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ARTICLE

DDG1 and G Protein α Subunit RGA1 Interaction Regulates Plant Height and Senescence in Rice (*Oryza sativa*)

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ABSTRACT

Many studies have already shown that dwarfism and moderate delayed leaf senescence positively impact rice yield, but the underlying molecular mechanism of dwarfism and leaf senescence remains largely unknown. Here, using map-based cloning, we identified an allele of *DEP2*, *DDG1*, which controls plant height and leaf senescence in rice. The *ddg1* mutant displayed dwarfism, short panicles, and delayed leaf senescence. Compared with the wild-type, *ddg1* was insensitive to exogenous gibberellins (GA) and brassinolide (BR). *DDG1* is expressed in various organs, especially in stems and panicles. Yeast two-hybrid assay, bimolecular fluorescent complementation and luciferase complementation image assay showed that DDG1 interacts with the α-subunit of the heterotrimeric G protein. Disruption of *RGA1* resulted in dwarfism, short panicles, and darker-green leaves. Furthermore, we found that *ddg1* and the *RGA1* mutant was more sensitive to salt treatment, suggesting that DDG1 and RGA1 are involved in regulating salt stress response in rice. Our results show that DDG1/DEP2 regulates plant height and leaf senescence through interacting with RGA1.

KEYWORDS

Oryza sativa; *DDG1*; plant height; senescence; *RGA1*

1 Introduction

Rice feed over two thirds of the world's population, and the 'Green Revolution' gene *sd1* has made a significant contribution increases in rice yields [1]. However, the reduction of plant height continues to be the main goal for increasing lodging resistance in rice breeding [2]. So far, many quantitative trait loci (QTLs) and genes that regulate plant height have been isolated and cloned, such as *D61*, *MIT1*, *D35*, and *D6* [3–6]. Among these QTLs and genes, a considerable number of genes are involved with plant hormones, especially GA (Gibberellin) and BR (Brassinosteroid). The 'Green Revolution' gene *sd1*

encodes gibberellin 20 oxidase 2 (GA20ox2), which converts GA_{12}/GA_{53} to the bioactive GA precursors GA_9/GA_{21} [7,8]. In addition, BR biosynthesis genes such as *D2*, *BRD2*, and *DWARF4* and signal pathway genes such as *D61*, *DLT*, and *GSK2* regulate rice plant height by controlling cell proliferation and cell expansion [9,10]. However, *sd1* is still the dwarf gene most used in rice breeding, and it is urgent to clone a new dwarf gene.

Senescence is the final stage of plant development, and premature leaf senescence negatively impacts rice yield stability [11,12]. Leaf senescence is a highly regulated process by both intrinsic regulators as well as environmental factors [13]. Many leaf senescence-related mutant genes have been isolated in various crops, including *OsNAP*, *OsNaPRT1*, *OsSRLK*, and *OsDOS* [12,14–16]. The *OsNAP* gene encodes an NAC (NAM, ATAF, and CUC2) transcriptional activator, and knock-down of *OsNAP* delays leaf senescence and increases yield [12]. OsNAP regulates the expression of chlorophyll degradation-related gene *stay-green* (*SGR*) and senescence-associated gene *Osh36* [12]. Similarly, *OsNaPRT1* affects the nicotinamide content and regulates the expression of senescence related genes [15]. However, plant leaf senescence is a very complex growth process, and the underlying molecular mechanism of leaf senescence remains largely unknown.

Here, we reported a new dwarfism and delayed leaf senescence mutant in rice, *dwarfism* and *darker green leaf 1* (*ddg1*). Map-based cloning showed that *DDG1* is a new allele of *DEP2*, which encodes a plant-specific protein without any known functional domain. We also found that *DDG1* was expressed in various rice tissues and the DDG1 protein was conserved in plants. In addition, we observed DDG1's interaction with RGA1 through yeast two-hybrid (Y2H) analysis, bimolecular fluorescent complementation (BiFC) and luciferase complementation image (LCI) assays. Finally, we found that *DDG1* positively regulated salt stress response in rice. Our results provide new insight into *DDG1*/*DEP2's* regulation rice growth and development.

2 Material and Methods

2.1 Plant Materials and Cultivation

The *ddg1* mutant that exhibited dwarfism and delayed leaf senescence was isolated from the mutant library of the *japonica* rice variety Kitaake by 1% ethyl methanesulfonate (EMS) in 2017. For EMS treatment, we soaked dry seeds of Kitaake in water for 24 h, added 1% EMS to soak for 10 h, and soaked in water for 24 h, then sowed seeds in the field. The *ddg1* mutant was crossed with the *indica* variety 93–11 to develop the gene mapping population. 521 extreme individuals selected from the F_2 population were used to map *ddg1* for dwarfism and delayed leaf senescence. The $RGA1^{KO}$ mutant used in this study was kindly provided by Professor Yidan Ouyang and Doctor Shengyuan Sun [17]. All the plants were grown in paddy fields in Huai'an (Jiangsu Province) and Lingshui (Hainan Province), China.

For salt treatment, we germinated seeds of both wild-type (WT, Kitaake) and *ddg1* in water for 48 h at 37°C in darkness, and then transferred them to a Yoshida rice nutrient salt mixture (Coolaber, NSP1040, Beijing, China) for 2 weeks, exchanging fresh solution every 3 days. Next, the 2-week-old seedlings were transferred to a hydroponic culture solution supplemented with 150 mM NaCl for 7 d. Finally, the rice seedlings were grown in climate chambers under 14/10 h light/dark and 30°C/25°C day/night conditions.

2.2 Scanning Electron Microscopy (SEM)

At the heading stage, the first internodes of the WT and the *ddg1* mutant were collected and fixed with 2.5% glutaraldehyde, and the processed samples were observed with a scanning electron microscope (JSM-840, JEOL).

2.3 Chlorophyll Determination

Chlorophyll was extracted from leaves of wild-type and *ddg1* at maturity. 0.1 g fresh leaves was cut and soaked in anhydrous ethanol for 48 h in darkness. The absorbance values at 649, and 665-nm wavelength

were measured using a spectrophotometer. Chlorophyll contents were determined according to as previously described method [18].

2.4 GA and BR Treatment

For GA treatment, we grew seeds of wild-type and *ddg1* in hydroponic media with different concentrations of GA for two weeks, and the seedling height of the WT and *ddg1* were measured. For BR sensitivity test, leaves with partial leaf blades and sheathes were cut from the 2-week-old seedlings of the wild type and *ddg1*. Leaves were put into a solution with different concentrations of 24-Epibrassinolide (E1641, Sigma) or control solution, and then incubated in the light for 3 d. Finally, lamina joint angles were measured using IMAGEJ software.

2.5 Map-Based Cloning

For map-based cloning of *DDG1*, we chose 521 recessive individual plants showing dwarfism and delayed leaf senescence similar to the phenotype of the *ddg1* mutant from an F_2 population derived from a cross between *ddg1*(♀) and the *indica* variety 93-11(♂). At heading stage, the mutant anthers were emasculated and subjected to the 9311 pollen. Simple sequence repeat (SSR) markers and the developed insertion/deletion (InDel) markers with Primer Premier 5 on chromosome 7 were used for fine mapping. Next, we amplified the corresponding fragments from *ddg1* and the WT, and compared sequences using the BioXM 2.6 software.

