The Nature of Green Revolution: A Re-evaluation

Zhang, Fan[1], J. Ali[2], Jian-Long Xu[1], Yong-Ming Gao[1]and Zhi-Kang Li[1,2]

[1]Institute of Crop Sciences/National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences, Beijing 100081, China; [2]The International Rice Research Institute, DAPO Box 7777, Metro Manila, the Philippines. E-mail: lizhk@caas.net.cn; z.li@cgiar.org

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. Many important questions remain largely unanswered regarding sd1, such as what genetic systems do the sd1 SRCs use to manage growth and development of rice plants? Does sd1 contribute to the yield plateau observed in most SRCs? To answer these questions, QTLs controlling nine growth and yield traits of the IR64/Azucena doubled haploid (DH) population which segregate at SD1, were characterized using a new molecular quantitative genetics model and the phenotypic data from 11 environments. Three genetic systems that control rice growth, development and productivity were revealed, including the SD1-mediated, SD1-repressed and SD1-independent pathways. 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 system comprised 64 FGUs that were independent of SD1. GR-I resulted from the overall differences between the SD1-mediated and SD1-repressed systems. 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. Two general strategies are proposed 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.

Keywords: Green revolution, *sd1*, genetic networks, rice productivity

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 of 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*) (Spielmeyer et al 2002; Sasaki et al 2002). Over the past 5 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 yield increases 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 towards this direction

by putting genes or traits for resistance to abiotic and biotic stresses 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 rice plant 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 wide-ranging analyses of data from extensive genetic mapping experiments using a new molecular quantitative genetics model (Zhang et al 2011). Our results 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

The materials used in our experiments 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 2003.4 cM and an average distance of 12.4 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 13.5º to 31.5º N and from 76º to 121.5º E as well as different cropping seasons in Asia (Li et al 2003). 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 plant height (PH in cm), heading date (HD in days), grain yield (GY, in g/plant), harvest index (HI), biomass (in g/plant), 1000-grain weight (GW in g), panicle number per plant (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

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 3 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). The DH population was further divided 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). Then, 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).

Furthermore, once the main and epistatic effects of *SD1* and its interacting FGUs were obtained, the functional relationships of *SD1* and its regulated downstream FGUs could be determined based on the predicted patterns of the main and epistatic effects of *SD1* and its downstream QTLs (Zhang et al 2011). 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, the IR64/ Azucena doubled haploid (DH) population showed highly significant variation among lines (G), among environments (E) and G x E interactions for nine measured growth and yield traits, on average, explaining respectively 32.9, 33.4, and 23.1% of phenotypic variation (Table 1). Mapped to the genomic region flanked by DNA markers RZ730 and RG810 on chromosome 1, subdivision of the population based on function (*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 (Table 2). Its effects on GY, SN and SF were small and inconsistent across the environments.

Three genetic systems underlying growth, development and productivity of rice

We identified 145 FGUs, each defined as either an 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 (Table 2). These included 108 M-QTLs and 37 pairs of E-QTLs, which are widely distributed on 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 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.

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 (Tables 3, 5, Figs. 1, 2).

This system contained 9 PH FGUs (*QPh3b*, *QPh4a*, *QPh4b*, QG_{Ph1}, *QPh10*, *QPh2b*, *QPh7b*, *QPh8c* and *QPh12a*), 7 HD FGUs (*QHd6b*, *QHd8a*, *QHd12a*, QG_{Hd1}, QG_{Hd2}, *QHd3a* and *QHd6a*), 3 biomass FGUs (*QBm3*, *QBm5* and *QBm7*), 4 PN FGUs (*QPn2a*, *QPn3a*, *QPn4a* and *QPn10*), 3 GY FGUs (*QGy2*, *QGy7a* and *QGy12*), 4 SF FGUs (*QSf2b*, *QSf3a*, *QSf4b* and *QSf7*), and 3 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 5 HI FGUs (*QHi6*, *QHi7a*, *QHi11*, *QHi12* and QG_{Hi1}) and 5 SN FGUs (*QSn2*, *QSn5a*, *QSn10*, *QSn11* and QG_{Sn1}). In these cases, all 8 M-QTLs had consistent pathway effects for reduced HI and SN, except 2 E-QTL pairs (QG_{Hi1} and QG_{Sn1}) which had pathway effects for increased HI and SN.

Table 1. Summarized ANOVA results of measured traits related to growth, development and yield in the IR64/Azucena DH population evaluated in nine (E1-E9) environments

Source of variation [a]	R^2 (MS) accounted for the total sum of phenotypic squares [b]							
	PH	HD	GW	Biomass	PN	SN	GY	SF
Genotype	71.1	19.9	60.9	8.5	21.3	41.2	12.2	28.4
Environment	18.2	56.8	15.6	46.3	38.2	25.1	46.2	20.4
G×E	9.5	21.4	17.8	27.6	21.3	22.3	27.3	37.2
Error	1.2	1.9	5.7	17.6	19.2	11.4	14.3	14.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] PH = plant height, HD = heading date, GW = 1000-grain weight, PN = panicle number per plant, SN = spikelet number per panicle, GY = grain yield per plant, and SF = spikelet fertility (data transformed). [b] Mean sums of squares of genotype (G), environment (E) and G x E interaction were all highly significant at $P < 0.0001$.

Table 2. Summary statistics of growth and yield related traits, plant height, harvest index, biomass, heading date, panicle number per plant, 1000-grain weight, grain yield, spikelet number per panicle, and spikelet fertility of the IR64/Azucena DH population and subpopulations evaluated in 11 environments and the pleiotropic effects of the mutant allele, *sd1*, at the *SD1* region on these traits

		E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11
		IR95	IR94	UAS	SCAUL	SCAUE	RRI	PAU	IARI	CNRRI	98-N	98-D
Plant height (PH, in cm)												
Whole	M	97.9± 17.9	103± 21.9	101.5± 16.5	106.7± 23.0	121.7± 24.6	114.8± 21.0	121.4± 22.8	116.6± 23.6	127.8± 24.3	94.1± 13.0	78.3± 10.5
	L	18.7	26.1	5.0	18.3	15.1	15.9	21.6	8.7	23.1	10.8	10.1
	E[a]	-13.0	-17.2	-8.5	-17.9	-17.7	-16.1	-16.9	-15.3	-18.7	-10.2	-7.3
sd1	M	84.4± 11.8	85.3± 12.3	92.7± 13.4	88.1± 13.6	103.4± 13.9	98.1± 11.7	103.8± 12.7	100.8± 15.1	108.5± 13.6	84.1± 7.8	70.7± 7.8
	R	59.3-110.7	58.8-108.0	66.7-125.3	61.65-118.0	73.4-139.6	76.5-134.3	78.9-127.4	69.46-138.0	81.3-143.4	67.8-94.8	57.5-86.3
SD1	M	110.4± 12.6	119.6± 14.8	109.6± 15.0	124.0± 15.0	138.8± 19.7	130.3± 15.1	137.7± 17.4	131.3± 20.3	145.9± 17.0	104.5± 8.1	85.3± 7.2
	R	86.7-139.7	91.2-157.0	73.1-136.5	92.0-157.9	84.2-181.4	98.3-160.0	104.2-178.0	75.0-176.5	115.0-183.9	89.5-117.7	73.0-100.3
Harvest index (HI)												
Whole	M	0.368± 0.088	NA	0.379± 0.098	0.406± 0.095	0.272± 0.118	0.396± 0.095	0.194± 0.068	0.386± 0.134	0.265± 0.106	NA	NA
	L	8.1	NA	2.7	2.4	11.2	2.8	7.5	4.1	4.2	NA	NA
	E	0.047	NA	0.017	0.020	0.052	0.021	0.030	0.030	0.030	NA	NA
sd1	M	0.417± 0.078	NA	0.397± 0.087	0.427± 0.083	0.327± 0.096	0.419± 0.098	0.226± 0.061	0.416± 0.145	0.297± 0.108	NA	NA
	R	0.228-0.538	NA	0.264-0.592	0.137-0.511	0.091-0.457	0.164-0.553	0.102-0.323	0.179-0.692	0.093-0.497	NA	NA
SD1	M	0.324± 0.073	NA	0.363± 0.105	0.388± 0.102	0.224± 0.114	0.378± 0.090	0.166± 0.062	0.357± 0.117	0.237± 0.098	NA	NA
	R	0.165-0.459	NA	0.200-0.772	0.184-0.728	0.019-0.459	0.168-0.562	0.036-0.295	0.102-0.566	0.027-0.427	NA	NA
Biomass (g/plant)												
Whole	M	86.3± 18.0	NA	61.0± 10.9	39.0± 9.6	43.1± 8.7	76.0± 18.5	56.9± 12.1	36.0± 17.2	58.7± 13.4	NA	NA
	L		NA		2.5		1.9	3.1	5.0	2.3	NA	NA
	E	-1.6	NA	-1.1	-2.6	-0.9	-5.6	-3.4	-6.4	-3.4	NA	NA
sd1	M	84.6± 16.2	NA	59.9± 9.3	35.2± 5.9	42.2± 8.3	69.8± 13.6	53.0± 8.0	28.1± 8.2	54.5± 11.0	NA	NA
	R	48.6-118.5	NA	39.1-78.8	18.3-44.1	24.4-62.5	42.8-109.8	38.0-72.4	15.5-52.4	33.2-85.8	NA	NA
SD1	M	87.8± 19.6	NA	62.0± 12.2	42.5± 11.0	44.0± 9.0	81.1± 20.5	60.2± 14.0	43.2± 20.0	62.4± 14.3	NA	NA
	R	52.4-128.7	NA	38.0-86.1	23.2-76.5	29.8-68.1	50.0-151.6	33.9-93.2	10.2-102.0	31.4-100.0	NA	NA
Heading date (HD, days)												
Whole	M	89.4± 11.3	98.7± 7.5	109.4± 8.1	104.2± 6.0	123.5± 7.1	106.6± 8.7	111.2± 5.0	102.4± 5.5	110.3± 6.6	91.2± 9.1	103.3± 10.3
	L		3.1	4.5	7.1	2.3	4.0				NA	NA
	E	-1.3*	-1.6	-2.2	-1.8	-1.7	-3.3	-0.8	-0.1	-1.0*	-1.0*	-1.0*
sd1	M	88.1± 9.7	97.0± 8.4	107.2± 8.3	102.4± 5.7	121.7± 5.6	103.0± 5.8	110.4± 4.2	102.3± 6.6	109.2± 6.1	90.3± 9.7	102.3± 11.7
	R	61.0-110.0	84.0-120.0	89.7-123.7	83.5-113.0	113.2-136.6	89.7-114.9	102.5-119.0	84.0-113.0	102.0-129.0	70.0-113.0	73.0-125.0
SD1	M	90.7± 12.5	100.4± 6.0	111.5± 7.4	106.0± 5.8	125.1± 7.8	109.6± 9.6	111.9± 5.5	102.5± 4.5	111.2± 6.9	92.2± 8.5	104.3± 8.7
	R	61.0-110.0	89.0-114.5	91.3-122.0	94.5-115.0	101.1-142.1	80.9-125.1	102.0-124.5	95.0-111.5	97.0-126.0	77.0-109.0	87.0-120.0
Panicle number per plant (PN)												
Whole	M	18.0± 4.4	11.9± 2.1	16.9± 4.7	9.9± 2.4	11.8± 2.9	15.3± 4.5	9.8± 1.7	9.1± 3.1	11.2± 2.2	NA	NA
	L	1.8	2.7	2.2	5.1	3.9	4.3	1.8		1.8	NA	NA
	E	1.9	1.0	1.1*	1.0	1.3	1.9	0.7	-0.1	0.8	NA	NA
sd1	M	20.0± 3.6	12.8± 2.1	18.0± 5.4	10.9± 2.5	13.2± 2.8	17.4± 4.3	10.5± 1.7	8.9± 2.3	12.0± 2.0	NA	NA
	R	12.1-27.1	9.1-17.8	9.1-32.1	6.1-17.7	7.6-20.1	11.3-29.1	7.1-13.2	5.0-17.3	7.1-15.8	NA	NA

