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ORIGINAL PAPER

OsGA20ox1, a candidate gene for a major QTL controlling seedling vigor in rice

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Abstract Seedling vigor is among the major determinants of stable stand establishment in direct-seeded rice (*Oryza sativa* L.) in temperate regions. Quantitative trait loci (QTL) for seedling vigor were identified using 250 recombinant inbred lines (RILs) derived from a cross between two *japonica* rice cultivars Kakehashi and Dunghan Shali. Seedling heights measured at 14 days after sowing were 20.3 and 29.4 cm for Kakehashi and Dunghan Shali, respectively. For the RILs, the height ranged from 14.1 to 31.7 cm. Four putative QTLs associated with seedling height were detected. *qPHS3-2*, the major QTL that was located on the long arm of chromosome 3, accounted for 26.2 % of the phenotypic variance. Using progeny of the near isogenic lines (NILs) produced by the backcross introduction of a chromosome segment carrying this major QTL into an elite cultivar Iwatekko, we fine-mapped *qPHS3-2* to a 81-kb interval between two markers, ID_CAPS_01 and RM16227. Within this mapped region, we identified the gene *OsGA20ox1*, which is related to gibberellin (GA) biosynthesis. The relative expression levels of *GA20ox1* in seedlings of Dunghan Shali and NILs were higher than that of Iwatekko. Concomitantly, the amount of endogenous active GA was higher in Dunghan Shali and the NILs compared to the level detected in Iwatekko. These results indicate that *OsGA20ox1* is a strong candidate gene for major QTL controlling seedling vigor in rice.

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Introduction

Direct seeding of rice (*Oryza sativa* L.) is becoming popular in Japan as it involves lower labor costs compared to the conventional transplanting of seedlings from nursery. However, yield loss mainly due to slow seedling growth is a common problem associated with direct seeding. Delayed emergence of rice seedlings from paddy water surface has been shown to greatly increase seedling mortality (Peterson et al. 1978), thereby causing significant reductions in yield. In addition, there is a higher risk of yield loss due to competition with weeds associated with direct seeding than transplanting (Rao et al. 2007). Seedling vigor is mainly determined by the rate of germination and rapid early growth of both the shoot and roots (Williams and Peterson 1973; Sasahara et al. 1986). However, this poses a major problem in temperate regions where low temperature limits early seedling growth (Redona and Mackill 1996a). As a

result, success in direct sowing of germinated seeds in puddled soils, which is becoming a common practice in countries like Japan, depends on having cultivars with good seedling vigor for stable stand establishment.

Many important agronomic traits, including seedling vigor, are generally controlled by complex interactions of multiple genes known as quantitative trait loci (QTLs) (Yano 2001; Yano and Sasaki 1997; Ashikari and Matsuoka 2006). Natural variation in QTLs accounts for a wide range of phenotypic differences (Yano and Sasaki 1997). To understand the genetic architecture of agronomic traits, separation of QTLs affecting complex traits into individual loci is important. Quantitative trait loci analysis aims to identify approximate positions of QTLs, and has been successfully used to identify loci or genes for complex traits (Yamamoto et al. 2009; Miura et al. 2011). Some QTLs for seedling vigor in rice have been identified by QTL analysis (Onishi et al. 2007; Redona and Mackill 1996b; Cui et al. 2002; Zhang et al. 2005a, b; Zhou et al. 2007). However, these results have not been always consistent in terms of the exact locations of the QTLs, although the QTLs have been mapped to specific regions in the genome.

The current study was initiated with the main aim of identifying QTLs for seedling vigor in the cultivar Dunghan Shali (*O. sativa* L. subsp. *japonica*). Dunghan Shali was identified as one of the few cultivars showing higher seedling vigor among our rice cultivars and breeding lines, and was then used as a potential donor parent to incorporate this important trait into elite cultivars. A set of recombinant inbred lines (RILs) derived from a cross between Kakehashi (*Oryza sativa* L. subsp. *japonica*) and Dunghan Shali was used to map QTLs affecting seedling vigor. We also produced NILs carrying a major QTL in an elite cultivar background and evaluated its contribution to the enhanced seedling vigor. Here, we report the chromosomal positions of the QTLs and the major QTL *qPHS3-2*, as well as identification of the main gene most likely responsible for the difference in seedling height between Dunghan Shali and Kakehashi.

Materials and methods

Plant materials

To produce RILs, we selected Kakehashi and Dunghan Shali as parental rice cultivars. Kakehashi, a temperate *japonica* variety, is adapted to the relatively cooler northern Japan climate. Dunghan Shali is a temperate *japonica* variety grown in Hungary and shows superior seedling vigor. The F_1 plants generated by crossing Kakehashi with Dunghan Shali using the former as female parent were self-pollinated, and ~300 F_2 seeds were obtained. Each F_2 seed was maintained as RIL by single-seed descent method, resulting in a total of 250 RILs of F_7 generation.

Development of NILs

We used the rice cultivar Iwatekko (*japonica*) as the recurrent parent to generate NILs harboring the target QTL region, *qPHS3-2*, from Dunghan Shali. Iwatekko is a promoted cultivar in Iwate prefecture (Japan), and is preferred for its superior food quality as well as for its tolerance to cool temperature at the booting stage. Iwatekko was first crossed to Dunghan Shali, and the resulting F_1 plants were backcrossed four times to Iwatekko using marker-assisted selection (Yano and Sasaki 1997; Yano 2001).

Evaluation of seedling height

The seeds of all lines used in this study were imbibed in water for 5 days at 12 °C and then forced into sprouting for 2 days at 25 °C. Fifteen seeds per line were sown on a cell plug tray (cell count: 8 × 16; tray size: 600 × 300 mm; depth of cell: 45 mm), and the plants were germinated and grown in a greenhouse where the temperature was controlled at 25 °C but under natural light conditions. Seedling height (length between the coleoptile node to the tip of longest leaf blade) was measured at 14 days after sowing.

