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QTLs identification for nitrogen and phosphorus uptake-related traits using ultra-high density SNP linkage

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ABSTRACT

To understand the genetic basis of nitrogen and phosphorus uptake in the cultivated rice, quantitative trait loci (QTL) analysis for 7 nitrogen and phosphorus uptake-related traits including above-ground biomass (AGB), leaf colour value (SPAD) in heading stage, grain nitrogen concentration (GNC), grain nitrogen content of the plant, total nitrogen content (TNC), grain phosphorus concentration, total phosphorus content (TPC) were conducted using SNP markers in a F2 population derived from a cross between GH128 and W6827. A total of 21 QTLs for nitrogen and phosphorus uptake-related traits distributed in 16 regions along 6 chromosomes were detected using a high density genetic map consisting of 1582 bin markers, with QTLs maximum explaining 8.19% of the phenotypic variation. Nine QTLs (42.9% of total QTLs) were detected on chromosome 2. Among them, two QTL clusters including AGB, TNC, TPC and GNC were also detected in the region bin 140 and bin 146 on the chromosome 2. The distance between the two clusters was only 4.1 cM. The presence of QTL clusters has important significance and could be useful in molecular marker assisted breeding. These genomic regions might be deployed for the simultaneous improving the use efficiency of nitrogen and phosphorus in rice breeding.

1. Introduction

Rice (*Oryza sativa L.*) is a staple food crop for over half of the population in the world. With the increase in population and the decrease in arable land, rice production is facing a huge challenge in the next 10 years. Nitrogen and phosphorus are the macronutrient elements for plant assimilating from soil. At present, nitrogen use efficiency (NUE, only about 30% in China) and phosphorus use efficiency (PUE, 14–22% in China) of rice evidently decline with increasing fertilizer application [1,2]. With over use of fertilizer for a long term and low both NUE and PUE, most of the N and P are lost through run-off, volatilization, immobilization *etc.*, thereby resulting in problems for environment and sustainable agriculture that indirectly affect human health [3,4]. Genetic improvement of rice for nitrogen and phosphorus uptake would reduce N and P input and improve N and P utilization, which would be an effective way to reduce costs of rice production and the environmental pollution [5,6].

Some researchers have reported that a number of crop populations detected many alleles for nitrogen or phosphorus use efficiency and grain yield using high density genetic map, such as maize [7,8], wheat [9,10], biennial crop Belgian endive [11], barley [12], *etc*. QTLs for rice NUE, grain yield and N-deficiency tolerance traits were identified using a set of recombinant inbred lines (RILs) [13–15]. Yamamoto et al [16] reported that 4 QTLs for nitrogen uptake were detected during ripening in the paddy yield, among them, QTL *qCHR1* is important for increasing nitrogen uptake and nitrogen partitioning to leaves during senescence. QTLs for PUE, P uptake efficiency (PUPE) were also detected and assigned into two QTL clusters in a chromosome region [7]. Su et al [9] found that 7 QTLs were detected repeatedly as controlling phosphorus uptake in Chinese winter wheat varieties. However, QTLs for nitrogen and phosphorus uptake in rice population are little reported.

It is well known that nitrogen or phosphorus use efficiency is divided into two components: N or P uptake efficiency and N or P utilization efficiency. It is reported that nitrogen uptake efficiency (NUPE) and nitrogen utilization efficiency (NUTE) are uncorrelated and may be improved independently, and NUPE contributed more to the variation in NUE than NUTE in rainfed upland condition [17]. Su et al [9] showed that positive linkages were observed between QTLs for P uptake (PUP) and utilization efficiency (PUTE), which implied the possibility of improving PUP and PUTE simultaneously. Nitrogen or phosphorus uptake is essential components of both N or P uptake efficiency and N or P utilization efficiency, contributing to improvement of nitrogen or

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phosphorus use efficiency. It is reported that both nitrogen and phosphorus uptakes were detected to have pleiotropic effects using RIL population of wheat varieties [18]. However, the genetic basis of nitrogen and phosphorus uptake-related traits in rice varieties, whether they can be improved simultaneously, remains largely unknown.

RAD-seq approach focuses on short fragments of DNA adjacent to each instance of a particular restriction enzyme recognition site in the genome and increased throughput, thus allowing efficient and high density SNP discovery and genotyping [19]. Based on RAD-seq approach, SNP discovery and genotyping are already essential to genetic mapping, which possess the most abundant type of genetic marker and high density makers, as well as a useful and cost-effective in a variety of organisms [20–22]. In this study, we detected QTLs for nitrogen and phosphorus uptake-related traits using ultra-high-density SNP map and analyzed the genetic characteristics of nitrogen and phosphorus uptake-related traits. The objective of this study was to provide references for molecular marker-assisted selection of N, P-efficient rice varieties.