		IR95	IR94	UAS	SCAUL	SCAUE	RRI	PAU	IARI	CNRRI	98-N	98-D
SD1	M	16.2± 4.4	10.9± 1.7	15.9± 3.6	9.0± 2.0	10.6± 2.3	13.5± 3.8	9.2± 1.4	9.2± 3.7	10.5± 2.2	NA	NA
	R	5.4-24.4	8.4-14.1	9.9-22.7	5.1-15.3	6.1-16.4	5.2-25.0	6.6-12.7	5.3-18.5	6.9-17.0	NA	NA
1000-grain weight (GW, in g)												
Whole	M	23.6± 3.4	26.7± 3.9	27.6± 3.7	25.1± 3.2	23.6± 3.3	27.0± 4.5	26.3± 3.3	22.3± 4.0	24.3± 3.4	24.0± 3.8	21.5± 4.1
	L	2.9	3.7		3.3	2.5	2.5	2.7	4.0	5.6	2.2	3.8
	E	-1.1	-1.5	-0.6*	-1.0	-0.9	-2.4	-1.1	-1.5	-1.5	-1.3	-2.2
sd1	M	22.5± 3.3	25.0± 3.6	27.0± 3.7	23.9± 3.1	22.4± 3.1	24.4± 3.8	25.1± 3.3	20.5± 3.3	22.5± 2.8	22.7± 3.6	20.0± 3.4
	R	16.9-30.2	18.8-32.5	17.7-34.7	18-32.4	17.5-32.1	18.1-33.9	18.3-33.5	14.2-31.1	17.8-30.5	16.8-32.2	13.4-27.1
SD1	M	24.6± 3.1	28.4± 3.4	28.1± 3.6	26.3± 2.9	24.6± 3.1	29.1± 4.0	27.4± 2.9	24.0± 3.8	25.8± 3.0	25.4± 3.5	24.6± 3.7
	R	13.7-29.8	22.5-39.6	19.4-35.4	21.0-34.0	15.1-31.8	20.4-41.3	20.1-34.3	16.2-35.2	20.8-34.3	18.3-30.9	18.4-32.8
Grain yield (GY, in g/plant)												
Whole	M	40.3± 12.6	35.5± 8.3	32.6± 13.1	25.4± 7.7	19.6± 10.0	41.0± 14.7	21.7± 8.4	19.6± 10.8	15.1± 6.3	NA	NA
	L	3.5			2.5	5.1			2.0		NA	NA
	E	4.1	2.3*	1.7*	2.2	3.7	1.0	1.0	-2.8	0.5	NA	NA
sd1	M	44.6± 10.7	37.2± 7.4	34.4± 15.1	26.7± 7.2	23.9± 9.5	42.0± 13.3	22.8± 8.7	16.6± 5.3	15.7± 6.0	NA	NA
	R	23.9-71.8	24.6-50.8	7.7-75.3	5.5-41.4	3.6-38.7	16.1-69.8	8.5-40.5	5.7-28.8	6.1-30.0	NA	NA
SD1	M	36.5± 13.0	33.7± 8.8	31.1± 10.8	24.2± 8.0	16.0± 9.0	40.1± 15.9	20.8± 8.1	22.3± 13.5	14.6± 6.6	NA	NA
	R	10.8-67.5	13.7-55.9	10.9-59.5	8.8-41.9	1.4-35.6	13.4-95.0	8.1-43.8	4.5-68.8	2.0-31.5	NA	NA
Spikelet number per panicle (SN)												
Whole	M	125.0± 27.3	149.1± 36.8	100.4± 22.8	153.5± 44.1	135.1± 32.4	146.2± 33.7	121.0± 35.7	144.3± 33.2	104.8± 26.5	NA	NA
	L	3.2				3.3			2.1		NA	NA
	E	8.5	2.1*	-2.6*	-2.3*	8.9	0.3	-2.1*	-5.0	2.4*	NA	NA
sd1	M	126.3± 26.6	151.2± 40.7	97.7± 22.7	150.6± 52.1	137.9± 40.4	146.5± 33.1	118.8± 30.2	138.6± 35.4	107.3± 29.2	NA	NA
	R	82.1-193.3	76-273.5	58.0-158.9	79.3-303.8	72.2-217.8	82.1-215.8	53.0-202.6	70.3-208	51.1-164.8	NA	NA
SD1	M	123.9± 28.2	146.9± 32.7	102.8± 22.9	156.1± 35.6	132.6± 23.7	146.0± 34.6	122.9± 40.1	149.5± 30.6	102.6± 24.0	NA	NA
	R	84.4-231.5	80.5-225.5	61.0-152.4	84.7-237.4	85.3-180.2	80.1-215.6	44.4-308.3	77.7-224.9	57.5-145.8	NA	NA
Spikelet fertility (SF, in %)												
Whole	M	77.5± 15.5	78.4± 9.9	71.1± 10.7	70.3± 13.2	53.6± 21.1	70.8± 13.2	71.9± 14.6	67.3± 13.5	55.4± 16.9	NA	NA
	L		23.1	2.9		4.9					NA	NA
	E	2.1*	5.5	1.8	2.6*	4.5	-1.2	2.6*	1.5	1.8*	NA	NA
sd1	M	80.8± 10.6	80.6± 9.1	72.3± 10.4	72.4± 13.0	60.4± 17.8	69.9± 13.5	73.4± 16.1	69.0± 12.9	56.5± 16.7	NA	NA
	R	50.6-100.0	54.9-91.1	40.9-86.4	34.4-88.0	13.7-88.4	34.4-88.2	26.8-91.9	34.0-90.7	19.8-85.0	NA	NA
SD1	M	76.7± 18.9	75.3± 9.9	68.6± 10.5	67.3± 14.3	46.1± 23.8	72.3± 12.7	68.2± 15.3	65.9± 14.2	52.8± 18.1	NA	NA
	R	42.2-100.0	49.8-90.3	47.1-84.4	31.5-91.6	4.1-93.3	34.2-90.1	27.8-92.3	41.2-96.9	11.1-90.6	NA	NA

M = Mean± SD; *L* = *sd1*-LOD; E = Effect; IR95 and IR94 = 1994 and 1995 seasons at IRRI; UAS – University of Agricultural Sciences, GKVK, Bangalore, India; SCAUL and SCAUE = late and early seasons at South China Agricultural University, China; RRI = Rice Research Institute, Bangkok, Thailand; PAU = Punjab Agricultural University, Ludhiana, India; IARI = Indian Agricultural Research Institute, New Delhi; CNRRI = China National Rice Research Institute, Hangzhou, China; 98normal and 98drought = 1998 and 1999 dry seasons at IRRI; [a] Effect was QTL main phenotypic effect estimated from mean trait values. The sign indicates the direction of the effect of the IR64 allele (*sd1*); * = marginally significant effects at P ≈ 0.05; NA = data not available.