To verify whether the *Os07g0616000* mutation was responsible for the phenotype of *ddg1*, For constructing the *Os07g0616000* complementary vector, the full-length coding sequence (CDS) was amplified and inserted into the p1390Ubi vector. Approximately 2 kb promoter sequence of *Os07g0616000* was amplified and substituted with ubiquitin promoter region of p1390Ubi vector. The recombinant was transformed into the calluses from the *ddg1* seeds by agrobacterium-infection methods. T_2 generation transgenic plants were used for phenotype analysis. The primers used in the map-based cloning and complementary vector construction were listed in Table S1.

2.6 RNA Extraction and qRT-PCR

Total RNAs were extracted using the TRIzol reagent (Invitrogen) from various tissues of the WT and *ddg1*, and cDNAs were synthesized using a HiScript® II 1st strand cDNA synthesis kit (#R211; Vazyme, Nanjing, China). qRT-PCR experiments were performed using a real-time PCR detection system (BIO-RAD, CFX96) with three biological repetitions using the rice *Ubiquitin* (*LOC_Os03g13170*) gene as the internal control. The primers used for qRT-PCR were given in Table S1.

2.7 Yeast Two-Hybrid (Y2H) Assay

The full-length CDS of *DDG1* and *RGA1* (primers listed in Table S1) were amplified and inserted into the pGBKT7 vector and the pGADT7 vector, respectively. Different combinations of plasmids were also transformed into *Saccharomyces cerevisiae* strain AH109 on the SD/-Trp/-Leu/-His/-Ade medium. The specific method of yeast transformation can be found in the manufacturer's instructions, which we followed carefully (http://www.clontech.com/).

2.8 Bimolecular Fluorescent Complementation (BiFC)

For BiFC assays, we inserted the full length CDS of *DDG1* and *RGA1* (lacking stop codon) into the p2YN and p2YC at *Pac*I and *Spe*I sites, respectively [19]. We next transformed the DDG1-nYFP and RGA1-cYFP plasmids or corresponding empty vectors into *Agrobacterium* strain EHA105, and then infiltrated this into leaves of *N. benthamiana* for 48 h. Fluorescences were then observed by laser confocal microscopy (Zeiss). The primers used for BiFC were listed in Table S1.

2.9 Luciferase Complementation Image (LCI) Assay

For LCI assays, the full-length CDS of *DDG1* and *RGA1* were amplified and fused to the pCAMBIA1300-nLUC and pCAMBIA1300-cLUC vector, respectively. These recombinants were co-transformed into *N. benthamiana* leaves by Agrobacterium infection. Empty pCAMBIA 1300-nLUC and pCAMBIA1300-cLUC vectors were used as negative controls. Primers used for LCI assays were listed in Table S1.

3 Results

3.1 Characterization of the ddg1 Mutant

We isolated a dwarf and dark-green leaves mutant, *ddg1*, from an ethyl methanesulfonate (EMS)-derived mutagenesis population in a *japonica* rice variety, Kitaake. Compared with WT, *ddg1* exhibited dwarfism and short panicles at maturity (Figs. 1A–1C). Each internode of *ddg1* was almost shortened (Fig. 1B). The seeds of *ddg1* were small and round (Fig. 1D).The plant height and panicle length of the *ddg1* mutant decreased by 30% and 27.8% (Figs. 1E, 1F). We observed no difference in the tiller number between the wild type and *ddg1* (Fig. 1G). However, *ddg1* showed delayed leaf senescence and had higher chlorophyll content than that of the WT at maturity (Figs. 2A and 2B). Similarly, two senescence-associated genes, *SGR* and *Osh36*, were significantly repressed in *ddg1* (Figs. 2C and 2D). These results suggest that *DDG1* regulates plant height and senescence.

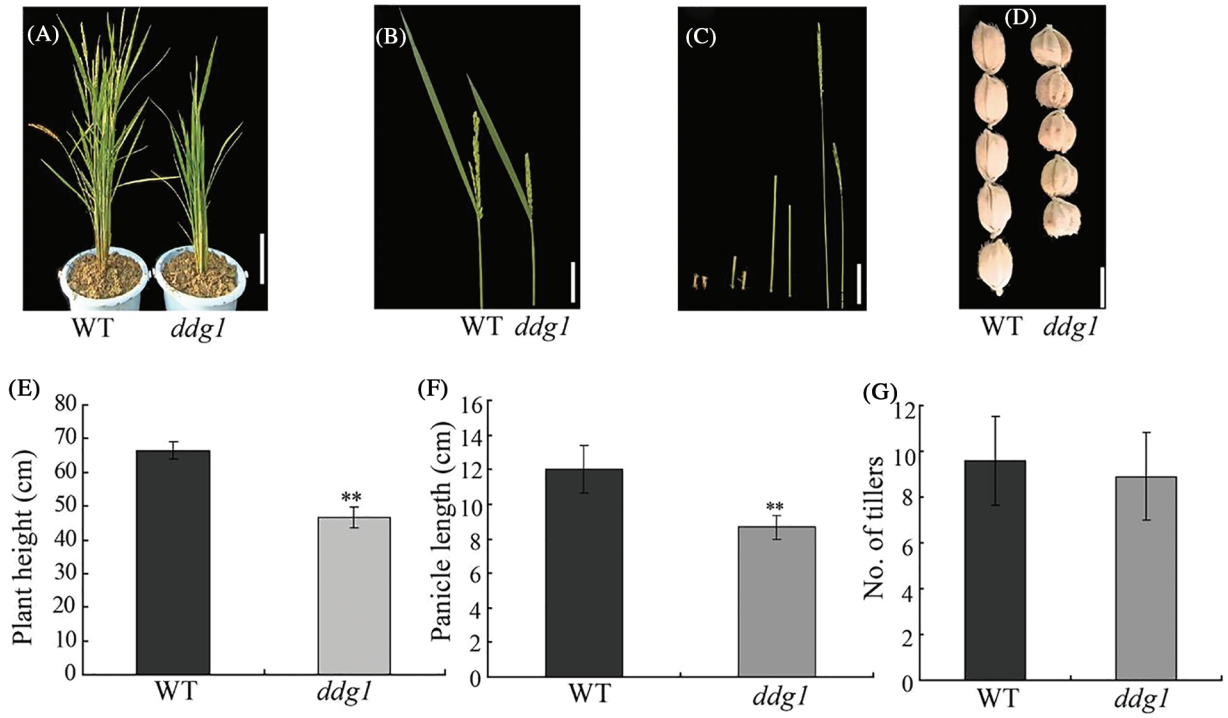

Figure 1: Plant height and panicle length were reduced in the *ddg1* mutant plants. (A) Phenotypes of the wild-type (WT) and the *ddg1* mutant at the heading stage. Bar = 10 cm. (B) Panicle morphology of WT and *ddg1*. Bar = 5 cm. (C) The internodes of the wild-type (left) and the *ddg1* mutant (right). Bar = 2 cm. (D) Grain morphology of WT and *ddg1*. Bar = 2 mm. (E) Plant height of the wild-type and the *ddg1* mutant. (F) Panicle length of WT and *ddg1*. (G) Tiller number of WT and *ddg1*. Data are shown as mean ± SD (n = 20). ** $p < 0.01$ by Student's t-test analysis

