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Identification and Validation of Quantitative Trait Loci for Grain Number in Rice (*Oryza sativa* L.)

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Abstract: Grains number is one of the most important agronomic traits in the determination of rice productivity. To explore the underlying genetic basis of grain number in rice, quantitative trait locus (QTL) analysis was performed using three recombinant inbred line populations derived from *indica* rice crosses of Teqing/IRBB lines, Zhenshan 97/Milyang 46, and Xieqingzao/Milyang 46, respectively. A total of 58 QTLs distributed on all 12 rice chromosomes were identified, including 22 for number of grains per panicle (NGP), 17 for number of spikelets per panicle, and 19 for spikelet fertility. The individual QTL counted for 1.5 to 22.1% of phenotypic variation. Among them, 15 QTLs shared by two or three populations and eight QTLs showed large effects with R^2 larger than 10%. Furthermore, three QTLs with minor effects for NGP, *qNGP5.5*, *qNGP9.1*, and *qNGP12.1*, were detected and validated by eliminating the segregation of major-effect QTL using four residual heterozygote-derived populations. These results not only enrich our understanding of the mechanism of grain number, but also provide a foundation for cloning and selecting candidate for marker-assisted selection breeding in rice.

Keywords: rice; grain number; QTL; minor effect; residual heterozygote

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, playing a key role in meeting the demand of food for a growing global population. During the last 60 years, grain yield has progressively increased in Yangtze River basin, the main *indica* rice-producing area in China, and such an increase is mainly attributed to the expanded sink size as a result of more number of spikelets per panicle (NSP), especially for the case of super rice [1]. Yang et al. analyzed the data of national rice regional trial in southern China from 1986 to 2002 and found that number of grains per panicle (NGP) played an important role in improving both the yield and rice quality based on suited number of effective panicles and grain weight [2]. Therefore, it is essential to understand the molecular mechanism of grain number in rice.

All three yield components—panicle number, grain number, and grain weight—are typical quantitative traits that are controlled by polygenes referred to as quantitative trait loci (QTLs) [3]. QTL mapping is an efficient strategy to dissect the molecular mechanisms of rice yield traits such as NGP [4]. Along with molecular marker technology development, hundreds of QTLs distributed on rice 12 chromosomes had been identified for NGP and NSP (www.gramene.org/archive/QTL data). Most of them were detected in different mapping populations such as F_2, doubled haploid lines, and recombinant inbred lines (RILs) [5–10]. Several dozens of QTLs for NGP and NSP have

been fine-mapped [11–17]. So far, eight QTLs have been cloned. *Gn1a* and *NOG1* regulate grain numbers [18,19]. *DEP1*, *IPA1*/*WFP*, *APO1*, and *GNP1* control panicle architecture and meristems [20–25]. *NAL1* mainly controls panicle size and plant architecture [26,27]. *Ghd7* presents large pleiotropic effect on NGP, heading date and plant height [28,29]. These findings have greatly promoted the dissection of genetic bases of rice grain number.

However, these studies mainly focused on major-effect QTLs, few studies investigated minor-effect QTLs. Recently, studies have shown that QTLs with minor effect also play a role in the regulation of important agronomic traits in rice [30]. Hence, identification of minor-effect QTLs would enrich our knowledge of genetic and molecular network regulating rice grain number. Usually, minor-effect QTLs are more sensitive to genetic background and environment than major-effect QTLs [31,32]. Identification of minor-effect QTLs would be facilitated by eliminating the effect of major-effect QTLs. The use of residual heterozygote (RH) to construct near-isogenic lines (NILs) or secondary segregation populations is an effective strategy for identification and verification of minor-effect QTLs [33,34]. Based on this approach, several QTLs for yield-related trait have been fine-mapped [35,36].

In the present study, QTL analysis for NGP, NSP, and spikelet fertility (SF) was performed using three RIL populations. Three minor-effect QTLs on chromosome 5, 9 and 12 were identified using one RH-derived population fixing the major-effect QTLs and further validated in the new secondary segregation population under more homozygous genetic background, respectively.

2. Materials and Methods

2.1. Plant Materials

A total of seven populations developed from crossing of *indica* rice were used. For primary mapping, the previously developed three RIL populations were already used to analyze grain quality and yield-related traits, including Teqing/IRBB lines (TI), Zhenshan 97/Milyang 46 (ZM) and Xieqingzao/Milyang 46 (XM), respectively [10,37–41]. Teqing, Zhenshan 97 and Xieqingzao are female parents, while IRBB lines and Milyang 46 are male parents. All of the parental lines have been widely used in commercial breeding and production of three-line hybrid rice in China, among which Zhenshan 97 and Xieqingzao are maintainer lines and used as early-season varieties, others are restorer lines and used as middle-season rice varieties in the middle-lower reaches of Yangtze River basin. For TI, phenotypic data of 203 lines in 2009 and 2010 and genetic maps with 127 markers, spanning 1198 cM were used to identify QTLs for yield heterosis [38]. In this study, extra two years' phenotypic data were added, and genetic linkage map had been updated to comprise 135 markers spanning 1345 cM [42]. For ZM, phenotypic data of 243 lines in 1999 and 2000 and genetic map with 158 makers, spanning 1288 cM were used to detect QTLs for yield traits [10]. In this work, phenotypic data for two more years were added. Genetic linkage map had been updated to consist of 256 markers and span 1815 cM [39]. For XM, phenotypic data of 209 lines was not used in any QTL mapping for NGP, NSP, and SF. A genetic map consisting of 240 markers and spanning 2080 cM [39], and phenotypic data of three years were used in this study.