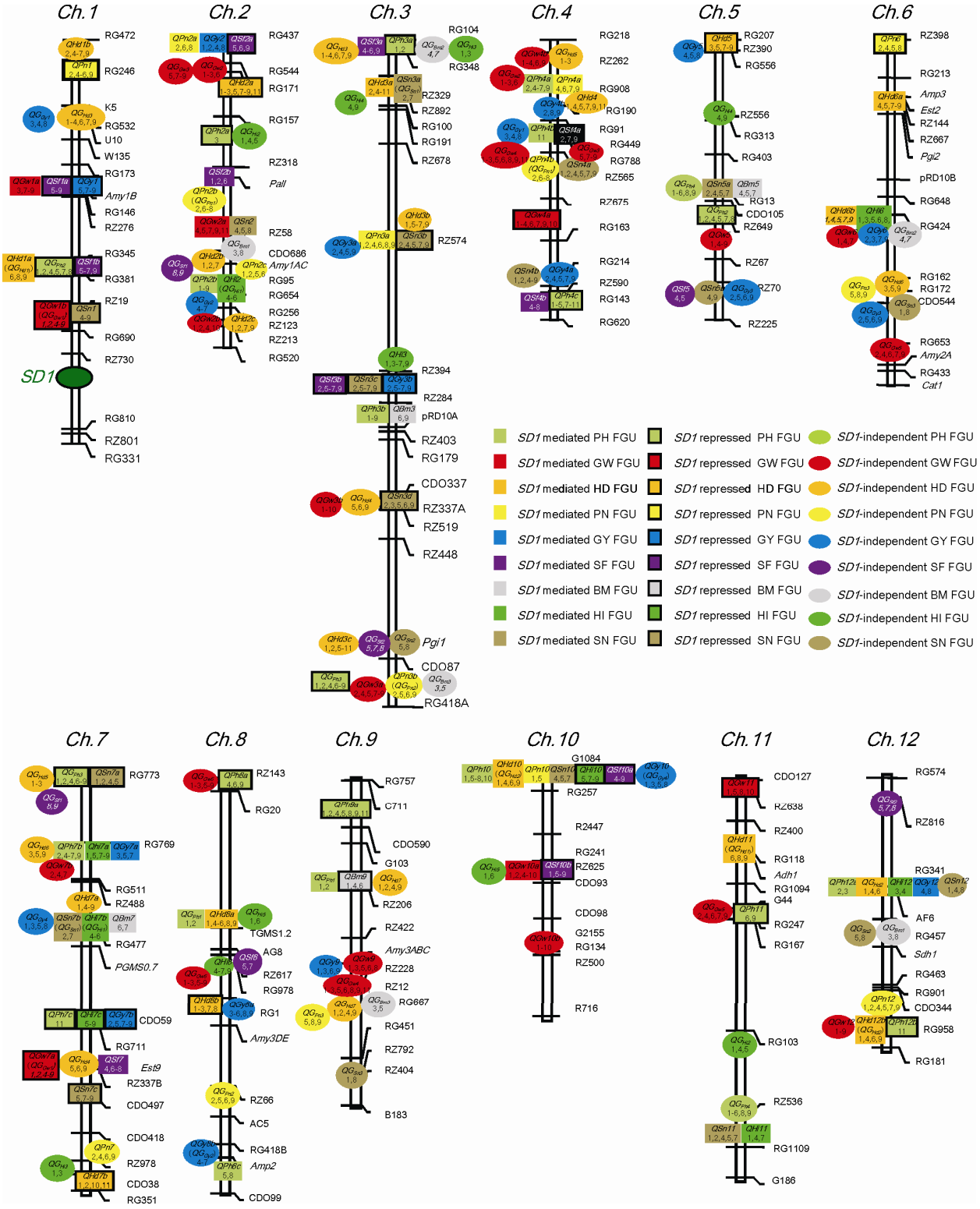

Figure 1. Genomic distribution of 183 QTLs including the SD1 gene in 145 functional genetic units affecting nine growth and yield traits detected in the IR64/Azucena DH population and its two subpopulations (with or without SD1) across 11 environments. Each box or oval represents an identified QTL and the numbers under each QTL represent the environments in which it was detected (Li et al 2003)

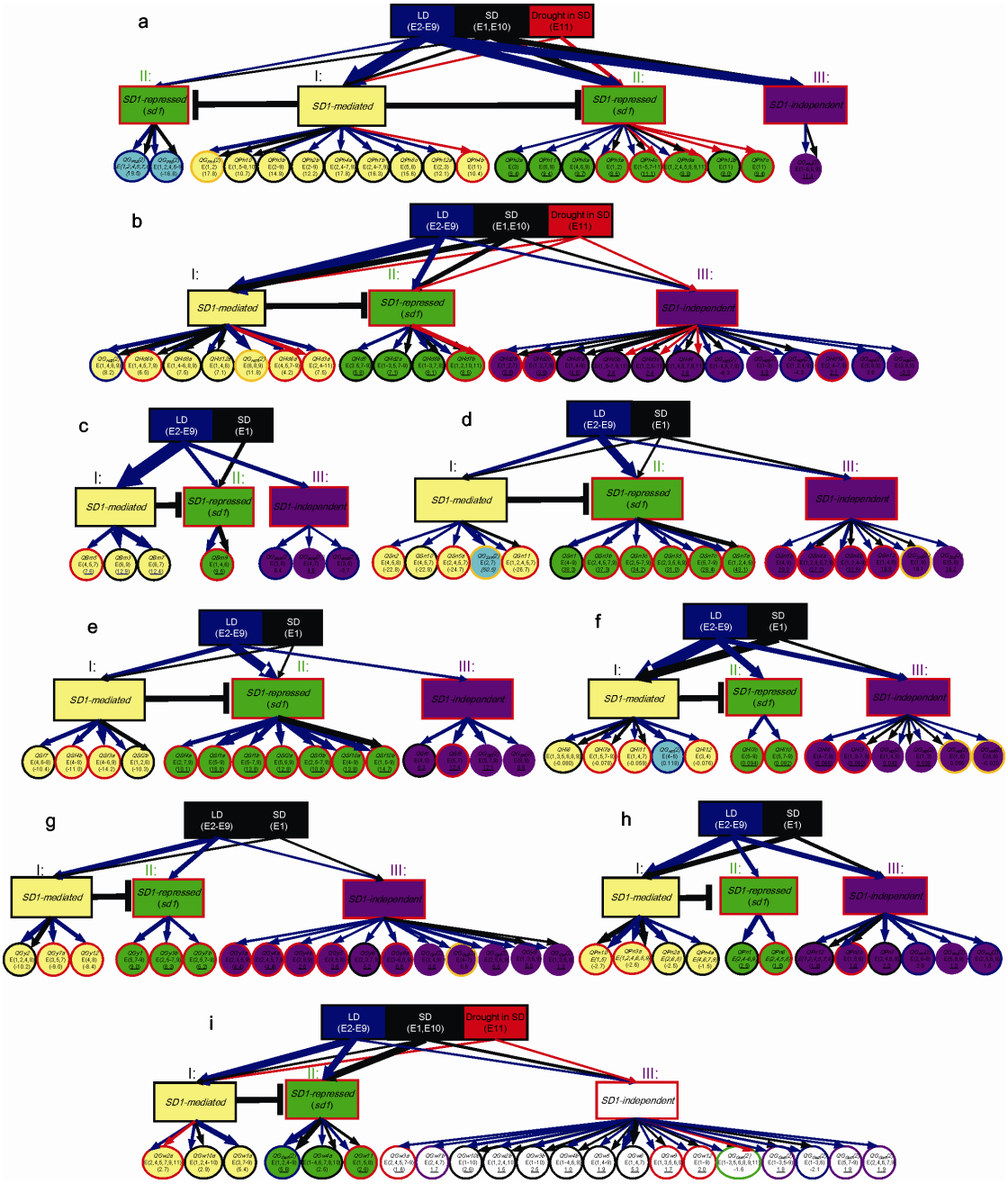

Figure 2. Putative genetic networks containing 145 identified functional genetic units (FGUs) and their estimated pathway effects on nine traits detected in the IR64/Azucena DH population across 11 environments. The traits include PH (a), HD (b), biomass (c), SN (d), SF (e), HI (f), GY (g), PN (h) and GW (i), respectively. Boxes on top of network for each trait are environments (E1-E11) classified into long-day (LD), short-day (SD) and drought environments. FGUs in each network were divided into 3 systems: (I) SD1-mediated pathways; (II) SD1-repressed pathways; and (III) SD1-independent pathways. Ovals at 3rd 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. 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 yellow outlines represent cases of the parental-type or recombinant-type increased trait values associated with parental digenic genotypes. FGUs without outlines are complementary epistatic loci pairs