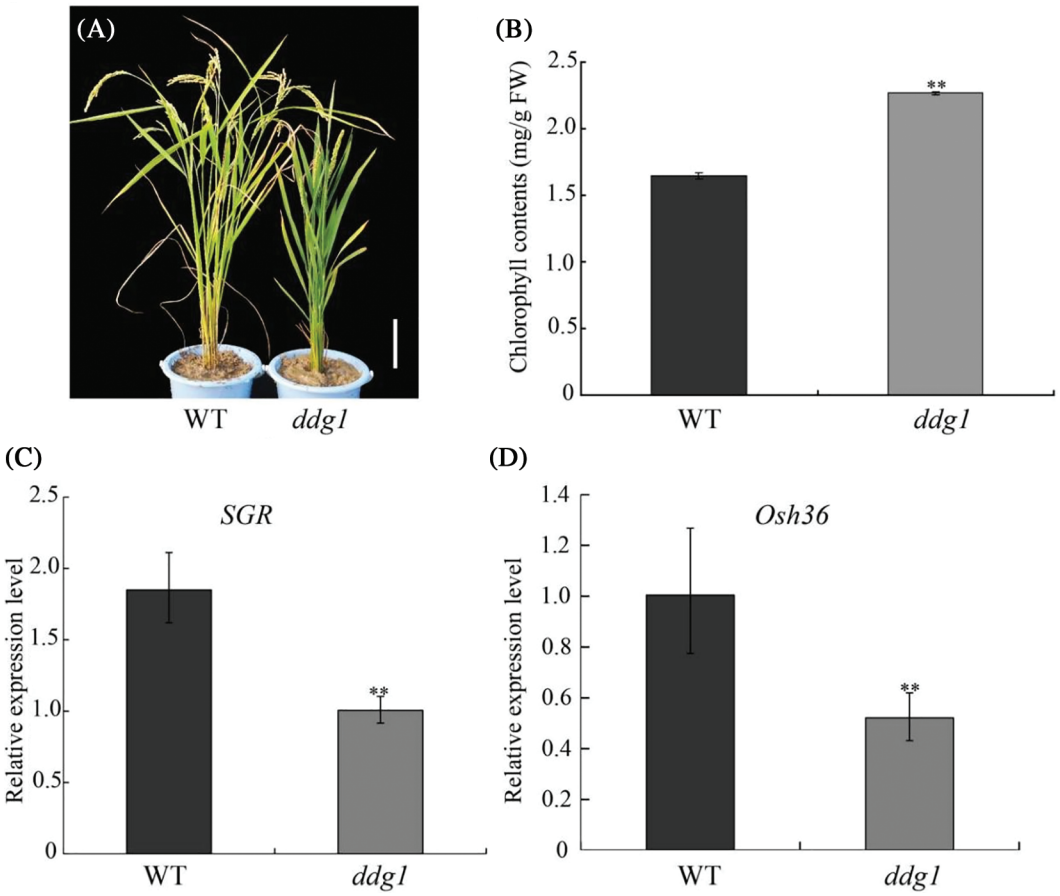

Figure 2: *DDG1* negatively affects leaf senescence. (A) Phenotypes of the wild type (WT) and *ddg1* at maturity. Bar = 10 cm. (B) Chlorophyll contents of the wild type and *ddg1* at maturity. (C and D) Expression analysis of *SGR* and *Osh36* in the wild type and *ddg1* by qRT-PCR. Data are shown as means ± SD (n = 10). Bar = 10 cm. ** represents a significant difference at $p < 0.01$

3.2 Altered Cell Proliferation in ddg1

Normal cell proliferation and cell expansion are responsible for plant organ size. Hence, in order to examine the dwarfism of *ddg1* in more detail, we examined the first internodes of the WT and *ddg1* at the heading stage using histological analysis. Our SEM observations showed that cell length and width were similar between the wild type and the *ddg1* mutant (Fig. S1), which suggests that the *DDG1* mutation does not influence cell expansion.

3.3 Decreased Sensitive to GA and BR in ddg1

Both GA and BR are essential for plant growth and developmental processes, including stem development, grain size, senescence, and seed germination [10]. In order to verify whether *DDG1* is involved in the GA and BR signaling pathways, we tested the sensitivity of *ddg1* to GA and 24-epibrassinolide. First, we cultured the WT and *ddg1* seedlings with different concentrations of GA and measured the seedlings' heights after 2 weeks of growth. As shown in Figs. 3A and 3B, the average seedling height of the wild type and *ddg1* increased significantly with an increase in GA concentration, but the rate of increase of seedling height for the *ddg1* mutant was significantly lower than that of the wild type, indicating that *ddg1* was less sensitive to GA. Secondly, for BR sensitivity test, we used 0, 0.1,

or 1 μM of 24-epiBL to treat excised leaf segments of the 2-week-old WT and *ddg1* mutant plants and then measured the lamina joint angle. Compared with the wild type, the rate of increase in the lamina joint angle in *ddg1* was remarkably reduced (Figs. 3C–3F). The above results indicate strongly that *DDG1* has a direct and critical involvement in the GA and BR signaling pathways.

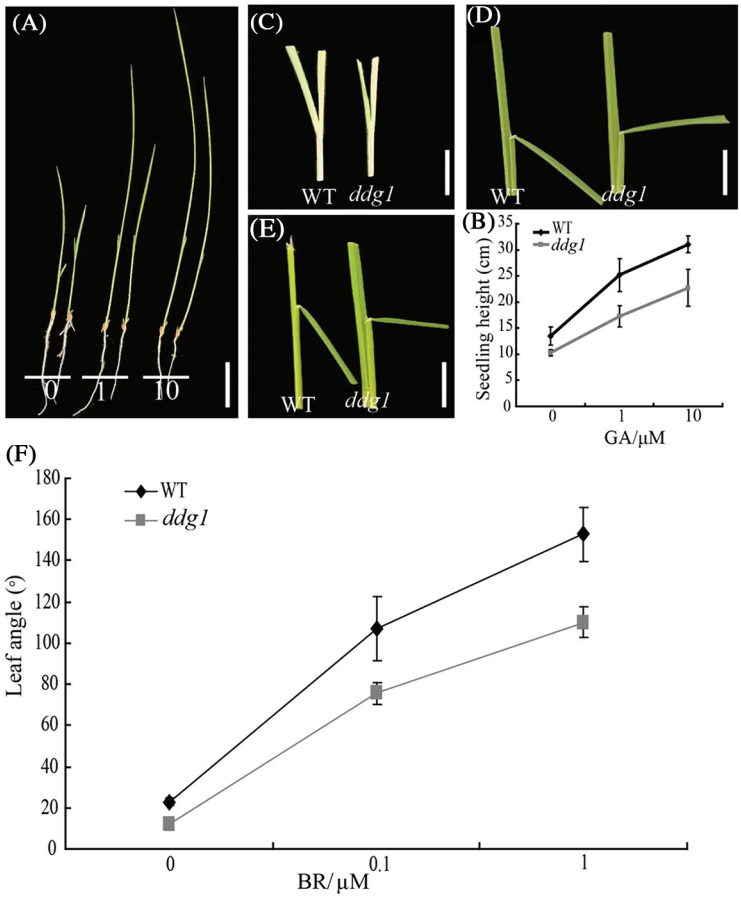

Figure 3: *ddg1* is less sensitive to GA and BR. (A) Phenotypes of the wild type (left) and *ddg1* (right) with GA treatment. Bar = 5 cm. (B) Seedling height of the wild type and *ddg1* after GA treatment. (C–F) Response to 0 (C), 0.1 (D), or 1 (E) μM of 24-epiBL of the lamina joint of the wild type and *ddg1*. Data represent the means of results from ten seedlings in each sample

