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ORIGINAL PAPER

Isolation and characterization of dominant dwarf mutants, *Slr1-d*, in rice

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Abstract *sd1* is known as the 'green revolution' gene in rice because its application in rice breeding has dramatically increased rice yield. Since the 'green revolution,' *sd1* has been extensively used to produce modern semi-dwarf varieties. The extensive use of limited dwarfing sources may, however, cause a bottleneck effect in the genetic background of rice varieties. To circumvent this problem, novel and useful sources of dwarf genes must be identified. In this study, we identified three semi-dominant dwarf mutants. These mutants were categorized as *dn*-type dwarf mutants according to the elongation pattern of internodes. Gibberellin (GA) response tests showed that the mutants were still responsive to GA, although at a reduced rate. Map-based cloning revealed that the dwarf phenotype in these mutants was caused by gain-of-function mutations in the N-terminal region of SLR1. Degradation of the SLR1 protein in these mutants occurred later than in the wild type. Reduced interaction abilities of the SLR1 protein in these mutants with GID1 were also observed using the yeast two-hybrid system. Crossing experiments indicated that with the use of an appropriate genetic background, the semi-dominant dwarf alleles identified in this study could be used to alleviate the deficiency of dwarfing genes for breeding applications.

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Introduction

Dwarfism is an agronomically important trait in breeding for resistance to damage by wind and rain (lodging resistance) and for stable high yields via increases in harvest index (Khush 2001). The two well-known dwarf genes that most significantly contributed to the history of crop breeding are *semi-dwarf1* (*sd1*) in rice and *reduced height1* (*Rht1*) in wheat (Hedden 2003). In the 1960s, IR8, a high-yielding semi-dwarf rice variety possessing the *sd1* gene was developed and distributed all over Asia. The introduction of high-yielding semi-dwarf varieties combined with the application of large amounts of nitrogen fertilizer led to a remarkable increase in rice production that marked the dawn of the 'green revolution' in rice (Hargrove and Cabanilla 1979; Khush 1999). Similarly, introduction of the *Rht1* gene in wheat resulted in a semi-dwarf phenotype. Adoption of the semi-dwarf wheat varieties possessing this gene greatly increased production and led to a parallel 'green revolution' (Hedden 2003). These landmarks in agricultural history clearly highlight the importance of the semi-dwarf trait in crop improvement. Since then, reducing plant height has become a predominant strategy in breeding high-yielding and non-lodging crop varieties (Hargrove and Cabanilla 1979). In fact, IR64, the most widely planted rice variety, possesses the *sd1* gene, and at least seven independent *sd1* alleles have been used to produce semi-dwarf rice varieties (Asano et al. 2007). Both *sd1* and *Rht1* are still widely used to produce modern varieties of rice and wheat, respectively (Hedden 2003).

Semi-dwarf rice varieties have been extensively used not only for the production of inbred, semi-dwarf varieties but

also as parents of F_1 hybrid varieties. Although F_1 hybrid rice varieties have greatly contributed in increasing crop production, the occurrence of excessively tall plants due to heterosis constrains the practical breeding of F_1 hybrids. To resolve this constraint, both the male and female parents must carry the same recessive semi-dwarf gene. Inevitably, this strategy led to the accumulation of several parental lines carrying the *sd1* gene that are available for hybrid breeding (Yuan 1996; Kikuchi and Futsuhara 1997). The extensive use of limited dwarfing sources may, however, cause a bottleneck effect in the genetic background when breeding for new varieties, and this may cause the eventual genetic vulnerability of crops to pests and diseases (Hargrove and Cabanilla 1979; Kikuchi and Futsuhara 1997). To circumvent this problem, novel and useful sources of dwarf genes must be identified. Of particular advantage would be the identification and the subsequent use of dominant dwarf mutants. When one parent carries a dominant dwarf allele, the other parent need not carry a dwarf gene. Consequently, the germplasm of the other parent would not be restricted. Moreover, F_1 seed production can be increased by selecting sterile parents that are 10–20 cm lower than the pollinator parent to increase pollen shedding on the female panicle (Virmani and Edwards 1983). Thus, introduction of a dominant dwarf allele into a sterile parent will increase seed production in the F_1 hybrid.