For further mapping and validation, four RH-derived populations were used, and they were derived from one RH of the cross TQ/IRBB52 as described below and illustrated in Figure 1. One F_7 plant carrying 13 heterozygous segments distributed on 10 chromosomes was selected and selfed to produce S_1 population consisting of 251 individuals, which was named Ti52-2. QTLs were determined from data generated from the $S_{1:2}$ and $S_{1:4}$ families of Ti52-2. QTLs for heading date had been detected using Ti52-2 in previous study [42].

Three RHs were selected from Ti52-2 covering *qNGP5.5*, *qNGP9.1* and *qNGP12.1*, respectively. The first one carried three heterozygous segments, which were RM18927−RM274 on chromosome 5, RM20731 on chromosome 6 and pTA248−RM5926 on chromosome 11. The second one included four heterozygous segments, which were RM12210 on chromosome 1, RM23662−RM1896 on chromosome 9, RM6704−RM7300 on chromosome 10 and RM1233−RM5926 on chromosome 11. The last one consisted

of two heterozygous segments, which were RM16252−RM335 on chromosome 4 and RM3246−RM511 on chromosome 12. They were selfed to produce three S_1 populations designated ZC5, ZC9 and ZC12 consisting of 216, 203 and 241 plants, respectively.

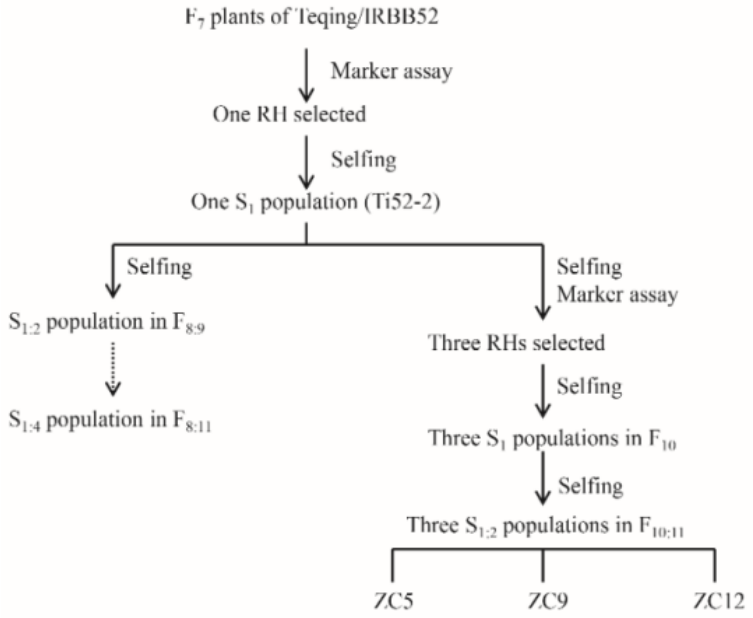

Figure 1. Construction of the rice populations used in this study. RH, residual heterozygote.

2.2. Field Trials and Phenotypic Evaluation

All the populations were grown from May to September in the paddy fields at the China National Rice Research Institute in Hangzhou, Zhejiang, China. A randomized complete block designed with two replications was used for all trials. In each replication, one line was grown as a single row of 12 plants. The planting density was 16.6 cm × 26.7 cm in all trials. Field management followed local agricultural practice. At maturity, five middle plants of each line were harvested in bulk for three RIL populations, and ten middle plants of each line for other populations. NGP, NSP, and SF were measured in three RIL populations and Ti52-2, whereas only NGP was measured in ZC5, ZC9 and ZC12 populations.

2.3. DNA Marker Analysis

Marker data and the linkage map have been available except for ZC5 [39,42]. For ZC5, A total of eight polymorphic DNA markers were employed, including three on chromosome 5, one on chromosome 6 and four on chromosome 11, respectively (Table S1). DNA was extracted using 2 cm long-leaf samples collected from the middle 10 plants of each line following the method of Zheng et al. [43]. PCR amplification followed the method of Chen et al. [44]. The products were visualized on 6% or 8% non-denaturing polyacrylamide gels by silver staining for seven simple sequence repeat markers, and 2% agarose gels using GelRed (Biotium, Fremont, CA, USA) staining for one sequence-tagged site marker. Linkage map was constructed using Mapmaker/Exp 3.0 (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) [45], Kosambi function was used to calculate the genetic distance.

2.4. Data Analysis

Phenotypic values of two replications were averaged for each line and used for data analysis in each trial. Basic descriptive statistics, including mean value, standard deviation, coefficient of variation, the minimum and maximum trait values, skewness, and kurtosis were computed in each trial. The mean value of each trait was used to calculate the correlation coefficients between each trait.

For three RIL and Ti52-2 populations, QTLs were determined using the MET (multi-environment trials) functionality in QTL IciMapping V4.1 software (Chinese Academy of Agricultural Sciences, Beijing, China), taking different years as different environments [46]. *LOD* thresholds were calculated with 1000 permutation tests ($p < 0.05$) for each trait and used to declare a putative QTL, which were 4.3–4.8, 4.3–4.8, and 4.4–4.6 for NGP, NSP, and SF in three RIL populations, as well as 2.8, 2.7 and 2.8 in Ti52-2, respectively. QTLs detected were designated following the rule proposed by McCouch and CGSNL [47].

For ZC5, ZC9 and ZC12 populations, QTLs were determined using CIM (composite interval mapping) approach in Windows QTL Cartographer 2.5 (North Carolina State University, Raleigh, NC, USA) [48]. A threshold of *LOD* > 2.0 was used for claiming a putative QTL.