3.4 Map-Based Cloning of DDG1

To isolate the *DDG1* gene responsible for the *ddg1* phenotype, we crossed *ddg1* with *indica* var 93–11 to construct the mapping population. Using 521 extreme individuals selected from the F_2 population, we mapped *DDG1* to a 140-kb region on rice chromosome 7 (Fig. 4A). We found that there were 18 genes in the 140-kb region (http://rice.plantbiology.msu.edu/) (Fig. 4A), and among these 18 genes, one was the *DEP2* (*Os07g0616000*) gene, which regulates panicle architecture and grain size [20,21]. We then sequenced the promoter and coding region of *DEP2* between the wild type and *ddg1* via Sanger sequencing [22] and found that there was a one-nucleotide deletion of *DEP2* in *ddg1*, compared with the wild type (Fig. 4B). One-nucleotide deletion of *DEP2* in *ddg1* led to premature termination of the DEP2 protein translation (Fig. S2). Next, in order to study further whether the *DEP2* mutation was responsible for the defects in *ddg1*, we conducted a genetic complementation test. Approximately 2 kb

promoter and full-length CDS of *DEP2* were transformed into the calluses from the *ddg1* mutant and found that the positive complementary plants were similar to the wild type (Fig. 4C), compared to the negative control plants (Fig. S3). Importantly, we found the specific mutant *DDG1* gene to be *DEP2*/*Os07g0616000*.

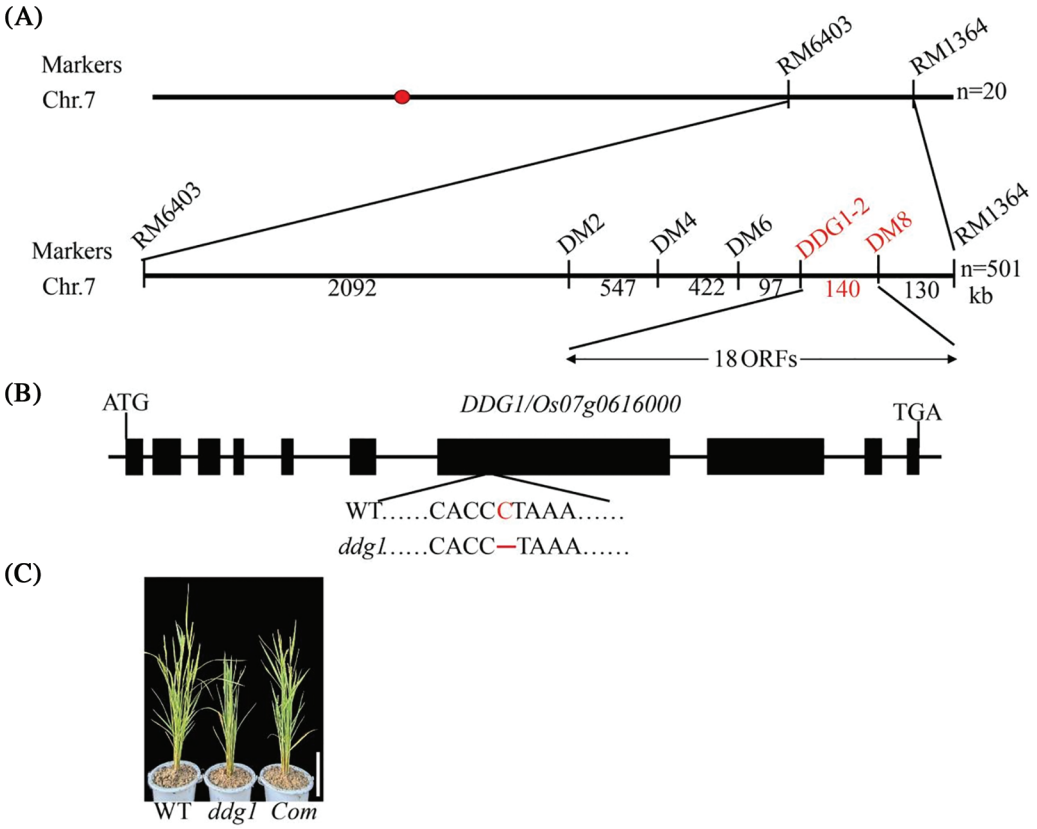

Figure 4: Map-based cloning of *DDG1*. (A) *DDG1* was fine-mapped in the 140-kb interval between InDel markers DDG1-2 and DM8 on chromosome 7. (B) *DDG1* gene structure. Black boxes represent exons, and a mutation site is shown in red. (C) Complementation of the *ddg1* mutant with the *DDG1*/*Os07g0616000* gene. Bar = 10 cm

3.5 Expression and Evolutionary Analysis of DDG1

To examine the expression pattern of *DDG1*, we first learned that *DDG1* was expressed in both vegetative and reproductive tissues by using the RiceXPro database (Fig. S4; https://ricexpro.dna.affrc.go.jp/). In addition, we performed qRT-PCR to analysis the expression pattern of *DDG1*. As shown in Fig. 5A, *DDG1* was expressed in roots, stem, leaves, panicles, and seeds. Interestingly, we observed that the expression level of *DDG1* was significantly repressed in *ddg1* (Fig. 5B).

DDG1 encodes a plant-specific protein without any known functional domain and that has 10 exons. To analyze the evolutionary relationship of the DDG1 protein, we performed a BLAST search in order to identify DDG1 homologs and found that DDG1 showed high amino acid sequence similarity to proteins in other species, including *Oryza brachyantha* (88%), *Brachypodium distachyon* (73%), *Triticum dicoccoides* (73%), and *Panicum virgatum* (68%; Fig. S5).

