

Mining Applicable Elite Alleles of Growing Duration, Plant Height and Panicle Number per Plant by Conditional QTL Mapping in Japonica Rice

JIANG Jian-hua¹, ZHAO Qi-bing², LIU Qiang-ming¹, CHEN Lan¹, CHEN Fu-long², QIAO Bao-jian², HONG De-lin¹

¹State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China;

²Fengshou Pure and Breed Corporation Limited of Jiangsu Province, Sihong County, Sihong 223905, China)

Abstract: Unconditional QTL mapping and conditional QTL mapping were conducted for growing duration (GD), plant height (PH) and effective panicle number per plant (PN) using a recombinant inbred line (RIL) population derived from a cross between Xiushui 79 and C Bao in japonica rice (*Oryza sativa* L.). The RIL population consisted of 254 lines was planted in two environments, Nanjing and Sihong, Jiangsu Province, China. Results showed that additive effects were major in all of QTLs for GD, PH and PN detected by the two methods, and the epistatic effects explained a small percent of phenotypic variation. No interactions were detected between additive QTL and environment, and between epistatic QTL pairs and environment. After growth duration was adjusted to an identical level, RM80-160bp was detected as an applicable elite allele for PN, with an additive effect of 0.71. After effective panicle number per plant was adjusted to an identical level, RM448-240bp was detected as an applicable elite allele for GD (shorten), with an additive effect of 4.64. After plant height was adjusted to an identical level, RM80-160bp was detected as an applicable elite allele for PN, with an additive effect of 0.62, and RM448-240bp was detected as an applicable elite allele for GD, with an additive effect of 3.89. These applicable elite alleles could be used to improve target traits without influencing the other two traits.

Key words: japonica rice; growth duration; plant height; effective panicle number per plant; unconditional QTL mapping; conditional QTL mapping; recombinant inbred line

After more than 40 years' studies on hybrid japonica rice in China, the area of hybrids planted annually is less than 6% of the total area of japonica rice with only 828 000 hm² (Deng et al, 2006; Tang et al, 2008). There are two critical reasons why the development of hybrid japonica rice is slow. One is that the adaptive ability of a single hybrid is narrow (Tang et al, 2008), and the other is that the competition heterosis of hybrids is not conspicuous compared with standard pure lines, due to less panicle number per plant (Li and Hong, 2004, 2005; Jin et al, 2005), despite of the larger panicle and more spikelets per panicle (Hua et al, 2007) in hybrids. In general, more spikelets per panicle was negatively correlated with more panicles per plant (Xu et al, 2005), and grain yield per unit area was positively correlated with growth duration (Su et al, 2003). DNA molecular basis of the complicated relationship has been analyzed by large number of QTL mapping research (Li et al, 1995, 2000; Lin et al, 1998; Maheswaran et al, 2000; Cao et al, 2001; Xing et al, 2001; Kennard et al,

2002; Yuan et al, 2003). However, most of these studies did not consider the influence of other traits on the QTL detecting of the target trait. Therefore, the elite alleles identified by above studies may not be applicable to genetic improvement, especially elite alleles of target trait detected in genetic separation populations derived from indica-japonica crosses. It is difficult to get results expected when these elite alleles were introduced into the materials to be improved, because of the influence of the loci for plant height (PH) and growth duration (GD). To mine applicable elite alleles for adaptive ability and panicle number per plant, unconditional QTL mapping and conditional QTL mapping for the traits of growth duration, plant height and effective panicle number per plant were conducted in two growth environments by using a recombinant inbred line (RIL) population.

MATERIALS AND METHODS

Rice materials

The population used in this study consisted of 254 RILs (F_{11:12}) was derived from a cross between two

japonica rice cultivars Xiushui 79 (japonica variety) and C Bao (japonica restorer line) by single-seed descend. The female parent, Xiushui 79, was bred by Jiaxing Institute of Agricultural Sciences, Zhejiang Province, China, and the male parent C Bao, was bred by Anhui Academy of Agricultural Sciences, Anhui Province, China.