3. Results

3.1. Phenotypic Variance

Descriptive statistics of the NGP, NSP, and SF in each trial of three RIL populations were presented in Table 1. The three traits were continuously distributed with low skewness and kurtosis, showing a typical pattern of quantitative variation. Generally, the mean values for all three traits were higher in TI than in ZM and XM. In terms of phenotypic differences between the female and male parents, significant differences ($p < 0.05$) were observed for NGP and NSP only in TI. The female parent TQ had higher values of NGP and NSP compared with the male parent.

Table 1. Phenotypic performance of number of grains per panicle (NGP), number of spikelets per panicle (NSP) and spikelet fertility (SF) in the three recombinant inbred line (RIL) populations.

Trait [a]	Population [b]	Year	Mean	SD	CV	Range	Skew	Kurt	Parental Mean		p [d]
									Female	Male [c]	
NGP	TI	2009	107.6	20.9	0.194	65.1–166.8	0.34	−0.16	187.8	80.9	
		2010	150.7	26.6	0.177	92.5–259.9	0.57	0.81	172.7	122.7	
		2011	166.5	25.0	0.150	112.4–232.4	0.35	−0.05	212.9	124.2	
		2016	161.5	30.5	0.189	54.3–308.3	0.35	2.35	187.7	131.3	0.011
	ZM	1999	90.5	25.1	0.278	39.5–185.5	0.68	0.70	114.4	117.2	
		2000	71.2	16.7	0.235	26.0–130.4	0.20	0.15	88.7	88.0	
		2003	73.7	16.1	0.219	22.8–117.7	−0.12	0.26	59.6	70.8	
		2016	104.0	26.9	0.259	33.9–183.1	0.17	−0.06	70.6	88.0	0.157
	XM	1999	84.2	22.3	0.265	39.4–173.2	0.76	1.09	101.3	117.2	
		2000	71.0	19.8	0.279	21.3–131.2	0.22	0.10	72.2	88.0	
		2003	79.9	15.3	0.191	41.4–122.9	0.34	0.08	70.2	70.8	0.170
NSP	TI	2009	121.2	24.2	0.200	73.3–190.0	0.38	−0.18	205.8	95.1	
		2010	194.0	32.5	0.168	128.4–318.3	0.75	1.00	226.7	159.3	
		2011	204.2	30.7	0.150	141.8–298.8	0.59	0.01	248.4	165.0	
		2016	188.6	35.9	0.191	87.6–351.2	0.65	1.88	225.9	141.6	0.002
	ZM	1999	129.7	31.6	0.244	61.9–242.2	0.66	0.75	126.2	138.5	
		2000	119.4	22.0	0.185	68.7–191.2	0.33	−0.03	118.4	123.3	
		2003	105.9	21.6	0.204	57.1–175.4	0.46	0.28	88.3	88.3	
		2016	132.0	28.2	0.213	62.5–226.0	0.40	0.18	105.4	112.9	0.094
	XM	1999	121.8	27.5	0.226	56.9–203.4	0.46	0.20	117.4	138.5	
		2000	113.6	20.9	0.184	56.0–193.6	0.32	0.97	101.2	123.3	
		2003	105.8	20.2	0.191	60.4–164.0	0.40	−0.17	99.2	88.3	0.423
SF	TI	2009	89.0	4.9	0.055	70.1–96.2	−1.05	1.28	91.4	88.7	
		2010	77.9	7.3	0.093	58.8–91.4	−0.55	−0.10	75.8	79.1	
		2011	81.8	7.0	0.085	60.5–94.7	−0.46	−0.23	85.7	79.8	
		2016	85.9	6.5	0.076	63.4–96.3	−0.95	0.82	82.9	86.4	0.856
	ZM	1999	69.9	10.1	0.145	40.7–92.6	−0.12	−0.26	90.7	84.6	
		2000	59.9	10.5	0.175	29.6–89.1	−0.33	0.22	73.4	70.8	
		2003	69.8	9.4	0.134	38.4–92.0	−0.43	0.17	67.5	80.1	
		2016	78.4	9.1	0.116	42.0–94.5	−0.99	1.01	75.7	79.1	0.682
	XM	1999	69.7	11.7	0.168	34.8–90.3	−0.37	−0.52	86.3	84.6	
		2000	62.1	11.9	0.191	28.7–89.4	−0.36	0.00	71.6	70.8	
		2003	75.9	7.6	0.100	53.7–93.9	−0.34	−0.30	70.6	80.1	0.579

[a] NGP, number of grains per panicle; NSP, number of spikelets per panicle; SF, spikelet fertility. [b] TI, Teqing/IRBB near isogenic lines, including 122 of Teqing/IRBB52, 77 of Teqing/IRBB59, two of Teqing/IRBB50, and each of Teqing/IRBB51, Teqing/IRBB54 and Teqing/IRBB55; ZM, Zhenshan 97/Milyang 46; XM, Xieqingzao/Milyang46. [c] Measured as the mean value of IRBB52 and IRBB59 in the TI population. [d] Two-tailed p value of Student's t test.

NGP was positively correlated with NSP and SF in all three RIL populations but the coefficients were obviously higher between NGP and NSP than between NGP and SF in each population (Figure 2). Non-significant correlation between NSP and SF was detected in three RIL populations except in TI.