2. Materials and methods

2.1. Mapping population

F2 populations derived from the cross between GH128 and W6827 was used in construction of genetic map and in the determination of nitrogen and phosphorus uptake-related traits. Among them, GH128 is an indica cultivar with high-nitrogen and -phosphorus uptake, higher biomass yield while W6827 is low-nitrogen and phosphorus uptake, which have been reported in the previous studies [23]. In this study, both parental lines and 262 F2 lines were used for the detection of genotyping and phenotyping.

2.2. Plant cultivation

In the early seasons of 2016, two parental lines and 262 F2 lines were cultivated in the experimental station of Guangdong Academy of Agricultural Sciences in Guangzhou, China. The soil type was loam with the following properties: pH 5.08, organic matter of 25.35 g kg^{-1}, total N of 1.13 g kg^{-1}, total P of 1.01 g kg^{-1}, total K of 9.20 g kg^{-1}, alkali-hydrolysable N of 171.64 mg kg^{-1}, Olsen-P of 73.3 mg kg^{-1}, available K of 61.02 mg kg^{-1}. Rice seeds were sown in the middle of March and transplanted in early April. The experiment used randomized block design. All plant materials were grown in a plastic container (10 cm diameter, 30 cm high) simulating normal field management practices.

2.3. Measurement of the nitrogen and -phosphorus uptake-related traits

At heading stage, the SPAD value (SVHS) was measured using the SPAD-502 chlorophyll meter (SPAD-502 Plus, Minolta, Japan). After maturity, plant samples were divided into filled grains, unfilled grains, branch and straw parts. All plant samples were oven-dried at 105℃ for 20 min, and at 75℃ for 2 days, and then weighed for dry matter content and ground to a powder for N (Kjeldahl method) and P concentrations (the molybdate-vanadate-phosphate method) [24]. Seven nitrogen and phosphorus uptake-related traits were analyzed including above-ground biomass (AGB), grain nitrogen concentration (GNC), grain nitrogen content of plant (GNCP), total nitrogen content (TNC), grain phosphorus concentration (GPC), total phosphorus content (TPC). Among them, AGB is above-ground dry weight per plant (g/plant), SVHS is flag leaf colour value of the main culm in heading stage, GNC is nitrogen concentration of the filled grain(g/kg), GNCP is filled grain nitrogen content per plant (g/plant), TNC is total nitrogen content including filled grains, unfilled gains, straw and branch (g/plant), GPC is phosphorus concentration of the filled grain (g/kg), TPC is total phosphorus content including filled grains, unfilled gains, straw and branch (g/plant). Correlation among the traits was calculated in IBM SPSS Statistics 21. Skewness and kurtosis were obtained to understand the nature of distribution of nitrogen and phosphorus uptake-related traits in the F2 populations [25].

2.4. DNA extraction and RAD-seq library preparation

Twenty-one days after transplanting, the fully expanding leaf were sampled. Genomic DNA of two parents and the F2 populations was extracted using the CTAB method. Genome-wide SNP (single nucleotide polymorphism) development and genotyping for the F2 populations were conducted by the Beijing Genomics Institute (BGI, Shenzhen, China) using RAD-seq (Restriction-site associated DNA sequencing) approach [21].

2.5. Statistical analyses and QTL mapping

Bin genotype was got from each individual and used for genetic map construction. A total of 25117 polymorphic SNP was obtained on the whole genome. Using a sliding window approach, a window size of 15 SNPs without break-point data for genotypng was labelled as a Bin corresponding to a marker. The Bin genetic map was constructed based on the SNP maskers using MSTMap software [26]. Data of the genetic map was imported into MapChart 2.2 software and composed of linkage map [27]. Genetic distances of each chromosome were documented. Composite interval mapping (CIM) method in Windows QTL cartographer 2.5 was used for QTLs detection, additive effect (a) and the phenotypic variance explained (R^2) were analyzed [28]. The significance threshold of LOD value for each trait was calculated based on the permutation test (1000 permutations) for CIM. The LOD value threshold at the significance level of 5% was used as the presence of a QTL. QTLs nomenclature was done according to the guidelines described by McCouch [29].