Field experiments

In 2009, the RILs and the two parents were grown in paddy fields at the Jiangpu Experiment Station, Nanjing Agricultural University (E1 environment) and Foundation Seed Production Farm in Sihong County in Jiangsu Province (E2 environment), China, respectively. The E1 environment, located in the northern bank of the Yangtz River (118°35' E and 32°05' N). The soil in the E1 environment is very sticky when it is wet and becomes very hard when it is dried, belonging to the soil type of paste and heavy-bodied in classification. During the rice-growing season (from May to October), rainfall is sufficient, and drainage and irrigation are convenient in the experiment paddy field. The E2 environment is in northern of the Huaihe River (118°15' E and 33°30' N). The soil in the E2 environment belongs to silt soil type, and the capacity of retention water is poor. During the rice growing season, sunshine is sufficient. The germinated seeds were sown in a seedling bed on 8 May, 2009 in E1 and 10 May, 2009 in E2, respectively. Seedlings of 35 days old were transplanted to a paddy field, with a single plant per hill. In each environment, parents and RILs were planted in two replications and each line was planted in three rows, each having 7? seedlings per hill?, in a density of 17 cm × 25 cm. Regular field management was carried out.

Phenotypic measurements

Growth duration (GD), plant height (PH) and effective panicle number per plant (PN) were recorded in five central plants in the second lines. GD means the days from sowing to heading. The date when the first panicle emerged 1 cm from the flag leaf collar in the selected individual plant was scored as the heading date of an individual. PH was the distance between the soil surface and the tip of the highest panicle (exclude awns). A panicle with at least five filled grains was

recorded as a productive panicle. The trait mean of five sampled plants from each plot was used in the data analysis, and the means of the two replications were used for QTL analysis.

Genetic linkage map construction

For the RIL population, a genetic map consisting of 91 SSR markers on 19 linkage groups was constructed in our previous report (Guo and Hong, 2010). The genetic map was 969 cM long, with an average distance of 10.6 cM between adjacent markers.

Statistical analysis

The phenotypic values for GD, PH and PN of the k -th RIL line in environment h were partitioned according to the following mixed linear model:

$$y_{hk} = \mu + a_i x_{A_{ik}} + a_j x_{A_{jk}} + aa_{ij} x_{AA_{ijk}} + u_{E_h} e_{E_h} + u_{A_i E_h} e_{A_i E_h} + u_{A_j E_h} e_{A_j E_h} + \sum_{f(h)} u_{M_{f(h)}} e_{M_{f(h)}} + \sum_{l(h)} u_{MM_{l(h)}} e_{MM_{l(h)}} + \varepsilon_{hk} \quad (1)$$

Where μ is the population mean; a_i and a_j are the additive main effects (fixed effects) of the two putative Q_i and Q_j , respectively; aa_{ij} is the additive × additive epistatic effect (fixed effect) between Q_i and Q_j ; $x_{A_{ik}}$, $x_{A_{jk}}$ and $x_{AA_{ijk}}$ are the coefficients of these genetic main effects; e_{E_h} is the random effect of environment h , with coefficient u_{E_h} ; $e_{A_i E_h}$ (or $e_{A_j E_h}$) is the random additive × environment interaction effect, with coefficient $u_{A_i E_h}$ (or $u_{A_j E_h}$) for Q_i (or Q_j); $e_{AA_{ij} E_h}$ is the random epistasis × environment interaction effect, with coefficient $e_{AA_{ij} E_h}$; $e_{M_{f(h)}}$ is the random effect of marker f nested within the h -th environment, with coefficient $u_{M_{f(h)}}$; $e_{MM_{l(h)}}$ is the random effect of the l -th marker × marker interaction nested within the h -th environment, with coefficient $u_{MM_{l(h)}}$; and ε_{hk} is the random residual effect. Marker factors $e_{M_{f(h)}}$ and $e_{MM_{f(h)}}$ in the model were used to absorb additive and epistatic effects of background QTLs for controlling noise.

Adjusted genetic effects were estimated by conditional mapping method of GD, PH and PN (Zhu, 1995; Ye et al, 2009). As with the Eq.(1), the conditional value $y_{hk(T_1|T_2)}$ could be partitioned as:

$$\begin{aligned}
 y_{hk(T_1|T_2)} = & \mu_{(T_1|T_2)} + a_{i(T_1|T_2)}x_{A_{ik}} + a_{j(T_1|T_2)}x_{A_{jk}} \\
 & + a_{ij(T_1|T_2)}x_{AA_{ijk}} + \mu_{E_{hk}}e_{E_h(T_1|T_2)} + \mu_{A_iE_{hk}}e_{A_iE_h(T_1|T_2)} \\
 & + \mu_{AA_jE_{hk}}e_{AA_jE_h(T_1|T_2)} + \sum_{f(h)} u_{M_{fk(h)}}e_{M_{f(h)}(T_1|T_2)} \\
 & + \sum_{l(h)} u_{MM_{lk(h)}}e_{MM_{l(h)}(T_1|T_2)} + \varepsilon_{hk(T_1|T_2)}
 \end{aligned}
 \tag{2}$$