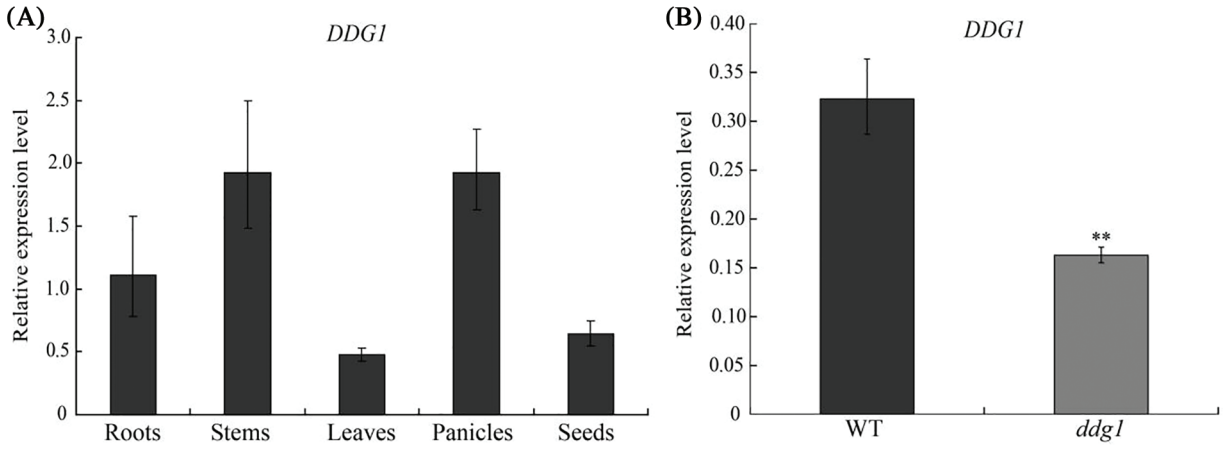

Figure 5: Expression pattern of *DDG1*. (A) Expression of *DDG1* in roots, leaves, panicles, stems and seeds of wild-type plants. (B) Expression level of *DDG1* in *ddg1* and the wild type leaves. ** represents a significant difference at $p < 0.01$

3.6 An Interaction between DDG1 and RGA1

Since the DDG1 protein has no known functional domain, we performed Y2H screening to identify its interacting proteins. To accomplish this, we fused the full-length CDS of *DDG1* to the pGBKT7 vector (DDG1-BD) and used it as bait to screen the interacting proteins against a rice leaf yeast library of 2-week-old Kitaake, and we were able to identify one candidate segment belonging to part of the *RGA1* gene. After this result, we fused the full-length CDS of *RGA1* to the pGADT7 vector (RGA1-AD). Next, DDG1-BD and RGA1-AD were co-transformed into the yeast cells, which grew well on our quadruple dropout media (Fig. 6A; Fig. S6). We then performed bimolecular fluorescent complementation assays to detect the interaction. As shown in Fig. 6B, the fluorescence signal was observed when the fusion constructs p2YN-DDG1 and p2YC-RGA1 were present, but not in the corresponding control. Using a LCI assay, we detected obvious LUC activity signal when DDG1-nLUC was co-expressed with RGA1-cLUC in *N. benthamiana* (Fig. 6C). Thus, we conclude that DDG1 interacts with RGA1. In addition, compared with the wild type, knock-out of *RGA1* showed dwarfism, short panicles, and darker-green leaves, which were similar to the phenotype of *ddg1* (Fig. 7).

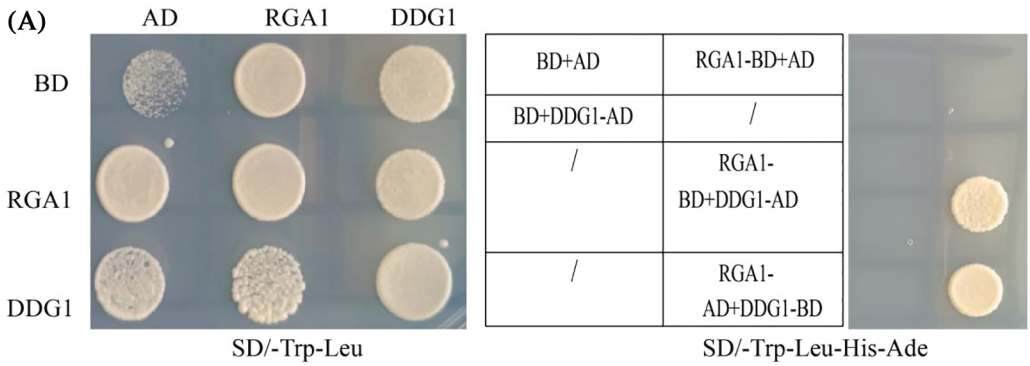

Figure 6: (Continued)

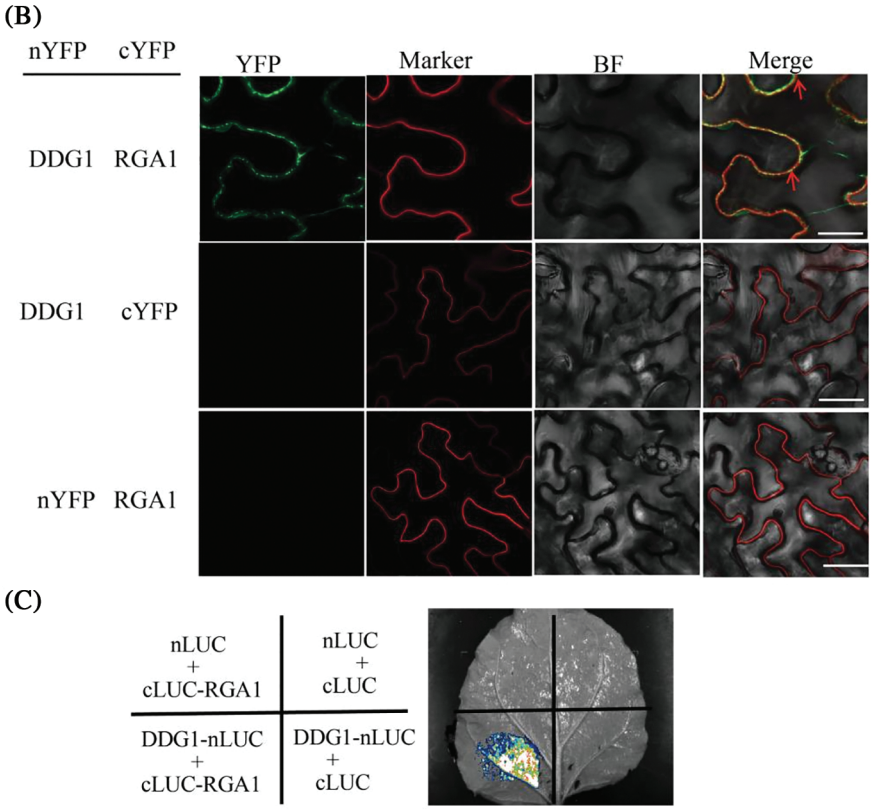

Figure 6: DDG1 interacts with the Gα protein RGA1. (A) Interaction of DDG1 and RGA1 using yeast two-hybrid assay. AD: GAL4 activation domain; BD: GAL4 binding domain. (B) BiFC assay. Bar = 10 μm. Plasma membrane protein OsCAMP1-mCherry was used as a marker. (C) LCI assays. LUC activity was detected 72 h after injection