To date, more than 60 rice dwarf genes have been identified (Futsuhara and Kikuchi 1997), although only a few have been identified as dominant, including *D53*, *Ssi1*, *Sdd*(*t*), and *Td1* (Sunohara and Kitano 2003; Sunohara et al. 2006; Wei et al. 2006; Liu et al. 2008). Although several dwarf genes in rice have been recently cloned through map-based cloning (Ashikari et al. 1999; Yamamuro et al. 2000; Komorisono et al. 2005; Ueguchi et al. 2005; Zou et al. 2006), the genes are recessive and are not available for practical use, especially since the mutants have abnormal phenotypes. In contrast, no study has reported on the cloning of dominant and useful dwarf alleles in rice, although some reports exist on the cloning and characterization of dominant dwarf mutants in other crops such as wheat, barley, and maize (Peng et al. 1999; Chandler et al. 2002). Here we report on the first map-based cloning of a semi-dominant dwarf allele and the elucidation of the mechanisms that govern the semi-dominant dwarf phenotype in rice.

Materials and methods

Plant materials and growth conditions

Wild-type rice plants (*Oryza sativa japonica* cvs. T65 and Kinmaze) and three mutant alleles of *Slr1-d* were used in this study. All alleles were identified in a mutant library generated using *N*-methyl-*N*-nitrosourea (MNU). These lines were grown under natural field conditions in the research field of Nagoya University, Togo, Aichi, Japan. Seeds of all the lines were immersed in water for 2 days and were sown in a nursery bed. One-month-old seedlings were transplanted to the paddy field at 20 × 35 cm spacing.

GA induction in shoot elongation

Seeds of the wild-type and mutant lines were sterilized with 2.5% NaClO for 30 min, washed five times in sterile distilled water, and then incubated at 4°C for 1 day. The seeds were then placed on 1% agar plates containing various concentrations of GA_3 and grown under continuous fluorescent light at 30°C. After 10 days, the length of the second leaf sheath of each plant was measured.

Mapping and sequencing of *Slr1-d*

To map *Slr1-d*, 634 F_2 plants derived from a cross between *Slr1-d3* and Kasalath, an indica cultivar, were used for map-based cloning. To identify the mutation sites in the *Slr1-d* alleles, *SLR1* was amplified using genomic DNA extracted from the three mutant lines. The amplified DNA fragments were sequenced directly using appropriate primers.

Plasmid construction, transformation, and growth condition

To construct *HA-SLR1* in a pActNos/Hm2 vector, each of the amplified *SLR1* and *HA* fragments containing the appropriate restriction sites were initially cloned into a pBluescript vector using an *Eco*RI site for *SLR1* and *Xba*I–*Sma*I sites for *HA*. To clone the *HA*, the pBluescript was modified to eliminate the *Eco*RI site in the vector by double digestion with *Eco*RV and PstI, followed by blunting the ends using T_4 polymerase and self-ligation. The *SLR1* clone was digested with *Eco*RI and inserted into HA at the *Eco*RI site. The *HA–SLR1* construct was digested with *Xba*I and *Sma*I, and inserted into pActNos/Hm2 at the *Xba*I and *Sma*I sites. The binary vectors were then introduced into *Agrobacterium tumefaciens* strain EHA 101 (Hood et al. 1986) by electroporation. Rice transformation was performed as described by Hiei et al. (1994) using T65, a *japonica* cultivar. Transgenic plants were selected on a medium containing 50 mg/L hygromycin. Hygromycin-resistant plants were transplanted to the soil and grown at 30°C under a 16-h light:8-h dark photoperiod.

Protein extraction and immunoblot analysis

Total proteins were extracted by grinding the calli with liquid nitrogen and then resuspending the ground tissue in

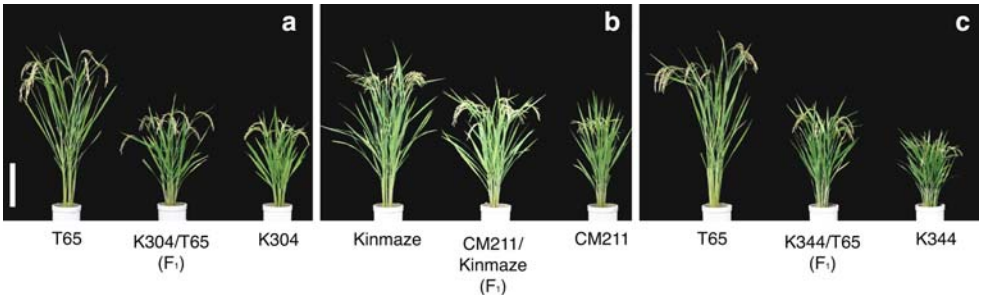

Fig. 1 Gross morphology of dominant dwarf mutants at harvest. *Each panel* shows the wild type (T65 for K304 and K344, Kinmaze for CM211) (*left*), F_1 plants derived from a cross between *Slr1-d* mutants and its wild type (*center*), and homozygous plants (*right*) of **a** K304 (*Slr1-d1*), **b** CM211 (*Slr1-d2*), and **c** K344 (*Slr1-d3*). *Bar* 30 cm