where T1|T2 denotes trait 1 conditioned on trait 2, which meant eliminating the phenotypic variation of trait 2. For example, PH|GD means the performance of PH when GD was adjusted to an identical level, or PN|PH means the performance of PN when PH was adjusted to an identical level. All parameters were defined as conditional effects as in the Eq.(1). Such as, $a_{i(T_1|T_2)}$ was the adjusted additive main effect of putative Q_i .

The trait measurement averaged over the two replications each environment was used for QTL mapping. Unconditional QTL mapping (namely conventional QTL mapping) was conducted for GD, PH and PN by the method of mixed liner model and computer program QTLNetwork2.0 (Yang et al, 2007). The remaining two traits were conducted by a conditional QTL mapping method when one of the traits was adjusted to an identical level. A threshold probability of $P=0.005$ was used to detect additive QTLs, additive \times additive QTL pairs and QTL \times environment (Q \times E) interactions. QTL nomenclature followed that of McCouch (1997).

Applicable elite alleles mean elite alleles were detected by unconditional and conditional QTL mapping methods at the same time, and accounted for 7% or more of the phenotypic variability.

RESULTS

Trait performance

The heading date of Xiushui 79 was 20 August in the two environments, and that of C Bao was 21 and

22 August in the E1 and E2 environments, respectively. The heading dates of the RIL population ranged from 4 August to 19 September in the E1 environment and 8 August to 19 September in the E2 environment, respectively. Both PH and PN showed significant differences between parents in the two environments. The plant height of Xiushui 79 in the E1 environment was higher than that in the E2 environment, and was similar for C Bao. Meanwhile, the plant height of C Bao was higher than that of Xiushui 79, which was consistence across two environments. The effective panicle number per plant of Xiushui 79 was more than that of C Bao across two environments, and the difference between environments was not significant. The phenotypic values of GD, PH and PN of the RIL population and its parents over two environments are presented in Table 1. GD, PH and PN of the RIL population segregated continuously and both skewness and kurtosis values were less than 1.0 except kurtosis values of GD in the E2 environment. The segregation of GD, PH and PN in the RIL population showed a normal distribution and transgressive segregations were observed (Table 1). Therefore, the data were suitable for QTL analysis.

Unconditional QTL analysis of GD, PH and PN

A total of 14 additive QTLs for GD, PH and PN were identified by the unconditional QTL mapping method in the two environments. Five QTLs were for GD, five for PH and four for PN, respectively. QTL \times environment interactions were not significant for additive QTLs (Table 2). Eight pairs of additive \times additive QTL interaction were detected for the three traits totally, including three interactions between main effect loci, four interactions between two loci having no main effect and one interaction between main effect loci and no main effect loci. QTL \times environment interactions were not significant for additive \times additive

Table 1. Phenotypic values of growth duration (GD), plant height (PH) and effective panicle number per plant (PN) for parents and RIL population across two environments.

Environment	Trait	Parent		Mean \pm SD	RIL population		
		Xiushui 79	C Bao		Range	Skewness	Kurtosis
Nanjing (E1)	GH (d)	108.0	109.0	108.9 \pm 9.3	86.0–132.0	0.13	-0.55
	PH (cm)	96.6	101.3	108.4 \pm 20.3	51.4–144.9	-0.42	-0.85
	PN	11.4	8.8	10.9 \pm 2.3	6.3–19.5	0.34	0.86
Sihong (E2)	GH (d)	108.0	110.0	113.2 \pm 10.8	90.0–135.0	0.10	-1.17
	PH (cm)	86.1	90.3	99.5 \pm 19.1	48.1–133.0	-0.43	-0.80
	PN	11.5	7.6	10.9 \pm 2.4	6.4–22.3	0.50	0.95

Table 2. Additive QTLs for three traits detected in RIL population derived from Xiushui 79 and C Bao.