Genotyping of RILs and NILs with DNA markers

For genotyping the RILs, we used a total of 88 Simple Sequence Repeat (SSR) markers that covered the 12 rice chromosomes from the lists provided by McCouch et al. (2002) and IRGSP (2005). Procedures for DNA extraction and SSR marker polymorphism detection were described previously (Matsubara et al. 2008; Hori et al. 2011). An additional set of 11 SSR (McCouch et al. 2002; IRGSP 2005), two insertions and deletions (InDels) and two cleavage amplified polymorphic sequence (CAPS) markers (Suppl. Table S1) located on the long arm of chromosome 3 and showing polymorphisms between Dunghan Shali and Iwatekko were used to develop the NILs.

Map construction and QTL mapping

The linkage map was constructed using Mapmaker/EXP 3.0 (Lander et al. 1987), and the Kosambi map function was used to calculate genetic distances (Kosambi 1943). Quantitative trait loci analysis was performed using composite interval mapping as implemented by the Zmapqtl program (model 6) provided by the software package QTL Cartographer version 2.5 (Basten et al. 2005; http://statgen.

ncsu.edu/qtlcart/WQTLCart.htm). A threshold value ($r = 0.05$) was determined by 1,000 permutation tests.

Gene expression analysis

Total RNA was isolated from embryo or shoots sampled from plants grown under continuous light at 7, 12, and 17 days after the start of imbibition (DAI) of the seeds using an RNA isolation kit (RNeasy plant mini, QIAGEN). This was followed by DNase treatment to remove contaminating DNA from the extracts. Two micrograms of total RNA were converted to cDNA with RTase (TOYOBO).

To quantify the expression levels of transcripts, real-time RT-PCR analysis was performed using SYBR green (QIAGEN) on a Step-One Plus Real-Time PCR system (Applied Biosystems). Expression levels were normalized with the *OsActin1* gene. The primer sequences used in this study are provided in Suppl Table S1.

Quantification of the endogenous gibberellins

About 80 mg fresh weight sample, which was composed of leaf sheath and the stem but without the leaf blades and the roots, was subjected to the analysis. The concentrations of gibberellins were measured with MS-probe modification method as previously described (Kojima et al. 2009). In brief, the extract was passed through an Oasis HLB column (Waters, Milford, MA, USA), and gibberellin-containing fraction was obtained by solid-phase extraction with an Oasis MCX column (Waters). After modification with MS-probe, the derivatized gibberellins were measured with a liquid chromatography–tandem mass chromatography system (AQUITY UPLC™ System/XEVO-TQS; Waters) with an ODS column (AQUITY UPLC BEH C_{18}, 1.7 μm, 2.1 × 100 mm, Waters). Data were processed by MassLynx™ software with QuanLynx™ (version 4.0, Waters).

Results

Phenotypic variation

A clear difference in seedling height was observed between the two parental lines grown at 25 °C for 14 days (Fig. 1a). On average, seedlings of Kakehashi attained a height of 20.3 cm (±1.3), whereas those of Dunghan Shali reached about 29.4 cm (±2.9). The RILs showed a continuous distribution with respect to seedling height from 14.1 to 31.7 cm, suggesting that this trait is controlled by multiple QTLs (Fig. 1b).

QTL analysis for plant height of seedling

We constructed a genetic linkage map, based on RILs of the F_6 generation, using a total of 88 SSR markers. The segregation ratios of the two genotype classes fit the expected Mendelian ratio of 1:1 in most loci. However, segregation distortion was observed for two loci located each on chromosome 3 (RM232–RM7365) and 11 (RM7221–RM5926), for which all RILs had the Kakehashi type alleles. Segregation distortion has been frequently observed in populations derived from intraspecific crosses of rice, and certain genomic regions related to segregation distortion have been uniquely reported to particular crosses (Matsubara et al. 2011). The region showing segregation distortion on chromosome 3 (RM232–RM7365) is the same location as the one identified in several previous studies (Matsubara et al. 2011). Nevertheless, the region on chromosome 11 has not been reported before.

Composite interval mapping was performed to identify the QTLs associated with seedling height, based on 250 RILs of the F_7 generation. A major QTL (LOD = 17.5) controlling plant height was identified on chromosome 3 with an average contribution of 26.2 % to the phenotypic variation (Fig. 2; Table 1). This QTL, designated as

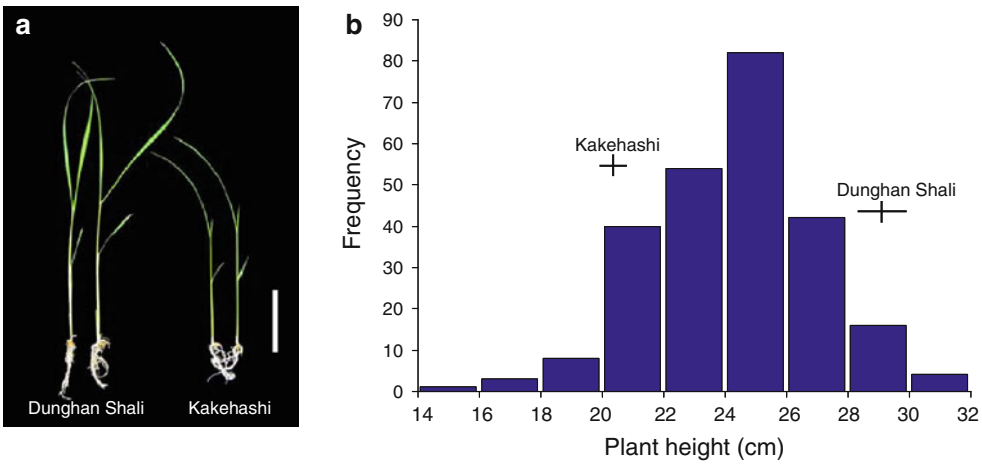

Fig. 1 Seedling vigor of Dunghan Shali, Kakehashi and RILs. **a** Gross morphology at 14 days after sowing. *Scale bar* 5 cm. **b** Frequency distribution of seedling height of 250 RILs germinated and grown at 25 °C for 14 days. *Vertical and horizontal bars* indicate the mean value and standard deviation, respectively