extraction buffer [20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.5% Tween 20, 1 mM EDTA, 1 mM dithiothreitol (DTT)] containing the Complete protease inhibitor cocktail (Roche, Switzerland). The extracts were cleared with two centrifugations at 20 min in a microcentrifuge. The protein concentrations of the soluble fractions were determined using Bradford reagent (Bio-Rad, USA) with bovine serum albumin as the standard. For immunoblot analysis, 10 μg of proteins was separated by SDS-PAGE and transferred to a Hybond ECL membrane (Amersham-Pharmacia Biotech, USA) by semi-dry blotting. Immunoblot analysis was carried out using a 1:10,000 dilution of a rabbit anti-SLR1 serum (Itoh et al. 2002) as the primary antibody and a horseradish peroxidase-conjugated goat anti-rabbit IgG (Medical and Biological Laboratories, Japan) as the secondary antibody. Peroxidase activity was detected following the manufacturer's instructions (Pierce, USA).

Yeast two-hybrid assay

The Matchmaker Two-Hybrid System (Clontech, USA) was used for the yeast two-hybrid assay. pGBKT7-GID1 served as the bait, and pGADT7-SLR1 as the prey. Plate assays (-His) and β-galactosidase (β-gal) liquid assays were performed according to the manufacturer's protocol (Clontech, USA) with the modification that the plate and liquid media either contained 10^{-4} M GA_3 or not. The yeast strain AH109 was used for growth tests on -His plates, and the strain Y187 was used for the detection of β-gal activity by liquid assay.

Results

Identification of semi-dominant dwarf mutants

Three semi-dominant dwarf rice mutants (K304, CM211, and K344) were screened from more than 2,000 M_2 lines mutagenized with MNU. K304 and K344 were derived from T65, whereas CM211 was derived from Kinmaze. All three mutants showed 50–70% reduction in plant height compared to the wild type, and had wide and dark green leaf blades (Fig. 1). Crossing K304, CM211, and K344 with their respective wild types yielded F_1 plants with intermediate plant height relative to both parents (Fig. 1). In the subsequent F_2 populations, the segregation ratio of dwarf to normal phenotype was 90:31 in K304, 69:23 in CM211, and 90:33 in K344, which corresponded to the expected 3:1 segregation ratio of single dominant genes ($\chi^2 = 0.003$, 0.014, and 0.133 in K304, CM211, and K344, respectively; Table 1). These results corroborate that dwarfism in K304, CM211, and K344 is controlled by a single dominant gene in each.

Table 1 Segregation of F_2 plants derived from a self-pollinated F_1 plant crossed between the dwarf mutants and their wild type

	Dwarf	Normal	χ^2 (3:1)
K304/T65	90	31	0.003 NS
CM211/Kinmaze	69	23	0.014 NS
K344/T65	90	33	0.133 NS

NS not significant

To investigate whether the genes responsible for the dwarf phenotypes in K304, CM211, and K344 were allelic, homozygous lines of the mutants (*K304/K304*, *CM211/CM211*, and *K344/K344*) were selected through progeny tests and were then crossed with each other using all possible cross-combinations. F_1 and F_2 populations derived from the crosses exhibited a dwarf phenotype without segregation (data not shown). The results indicated that the genes conferring the dwarf phenotype in K304, CM211, and K344 were allelic.

Characterization of dominant dwarf mutants in rice

The dwarf phenotype in rice is generally caused by a reduction in the length of the culm. Based on the elongation pattern of internodes, Takeda (1977) classified the dwarf phenotypes in rice into six types: *N*-, *dn*-, *dm*-, *d6*-, *nl*-, and

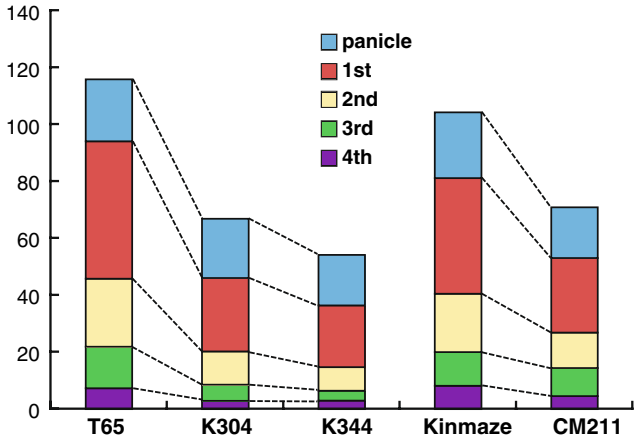

Fig. 2 Diagram of plant height and internode length of T65, Kinmaze, and dwarf mutants