Trait	QTL	Marker interval ^a	Position (cM) ^b	LOD value	A ^c	P	H _a ² (%)
GD	<i>qGD-2-1</i>	RM1358 –RM145	3.0	11.25	-1.92	<0.0001	5.67
	<i>qGD-3-1</i>	RM7097– RM448	10.7	29.27	-4.43	<0.0001	11.79
	<i>qGD-8-1</i>	RM22899 –RM22957	3.5	11.02	-2.38	<0.0001	6.17
	<i>qGD-10-1</i>	RM5629– RM171	0.3	8.78	-1.54	<0.0001	1.12
	<i>qGD-11-1</i>	RM7120– RM287	0.1	9.55	-1.60	<0.0001	1.93
GD PH	<i>qGD-2-2</i>	RM5356– RM1358	2.6	12.34	-1.92	<0.0001	4.88
	<i>qGD-3-1</i>	RM7097– RM448	11.7	21.56	-3.89	<0.0001	10.03
	<i>qGD-9-1</i>	RM5652 –RM410	0.0	8.34	1.77	<0.0001	2.29
GD PN	<i>qGD-10-1</i>	RM5629– RM171	0.3	13.27	-1.58	<0.0001	1.52
	<i>qGD-2-1</i>	RM1358 –RM145	4.0	11.37	-1.86	<0.0001	6.27
	<i>qGD-3-1</i>	RM7097– RM448	11.7	26.39	-4.64	<0.0001	11.86
	<i>qGD-6-1</i>	RM162 –RM5753	0.0	8.04	1.82	<0.0001	4.08
	<i>qGD-8-1</i>	RM22899 –RM22957	3.0	9.35	-1.86	<0.0001	6.29
PH	<i>qGD-10-1</i>	RM5629– RM171	0.3	10.77	-1.41	<0.0001	1.04
	<i>qGD-11-1</i>	RM7120– RM287	1.1	8.09	-1.91	<0.0001	2.00
	<i>qPH-1-1</i>	RM3453– RM490	4.0	37.56	7.64	<0.0001	19.15
	<i>qPH-3-1</i>	RM7097– RM448	6.7	8.31	-3.56	<0.0001	1.34
	<i>qPH-8-1</i>	RM22899 –RM22957	1.0	10.11	-3.31	<0.0001	6.87
PH GD	<i>qPH-8-2</i>	RM80 – RM281	0.0	8.77	4.23	<0.0001	7.31
	<i>qPH-9-1</i>	RM257 –OSR28	3.0	51.83	-7.97	<0.0001	16.71
	<i>qPH-1-1</i>	RM3453– RM490	5.0	39.40	7.93	<0.0001	19.77
	<i>qPH-8-2</i>	RM80 –RM281	1.0	14.96	3.52	<0.0001	9.23
	<i>qPH-9-2</i>	RM6570 –RM5652	9.0	65.76	-10.26	<0.0001	15.35
PH PN	<i>qPH-1-1</i>	RM3453– RM490	5.0	38.49	8.43	<0.0001	18.91
	<i>qPH-3-1</i>	RM7097– RM448	8.7	9.37	-3.35	<0.0001	1.51
	<i>qPH-8-1</i>	RM22899 –RM22957	0.0	9.65	-2.01	0.0011	7.22
PN	<i>qPH-9-1</i>	RM257 –OSR28	2.0	62.10	-8.06	<0.0001	17.84
	<i>qPN-1-1</i>	RM495 –RM84	4.0	10.10	0.46	<0.0001	3.75
	<i>qPN-2-1</i>	RM145 –RM1313	11.0	7.71	-0.83	<0.0001	5.05
PN GD	<i>qPN-8-1</i>	RM1235 –RM331	18.0	8.15	0.44	0.002	1.21
	<i>qPN-8-2</i>	RM80 –RM281	4.0	12.96	-0.71	<0.0001	9.04
	<i>qPN-1-1</i>	RM495 –RM84	4.0	10.87	0.45	<0.0001	3.98
	<i>qPN-2-1</i>	RM145 –RM1313	10.0	9.47	-0.87	<0.0001	5.55
	<i>qPN-8-2</i>	RM80 –RM281	4.0	14.27	-0.71	<0.0001	9.17
PN PH	<i>qPN-8-3</i>	RM152 –RM1235	0.0	7.67	0.29	0.0009	1.79
	<i>qPN-1-1</i>	RM495 –RM84	4.0	10.48	0.45	<0.0001	3.90
	<i>qPN-2-1</i>	RM145 –RM1313	9.0	8.70	-0.70	<0.0001	5.59
	<i>qPN-8-2</i>	RM80 –RM281	4.0	10.75	-0.62	<0.0001	7.42
	<i>qPN-9-1</i>	RM6570 –RM5652	9.0	10.58	-0.55	<0.0001	7.55

^a Bold letters indicate the nearest marker; ^b Distance from the nearest marker to putative QTL; ^c '+' and '-' mean that the positive alleles come from Xiushui 79 and C Bao, respectively.