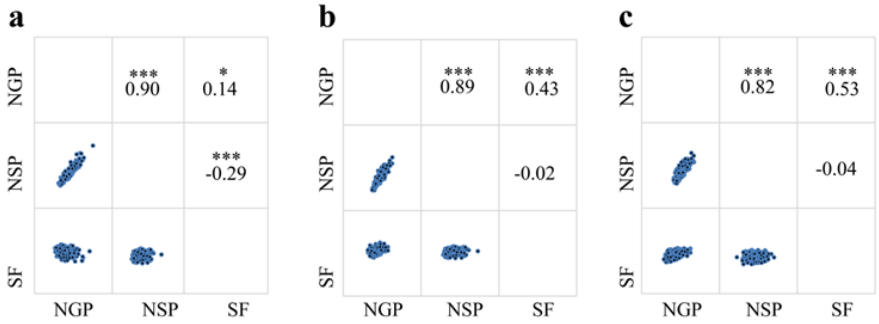

Figure 2. Correlation between different traits using mean value in Teqing/IRBB lines (TI) population (**a**), Zhenshan 97/Milyang 46 (ZM) population (**b**) and Xieqingzao/Milyang 46 (XM) population (**c**). The upper panel contains the correlation coefficients, and the lower panel contains the distributions of the three traits. * and *** represent significant level at 0.05 and 0.001, respectively.

In TI, the standard deviations of NGP were 20.9, 26.6, 25.0, and 30.5 in 2009, 2010, 2011, and 2016, respectively (Table 1), while the standard deviation values of the Ti52-2 originated from one RH of TI were reduced to 17.0 and 12.3 in 2016 and 2017, respectively (Table S2). Furthermore, the standard deviation values of ZC5, ZC9 and ZC12 derived from three RHs of Ti52-2 were decreased to 8.5, 9.6 and 9.9 in 2017, respectively (Table S2). Likewise, the coefficients of variation decreased from 0.150~0.194 to 0.118 and 0.079, then to 0.056~0.063 (Table 1 and Table S2). A similar trend was also observed between TI and Ti52-2 for NSP. These results were in line with the expectation that the background variations get more homozygous from TI to Ti52-2 and then to ZC5, ZC9, and ZC12.

3.2. QTLs Detected in Three RIL Populations

A total of 58 QTLs were detected in three RIL populations and distributed on all 12 rice chromosomes, including 22 for NGP, 17 for NSP, and 19 for SF (Table 2).

Table 2. Quantitative trait locus (QTLs) detected for NGP, NSP, and SF in three RIL populations.

Trait	QTL [a]	TI Population				ZM Population				XM Population			
		Interval	*LOD*	*A*[b]	R^2 (%) [c]	Interval	*LOD*	*A*	R^2 (%)	Interval	*LOD*	*A*	R^2 (%)
NGP	*qNGP1.1*					RG532-RM151	29.1	6.13	15.2	RG532-RM1195	10.1	4.32	6.6
	qNGP1.2	Wn34352-RM11869	4.9	−3.17	1.9	RM315-RZ538	7.4	2.66	2.5				
	qNGP2.1					A5-RM71	5.3	−2.85	2.5				
	qNGP2.2	RM6-RM240	48.9	−10.78	20.6								
	qNGP3.1	RM15303-RM16	25.9	−7.90	11.4								
	qNGP3.2									RM85-RG418A	5.7	−3.60	4.6
	qNGP4	RM349-RM3333	8.7	4.00	3.4	RG776A-RG620	5.4	−2.60	2.9				
	qNGP5.1									CDO82-RG182	4.6	−2.78	2.6
	qNGP5.2	RM146-RM164	7.5	3.25	2.9	RG13-RM164	5.5	−2.72	2.6				
	qNGP5.3					RG573-RG470	5.8	−2.70					
	qNGP5.4	RM274-RM334	9.3	3.81	3.2								
	qNGP6.1					RM508-RM190	6.8	2.19	4.4				
	qNGP6.2					RZ398-RM204	19.2	−4.46	12.4	RZ398-RM217	6.5	−3.16	3.6
	qNGP6.3	RM276-RM549	9.2	−4.43	3.9								
	qNGP6.4	RM3827-RM20361	4.7	−2.69	1.5								
	qNGP7.1					RM3859-RG678	9.3	2.54	5.4				
	qNGP7.2	RM70-RM18	7.8	−3.97	2.7								
	qNGP9.1	RM8206-RM219	5.8	−3.00	1.6								
	qNGP9.2					RM242-RM108	5.4	2.58	2.5				
	qNGP11					RG167-RM287	6.5	2.99	2.9				
	qNGP12.1	RM511-RM28313	9.5	4.35	3.9								
	qNGP12.2	RM28597-RM17	4.9	−3.09	2.1								
NSP	*qNSP1*					RG532-RM151	33.6	7.41	15.0	RG532-RM1195	15.4	6.60	11.1
	qNSP2.1	RM3732-RM71	4.8	3.67	1.8					RZ742-RZ512	7.4	−4.58	5.2
	qNSP2.2	RM6-RM240	53.7	−13.37	22.1	RM240-RZ123	4.8	−3.07	2.1				
	qNSP2.3	Tw35293-RM207	5.2	−3.63	1.9								
	qNSP3	RM15303-RM16	9.0	−5.19	3.3								
	qNSP4	RM3474-RM6992	8.7	4.36	3.0	RG776A-RG620	7.4	−3.41	2.9				
	qNSP5.1	RM164-RM18927	5.4	3.13	1.9	RM164-RM163	6.7	−3.28	2.9	RM163-RG470	4.5	−3.44	2.9
	qNSP5.2					RG573-RG470	5.1	−2.93	2.7				
	qNSP5.3	RM274-RM334	7.5	4.27	2.5								
	qNSP6.1									RZ398-RM217	7.5	−3.79	6.5
	qNSP6.2	RM276-RM549	22.6	−8.35	8.6	RM253-RM276	24.3	−7.30	13.6				
	qNSP7.1					RM1243-RM3859	7.2	2.93	4.6	RM1243-RM3859	7.9	3.72	6.4
	qNSP7.2	RM70-RM18	11.2	−5.86	4.1					RZ264-RZ626	5.4	−4.03	3.4
	qNSP10									RM1859-RM184	5.0	−3.42	4.2
	qNSP11.1					RG118-RM202	5.1	2.85	2.0				
	qNSP11.2									RZ797-RG103	6.9	4.09	5.5
	qNSP12	RM511-RM28313	18.4	7.24	6.8								