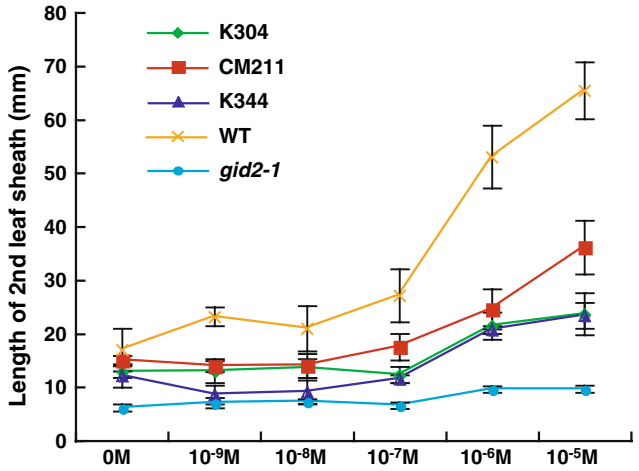

Fig. 3 Elongation of the second leaf sheath in response to GA_3 treatment. Wild type (*WT*) and GA-insensitive mutant (*gid2-1*) were used as controls. *Error bars* the SD from the mean ($n = 10$)

sh-. Of these, the *dn*-type is defined by a reduction in the length of all internodes. The mutants K304, CM211, and K344 exhibited a reduction in the length of all internodes, characteristic of the *dn*-type dwarf (Fig. 2, Table 2).

Dwarfism in plants may arise due to various reasons, although defects in gibberellin (GA) biosynthesis and perception are the primary determinants of plant height. GAs comprise a large family of tetracyclic diterpenoid plant hormones that induce a wide range of plant growth responses including seed germination, stem elongation, leaf expansion, induction of flowering, and pollen maturation (Richards et al. 2001; Thomas et al. 2005). GA-related mutants exhibit dwarfism without any additional aberrant morphology. Often, leaf blades of the GA-related mutant plants are shorter, wider, and darker in color than those of the wild-type plants (Sakamoto et al. 2004). In the present study, the dominant mutants exhibited phenotypes that were reminiscent of GA-related mutants, and hence, the effects of GA application on the second leaf sheath elongation of the mutants were examined. Elongation of the second leaf sheath of the wild type was observed with the application of 10^{-8} to 10^{-7} M GA_3. No such effects were observed in K304, CM211, and K344 with the application of $\sim 10^{-7}$ M GA_3 (Fig. 3). Treatment with 10^{-5} M GA_3 resulted in the elongation of the second leaf sheath of the wild type up to 65 mm, whereas the same treatment only resulted in the elongation of the second leaf sheath of the mutants up to between 10 and 30 mm (Fig. 3). The results indicated that the mutants were still responsive to GA, albeit at a reduced rate.

Map-based cloning of the dwarf gene

To elucidate the molecular mechanism of the dominant genes conferring dwarfism in the mutants, map-based cloning of the dominant gene controlling dwarfism in K344, which showed the most severe phenotype, was carried out. In total, 634 F_2 plants generated by crossing K344 with Kasalath were used to map the location of the gene. Plant height of the F_2 population showed continuous and transgressive segregation (data not shown), so only plants exhibiting a severe dwarf phenotype were selected and used for mapping. Linkage analysis indicated that the dominant dwarf gene was tightly linked with one STS marker, TG1093, and was localized between the STS markers, ddw77 and ddw132, on the long arm of chromosome 3 (Fig. 4a). Cross-referencing in the Rice Annotation Project Database (Rice Annotation Project 2008) showed more

Table 2 Length of each internode of the mutants and their wild type

	Panicle (cm)	First internode (cm)	Second internode (cm)	Third internode (cm)	Fourth internode (cm)
T65	21.77 (±1.81)	48.34 (±1.98)	23.84 (±1.27)	14.60 (±2.25)	7.34 (±2.54)
K304	20.83 (±0.95)	25.75 (±0.93)	11.72 (±0.44)	5.68 (±1.16)	2.91 (±0.66)
K344	17.73 (±0.96)	21.76 (±1.19)	8.21 (±0.82)	3.50 (±1.29)	3.00 (±0.71)
Kinmaze	23.08 (±1.01)	40.63 (±2.23)	20.51 (±1.89)	11.86 (±1.89)	8.20 (±1.95)
CM211	17.89 (±1.60)	26.19 (±1.19)	12.40 (±0.66)	9.88 (±3.76)	4.59 (±1.10)

Numbers in parentheses indicate standard deviation ($n = 5$)