GD, Growth duration; PH, Plant height; PN, Effective panicle number per plant; GD|PH, Growth duration after plant height was adjusted to an identical level; GD|PN, Growth duration after effective panicle number per plant was adjusted to an identical level; PH|GD, Plant height after growth duration was adjusted to an identical level; PH|PN, Plant height after effective panicle number per plant was adjusted to an identical level; PN|GD, Effective panicle number per plant after growth duration was adjusted to an identical level; PN|PH, Effective panicle number per plant after plant height was adjusted to an identical level. The same for table 3.

QTL pairs (Table 3).

Among the five additive QTLs detected for GD, *qGD-2-1*, *qGD-3-1*, *qGD-8-1*, *qGD-10-1* and *qGD-11-1* explained 5.67%, 11.79%, 6.17%, 1.12% and 1.93% of the phenotypic variation. The alleles from C Bao at these five loci showed positive effects (Table 2). One pair of additive × additive QTL interaction between main effect loci and one interaction between two loci having no main effect were detected for GD, accounting for 4.09% and 5.75% of the phenotypic variation in the population, respectively (Table 3). Both of the two interactions had positive effects on GD.

Among the five additive QTLs detected for PH, *qPH-1-1* and *qPH-8-2* explained 19.15% and 7.31% of the phenotypic variation, respectively. The alleles from Xiushui 79 at these two loci showed positive effects. *qPH-3-1*, *qPH-8-1* and *qPH-9-1* explained 1.34%, 6.87% and 16.71% of the phenotypic variation, respectively. The alleles from C Bao at these three loci showed positive effects. One pair of additive × additive QTL interaction between main effect loci and no main effect loci and two interactions between two loci having no main effect were detected for PH, accounting for 9.58% of the total phenotypic variation

Table 3. Additive×additive interactive effects on the three traits detected in RIL populations derived from Xiushui 79 and C Bao.

