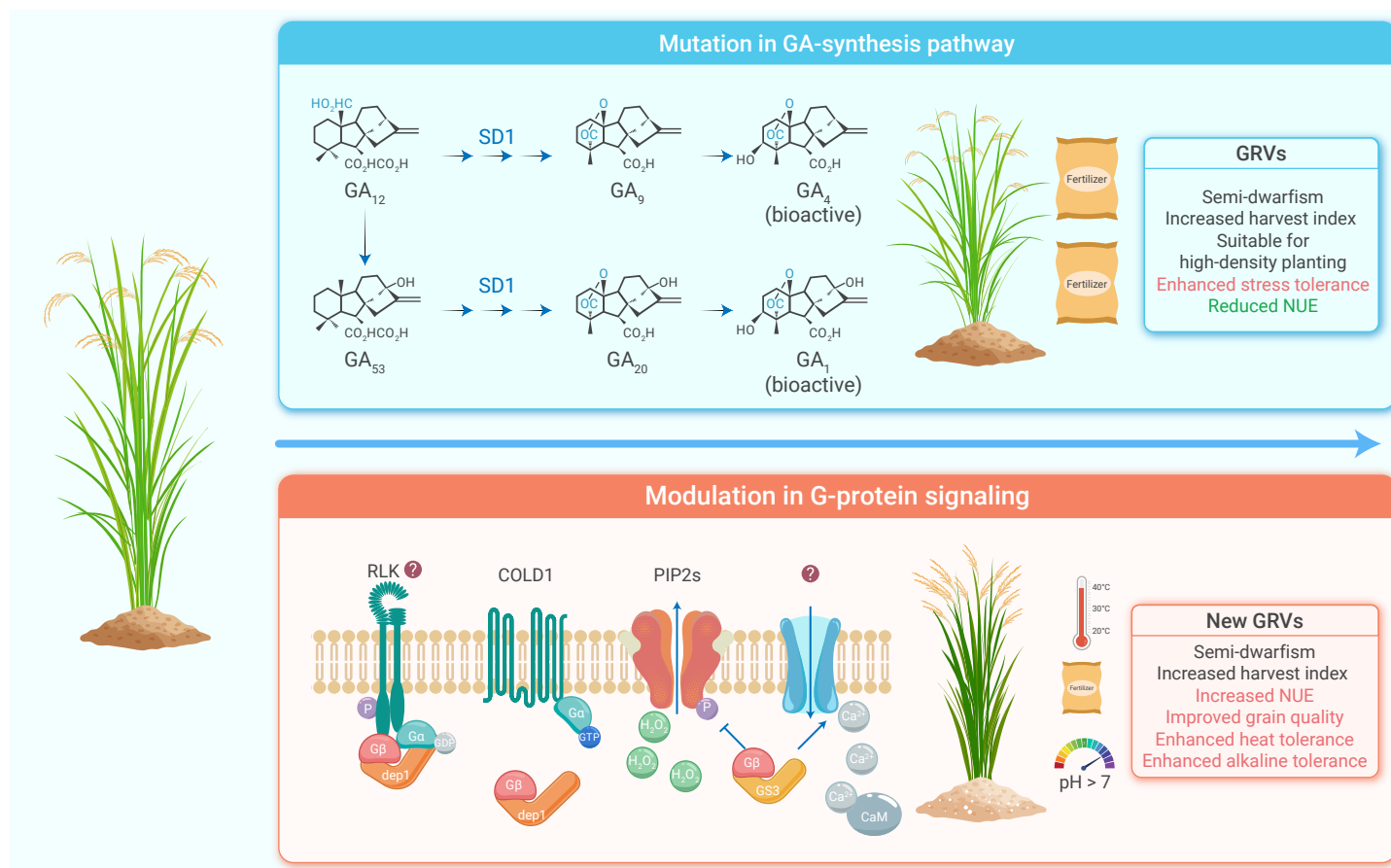


Figure 1. Manipulation of G proteins enables future sustainable Green Revolution.

