Identification of Heterosis QTLs for Yield and Yield-Related Traits in *Indica-Japonica* Recombinant Inbred Lines of Rice (*Oryza sativa* L.)

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ABSTRACT Supplying sufficient rice to growing populations is a global challenge. Hybrid *indica* rice varieties exploiting heterosis have increased yields, but inter-subspecific crosses between *indica* and *japonica* varieties are hampered by sterility. Examination and genetic understanding of yield heterosis in *indica/japonica* crosses addressing yield barriers are basic requirements. In this study, QTLs for heterosis of yield traits were identified in *indica-japonica* recombinant inbred lines (RILs) using a total of 178 RILs originating from Dasanbyeon (*indica*) × TR22183 (*japonica*) (DT-RILs) and their backcrossed populations. Nine of sixty-six major quantitative trait loci (QTLs) identified in DT-RILs exhibited heterosis. Heterosis QTLs clustered with other traits on chromosomes 1, 4, and 8, and clusters were conserved between different RILs. The clusters contained several known yield enhancement genes/QTLs. Specific heterotic allele combinations contributed to four major heterosis QTLs, particularly for panicle and spikelet number traits. Heterosis for yield and yield-related traits was explained by the harmonized effects of overdominance, dominance, and epistatic interactions in inter-subspecific breeding populations.

Keywords Rice, Heterosis, Yield, QTL

INTRODUCTION

Rice is one of the most important staple foods worldwide. Yearly global rice consumption is estimated to reach 500 million tonnes by 2050 (Abdullah *et al.* 2008). Arable land area per capita has declined substantially in recent decades, from 0.37 ha in 1961 to 0.196 ha in 2014 (<http://data.worldbank.org/indicator/%20AG.LND.ARB.L.HA.PC>), and supplying sufficient food to a growing

population is a major challenge for future human development. Utilization of high yield potential traits will be needed to facilitate the development of high yield rice production systems to overcome the global rice deficit. One of the most effective approaches was used in China, where hybrid rice programs utilizing heterosis contributed to increased yields of up to 13.5 tonnes/ha in 2012 (FAORAP and APSA 2014).

Heterosis, also known as hybrid vigor, is observed in

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plants, animals, and fungi and is characterized by the superior phenotypic performance of a hybrid compared with its parents. Hybrid vigor is observed in a range of environmental conditions, and phenotypic performance is stably observed. Despite substantial research on heterosis, the genetic underpinnings of the phenomenon are not yet fully understood. Dominance, overdominance, and epistasis (or digenic interactions) are three major genetic components involved in heterosis (Li *et al.* 2001; Luo *et al.* 2001; Li *et al.* 2008; Shen *et al.* 2014). Dominance and overdominance are interactions of different alleles at the same locus, and epistasis describes interactions between different loci.

Currently available commercial rice hybrids utilizing heterosis optimize the yield potential of crosses between *indica* varieties. Several recent studies investigated the potential of *indica-japonica* hybrids (Chin *et al.* 2011; Xin *et al.* 2011; Chu *et al.* 2012; Dan *et al.* 2014); however, severe hybrid sterility was observed, creating a major barrier to developing super high-yielding hybrid rice (Chu *et al.* 2012; Dan *et al.* 2014). The relationship between hybrid sterility and heterosis is unclear, but yield decreases in F₁ plants of inter-subspecific crosses were attributed to severely low seed set. A method of yield adjustment, namely, presupposition yield, was suggested as a means of experimentally minimizing the effect of hybrid sterility (Chu *et al.* 2012).

Broad-sense heritability of yield-related traits was 21.47-79.85% for seven yield-related traits of testcross populations derived from *indica-japonica* chromosome segment substitution lines (Tao *et al.* 2016). The contribution of additive effects (narrow sense heritability) to heterosis was particularly large for grain yield and thousand grain weight (TGW). Dominant effects for spikelet and panicle number also had a major impact on heterosis.

In this study, two recombinant inbred lines (RILs) derived from *indica* (Korean Tongil type) / *japonica* crosses were used to investigate the genetic contributions of dominance, overdominance, and epistasis to yield and yield-related traits. Major quantitative trait loci (MQTLs) and EpQTLs (epistatic QTLs or digenic interactions) were identified for each RIL. Comparisons of QTLs/genes in two RILs allowed identification of putative heterosis QTL

hotspots. MQTL analysis used average values for the three heterotic allele types (AiAi/AiAj, AiAj/AiAi, and AiAj/AiAj) present in a BC₁F₁ population. The genetic effects of each allele type were then analyzed separately to understand which heterotic allele types were the major contributors to yield and yield-related traits. Lastly, the direct effects of yield-related traits on yield heterosis QTLs were determined.

MATERIALS AND METHODS

Plant materials

A total of 179 F₈ RILs derived using the single-seed descendant method from a cross between Dasanbyeo (Tongil type, *indica*) and TR22183 (temperate *japonica*) developed by Seoul National University in Korea (DT-RILs) were used in this study (Chin *et al.* 2011; Jiang *et al.* 2011). Each RIL was crossed with Dasanbyeo as the female parent, and 178 BC₁F₁ lines (except for line 40) were produced (BC₁F₁ line). F₁ plants of Dasanbyeo and TR22183 were generated and cultivated alongside the BC₁F₁ line to allow identification of specific heterosis traits.

Experiment design and phenotyping

All plant materials were grown at Seoul National University Experimental Farm. Field preparation and crop cultivation were as described previously (Chu *et al.* 2012). Nine traits were measured and scored: culm length (CL, cm), days to heading (DTH, days), panicle number per plant (PN), panicle length (PL, cm), spikelet number per panicle (SN), spikelet fertility (SF, %), TGW (g), grain yield per plant (GYP), and presupposition yield per plant (PYP). Of these, PN, SN, SF, and TGW were regarded as yield-component traits. To remove the effect of hybrid sterility, PYP was estimated and applied where substantial hybrid sterility was observed in BC₁F₁ lines (Chu *et al.* 2012). PYP was calculated if the specific BC₁F₁ line exhibited fertility < 90%. As SF had a proportional effect on GYP, an adjustment for grain yield value was used (Supplementary Fig. S1). The equation used was $PYP = GYP \times 90 / SF$. Phenotyping results of RILs and BC₁F₁s and calculated mid-parent heterosis (MPH) values are

described as the ‘RIL’, ‘BCF1’, and ‘MPH’ datasets, respectively.

Linkage map construction and QTL mapping analysis

Genomic DNA was extracted from 2 cm fresh leaves from seedlings at the three-leaf stage using cetyltrimethylammonium-bromide (CTAB) as previously described (Rogers and Bendich 1988). Across the 12 chromosomes, 162 SSR and subspecies-specific STS markers (Chin *et al.* 2007) that showed polymorphisms between Dasanbyeo and TR22183 were used to construct a linkage map with JoinMap version 3.0 software (Stam 1993). Only markers with LOD scores > 3.0 were used.