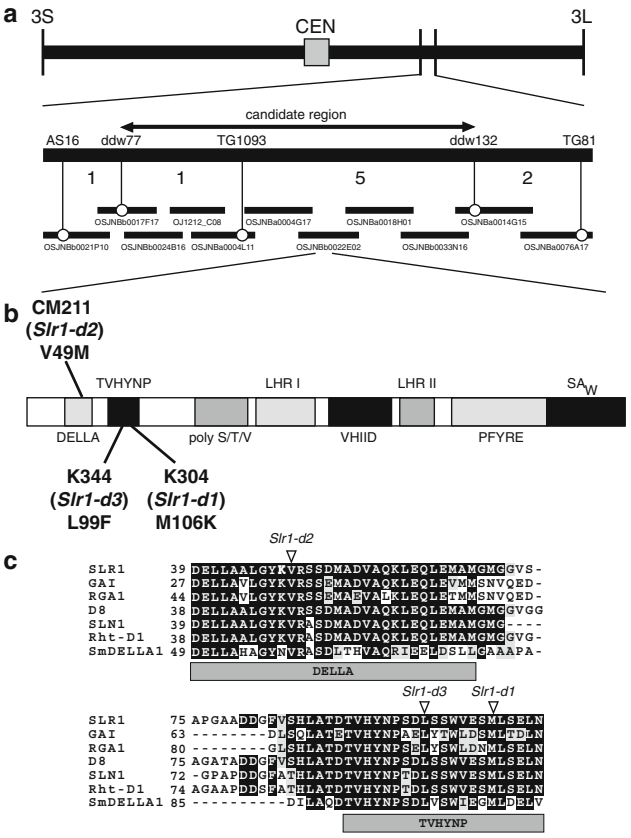

Fig. 4 Map-based cloning of *K344* and sequence comparison of DELLA proteins. **a** High-resolution linkage and physical map of the *K344* gene. *Horizontal lines* represent chromosome 3 and a physical map around the *K344* locus of chromosome 3, near 126 cM. The *vertical bars* represent the molecular markers, and the *numbers* of recombinant plants are indicated between markers. **b** Structure of the *SLR1* gene and mutation sites of the *Slr1-d* alleles. **c** Comparison of the deduced amino acid sequences of DELLA proteins. Amino acid sequences of DELLA proteins from rice (SLR1), *Arabidopsis* (GAI1 and RGA1), maize (D8), barley (SLN1), wheat (Rht1-D1), and lycophyte (SmDELLA1) were aligned using ClustalW, followed by manual alignment. The *open triangles* indicate mutation sites of *Slr1-d* mutants

than 120 predicted genes in this region. Survey of the predicted proteins within this candidate region showed that one gene encodes the DELLA protein, SLR1. DELLA proteins encoded by *SLR1* and its orthologs in *Arabidopsis* (*GAI*, *RGA*, *RGL1*, *RGL2*, and *AGL3*), maize (*d8*), barley (*SLN1*), and grape (*VvGAI*) have a conserved function as repressors of GA signaling (Sun and Gubler 2004). Previous studies showed that in-frame deletion and specific point mutations within the N-terminal domain (DELLA and TVHYNP motifs and nearby sequences) result in constitutive DELLA protein function, thus conferring a dominant and GA-insensitive dwarf phenotype (Peng et al. 1997, 1999). Since the dwarf mutants in the current study were controlled by a dominant gene and had reduced GA responsiveness, the *SLR1* gene was considered a very likely candidate for the dominant gene controlling dwarfism in K304, CM211, and K344.

Molecular analysis of the dominant dwarf mutants

To verify whether the dwarf phenotypes were caused by mutations within *SLR1*, DNA sequence analysis of *SLR1* in the mutants was carried out. As expected, all mutants had a single nucleotide substitution in the N-terminal domain of *SLR1*. Based on these results and those of subsequent transgenic analysis, we renamed K304 as *Slr1-d1*, CM211 as *Slr1-d2*, and K344 as *Slr1-d3*. These 1-bp substitutions induced an amino acid substitution of Val-49 to Met in the DELLA domain of *Slr1-d2* (CM211), and Leu-99 to Phe and Met-106 to Lys in the TVHYNP domain of *Slr1-d3* (K344) and *Slr1-d1* (K304), respectively (Fig. 4b). All amino acid residues substituted in *Slr1-d* mutants are likely to be important for DELLA protein function because they are completely conserved in all of the DELLA proteins from different plant species (Fig. 4c). To confirm that these mutations cause the dwarf phenotypes, transgenic plants that highly and constitutively express mutant SLR1 protein under the control of rice *Actin1* promoter were generated and compared in terms of plant height. All transgenic plants expressing the mutant SLR1 proteins showed a more severe dwarf phenotype than those expressing the wild-type SLR1 protein (Fig. 5). The severity of the dwarf phenotype in the transgenic plants was correlated with the severity of dwarfism in the mutants (compare Figs. 5, 1), thereby confirming that the dominant dwarf mutants were caused by the gain-of-function of rice DELLA protein, SLR1.