qPHS3-2 (QTL for plant height of seedling 3-2), was located within a 12.2-cM interval flanked by two SSR markers, RM1373 and RM7389, on the distal end of the long arm of chromosome 3. Three additional QTLs, *qPHS1* on chromosome 1, *qPHS3-1* on chromosome 3 and *qPHS4* on chromosome 4, were detected each accounting for 7.2, 6.6 and 5.2 % of the total phenotypic variation, respectively (Fig. 2; Table 1).

Production of NILs and fine-mapping of *qPHS3-2*

To evaluate the effects of individual QTLs separately, it is necessary to produce NILs for each QTL in a common genetic background (Paterson et al. 1988). We produced NILs carrying the major QTL from Dunghan Shali in an Iwatekko genetic background by a repeated backcrossing to Iwatekko. By means of marker-assisted selection using a total of 88 SSR markers, we developed nine BC_4F_3 NILs harboring different portion of Dunghan Shali segments in the long arm of chromosome 3 (Fig. 3). The rest of the genome was Iwatekko type for all the nine NILs. Using these NILs, we fine-mapped the major QTL, *qPHS3-2*. The eight NILs (17, 19, 28, 92, 86, 32, 33 and 114) harboring Dunghan Shali segments at the distal region of the long arm of chromosome 3 in an Iwatekko background had significantly larger plant height than Iwatekko (Fig. 3). Among the eight NILs, NIL-114 has the shortest DNA segment (797-kbp) derived from Dunghan Shali at the marker interval RM2187–RM16245. On the other hand, the seedling height of NIL-11, which contained only a short Dunghan Shali segment at its proximal-end and the largest segment of Iwatekko within the candidate region for *qPHS3-2*, was not significantly different from that of Iwatekko seedlings. These results reveal that the *qPHS3-2*

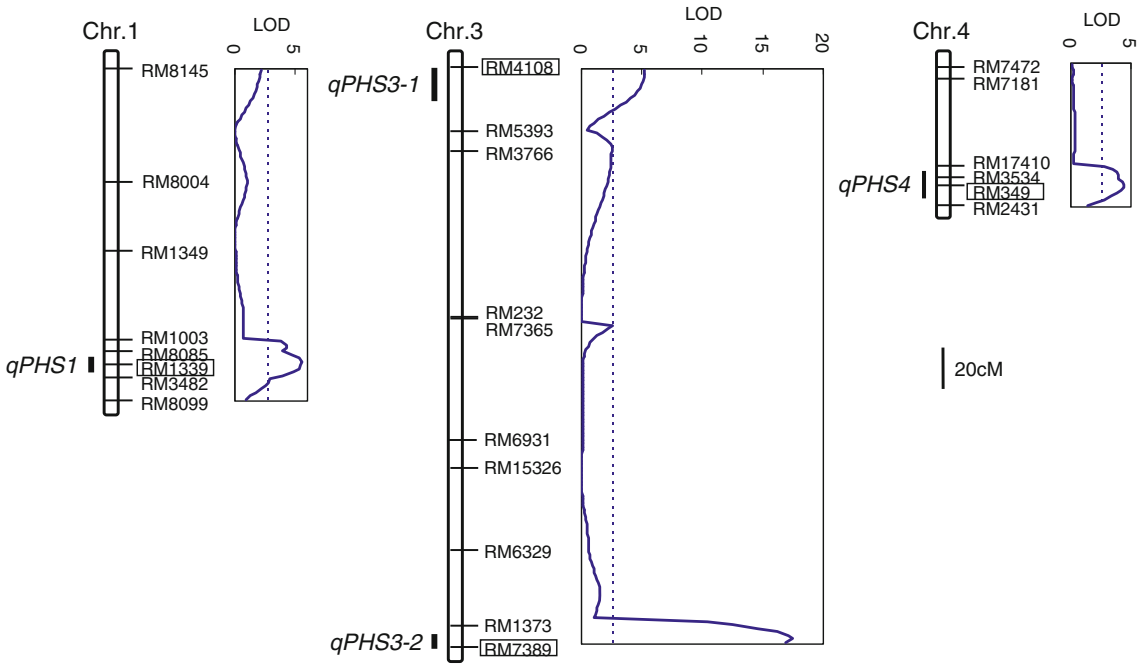

Fig. 2 Linkage map of the RILs derived from a cross between Kakehashi and Dunghan Shali showing location of the putative QTLs with LOD score. The markers in *boxes* represent those nearest to their respective QTLs

Table 1 QTLs for height of seedlings detected in the RILs derived from a cross between Kakehashi and Dunghan Shali

QTL	Chr.	Marker interval	Nearest marker	LOD	PVE	AE (cm)
qPHS1	1	RM8085–RM1339	RM1339	5.5	7.2	0.8
qPHS3-1	3	RM4108–RM5393	RM4108	5.3	6.6	0.7
qPHS3-2	3	RM1373–RM7389	RM7389	17.5	26.2	1.5
qPHS4	4	RM3534–RM349	RM349	4.4	5.2	0.6

All genetic parameters were calculated by QTL Cartographer Version 2.5

The LOD threshold for detection of QTL in the composite interval mapping procedure was 2.6

LOD Log-likelihood value, *PVE* percentage of total phenotypic variance explained by the QTL, *AE* additive effect of Dunghan Shali allele for plant height of seedling