Trait	QTL <i>i</i>			QTL <i>j</i>			LOD value	AA ^c	P	Haa ² (%)
	Locus	Marker interval ^a	Position (cM) ^b	Locus	Marker interval ^a	Position (cM) ^b				
GD	<i>qGD-2-1</i>	RM1358 –RM145	3.0	<i>qGD-10-1</i>	RM5629– RM171	0.3		-2.11	<0.0001	4.09
	6-3	RM454– RM162	4.2	10-1	RM5095 –RM311	11.0	21.33	-6.54	<0.0001	5.75
GD PH	<i>qGD-2-2</i>	RM5356– RM1358	2.6	<i>qGD-10-1</i>	RM5629– RM171	0.3		-2.02	<0.0001	4.84
	2-5	RM1313– RM327	6.4	<i>qGD-6-1</i>	RM162– RM5753	13.9	16.47	2.75	<0.0001	3.72
	2-8	RM262 –RM525	11.0	12-4	RM5479– RM1227	0.5	18.67	3.01	<0.0001	6.09
	<i>qGD-3-1</i>	RM7097– RM448	11.7	<i>qGD-9-1</i>	RM5652 –RM410	0.0		-2.33	<0.0001	2.24
	<i>qGD-6-1</i>	RM162– RM5753	13.9	10-1	RM5095 –RM311	8.0	12.29	-2.92	0.0001	3.92
GD PN	<i>qGD-2-1</i>	RM1358 –RM145	4.0	<i>qGD-10-1</i>	RM5629– RM171	0.3		-2.12	<0.0001	4.12
	5-4	RM305 –RM161	0.0	10-1	RM5095 –RM311	1.0	10.85	-1.89	<0.0001	3.36
	<i>qGD-6-1</i>	RM162– RM5753	18.9	10-1	RM5095 – RM311	13.9	20.38	-6.13	<0.0001	5.32
	<i>qGD-8-1</i>	RM22899 –RM22957	3.0	<i>qGD-11-1</i>	RM7120– RM287	1.1		-1.23	0.0033	0.98
PH	1-2	RM84– RM1003	0.8	2-1	RM7288 –RM5356	0.0	14.46	3.89	<0.0001	3.73
	6-5	RM5753– RM345	0.6	7-3	RM180 –RM214	6.0	11.13	-4.20	<0.0001	2.80
	<i>qPH-9-1</i>	RM257 –OSR28	3.0	11-1	RM3133 –RM7120	1.0	8.05	2.83	<0.0001	3.05
PH GD	1-2	RM84– RM1003	0.0	2-1	RM7288 –RM5356	2.0	11.41	3.63	<0.0001	4.62
	6-5	RM5753– RM345	0.6	12-4	RM5479– RM1227	0.5	20.11	3.08	<0.0001	2.39
	6-5	RM5753– RM345	0.6	7-3	RM180 –RM214	10.0	12.58	-4.11	<0.0001	3.39
	8-11	RM6948– RM433	0.1	<i>qPH-9-1</i>	RM257 –OSR28	3.0	9.44	-3.98	<0.0001	2.40
	9-5	RM410– RM257	0.8	11-1	RM3133 –RM7120	0.0	14.48	3.43	<0.0001	6.38
PH PN	1-3	RM1003 –RM3456	0.0	2-1	RM7288 –RM5356	0.0	8.86	2.79	<0.0001	3.34
	6-5	RM5753– RM345	0.6	7-2	RM125– RM180	8.8	9.52	-4.06	<0.0001	3.08
	8-11	RM6948–RM433	3.0	11-1	RM3133 –RM7120	1.0	8.41	-3.32	<0.0001	2.99
	<i>qPH-9-1</i>	RM257 –OSR28	2.0	11-1	RM3133 –RM7120	2.0	10.87	3.52	<0.0001	3.48
PN	<i>qPN-1-1</i>	RM495 –RM84	4.0	<i>qPN-8-2</i>	RM80 –RM281	4.0		-0.27	0.0083	1.18
	<i>qPN-2-1</i>	RM145 –RM1313	11.0	<i>qPN-8-2</i>	RM80 –RM281	4.0		0.50	0.0003	1.76
	3-8	RM7097 –RM448	0.0	6-5	RM5753– RM345	0.6	10.77	-0.53	<0.0001	4.28
PN GD	<i>qPN-1-1</i>	RM495 –RM84	4.0	<i>qPN-8-2</i>	RM80 –RM281	4.0		-0.21	0.0395	0.74
	<i>qPN-2-1</i>	RM145 –RM1313	11.0	<i>qPN-8-2</i>	RM80 –RM281	4.0		0.56	<0.0001	2.40
	3-8	RM7097 –RM448	0.0	6-5	RM5753– RM345	0.6	11.43	-0.53	<0.0001	4.18
	<i>qPN-1-1</i>	RM495 –RM84	4.0	<i>qPN-8-2</i>	RM80 –RM281	4.0		-0.25	0.0144	0.95
PN PH	<i>qPN-2-1</i>	RM145 –RM1313	9.0	<i>qPN-8-2</i>	RM80 –RM281	4.0		0.45	0.0006	1.55
	3-8	RM7097 –RM448	0.0	6-5	RM5753 –RM345	1.0	11.20	-0.48	<0.0001	3.47

^a Bold letters indicate the nearest marker; ^b Distance from the nearest marker to putative QTL; ^c ‘+’ and ‘-’ mean additive × additive interactions had positive and negative effects on traits, respectively.

in the population. One interaction (6-5/7-3) had positive effects on PH, while the remaining two interactions had negative effects on PH.