Accumulation of SLR1 protein in *Slr1-d* mutants

The DELLA protein acts as a repressor of GA signaling, and its GA-dependent degradation is an essential event in GA signaling. Previous studies showed that gain-of-function mutation in the N-terminal domain of the DELLA

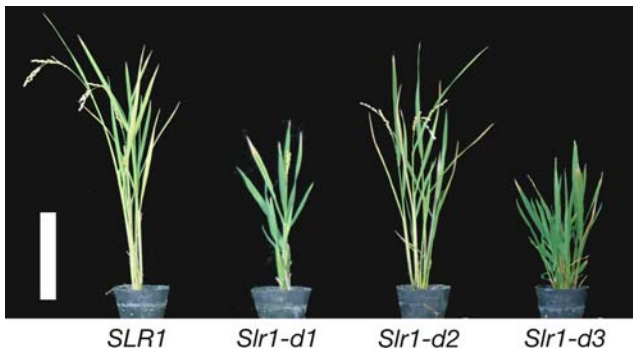

Fig. 5 Gross morphology of transgenic plants overproducing mutant SLR1 protein. From *left* to *right*, transgenic plants highly express SLR1 from the wild type, *Slr1-d1*, *Slr1-d2*, and *Slr1-d3*. *Bar* 20 cm

protein, such as RGA and GAI in *Arabidopsis* and SLN1 in barley, gives the mutant protein resistance to GA-dependent degradation resulting in a GA-insensitive dwarf phenotype (Dill et al. 2001; Gubler et al. 2002). To examine the extent of GA responsiveness of Slr1-d proteins compared to the wild-type SLR1, immunoblot analysis using polyclonal anti-SLR1 antibodies was performed. Slr1-d calli were used for the experiment since rice callus cells contained very low levels of bioactive GA (Itoh et al. 2005), thus eliminating the need to pretreat these materials with a GA synthesis inhibitor to exclude the possibility of differences in GA content between varieties. Concordant with the results presented by Itoh et al. (2005), the GA-dependent degradation of SLR1 triggered by GA_3 occurred within 60 min in the wild-type callus (Fig. 6). In the *Slr1-d* mutants, however, complete degradation of SLR1 proteins was not observed within 60 min. Furthermore, the rate of degradation corresponded to the severity of dwarfism in the mutants; that is, degradation of SLR1 protein in *Slr1-d2*, the mutant with the mildest dwarf phenotype, occurred the earliest, followed by that in *Slr1-d1* and finally by that in *Slr1-d3*, the mutant exhibiting the most severe dwarfism (compare Figs. 6, 1). These results indicate that mutations in the N-terminal domain of SLR1 confer resistance against GA-dependent degradation, making the mutants only moderately sensitive to GA.

Interaction between Slr1-d and GID1

Recent studies showed that DELLA proteins interact with the GA receptor, GID1, in a GA-dependent manner. The interaction occurs as a first step in GA signaling and is necessary for subsequent degradation of DELLA proteins. GID1 proteins in rice and *Arabidopsis* can interact with DELLA proteins in a GA-dependent manner in yeast cells (Ueguchi-Tanaka et al. 2005, 2007; Griffiths et al. 2006; Nakajima et al. 2006; Willige et al. 2007). A yeast two-hybrid assay was used to determine whether the interaction between Slr1-d and GID1 was affected in the *Slr1-d* mutants. Slr1-d proteins were expressed as GAL4 transactivation domain (AD) fusion proteins and the GID1 as GAL4 DNA-binding domain (BD) fusion proteins in yeast. Growth tests on -His plates using the yeast strain AH109, with or without 10^{-4} M GA_3, were performed. All yeast cells expressing Slr1-d and GID1 grew on -HIS plates in the presence of GA_3, whereas none of the yeast cells grew on -HIS plates without GA_3. No apparent differences in the growth of yeast cells expressing the wild-type SLR1 and Slr1-d were observed, indicating that all of the Slr1-d proteins interacted with the GID1 protein in a GA-dependent manner (Fig. 7b). To assess the extent of interaction between Slr1-d and GID1, β-gal activity was measured by liquid assay using yeast strain Y187, with or without 10^{-4} M GA_3. All Slr1-d weakly interacted with GID1 relative to the wild type (8.6, 0.22, 6.8, and 15.8 Miller units in Slr1-d1, Slr1-d2, Slr1-d3, and the wild type, respectively) (Fig. 7a). Results of the yeast two-hybrid assays confirmed that the extent of interaction between Slr1-d proteins and GID1 was reduced in the mutants.