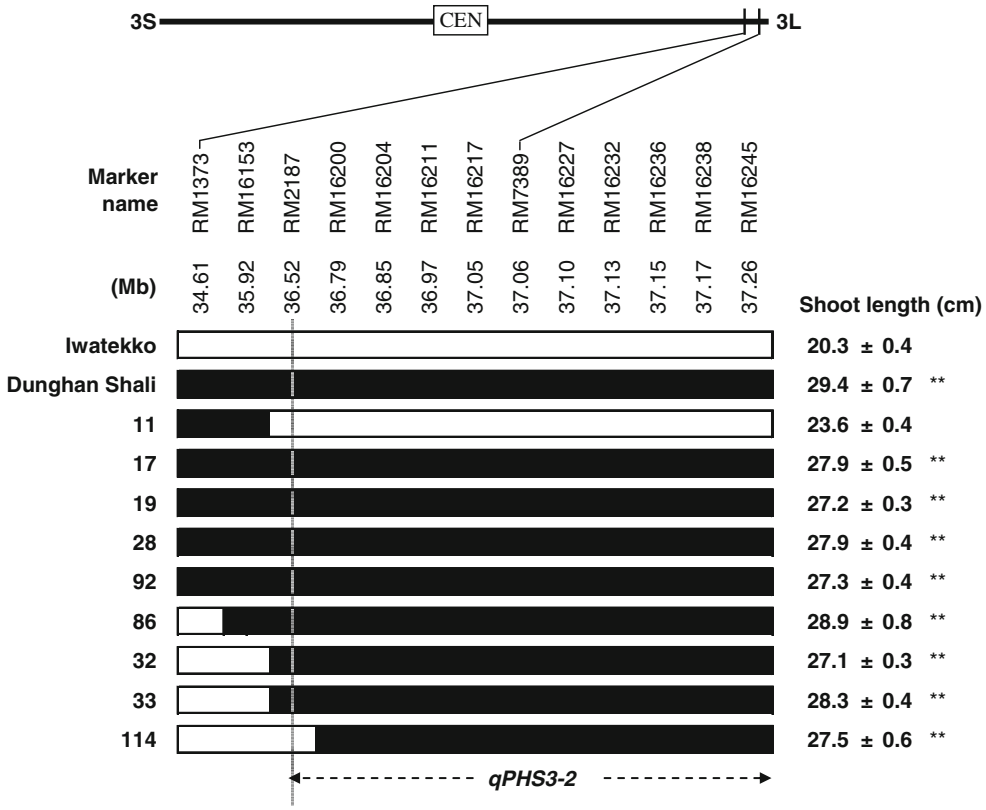

Fig. 3 Graphical genotypes of near isogenic lines (NILs) of BC_4F_3 generation and location of *qPHS3-2*. *White and black boxes* indicate homozygous regions for the Iwatekko and Dunghan Shali, respectively. Mean shoot length and standard deviation values were included. *Double asterisks* indicate significant difference ($P < 0.01$) between Iwatekko and Dunghan Shali or NILs by Dunnett's multiple comparison test

is localized within a 797-kbp region in the distill region of the long arm of chromosome 3 (Fig. 3).

To narrow down the *qPHS3-2* region, we increased the number of NILs that harbor recombinations between RM16200 and RM16245. Two BC_4F_4 lines, NIL-115-18 and NIL-116-66, had different segments of Dunghan Shali chromosome within the interval RM2187–RM16245 (Fig. 4a). Plant heights of the progeny derived from selfing of these two lines (BC_4F_5) did not show significant difference from that of Iwatekko (Fig. 4a). Besides, we obtained an additional NIL, NIL-116-43-497, which had Dunghan Shali genome segment in the marker interval between RM16217 and RM7389 in a heterozygous state (Fig. 4b). The BC_4F_6 obtained by selfing of this NIL exhibited segregation for plant height among the seedlings (Fig. 4c). Progeny with Dunghan Shali genome segment in this region in homozygous or heterozygous states showed larger seedling heights than those with Iwatekko-type segment in homozygous state. This result suggested that Dunghan Shali-type allele of the QTL might be dominant over the Iwatekko allele (Fig. 4c).

Furthermore, NIL-497-8 with Dunghan Shali genome segment in the marker interval between ID_INDEL_02 and ID_CAPS_02 was selected from NIL-116-43-497 at BC_4F_6 generation. NIL-497-8 harboring only 81 kb segment of Dunghan Shali genome in the Iwatekko background showed a significantly larger seedling height than Iwatekko (Fig. 5a). This result strongly suggests that *qPHS3-2* resides in the interval between ID_CAPS_01 and RM16227 with a distance of 81 kb (Fig. 5).

Expression of *OsGA20ox1* in seedlings

The 81-kb interval between markers ID_CAPS_01 and RM16227 is predicted to contain ten genes including *OsGA20ox1*, a gene that encodes gibberellins 20-oxidase-1 (Fig. 5b). The gibberellins (GAs) are plant hormones that play important roles in many aspects of plant growth and development, such as seed germination, stem elongation, and flower development (Yamaguchi and Kamiya 2000; Olszewski et al. 2002). Loss-of-function mutations of the *OsGA20ox2/sd1* gene that is located on chromosome 1 cause semi-dwarfism, resulting in lodging resistance and increased grain yields, and thus are responsible for rice 'Green Revolution' (Sasaki et al. 2002). It is interesting that *OsGA20ox1* is located within the 81-kb candidate genomic region of the major QTL for plant height, *qPHS3-2*, detected in the present study (Fig. 5b). To evaluate the relationship between seedling height and *OsGA20ox1*, real-time RT-PCR analysis of *OsGA20ox1* gene expression was performed using Iwatekko, Dunghan Shali, NIL-114 and NIL-497-8 (see Figs. 4a, 5a) during the initial growth stage. Relative expression levels of *OsGA20ox1* in embryo of seeds maintained at 25 °C for seven DAI (days after

