Theor Appl Genet (2013) 126:1011–1024
DOI 10.1007/s00122-012-2033-1

ORIGINAL PAPER

Three genetic systems controlling growth, development and productivity of rice (*Oryza sativa* L.): a reevaluation of the 'Green Revolution'

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Received: 15 May 2012 / Accepted: 6 December 2012 / Published online: 12 February 2013
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Abstract The Green Revolution (**GR-I**) included worldwide adoption of semi-dwarf rice cultivars (SRCs) with mutant alleles at *GA20ox2* or *SD1* encoding gibberellin 20-oxidase. Two series of experiments were conducted to characterize the pleiotropic effects of *SD1* and its relationships with large numbers of QTLs affecting rice growth, development and productivity. The pleiotropic effects of *SD1* in the IR64 genetic background for increased height, root length/mass and grain weight, and for reduced spikelet fertility and delayed heading were first demonstrated using large populations derived from near isogenic IR64 lines of *SD1*. In the second set of experiments, QTLs controlling nine growth and yield traits were characterized using a new molecular quantitative genetics model and the phenotypic data of the well-known IR64/Azucena DH population evaluated across 11 environments, which revealed three genetic systems: the *SD1*-mediated, *SD1*-repressed and *SD1*-independent pathways that control rice growth, development and productivity. The *SD1*-mediated system comprised 43 functional genetic units (FGUs) controlled by GA. The *SD1*-repressed system was the alternative one comprising 38 FGUs that were only expressed in the mutant *sd1* backgrounds. The *SD1*-independent one comprised 64 FGUs that were independent of *SD1*. **GR-I** resulted from the overall differences between the former two systems in the three aspects: (1) trait/environment-specific contributions; (2) distribution of favorable alleles for increased productivity in the parents; and (3) different responses to (fertilizer) inputs. Our results suggest that at 71.4 % of the detected loci, a QTL resulted from the difference between a functional allele and a loss-of-function mutant, whereas at the remaining 28.6 % of loci, from two functional alleles with differentiated effects.

Communicated by Y. Xu.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-012-2033-1) contains supplementary material, which is available to authorized users.

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Our results suggest two general strategies to achieve **GR-II** (1) by further exploiting the genetic potential of the *SD1*-repressed and *SD1*-independent pathways and (2) by restoring the *SD1*-mediated pathways, or 'back to the nature' to fully exploit the genetic diversity of those loci in the *SD1*-mediated pathways which are virtually inaccessible to most rice-breeding programs worldwide that are exclusively based on *sd1*.

Introduction

A key element of the Green Revolution (**GR-I**) was the rapid adoption of semi-dwarf rice cultivars (SRCs) that almost tripled worldwide rice production and greatly enhanced food security (Tilman 1998). The high yield potentials of modern SRCs are attributed primarily to their improved harvest index, lodging resistance and "responsiveness" to high inputs (primarily nitrogen and water) (Khush 1995; Matson et al. 1997), contributing to their adoption in irrigated areas that occupy ~57 % of world rice lands. The spreading of SRCs has been accompanied by steadily increased uses of inputs such as chemical fertilizers and pesticides, and by associated increases in major biotic stresses and environmental problems (Vitousek et al. 1997).

Genetically, the short stature of modern SRCs is due to *sd1*, a single locus at which various mutant alleles in the gene encoding gibberellin 20-oxidase (*GA20ox2*), a key enzyme functioning in gibberellic acid (GA) biosynthesis, result in greatly reduced content of the bioactive molecule, GA_1, in SRCs (Spielmeyer et al. 2002; Sasaki et al. 2002). Interestingly, there are two functional alleles, *SD1-GR* and *SD1-EQ* at *SD1* in rice. The former is predominant in ssp. *Indica* landraces and has a greater effect for increased height than the latter, which is fixed in ssp. *Japonicas* of rice (Asano et al. 2011). Over the past five decades, rice-breeding programs worldwide have predominantly used *sd1* backgrounds and yield potentials of both inbred and hybrid SRCs have plateaued, with diminishing responses to ever increasing inputs (Cassman 1999; Peng et al. 1999). Meanwhile, ~50 % of rice grown in the rainfed ecosystems of Asia and Africa that occupy ~30 % of world rice lands remain tall landraces, because most high-yielding SRCs are poorly adapted to abiotic stresses such as drought, submergence, low soil fertility, and problem soils common in rainfed production (Mackill et al. 1996).

To achieve sustainable increase in yield that may help to lift poor rice farmers in rainfed areas of Asia and Africa out of poverty, there has been a call for Doubly **GR** or **GR-II** rice that better balances ecological stewardship, conservation and productivity, and for further increasing the yield potential of SRCs (Conway 1999). Appreciable breeding progress has been made toward this direction by putting genes/traits for abiotic/biotic stress resistances into *sd1* backgrounds (Ali et al. 2006; Lafitte et al. 2006). However, many important questions remain largely unanswered regarding *sd1*. For example, what genetic system(s) do the *sd1* SRCs use to manage their growth and development? Does *sd1* contribute to the yield plateau observed in most SRCs? Indeed, it remains unclear if *sd1* truly contributes to improved productivity of SRCs in ways other than short stature and improved lodging resistance, or if *sd1* affects the low fertilizer use efficiency and vulnerability of most SRCs to abiotic stresses (Paterson and Li 2011).

Here, we tried to answer these questions by extensive analyses of data from two large sets of genetic-mapping experiments using a new molecular quantitative genetics model (Zhang et al. 2011). Our results provided direct evidence for the pleiotropic effects of *SD1* and revealed three genetic systems controlling rice growth, development and productivity that underlie **GR-I**, which have important implications for future rice improvement.

Materials and methods

Plant materials, genotyping and phenotyping

Two series of experiments were carried out in this study. The materials used in the first series of experiments included 808 BC_4F_2 plants and their 720 derived BC_4F_3 progenies from a cross between IR64 and PB24 (IR74418-910-2). PB24 is a near isogenic line (BC_3F_4 line) of IR64 carrying a single large (44.0 cm) Azucena (donor) segment flanked by RM543 (145.6 cm) and RM14 (189.6 cm) of chromosome 1 harboring the *SD1* locus derived from three rounds of backcrosses between IR64 (recurrent parent) and one of the IR64/Azucena DH line, P0055 (donor). P0055 has a strong root system like Azucena and carries a major QTL for deep roots near the *sd1* locus (Shen et al. 2001). The first series of three phenotyping experiments were designed to characterize the pleiotropic effects of *sd1*/*SD1* in the IR64 genetic background. The first one was to evaluate all 808 BC_4F_2 plants for growth- and yield-related traits: plant height (PH), number of tillers (TN), panicle number per plant (PN), panicle length (PL), panicle exertion, number of filled and unfilled spikelets, 100-grain weight, flag leaf angle, length and width in the IRRI screenhouse during 2000–2001 dry-season (Dec 2000–April 2001).

In the second experiment, IR64, Azucena, P0055, PB24, and 720 F_3 lines were evaluated in three environments: the lowland, upland and greenhouse conditions during the 2001 dry (Dec 2000–April 2001) and wet seasons (June–Oct 2001). To reduce competition between tall and short lines, the whole population of 720 F_3 lines was divided into

tall (*SD1*) and short (*sd1*) subpopulations. Tall plants were planted in 24 blocks and short plants into 19 different blocks in the field. The two subpopulations were arranged separately in the field using an incomplete block design (alpha 0, 1 lattice) for the tall lines (13 × 24) and an alpha lattice (13 × 19) for the short lines with three replications for each line. Germinated seeds of each line from trays were directly sowed to the field with three seedlings per hill at a spacing of 20 × 25 cm. Each row plot contained 13 hills. The first five hills in each plot were counted 1 week after dibbling of seedlings in the field. The traits on the first five hills were measured, which included: final PH up to the panicle node, PL, TN, PN in first five hills, heading date (HD), and grain filling and yield scores. In addition, the main culms of three plants or hills in each plot (marked using paper clips) were measured for PH and HD.

The third experiment included the 720 F_3 progenies along with IR64, PB24 and Azucena grown in a randomized complete design with three replications for each line in irrigated lowland field of IRRI. The 25-day-old seedlings were transplanted into single-row plots with a single plant per hill and 12 plants per F_3 line in the field. The spacing was 20 cm between plants within a row and 25 cm between rows. A single row of IR64 was inserted as checks in every 20 rows of the F_3 lines. The following data were collected from five plants in each plot: PH, PN, HD and 1,000-grain weight (GW).