Table 2. *Cont.*

Trait	QTL [a]	TI Population				ZM Population				XM Population			
		Interval	*LOD*	*A*[b]	R^2 (%) [c]	Interval	*LOD*	*A*	R^2 (%)	Interval	*LOD*	*A*	R^2 (%)
SF	*qSF1.1*					RM294A-RM294B	6.6	1.31	4.8				
	qSF1.2	RM12178-RM12210	7.0	−0.95	3.1								
	qSF2.1					RZ318-RM263	6.4	−0.74	3.6				
	qSF2.2	RM6-RM240	6.5	0.87	3.3								
	qSF3.1	RM15303-RM16	13.1	−1.45	8.2								
	qSF3.2									R1927-RM143	4.9	−1.25	3.2
	qSF3.3					RZ613-RM85	11.4	−1.80	7.4	RM85-RG418A	6.1	−1.99	7.6
	qSF4.1					RM551-RM261	4.8	0.93	3.3				
	qSF4.2	Fo13346-RM303	8.6	−1.07	5.1								
	qSF5.1									RM13-RM267	6.0	−1.99	5.8
	qSF5.2	RM18038-RM18189	22.2	1.96	13.9								
	qSF6.1									RM190-RZ516	7.3	1.83	7.9
	qSF6.2	RM6119-RM276	7.5	0.93	3.1	RG138-RM111	5.3	0.88	4.2				
	qSF6.3	RM340-RM20731	9.0	1.19	5.4								
	qSF8	RM23001-RM210	6.4	0.95	3.2								
	qSF9					RM105-RM3700	7.0	1.35	4.0				
	qSF10.1									RM3229B-RM1376	5.0	1.13	5.6
	qSF10.1	RM3773-RM3123	5.4	0.83	2.9								
	qSF12	RM28313-RM28597	6.8	−0.94	2.9								

[a] QTL are named as proposed by McCouch and CGSNL (2008). [b] A, additive effect of replacing a maternal allele with a paternal allele. [c] R^2, proportion of phenotypic variance explained by the QTL effect.

For NGP, 12, 11, and four QTLs were identified in TI, ZM, and XM, respectively. Among them, five QTLs were shared between two populations and 17 were identified in single population (Table 2 and Figure 3a). In TI, *qNGP2.2* and *qNGP3.1* explained the highest two proportions of phenotypic variance with the value of 20.6% and 11.4%, respectively. The other 10 QTLs had the phenotypic variance explanation ranging from 1.5 to 3.9%, of which three QTLs: *qNGP1.2*, *qNGP4*, and *qNGP5.2* were shared with ZM. In ZM, *qNGP1.1* and *qNGP6.2* were revealed to have the highest two R^2 of 15.2% and 12.4%, which were also detected in XM. The R^2 values of other QTLs were much smaller ranging as 2.5–5.4%. In XM, all four QTLs showed small R^2 values ranging from 2.6 to 6.6%. No QTL was found in both of TI and XM. The overall R^2 for NGP were much higher in TI and ZM than in XM, which were 59.0%, 56.3%, and 17.4%, respectively. Significant genotype-by-environment (GE) interaction was observed for *qNGP1.1* and *qNGP6.2* only in ZM (Table S3).

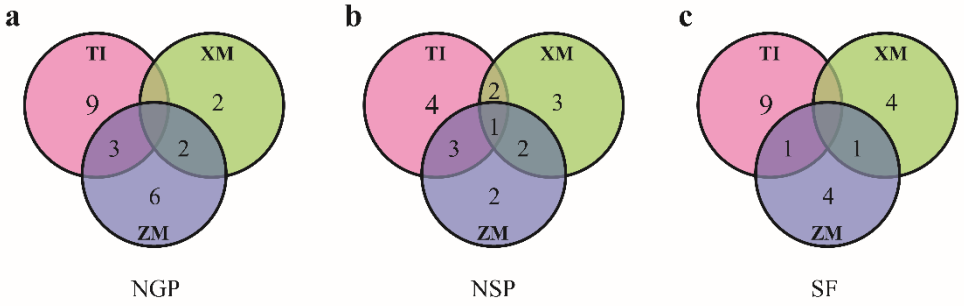

Figure 3. Number of QTLs detected in three RIL populations for NGP (**a**), NSP (**b**) and SF (**c**), respectively.

For NSP, ten, eight and eight QTLs were detected in TI, ZM and XM, respectively. Among them, one QTL was shared within all three populations, seven QTLs were contributed by two populations and nine QTLs were identified in single population (Table 2 and Figure 3b). *qNSP5.1*, the only one stably detected in three populations, lay in the interval of RM164–RM18927 on chromosome 5 in TI and showed minor effect in all populations. *qNSP2.2* displayed the largest R^2 of 22.1% in TI but nearly the smallest R^2 of 2.1% in ZM, indicating divergent effects produced in different genetic background. In ZM, *qNSP1* and *qNSP6.2* had the highest two R^2 of 15.1% and 13.6%, the other six QTLs had R^2 ranging from 2.0 to 4.6%. In XM, *qNSP1* also had the highest contribution of 11.1%, and the other seven QTLs had R^2 ranging from 2.9 to 6.5%. The overall R^2 of the QTLs were 55.9%, 45.8% and 45.1% in TI, ZM and XM, respectively. Except *qNSP2.3*, *qNSP10*, and *qNSP11.2*, all QTLs for NSP were simultaneously shared the same intervals as QTLs for NGP, in accordance with high positive correlation between NGP and NSP in three populations. In addition, one significant GE interaction was found for *qNSP1* in ZM (Table S3).