3. Results

3.1. Sequence analysis and construction of bin-map

Two parents and 262 F2 populations were used for RAD-seq and results in a total of 29.91 Gb raw data, with an average of 111.61Mb for each individual (Fig. 1A). Sequences of individual lines were matched with the reference genome (IRGSP-1.0_genome), and coverage on average of 5.05%, the depth on average of 3.21X, and matching rate of 83.88% were obtained (Fig. 1B**)**. A window size of 15 SNPs was chosen following the procedure of sliding-widow method [30]. One SNP was slidden each time and the genotype of that window was got, thus obtaining the genotypes of each individual and eventually getting a total of 1582 bins markers (Table 1). The bin markers length ranged from 19 kb to 15.9 Mb, with an average of 230.4 kb (Supplementary Figure S1). In total, 95.7% of bin marker length were less than 1.0 Mb, among them, 9 bins larger than 5 Mb in size and 1 large bin over 10 Mb on chromosomes 12 (chr12-bin55) (Fig. 1C, Supplementary Figure S1). SNPs and bins per chromosome were shown in Table 1. A high-density genetic linkage map with a total distance of 1255.248 cM using 1582 bin markers were constructed (Table 1). The bin marker numbers on different chromosomes ranged from 86 to 215. The average distance between two bin markers across the map was 0.79 cM. For each chromosome, the average genetic distance between adjacent bins ranged from 0.74 cM to 0.98 cM (Table 1).

A: base counts of the individual lines; B: coverage distribution, distribution of sequencing depth and matching rate in the F2 populations; C: Heat map distribution of SNPs on each chromosome of the rice genome.

3.2. Distribution of nitrogen and phosphorus uptake related traits

There are considerable variances between two parental lines (GH128 and W6827) regarding the above-ground biomass, SPAD value