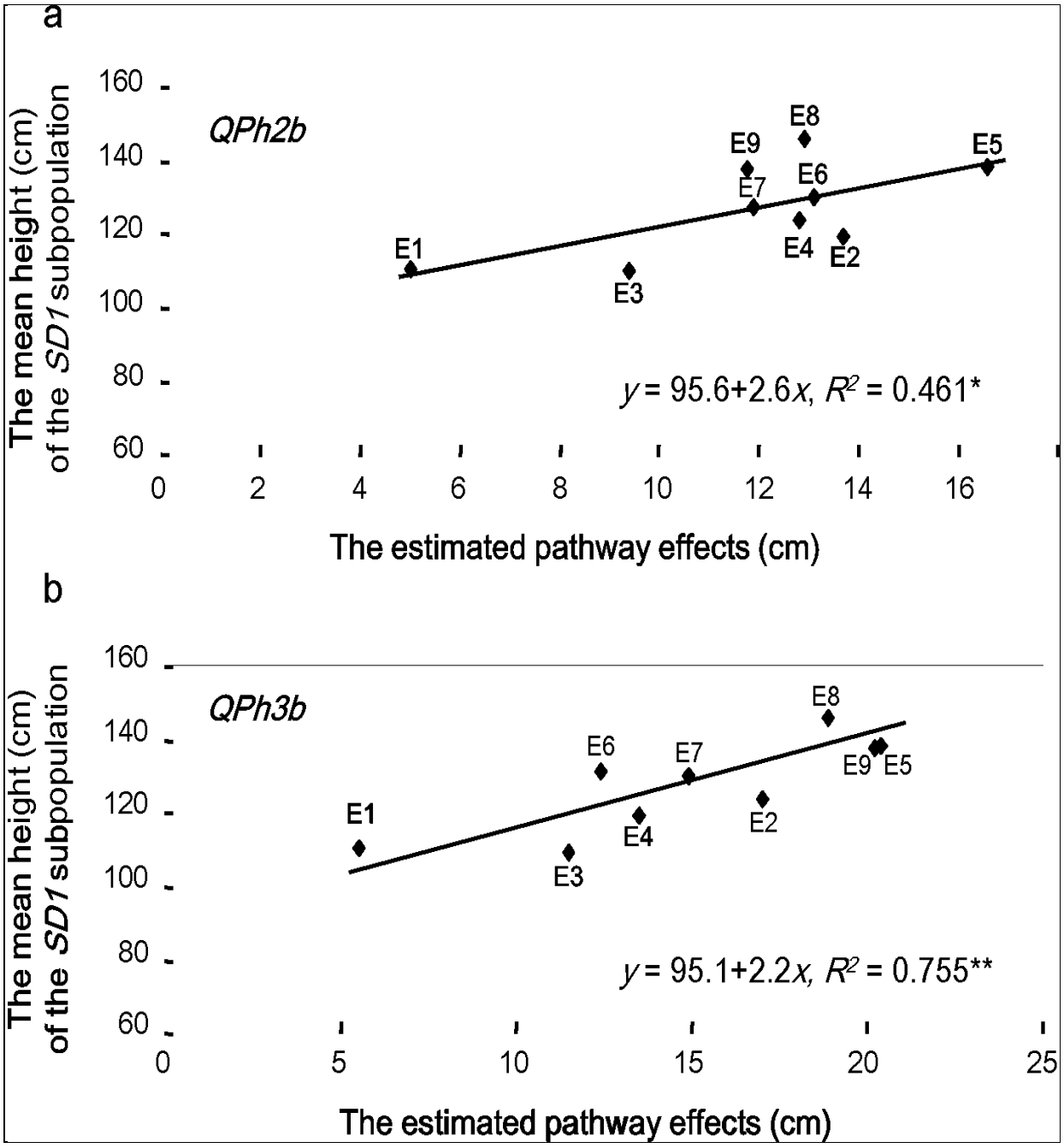

Figure 3. Positive correlations between the pathway effects of two SD1-mediated downstream pathways QPh2b (a) and QPh3b (b) with the environmental effects (measured as the mean values of the SD1 subpopulation for PH, see details in Table 3). E1-E9 are defined in Li et al (2003).

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) 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, $P < 0.05$).

Table 3. Forty-three *SD1*-mediated FGUs (48 QTLs) (only detected in the *SD1*-subpopulation) affecting growth and yield related traits, plant height (PH, cm),harvest index (HI), biomass (BM, g/plant), heading date (HD, days), panicle number per plant (PN), 1000-grain weight (GW, in g), grain yield (GY, in g/plant), spikelet number per panicle (SN), and spikelet fertility (SF, in %), detected in the IR64/Azucena doubled haploid population across 11 environments

Chro Mo some	Marker Interval	Trait	FGU	Parameter[a]	Environments											
					E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	
1	*Amy1B*	RG146	GW	*QGw1a*	F			19.0				24.6	33.4	24.9		
					Ef			5.2				4.6	6.9	4.9		
1	RG345	RG381	HD	*QHd1a*(QG_{Hd1})	F	135.8			68.4		67.7	43.8	41.4	37.6		
					Ef	15.8			5.6		9.3	4.4	3.5	5.1		
2	RG437	RG544	PN	*QPn2a*	F		38.4				23.0		36.0			
					Ef		-1.6				-2.7		-3.2			
			GY	*QGy2*	F	18.8	28.6		21.7				14.1			
					Ef	-12.0	-10.3		-7.4				-11.1			
2	RZ318	*Pall*	SF	*QSf2b*	F	12.0	26.3				22.5					
					Ef	-10.3	-9.8				-10.8					
2	RZ58	CDO686	GW	*QGw2a*	F				19.0	34.5		22.8		25.6		51.5
					Ef				1.8	2.5		1.9		2.2		5.2
			SN	*QSn2*	F				9.0	18.0			10.5			
					Ef				-24.0	-21.5			-22.9			
2	RG95	RG654	PH	*QPh2b*	F	20.0	40.6	42.0	32.4	64.6	47.6	42.8	95.8	28.8		
					Ef	5.0	13.7	9.6	12.8	16.6	13.1	11.9	14.3	12.9		
			HI	*QHi2*(QG_{Hi1})	F				20.8			17.0		8.5		
					Ef				-0.093			-0.053		-0.061		
3	RG104	RG348	SF	*QSf3a*	F				13.8	15.8	21.9			28.9		
					Ef				-10.2	-17.9	-11.0			-17.6		
3	RZ892	RG100	HD	*QHd3a*	F		71.4		107.1	38.6	73.4	58.3	85.0	73.0	61.8	42.3
					Ef		5.6		5.7	5.0	8.9	4.2	4.2	5.8	8.3	7.2
			SN	*QSn3a*(QG_{Sn1})	F				17.9	9.3		9.3				
					Ef				-33.2	-16.7		-28.2				
3	RZ574	RZ394	PN	*QPn3a*	F	29.7	37.6		30.1		44.2		38.0	61.7		
					Ef	-3.6	-1.5		-1.6		-3.2		-3.2	-2.3		
3	pRD10A	RZ403	PH	*QPh3b*	F	21.2	64.0	58.4	144.0	211.2	108.8	153.4	37.0	168.8		
					Ef	5.5	13.5	11.5	17.0	20.4	14.9	20.2	12.4	18.9		
			BM	*QBm3*	F						15.7			26.8		
					Ef						13.8			12.1		
4	RG908	RG190	PH	*QPh4a*	F		36.8		57.2	162.4	97.2	163.2		82.4		
					Ef		12.1		14.8	22.4	17.8	22.4		17.0		
			PN	*QPn4a*	F				23.0		20.5	25.7		22.2		
					Ef				-1.3		-2.4	-1.0		-1.4		
4	RG449	RG788	PH	*QPh4b*	F											18.8
					Ef											10.4
4	RG143	RG620	SF	*QSf4b*	F				14.8	11.5	16.3	11.8	14.2			
					Ef				-10.2	-15.2	-9.4	-9.8	-10.4			
5	RG13	CDO105	SN	*QSn5a*	F		11.4		18.0	10.3		11.6				
					Ef		-25.5		-29.9	-15.6		-27.9				
			BM	*QBm5*	F				17.0	16.2		15.0				
					Ef				7.6	6.1		9.2				
6	RZ667	Pgi-2	HD	*QHd6a*	F				38.0	42.5		63.6	37.8	78.0		
					Ef				3.5	5.0		4.2	2.8	5.7		
6	RG648	RG424	HD	*QHd6b*	F	92.0			53.2	50.2		67.8		123.8		
					Ef	11.1			4.3	5.6		4.5		7.0		
			HI	*QHi6*	F	9.8		13.7		16.5	11.2		8.3	23.1		
					Ef	-0.053		-0.087		-0.100	-0.068		-0.075	-0.096		
7	RG769	RG511	PH	*QPh7b*	F		30.0		47.0	88.8	49.8	86.6		36.0		
					Ef		14.1		16.3	19.8	15.0	18.7		14.1		
			HI	*QHi7a*	F	19.7				13.4		11.9	16.1	11.4		
					Ef	-0.071				-0.091		-0.055	-0.103	-0.072		
			GY	*QGy7a*	F			15.9		21.1		16.8				
					Ef			-10.7		-8.6		-7.6				
7	RG477	PGMS0.7	SN	*QSn7b*(QG_{Sn1})	F	11.1	14.5		9.1			9.9				
					Ef	-21.2	-29.0		-23.3			-27.3				
			HI	*QHi7b*(QG_{Hi1})	F					34.0	13.0			19.7		
					Ef					-0.127	-0.068			-0.086		
			BM	*QBm7*	F						17.3	18.7				
					Ef						14.4	10.5				
7	Est9	RZ337B	SF	*QSf7*	F				22.0		10.8	10.8	19.3			
					Ef				-12.2		-8.1	-9.5	-11.8			
8	TGMS1.2	AG8	PH	*QPh8b*(QG_{Ph1})	F	75.4	97.8									
					Ef	12.7	14.5									
			HD	*QHd8a*	F	80.4			102.8	55.7	102.9		75.0	44.1		
					Ef	12.9			6.1	6.4	10.2		4.3	5.1		