MPH values were calculated for each RIL using the following equation, as given in Chu *et al.* (2012): (value of MPH) = (average value of the corresponding BC₁F₁) – (average value of RIL and Dasanbyeo). All datasets were analyzed using QTLmapper version 1.6 for the identification of MQTLs and QTLmapper version 2.0 for EpQTLs (Wang *et al.* 1999). For MQTL analysis, interval mapping using a mixed model approach was applied. For EpQTLs (digenic interactions), only significant two-locus interactions below $P < 0.001$ were used for further analysis.

Raw genotyping and phenotyping data from another set of *indica-japonica* RILs, MT-RILs (Milyang 23 × Tong 88-7) (Chu *et al.* 2012), were re-analyzed using the same software and options to allow comparative analysis with DT-RILs.

Determination and detection of heterosis QTLs

The genetic components of heterosis were suggested to be overdominance, dominance, and epistasis (Li *et al.* 2008). Different types of dominance (overdominance, partial-to-complete dominance, and underdominance) can be determined by estimating additive effect (a) and dominance effect (d) in RIL and MPH datasets, respectively (Supplementary Fig. S1). Theoretically, sum of dominance and additive effects (a + d) estimated from hetero alleles of linked loci can act as another indicator of heterosis QTLs in the BCF1 dataset. The QTLs detected in both the BCF1 and MPH datasets were regarded as candidates for heterosis QTLs. QTLs detected only in RILs and/or the BC₁F₁ population were classified as additive

Table 1. Phenotypic performance and heterosis of traits in parents: F₁s, RILs, and BCF1 populations of DT-RIL.

	DTH ²⁾	CL	PL	PN	SN	SF	TGW	GYP	PYP
P ₁ (Dasanbyeo)	113.0 ± 0.7 a	58.2 ± 2.6 a	24.9 ± 1.1 a	13.4 ± 1.8 a	156.6 ± 10.4 a	82.0 ± 7.5 a	21.8 ± 0.7 a	27.6 ± 2.0 a	30.3 a
P ₂ (TR22183)	88.0 ± 1.0 b	71.4 ± 4.1 b	25.4 ± 1.5 a	6.8 ± 1.1 b	179.3 ± 16.2 b	89.0 ± 7.6 a	22.2 ± 0.7 a	28.7 ± 4.8 a	29.0 a
MP = (P ₁ + P ₂) / 2	100.5 a	64.8 a	25.2 a	10.1 a	168.0 a	85.5 a	22.0 a	28.2 a	29.7 a
F ₁	100.0 ± 1.0 a	81.7 ± 3.3 b	27.4 ± 0.9 b	8.9 ± 2.6 b	236.3 ± 12.6 b	47.3 ± 9.0 b	30.5 ± 0.2 b	33.0 ± 2.3 b	54.1 b
MPH ³⁾ = F ₁ – MP	–0.5	16.9	2.3	–1.2	65.9	–28.0	6.6	5.5	82.5
RILs	101.3 ± 8.3 (88.0–130.0)	69.1 ± 12.0 (44.2–113.8)	24.2 ± 2.8 (18.2–32.3)	10.3 ± 2.4 (6.0–16.6)	175.3 ± 41.8 (68.0–313.8)	80.3 ± 11.1 (37.4–96.8)	24.2 ± 3.1 (16.8–31.8)	27.9 ± 6.8 (15.1–48)	31.8 ± 6.8 (19.2–56.1)
BCF ₁ s (P ₁ × RILs)	106.6 ± 6.1 (97.0–127.0)	80.0 ± 7.2 (65.0–107.9)	25.9 ± 1.6 (22.4–30.3)	9.8 ± 1.6 (7.0–15.4)	201.0 ± 29.3 (94.0–266.8)	81.9 ± 14.8 (24.8–96.0)	27.7 ± 1.6 (23.5–31.8)	37.6 ± 6.2 (16.5–52.3)	41.9 ± 6.0 (30.9–70.0)
BC MPH ³⁾ = BCF ₁ – (RIL + P ₁) / 2	–0.2 ± 6.0 (–18.5–28.5)	16.4 ± 5.2 (–3.3–38.5)	1.3 ± 1.5 (–1.7–5.7)	–2.0 ± 1.3 (–4.7–2.6)	35.0 ± 24.9 (–53.6–91.3)	–0.1 ± 15.1 (–84.7–24.0)	4.7 ± 1.4 (0.2–9.1)	9.5 ± 6.8 (–26.8–26.0)	11.1 ± 5.7 (–1.2–34.9)

²⁾DTH (days to heading, days), CL (culm length, cm), PL (panicle length, cm), PN (panicle number per plant), SN (spikelet number per panicle), SF (fertility, %), TGW (thousand grain weight), GYP (grain yield per plant), PYP (presupposition yield per plant); See the text for the detailed information.

³⁾MPH: mid-parent heterosis; see the main text for the detailed information.

QTLs ('A' type). Dominant QTLs ('D' type) and overdominant QTLs ('OD' type) were determined using the values of d/a and $a + d$. QTLs with $0 < d/a < 1$ or $(a + d) \geq 2d$ were designated as 'D' type QTLs. QTLs with $d/a > 1$, or $2d > a + d$, or those detected only in MPH datasets, were designated as 'OD' type QTLs (Mei *et al.* 2005; Chu *et al.* 2012). QTLs detected in MPH datasets but showing negative 'd' values were defined as underdominance QTLs ('UD' type). Of the detected QTLs, only 'D', 'OD', and 'UD' type QTLs were used for subsequent comparative studies and direct effect analysis.

The allele types that contributed to heterosis in the three heterotic combinations (homo/hetero, hetero/homo, and hetero/hetero) were identified for MQTLs for GYP and PYP. The additive values of yield and yield-related traits (PN, SN, SF, and TGW) of the three heterotic allele types in BC₁F₁ populations were calculated and compared. All statistical analyses were conducted using Minitab version 14.

Comparative mapping study and direct effect analysis

Re-analyzed QTL results and the map of MT-RILs reported by Chu *et al.* (2012) were compared with those of DT-RILs. Primer sequences of linked markers for all the

identified heterosis QTLs were searched using GRAMENE Blast tools (<http://ensembl.gramene.org>, December 15, 2015) to identify their physical positions in the Nipponbare genome. QTLs anchored in those regions were compared with the heterosis QTLs from this study using the QTARO database (<http://qtaro.abr.affrc.go.jp/>).

SPSS-AMOS version 21 was used to estimate the standardized direct and indirect effects of yield-related traits (PN, SN, SF, and TGW). The contribution fractions of MQTLs and EpQTLs for each trait were calculated and analyzed together with the standardized direct effects of the yield-related trait components. For each GYP and PYP QTL in two RILs, the traits with the strongest positive effects were proposed if the standardized direct effect value was > 0.5 .