The greenhouse experiment

The fourth experiment was conducted in the greenhouse using a subset of 100 BC_4F_3 lines selected to represent different genotypes at the *sd1* region. IR64 (4 entries), Azucena, PB24, and P0055 were used as checks. On June 7 of 2001, a single plant of each line or check was planted in the aerobic conditions of well-drained plastic bags set into a PVC tube of 1.0-m long and 0.2 m in diameter under two water treatments: the well-watered and water-stress conditions with five replications for each line under each treatment. The experiment was laid out in a randomized complete block design. The soil strength in the PVC tubes was monitored using a penetrometer. The cone penetrometer resistances across different tubes were maintained uniform to minimize constraints to root growth. For the water treatments, the 100 tested BC_4F_3 lines and checks were well watered up to 60 days after sowing and then, watering was withheld until maturity such that all materials experienced drought at the reproductive stage. The experimental design was a 9 × 12 completely randomized alpha lattice design. Four replications of one plant per line were distributed over two runs of two replications each, staggered in time because of space constraints. Individual progeny were grown in aerobic conditions in well-drained plastic bags set into PVC tubes of 75-cm long and 0.2-m diameter. The soil strength in the tubes was maintained uniform to limit constraints to root growth. The plants were uprooted carefully for measuring root traits after 35 days of sowing. The following traits were recorded on each plant at maturity: PH, TN, fresh and dry shoot weight, root length and thickness (measured on 10 nodal roots at 2 cm below the crown base region in the 0–25 cm sections at the tillering peak stage using micrometer), root number (in 0–25 cm section), root dry weight (the soil column of each sampled plant was cut in three sections of 25 cm each, roots from each section were carefully washed and oven dried for 72 h at 55 °C and weighed).

The materials used in the second series of experiment set were 126 doubled-haploid (DH) lines from a cross between IR64 (*indica*) and Azucena (upland *japonica*), as described previously (Li et al. 2003). The genotypic data of the DH lines included 173 markers (143 RFLPs, 8 isozymes, 10 RAPDs and 12 cloned genes) and the linkage map was constructed with a total genome size of 1,789.4 cm and an average distance of 11.1 cm between adjacent markers. Phenotyping of the DH population were conducted across 11 environments in experiments during 1994 and 1995, as described previously (Li et al. 2003). The geography of the environments covered a wide range of latitudes and longitudes from 13.5° to 31.5°N and from 76° to 121.5°E as well as different cropping seasons in Asia (Online resource 1). The randomized block design with 2–3 replications for each tested DH line was used in all environments (Li et al. 2003). Nine traits, including PH (in cm), HD (in days), grain yield (GY, in g/plant), harvest index (HI), biomass (in g/plant), GW (in g), PN, spikelet number per panicle (SN), and spikelet fertility (SF in %) were measured on five representative plants in each plot, as described previously (Li et al. 2003).

Data analyses

QTLs associated with the measured traits within the *SD1* region were detected and mapped using the genotypic data of 11 linked SSR markers covering the *SD1* locus in the 808 BC_4F_2 and 720 BC_4F_3 using the interval mapping method (Zeng 1994). The pleitropic effect of *SD1* on a specific trait was determined and estimated when the LOD peak was mapped to the exact location of *SD1*. Analysis of variance (ANOVA) was performed to partition the variance components for measured traits due to the genotypic differences of individual DH lines, environments and genotype × environment interactions (GEI) using the SAS PROC GLM (SAS Institute 2004). The genetic network affecting all measured traits was constructed according to genetic expectations of the identified QTLs using the molecular-quantitative genetic model (Zhang et al. 2011)

in three steps. First, main-effect (M-QTLs) and digenic epistatic QTLs (E-QTL pairs) affecting each trait in the DH population were identified by ANOVA using SAS PROC GLM (SAS Institute 2004) with the mean trait data in each environment and marker genotype of the DH lines as input data. The statistical threshold to claim an M-QTL or E-QTL pair was $P < 0.005$ in at least two environments. All identified QTL were confirmed in the multiple QTL model by the interval mapping approach using QTLmapper (Wang et al. 1999). When a QTL was detected with the selected threshold, its presence in other environments was re-examined and results were presented if it was also detected with the minimum threshold of $P < 0.05$ in any additional environment. Because *sd1* was detected in the DH population across all environments with the largest effect on PH and pleiotropic effects on many other traits, we divided the DH population into the *sd1* subpopulation (60 lines) and the *SD1* subpopulation (66 lines) based on the genotypes of RZ730 and RG810 flanking *sd1*. All identified M-QTLs and E-QTLs identified in the whole population were reanalyzed in the two subpopulations by ANOVA using SAS PROC GLM (SAS Institute 2004). Second, the relationships between the identified QTLs and *SD1* were determined based on their epistatic relationships and the magnitudes of their QTL main effects using a new molecular-quantitative genetics model (Zhang et al. 2011). Based on their relationships with *SD1*, all identified QTLs or functional genetic units (FGUs) could be attributed to three groups or genetic systems: the *SD1*-mediated, *SD1*-repressed and *SD1*-independent FGUs. We previously defined a group of functionally dependent genes acting at each level of a signaling pathway as a FGU within which functional alleles of all constituent loci are required for the FGU to function normally and have an effect on phenotype (Zhang et al. 2011). For example, the *SD1*-mediated FGUs included QTLs and epistatic QTL pairs that interacted strongly with *SD1* and were detectable only in the whole population and the *SD1* subpopulation, but not in the *sd1* subpopulation. The *SD1* repressed FGUs contained those QTLs and epistatic QTL pairs that were detected only in the whole population and the *sd1* subpopulation, but not in the *SD1* subpopulation. Those FGUs detectable in the whole and both the *SD1* and *sd1* subpopulations belonged to the *SD1*-independent pathways. Furthermore, once the main and epistatic effects of *SD1* and its interacting FGUs were obtained, the functional (regulatory) relationships of *SD1* and its regulated downstream FGUs could be determined based on the predicted patterns of the main effects and epistatic of *SD1* and its downstream QTLs (Zhang et al. 2011). Then, the pathway effects regulated by *SD1* were estimated based on the genetic expectations of the multi-locus genotypes of interacting QTLs using the following formula (Zhang et al. 2011):

$$A_i = F^{r_{SD1}-1} \sum_{i=1}^{m} F^{r_{B_{ij}}} \left[\frac{1}{2} a_{ij}\right] \text{ for any loci in the } SD1 \text{-mediated pathways;} \quad (1)$$

$$A_{ij} = F^{r_{SD1}} \sum_{i=1}^{m} F^{r_{B_{ij}}-1} \left[\frac{1}{2} a_{ij}\right] \text{ for any loci in the } m\,B_{ij} \text{ units,} \quad (2)$$

where A_i is the QTL main additive effects of the interacting loci, a_{ij} is the expected pathway effects of the FGUs (QTLs), $\mathbf{B}_{ij}$ downstream of *SD1*, $F^{r_{SD1}}$ and $F^{r_{B_{ij}}}$ are the frequencies of the functional genotypes at the ***SD1*** and its regulated FGU unit, respectively, which is $1/2$ in the DH population, r_{SD1}, and $r_{B_{ij}}$ are the numbers of segregating loci at the corresponding ***SD1***, ***B*** FGU units (Zhang et al. 2011). The pathway effects of any independent QTL groups (E-QTL pairs) could be estimated similarly using the above formula, while those single independent QTLs in the *SD1*-independent group or *SD1*-repressed group could not be predicted if the FGU was not interacting with any other FGU. Finally, the putative genetic networks underlying all measured traits were constructed according to the relationships between the identified QTLs and *SD1* based on the principle of hierarchy and the total contribution of each of the identified three genetic systems (the *SD1*-mediated, *SD1*-repressed and *SD1* independent pathways) to the total genotypic variance (R^2) of each trait was estimated using the formula derived in the molecular-quantitative genetics theory (Zhang et al. 2011).