For SF, ten, six and five QTLs were identified in TI, ZM and XM, respectively. Only two QTLs were shared between two populations (Table 2 and Figure 3c). *qSF3.3* was simultaneously identified in ZM and XM, of which the R^2 reached almost the highest with value of 7.4% and 7.6%, respectively. *qSF6.2* was detected in TI and ZM, with minor effect to SF in both populations. In TI, *qSF5.2* appeared the highest R^2 of 13.9% and the remaining 16 QTLs had the R^2 ranging from 2.9 to 8.2%. The overall R^2 of the QTLs were 51.1%, 27.3% and 30.1% in TI, ZM and XM, respectively. Only one QTL, *qSF2.1*, showed significant GE interaction in ZM (Table S3).

3.3. QTLs Detected in Ti52-2 Population

A total of five, three and four QTLs for NGP, NSP, and SF were detected in Ti52-2 population, respectively and distributed on rice chromosome 3, 5, 6, 9, 11 and 12 (Table 3). No significant GE interaction was observed.

Table 3. QTLs detected for NGP, NSP, and SF in Ti52-2 population.

Trait	QTL	Interval	*LOD*	*A* [a]	R^2 (%) [b]
NGP	*qNGP3.3*	RM232	3.2	2.29	4.1
	qNGP5.5	RM18927-RM3321	4.1	−2.46	4.9
	qNGP6.1	RM469-RM589	5.8	2.31	9.9
	qNGP9.1	RM5688-RM219	3.5	−2.18	5.6
	qNGP12.1	Pita-RM511	3.1	2.19	4.8
NSP	*qNSP3.2*	RM232	2.6	1.89	2.2
	qNSP5.4	RM18927-RM3321	5.5	−3.44	5.3
	qNSP12	RM3246-Pita	6.8	3.73	7.8
SF	*qSF3.4*	RM14303-RM14383	3.8	0.53	7.3
	qSF6.1	RM587-RM584	7.8	1.08	16.3
	qSF9.2	RM5688-RM219	6.1	−0.98	11.3
	qSF11	RM224-RM5926	6.0	0.97	9.0

[a] A, additive effect of replacing a maternal allele with a paternal allele. [b] R^2, proportion of phenotypic variance explained by the QTL effect.

Ti52-2 was derived from TI carrying 13 segregating regions (Figure 4). In Ti52-2, those regions involving QTLs with major effects for NGP, NSP, and SF in TI and other two RIL populations had become homozygous, such as RG532–RM151 interval containing *qNGP1.1* and *qNSP1*, RM6–RM240 interval containing *qNGP2.2* and *qNSP2.2*, RM15303–RM16 interval containing *qNGP3.1* and *qSF3.1*, RM276–RM549 interval containing *qNGP6.2* and *qNSP6*.2, RM18038–RM18189 interval containing *qSF5.2*. Therefore, Ti52-2 is a good candidate to detect minor-effect QTL for NGP, NSP, and SF.

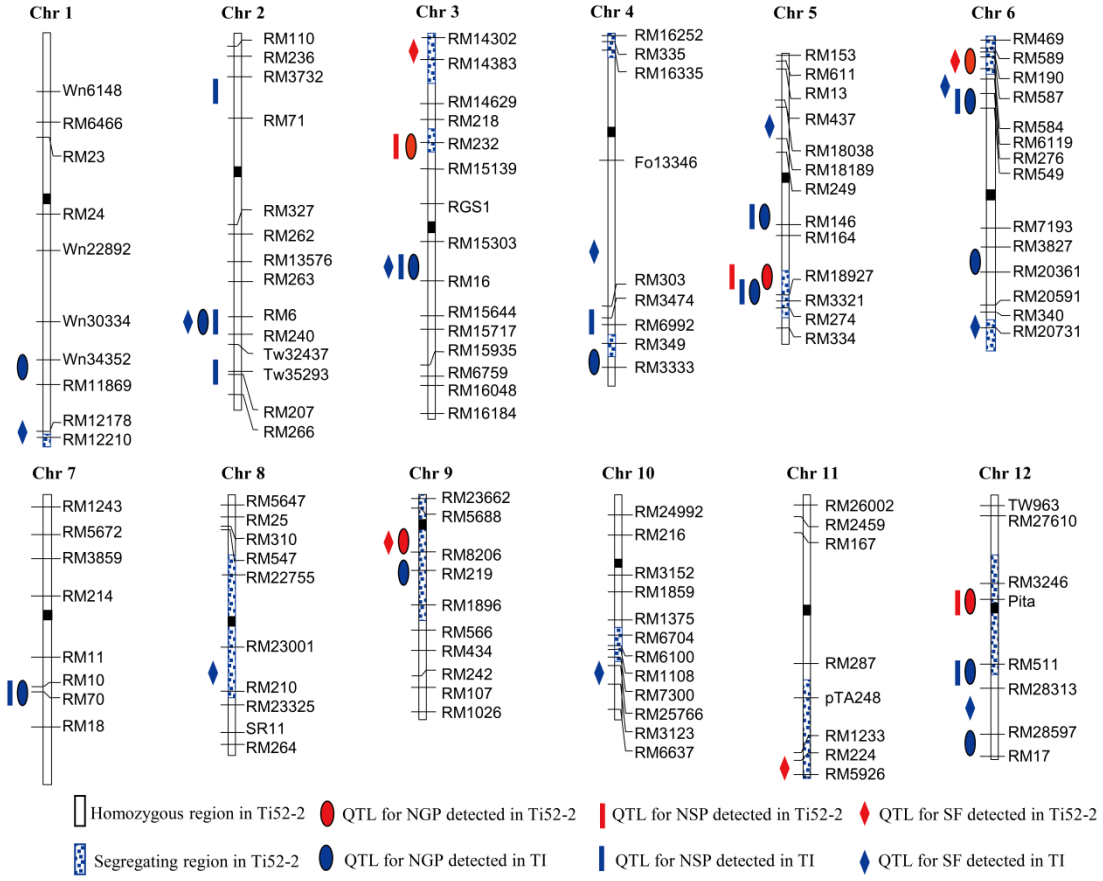

Figure 4. Distribution of QTLs for NGP, NSP, and SF detected in Ti52-2 and TI populations.