RESULTS

Trait performance with respect to yield and yield-related traits

Significant differences were observed between Dasanbyeo (P1) and TR22183 (P2) for four traits (DTH,

Fig. 1. Phenotypic distribution of yield and yield-related traits of Dasanbyeo (P1), TR22183 (P2), their respective F₁ values, and their recombinant inbred lines (RILs). (a) Culm length, (b) days to heading, (c) panicle number, (d) panicle length, (e) spikelet number per panicle, (f) spikelet fertility, (g) thousand grain weight, (h) grain yield per plant, and (i) presupposition yield per plant.

CL, PN, and SN), with no significant differences seen for the remaining traits (PL, SF, TGW, GYP, and PYP) (Table 1). TR22183 exhibited earlier heading (88 days after transplanting) than Dasanbyeo but maintained the same productivity. Values of CL, PL, SN, TGW, GYP, and PYP were significantly elevated in F₁ plants, but very low fertility was observed. In DT-RILs, transgressive segregation was observed in CL, PL, SN, SF, TGW, GYP, and PYP. With the exception of PN and SF, the trait value distributions differed between the BC₁F₁ and RIL populations (Fig. 1). The median panicle trait values were smaller for BC₁F₁ than for F₁ plants in SN, PL, TGW, and PYP. After fertility recovery by backcrossing, median SF and GYP values were larger in BC₁F₁ than in F₁ plants.

MQTL identification

A total of 66 QTLs (15 QTLs for yield traits and 51 QTLs for the other traits) were identified in the RIL, BCF₁, or MPH datasets for the traits used in this study (Table 2). Of these, 19 and 14 QTLs were identified in the BCF₁ and MPH datasets, respectively.

Culm length (CL)

Eight QTLs in RIL, four QTLs in BCF₁, and one QTL in MPH explained 81.4%, 36.4%, and 21.6% of total variance, respectively. No common QTLs were identified across all datasets, but two QTLs of BCF₁ (*qCL1.5* and *qCL4.3*) were adjacent to RIL QTLs (*qCL1.4* and *qCL4.2*). One overdominance ('OD' type) QTL, which had the effect of extending CL by 2.11 cm, was detected on chromosome 9 (*qCL9.1*). All other QTLs were classified as additive ('A' type) QTLs.

Days to heading (DTH)

Four QTLs in RIL and one QTL in BCF₁ explained 42.3% and 12.4% of total variance, respectively. No QTLs showing the effects of heterotic alleles were identified.

Panicle number per plant (PN)

Three QTLs in RIL and one QTL in BCF₁ explained 23.5% and 16.3% of total variance, respectively. No heterosis QTLs were identified for this trait.

Panicle length (PL)

Three QTLs in RIL, two QTLs in BCF₁, and two QTLs in MPH were responsible for 59.1%, 16.4%, and 24.8% of total variance, respectively. Two heterosis QTLs were

identified in MPH. One dominance ('D' type) QTL with relatively large additive (36.8% phenotypic variance explained; PVE) and dominance effects (15.4% PVE) was identified in RIL on chromosome 8 (*qPL8.1*). Another overdominance QTL (*qPL4.1*), which had a smaller dominance effect (9.4% PVE) than *qPL8.1*, was identified in MPH.

Spikelet number per panicle (SN)

Five QTLs in RIL, two QTLs in BCF₁, and one QTL in MPH explained 43.9%, 17.9%, and 10.2% of total phenotypic variance, respectively. One overdominance heterosis QTL (*qSN9.1*), which resulted in a 6.2% increase in SN, was identified on chromosome 9 in MPH.

Spikelet fertility (SF)

Two QTLs in RIL and two QTLs in BCF₁ were responsible for 13.7% and 27.6% of total phenotypic variance, respectively. For SF, examination of hybrid sterility in BCF₁ is more meaningful than consideration of heterosis QTL. Two QTLs in BCF₁ (*qSF4.1* and *qSF6.1*) contributed to 7.86% and 19.69% reductions in SF, respectively.

Thousand grain weight (TGW)

Eight QTLs in RIL, two QTLs in BCF₁, and six QTLs in MPH explained 50.4%, 22.4%, and 43.9% of total phenotypic variance, respectively. Three overdominance heterosis QTLs (*qTGW3.3*, *qTGW4.1*, and *qTGW10.1*) and two dominance heterosis QTLs (*qTGW1.1* and *qTGW7.1*) were identified. One underdominance QTL (*qTGW4.2*) with a negative dominance effect (−0.41) was identified on chromosome 4, adjacent to *qSF4.1*. Excepting *qTGW4.2*, the total PVE of the five heterosis QTLs was 41.7%.

Grain yield per plant (GYP)

Three QTLs in RIL, three QTLs in BCF₁, and one QTL in MPH were responsible for 30.7%, 34.4%, and 16.0% of total phenotypic variance, respectively. No heterosis QTLs were identified for GYP in DT-RIL. One identified QTL on chromosome 5 (*qGYP5.1*) showed a negative dominance effect (−2.72%).

Presupposition yield per plant (PYP)

Three QTLs in RIL, two QTLs in BCF₁, and three QTLs in MPH explained 27.3%, 15.1%, and 23.4% of total phenotypic variance. Two QTLs (*qPYP8.1* and *qPYP9.1*) were also QTLs for GYP (*qGYP8.1* and *qGYP9.1*). One

Table 2. Continued 1.