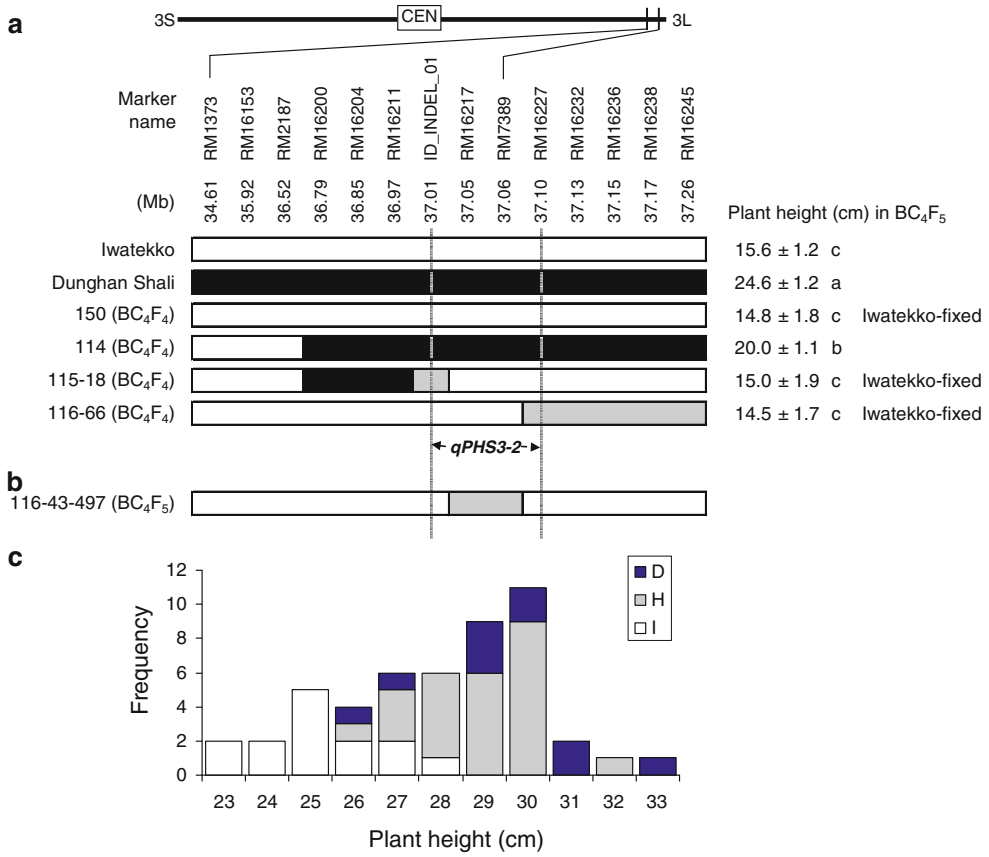

Fig. 4 Fine-mapping of *qPHS3-2*. **a** Graphical genotype of Iwatekko, Dunghan Shali and NILs of BC_4F_4 generation. Positions of the markers in rice genome sequence (Build 5.0; IRGSP 2005) are given. *White and black boxes* are homozygous for the Iwatekko- and Dunghan Shali-type chromosome segments, respectively. *Gray box* indicates heterozygous region. Plant height (mean ± SD) was measured in BC_4F_5 progeny that were obtained by self-pollination of each NILs. *Letters* indicate significant difference among the six lines ($P < 0.01$, Tukey's multiple comparison tests). **b** Graphical genotype of NIL-116-43-497 in BC_4F_5. *White and gray boxes* indicate homozygous and heterozygous for the Iwatekko-type segment, respectively. **c** Frequency distribution of seedling height in the BC_4F_6 progeny that was obtained by self-pollination of NIL-116-43-497. *D*, *H* and *I* indicate Dunghan Shali-type homozygous, heterozygous, and Iwatekko-type homozygous, respectively, for RM16217 and RM7389 markers

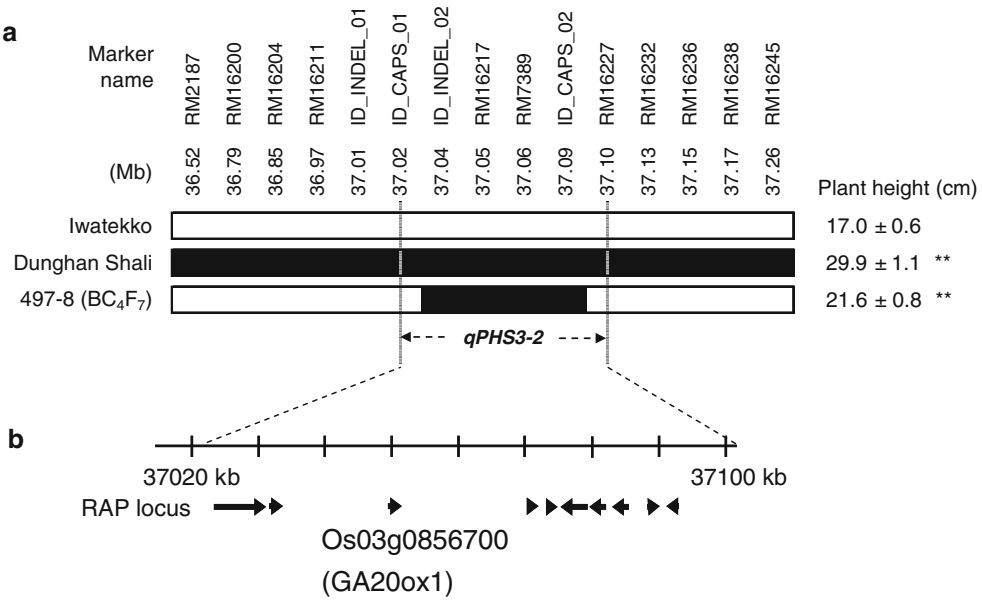

Fig. 5 High-resolution mapping of *qPHS3-2*. **a** Graphical genotype of Iwatekko, Dunghan Shali and NILs of BC_4F_7 generation with plant height (mean ± SD) at seedling stage. *White and black boxes* are homozygous for the Iwatekko- and Dunghan Shali-type chromosome segments, respectively. *Double asterisks* indicate significant difference ($P < 0.01$) between Iwatekko and Dunghan Shali or NILs by Dunnett's multiple comparison test. **b** Predicted genes, including *GA20ox1*, in the *qPHS3-2* candidate region according to the Rice Annotation Project Database (RAP-DB) (Ohyanagi et al. 2006)