Chr	Mark	Trait	FGU	*Para*	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	
8	Amp-2	CDO99	PH	*QPh8c*	F					41.0			98.2			
					Ef					14.7			16.5			
9	RZ206	RZ422	PH	*QPh9b*(QG_{Ph1})	F	23.0	36.8									
					Ef	7.0	8.8									
10	G1084	RG257	PH	*QPh10*	F	17.2				16.0	21.2	22.4	21.0		11.4	
					Ef	9.5				10.4	12.5	12.8	11.9		6.9	
			HD	*QHd10*(QG_{Hd2})	F	43.4	56.2	42.5	65.2							
					Ef	8.8	4.8	5.1	4.7							
			PN	*QPn10*	F	36.0				26.0						
					Ef	-3.7				-1.6						
			SN	*QSn10*	F				11.1	10.9		10.7				
					Ef				-24.4	-16.1		-28.0				
10	RZ625	CDO93	GW	*QGw10a*	F	71.4	19.6		96.8	45.6	33.4	32.7	53.8	36.7	24.8	
					Ef	3.4	2.4		3.4	2.7	3.1	2.2	3.8	2.5	3.0	
11	RG118	*Adh1*	HD	*QHd11*(QG_{Hd1})	F	67.4			47.3				74.9	81.5		51.9
					Ef	12.6			4.9				4.6	7.3		8.5
11	RG103	RG1109	SN	*QSn11*	F	23.4	16.8		20.7	10.2		10.3				
					Ef	-27.6	-		-32.7	-16.3		-27.7				
							29.2									
			HI	*QHi11*	F	20.8			8.7			10.9				
					Ef	-			-			-				
						0.070			0.065			0.044				
12	RG341	AF6	PH	*QPh12a*	F		29.4	114.6								
					Ef		9.5	14.6								
			HD	*QHd12a*	F	76.4			56.3		41.9					
					Ef	10.9			4.2		6.2					
			HI	*QHi12*	F			16.8	8.3							
					Ef			-	-							
								0.089	0.063							
			GY	*QGy12*	F				14.8				12.9			
					Ef				-6.1				-10.6			
12	RG958	RG181	HD	*QHd12b*(QG_{Hd2})	F		86.9	61.1				78.5		37.4		
					Ef		6.1	5.3				4.4		4.2		

[a] Effect is the pathway phenotypic effect estimated from mean trait values. The sign indicates the direction of the genetic pathway and the threshold for claiming a significant QTL is 0.0001 for PH, HD, GW and PN, 0.005 for SN, BM, SF, HI and GY, respectively.

Table 4. 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^2 [a]	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

[a] V_G = the total genetic variances accounted by all detected FGUs in each environment. 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, and SF = sikelet fertility

Table 5. Five *SD1*-mediated FGUs of epistatic QTL pairs affecting plant height (PH, cm), harvest index (HI), heading date (HD, days), and spikelet number per panicle (SN), detected in the IR64/Azucena doubled haploid population across nine environments (E1-E9)

Marker *i*	QTL *i*	Marker *j*	QTL *j*	FGU	Parameter[a]	E1	E2	E3	E4	E5	E6	E7	E8	E9
TGMS1.2	*QPh8b*	RZ422	*QPh9b*	QG_{Ph1}	F	24.6	27.0							
					Ef	16.1	19.4							
RG345	*QHd1a*	RG118	*QHd11*	QG_{Hd1}	F						34.4		43.6	45.7
					Ef						12.1		8.9	14.3
G1084	*QHd10*	RG958	*QHd12b*	QG_{Hd2}	F	31.3			42.8		37.9			30.0
					Ef	12.3			6.0		8.7			6.0
RG654	*QHi2*	RG477	*QHi7b*	QG_{Hi1}	F				12.1	13.2	9.5			
					Ef				0.118	0.144	0.093			
RZ892	*QSn3a*	RG477	*QSn7b*	QG_{Sn1}	F		16.54					10.2		
					Ef		53.2					47.7		

[a] The effect is the pathway effect and the sign indicates the direction of the pathway effect.

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 4). 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; and for SF in E2 and E4 (Table 4).

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* (Tables 6-7, Figs. 1, 2). The *SD1*-repressed pathways included 10 PH FGUs (*QPh2a*, *QPh3a*, *QPh4c*, *QPh7c*, *QPh8a*, *QPh9a*, *QPh11*, *QPh12b*, QG_{Ph2} and QG_{Ph3}), 4 HD FGUs (*QHd2a*, *QHd5*, *QHd7b*, and *QHd8b*), 1 biomass FGU (*QBm9*), 2 PN FGUs (*QPn1* and *QPn6*), 2 HI FGUs (*QHi7c* and *QHi10*), 3 GY FGUs (*QGy1*, *QGy3b* and *QGy7b*), 6 SN FGUs (*QSn1*, *QSn3b*, *QSn3c*, *QSn3d*, *QSn7c* and *QSn7a*), 7 SF FGUs (*QSf1a*, *QSf1b*, *QSf2a*, *QSf3b*, *QSf4a*, *QSf10a* and *QSf10b*), and 3 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 (Table 5). 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 4). 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 (short-day); 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; and for SF in E1 and E5-E9 (Table 4).

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 and Tables 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.

Table 6. Thirty six *SD1*-repressed main-effect FGUs (detectable only in *sd1*-subpopulation) affecting growth and yield related traits, plant height (PH, cm), harvest index (HI), biomass (BM, g/plant), heading date (HD, days), panicle number per plant (PN), 1000-grain weight (GW, in g), grain yield (GY, in g/plant), spikelet number per panicle (SN), and spikelet fertility (SF, in %), detected in the IR64/Azucena doubled haploid population across 11 environments

Chr	Interval		Trait	FGU	Parameter[a]	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11
1	RG472	RG246	PN	*QPn1*	F		34.0		17.9	29.3	69.4			51.2		
					Ef		1.8		1.5	2.2	4.9			2.0		
1	*Amy1B*	RG146	GY	*QGy1*	F					30.6		13.9	10.0	13.4		
					Ef					15.1		9.5	4.7	6.5		
			SF	*QSf1a*	F					19.7	16.1	23.7	11.0	17.8		
					Ef					20.4	14.2	19.9	10.9	18.4		
1	RG345	RG381	SF	*QSf1b*	F					47.1	12.3	13.5		15.9		
					Ef					20.8	9.6	11.7		13.0		
1	RZ19	RG690	GW	*QGw1b*(*QG$_{Gw1}$*)	F	63.0	25.5		41.9	60.3	18.3	43.5	33.9	36.1	52.1	
					Ef	4.5	3.7		3.6	4.2	3.1	3.9	3.6	3.0	5.2	
			SN	*QSn1*	F				15.7	17.5	17.3	10.5	17.1	10.6		
					Ef				55.2	44.7	37.1	27.0	39.4	26.3		
2	RG437	RG544	SF	*QSf2a*	F					15.9	10.6			22.4		
					Ef					14.5	9.2			14.8		
2	RG171	RG157	HD	*QHd2a*	F	184.6	74.1	47.5		81.5		57.3	46.6	50.0		39.8
					Ef	12.0	8.8	7.8		6.1		3.6	4.8	4.4		9.3
2	RG157	RZ318	PH	*QPh2a*	F			29.4								
					Ef			8.4								
3	RG348	RZ329	PH	*QPh3a*	F	47.0	30.2									
					Ef	8.6	8.6									
3	RZ574	RZ394	SN	*QSn3b*	F		22.9		21.1	27.5		9.3		11.6		
					Ef		43.3		50.9	45.7		22.3		24.1		
3	RZ284	pRD10A	SN	*QSn3c*	F		16.0			41.5	12.2	12.4		29.2		
					Ef		37.4			49.9	26.6	24.3		32.8		
			GY	*QGy3b*	F		21.7			23.5	13.9	10.5		35.6		
					Ef		7.3			9.9	11.4	6.1		7.0		
			SF	*QSf3b*	F		11.7			11.9	11.6	11.5		16.6		
					Ef		6.2			12.6	9.8	11.4		13.3		
3	RZ337A	RZ519	SN	*QSn3d*	F		23.1	8.9		19.9	23.5			13.8		
					Ef		41.3	15.9		38.6	34.7			24.5		
4	RG449	RG788	SF	*QSf4a*	F		10.9					10.6		17.0		
					Ef		5.9					10.8		13.7		
4	RG163	RG214	GW	*QGw4a*	F	22.7	20.0	22.7	26.6		20.8	19.5		25.9	20.6	
					Ef	2.5	2.5	2.7	2.4		2.7	2.3		2.1	2.9	
4	RG214	RZ590	PH	*QPh4c*	F	36.8	50.0	37.4	80.0	67.2		40.8	62.8	41.6	11.4	21.4
					Ef	9.8	11.4	10	14.8	14.4		10.6	12.8	10.4	6.8	9.8
5	RZ390	RG556	HD	*QHd5*	F			51.4		83.5		166.8	141.9	67.3		
					Ef			6.3		5.2		5.0	7.5	5.2		
6	RZ398	RG213	PN	*QPn6*	F		19.8		36.5	29.1			18.0			
					Ef		1.4		2.1	2.2			1.5			
7	RG773	RG769	SN	*QSn7a*	F	15.9	30.4		9.4	9.0						