Results

Phenotypic variation in the IR64/Azucena DH population and the pleiotropic effects of *sd1*

Across 11 diverse environments (Online resource 1), a DH population of a cross between an SRC, IR64, and a stress tolerant conventional rice cultivar, Azucena, showed highly significant variation among lines (G), among environments (E) and G × E interactions for nine measured growth and yield traits, on average, explaining, respectively, 32.9, 33.4, and 23.1 % of phenotypic variation (Online resource 2). Mapped to the genomic region flanked by DNA markers RZ730 and RG810 on chromosome 1, subdivision of the population based on functional (*SD1*) or loss-of-function (*sd1*) alleles for the **GR** gene revealed a suite of phenotypic effects. The loss-of-function (*sd1*) allele was consistently associated with reduced PH by 14.4 ± 4.1 cm, early HD (1.6 ± 0.8 days), increased HI (3.1 ± 1.3 %), reduced biomass (3.1 ± 2.0 g/plant), reduced GW (1.5 ± 0.5 g), and increased PN (1.2 ± 0.5/plant) across

all environments (Online resource 3). Its effects on GY, SN and SF were small and inconsistent across the environments.

Significant differences in height and many root traits existed between IR64 and its isogenic line PB24 which carries a single large Azucena fragment harboring *SD1* (Table 1). In IR64, *SD1* increased height by 43 cm under the irrigated conditions of the screenhouse and lowland field and by 17.2 cm under the upland drought conditions. In greenhouse pot experiments, *SD1* was associated with increases of 22.8 % in root thickness, 16.7 % in root dry-weight, a remarkable 317.5 % in 'deep' root weight (i.e., 50- to 75-cm depth), and 57.7 % reduction in shallow root weight.

Based on the fine mapping using a large F_2 and F_3 populations derived from a cross between IR64 and PB24, *SD1* had an additive effect of 15.9 cm and a dominance effect of 11.0 cm for increased height estimated from the F_2 population, which were 14.9 and 9.4 cm estimated in the F_3 population under the lowland field (Table 2). Interestingly, *SD1* was associated with increased GW by 2.0 g and 4.7 % reduced fertility under rainfed upland conditions (Table 2). In greenhouse pot experiments, *SD1* had an additive effect of 32 mg of increased total dry root weight (DRW) and a dominance effect of 22.3 mg for reduced DRW, ~50 % of the effects were on the deep roots (50- to 75-cm depth) (Table 2).

Three genetic systems underlying growth, development and productivity of rice

We identified 145 FGUs, each defined as either a M-QTL or an E-QTL affecting the nine measured traits in the whole DH population and/or in the '*SD1*' and '*sd1*' subpopulations across 11 environments (Online resources 4–9). These included 108 M-QTLs and 37 pairs of E-QTLs, which are widely distributed on the 12 rice chromosomes (Fig. 1). Based on the relationships between *SD1* and these FGUs, genetic networks containing all FGUs affecting the nine measured traits (Fig. 2) were constructed based on the expected pathway effects of the estimated main and epistatic effects of the identified QTLs (Zhang et al. 2011), revealing three genetic systems underlying the growth, development and productivity of rice.

Table 1 Phenotypic values of IR64, its isogenic lines PB24 and P0055, and donor (Azucena) for plant height (PH, in cm), and root traits measured under four environments

Traits[a]	Environment[b]	IR64	PB24	P0055	Azucena
PH (cm)	SH	92.0	135.0***	122.0***	132.3***
PH (cm)	LL	90.0	133.0***	–	129.8***
PH (cm)	UL	75.0	92.2***	89.6***	105.0***
RT (mm)	GH	1.01	1.24**	1.20**	1.29***
RN	GH	133.7	121.5	72.5*	92.5*
TDRW (mg)	GH	215.0	250.8	202.8	225.2
DRW-A (mg)	GH	173.3	100.0*	79.1*	76.6*
DRW-B (mg)	GH	40.9	134.1**	92.1**	107.9**
DRW-C (mg)	GH	6.6	16.7*	31.8**	17.8**

[a] RT = mean thickness of roots within 2 m under soil surface in the soil column of PVC tubes; RN = number of roots counted within 25 cm in the soil column of PVC tubes; DRW-A = dry root weight in the up (proximal) 25 cm section of the soil column in the PVC tubes; DRW-B = dry root weight in the middle 25 cm section of the soil column in the PVC tubes; DRW-C = dry root weight in the low (distal) 25 cm section of the soil column in the PVC tubes; TDRW = total dry root weight = (A + B + C)

[b] SH, LL, UL and GH are the testing environments of the screenhouse, lowland field, upland field and greenhouse at IRRI, respectively

*, ** and *** indicate the significance levels of $P \leq 0.05$, 0.01 and 0.001, as compared to IR64 based on *t* tests

Table 2 The phenotypic effects associated with the *SD1* region on plant height (PH), heading date (HD), spikelet fertility (SF), thousand grain weight (GW) and root traits in F_2 and F_3 progenies derived from a cross between IR64 and its isogenic line, PB24 evaluated under four different environments

Traits[a]	E[b]	Gen.	N	Marker interval	LOD	*A*	*D*
PH (cm)	SH	F_2	805	*SD1*	160.30	15.9	11.0
PH (cm)	UL	F_3	720	*SD1*	131.49	8.1	7.8
PH (cm)	LL	F_3	720	*SD1*	197.58	14.9	9.4
HD (days)	UL	F_3	720	RM315-RM472	35.59	2.7	1.3
HD (days)	LL	F_3	720	RM315-RM472	24.38	1.7	1.1
SF (%)	LL	F_3	720	*SD1*	4.67	−1.4	−1.3
SF (%)	UL	F_3	720	*SD1*	21.64	−4.7	−6.5
GW (g)	UL	F_3	720	RM486-RM265	4.61	2.0	−0.16
TDRW (mg)	GH	F_3	100	*SD1*	2.41	32.0	−22.3
DRW-A (mg)	GH	F_3	100	*SD1*	2.08	15.3	−9.7
DRW-B (mg)	GH	F_3	100	*SD1*	4.12	14.5	−11.7
DRW-C (mg)	GH	F_3	100	*SD1*	2.34	2.3	−1.1

Superscripts a and b are the same as Table 1

A the additive effect associated with the functional Azucena allele at the *SD1* locus, *D* the dominance effect associated with the heterozygote at the *SD1* locus