Guanine nucleotide-binding proteins (G proteins) are cell membrane-anchored glycoproteins that transmit extracellular signals to intracellular effectors to regulate plant growth. In G-protein signaling, the guanosine diphosphate (GDP)-bound Ga subunit interacts with the Gβγ dimer to form an inactive heterotrimer. After GDP is exchanged for guanosine triphosphate (GTP), the GTP-Ga monomer is activated and separates from the Gβγ dimer to initiate the downstream signaling cascade (Figure 1). The rice genome encodes a single subunit each of Ga (RGA1) and Gβ (RGA1), two typical Gy subunits (RGG1 and RGG2), and three atypical Gy subunits (GS3, DEP1, and OsGGC2). Recent research on rice G-protein signaling indicated that G proteins have the potential to be engineered to: (i) further increase grain yield and harvest index by breaking the link between the semi-dwarfism trait and decreased grain number per panicle in GRVs, (ii) synergistically improve grain

quality and yield, (iii) coordinate plant growth and metabolism to obtain higher yields with lower applications of nitrogen (N) fertilizer, (iv) enable crop production on marginal lands, and (v) enhance crop resilience to the abiotic stresses associated with climate change.

RETAINING SEMI-DWARFISM AND INCREASING GRAIN PRODUCTION

Introducing the semi-dwarfism trait into crops was a revolutionary plant architecture modification, as it contributed to lodging-resistance and harvest index, greatly increasing crop yields during the Green Revolution. The semi-dwarf genes, *Rht-1* in wheat encoding a suppressor DELLA protein in gibberellin (GA) signaling, and *sd1* in rice conferring loss of function in GA biosynthesis (Figure 1), led to DELLA accumulation and the resulting semi-dwarf phenotype, but coupling with lower N-use efficiency (NUE) and lower

grain number per panicle. Thus, how to breed semi-dwarf GRVs that can bypass the lower grain number caused by DELLA accumulation is critical for crop improvement.

The rice semi-dwarf gene *sd1* originated mainly from *indica* varieties in Southern China and then spread around the world. Notably, most *japonica* rice varieties, especially in Northeast China and the Yangtze River Valley, carry a different semi-dwarf allele *dep1-1* encoding a Gy protein with C-terminal truncation. Thus, semi-dwarfism in *japonica* rice is mainly dependent on G-protein function rather than the GA signaling. *DEP1* was first reported to regulate panicle branching and grain number. The dominant *dep1-1* allele conferred erect and dense panicle, thus significantly enhancing grain number and yield. Near-isogenic lines (NILs) harboring *dep1-1* allele were also shorter in stature, with thicker and sturdier stems, and improved canopy photosynthesis and NUE.¹ The linkage between semi-dwarfism and decreased panicle size in *sd1*-containing GRVs was absent in *dep1-1*-containing varieties. Consequently, the grain yield and harvest index of NILs carrying *dep1-1* outperformed those of NILs with *sd1*.¹ Thus, the *dep1-1* allele is a promising new tool for rice improvement.

SYNERGISTICALLY IMPROVING GRAIN YIELD AND QUALITY

Overcoming the trade-off between grain yield and quality is a major challenge for rice breeders. Grain size, especially grain length, plays an important role in yield potential and appearance quality. Therefore, increasing grain length is considered to be the simplest way to simultaneously improve rice yield and quality. All of the rice G-protein components appear to regulate grain size. RGA1 positively regulates grain length, whilst both down- and up-regulation of *RGB1* lead to small grains. The loss-of-function mutation of *GS3* confers longer grains, leading to improved appearance quality and thousand-grain weight. Both *OsGGC2* and *DEP1* have a longer C-terminus than *GS3* and play positive roles in grain length, while truncated *dep1-1* protein leads to short grains. A newly discovered natural allele, *dep1-2*, also encodes a functionally truncated *DEP1* protein that contains an additional von Willebrand factor type C (vWFC) domain and is associated with long grains, resulting in synergistic improvement of yield and quality. Moreover, *dep1-2* partially suppresses the negative function of *GS3* in grain size, thus combining *dep1-2* with the elite *gs3* allele represents a promising breeding strategy to simultaneously improve grain quality and yield in rice.²

COORDINATING PLANT GROWTH AND METABOLISM TO IMPROVE NUE

The accumulation of DELLA proteins in *sd1*-harboring rice confers decreased NUE, thus increasing N fertilizer needs, and raising the question of how to improve NUE in GRVs. Generally, plant height and tiller number are positively correlated with the N input level. *DEP1* was identified as a candidate gene related to N-mediated growth response. Similar to *sd1*, *dep1-1* also affected N utilization, but *dep1-1* led to improved N metabolism, thus enhancing grain yield even under moderate levels of N fertilization.¹

DEP1 cooperates with Ga and Gβ in N response. Downregulating *RGA1* and upregulating *RGB1* also led to N-insensitive dwarf plants and enhanced NUE. *DEP1*, as a plant-specific Gy subunit, directly interacts with and inhibits the activity of Ga subunit through its C-terminal vWFC domain.¹ Thus, manipulating heterotrimeric G proteins may provide a new breeding strategy to simultaneously improve crop yield and NUE for sustainable agriculture.