Chr	Interval		Trait	FGU	Parameter[a]	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11
					Ef	29.2	63.4		44.2	35.7						
7	CDO59	RG711	PH	*QPh7c*	F											23.0
					Ef											9.4
			HI	*QHi7c*	F					21.8	18.4	15.2	11.3	28.7		
					Ef					0.096	0.094	0.054	0.110	0.116		
			GY	*QGy7b*	F		8.4			15.7		11.5	15.1	29.8		
					Ef		5.2			8.3		6.7	4.4	6.7		
7	RZ337B	CDO497	GW	*QGw7a*(*QG$_{Gw1}$*)	F		19.1	30.5	39.7	18.0	29.5	21.4			21.8	
					Ef		2.6	3.1	2.8	2.0	3.1	2.3			3.0	
			SN	*QSn7c*	F					9.3		17.3	20.5	12.5		
					Ef					27.8		27.2	35.5	23.1		
7	CDO38	RG351	HD	*QHd7b*	F	46.1	71.9								50.2	65.1
					Ef	7.2	7.5								8.1	11.1
8	RZ143	RG20	PH	*QPh8a*	F				36.4		56.2			28.8		
					Ef				9.4		11.4			8.2		
8	RG1	*Amy3DE*	HD	*QHd8b*	F	60.4	53.7	69.5				65.4	186.0			
					Ef	9.4	8.2	8.9				4.2	9.9			
9	C711	CDO509	PH	*QPh9a*	F	32.0	29.8		24.8	28.4			27.4	49.0		17.2
					Ef	8.8	8.6		8.0	9.0			8.2	11.0		8.2
9	G103	RZ206	BM	*QBm9*	F	18.0			21.5		24.3					
					Ef	12.4			4.6		11.9					
10	G1084	RG257	HI	*QHi10*	F					21.5		14.5	10.8	8.3		
					Ef					0.095		0.052	0.110	0.070		
			SF	*QSf10a*	F				11.7	37.6	32.8	27.8	15.1	19.2		
					Ef				8.7	19.6	14.5	16.1	10.1	13.8		
10	RZ625	CDO93	SF	*QSf10b*	F	10.8				15.7	20.7	32.6	19.7	21.5		
					Ef	11.7				14.8	13.3	19.2	13.0	16.3		
11	RZ638	RZ400	GW	*QGw11*	F	25.1				23.1			23.4		22.1	
					Ef	2.5				2.3			2.7		2.8	
11	RG247	RG167	PH	*QPh11*	F						15.8			24.0		
					Ef						8.4			10.4		
12	RG958	RG181	PH	*QPh12b*	F											17.0
					Ef											8.0

[a] Effect is the estimated pathway phenotypic effect (2 x QTL effect), because no epistasis is involved in these QTLs, the directions (positive or negative) of these pathway effects can not be determined based on the available data

Table 7. The *SD1*-repressed FGUs consisting of 2 interacting loci and their pathway effects on plant height (PH, cm) and 1000-grain weight (GW, in g) detected in the IR64/Azucena doubled haploid population across nine environments

Marker i	QTL i	Marker j	QTL j	FGU	Parameter[a]	E1	E2	E3	E4	E5	E6	E7	E8	E9
RG381	*QPh1*	RZ649	*QPh5*	*QG$_{Ph2}$*	F	23.8	50.0		44.4	33.4		31.8	90.8	
					Effect	14.6	19.5		16.4	11.7		13.1	19.6	
RG418a	*QPh3c*	RG773	*QPh7a*	*QG$_{Ph3}$*	F	43.2	66.2		31.6		21.4	19.4	91.2	30.8
					Effect	-15.0	-16.8		-13.3		-9.4	-14.0	-16.3	-12.7
RZ19	*QGw1b*	RZ337B	*QGw7a*	*QG$_{Gw1}$*	F	20.5	32.1		29.9	41.5	45.0	20.7	43.8	34.7
					Effect	5.6	5.8		4.5	6.1	5.8	4.8	7.3	4.9

[a] The effect is the pathway effect and the sign indicates the direction of the pathway effect

Table 8. Forty *SD1*-independent main-effect QTLs (detected in both subpopulations) affecting growth and yield related traits, harvest index (HI), heading date (HD, days), panicle number per plant (PN), 1000-grain weight (GW, in g), grain yield (GY, in g/plant), and spikelet number per panicle (SN), detected in the IR64/Azucena doubled haploid population across 11 environments (E1-E11)

Ch	Interval	Trait	FGU	Parameter[a]	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11
1	RG472-RG246	HD	*QHd1b*	F		20.0		14.3	12.1	22.9	16.4		44.3		
				Ef		3.4		-3.8	-1.8	-4.2	-2		-4.4		
2	*Pall*-RZ58	PN	*QPn2b*(*QG*$_{Pn1}$)	F	32.0	23.6			19.9	13.7					
				Ef	1.7	2.8			1.2	1.1					
2	*Amy1AC*-RG95	PN	*QPn2c*	F	37.8				36.9	23.2					
				Ef	-1.8				-1.9	-1.6					
2	RZ123-RZ213	GW	*QGw2b*	F	15.2	21.6		30.6						25.2	
				Ef	0.72	1.80		1.14						2.32	
2	CDO686-Amy1AC	HD	*QHd2b*	F	41.0	74.4					48.9				
				Ef	-4.4	-4.0					-2.2				
2	RZ123-RZ213	HD	*QHd2c*	F	50.9	55.2					116.3		37.7		
				Ef	-5.0	-3.6					-3.4		-2.4		
3	RZ574-RZ394	GY	*QGy3a*	F		24.5		8.8	14.8				9.4		
				Ef		-6.2		-2.8	-5.0				-3.6		
3	RZ394-RZ284	HI	*QHi3*	F	29.5		7.9	26.4	46.5	30.1	18.8		35.5		
				Ef	-0.062		-0.036	-0.058	-0.092	-0.064	-0.038		-0.074		
3	CDO87-RG418A	GW	*QGw3a*	F		21.5		55.0	38.1		23.4	21.5	45.2		
				Ef		-1.6		-1.8	-1.6		-1.4	-1.6	-1.8		
		PN	*QPn3b*(*QG*$_{Pn2}$)	F		19.8		40.5	31.2	23.8			39.6		
				Ef		1.0		1.4	1.4	4.2			1.2		
3	RZ678-RZ574	HD	*QHd3b*	F	17.9				42.9	15.7	16.4		11.4		
				Ef	5.0				4.4	3.8	4.4		1.8		
3	RZ337A-RZ519	GW	*QGw3b*	F	33.5	40.3	84.7	27.9	60.8	40.7	26.1	28.8	30.6	51.4	
				Ef	2.14	2.58	3.04	2.36	3.02	2.12	2.26	2.00	2.62	2.92	
3	RZ448-*Pgil*	HD	*QHd3c*	F	10.0	17.1			62.8	20.0	15.0	15.7	32.1	25.0	13.6
				Ef	2.2	3.6			6.0	6.2	3.8	3.8	5.0	-5.2	-3.8
4	RG788-RZ565	SN	*QSn4a*	F	43.9	24.3		39.3	26.9		36.5		43.8		
				Ef	-25.8	-28.2		-36.2	-24.4		-26.2		-22.0		
4	RZ565-RZ675	PN	*QPn4b*(*QG*$_{Pn1}$)	F	30.9	36.3		51.2	42.6	22.4	52.7	19.2	36.9		
				Ef	2.4	1.4		1.6	1.6	2.0	1.2	1.2	1.2		
4	RZ590-RG143	SN	*QSn4b*	F	20.0	43.5		89.8	97.4	59.1	48.6	38.1	86.2		
				Ef	-17.8	-36.2		-49.4	-39.2	-36.2	-28.4	-27.4	-27.2		
		GY	*QGy4a*	F		17.8		34.1	9.4		17.6		14.5		
				Ef		-5.4		-5.2	-3.8		-4.4		-3.0		
4	RZ262-RG908	GW	*QGw4b*	F	19.8	15.1	16.4	19.4		30.6			31.9		
				Ef	1.46	0.80	0.62	1.04		0.94			1.12		
4	RG908-RG190	HD	*QHd4*	F	21.4			20.0	10.7		17.9		20.7		16.4
				Ef	5.4			2.0	1.8		2.0		3.2		2.6
4	RG190-RG91	GY	*QGy4b*	F		13.6						25.2	15.0		
				Ef		-6.6						-5.6	4.6		
5	RZ390-RG556	GY	*QGy5*	F				18.8	14.4			11.7			
				Ef				-3.8	-3.2			-3.8			
5	RZ649-RZ67	GW	*QGw5*	F	17.8			16.2	31.5	24.4	37.9	26.7	24.5		
				Ef	1.50			1.82	2.18	1.56	3.02	1.26	2.18		
5	RZ70-RZ225	SN	*QSn5b*	F				11.2					10.8		
				Ef				-12.8					-7.2		
		SF	*QSf5*	F				12.8	17.8						
				Ef				6.2	10.4						