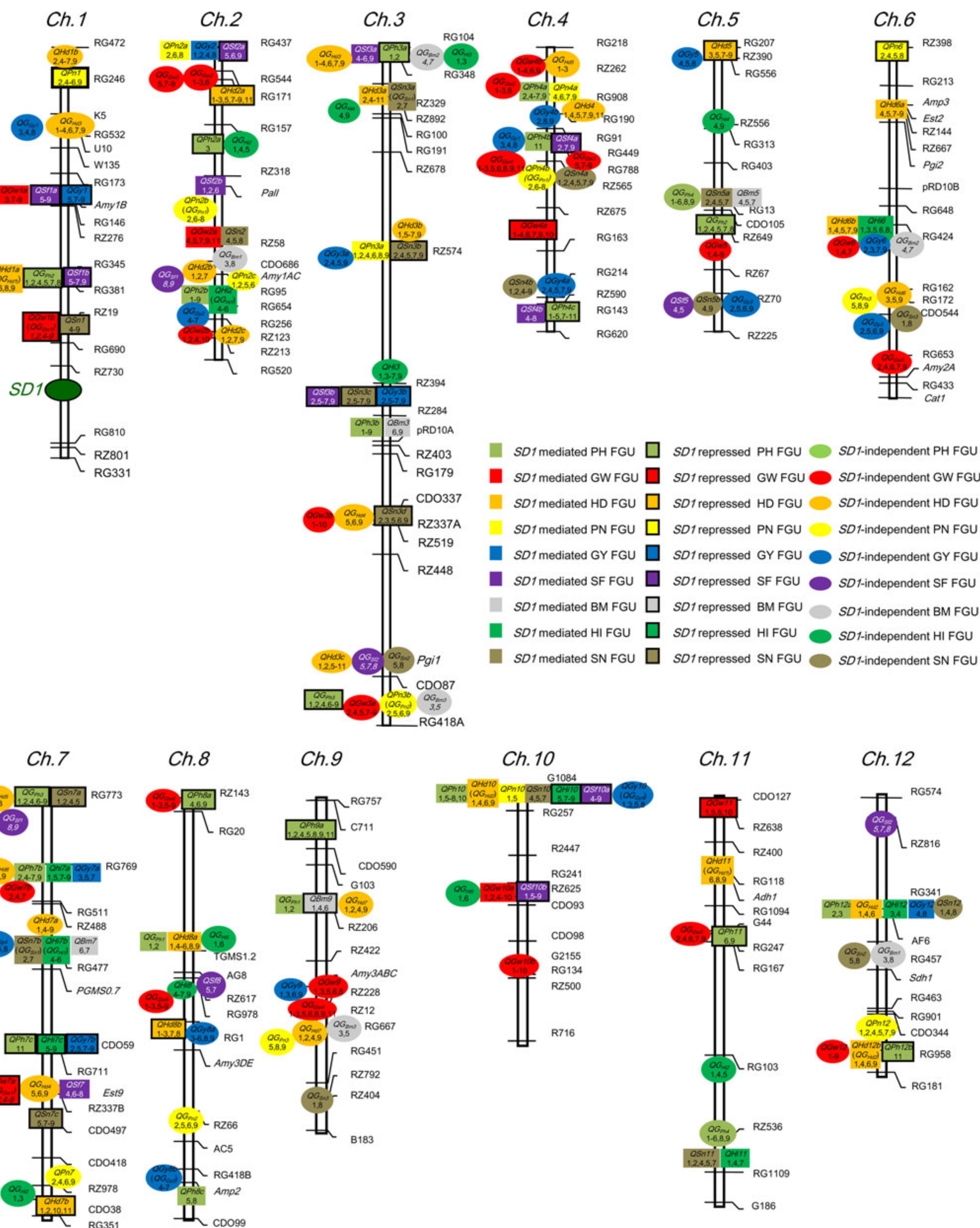


Fig. 1 Genomic distribution of 183 QTLs including the *SD1* gene in 145 functional genetic units affecting nine growth and yield traits detected in the IR64/Azucena DH population and its two subpopulations (with or without *SD1*) across 11 environments. Each *box* or *oval* represents an identified QTL and the numbers under each QTL represent the environments in which it was detected (Online resource 1)

The SD1-mediated pathways

Of the 145 identified FGUs, 43 (29.7 %) interacted strongly with *SD1* and were detectable only in the whole population and *SD1* subpopulation, but not in the *sd1* subpopulation, forming the *SD1*-mediated pathways (Figs. 1, 2; Online resources 4, 5). This system contained nine PH FGUs (*QPh3b*, *QPh4a*, *QPh4b*, QG_{Ph1}, *QPh10*, *QPh2b*, *QPh7b*, *QPh8c*, and *QPh12a*), seven HD FGUs (*QHd6b*, *QHd8a*, *QHd12a*, QG_{Hd1}, QG_{Hd2}, *QHd3a*, and *QHd6a*), three biomass FGUs (*QBm3*, *QBm5*, and *QBm7*), four PN FGUs (*QPn2a*, *QPn3a*, *QPn4a*, and *QPn10*), three GY FGUs (*QGy2*, *QGy7a* and *QGy12*), four SF FGUs (*QSf2b*, *QSf3a*, *QSf4b* and *QSf7*), and three GW FGUs (*QGw1a*, *QGw2a*, and *QGw10a*) with consistent pathway effects for increased PH, biomass and GW, delayed HD, reduced PN, and reduced GY across the test environments. This system also mediated five HI FGUs (*QHi6*, *QHi7a*, *QHi11*, *QHi12*, and QG_{Hi1}) and five SN FGUs (*QSn2*, *QSn5a*, *QSn10*, *QSn11*, and QG_{Sn1}). In these cases, all eight M-QTLs had consistent pathway effects for reduced HI and SN, except two E-QTL pairs (QG_{Hi1} and QG_{Sn1}) which had pathway effects for increased HI and SN. The Azucena alleles were predicted to be functional (positively regulated by *SD1*) for increased trait values at 25 of the 48 loci in the *SD1*-mediated pathways (Fig. 2; Online resource 10) and to be non-functional mutants at the remaining 23 loci (Zhang et al. 2011). Two QTLs, *QPh2b* and *QPh3b*, were stably detected in nine environments (E1–E9) with pathway effects significantly and positively correlated to the mean PH values of the *SD1* subpopulation (Fig. 3), suggesting their positive responses to the overall soil fertility across the environments as the same rice plants in more fertile soils grow taller. A third QTL, *QPh4a*, detected in six environments also had pathway effects positively correlated with the environmental effects ($r = 0.694$, $P < 0.05$).

Collectively, the *SD1*-mediated pathways explained 37.9 % (R^2) of the total genotypic variation of the measured traits, although differing widely among traits and environments (Table 3). On average, the *SD1*-mediated pathways had the greatest effects on PH (54.8 %), biomass (48.7 %) and HI (54.6 %) and minimal effect on SN (16.0 %). The *SD1*-mediated pathways showed large trait-specific GE interactions with large R^2 for PH in E2–E7, E9, E10; for HD in E1, E4, E6, E9 and E10; for PN in E1 and E8; for GY in E1, E3 and E8; for GW in E3, E8 and E11; for biomass in E4–E7 and E9; for HI in E1 and E4–E6; for SN in E7; for SF in E2 and E4 (Table 3).