Trait	Name	Chr. ²⁾	Interval	RIL			BCF ₁			MPH				QTL type ^{u)}	d / a	2d / a + d
				LOD ^{y)}	a ^{x)}	R ² (%)	PVE ^{w)} (%)	LOD	a + d	R ² (%)	PVE (%)	LOD	d ^{v)}	R ² (%)	PVE (%)	
SN	<i>qSN1.1</i>	1	RM297-S01140				43.9	2.8	-7.39	6.24	17.9				10.2	A
	<i>qSN1.2</i>	1	S01140-S01143A	7.54	-15.51	13.1										A
	<i>qSN1.3</i>	1	S01167-S01181	4.41	9.84	5.3										A
	<i>qSN4.1</i>	4	S04077A-RM451	4.32	-10.24	5.7										A
	<i>qSN4.2</i>	4	RM451-S04097B					6.28	10.10	11.66						A
	<i>qSN8.1</i>	8	RM25-RM72	9.23	16.41	14.7										A
	<i>qSN9.1</i>	9	S09000A-S09006									2.56	6.38	6.2		OD
	<i>qSN10.1</i>	10	S10071-RM590	6.67	13.13	9.4										A
	<i>qSF1.1</i>	1	S01143A-S01157B	4.39	2.32	4.8	13.7				27.6				No analysis	A
	<i>qSF4.1</i>	4	S04128-RM127					4.98	-4.17	7.86						A
TGW	<i>qSF6.1</i>	6	RM253-S06031					9.14	-6.60	19.69						A
	<i>qSF10.1</i>	10	S10058A-S10071	7.25	3.16	8.9										A
	<i>qTGW1.1</i>	1	RM297-S01140	13.6	0.92	8.8	50.4				22.4	6.12	0.53	13.4	43.9	D
	<i>qTGW1.2</i>	1	S01167-S01181	4.72	-0.56	3.3		5.46	0.44	8.39						A
	<i>qTGW3.1</i>	3	S03002-S03010B	5.42	0.59	3.6										A
	<i>qTGW3.2</i>	3	S03046-S03048	11.11	-1.00	10.5										A
	<i>qTGW3.3</i>	3	S03099-S03115													A
	<i>qTGW4.1</i>	4	S04077A-RM451									2.8	0.47	10.4		OD
	<i>qTGW4.2</i>	4	RM127-S04129B									3.02	0.39	7.2		OD
	<i>qTGW5.1</i>	5	S05004A-S05009	4.66	-0.59	3.7						3.44	-0.41	7.9		UD
GYP	<i>qTGW5.2</i>	5	S05080-S05101	13.63	0.99	10.3		7.89	-0.57	13.96						A
	<i>qTGW7.1</i>	7	RM248-RM172	7.13	0.69	4.9										A
	<i>qTGW10.1</i>	10	RM258-S10058A									2.41	0.30	4.3		D
	<i>qTGW12.1</i>	12	RM4A-S12011B	11.14	0.91	8.7						3.02	0.36	6.4		OD
	<i>qGYP5.1</i>	5	S05014B-RM267				30.7				34.4	7.74	-2.72	16.0	16	UD
	<i>qGYP6.1</i>	6	RM584-RM204					2.56	-1.63	6.67						A
	<i>qGYP8.1</i>	8	RM25-RM72	20.8	3.18	22.4										A
	<i>qGYP9.1</i>	9	S09073-S09075A	8.21	1.71	6.5										A
	<i>qGYP10.1</i>	10	S10019-S10026C					6.71	-2.88	20.67						A
	<i>qGYP11.1</i>	11	S11078-RM229	2.17	-0.90	1.8										A
	<i>qGYP11.2</i>	11	RM21-S11117					2.29	-1.68	7.06						A

Table 2. Continued 2.

Trait	Name	Chr. ²⁾	Interval	RIL			BCF ₁			MPH			QTL type ^{u)}	d / a	2d / a + d
				LOD ^{y)}	a ^{x)}	R ² (%)	PVE ^{w)} (%)	LOD	a + d	R ² (%)	PVE (%)	d ^{v)}			
PYP	<i>qPYP1.1</i>	1	S01140-S01143A				27.3						OD		
	<i>qPYP2.1</i>	2	S02026-RM71	2.92	1.47	4.5							A		
	<i>qPYP7.1</i>	7	RM234-S07101										OD		
	<i>qPYP8.1</i>	8	RM25-RM72	9.44	2.84	16.7							A		
	<i>qPYP8.2</i>	8	S08090-S08106										OD		
	<i>qPYP8.3</i>	8	S08106-S08107										A		
	<i>qPYP9.1</i>	9	S09075A-S09093A	5.27	1.72	6.1		4.18	1.69	7.96			A		
	<i>qPYP11.1</i>	11	S11055A-S11064					2.48	1.60	7.14			A		

²⁾Chr: chromosome. ^{y)}LOD: logarithm of odds numbers. ^{x)}a: additive effect. ^{w)}PVE: phenotypic variance explained. ^{v)}d: dominant effect. ^{u)}A: additive QTL, D: dominant QTL, OD: overdominant QTL, UD: underdominant QTL. See the main text for the details.

QTL for GYP on chromosome 11 (*qGYP11.1*), which showed a negative additive effect (−0.9), was not detected in the PYP dataset, as expected. One QTL on chromosome 2 (*qPYP2.1*) was not found for GYP. Three overdominance heterosis QTLs were identified on chromosomes 1, 7, and 8. The removal of hybrid sterility effects allowed possible QTLs for GYP to be identified.

The QTLs identified only in the BCF₁ population were not classified as heterosis QTLs in this study. However, many of these QTLs showed positive values of combined additive and dominant effects: all four QTLs for CL; one QTL for DTH; one QTL for PN; one of two QTLs for PL (*qPL9.1*); one of two QTLs for SN (*qSN4.2*); one of two QTLs for TGW (*qTGW1.2*), and both QTLs for PYP.

Many of the QTLs were clustered within specific regions on chromosome 1 (RM297-S01143A), chromosome 4 (S04120-S04129B), and chromosome 8 (RM25-RM72) (Fig. 2, left chromosome shown).

EpQTL identification

When all traits were considered, 23 EpQTLs in RIL, 15 EpQTLs in BCF₁, and two EpQTLs in MPH were identified in this study (Table 3). The total PVE of EpQTLs for each trait was 10.7-27.5% for RIL, 11.2-26.8% for BCF₁, and 7.9-22.0% for MPH. No EpQTLs were identified for SN and PYP in RIL or for PN and TGW in BCF₁.

For grain yield traits, different EpQTLs for GYP were identified in RIL and BCF₁. Only one EpQTL, between chromosome 1 (S01143A-S01157B, *qSF1.1* of RIL) and chromosome 8 (RM72-S08052B, *qGYP8.1* of RIL), contributed positively to higher grain yield (1.75 g/plant). For PYP, one EpQTL between chromosome 3 (S03010B-S03020) and chromosome 10 (S10071-RM590, *qSN10.1* of RIL) had positive additive effects.

One EpQTL for PL and one for SN were identified in the MPH dataset. No MQTLs were identified within the chromosomal regions linked with EpQTLs for PL. However, for SN, *qPL12.1* in RIL interacted with the region on chromosome 4.

Trait contribution to heterosis QTLs for grain yield

First, to identify the yield-component traits contributing

Fig. 2. Comparative linkage maps of DT-RILs (left, DT) and MT-RILs (right, MT) for the 12 rice chromosomes. Vertical gray bars represent chromosomes. Black dots on each chromosome represent the estimated position of the centromere. QTLs for each trait identified in this study are located next to the linked markers on each chromosome. The legend box below the chromosomes indicates the symbols corresponding to each trait. Vertical dotted lines divide the QTL groups identified in different populations (RILs, BCF₁, and MPH). QTLs with black symbols were identified in MPH, gray in BCF₁, and blank in RILs. Upward or downward arrows next to QTLs designate the QTLs as overdominant or underdominant, respectively. Dotted lines connect the same markers used in the two RILs.

Table 3. EpQTLs of DT-RIL and BCF1 populations and MPH datasets.