Ch	Interval	Trait	FGU	Parameter[a]	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11
6	RG424-RG162	GY	*QGy6*	F		20.5	18.3				11.1		22.4		
				Ef		7.2	2.8				2.7		8.2		
		GW	*QGw6*	F	49.2			26.7			17.3				
				Ef	6.80			4.80			4.20				
7	RG769-RG511	GW	*QGw7b*	F		32.0		19.6			17.6				
				Ef		0.98		2.16			1.92				
7	CDO418-RZ978	PN	*QPn7*	F		13.0		33.1		41.8			36.5		
				Ef		1.8		4.0		1.8			1.7		
7	RZ488-RG477	HD	*QHd7a*	F	35.5			124.6	200.7	88.9	131.3	149.0	160.9		
				Ef	4.4			4.0	6.2	5.2	3.8	4.0	4.8		
8	RZ617-RG978	HI	*QHi8*	F				11.8	45.8	23.4	23.7		30.8		
				Ef				-0.044	-0.102	-0.064	-0.046		-0.078		
8	RZ617-RG978	SF	*QSf8*	F					27.4		42.1				
				Ef					-13.2		-11.6				
8	RG1-*Amy3DE*	GY	*QGy8a*	F			14.0	39.5	40.8	25.6		25.8	27.1		
				Ef			-3.2	-7.8	-9.0	-4.8		-3.4	-12.6		
8	RG418B-*Amp2*	GY	*QGy8b*(QG_{Gy2})	F		29.6			14.0	19.1	19.4	11.5	15.6		
				Ef		9.6			5.7	2.6	6.6	4.0	4.8		
9	Amy3ABC-RZ228	GY	*QGy9*	F	22.5		20.4			21.5			19.9		
				Ef	3.6		3.2			4.8			10.2		
		GW	*QGw9*	F	19.8		36.0		28.8	34.0			30.7		
				Ef	-1.50		-1.80		-1.62	-1.92			-1.76		
10	RG134-RZ500	GW	*QGw10b*	F	60.2	84.4	33.3	155.6	64.7	71.1	70.4	81.5	82.2	32.3	
				Ef	2.0	3.0	1.8	3.0	2.0	5.2	2.4	2.8	2.4	2.2	
10	G1084-RG257	GY	*QGy10*(QG_{Gy4})	F		11.1			19.3				17.7		
				Ef		-4.2			-5.2				-4.6		
12	RG341-AF6	SN	*QSn12*	F	7.8			14.9				11.3			
				Ef	-13.8			-27.6				-18.4			
12	RG958-RG181	GW	*QGw12*	F	39.4	18.4	72.3	24.1	64.9	41.3	16.6	40.6	60.0		
				Ef	-2.18	-1.56	-2.40	-1.50	-2.38	-1.80	-0.90	-2.38	-2.86		
12	CDO344-RG958	PN	*QPn12*	F	23.2	59.3		95.6	107.8		53.9		116.8		
				Ef	2.0	1.6		2.0	2.4		1.0		2.0		

[a] Effect is FGU pathway effect estimated from mean trait values. The sign indicates the direction of the effect of the IR64 allele and the directions (positive or negative) of these pathway effects can not be determined based on the available data

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 and Table 9), implying that the parental alleles at each of the 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 (Table 8).

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 4). 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%).

Table 9. Twenty-nine *SD1*-independent E-QTL pairs affecting plant height (PH in cm), heading date (HD), biomass (BM, in g/plant), harvest index (HI), spikelet number per plant (SN), spikelet fertility (SF, %), grain yield (GY, g/plant), panicle number per plant (PN), grains per panicle (GN) and 1000 grain weight (GW in g) detected in the IR64/Azucena DH population across 11 environments (E1-E11)

Ch.	Flanking Marker *i*	Ch.	Flanking Marker *j*	Trait	FGU	Parameter[a]	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11
5	RG13	*11*	RZ536	PH	QG_{Ph4}	F	58.3	46.6	36.2	39.7	38.5	38.5		80.5	46.6		
						Ef	9.4	9.2	6.6	11.4	11.2	12.4		16.8	14.0		
1	U10	*3*	RG348	HD	QG_{Hd3}	F	19.0	54.7	34.3	27.7		16.8	48.1		51.8		
						Ef	-7.2	-7.2	-6.9	-6.0		-7.1	-3.9		-5.4		
3	RZ337A	*7*	RZ337B	HD	QG_{Hd4}	F					35.7	15.3			16.0		
						Ef					5.5	2.2			4.1		
4	RZ262	*7*	RG773	HD	QG_{Hd5}	F	22.6	41.5	16.8								
						Ef	5.6	4.4	2.8								
6	RG172	*7*	RG769	HD	QG_{Hd6}	F			16.0		16.8				35.0		
						Ef			-2.4		-3.8				-3.6		
9	RG667	*9*	RZ206	HD	QG_{Hd7}	F	18.2	18.2		35.0					43.0		
						Ef	-7.5	-4.1		-3.4					-2.2		
2	RZ58-CDO686	*12*	RG457-*Sdh1*	BM	QG_{Bm1}	F			24.2					22.9			
						Ef			4.7					8.2			
3	RG104-RG348	*6*	RG424-RG162	BM	QG_{Bm2}	F				17.4			17.5				
						Ef				5.1			4.0				
3	CDO87-RG418A	*9*	RZ12-RG667	BM	QG_{Bm3}	F			22.9		35.9						
						Ef			-3.3		-4.1						
2	RG157-RZ318	*11*	RG103-RG1109	HI	QG_{Hi2}	F	15.7			12.4	13.2						
						Ef	-0.036			-0.048	-0.036						
3	RG104-RG348	*7*	RZ978-CDO38	HI	QG_{Hi3}	F	26.0		18.7								
						Ef	-0.040		-0.036								
3	RZ892-RG100	*5*	RZ556-RG313	HI	QG_{Hi4}	F				15.8					36.7		
						Ef				-0.026					-0.014		
8	RG20-TGMS1.2	*10*	RZ625-CDO93	HI	QG_{Hi5}	F	17.5					19.1					
						Ef	0.036					0.075					
3	RZ448-*Pgil*	*12*	RG457-*Sdh1*	SN	QG_{Sn2}	F					17.7			21.7			
						Ef					11.7			20.7			
6	CDO544-RG653	*9*	RZ792-RZ404	SN	QG_{Sn3}	F	19.2							16.1			
						Ef	21.3							16.9			
2	Amy1AC-RG95	*7*	RG773-RG769	SF	QG_{Sf1}	F								26.8	27.6		
						Ef								6.7	10.8		
3	RZ448-*Pgil*	*12*	RZ816-RG341	SF	QG_{Sf2}	F					48.6		31.9	27.1			
						Ef					-19.7		-8.5	-8.3			
1	U10-W135	*4*	RG91 – RG449	GY	QG_{Gy1}	F			13.4	9.3				7.5			
						Ef			3.3	3.7				3.5			
2	RG654-RG256	*8*	RG418B-*Amp2*	GY	QG_{Gy2}	F				28.2	9.3	11.6	8.5				
						Ef				6.2	6.3	9.6	3.9				

Ch.	Flanking Marker *i*	Ch.	Flanking Marker *j*	Trait	FGU	Parameter[a]	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11
5	RZ70-RZ225	*6*	CDO544-RG653	GY	QG_{Gy3}	F		15.9			12.3	20.4			14.7		
						Ef		-8.5			-3.9	-6.3			-7.5		
7	RG477-PGMS07	*10*	G1084-RG257	GY	QG_{Gy4}	F	8.8		14.1		12.9			12.1			
						Ef	4.6		-1.7		-2.9			-5.1			
2	Pall-RZ58	*4*	RG788-RZ565	PN	QG_{Pn1}	F		23.0				28.6	24.4	18.5			
						Ef		1.9				2.3	1.1	2.8			
3	CDO87-RG418A	*8*	RZ66-AC5	PN	QG_{Pn2}	F		19.1			45.8	37.7			22.9		
						Ef		0.9			1.9	2.3			1.2		
6	RG172-CDO544	*9*	RG667-RG451	PN	QG_{Pn3}	F					28.9			29.6	28.5		
						Ef					1.3			1.26	1.22		
2	RG544-RG171	*4*	RZ262-RG908	GW	QG_{Gw2}	F	21.6	20.5	66.6			23.5					
						Ef	-1.46	-2.13	-1.48			-3.49					
2	RG544-RG171	*4*	RG449-RG788	GW	QG_{Gw3}	F					26.3		23.3	16.9	19.5		
						Ef					-1.62		-2.02	-1.80	-2.00		
4	RG449-RG788	*9*	RZ228-RZ12	GW	QG_{Gw4}	F	15.9	22.3	32.9		36.8	22.4		33.8	26.9		36.4
						Ef	-1.39	-2.19	-1.05		-1.58	-0.91		-1.15	-2.17		-2.55
6	RG653-*Amy2A*	*11*	G44-RG247	GW	QG_{Gw5}	F		18.4		23.3		19.5	19.0		16.8		
						Ef		1.24		2.00		1.64	2.56		2.14		
8	RZ143-RG20	*8*	RG978-RG1	GW	QG_{Gw6}	F	20.6	20.2	14.0		29.7	16.9	15.1	23.3	29.4		
						Ef	2.02	1.64	1.26		1.78	1.34	1.58	2.16	2.22		

[a] Effect is FGU pathway effect estimated from mean trait values. The sign indicates the direction of the pathway effect

Table 10. Parental distribution of favorable alleles for increased trait values at 148 loci affecting nine traits related to rice growth, development and productivity detected in IR64/Azucena DH population

Traits	*SD1*-mediated pathways		*SD1*-repressed pathways		*SD1*-independent pathways		Total	
	Azucena	IR64	Azucena	IR64	Azucena	IR64	Azucena	IR64
HD	3(3)[a]	2(1)	1	3	3	4(6)	7(3)	9(7)
PH	1(1)	7(1)	4	4(4)	0	0	5(1)	11(5)
PN	2	2	1	1	1	2(4)	4	5(4)
Biomass	1	2	1	0	(4)	0	2(4)	2
HI	3(2)	1	2	0	2(2)	(2)	7(4)	1(2)
Yield	2	1	3	0	5(1)	1(1)	10(1)	2(1)
GW	1	2	1(2)	1	3(3)	6(1)	5(5)	9(1)
SN	3(1)	1(1)	5	1	4(1)	(1)	12(2)	2(2)
SF	2	2	7	0	1	1	10	3
Average (1)[b]	18(7)	20(3)	25(2)	10(4)	19(11)	14(15)	62(20)	44(22)
Average (2)	14(3)	11(1)	20(2)	3	16(11)	10(9)	50(16)	24(10)