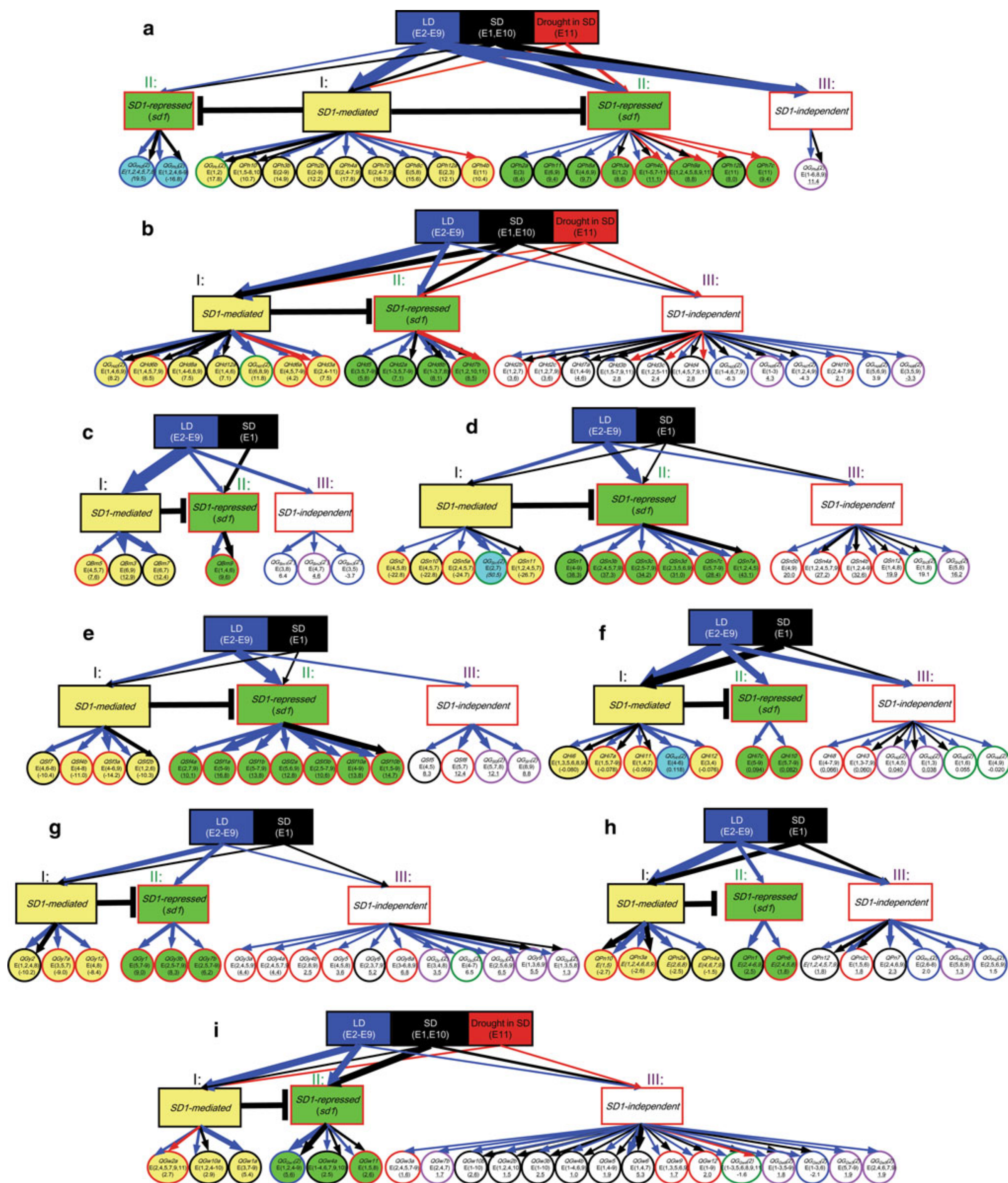
The SD1-repressed pathways

The second system contained 38 identified FGUs (37 M-QTLs and 3 E-QTL pairs) that were detected only in the whole population and *sd1* subpopulation, but not in the *SD1* subpopulation, indicating these FGUs were repressed by *SD1* (Figs. 1, 2; Online resources 6, 7). The *SD1*-repressed pathways included ten PH FGUs (*QPh2a*, *QPh3a*, *QPh4c*, *QPh7c*, *QPh8a*, *QPh9a*, *QPh11*, *QPh12b*, QG_{Ph2}, and QG_{Ph3}), four HD FGUs (*QHd2a*, *QHd5*, *QHd7b*, and *QHd8b*), one biomass FGU (*QBm9*), two PN FGUs (*QPn1* and *QPn6*), two HI FGUs (*QHi7c* and *QHi10*), three GY FGUs (*QGy1*, *QGy3b*, and *QGy7b*), six SN FGUs (*QSn1*, *QSn3b*, *QSn3c*, *QSn3d*, *QSn7c*, and *QSn7a*), seven SF FGUs (*QSf1a*, *QSf1b*, *QSf2a*, *QSf3b*, *QSf4a*, *QSf10a*, and *QSf10b*), and three GW FGUs (QG_{Gw1}, *QGw4a* and *QGw11*). Directions of the pathway effects of these *SD1*-repressed FGUs could be determined only for three pairs of these QTLs (Zhang et al. 2011), affecting PH and GW (QG_{Ph2}, QG_{Ph3}, and QG_{Gw1}). QG_{Ph2} had a mean pathway effect of 19.5 cm for increased PH. QG_{Ph3} (*QPh3c* and *QPh7a*) had a mean pathway effect of 16.8 cm for reduced PH. QG_{Gw1} had a mean pathway effect of 5.6 g for increased GW. The Azucena alleles were associated with increased trait values at 27 of the 41 loci involved in the *SD1*-repressed pathways and with the reduced trait values at the remaining 14 loci (Online resource 10).

Together, the *SD1*-repressed pathways explained 35.6 % of the total genotypic variation of the nine measured traits, again differing widely among traits and environments (Table 3). Importantly, the *SD1*-repressed pathways had the greatest effects on SN (59.3 %) and SF (56.6 %) and minimal effects on PN (18.6 %) and biomass (18.3 %). The *SD1*-repressed pathways also showed large trait-specific GE interactions with large R^2 for PH in E8 and E11 (the short-day environments); for HD in E2, E3, E8 and E11; for PN in E6; for GY in E5 and E7; for GW in E2 and E5; for biomass in E1; for HI in E8 and E9; for SN in E2–E6, E8 and E9; for SF in E1 and E5–E9 (Table 3).

The SD1-independent pathways

This system consisted of 64 FGUs (40 M-QTLs and 29 E-QTL pairs) that were detectable in both the *SD1* and *sd1* subpopulations as well as in the whole population, indicating that they were independent of *SD1* (Figs. 1, 2; Online resources 8, 9). These included 1 PH FGU (QG_{Ph4}), 3 biomass FGUs (QG_{Bm1}, QG_{Bm2}, and QG_{Bm3}), 12 HD FGUs (*QHd1b*, *QHd2b*, *QHd2c*, *QHd3b*, *QHd3c*, *QHd4*, *QHd7a*, QG_{Hd3}, QG_{Hd4}, QG_{Hd5}, QG_{Hd6}, and QG_{Hd7}), 6 PN FGUs (*QPn2c*, *QPn7*, *QPn12*, QG_{Pn1}, QG_{Pn2}, and QG_{Pn3}), 11 GY FGUs (*QGy3a*, *QGy4a*, *QGy4b*, *QGy5*, *QGy6*, *QGy8a*, *QGy9*, QG_{Gy1}, QG_{Gy2}, QG_{Gy3}, and QG_{Gy4}), 15 GW FGUs (*QGw2b*, *QGw3a*, *QGw3b*, *QGw4b*, *QGw5*, *QGw6*, *QGw7b*, *QGw9*, *QGw10b*, *QGw12*, QG_{Gw2}, QG_{Gw3}, QG_{Gw4}, QG_{Gw5}, and QG_{Gw6}), 6 HI FGUs (*QHi3*, *QHi8*, QG_{Hi2}, QG_{Hi3}, QG_{Hi4}, and QG_{Hi5}), 6 SN FGUs (*QSn4a*,



QSn4b, *QSn5b*, *QSn12*, QG_{Sn2}, and QG_{Sn3}), and 4 SF FGUs (*QSf5*, *QSf8*, QG_{Sf1}, and QG_{Sf2}). Again, the directions of the pathway effects of the 40 M-QTLs could not be determined based on the available QTL information due to lack of epistasis found among them. Of the identified 29 E-QTLs identified in this system, the directions of pathway effects of 13 E-QTLs (QG_{Hd3}, QG_{Hd4}, QG_{Hd7}, QG_{Bm1}, QG_{Bm3}, QG_{Pn1}, QG_{Pn2}, QG_{Hi4}, QG_{Hi5}, QG_{Gy2}, QG_{Sn3}, QG_{Gw2}, and QG_{Gw4}) could be determined (Fig. 2; Online resource 9), implying that the parental alleles at each of the

◀ **Fig. 2** Putative genetic networks containing 145 identified functional genetic units (FGUs) and their estimated pathway effects on nine traits detected in the IR64/Azucena DH population across 11 environments (Online resources 4–9). The traits include PH (**a**), HD (**b**), Biomass (**c**), SN (**d**), SF (**e**), HI (**f**), GY (**g**), PN (**h**) and GW (**i**), respectively. *Boxes shaded in blue*, *black* and *red* on *top* of network for each trait are environments (E1–E11) classified into long-day (LD), short-day (*SD*) and drought environments (Online resource 1), respectively. FGUs in each network were divided into three systems: (*I*) *SD1*-mediated pathways (*yellow boxes* and *ovals*); (*II*) *SD1*-repressed pathways (*green boxes* and *ovals*); (*III*) *SD1*-independent pathways (*white boxes* and *ovals*). *Ovals* at the third level of each network represent the FGUs identified, within each of which the name of FGU is on the *top*, the environments where detected in the *middle*, and the estimated mean pathway effect in the *bottom*. The FGUs of the third level with the effects in the opposite direction relative to their upstream FGU's (*SD1*/*sd1* level indicated by *yellow*/*green boxes*) were highlighted in *pale blue ovals*. *Blue*, *black* and *red arrow-shaped lines* indicated that the FGUs were identified in the LD, SD and drought environments, respectively. The line weights were directly proportional to the effects of FGUs indicated by the *arrows*. *Underlined numbers* in the FGUs of (*II*) and (*III*) pathways represent the absolute values of their estimated pathway effects and the directions of the pathway effects could not be determined based on available information. FGUs with *red* or *black outlines* indicated the Azucena alleles at FGUs for increased or decreased trait values, while FGUs with *blue* or *green outlines* represent cases of the parental-type or recombinant-type increased trait values associated with parental digenic genotypes. FGUs with *purple outlines* are complementary epistatic loci pairs

26 loci consisted of a functional allele (the expressed gene) with an effect in the same direction of the pathway effect and a mutant allele with little or no effect. The remaining 16 E-QTLs occurred between pairs of complementary loci which had no detectable main effects, indicating that parental alleles at these interacting loci pairs were co-adapted (Zhang et al. 2011). Together, the Azucena alleles were associated with increased trait values at 30 of the 59 loci in the *SD1*-independent pathways and with the reduced trait values at the remaining 29 loci (Online resource 10).

Collectively, the *SD1*-independent pathways explained 26.5 % (R^2) of the total genotypic variation of the nine measured traits with large trait specific GE interactions (Table 3). On average, the *SD1*-independent pathways had large R^2 on PN (45.2 %), GY (41.1 %) and GW (43.3 %), and minimal effects on PH (6.0 %) and SF (7.8 %).