Distribution of culm length in another genetic background

The culm length of *Slr1-d2*, the mutant with the mildest dwarf phenotype, was about 50 cm (Fig. 2, Table 2) and therefore may be too short for direct practical use. However, because *Slr1-d* mutants showed no other unfavorable phenotype except for low fertility in *Slr1-d3*, if crossed with tall varieties, these mutants might be useful as new dwarf gene resources. To test whether *Slr1-d2* would have progeny with plant heights suitable for breeding purposes

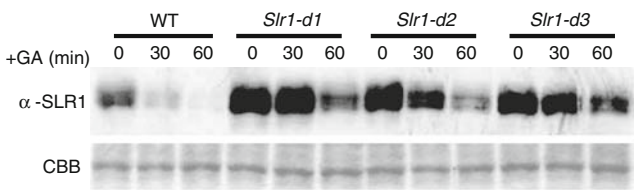

Fig. 6 Degradation of SLR1 induced by GA_3 treatment of the *Slr1-d* protein. Gel-blot analysis of SLR1 in the callus of the wild type, *Slr1-d1*, *Slr1-d2*, and *Slr1-d3*. These calluses were incubated with 10^{-5} M GA_3 for the indicated times. A 10-μg aliquot of protein extract was loaded per lane and probed with anti-SLR1 antibody. The loading control of Coomassie Brilliant Blue staining is shown

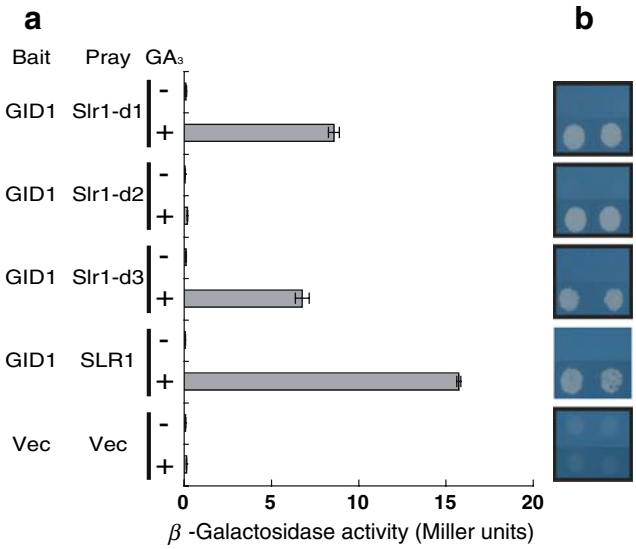

Fig. 7 Interaction between Slr1-d and GID1 in vitro β-gal activity detected in a liquid assay with or without 10^{-4} M GA_3 with yeast strain Y187 (**b**). *Error bars* the SD from the mean ($n = 3$). Growth of yeast strain AH109 on -HIS plate with or without 10^{-4} M GA_3 (**a**)

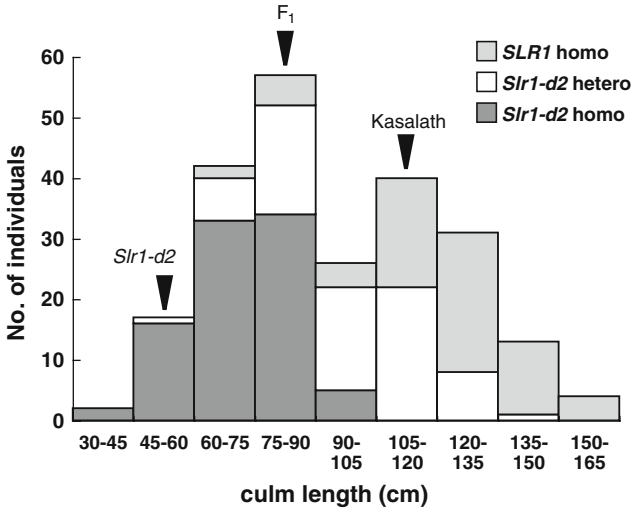

Fig. 8 Frequency distribution of culm length observed in the F_2 population from the cross between *Slr1-d2* and Kasalath

when crossed with a variety of different genetic backgrounds, *Slr1-d2* was crossed with Kasalath, one of the tall *indica* varieties. Culm length showed a continuous and transgressive segregation in F_2 populations (Fig. 8). We observed plants showing suitable culm length (around 70 cm) for practical use in both F_1 and F_2 populations.