Both of *qNGP9.1* and *qNGP12.1* were validated in Ti52-2 and the direction of additive effects remained unchanged between TI and Ti52-2. Moreover, the R^2 increased from 1.6 to 5.6% and 3.9 to 4.8%, respectively. In TI, *qNGP5.4* was detected in the interval of RM274−RM334 on chromosome 5 and the IRBB52 allele increased NGP 3.81. In Ti52-2, the locus of RM334 had got homozygous, while RM274 remained be segregating. However, a QTL was found in the interval of RM18927−RM3321 upstream of *qNGP5.4* with the TQ allele increasing NGP 2.46. Hence, the new QTL was named *qNGP5.5*. Among the five QTLs for NGP detected in Ti52-2, *qNGP6.1* with the highest R^2 was undetected previously in TI but mapped in the similar interval of RM508−RM190 in ZM, while *qNGP3.3* with the lowest R^2 was not identified in previous three RIL populations.

Amid of the three QTLs for NSP detected in Ti52-2, *qNSP12* was validated with the largest R^2 of 7.8% and the enhancing allele was from IRBB52 in both TI and Ti52-2. *qNSP5.4*, a new QTL with an allelic effect in opposite direction to *qNSP5.3* in the interval of RM274−RM334 detected in TI, was detected in the RM18927−RM3321 region. In addition, *qNSP3.2* having the marginal LOD threshold and the smallest R^2 was not previously detected in TI, ZM and XM.

Out of the four QTLs for SF detected in Ti52-2, *qSF6.1* with the highest R^2 of 16.3% was mapped in RM586−RM584 region, which was undetected in TI and lay in a similar interval of RM190−RZ516 detected in XM. The others, *qSF9.2*, *qSF3.4,* and *qSF11*, were not identified previously in three RIL populations. Either position or additive effect direction of *qSF9.2* was different from *qSF9* identified in ZM. Thus *qSF9.2* was a new QTL and *qSF9* was renamed as *qSF9.1*.

3.4. Validation of QTLs for NGP in ZC5, ZC9, and ZC12 Populations

The three QTLs, *qNGP5.5*, *qNGP9.1* and *NGP12.1*, which had shown significant effects in Ti52-2, were well validated in the new secondary segregation population, respectively (Table 4).

Table 4. QTLs detected for NGP in the ZC5, ZC9, and ZC12 populations.

QTL	Population	Interval	*LOD*	*A* [a]	*D* [b]	R^2 (%) [c]
qNGP5.5	ZC5 $S_{1:2}$	RM18927-RM3321	2.72	−1.94	3.42	6.3
qNGP9.1	ZC9 $S_{1:2}$	RM219-RM1896	2.22	−2.96	1.00	5.1
qNGP12.1	ZC12 $S_{1:2}$	RM3246-Pita	9.06	5.68	0.43	16.0

[a] A, additive effect of replacing a maternal allele with a paternal allele. [b] *D*, dominance effect. [c] R^2, proportion of phenotypic variance explained by the QTL effect.

For *qNGP5.5* and *qNGP9.1*, TQ allele increased NGP about 2.46 and 2.18 and explained 4.9% and 5.6% of phenotypic variance in Ti52-2, respectively. In ZC5 and ZC9, TQ allele also increased NGP about 1.94 and 2.96, explaining 6.3% and 5.1% of phenotypic variance, respectively. For *qNGP12.1*, the additive effect was doubled and the R^2 values were increased by 3.3 times in ZC12.

4. Discussion

Grain number is one of the most important agronomic traits in the determination of rice productivity, which is governed by multiple QTLs with major or minor effect. In the present study, a total of 58 QTLs were detected in three RIL populations, including 22, 17, and 19 for NGP, NSP, and SF, respectively. The individual QTL counted for 1.5% to 22.1% of phenotypic variation. Among 58 QTLs, 15 with various effects shared by two or three populations and eight showed large effects with R^2 larger than 10%. When major-effect QTLs were fixed, three minor-effect QTLs for NGP were further detected and validated using secondary segregation populations derived from one RH of the cross TQ/IRBB52 under increment homogeneous backgrounds.

Among the three minor-effect QTLs for NGP, *qNGP9.1* and *qNGP12.1* were stably identified in TI, Ti52-2, ZC9, and ZC12 populations, having the same direction of additive effects and similar value. Moreover, the regions of RM8206−RM219 containing *qNGP9.1* and RM511−RM28313 containing *qNGP12.1* were previously reported to affect NGP. *qFG9* and *Gpp12.2* were detected in the region

of RM219−RM342 on chromosome 9 and RG869−RM277 on chromosome 12, respectively [49,50]. It proves that *qNGP9.1* and *qNGP12.1* are good targets for underlying the genetic basis of grain number.

qNGP5.5 was a new QTL identified in Ti52-2. This QTL was well verified in ZC5, the direction of allelic effect remaining consistent and R^2 increased from 4.9 to 6.3%. Actually, *qNGP5.4* was detected in TI with an allelic effect in the opposite direction to *qNGP5.5*. Comparing the two loci, we found that *qNGP5.5* was located in RM18927−RM3321, while the *qNGP5.4* was detected in RM274−RM334. The segregation region of Ti52-2 population covered RM274 but uncovered RM334. In previous studies, two QTLs with opposite allelic direction for grain weight were detected in RM18927−RM334 [37,51]. Hence, there might be two tightly linked QTLs with opposite direction of additive effect in the region of RM18927−RM334.