Among the four additive QTLs detected for PN, *qPN-1-1* and *qPN-8-1* explained 3.75% and 1.21% of the phenotypic variation, respectively. The alleles from Xiushui 79 at these two loci showed positive effects. *qPN-2-1* and *qPN-8-2* explained 5.05% and 9.04% of the phenotypic variation, respectively. The alleles from C Bao at these two loci showed positive effects. Two pairs of additive × additive QTL interaction between main effect loci and one interaction between two loci having no main effect were detected for PN, accounting for 7.22% of the total phenotypic variation in the population. One main effect loci interaction (*qPN-2-1/qPN-8-2*) had positive effects on PH, while the other two interactions had negative effects on PN

Conditional QTL analysis of GD, PH and PN

Four additive QTLs for PN were detected when

GD was adjusted to an identical level (Table 2). And *qPN-1-1*, *qPN-2-1* and *qPN-8-2* were detected by the unconditional mapping method also. This indicated that differential expression of alleles at these three loci was not influenced by GD. In other words, if we substituted the allele linked to RM495-130bp for the allele linked to RM495-145bp from C Bao on rice chromosome 1, it can increase 0.45 of PN, and the GD of substituted material was not affected. Similarly, RM145-180bp and RM80-160bp were also elite alleles for increasing PN. The conditional QTL-*qPN-8-3* was undetected by the unconditional QTL mapping method, indicating differential expression of the alleles at this locus was influenced by GD. Meanwhile, the unconditional QTL-*qPN-8-1* was undetected by the conditional QTL mapping method, indicating that *qPN-8-1* was a false positive locus, the discrepancy of PN might due to the difference of GD. Two elite alleles of RM490-110bp and RM80-180bp for PH were detected when GD was

adjusted to an identical level. And if the allele linked to RM490-105bp were substituted by the allele linked to RM490-110bp, the plant height would increase 7.93 cm.

Four additive QTLs for GD were detected when PH was adjusted to an identical level (Table 2). And *qGD-3-1* and *qGD-10-1* were detected by the unconditional mapping method also. This indicated that the differential expression of alleles at these two loci was not influenced by PH. This meant that if the allele linked to RM448-245bp was substituted by the allele linked to RM448-240bp, the growth duration would shorten 3.89 d, and if RM171-155bp were substituted by RM171-150bp, the growth duration would shorten 1.58 d, without affecting the PH of the improved material. When PH was adjusted to an identical level, three elite alleles, i.e. RM495-130bp, RM145-180bp and RM80-160bp, for PN were detected. And if we substituted the allele linked to RM80-180bp for the allele linked to RM80-160bp, panicle number per plant would increase 0.62.

Six additive QTLs for GD were detected when PN was adjusted to an identical level (Table 2). And *qGD-2-1*, *qGD-3-1*, *qGD-8-1*, *qGD-10-1* and *qGD-11-1* were detected by the unconditional mapping method also. This meant that the differential expressions of alleles at these five loci were not influenced by PN. The five elite alleles at the five loci were RM1358-190bp, RM448-240bp, RM22899-160bp, RM171-150bp and RM287-150bp, respectively. If the allele linked to RM448-245bp were substituted by the allele linked to RM448-240bp, the growth duration would shorten 4.64 d. After PN was adjusted to an identical level, four additive QTLs were detected for PH. The elite alleles of the four loci were RM490-110b, RM448-245bp, RM22899-175bp and RM257-150bp, respectively. And if we substituted the allele linked to RM490-105bp and RM257-160bp for the allele linked to RM490-110bp and RM257-150bp, respectively, the plant height would increase 8.43 cm and 8.06 cm, respectively.

QTL×environment interactions were not significant for all additive loci mentioned above.

Twenty pairs of additive × additive QTL interactions were detected by the conditional mapping method, explained 0.74%–6.38% of the phenotypic variation.

Among the 20 pairs of additive × additive QTL interactions, 6 interactions occurred between main effect loci, 10 interactions between two loci having no main effect and 4 interactions between main effect loci and no main effect loci (Table 3). Interactions between additive × additive QTL pairs and environment were not significant.

For GD trait, eight pairs of additive × additive QTL interactions were detected among the 20 pairs of interactions. Out of the 8 interactions, only one interaction between *qGD-2-1* and *qGD-10-1* was detected by both unconditional mapping method and conditional method that PN was adjusted to an identical level. Interaction between *qGD-2-1* and *qGD-10-1* will prolong 2.12 d of GD. Nine pairs of additive × additive QTL interactions were detected for PH. Among them, 5 pairs of additive × additive QTL interactions were detected when GD was adjusted to an identical level. Two pairs of interactions between *l-2* and *2-1*, and between *6-5* and *7-3* were also detected by the unconditional mapping method. The interaction between *l-2* and *2-1* would reduce 3.63 cm of PH, whereas the interaction between *6-5* and *7-3* would increase 4.11 cm of PH. In the event of PN was adjusted to an identical level, 4 pairs of additive × additive QTL interactions were detected for PH, only one pair of interaction (between *qPN-9-1* and *11-1*) was also detected by the unconditional mapping method. The interaction will reduce 3.52 cm of PH. Three pairs of additive × additive QTL interactions were detected for PN. These three interaction pairs were all detected when GD or PH were adjusted to an identical level. And they were also detected by the unconditional mapping method. This meant that these interaction pairs for PN were not affected by GD or PH. Among the three interactions, the interactions between *qPN-1-1* and *qPN-8-2* and between *3-8* and *6-5* could reduce 0.25 and 0.48 of PN, respectively. And the interaction between *qPN-2-1* and *qPN-8-2* could increase 0.45 of PN.