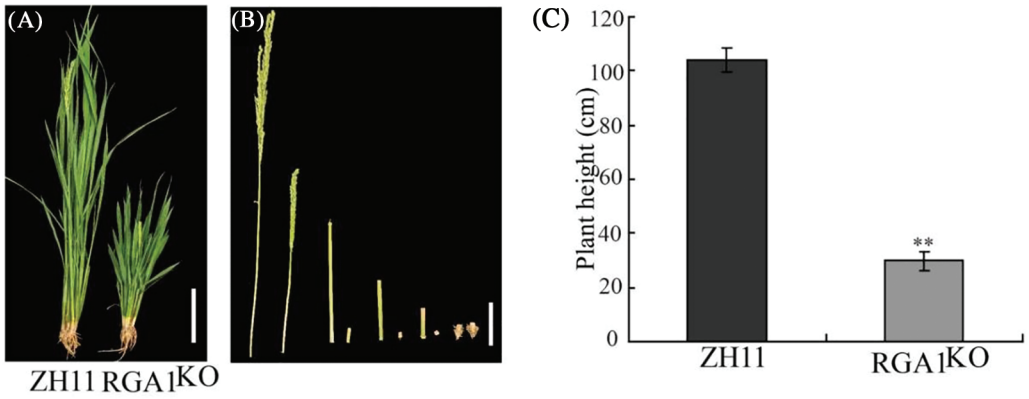

Figure 7: (Contined)

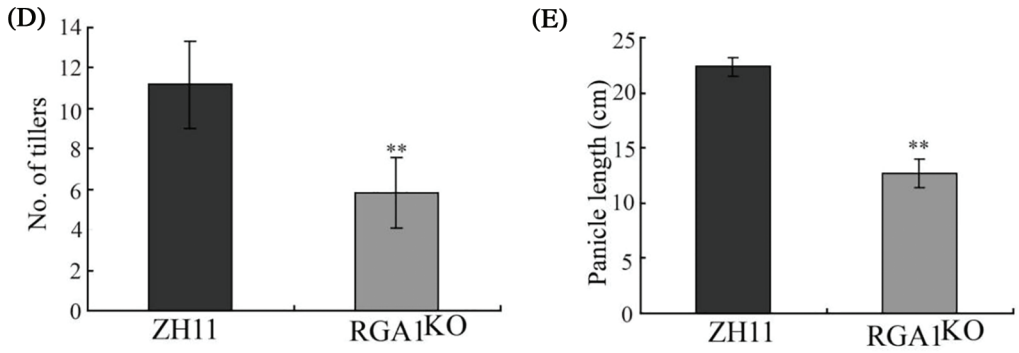

Figure 7: The *RGA1*^{KO} mutant showed dwarfism and short panicles. (A) Phenotypes of Zhonghua 11 (ZH11) and the *RGA1*^{KO} mutant at the heading stage. Bar = 10 cm. (B) The internodes of ZH11 (left) and the mutant (right). Bar = 2 cm. (C–E) Plant height, tiller number, and panicle morphology of ZH11 and the mutant. Data are shown as mean ± SD (n = 20). ** $p < 0.01$ by Student's t-test analysis

3.7 Decreased Tolerance of ddg1 to Salt Stress Response

Microarray analysis showed that 889 up-regulated and 841 down-regulated genes related-salt stress between control and the *RGA1* mutant *d1*, indicating that *RGA1* may be involved in the regulation of salt stress in rice [23]. Therefore, we speculated that *DDG1* might also be involved in the regulation of salt stress response in rice. To test this hypothesis, we treated the *ddg1* mutant and wild type seedlings with 150 mM NaCl. Compared with the wild type, *ddg1* seedlings were more sensitive to salt stress, and the survival frequencies of were approximately 22% and 62% under salt stress conditions in *ddg1* and WT plants, respectively (Figs. 8A–8C). Consistent with the salt sensitivity of *ddg1*, knock-out of *RGA1* resulted in a lower survival rate than that of wild-type plants (Fig. S7). Compared with ZH11, the expression level of *RGA1* in knock-out of *RGA1* plants was decreased (Fig. S8). These results showed that *DDG1* and *RGA1* both play important roles in rice salt tolerance.

4 Discussion

Dwarfism and moderate delayed leaf senescence are essential for increasing rice yield [12]. To date, many genes related to plant height and leaf senescence have already been cloned, but few can be directly applied to rice breeding. In our study, we cloned a novel allele of *DEP2*, *DDG1*, which controls plant height and leaf senescence in rice. The *DEP2*/*EP2* allele was first reported to be involved in the regulation of rice panicle erectness [21,24], and it encodes a novel, unknown plant-specific protein and is highly expressed in young panicles. In addition, another allele *SRS1*, has already been isolated as a regulator of grain size in rice [20]. The *srs1* mutant exhibited small and round seeds, and the reduction of cell length and cell number in *srs1* have been classified as its phenotype [20]. Along similar lines, our study showed that the reduction of cell number in stems leads to the dwarfism of *ddg1*. These results indicate that *DEP2*/*EP2*/*SRS1*/*DDG1* all regulate cell expansion and cell proliferation. Recently, a cleistogamy gene *cl7(t)*, which is an allele of *DEP2*, was identified by map-based cloning [25]. *cl7(t)* showed cleistogamy and closed spikelets, which might be used for the selection of ecologically safe genetically modified crops. Five *DEP2* alleles have pleiotropic effects on rice growth and development.