	Trait ⁽²⁾	Chr.	Interval i	Chr.	Interval j	LOD	AI ^(y)	Aj	AAij	H ² (Ai)	H ² (Aj)	H ² (AAij)	PVE ⁽³⁾ (%)
RIL	CL	3	S03002-S03010B	9	S09000A-S09006	9.92	1.33**		2.48	0.007		0.026	15.8
		4	S04048-S04058	4	S04097B-RM348	4.79			2.34			0.023	
		5	S05036-RM289	12	RM235-RM17	7.12			2.96			0.037	
		7	RM336-S07076	11	S11028-S11045	10.96	1.9***		2.14	0.015		0.019	
	DTH	7	RM248-RM172	9	S09065-S09073	11.51	1.03*		-3.57	0.004		0.054	
		8	S08107-S080120	10	S10071-RM590	6.42			2.95			0.107	10.7
	PN	6	S06035-RM5963	8	S08075-S08090	7.21			-0.78			0.096	17.1
		8	S08052B-S08066	8	S08090-S08106	4.8			-0.72			0.075	
	PL	1	S01167-S01181	9	S09065-S09073	5.61			0.67			0.033	16.8
		3	S03099-S03115	10	S10058A-S10071	4.66			0.62			0.028	
	SF	5	S05009-S05014B	6	RM469-RM225	5.08			0.63			0.029	
		6	S06074-RM30	7	RM336-S07076	8.63		0.78***	-0.76		0.045	0.043	
		7	RM346-S07084	8	S08107-S080120	6.02	0.87*		-0.69	0.056		0.035	
		1	RM488-RM246	2	S02085-RM318	5.21			3.38			0.076	27.5
	TGW	6	RM469-RM225	9	S09075A-S09093A	9.75			4.58			0.140	
		8	RM25-RM72	8	S08090-S08106	7.37	1.91**		2.99	0.024		0.059	
		2	RM71-RM438	2	S02085-RM318	7.07	-0.5***		-0.56	0.021		0.023	25.8
		3	S03120-S03130	8	S08090-S08106	11.5			1.02			0.085	
BCF ₁	GYP	6	RM3183-S06074	8	S08075-S08090	10.25			0.89			0.065	
		8	RM25-RM72	10	S10026C-RM258	13.43	0.37**	0.99***	0.61	0.011	0.080	0.031	
		11	S11028-S11045	12	S12091-RM270	7.42			-0.81			0.054	
		5	S05054-S05064	8	RM25-RM72	16.63		2.96***	1.52		0.155	0.041	15.1
	CL	6	RM527-RM3183	11	RM229-RM21	8.15			2.49			0.110	
		1	S01157B-S01160	12	RM277-S12091	8.45	-1.93***		-1.88	0.048		0.046	11.2
	DTH	5	S05009-S05014B	9	S09000A-S09006	8.27		-2.47***	2.27		0.079	0.067	
		2	RM475-S02085	11	S11018-RM332	4.77	-1*		2.01	0.017		0.068	13.7
	PL	6	S06053-RM527	11	RM229-RM21	6.98		-1.99***	2.04		0.066	0.070	
		1	RM128-RM297	8	RM25-RM72	5.46	0.44***		0.53	0.055		0.080	22.8
	SN	3	S03099-S03115	4	RM348-S04120	6.53			-0.72			0.148	
		1	S01143A-S01157B	8	S08052B-S08066	5.43			10.93			0.148	14.8
	SF	1	RM128-RM297	11	RM21-S11117	4.62	3.32**	3.04*	-5.62	0.021	0.017	0.059	14.7
		1	RM297-S01140	11	RM21-S11117	5.92	4.47***	3.58**	-6.01	0.037	0.024	0.068	
		4	S04128-RM127	11	S11045-S11055A	5.95	4.05***		-3.25	0.031		0.020	

Table 3. Continued.

Trait ^{a)}	Chr.	Interval i	Chr.	Interval j	LOD	Ai ^{b)}	Aj	AAij	H ² (Ai)	H ² (Aj)	H ² (AAij)	PVE ^{c)} (%)
GYP	1	S01143A-S01157B	8	RM72-S08052B	4.75	-0.84*		1.75	0.013		0.056	26.8
	2	RM475-S02085	3	S03120-S03130	5.46	1.22**		-2.22	0.027		0.089	
	5	S05036-RM289	6	RM469-RM225	4.77			-2.6			0.123	
PYP	3	S03010B-S03020	10	S10071-RM590	4.79			2			0.114	21.3
	4	RM348-S04120	9	S09062B-S09065	4.87			-1.86			0.099	
MPH	1	RM128-RM297	7	S07024-S07038	4.8			0.39			0.079	7.9
SN	4	S04048-S04058	12	S12091-RM270	5.62			12.94			0.220	22.0

^{a)}See Table 1 for the trait abbreviations.

^{b)}Ai and Aj are the main effects of locus i and locus j. AAij is the epistatic effect between loci i and j, as defined by Mei *et al.* (2005).

Significance level at * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

^{c)}Percentage of the total variation explained by AAij. Significant at $P \leq 0.001$.

substantially to the three overdominance heterosis QTLs and one underdominance QTL for grain yield in DT-RIL, the additive effects of each yield-component trait on the QTLs were presented in radar charts (Fig. 3). Interval mapping through backcrossing was used to identify QTLs between markers. There were three types of heterotic allele combination for each marker (AiAi/AiAj, AiAj/AiAi, or AiAj/AiAj), and the additive effects of each allele type were calculated by dividing the value of the corresponding allele type with that of the AiAi/AiAi (homo) allele genotype. Additive effects identified in this study were in the -15-15% range (Table 4).

For *qPYP1.1*, the AiAi/AiAj (S01140/S01143A) genotypes did not contribute to heterosis. However, AiAj/AiAi genotypes had a significantly larger PN, and significantly or highly significantly smaller SN, SF, and GYP, than homoallele genotypes. Likewise, AiAj/AiAj genotypes had significantly higher PN and PYP values than homoallele genotypes. This suggested that the PN of AiAj/AiAj genotypes might be the major factor explaining the significant heterotic effect on PYP.

For *qPYP7.1*, AiAi/AiAj (RM234/S07101) genotypes showed negative additive effects on PYP. The AiAj/AiAi and AiAj/AiAj genotypes had significantly higher PYP values than homoallele genotypes. The additive effect of AiAj/AiAi on SN might explain the significantly positive additive effect of PYP. Although a significant positive additive effect of SF was found for AiAi/AiAj, the PYP values of the genotypes were negative, thus demonstrating that SF alone could not be a factor of heterosis.

For *qPYP8.2*, AiAi/AiAj (S08090/S08106) and AiAj/AiAj showed significant additive effects for PYP, which may have been caused by low hybrid fertility. AiAj/AiAj genotypes showed a positive effect of SF, but PYP did not show additive effects.

Unlike the PYP cases, no heterosis QTLs were identified in GYP. However, one underdominance GYP QTL (*qGYP5.1*) was analyzed to see which allele types were contributory. Only AiAj/AiAj (S05014B/RM267) showed a significant negative effect on SF and GYP. Two other heterotic allele genotypes of linked markers (AiAj/AiAi and AiAi/AiAj) contributed to significantly higher SF and GYP values. This result indicates the importance of

Fig. 3. Phenotypic additive performance (%) of three different heterotic allele types on yield heterosis QTLs identified in DT-RILs. The radial charts for each yield heterosis QTL show two yield (GYP and PYP) and four yield-related traits (PN, SN, SF, and TGW). Blue (AiAi/AiAj), orange (AiAj/AiAi), and gray (AiAj/AiAj) lines indicate the additive value in percentage over the value AiAi/AiAi (Dasanbyeon) type (dotted closed hexagonal line). Asterisks represent the statistical significance level (***) for $P < 0.001$; ** for $0.001 < P < 0.01$; and * for $0.01 < P < 0.05$. The upward/downward arrows indicate the significant additive values of the corresponding heterotic allele types.

studying different heterotic allele combinations of linked markers for underdominance QTLs.