Verification of three minor-effect QTLs was benefited from the fixation of the major-effect QTLs and increment homozygous of genetic background. Along with the background became more homogenous from TI to Ti52-2 then to ZC5, ZC9 and ZC12, the phenotypic variations were getting smaller, and the power of detection of QTLs was further improved. For instance, the standard deviation values and coefficient of variation of NGP and NSP has been decreasing with background became more homogenous (Table 1 and Table S2). Out of the 12 QTLs for NGP detected in TI, only *qNGP4*, *qNGP5.4*, *qNGP9.1* and *qNGP12.1* were located in the segregation region of Ti52-2. Except *qNGP4* with the smallest R^2, other three QTLs were detected in Ti52-2. In addition, two more QTLs *qNGP3.3* and *qNGP5.5* were detected in Ti52-2. These results supported that RHs are efficient materials in the establishment of NILs and secondary mapping populations for QTL validation and fine-mapping especially for minor-effect QTLs.

Interestingly, among the five QTLs for NGP identified in Ti52-2, *qNGP3.3*, *qNGP5.5,* and *qNGP12.1* were located in the same regions with *qNSP3.2*, *qNSP5.4,* and *qNSP12*, respectively. The regions of *qNGP6.1* and *qNGP9.1* showed the same intervals with *qSF6.1* and *qSF9.2*. The results suggest that the five QTLs for NGP detected in Ti52-2 is a result of the integration of NSP and SF. Usually, NGP is determined by NSP multiply SF. Thus, these QTL regions sharing for both NGP and NSP or SF might be due to a pleiotropic effect of one QTL rather than the close linkage of different QTLs.

The eight QTLs with large effects detected in three RIL populations were located in six regions on chromosome 1, 2, 3, 5 and 6. Except for *qSF5.2*, all QTLs controlled NGP and/or NSP.

The region of RG532−RM151 on chromosome 1 containing *qNGP1.1* and *qNSP1* showed the largest R^2 for NGP and NSP in both of ZM and XM, while the interval of RM6−RM240 on chromosome 2 covering *qNGP2.2* and *qNSP2.2* showed the largest R^2 for NGP and NSP in TI. The special significance demonstrated in the given population might reveal the different mechanism of variant ecological type of rice varieties. *Gn1a*, the first cloned QTL for grain number in rice, is located in the former region [18], whereas no QTL for grain number was found to be fine-mapped or cloned in the latter interval. By comparing the physical position, we found a heading date QTL, *DTH2*, situated within this interval [52]. Anyway, no QTL controlling heading date was detected around this region in previous studies [42], which indicated that *DTH2* could not the candidate gene of *qNGP2.2* and *qNSP2.2*. In addition, we found that *GS2*, a major QTL for grain size was lain 0.7 Mb upstream of RM6. Hu et al. reported that no significant differences of NGP were found between the recurrent parents and NILs [53], suggesting it would not probably be the pleiotropic effect of *GS2*. Hence, this locus might be a new QTL controlling the grain number. Fine-mapping of *qNGP2.2* is to be undertaken.

The region of RM15303−RM16 on chromosome 3 was another one for both of NGP and NSP identified in TI. However, it presented the second largest R^2 for NGP, but much smaller effect on NSP. *GL3.1*, a cloned QTL for grain length, was positioned 1.9 cM downstream of RM16 and was reported to have a significant effect on NGP [54]. More work is needed to confirm whether this locus is a new QTL for grain number or just a pleiotropic effect of *GL3.1*.

The region of RZ398−RM217 on chromosome 6 exhibited the second largest R^2 for NGP but no significant effect on NSP in ZM. It also displayed minor effect on NGP and NSP in XM. No QTL controlling grain number had been cloned or fine-mapped in this interval. In the other hand, the

region of RM253−RM276 downstream of RZ398−RM217 exhibited the second largest R^2 for NSP but no significant effect on NGP in ZM. It was noteworthy that it also displayed the second largest R^2 for NSP and minor effect on NGP in TI. It was previously reported that a QTL controlled NGP and yield-related traits in the same region [55]. Hence, these QTLs provided new candidates for gene cloning and marker-assisted breeding.

5. Conclusions

A total of 58 QTLs for NGP, NSP, and SF were detected in three RIL populations of *indica* rice. Among them, eight QTLs showed large effects with R^2 larger than 10%. Three QTLs with minor effects for NGP, *qNGP5.5*, *qNGP9.1,* and *qNGP12.1,* were further detected and validated using segregation populations derived from one RH of the cross TQ/IRBB52. These results proved that the use of residual heterozygotes to construct secondary mapping populations is an efficient strategy to detect minor-effect QTLs for complex traits. The results also enrich our understanding of the mechanism of grain number and provide foundation for cloning and selecting candidate for marker-assisted selection breeding in rice.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/2/180/s1, Table S1: DNA markers used in the ZC5 population. Table S2: Phenotypic performance of NGP and NSP in the Ti52-2, ZC5, ZC9 and ZC12 populations. Table S3: Genotype-by-environment interaction (GE) detected in the RIL populations.

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