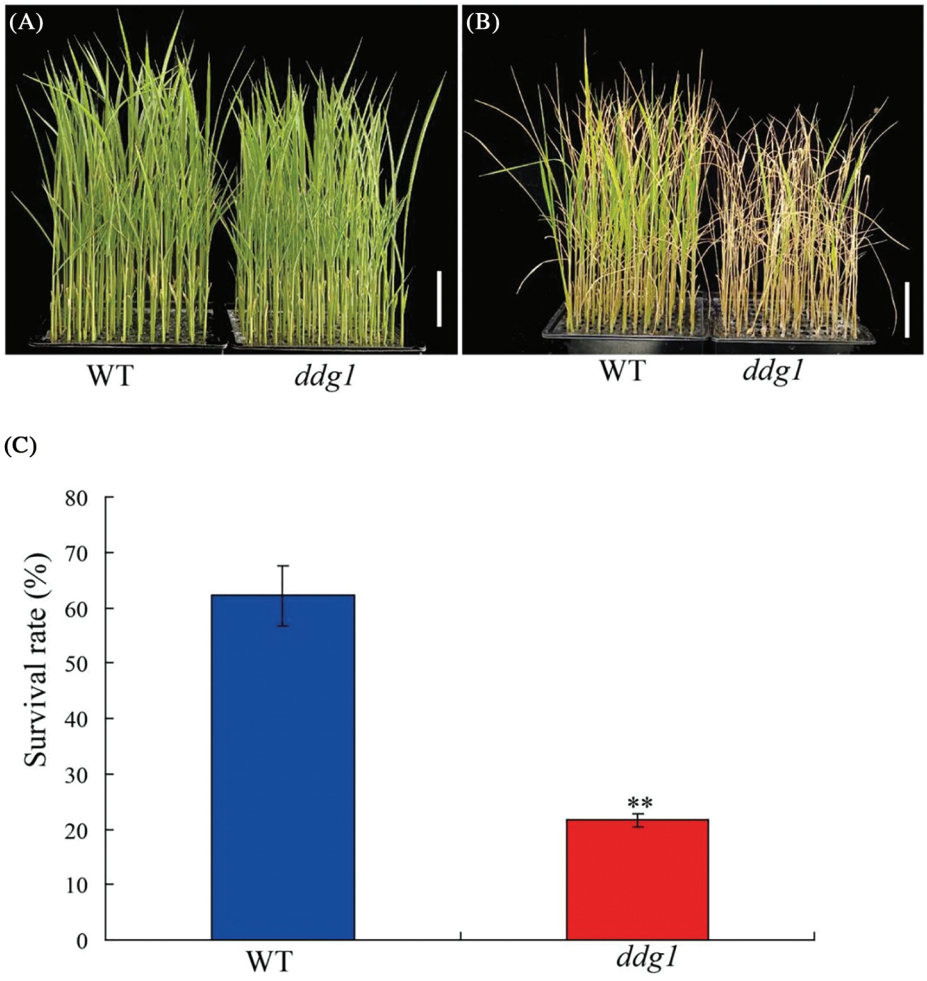

Figure 8: The *ddg1* mutant was sensitive to salt stress at the seedling stage. (A and B) Phenotypes of the wild type (WT) and *ddg1* before or after 150 mM NaCl treatment. Bar = 5 cm. (C) Survival rates of the WT and *ddg1* after salt stress. Three biological repeats were performed with 96 individual plants per repeat. ** indicates a significant difference between WT and *ddg1* at $p < 0.01$

Heterotrimeric G proteins play an important role in plant growth and developmental processes, including hormone responses, plant height, nitrogen-use efficiency, and seed size [26–28]. In *Arabidopsis thaliana*, there are one G protein α subunit (GPA1), one G protein β subunit (AGB1), and over three G protein γ subunits (AGG1, AGG2 and AGG3), but rice has one Gα gene (*RGA1*), one Gβ gene (*RGB1*) and five Gγ homologous genes (*RGG1*, *RGG2*, *GS3/TT2*, *qPE9-1/DEP1* and *GGC2*) [29–31]. In addition, abnormal GPA1, AGB1 and AGG3 can lead to defects in *Arabidopsis* growth and development [32,33]. In rice, seven G protein subunits are well characterized and are mainly involved in regulating plant architecture, grain size, and stress responses [27,30,31,34]. RGA1/D1 was the first reported G protein in rice [35,36], and the *RGA1* mutant *d1* has a dwarf, darker-green leaves, and small-seed phenotype and is less sensitive to 24-epiBL and GA [37]. In our study, the phenotype of *ddg1* was very similar to that of *rga1*/*d1*. Compared with the wild type plants, *ddg1* was less sensitive to GA xand BR treatments, similar to *rga1*/*d1*. Furthermore, we found that DDG1 interacts with RGA1 from the results of our Y2H, BiFC

and LCI assays. The interaction between DEP2 and RGA1 may lead to disruption of the function of the G protein complex, which is our next research focus.

In addition to its function that regulates plant height and senescence, *DDG1* seems to play a vital role in controlling salt stress response due to the observed salt-stress-sensitive phenotype of the *ddg1* mutant. The $RGA1^{KO}$ mutant was slightly more sensitive to salt stress than the wild type. Based on the DDG1-RGA1-mediated pathway having dual roles in regulating plant growth and salt stress response, it may be possible to manipulate these components to enhance rice yield under stress conditions. Conceivably, *DDG1* has multiple biological functions and some unknown interacting proteins of *DDG1* possibly still need to be identified in the future.

In conclusion, we isolated a new allele of *DEP2*, *DDG1*, which controls plant height and leaf senescence in rice. Y2H, BiFC and LCI assays showed that DDG1 interacted with RGA1. Compared with the wild type, *ddg1* was more sensitive to salt stress. In future work, we intend to focus on the interaction proteins of DDG1 to clarify its mechanism of regulating rice's salt stress response.

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Availability of Data and Materials: Sequence data from this article can be found in the GenBank/EMBL data libraries under the following accession numbers: *DDG1/DEP2* (*Os07g0616000*); *RGA1* (*Os05g0333200*); *SGR* (*Os09g0532000*); *Osh36* (*Os05g0475400*).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Terao, T., Hirose, T. (2015). Control of grain protein contents through *SEMIDWARF1* mutant alleles: *Sd1* increases the grain protein content in Dee-geo-woo-gen but not in Reimei. *Molecular Genetics and Genomics, 290(3),* 939–954.
2. Ishimaru, K., Togawa, E., Ookawa, T., Kashiwagi, T., Madoka, Y. et al. (2008). New target for rice lodging resistance and its effect in a typhoon. *Planta, 227(3),* 601–609.
3. Sentoku, N., Sato, Y., Kurata, N., Ito, Y., Kitano, H. et al. (1999). Regional expression of the rice KN1-type homeobox gene family during embryo, shoot, and flower development. *Plant Cell, 11(9),* 1651–1664.
4. Yamamuro, C., Ihara, Y., Wu, X., Noguchi, T., Fujioka, S. et al. (2000). Loss of function of a rice *brassinosteroid insensitive1* homolog prevents internode elongation and bending of the lamina joint. *Plant Cell, 12(9),* 1591–1606.
5. Itoh, H., Tatsumi, T., Sakamoto, T., Otomo, K., Toyomasu, T. et al. (2004). A rice semi-dwarf gene, *Tan-Ginbozu* (*D35*), encodes the gibberellin biosynthesis enzyme, ent-kaurene oxidase. *Plant Molecluar Biology, 54(4),* 533–547.