start of imbibition) were clearly different between Iwatekko and the other three lines (Fig. 6a). All the four lines began to germinate at this stage, but the initiation of germination in Dunghan Shali was clearly earlier as compared to Iwatekko and NIL-114 (Fig. 7), as well as compared to NIL-497-8 (data not shown). In addition, the relative expression levels of *OsGA20ox1* in seedlings of Dunghan Shali, NIL-114 and NIL-497-8 at 12 DAI and 17 DAI were significantly higher than that of Iwatekko (Fig. 6b). However, *OsGA20ox1* expression levels in seedlings (Fig. 6b) were lower than the levels detected in embryo at germination stage (Fig. 6a).

We also compared the genomic *OsGA20ox1* sequence between Iwatekko and Dunghan Shali alleles. There was no nucleotide change in the coding region, but there were four DNA changes within the 10-kb region upstream of the translation start site (Fig. 8).

Quantification of endogenous GAs in seedling

Gibberellic Acid$_1$ (GA_1) is the primary active GA in the vegetative shoots of rice, and the early-13-hydroxylation pathway is considered to be predominant (Kobayashi et al. 2000). In addition, the rice *OsGA20ox1* enzyme prefers GA_{53} to GA_{12} as a substrate (Toyomasu et al. 1997). Therefore, we measured endogenous concentrations of the 13-hydroxylated GAs (GA_{53}, GA_{44}, GA_{19}, GA_{20}, GA_1 and GA_8) in rice seedlings without leaves and roots sampled at 18 DAI. Compared with Iwatekko, we detected significantly higher levels of GA_{20}, the final product of GA20ox activity, in Dunghan Shali and NIL-114, which is consistent with the *GA20ox1* expression levels in these lines (Fig. 9). In Dunghan Shali and NIL-114, the levels of

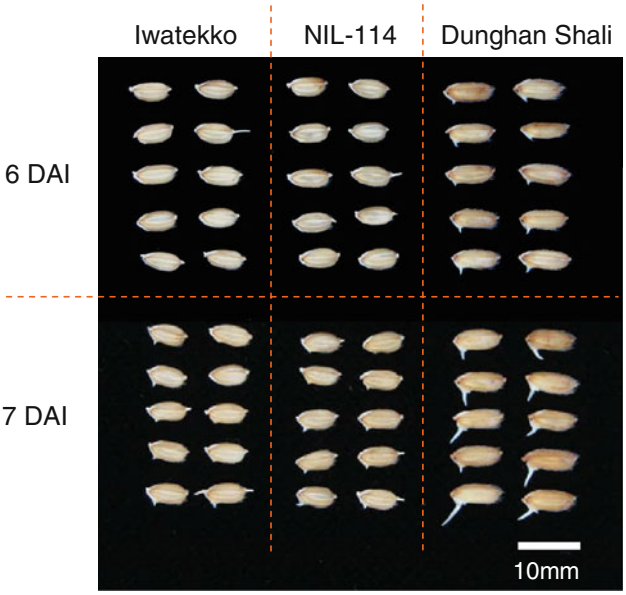

Fig. 7 Variation in germination rate between Dunghan Shali, Iwatekko and NIL-114. The germination in Dunghan Shali was visible from about six DAI. No difference in germination rate was observed between Iwatekko and NIL-114

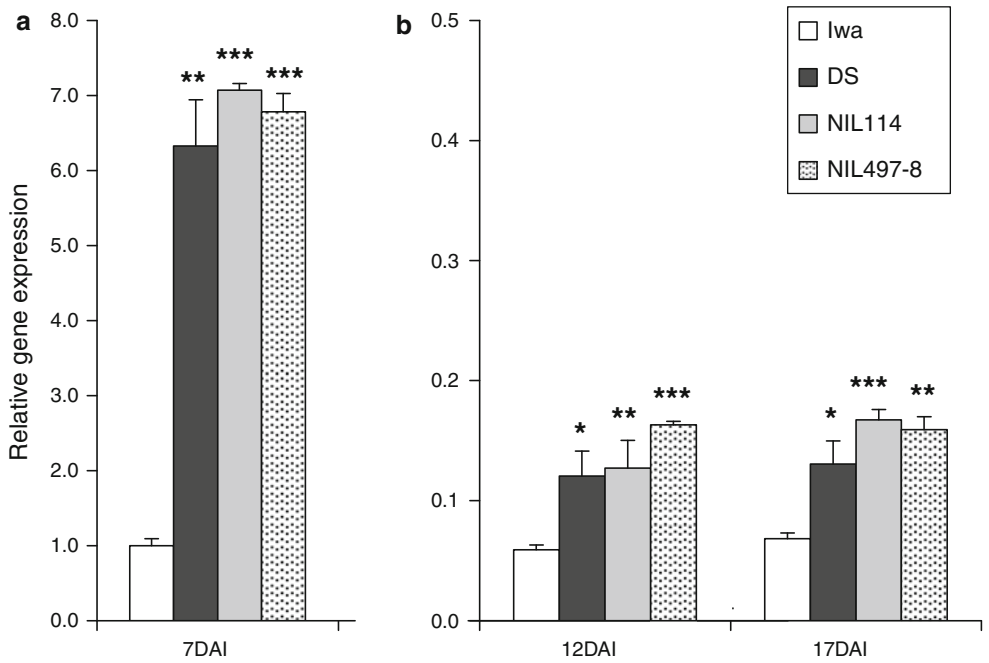

Fig. 6 Relative expression levels of *OsGA20ox1* during shoot elongation at **a** 7 days, and **b** 12 and 17 days after start of imbibition (DAI). Transcript abundance was determined by real-time RT PCR and normalized to the abundance in Iwatekko at seven DAI (mean ± SD, $n = 3$). *OsActin1* was used as an internal control for normalization. *Asterisks* indicate significant differences from Iwatekko (Student's *t* test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$)