Discussion

To date, more than 60 dwarf mutants of rice have been identified (Kikuchi and Futsuhara 1997) and some of them have been cloned through map-based cloning (Ashikari et al. 1999; Yamamuro et al. 2000; Komorisono et al. 2005; Ueguchi-Tanaka et al. 2005; Zou et al. 2006). However, because these mutants show malformed phenotypes such as short grain, extreme dwarfism, and sterility, *sd1* is still the gene that is predominantly being used to produce semi-dwarf varieties (Hedden 2003; Asano et al. 2007). Identification of dominant dwarf genes would provide a new genetic resource that can be tapped for producing inbred semi-dwarf varieties and also for generating F_1 hybrids. Cloning of the 'green revolution' genes, *sd1* and *Rht1*, revealed that both are related to GA. The *SD1* gene encodes the GA biosynthetic enzyme, GA20 oxidase 2, and mutations in this gene cause a reduction in the endogenous GA level, consequently resulting in a semi-dwarf phenotype in *sd1* mutants (Ashikari et al. 2002; Sasaki et al. 2002; Spielmeyer et al. 2002). In contrast, *Rht1* encodes a GA signal suppressor DELLA protein and deletion in the N-terminal region of the native RHT1 constitutively suppresses GA signaling, consequently resulting in a dominant semi-dwarf phenotype (Peng et al. 1997, 1999). Both cases underline the importance of GA in the regulation of developmental processes critical to agriculture, making the GA pathway a prime target for improving crop yield (Hedden 2003).

In this study, novel dominant mutants were identified: *Slr1-d1*, *Slr1-d2*, and *Slr1-d3*. All *Slr1-d* mutants had reduced plant height via the inhibition of elongation of all internodes, which is characteristic of *dn*-type dwarf rice mutants. Based on GA response tests, we concluded that *Slr1-d* mutants were still responsive to GA, albeit at a reduced rate. Cloning of the gene revealed that all *Slr1-d* mutants had a 1-bp substitution in the rice DELLA gene, *SLR1*, and transgenic analysis confirmed that these mutations were responsible for the dominant dwarf phenotype. Immunoblot analysis revealed that degradation of the SLR1 protein in *Slr1-d* mutants occurs later than in the wild type, with the rate of degradation corresponding to the severity of dwarfism in the mutants. DELLA proteins interact with the GA receptor, GID1, and this interaction eventually leads to the induction of DELLA protein degradation via the 26S proteasome pathway (McGinnis et al. 2003; Sasaki et al. 2003). Reduced interaction abilities of the SLR1 protein in the *Slr1-d* mutants with GID1 were confirmed using the yeast two-hybrid system. However, the extent of interaction did not correspond to the severity of dwarfism in the mutants. In particular, even under the presence of GA, SLR1 from *Slr1-d2*, the mutant with the mildest dwarf phenotype, showed no or very weak interaction with GID1. This may have been due to the unstable or low expression level of SLR1 protein of *Slr1-d2* in Y187. Previous studies using a deletion series of the DELLA protein in yeast two-hybrid analysis also revealed that DELLA proteins and GID1 interact via the N-terminal of the DELLA protein (Ueguchi-Tanaka et al. 2007; Griffiths et al. 2006; Nakajima et al. 2006; Willige et al. 2007). In this study, results of interaction studies between GID1 and the SLR1 proteins from the *Slr1-d* mutants having single amino acid substitutions in the DELLA and TVHYNP domain reconfirmed the essential role of the N-terminal domain in the interaction between SLR1 and GID1. Based on these results, we concluded that the dwarf phenotypes of the *Slr1-d* mutants are caused by the inefficient GA-dependent degradation of the SLR1 protein due to the reduced interaction with GID1.

Fu et al. (2001) produced transgenic rice plants expressing the mutant form of *Arabidopsis* DELLA protein, gai, a protein resistant to GA-dependent degradation due to its lack of 17 amino acids in the DELLA domain. The authors pointed out the possible application of gai transgenic plants in breeding. However, because cultivation of transgenic plants in the field is restricted, identification and subsequent application of novel dwarf genes would still be more advantageous compared to the transgenic approach. *Slr1-d2*, the mutant with the mildest dwarf phenotype, may be too short for direct practical use. However, crossing

experiments between *Slr1-d2* and Kasalath revealed that culm length showed continuous and transgressive segregation in the resultant F_2 populations, and plants with suitable culm length (around 70 cm) for practical use were also observed in F_1 and F_2 populations. The results indicate that with the use of an appropriate genetic background, the dominant dwarf genes identified in this study could provide a powerful tool to resolve the deficiency in dwarf genes for breeding. Alternatively, exploiting milder *Slr1-d* alleles using a more extensive screening approach or the tilling method (Suzuki et al. 2008) may yield new genetic sources for dwarf breeding. The practical application of this gene in breeding is currently under way.

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