6. Liu, X., Hu, Q., Yan, J., Sun, K., Liang, Y. et al. (2020). ζ-Carotene isomerase suppresses tillering in rice through the coordinated biosynthesis of strigolactone and abscisic acid. *Molecualr Plant, 13(12),* 1784–1801.
7. Sasaki, A., Ashikari, M., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A. et al. (2002). Green revolution: A mutant gibberellin-synthesis gene in rice. *Nature, 416(6882),* 701–702.
8. Spielmeyer, W., Ellis, M. H., Chandler, P. M. (2002). *Semidwarf* (*sd-1*), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. *Proceedings of the National Academy of Sciences of the United States of America, 99(13),* 9043–9048.
9. Belkhadir, Y., Jaillais, Y. (2015). The molecular circuitry of brassinosteroid signaling. *New Phytologist, 206(2),* 522–540.
10. Saini, S., Sharma, I., Pati, P. K. (2015). Versatile roles of brassinosteroid in plants in the context of its homoeostasis, signaling and crosstalks. *Frontiers in Plant Science, 6,* 950.
11. Morita, R., Sato, Y., Masuda, Y., Nishimura, M., Kusaba, M. (2009). Defect in non-yellow coloring 3, an α/*β* hydrolase-fold family protein, causes a stay-green phenotype during leaf senescence in rice. *Plant Journal, 59(6),* 940–952.
12. Liang, C., Wang, Y., Zhu, Y., Tang, J., Hu, B. et al. (2014). OsNAP connects abscisic acid and leaf senescence by fine-tuning abscisic acid biosynthesis and directly targeting senescence-associated genes in rice. *Proceedings of the National Academy of Sciences of the United States of America, 111(27),* 10013–10018.
13. Lim, P. O., Woo, H. R., Nam, H. G. (2013). Molecular genetics of leaf senescence in *Arabidopsis*. *Trends in Plant Science, 8(6),* 272–278.
14. Kong, Z., Li, M., Yang, W., Xu, W., Xue, Y. (2006). A novel nuclear-localized CCCH-type zinc finger protein, OsDOS, is involved in delaying leaf senescence in rice. *Plant Physiology, 141(4),* 1376–1388.
15. Wu, L., Ren, D., Hu, S., Li, G., Dong, G. et al. (2016). Down-regulation of a nicotinate phosphoribosyltransferase gene, *OsNaPRT1*, leads to withered leaf tips. *Plant Physiology, 171(2),* 1085–1098.
16. Shin, N. H., Trang, D. T., Hong, W. J., Kang, K., Chuluuntsetseg, J. et al. (2019). Rice senescence-induced receptor-like kinase (*OsSRLK*) is involved in phytohormone-mediated chlorophyll degradation. *International Journal of Molecular Sciences, 21(1),* 260.
17. Sun, S. Y., Wang, L., Mao, H., Shao, L., Li, X. et al. (2018). A G-protein pathway determines grain size in rice. *Nature Communications, 9(1),* 851.
18. Liu, X., Xu, Z. Y., Yang, Y. R., Cao, P. H., Cheng, H. et al. (2021). Plastid caseinolytic protease OsClpR1 regulates chloroplast development and chloroplast RNA editing in rice. *Rice, 14(1),* 45.
19. Kudla, J., Bock, R. (2016). Lighting the way to protein-protein interactions: Recommendations on best practices for bimolecular fluorescence complementation analyses. *Plant Cell, 28(5),* 1002–1008.
20. Abe, Y., Mieda, K., Ando, T., Kono, I., Yano, M. et al. (2010). The *SMALL AND ROUND SEED1* (*SRS1*/*DEP2*) gene is involved in the regulation of seed size in rice. *Genes & Genetic Systems, 85(5),* 327–339.
21. Li, F., Liu, W., Tang, J., Chen, J., Tong, H. et al. (2010). Rice *DENSE AND ERECT PANICLE* 2 is essential for determining panicle outgrowth and elongation. *Cell Research, 20(7),* 838–849.
22. Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A. R. et al. (1977). Nucleotide sequence of bacteriophage φX174 DNA. *Nature, 265(5596),* 687–695.
23. Jangam, A. P., Pathak, R. R., Raghuram, N. (2016). Microarray analysis of rice d1 (*RGA1*) mutant reveals the potential role of G-protein alpha subunit in regulating multiple abiotic stresses such as drought, salinity, heat, and cold. *Frontiers in Plant Science, 7,* 11.
24. Zhu, K., Tang, D., Yan, C., Chi, Z., Yu, H. et al. (2010). *Erect panicle2* encodes a novel protein that regulates panicle erectness in *indica* rice. *Genetics, 184(2),* 343–350.
25. Ni, D. H., Li, J., Duan, Y. B., Yang, Y. C., Wei, P. C. et al. (2014). Identification and utilization of cleistogamy gene *cl7(t)* in rice (*Oryza sativa* L.). *Journal of Experimental Botany, 65(8),* 2107–2117.
26. Jin, X., Wang, R. S., Zhu, M., Jeon, B. W., Albert, R. et al. (2013). Abscisic acid-responsive guard cell metabolomes of *Arabidopsis* wild-type and *gpa1* G-protein mutants. *Plant Cell, 25(12),* 4789–4811.

27. Sun, H., Qian, Q., Wu, K., Luo, J., Wang, S. et al. (2014). Heterotrimeric G proteins regulate nitrogen-use efficiency in rice. *Nature Genetics, 46(6),* 652–656.
28. Miao, J., Yang, Z., Zhang, D., Wang, Y., Xu, M. et al. (2019). Mutation of *RGG2*, which encodes a type B heterotrimeric G protein γ subunit, increases grain size and yield production in rice. *Plant Biotechnology Journal, 17(3),* 650–664.
29. Temple, B. R., Jones, A. M. (2007). The plant heterotrimeric G-protein complex. *Annual Review of Plant Biology, 58,* 249–266.
30. Tao, Y., Miao, J., Wang, J., Li, W., Xu, Y. et al. (2020). *RGG1*, involved in the cytokinin regulatory pathway, controls grain size in rice. *Rice, 13,* 76.
31. Kan, Y., Mu, X. R., Zhang, H., Gao, J., Shan, J. X. et al. (2022). *TT2* controls rice thermotolerance through SCT1-dependent alteration of wax biosynthesis. *Nature Plants, 8(1),* 53–67.
32. Ullah, H., Chen, J. G., Young, J. C., Im, K. H., Sussman, M. R. et al. (2001). Modulation of cell proliferation by heterotrimeric G protein in *Arabidopsis*. *Science, 292(5524),* 2066–2069.
33. Li, S., Liu, Y., Zheng, L., Chen, L., Li, N. et al. (2012). The plant-specific G protein γ subunit AGG3 influences organ size and shape in *Arabidopsis thaliana*. *New Phytologist, 194(3),* 690–703.
34. Swain, D. M., Sahoo, R. K., Srivastava, V. K., Tripathy, B. C., Tuteja, R. et al. (2017). Function of heterotrimeric G-protein γ subunit RGG1 in providing salinity stress tolerance in rice by elevating detoxification of ROS. *Planta, 245(2),* 367–383.
35. Seo, H. S., Kim, H. Y., Jeong, J. Y., Lee, S. Y., Cho, M. J. et al. (1995). Molecular cloning and characterization of *RGA1* encoding a G protein α subunit from rice (*Oryza sativa* L. IR-36). *Plant Molecluar Biology, 27(6),* 1119–1131.
36. Ashikari, M., Wu, J. Z., Yano, M., Sasaki, T., Yoshimura, A. (1999). Rice gibberellin-insensitive dwarf mutant gene *Dwarf 1* encodes the α-subunit of GTP-binding protein. *Proceedings of the National Academy of Sciences of the United States of America, 96(18),* 10284–10289.
37. Wang, L., Xu, Y. Y., Ma, Q. B., Li, D., Xu, Z. H. et al. (2006). Heterotrimeric G protein α subunit is involved in rice brassinosteroid response. *Cell Research, 16(12),* 916–922.

Appendix

Supplementary Figure S1: Scanning electron microscope observation of the wild type and *ddg1* stems

Supplementary Figure S2: Amino acid sequence alignment of DDG1 and ddg1

Supplementary Figure S3: The phenotype of transgenic negative plant

Supplementary Figure S4: Expression pattern of *DDG1* based on the RiceXPro (https://ricexpro.dna.affrc.go.jp/Zapping/)

Supplementary Figure S5: Amino acid sequence alignment of DDG1 and its homologous proteins

Supplementary Figure S6: DDG1 interacts with RGA1 in yeast cell

Supplementary Figure S7: The *RGA1* mutant was sensitive to salt stress in rice

Supplementary Figure S8: The expression level of *RGA1* in the mutant

Supplementary Table S1: Primers used in this study