Second, the direct effects of each trait on grain yield were analyzed using the path analysis module of SPSS-AMOS software (Table 5). Only DTH, PN, SN, SF, and TGW were considered as yield-related traits for this analysis, because CL was a vegetative trait and PL was highly correlated with SN. In each heterosis QTL for grain yield, the standardized direct effects of each trait > 0.5 or < -0.5 were considered as positive effect QTLs. As expected from the radial chart analysis, PN and SN were the most important traits explaining heterosis of *qPYP1.1*. Likewise, PN, SN, and TGW variously contributed to *qPYP7.1* for each allele combination. *qPYP8.2* was related to PN and SN for the AiAj/AiAi type only. PN, SN, and SF showed direct effects on GYP via the contribution of the AiAj/AiAi and AiAi/AiAj genotypes at *qGYP5.1*. SF was a negatively effective trait in all heterosis QTLs for PYP.

DISCUSSION

Heterosis QTLs of intra- and inter-locus interactions

Heterosis is governed by various genetic effects including overdominance effects, dominance effects, additive effects, and their interactions. In this study, *indica-japonica* RILs were used to identify heterosis QTLs for grain yield and its related traits. Theoretically, heterotic effects (inter-locus heterosis) should be explained solely by the genetic interactions between different recombinant blocks originating from different parents. However, two-QTL interactions (or digenic interactions) are difficult for plant breeders to utilize due to their complexity and the difficulties in dealing with the large numbers of samples in breeding programs. Accessing heterosis through intra-locus interactions between different alleles (dominance and overdominance) may thus be more amenable. Intra- and inter-locus heterosis can be assessed using BC₁F₁ populations, which were used to study overdominance and dominance effects in this study. Inter-locus heterogeneity is more prevalent in RIL rather than BC₁F₁ populations,

Table 4. Percent additive effect of each yield-related trait to its parent haplotype (AiAi) and yield traits in BCF1 of DT-RIL.

QTL	Marker	Haplotype	n	PN		SN		SF		TGW		GYP		PYP	
				A ²⁾ (%)	P ³⁾ (T < t)	A (%)	P (T < t)	A (%)	P (T < t)	A (%)	P (T < t)	A (%)	P (T < t)	A (%)	P (T < t)
<i>qPYP1.1</i>	S01140	AiAi	9	-2.34	0.170	-1.24	0.358	-1.20	0.394	-0.89	0.368	-1.52	0.391	-1.32	0.463
		AiAj	12	12.13	0.021	-11.75	0.046	-8.75	0.048	0.92	0.180	-8.44	0.004	0.41	0.459
		AiAj	50	9.69	0.001	-2.13	0.188	-8.87	0.008	0.78	0.220	-0.63	0.430	8.39	0.002
<i>qGYP5.1</i>	S05014B	AiAi	22	4.24	0.170	-5.95	0.022	6.65	0.000	1.49	0.145	6.27	0.043	-0.97	0.386
		AiAj	9	5.08	0.193	2.97	0.277	5.76	0.032	-0.04	0.493	9.32	0.005	3.82	0.207
		AiAj	46	2.59	0.195	-0.20	0.471	-11.18	0.004	2.16	0.013	-9.55	0.004	2.69	0.177
<i>qPYP7.1</i>	RM234	AiAi	11	5.04	0.197	-4.87	0.092	6.17	0.013	-1.01	0.352	3.51	0.180	-2.90	0.180
		AiAj	10	4.62	0.199	8.82	0.042	-6.38	0.165	2.12	0.233	2.59	0.338	10.10	0.029
		AiAj	72	0.16	0.476	3.24	0.098	-4.57	0.069	-0.37	0.324	0.65	0.413	6.17	0.006
<i>qPYP8.2</i>	S08090	AiAi	20	5.29	0.056	1.56	0.349	-7.72	0.068	0.31	0.405	-0.17	0.483	11.92	0.011
		AiAj	35	2.95	0.202	-1.99	0.270	1.91	0.163	0.18	0.440	1.53	0.281	-0.15	0.475
		AiAj	57	4.49	0.078	0.03	0.496	-9.38	0.004	-1.12	0.147	-3.65	0.145	7.70	0.002

²⁾Percent additive effect of the corresponding haplotype to its parent haplotype (AiAi). ³⁾Probability of significance assuming $H_0 = 0$ by Student's t-test.

and estimation of intra-locus heterosis may be improved using BCF1 and MPH datasets. The heterotic effects on inter-locus and intra-locus interactions governing grain yield may not be identified at the same loci in different populations. For example, two EpQTLs for GYP in RIL were unidentified in the BCF1 and MPH datasets (Tables 2 and 3). On the other hand, two EpQTLs for GYP and two EpQTLs for PYP in MT-RILs (RILs derived from Milyang 23 and Tong 88-7; Chu *et al.* 2012) were not identified in heterosis MQTL analysis using BCF1 and MPH datasets in DT-RILs (Supplementary Tables S1 and S2).

In DT-RIL, there were nine heterosis MQTLs for all traits and one underdominance heterosis MQTL for GYP (Table 2). EpQTLs for heterosis are rarely detected if the population size is small, because the effects of multi-locus allele combinations require a large number of samples and meaningful statistical analysis for detection. Thus, the subsequent heterosis QTL analyses in this study were conducted using only MQTL results. Nevertheless, there were clearly no EpQTLs for GYP or PYP found using MPH datasets in DT-RIL. Conversely, in MPH datasets of MT-RIL, three overdominance MQTLs for GYP and two for PYP were identified (Supplementary Table S1).

Common heterosis QTL clusters in two *indica-japonica* RILs indicating relatedness of heterosis for yield and for yield-related traits

MQTLs for different traits were co-located at some chromosomal regions (Fig. 2), and several QTL clusters were observed in MT-RILs, on chromosome 1 (S01143A-S01157B), chromosome 4 (RM348-S04128), chromosome 6 (S06015-S06019B, RM50-RM527, and S06087-S06100), and chromosome 7 (S07035-S07055B). In both RILs, QTL clusters included QTLs identified in RIL, BCF1, and MPH datasets for several yield-related traits. Thus, an additive effect of preferred alleles in RILs would be expected in marker-assisted breeding programs that utilized the heterosis QTLs in the QTL clusters. This may explain the plant growth vigor seen in recombinant plants generated through introgression of donor QTLs in some generations of breeding programs.

Heterosis QTLs for each trait were compared between the two RILs (Supplementary Table S3), and no common

Table 5. Standardized direct effect of yield-related traits to yield in DT-RILs.