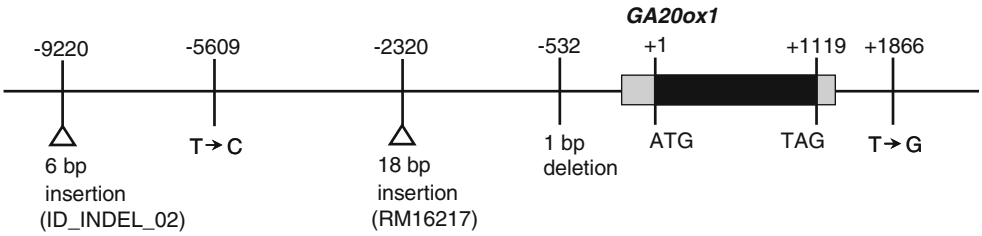

Fig. 8 Comparison of the DNA sequences of *GA20ox1* between Dunghan Shali and Iwatekko. Nucleotide changes are shown for Dunghan Shali using Iwatekko as the reference

active GA (GA_1) and its catabolic GA (GA_8) were also significantly higher than in Iwatekko (Fig. 9).

Effects of *qPHS3-2* on the plant characteristics at maturity

Enhanced seedling growth of NILs harboring *qPHS3-2* prompted us to study its effect on plant gross morphology at the mature stage. Thus, we measured culm length (plant height), panicle length, and panicle number in NIL-114 and Iwatekko in two consecutive seasons, 2010 and 2011 (Table 2). We also made additional measurements on internode length, number of grains per panicle in 2011. Culm length of NIL-114 was longer than that of Iwatekko in both seasons, but the difference was statistically significant only in the 2011 experiment. The longer culm length in NIL-114 appears to be due to the proportional

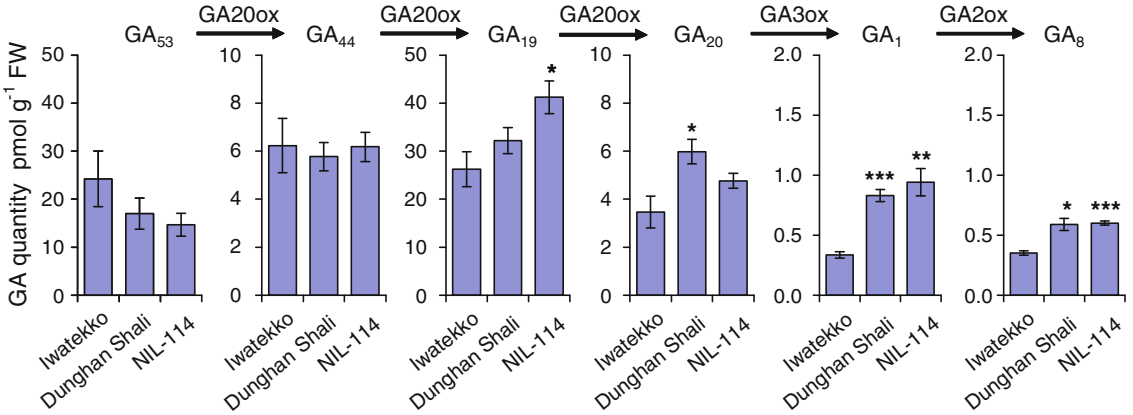

Fig. 9 Endogenous concentration of GAs in seedlings without leaves and roots at 18 days after start of imbibition. The GA biosynthesis pathway (Olszewski et al. 2002) is shown above graphs. Gibberellic acid values are mean ± SE ($n = 4$). *Asterisks* indicate significant differences from Iwatekko (Student's *t* test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$)

Table 2 Phenotypic characteristics of NIL-114 at the mature stage

	Culm length (cm)[a]	Internode length (cm)[a,b]					Panicle length (cm)[a]	No. of panicles per hill	No. of grains per panicle
		I	II	III	IV	V			
2010 ($n = 8$)									
NIL-114 (BC_4F_6)	85.0 ± 1.1	–	–	–	–	–	20.9 ± 0.6	10.4 ± 0.5	–
Iwatekko	82.5 ± 1.9	–	–	–	–	–	18.9 ± 0.3	10.6 ± 0.7	–
	ns	–	–	–	–	–	**	ns	–
2011 ($n = 10$)									
NIL-114 (BC_4F_7)	91.4 ± 0.5	39.2 ± 0.8	24.5 ± 0.4	16.3 ± 0.4	9.5 ± 0.4	2.0 ± 0.5	19.5 ± 0.2	16.9 ± 0.8	84.7 ± 1.4
Iwatekko	85.0 ± 1.0	38.4 ± 0.7	23.1 ± 0.5	14.7 ± 0.7	7.8 ± 0.6	1.0 ± 0.3	20.7 ± 0.4	15.6 ± 0.7	83.4 ± 1.7
	***	ns	*	ns	*	ns	*	ns	ns

[a] Measurements of culm, internode and panicle lengths were made on the tallest tillers each sampled from hills showing optimum growth. In 2010 and 2011, one and three plants were planted per hill, respectively. Asterisks indicate significant differences (Student's *t* test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$)

[b] Each internode is numbered from top to bottom, such that the uppermost internode just below the panicle is the first (I)

elongation of all the internodes, although only the contributions of the second and fourth internodes were significant. Although the differences are subtle, this result suggests that Dunghan Shali allele of *qPHS3-2* enhances not only growth at the seedling stage but also promotes internode elongation, thereby resulting in longer culms at the mature stage. On the other hand, we could not find significant differences between NIL-114 and Iwatekko with respect to the other traits measured, i.e., panicle length, panicle number and grain number (Table 2).