DISCUSSION

QTL mapping for GD, PH and PN were conducted by using unconditional and conditional QTL mapping methods in japonica rice in this study.

Results showed that these loci or pairs for the three traits existed genetically independently and were influenced by the other two traits as well. Additive effects were principal in all of the QTLs for GD, PH and PN in japonica rice, and epistatic effects explained a small percent of phenotypic variation. No interactions were detected between additive QTL (epistatic QTL pairs) and environment. Loci or pairs detected only by the unconditional QTL mapping method might be false positive.

Growth duration is a major constraint for wide adaptive ability of a single japonica hybrid rice. Hybrids with high-yielding potential in southern Jiangsu could not mature safely when planted in middle and northern Jiangsu province in China. High-yielding hybrids in northern Jiangsu could be planted in middle and southern Jiangsu and matured naturally, but the yield per unit area decreased and heterosis disappeared due to shortened growth duration. According to the results obtained by the unconditional QTL mapping method and conditional QTL mapping method, we found that if the allele linked to RM448-240bp on chromosome 3 were pyramided with the allele linked to RM171-150bp on chromosome 10, growth duration would shorten 5.5 d, and PN and PH of the improved material were not influenced. If alleles linked to RM1358-190bp, RM448-240bp, RM22899-160bp and RM287-150bp were pyramided, growth duration would shorten 10 d, and PN of the improved material was not influenced. Similarly, if the allele linked to RM1358-190bp on chromosome 2 were substituted by the allele linked to RM171-150bp on chromosome 10, growth duration would shorten 10 d. Meanwhile, if the allele linked to RM171-150bp were pyramided with the allele linked to RM1358-190bp into a single plant, the growth duration would prolong two more days due to the interaction between these two loci.

It is reported that there exist significant negative correlations between PN and PH, and between PN and GD (Cui et al, 2004). In this study, however, by using the unconditional QTL mapping and conditional QTL mapping methods, some of loci for PN were correlated with GD and PH, and most of loci for PN inherited independently, without affecting GD and PH. Meanwhile, all pairs of additive \times additive interactions

between loci detected in this study were independent of the other two traits. Three elite alleles for PN, i.e. RM495-145bp, RM145-180bp and RM80-160bp, were detected when GD was adjusted to an identical level. So we can cumulate the allele linked to RM495-145bp on chromosome 1 and the allele linked to RM145-180bp on chromosome 2, and make PN increase by 1.3. Also, the GD was not influenced by this accumulation. The panicle number per plant would increase 2 if we accumulate the allele linked to RM145-180bp on chromosome 2 and the allele linked to RM80-160bp on chromosome 8, due to the interaction between these two loci. However, the three elite alleles cannot cumulate simultaneously because the accumulation of the allele linked to RM495-145bp and the allele linked to RM80-160bp will decrease 0.25 of PN. Similarly, the three elite alleles designated as RM495-130bp, RM145-180bp and RM80-160bp for PN were detected when PH was adjusted to an identical level. If we cumulate the allele linked to RM145-180bp and the allele linked to RM80-160bp, the panicle number per plant would increase by 2, and GD and PH were not affected by the accumulation.

ACKNOWLEDGEMENTS

This study was supported by the Program of National High Technology Research and Development, Ministry of Science and Technology, China (Grant No. 2010AA101301), the Program of Introducing Talents of Discipline to University in China (Grant No. B08025), the Program of Introducing International Advanced Agricultural Science and Technology in China (Grant No. 2006-G8 [4]-31-1) and the Program of Science-Technology Basis and Conditional Platform in China (Grant No. 505005).

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