QTL	Allele type	DTH ²⁾	PN	SN	SF	TGW	Most positively effective trait (>0.5 or <-0.5)
<i>qPYP1.1</i>	AiAi/AiAi	0.083	0.512	0.413	-0.436	0.108	PN
	AiAi/AiAj	-0.287	0.241	0.242	-0.686	0.149	(SF) ^a
	AiAj/AiAi	-0.345	0.048	0.548	-0.499	0.124	SN
	AiAj/AiAj	0.114	0.523	0.505	-0.542	0.191	PN and SN
<i>qPYP7.1</i>	AiAi/AiAi	-0.059	0.506	0.681	-0.275	0.14	PN and SN
	AiAi/AiAj	0.324	1.633	1.051	0.258	0.538	PN, SN and TGW
	AiAj/AiAi	-0.046	0.357	0.631	-0.272	0.047	SN
	AiAj/AiAj	-0.005	0.551	0.281	-0.68	0.191	PN, (SF)
<i>qPYP8.2</i>	AiAi/AiAi	-0.225	0.719	0.574	-0.058	0.212	PN and SN
	AiAi/AiAj	-0.333	0.219	0.246	-0.828	0.131	(SF)
	AiAj/AiAi	0.126	0.852	0.831	-0.068	0.146	PN and SN
	AiAj/AiAj	0.289	0.486	0.317	-0.5	0.168	(SF)
<i>qGYP5.1</i>	AiAi/AiAi	0.03	0.592	0.51	0.682	0.161	PN, SN and SF
	AiAi/AiAj	0.24	0.759	0.266	0.199	0.256	PN
	AiAj/AiAi	-0.264	0.842	1.042	0.623	0.252	PN, SN and SF
	AiAj/AiAj	-0.074	0.331	0.344	0.961	0.095	SF

²⁾DTH: days to heading, PN: panicle number per plant, SN: spikelet number per panicle, SF: fertility, TGW: thousand grain weight.

heterosis QTLs were found. However, the QTL clusters may represent common loci for heterosis (Fig. 2). QTL clusters in DT-RIL (RM297-S01143A) and MT-RIL (S01143A-S01157B) were at adjacent regions on chromosome 1, and might share common genetic elements for heterosis. This was also observed for S04120-S04129B (CL, SF, and TGW) in DT-RIL and RM348-S04128 (PL, GYP, and SN) in MT-RIL on chromosome 4. Three QTL regions linked to grain yield traits are close in comparative linkage maps and should be studied further to see whether common regions for heterosis are shared between the RILs (Fig. 2): RM584-RM204 in DT-RIL and S06015-S06018 in MT-RIL on chromosome 6, RM253-S06031 (SF in DT-RIL) and RM50-RM527 (PYP in MT-RIL) on chromosome 6, and RM21-S11117 (GYP in DT-RIL) and S11112-S11117B (PYP in MT-RIL) on chromosome 11.

Previously reported QTLs that are linked to the identified heterosis QTLs in DT and MT-RILs and are linked to a range of agronomic traits are listed in Supplementary Table S4. QTL locations were compared between the physical map locations of the markers in this study and the linked markers from various mapping populations. In DT- and MT-RILs, *qTGW4.2*, *qGYP8.1*, *qSN8.1*, and

qCL9.1/qSN9.1 were not previously reported as yield or yield-related QTLs. With the exception of these, all the QTL regions in this study were previously linked to yield or yield-related traits. For example, *qSN1.1* of MT-RIL was co-located with *gpla* in F₂ (grains per panicle, Shen *et al.* 2003), *qPNI* in BC₄F₄ (panicles per plant, Tian *et al.* 2006), and others. Thus, some previously identified QTLs linked to different traits may be co-located with or the same as QTLs identified in this study. This may explain the significant positive correlations observed between some yield traits. For example, with respect to the linked QTLs given above, grain number (*gpla*) is correlated with spikelet number (*qSN1.1*) and panicle number (*qPNI.1*).

Previous studies of RILs rarely discussed whether these genes/QTLs exhibited heterosis. Some previously reported QTLs/genes were co-located with the heterosis QTLs identified in this study, and further analysis is needed to determine whether heterosis is seen in heterotic alleles. For DT-RIL, these were as follows: *qTGW3.3* (*gw3a*, grain weight, Yu *et al.* 1997); *qGYP5.1* (*S31*, hybrid sterility, Li *et al.* 2005; *qSF5.2*, hybrid sterility, Reflinur *et al.* 2012); *qPYP7.1* (*qPLL7*, pollen fertility, Yan *et al.* 2003; *hd7a*, heading date, Yu *et al.* 2002); *qTGW7.1* (*S23*, hybrid

sterility, Sobrizal *et al.* 2000); *qPL8.1* (RH8, heading date, Li *et al.* 2016; *qPL8*, *Hd5*, heading date, Lin *et al.* 2003; PL, Miyata *et al.* 2007); and *qPYP8.2* (*WFP*, panicle branching and yield, Miura *et al.* 2010). For MT-RIL, these were *qCL1.2* (*sd1*, plant height, Li *et al.* 2003); *qGYP6.1* (*DPL2*, sterility, Mizuta *et al.* 2010; *qSPTF6*, SF, Yan *et al.* 2003); *qPYP6.1* (*spf6*, SF, Song *et al.* 2005; *S5*, SF, Yan *et al.* 2000); *qCL7.2* (*Hd4*, DTH, Lin *et al.* 2003); and *qPYP11.1* (*FON3*, floral organ number, Jiang *et al.* 2007). These QTLs/genes were reported in various population types as well as in backcrossed populations, implying that QTLs showing additive effects in homozygous alleles may give heterotic effects.

Comparative analysis indicated that the negative additive values of some QTLs of DT- and MT-RILs could be partially explained by hybrid sterility QTLs/genes. *qGYP5.1*, *qGYP6.1*, and *qGYP10.1* were co-located with known sterility QTLs (*qSF5.2* and *S31*) and QTLs for pollen fertility (Doi *et al.* 1998; Tan *et al.* 1998). However, there were no known hybrid sterility QTLs/genes for *qTGW4.2*, *qSN8.1*, *qTGW9.1*, and *qGYP10.1*. Conversely, *qPYP6.1* in MT-RIL did not show negative additive values, indicating a lack of allele-induced hybrid sterility between Milyang 23 and Tong 88-7.

Tao *et al.* (2016) also reported some heterosis QTLs from testcrossed CSSLs (Chromosome Segment Substitution Lines). The QTL hotspots on chromosomes 1, 6, and 8 identified in this study were also identified by Tao *et al.* (2016).

Three heterotic allele genotypes on heterosis QTLs of MT-RILs

For QTL determination, the effects of three heterotic allele combinations (AiAi/AiAj, AiAj/AiAi, and AiAj/AjAj) were combined, hampering the identification of the allele types that had the most substantial impact on phenotypic values (Fig. 3 and Supplementary Fig. S2).