Discussion

Seedling vigor is one of the major determinants for stable stand establishment in direct seeding of rice in temperate regions. Previous genetic analyses have revealed that seedling vigor is controlled by several genes (Onishi et al. 2007; Redona and Mackill 1996b; Cui et al. 2002; Zhang et al. 2005a, b; Zhou et al. 2007). However, the genes responsible for the enhanced growth of Dunghan Shali seedlings have not been addressed yet. In this study, we identified QTLs for seedling vigor, and demonstrated that the QTL, *qPHS3-2*, that was located on chromosome 3 explains a major part (26.2 %) of the total phenotypic variation in the RILs population that was derived from a cross between Kakehashi and Dunghan Shali (Fig. 2; Table 1).

Our results show that the height of seedlings in Dunghan Shali is controlled by a QTL with a major effect, *qPHS3-2*, and additional QTLs with minor effects, *qPHS1*, *qPHS3-1* and *qPHS4* (Table 1). The major QTL, *qPHS3-2*, was localized to a 12.2-cM interval between the markers RM1373 and RM7389 on chromosome 3. Four QTLs for plant height have been identified by studies involving a cross between Lemont (*O. sativa* L. subsp. *japonica*) and Teqing (*O. sativa* L. subsp. *indica*) (Zhang et al. 2005b). Of these, one QTL was detected near the marker RM148 that was located on chromosome 3, and the Teqing allele in this QTL is associated with the larger plant height (Zhang et al. 2005b). In addition, another QTL, *q2LSL3* (leaf sheath length at seedling stage) was detected using RILs derived from a cross between *O. rufipogon* (from India) and a *japonica* strain (Onishi et al. 2007). Based on their chromosomal locations, these QTLs appear to correspond to the *qPHS3-2* detected in this study. However, further analyses are required to prove the allelic relationship between these QTLs.

In this study, we used a map-based cloning strategy to identify the location of *qPHS3-2* and narrow down its region to 81-kb interval on the long arm of chromosome 3 (Figs. 4, 5). In this region, there are ten putative genes that were annotated by RAP-DB (Ohyanagi et al. 2006) including *OsGA20ox1* (Fig. 5). Gibberellin 20-oxidase (GA20ox) is an enzyme that normally catalyzes the penultimate steps in GA biosynthesis. In rice, four genes encoding isoforms of GA20ox (*OsGA20ox1*, *OsGA20ox2*, *OsGA20ox3* and *OsGA20ox4*) have been identified. *OsGA20ox2*, located on chromosome 1, is well known as 'Green Revolution gene', and loss-of-function mutation in this gene, *sd1*, causes semi-dwarf phenotype (Sasaki et al. 2002; Ashikari et al. 2002). In addition, a previous study reported that rice plant statue is regulated not only by *OsGA20ox2* (*SD1*) but also by *OsGA20ox1* (Oikawa et al. 2004). The expression levels of *OsGA20ox1* in seedlings of Dunghan Shali, NIL-114 and NIL-497-8, an Iwatekko NIL with a replacement of *qPHS3-2* by Dunghan Shali allele, were significantly higher than that of Iwatekko (Fig. 6). Corroborating to this observation, the endogenous GA_1 levels of seedlings in Dunghan Shali and NIL-114 are higher than that of Iwatekko (Fig. 9). These results strongly suggest that the major QTL, *qPHS3-2*, that is involved in the control of height of seedlings corresponds to *OsGA20ox1*. However, we could not detect any nucleotide substitutions in the coding region of *OsGA20ox1* between Dunghan Shali and Iwatekko (Fig. 8). We hypothesize that *qPHS3-2* is caused by an unidentified DNA sequence change (s) between Dunghan Shali and Iwatekko that results in differential expression levels of *OsGA20ox1*. Consistent with this hypothesis, a previous study showed that T-DNA insertion in the upstream of *OsGA20ox1* gene (*O. sativa* L., cv. Dontokoi) enhanced its transcription, resulting in the increase in the endogenous GA_1 levels (Oikawa et al. 2004). We detected several nucleotide substitutions as well as an insertion/deletion in the region upstream of the *OsGA20ox1*, which might have caused the observed differences in expression levels of the gene (Fig. 8).

A GUS activity analysis using the rice cultivar Nipponbare (*japonica*) has revealed that the promoter of *OsGA20ox1* produces low GUS activity in young seedlings (Kaneko et al. 2003). In this study, however, we observed the existence of varietal differences in the expression level of *OsGA20ox1* in rice seedlings. Recently, Yano et al. (2012) conducted a QTL analysis for plant height at the initial growth stage using backcross inbred lines of Koshihikari (*japonica*) and Habataki (*indica*). Combining QTL analysis with microarray expression profiling, they detected two major QTLs, *qEPD1* and *qEPD2*, corresponding to *OsGA20ox2* and *OsGA20ox1*, respectively. They concluded that *OsGA20ox1* and *OsGA20ox2* (*SD1*) function during initial growth, but *OsGA20ox1* plays a dominant role in increasing plant height at this stage. Our present result corroborates with the finding of Yano et al. (2012). Together, we propose that *qPHS3-2*, most probably corresponding to *OsGA20ox1*, could be used for enhancing

yield rice grown by direct seeding method. The Dunghan Shali allele of *qPHS3-2*, the molecular markers and the NIL developed in this study should be useful to improve seedling vigor in rice breeding programs in temperate regions worldwide.

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