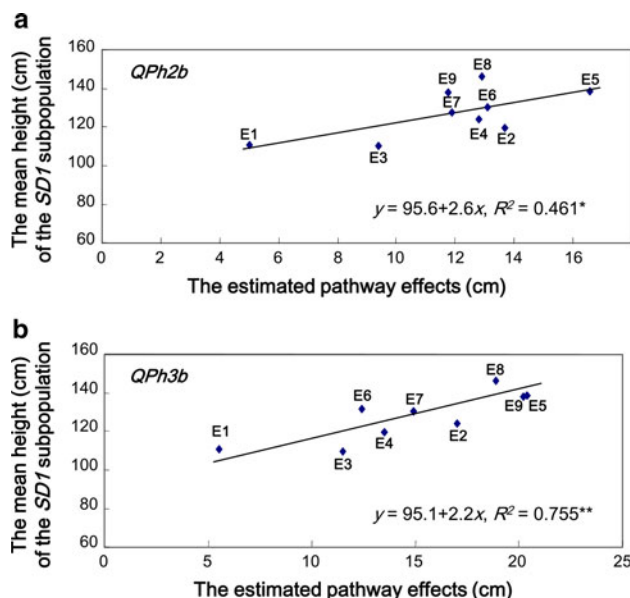


Fig. 3 Positive correlations between the pathway effects of two *SD1*-mediated downstream pathways *QPh2b* (**a**) and *QPh3b* (**b**) with the environmental effects (measured as the mean values of the *SD1* subpopulation for PH, see details in Online resource 4). E1–E9 are defined in Online resource 1

Discussion

Further increasing the high productivity of SRCs has been a challenge to rice scientists for more than four decades since **GR-I.** While it has been generally agreed that **GR-II** is needed for sustainable grain production and global food security (Conway 1999; Zhang 2007), there is less consensus about how it can be achieved. Many believe that **GR-II** might be simply built upon **GR-I**, i.e., to increase efforts to stack new genes for 'green' traits into the modern SRCs to make them better and more versatile (Khush 2001; Zhang 2007). Our results provide insights into the nature of **GR-I** and offer alternative breeding strategies for achieving **GR-II**.

The nature of GR in rice

Recent efforts in genetic and molecular dissection of complex traits have resulted in cloning of many QTL genes of large effect, many of which are regulatory genes with pleiotropic effects on several traits (Jiang et al. 2012). While it was obvious why those QTL of large effect were the targets of cloning experiments, our results suggest that functional attributions of these regulatory QTL genes such as *SD1* at cellular levels may be misleading and their phenotypic effects may vary considerably in different genetic backgrounds (Mei et al. 2006; Zheng et al. 2011). Historically, **GR-I** has been attributed to the mutants at a single locus, *SD1*. Indeed, we were able to show that the *sd1* had pleiotropic but relatively small effects on many other traits in addition to short height when characterized in the largely homogeneous IR64 genetic background. Using the new molecular-quantitative genetics theory (Zhang et al. 2011), we reveal the *SD1*-mediated, *SD1*-repressed, and *SD1*-independent pathways that control rice growth, development and productivity. This is consistent with the current knowledge that different plant hormones are known to regulate similar processes through largely

Table 3 Relative contributions of the *SD1*-mediated, *SD1*-repressed and *SD1*-independent pathways to the total trait genetic variation of the IR64/Azucena DH population for nine traits measured across 11 environments

Trait	R^{2a}	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	Average
PH	$V_{SD1\text{-mediated}}/V_G$	0.343	**0.535**	**0.671**	**0.497**	**0.776**	**0.712**	**0.765**	0.399	**0.570**	**0.507**	0.255	0.548 ± 0.172
	$V_{SD1\text{-repressed}}/V_G$	**0.582**	0.427	0.262	**0.434**	0.173	0.188	0.235	**0.455**	0.319	**0.493**	**0.745**	0.392 ± 0.177
	$V_{SD1\text{-independent}}/V_G$	0.075	0.038	0.067	0.069	0.051	0.100	0.000	0.146	0.111	0.000	0.000	0.060 ± 0.048
HD	$V_{SD1\text{-mediated}}/V_G$	**0.506**	0.084	0.000	**0.651**	0.370	**0.742**	0.279	0.373	**0.659**	**0.425**	0.181	0.388 ± 0.241
	$V_{SD1\text{-repressed}}/V_G$	0.257	**0.540**	**0.745**	0.000	0.195	0.000	0.279	**0.536**	0.080	**0.406**	**0.723**	0.342 ± 0.269
	$V_{SD1\text{-independent}}/V_G$	0.237	0.377	0.255	0.349	**0.435**	0.258	**0.443**	0.091	0.261	0.169	0.096	0.270 ± 0.122
PN	$V_{SD1\text{-mediated}}/V_G$	**0.785**	0.236	NA	0.135	0.098	0.372	0.295	**0.634**	0.345	NA	NA	0.363 ± 0.238
	$V_{SD1\text{-repressed}}/V_G$	0.000	0.266	NA	0.219	0.363	**0.374**	0.000	0.071	0.191	NA	NA	0.186 ± 0.150
	$V_{SD1\text{-independent}}/V_G$	0.215	**0.498**	NA	**0.645**	**0.539**	0.253	**0.705**	0.295	**0.464**	NA	NA	0.452 ± 0.182
GY	$V_{SD1\text{-mediated}}/V_G$	**0.809**	0.252	**0.732**	0.361	0.112	0.000	0.212	**0.632**	0.000	NA	NA	0.346 ± 0.310
	$V_{SD1\text{-repressed}}/V_G$	0.000	0.191	0.000	0.000	**0.596**	0.423	**0.633**	0.111	0.241	NA	NA	0.244 ± 0.252
	$V_{SD1\text{-independent}}/V_G$	0.191	**0.558**	0.268	**0.639**	0.293	**0.577**	0.154	0.256	**0.759**	NA	NA	0.411 ± 0.222
GW	$V_{SD1\text{-mediated}}/V_G$	0.089	0.068	**0.443**	0.151	0.146	0.088	0.257	**0.404**	0.306	0.205	**0.807**	0.269 ± 0.219
	$V_{SD1\text{-repressed}}/V_G$	0.337	**0.471**	0.119	0.265	**0.457**	0.373	0.246	**0.395**	0.247	0.369	0.000	0.298 ± 0.143
	$V_{SD1\text{-independent}}/V_G$	**0.575**	**0.461**	**0.438**	**0.585**	0.398	**0.540**	**0.497**	0.201	**0.447**	**0.426**	0.193	0.433 ± 0.131
BM	$V_{SD1\text{-mediated}}/V_G$	0.000	NA	0.000	**0.548**	**0.686**	**0.737**	**0.923**	0.000	**1.000**	NA	NA	0.487 ± 0.426
	$V_{SD1\text{-repressed}}/V_G$	**1.000**	NA	0.000	0.201	0.000	0.263	0.000	0.000	0.000	NA	NA	0.183 ± 0.347
	$V_{SD1\text{-independent}}/V_G$	0.000	NA	**1.000**	0.251	0.314	0.000	0.077	**1.000**	0.000	NA	NA	0.330 ± 0.430
HI	$V_{SD1\text{-mediated}}/V_G$	**0.614**	NA	NA	**0.857**	**0.728**	**0.504**	0.369	0.351	0.401	NA	NA	0.546 ± 0.194
	$V_{SD1\text{-repressed}}/V_G$	0.000	NA	NA	0.000	0.000	0.236	0.246	**0.397**	**0.599**	NA	NA	0.211 ± 0.231
	$V_{SD1\text{-independent}}/V_G$	0.386	NA	NA	0.143	0.272	0.260	**0.385**	0.252	0.000	NA	NA	0.243 ± 0.136
SN	$V_{SD1\text{-mediated}}/V_G$	0.235	0.281	0.000	0.197	0.090	0.000	**0.532**	0.102	0.000	NA	NA	0.160 ± 0.175
	$V_{SD1\text{-repressed}}/V_G$	0.263	**0.583**	**1.000**	**0.478**	**0.743**	**0.715**	0.295	**0.547**	**0.709**	NA	NA	0.593 ± 0.232
	$V_{SD1\text{-independent}}/V_G$	**0.502**	0.136	0.000	0.325	0.167	0.285	0.173	0.351	0.291	NA	NA	0.248 ± 0.146
SF	$V_{SD1\text{-mediated}}/V_G$	0.437	**0.567**	NA	**0.758**	0.181	0.312	0.103	0.330	0.157	NA	NA	0.356 ± 0.223
	$V_{SD1\text{-repressed}}/V_G$	**0.563**	0.433	NA	0.161	**0.599**	**0.688**	**0.782**	**0.519**	**0.784**	NA	NA	0.566 ± 0.205
	$V_{SD1\text{-independent}}/V_G$	0.000	0.000	NA	0.082	0.220	0.000	0.115	0.151	0.060	NA	NA	0.078 ± 0.081

NA not available, *PH* plant height, *HD* heading date, *PN* panicle number per plant, *GY* grain yield, *GW* thousand grain weight, *BM* biomass, *HI* harvest index, *SN* spikelet number per panicle, *SF* sikelet fertility

[a] V_G = the total genetic variances accounted by all detected FGUs in each environment

Bold values indicate large trait specific GE interactions with large R^2

non-overlapping transcriptional responses (Nemhauser et al. 2006). There are two major types of bioactive GAs in rice, GA_1 and GA_4 (Hooley 1994; Hedden and Phillips 2000; Hedden 2003; Ma et al. 2011) but it remains unclear whether the bioactive GA(s) produced by *SD1* (*GA20ox2*) in most tall rice landraces are GA_1 or GA_4 or both. Our results showed that the bioactive GA produced by *SD1* acted as an activator and a repressor in controlling rice growth, development and productivity by regulating many downstream pathways. The *SD1*-mediated pathways are the predominant ones favored by natural selection, because the *SD1*-repressed pathways are expressed in the absence of *SD1* and function only in the *sd1* genetic backgrounds. Thus, the nature of **GR-I** is the overall differences between the two systems, as reflected in the following aspects.