The range of additive values was larger for MT-RILs than for DT-RILs, indicating that the effect of MQTLs was larger for MT-RILs than for DT-RILs. Five QTLs for GYP and two QTLs for PYP were investigated in this study (Supplementary Fig. S2 and Supplementary Table S5). For *qGYP1.2* (RM259/RM600), the heterotic allele of RM259

contributed to the additive effect of SF and heterosis of GYP. The heterotic alleles linked to both the markers contributed to the increase of PN. A QTL for panicle number (*ppp1.1*) was reported on *qGYP1.2* (Cho *et al.* 2007). This was not significant ($P = 0.051$), but a significant positive effect was seen for heterotic allele type for SF contribution to *qGYP5.1*. On *qPYP6.1* (S06015/S06018), all three heterotic allele types exhibited similar contributions to heterosis of PYP. SN was the most important contributory trait for PYP increase, though SF was significantly low for all three types. A QTL for SN was identified on *qPYP6.1* (Zhuang *et al.* 2001). At *qGYP8.1* (S08059/S08066), heterotic allele effects of S08059 and S08066 gave contrasting effects for SF and GYP. S08059 heterotic allele type positively impacted the additive effect of GYP. On *qPYP11.1* (S11112/S11117B), the AiAi/AiAj and AiAj/AiAj types showed significantly low SF but high PYP. The presupposition yield calculation was influenced by S11117B heterotic allele type. *FON3* (Jiang *et al.* 2007) reported a QTL for floral organ numbers in the F₂ population on the same region. However, it was unclear which trait contributed to the PYP increase of *qPYP11.1* in this population. Low SF on *qGYP6.1* (RM50/RM527) was impacted by RM527 heterotic allele type, giving significant positive additive effects of PYP and significant negative effects of GYP. Likewise, *qGYP10.1* (S10019/S10026C) also showed an underdominance heterosis type.

Direct effect analysis for MQTLs and EpQTLs

It is desirable to identify the yield-related traits or yield-component traits that are most influential on yield. In this study, to simplify the complicated mediation networks, only the standardized direct effect of each yield-related trait on PYP and GYP was estimated by path analysis (Table 5).

The direct effect of each trait was estimated for each different allele type. One homoallele type (AiAi/AiAi) in RIL and three heterotic allele types (AiAi/AiAj, AiAj/AiAi, and AiAj/AjAj) in BCF₁ populations were analyzed separately. PN and SN were the yield-related traits with most direct impact on heterosis QTLs in DT-RILs. For *qPYP1.1*, the strongest trait, with an estimated direct effect >0.5, was SN, particularly for

AiAj/AiAi and AiAj/AiAj types (62 of 179 RILs; 34.6% (Table 4). However, for the same locus, PN was the most effective trait for *qPYP1.1* with respect to the homoallele (AiAi/AiAi) and complete heterotic allele (AiAj/AiAj) types. The same approach could be applied to other heterosis yield QTLs. Different combinations of yield-related traits provided direct effects for each *qPYP7.1* allele type. Only the AiAj/AiAi allele type of *qPYP8.2* was linked with PN and SN in BCF1. Conversely, for *qGYP5.1*, only the AiAi/AiAj type was directly impacted by PN, without the negative effect of SF.

A similar approach was used for MT-RILs (Supplementary Table S6). Unlike with DT-RILs, the positive direct effect on SF was apparent across many QTLs. A direct negative effect of SF was found only for *qPYP6.1* in all allele types. *qGYP1.2*, *qGYP5.1*, and *qGYP10.1* all positively impacted SF. Conversely, SN had the most positive effect in AiAj/AiAj of *qGYP1.2*, AiAj/AiAj of *qPYP6.1*, AiAj/AiAi of *qGYP6.1*, and AiAj/AiAj of *qGYP10.1*. TGW was influenced by AiAi/AiAj of *qPYP6.1* and AiAj/AiAi of *qGYP6.1*. PN and DTH directly affected *qPYP6.1* in partial heterotic allele types (AiAi/AiAj or AiAj/AiAi).

In brief, the standardized direct effects of different

heterotic allele types on yield-related traits differed between two *indica-japonica* RILs. Heterosis effects were identified using the accumulated additive effects of PN, SN, SF, and TGW (Dan *et al.* 2015). For each QTL, the yield-related trait with the most positive impact could be identified by this analysis. Followed by the development of near-isogenic lines (NILs) for each QTL, the most important yield-related traits could next be studied to estimate the consequent yield heterosis.

The contribution of MQTLs and EpQTLs to heterosis can be estimated by the sum of total PVEs for the identified QTLs. Fig. 4 shows the total effects of PVE of each yield-related trait (center), PYP (outer sides), and GYP (inner sides) connected by arrows representing the direct effects. The total PVEs of yield-related traits were larger in DT-RILs than in the BCF1 population. However, total PVEs of GYP and PYP were larger in the BCF1 population than in RILs. In the DT-BCF1 population, contributions of EpQTLs for GYP (61.2%) and PYP (36.4%) were large, and consequently, the heritability (total PVE) of yield heterosis was larger than GYP (45.8%) and PYP (27.3%) in RILs. This differed for MT-RILs (Supplementary Fig. S3), where heritability of yield heterosis was smaller than in RILs. This implies that the contribution of the dominance

Fig. 4. Contribution of MQTL and EpQTL for each trait to yield in DT-RILs. Pie chart size for each trait represents the sum of total PVE of MQTL (dark gray) and EpQTL (light gray) identified in this study. Five yield-related traits were connected by arrows with numbers (standardized direct effect) to GYP (inner side) and PYP (outer side). The direct effects of each trait were shown in two populations: RIL (left) and BCF1 (right).

effect was larger than that of epistasis between Dasanbyeo (*indica*) and TR22183 (*japonica*).

Suggestions for plant breeding strategies utilizing heterosis QTLs

Heterosis QTLs identified by current software provide average values for different allele types of heterotic alleles. The additive values of different heterotic allele combinations could be calculated from the identified QTLs to identify the allele type that contributed to the preferable yield heterosis. Hybrid sterility, which is common in *indica-japonica* crosses in rice, hampers the interpretation of heterotic effects of yield traits in specific heterotic allele types at those loci. The breakdown of allele types may help resolve this.

The heterosis QTLs identified in the two RILs in this study mostly coincided with previously reported yield and yield-related traits. This similarity was also noted when specifically expressed genes of F₁ plants were compared to the linkage map of Nipponbare/93-11 (Venu *et al.* 2014). This also showed that heterosis could be explained by certain specific yield-related traits associated with specific loci. These loci might be detected as MQTLs in conventional QTL studies.

The identified EpQTLs explained the presence of epistatic interactions between loci for heterosis. To utilize EpQTLs for plant breeding, marker-assisted recurrent selection or genomic selection strategies would be more effective rather than the simple marker-assisted backcrossing strategies used for MQTLs.

Common heterosis QTLs identified across different populations and studies would be highly valuable for heterosis breeding programs; however, further quantitation of the complex genetic networks for each yield-related trait is needed to design breeding programs with high predictability.

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