INCREASING CROP RESISTANCE TO ALKALINE STRESS

Exploiting marginal lands is important for increasing the annual crop production with limited arable land. Sorghum has abundant genetic resources for tolerance to alkaline stress. The *SbAT7* gene encoding a GS3 homolog accounts for the alkali tolerance in sorghum. Knocking out *SbAT7* enhanced tolerance to alkaline soils. The natural *Sbat1* allele encodes a truncated Gy protein that is exactly homologous to the rice GS3-4 protein (from Chuan 7 accession) lacking a cysteine-rich tail, resulting in reduced tolerance to alkaline stress. Accordingly, overexpressing *GS3-4* conferred not only shorter grains but also more severe alkaline sensitivity than overexpressing *GS3* in rice,³ suggesting that cereal crops evolved a conserved G-protein signaling pathway to regulate growth and stress response.

In rice, GS3 interacts with the aquaporin PIP2s and inhibits its hydrogen peroxide (H₂O₂) export activity, resulting in overaccumulation of reactive oxygen species (ROS) and consequently conferring alkaline sensitivity

(Figure 1). The nonfunctional *gs3* protein does not inhibit PIP2s and thus enhances alkaline tolerance,³ indicating that the *gs3* allele, in addition to improving grain yield and quality, can also enhance rice tolerance to alkali soil, potentially expanding the cereal planting area.

ENHANCING CROP RESILIENCE TO CLIMATE CHANGE

Climate change, especially global warming, seriously threatens agriculture worldwide. An investigation of the molecular mechanism underlying thermotolerance in African rice identified a QTL for thermotolerance caused by natural variations in the G-protein-encoding gene *GS3*. Upon heat stress, *GS3* facilitates cytosolic Ca²⁺ elevation and promotes the downstream interaction between calmodulin CaM and the transcription factor SCT1, which suppresses *OsWR2* gene expression and diminishes the cuticular wax content, consequently leading to greater thermosensitivity. In the absence of *GS3*, the resulting Ca²⁺ accumulation and CaM–SCT1 interaction are blocked, which promotes the deposition of cuticular wax and endows plant thermotolerance.⁴ Thus, the loss-of-function *gs3* allele can significantly enhance rice yield under high temperature conditions.

In addition to heat, cold stress also limits crop yields. Generally, *japonica* rice exhibits better cold tolerance than *indica*. A major QTL related to chilling tolerance in *japonica* was identified, and the causal gene *COLD1* encodes a regulator of G-protein signaling (RGS) with nine-transmembrane domains. Under cold treatment, the *japonica*-derived *COLD1*^{ja} protein interacts with Ga subunit and increases its GTPase activity, triggering Ca²⁺ elevation and conferring cold tolerance.⁵ Unlike mammals, the plant Ga has constitutive and weak GTPase activity. Modulating its GTPase activity is critical to the activation-deactivation cycle of G proteins in plants. Thus, modulating G-protein signaling may be the key to cultivate thermotolerant GRVs to cope with future global climate changes.

CONCLUSION AND PERSPECTIVES

Although the modulation of GA signaling has played an indispensable role in the 1960s' Green Revolution, the alleles account for semi-dwarf trait are associated with serious limitations, such as lower grain number per panicle and NUE. Heterotrimeric G proteins may be better targets for enhancing crop productivity because of their beneficial roles in semi-dwarfism and environment adaptation. No canonical G-protein-coupled receptor (GPCR) has been reported in plants, but the receptor-like protein kinase (RLK) has been demonstrated to interact with Ga proteins in maize (Figure 1). Thus, an in-depth understanding of RLK functions in G-protein signaling may shed light on the corresponding mechanism in rice. Based on the explored mechanism, some better dominant alleles will be created through the de novo design or direction evolution of G proteins, thus accelerating the cultivation of new GRVs with improved agronomic traits for sustainable agriculture.

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DECLARATION OF INTERESTS

The authors declare no competing interests.