A key difference between the *SD1*-mediated and *SD1*-repressed pathways is their contrasting contributions to specific traits in specific environments. For example, the *SD1*-mediated pathways contributed much to PH, HI and biomass (the vegetative traits) but little to SN and SF (the reproductive traits), particularly under the long-day (LD) environments (Fig. 2). In contrast, the *SD1*-repressed pathways contributed much to SN and SF but little to PH, HI, or biomass. The contribution of each system to each trait varied considerably among environments (Table 3), suggesting that extrinsic factors and plant hormones jointly control rice growth, development and productivity by modulating various downstream loci and pathways.

Further, allelic diversity at loci involved in the three genetic systems is very different in the parents. For

example, the Azucena alleles were inferred to be functional (Zhang et al. 2011) and confer increased trait values at 25 of the 48 loci involved in the *SD1*-mediated pathways, and were non-functional mutants for reduced values at the remaining 23 (Online resource 10). In contrast, the IR64 alleles were associated with increased trait values at 14 (11 QTL associated with PH and HD) of the 41 loci involved in the *SD1*-repressed pathways, but with reduced trait values at the remaining 27 loci. Favorable alleles at loci involved in the *SD1*-independent pathways were approximately equally distributed in the parents (Online resource 10). Perhaps this is because IR64 was developed in the early 1980s, when loci acting in the *SD1*-repressed and *SD1*-independent pathways still had more allelic diversity available to be exploited for increasing yield potential, as has been demonstrated recently (Guan et al. 2010). However, the yield plateaus of modern SRCs with *sd1* suggest that intensive selection of more than two decades for increased productivity may have reduced allelic diversity at some loci involved in the *SD1*-repressed and *SD1*-independent pathways.

Although most SRCs have high yields under high input environments that are primarily attributable to their increased HI and lodging resistance, they have apparent drawbacks. For example, *sd1* reportedly reduced SN and GW (Murai et al. 2002). The poor adaptability of most SRCs to rainfed environments is partially attributable to the association of *sd1* with reduced root length (drought avoidance) as we observed that most drought-tolerant IR64 introgression lines were significantly taller with longer roots than IR64 (Table 1) (Lafitte et al. 2007). In addition, *sd1* was at least partially responsible for the poor responses of most SRCs to nutrient inputs, evidenced by the minimal effects of the *SD1*-repressed pathways on biomass and PN (Table 3). This is in contrast to the positive responses of *SD1*-mediated downstream PH pathways (*QPh2b, QPh3b* and *QPh4a*) to the overall fertility of the test environments (Fig. 3). SRCs with *sd1* are also associated with reduced basal culm strength that leads to lodging (Ookawa et al. 2010). Evidence based on cloned *sd1* indicates its negative effects on yield and other important traits such as much shorter seeds, reduced panicle length, and possible vulnerability to diseases and insects (Kuroda et al. 1989; Cho et al. 1994; Monna et al. 2002; Evenson and Gollin 2003).

Our results lent a strong support for the newly developed molecular-quantitative genetics theory (Zhang et al. 2011) for the fundamental importance of signaling pathways as the molecular basis of complex traits and provided insights into the nature of QTL genes. Based on their theoretical expectations (Zhang et al. 2011), it can be predicted that there is a loss-of-function mutant allele at each of the 80 loci of the 59 FGUs. In other words, 71.4 % of the QTLs affecting the nine measured traits in the DH population were attributable to the difference between a loss-of-function allele and a functional one, whereas the remaining 28.6 % to two functional alleles of differentiated effects in co-adapted gene complexes. This figure corresponds closely to the situation of the 26 cloned QTLs in both plants and animals (Zhang et al. 2011). Furthermore, we were able to determine the pathway effect directions for 59 FGUs (80 loci) of the 151 identified FGUs (all 48 GA regulated loci, 3 pairs of GA repressed loci and 13 pairs of GA-independent loci). In these cases, the additive effects of individual QTLs estimated by the classical QTL-mapping approach have tremendously underestimated their involved pathway effects because of epistasis. However, we were unable to do so for the remaining 76 single-locus FGUs, because epistasis was not detectable in the small population size of the DH population. Our results also indicate that the GEI of the measured traits resulted primarily from the differential expression of the QTL genes in different environments, consistent with the previous reports (Zhuang et al. 1997; Xing et al. 2002; Li et al. 2003). Hormones such as GAs and extrinsic factors jointly determined the expression of most downstream pathways and appear responsible for the observed GEI of complex traits (Yamaguchi 2008; Online resource 2). Nevertheless, our results were consistent with the theoretical expectation that the statistically detectable epistasis occurs only between or among genes involved in positively regulated signaling pathways (Zhang et al. 2011).

Then, an interesting question arises: what are the *SD1*-mediated and *SD1*-repressed pathways? A hypothesis that they might represent the GA_1-mediated pathways and GA_4-mediated pathways is suggested by combined evidence (Fig. 4). First, all *indica* SRCs carrying the defective *sd1* allele contain much reduced GA_1 insufficient to interact with GID1 (the GA receptor) but a sufficient amount of GA_4 to interact with GID1 (Sasaki et al. 2002; Hirano et al. 2008; Yamaguchi 2008; Ma et al. 2011), which are presumably produced by functional GA20 oxidase isozymes encoded by *GA20ox1, GA20ox3* and *GA20ox4* located on rice chromosomes 3, 5, and 7 (Sakamoto et al. 2004). In other words, the repression of GA_1 by GA_4 in the *sd1* mutant backgrounds was achieved by its competitive binding to GID1. Thus, the *SD1*-repressed pathways detected in the *sd1* genetic backgrounds are presumably the GA_4-mediated pathways, supported by the observations that accumulated biomass of young rice seedlings in the *sd1* SRCs is correlated positively with the GA_4 content, but negatively with the GA_1 content (Ma et al. 2011), and that GA_4 is normally present in large amounts in the reproductive tissues, but in low quantity in the vegetative parts of rice (Choi et al. 1995). This is consistent with the contrasting effects of the *SD1*-mediated and *SD1*-repressed pathways on the vegetative and reproductive traits