[a] The number in the parenthesis is the number of loci involved in epistasis; [b] Average (1) is the total average; and Average (2) is the average value of the traits directly related to yield (no HD and PH)

Table 11. Phenotypic variation for plant height in the subpopulations with fixed alleles at the *sd1* locus in of IR64/Azucena doubled-haploid (DH) population

Envir.	Mean			Range		Variance[a]		
	sd1	*SD1*	*Diff.*	*sd1*	*SD1*	*sd1*	*SD1*	*F value*
E1	84.4	110.4	25.9	59.3-110.7	86.7-139.7	140.4	156.1	1.11
E2	85.3	119.5	34.2	58.8-108.0	91.2-157.0	151.2	213.9	1.41
E3	92.7	108.9	16.2	66.7-125.3	73.1-136.5	179.6	243.5	1.36
E4	88.1	123.9	35.8	61.6-118.0	92.0-157.9	183.9	220.2	1.20
E5	103.4	138.5	35.1	73.4-139.6	84.2-181.4	192.7	383.0	1.99*
E6	98.1	130.3	32.1	76.5-134.3	98.3-160.0	136.3	222.2	1.63*
E7	103.8	137.3	33.5	78.9-127.4	104.2-178.0	160.2	302.3	1.89*
E8	100.8	131.2	30.4	69.5-138.0	75.0-176.5	226.7	404.9	1.79*
E9	108.5	145.4	36.9	81.3-143.4	115.0-183.9	183.8	292.8	1.59*
E10	84.1	104.7	20.6	67.8-94.8	89.5-117.7	60.8	64.4	1.06
E11	70.7	85.4	14.7	57.5-86.3	73.0-100.3	61.0	51.1	1.19
Mean			28.7			152.4	232.2	

[a] * indicates the significance at the level of $p < 0.05$ by *F* tests.

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 Green Revolution in rice

Historically, GR-I has been attributed to the mutants at a single locus, *SD1*. Indeed, we were able to show that *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 have revealed 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 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 GAs 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 both 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 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 (Table 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 (Table 9). 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 (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 6). 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 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) (Table 1). Nevertheless, our results were consistent with the theoretical expectation that statistically detectable epistasis occurs only between or among genes involved in positively regulated signaling pathways (Zhang et al 2011).

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 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 4). 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 (Table 11). Likewise, the feasibility of semi-dwarf rice with functional *SD1*-mediated pathways is 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 1970s and 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 4) indicate that different breeding strategies should be taken for the same target traits in different target environments. In rice with widespread use of both 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.

REFERENCES

Ali AJ, Xu JL, Ismail AM, Fu BY, Vijaykumar CHM, Gao YM, Rey JD, Maghirang R, Yu SB, Gregorio GB, Yanagihara S, Cohen M, Mackill DJ and Li ZK. 2006. Hidden diversity for abiotic stress tolerances in the primary gene pool of rice revealed by a large backcross breeding program. *Field Crops Res* 97: 66-76.

Cassman KG. 1999. Ecological intensification of cereal production systems: yield potential, soil quality, and precision agriculture. *Proc Natl Acad Sci USA* 96:5952–5959.

Cho YG, Eun MY, McCouch SR and Chae YA. 1994. The semidwarf gene, *sd-1*, of rice (*Oryza sativa* L.). II. Molecular mapping and marker-assisted selection. *Theor Appl Genet* 89:54–59.

Conway G. 1999. *The Doubly Green Revolution: Food for All in the Twenty-First Century.* Cornell University Press, Ithaca

Evenson RE and Gollin D. 2003. Assessing the impact of the green revolution, 1960 to 2000. *Science* 300:758–762.

Guan YS, Serraj R, Liu SH, Xu JL, Ali J, Wang WS, Venus E, Zhu LH and Li ZK. 20103 Simultaneously improving yield under drought stress and non-stress conditions: a case study of rice (*Oryza sativa* L.). *J Exp Bot* 61:4145–4156.

Hedden P. 20033. The genes of the Green Revolution. *Trends Genet* 19:5–9

Hedden P and Phillips AL. 2000. Gibberellin metabolism: new insights revealed by the genes. Trends Plant Sci 5:523–530

Hooley R. 1994. Gibberellins: perception, transduction and responses. *Plant Mol Biol* 26:1529–1555.

Khush GS. 1995. Modern varieties – their real contribution to food supply and equity. *Geo J* 35:275–284.

Khush GS. 2001. Green revolution: the way forward. *Nat Rev Genet* 2:815–822.

Kuroda E, Ookawa T and Ishihara K. 1989. Analysis on difference of dry matter production between rice cultivars with different plant height in relation to gas diffusion inside stands. *Japanese J Crop Sci* 58:374–382.

Lafitte HR, Guan YS, Shi Y and Li ZK. 2007. Whole plant responses, key processes, and adaptation to drought stress: the case of rice. *J Exp Bot* 58:169–175.

Lafitte HR, Li ZK, Vijayakumar CHM, Gao YM, Shi Y, Xu JL, Fu BY, Yu SB, Ali AJ, Domingo J, Maghirang R, Torres R and Mackill DJ. 2006. Improvement of rice drought tolerance through backcross breeding: evaluation of donors and selection in drought nurseries. *Field Crop Res* 97:77–86.

Li ZK, Yu SB, Lafitte HR, Huang L, Courtois B, Hittalmani S, Vijayakumar CHM, Liu GF, Wang GC, Shashidhar HE, Zhuang JY, Zheng KL, Singh VP, Sidhu JS, Srivantaneeyakul S and Khush GS. 2003. QTL · environment interactions in rice. I. Heading date and plant height. *Theor Appl Genet* 108:141–153

Ma Q, Hedden P and Zhang Q. 2011. Heterosis in rice seedlings: its relationship to gibberellin content and expression of gibberellin metabolism and signaling genes. *Plant Physiol* 156(4):1905-20.

Mackill DJ, Coffman WR and Garrity DP. 1996. *Rainfed lowland rice improvement*. International Rice Research Institute, Los Baños, Metro Manila, the Philippines

Matson PA, Parton WJ, Power AG and Swift MJ 1997. Agricultural intensification and ecosystem properties. *Science* 277:504–509.

Mona, L., N. Kitazawa, R. Yoshino, J. Suzuki, H. Masuda, Y. Maehara, M and Tanji, M. 2002. Positional cloning of rice semidwarfing gene, *sd-1*: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. *DNA Res* 9:11–17.

Murai M, Takamure I, Sato S, Tokutome T and Sato Y. 2002. Effects of the dwarfing gene originating from 'Dee-geo-woo-gen' on yield and its related traits in rice. *Breed Sci* 52:95–100.

Nemhauser JL, Hong F and Chory J. 2006. Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. *Cell* 126:467–475.

Ookawa T, Hobo T, Yano M, Murata K, Ando T, Miura H, Asano K, Ochiai Y, Ikeda M, Nishitani R, Ebitani T, Ozaki H, Angeles ER, Hirasawa T and Matsuoka M. 2010. New approach for rice improvement using a pleiotropic QTL gene for lodging resistance and yield. *Nat Comms* 1: 132. doi: 10.1038/ncomms1132

Paterson AH and Li ZK. 2011. Paleo-green revolution for rice. *Proc Natl Acad Sci USA* 108:10931–10932.

Peng S, Cassman KG, Virmani SS, Sheehy J and Khush GS. 1999 Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. *Crop Sci* 39:1552–1559.

SAS Institute. 2004. *SAS/STAT 9.1 User's Guide.* SAS Institute Inc., Cary, NC, USA

Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H and Matsuoka M. 2002. A mutant gibberellin-synthesis gene in rice. *Nature* 416:701–702.

Spielmeyer W, Ellis MH and Chandler PM. 2002. Semidwarf (*sd-1*), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. *Proc Natl Acad Sci USA* 99:9043–9048.

Tilman D. 1998. The greening of the green revolution. *Nature* 396:211–212.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH and Tilman DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecol Appl* 7:737–750.

Wang DL, Zhu J, Li ZKL and Paterson AH. 1999. Mapping QTLs with epistatic effects and QTL×environment interactions by mixed linear model approaches. *Theor Appl Genet* 99:1255-1264.

Xing YZ, Tan Y F, Hua JP, Sun XL, Xu CG and Zhang Q. 2002. Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theor Appl Genet* 105, 248-257.

Yamaguchi S. 2008. Gibberellin Metabolism and its Regulation. *Annu Rev Plant Biol* 59:225–251

Zhang F, Zhai HQ, Paterson AH, Xu JL, Gao YM, Zheng TQ, Wu RL, Fu BY, Ali JH and Li ZK . 2011. Dissecting genetic networks underlying complex phenotypes: the theoretical framework. *PLoS ONE* 6:e14541

Zhang Q. 2007. Strategies for developing green super rice. *Proc Natl Acad Sci USA* 104:16402–16409

Zhuang JY, Lin HX, Lu J, Qian HR, Hittalmani S, Huang N and Zheng KL. 1997. Analysis of QTL 9 environment interaction for yield components and plant height in rice. *Theor Appl Genet* 95:799–808

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