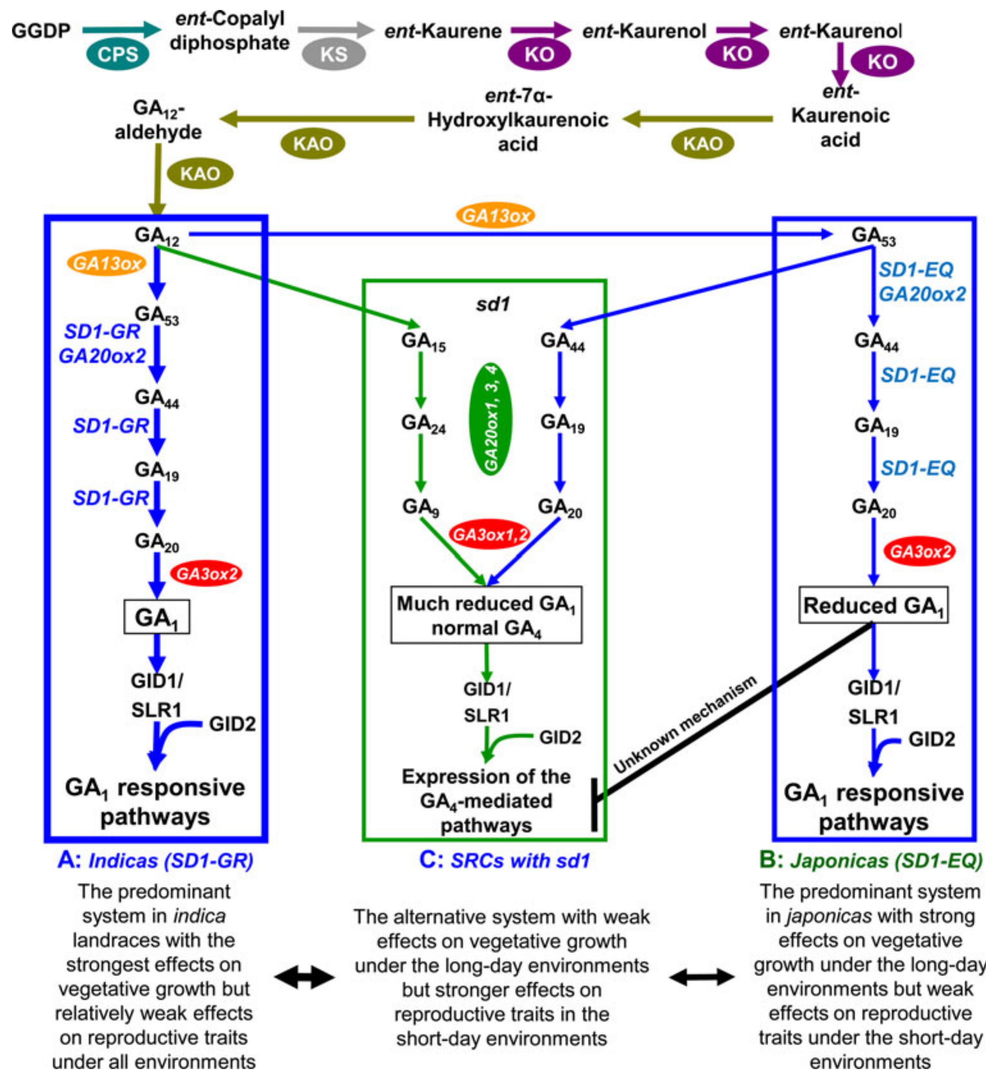


Fig. 4 A hypothesis that the allelic differences at *GA20ox2* (*SD1*) leading to Green Revolution and contributed greatly to the sub-specific differentiation in rice. The functional allele, *SD1-GR*, predominant in *Indica* landraces and *O. rufipogon* encodes a GA20 oxidase that has a high catalytic ability to convert GA_{53} into GA_{43}, GA_{44} and GA_{19}, and then to GA_1 by *GA3ox2* through the 13-hydroxylated pathway; the functional allele, *SD1-EQ* fixed in *Japonicas* encodes a GA20 oxidase isozyme that has a low catalytic ability to convert GA_{53} into GA_{44}, GA_{19} and GA_{20}, then to a much reduced mount of GA_1 through the same pathway (Asano et al. 2011; Ma et al. 2011); While in the *sd1* SRCs, much reduced GA_1 which is insufficient to induce the GA_1-mediated pathways (Yamaguchi 2008). There is a normal amount of GA_4 in the SRCs that are produced by other GA20 oxidase isozymes encoded presumably by *GA20ox1*, *GA20ox3*, and/or *GA20ox4* on chromosomes 3, 5 and 7 (Sakamoto et al. 2004). The alternative (*SD1-repressed*) genetic system detected in the *sd1* genetic backgrounds is presumed to primarily be the GA_4-mediated system which is normally repressed in the vegetative tissues by the (*SD1*) GA_1-mediated pathways achieved an unknown mechanism

discussed above (Table 3). Second, the Azucena *SD1* allele is inferred to be *SD1-EQ* (fixed in *ssp. Japonicas*) which has a low catalytic activity in the hydroxylated pathway leading to reduced GA_1 and is associated with reduced height as compared to *SD1-GR* which is predominant in *ssp. Indica* landraces and the wild rice species, *O. rufipogon* (Asano et al. 2011). Third, the fact that the *SD1*-mediated and *SD1*-repressed pathways affect the same suite of traits and the latter was repressed by the former suggests a qualitative difference in their regulatory function and high level of similarity in their phenotypic effects. Thus, it appears that the allelic diversity at *SD1* might have resulted in a quantitative difference in GA_1-mediated pathways that contributed greatly to subspecific differentiation of rice and a qualitative difference in the GA-mediated downstream pathways that led to **GR-I** (Paterson and Li 2011). Of cause, the fundamental assumption of this hypothesis is that the two bioactive GAs in rice, GA_1 and GA_4, are mediating different downstream pathways, which remains to be actively tested.

Implications to achieve GR-II

The presence of three alternative systems for growth, development and productivity, no matter what their natures are, suggests that a significant portion of the total genetic diversity at loci involved in the *SD1*-mediated pathways in the primary gene pool of rice cannot be utilized in the *sd1* SRC backgrounds. As proposed by Conway (1999) and agreed by many (Zhang 2007), if **GR-II** refers to "green super cereal varieties that can produce high and stable yields with less inputs (water, fertilizer and pesticides) and thus are more environment-friendly" (Zhang 2007), then, our results suggest two general strategies to achieve this goal. One would be to add new genes for 'green' traits into the current high-yielding SRCs to further exploit the genetic potential of the *SD1*-repressed and *SD1*-independent pathways, as breeders have been doing with considerable success (Khush 2001; Ali et al. 2006; Lafitte et al. 2006; Paterson and Li 2011). Our results suggest that this strategy should be more effective for high input irrigated systems and/or the short-day season of the tropics where the *SD1*-repressed system tends to express more strongly (Table 3). For example, the discovery of a downstream pathway for reduced height (QG_{Ph3}) provided direct information of the presence of the dominant pathway for semi-dwarfism and its potential uses in developing semi-dwarf rice hybrids. However, our results suggest that the exclusive use of *sd1* in the worldwide rice-breeding program will inevitably encounter some of the dangers inherent in monoculture.

An alternative strategy would be restoring the *SD1*-mediated pathways, or 'back to the nature of rice before **GR-I**'. This is critically important for future rice improvement, because the *SD1*-mediated pathways account for the genetic diversity at a significant portion of loci in the rice genome that affect many important rice traits, which are now virtually inaccessible to most rice-breeding programs worldwide. Our results suggest that this strategy may be advantageous for developing new varieties with high nutrient use efficiency and better abiotic-stress tolerance for rainfed systems. This can be achieved by keeping *SD1* but precisely manipulating its downstream pathways (loci) with molecular and genomic technology, to develop superior rice cultivars of different heights suitable for different low-input rainfed environments. While *sd1* dramatically and quickly reduced plant height, our results and many others show rich quantitative variation that may permit the semi-dwarf phenotype to be recapitulated without knocking out *SD1*, as many lines in the *SD1* subpopulation had the semi-dwarf phenotype (Online resource 11). Likewise, the feasibility of semi-dwarf rice with functional *SD1*-mediated pathways is as also suggested by development and commercialization of some very high-yielding *Japonica* SRCs without *sd1* such as Mahsuri which was very popular under rainfed conditions in India, Bangladesh, Nepal and Burma in the 1970–1980s. However, whether the *SD1*-mediated pathways can be used for developing high-yielding and N-use efficient SRCs under normal input conditions remains to be explored.

Finally, the strong trait-specific genotype-environment interactions observed in this study (Table 3) indicate that different breeding strategies should be taken for the same target traits in different target environments. In rice with widespread use of irrigated and rainfed production systems, genetic improvement must be targeted to the specific production system to maximize rates of genetic gain. Tremendous efforts remain necessary to fully characterize and understand the differences between the three (or more) genetic systems underlying growth, development and productivity of rice, with regard to their specific signaling pathways and loci involved, and the ways they interact with environments.

Acknowledgments We thank the co-authors in Li et al. (2003) for their contributions in generating the original data of the DH population (Li et al. 2003). This work was funded by the "863" project (2012AA101101) from the Ministry of Science and Technology of China, the "948" Project (#2011-G2B) from the Ministry of Agriculture of China, a program of the International S & T Cooperation (S2012ZR0160), the Generation Challenge Program project (#12) of CGIAR, and the Bill & Melinda Gates Foundation project (OPP51587). Fan Zhang was also supported by the Monsanto's Beachell-Borlaug International Scholars Program. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest The authors declare no conflict of interest.

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