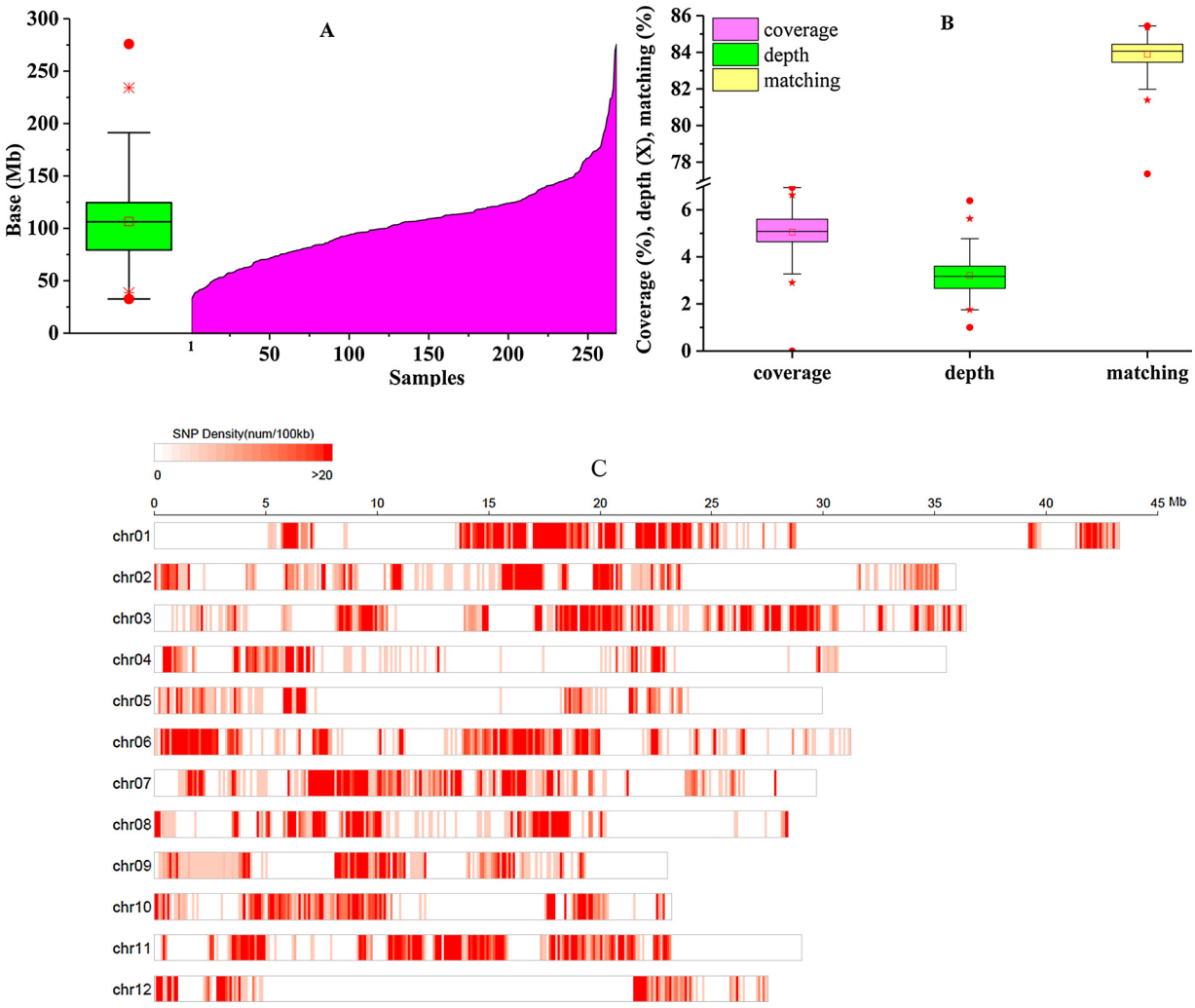

Fig. 1. Sequencing results for the F2 populations.

Table 1
SNP number and bins per chromosome in the GH128 × W6827 F2 populations.

Chromosome	SNP Number in population	bin numbers	Length of genetic distance (cM)
Chr01	3390	185	142.952
Chr02	2340	173	139.421
Chr03	3002	215	159.793
Chr04	1191	86	84.54
Chr05	947	126	90.569
Chr06	2722	137	123.615
Chr07	2694	135	103.385
Chr08	1878	117	89.926
Chr09	1509	92	70.83
Chr10	1648	88	75.942
Chr11	2731	128	87.586
Chr12	1065	100	86.689
Total	25,117	1582	1255.248

in heading stage, grain nitrogen concentration, grain nitrogen content of plant, total nitrogen content, grain phosphorus concentration, total phosphorus content (Table 2). Taken together, W6827 is a variety with low nitrogen or phosphorus uptake, whereas GH128 is featured by high nitrogen or phosphorus uptake. All the seven traits displayed a normal distribution in the F2 populations. Most of them showed transgressive segregation (Fig. 2). Both the degree of kurtosis was mostly 3 and the degree of skewness were mostly less than 1, indicating their nature as quantitative traits controlled by multiple genes.

3.3. Trait correlations

The correlations between nitrogen, phosphorus uptake related traits were evaluated by regressing phenotypic values. The correlation coefficients among traits are shown in Table 3. For instance, above-ground biomass was positively correlated with grain nitrogen content per plant, total nitrogen concentration, total phosphorus content, negatively correlated with SPAD value in heading stage, grain nitrogen concentration, grain phosphorus concentration. Grain nitrogen concentration was positively correlated with grain phosphorus concentration, negatively correlated with grain nitrogen content per plant, total nitrogen concentration, total phosphorus content. Grain nitrogen content per plant was positively correlated with total nitrogen concentration, total phosphorus content. Total nitrogen content was positively correlated total phosphorus content. Grain phosphorus concentration was positively correlated with total phosphorus content.

3.4. Detection of nitrogen and phosphorus uptake related QTLs

A total of 21 nitrogen, phosphorus uptake related QTLs in the population were detected. Significant QTLs from seven nitrogen, phosphorus uptake-related traits were summarized in Table 4 and Fig. 3.

Five QTLs for nitrogen, phosphorus uptake-related traits were associated with above-ground biomass. Alleles from GH128 contributed positively to the trait AGB. They were located on the chromosomes 2 and 5, respectively, terming *qAGB2.1*, *qAGB2.2*, *qAGB5.1*, *qAGB5.1*,

Table 2
Means of traits for the parental lines and the F2 populations.

Traits	Parent		F2 Populations			
	W6827	GH128	Mean ± SE	Range	Kurtosis	Skewness
AGB	24.30	31.75*	24.62 ± 4.07	7.92~35.46	1.79	−0.55
SVHS	43.99*	35.42	39.66 ± 2.36	33.07~45.20	−0.15	−0.45
GNC	11.67*	10.61	10.54 ± 1.04	7.48~15.25	3.13	1.17
GNCP	128.41	143.73*	118.60 ± 25.44	14.82~188.32	4.25	−1.47
TNC	206.82	239.12*	197.44 ± 28.50	66.42~282.63	2.59	−0.66
GPC	3.81*	3.21	3.43 ± 0.47	2.31~5.54	2.09	0.85
TPC	61.55	70.18*	59.24 ± 10.68	16.85~88.86	1.41	−0.33

Note: * indicate significant difference at the 0.05 level between GH128.

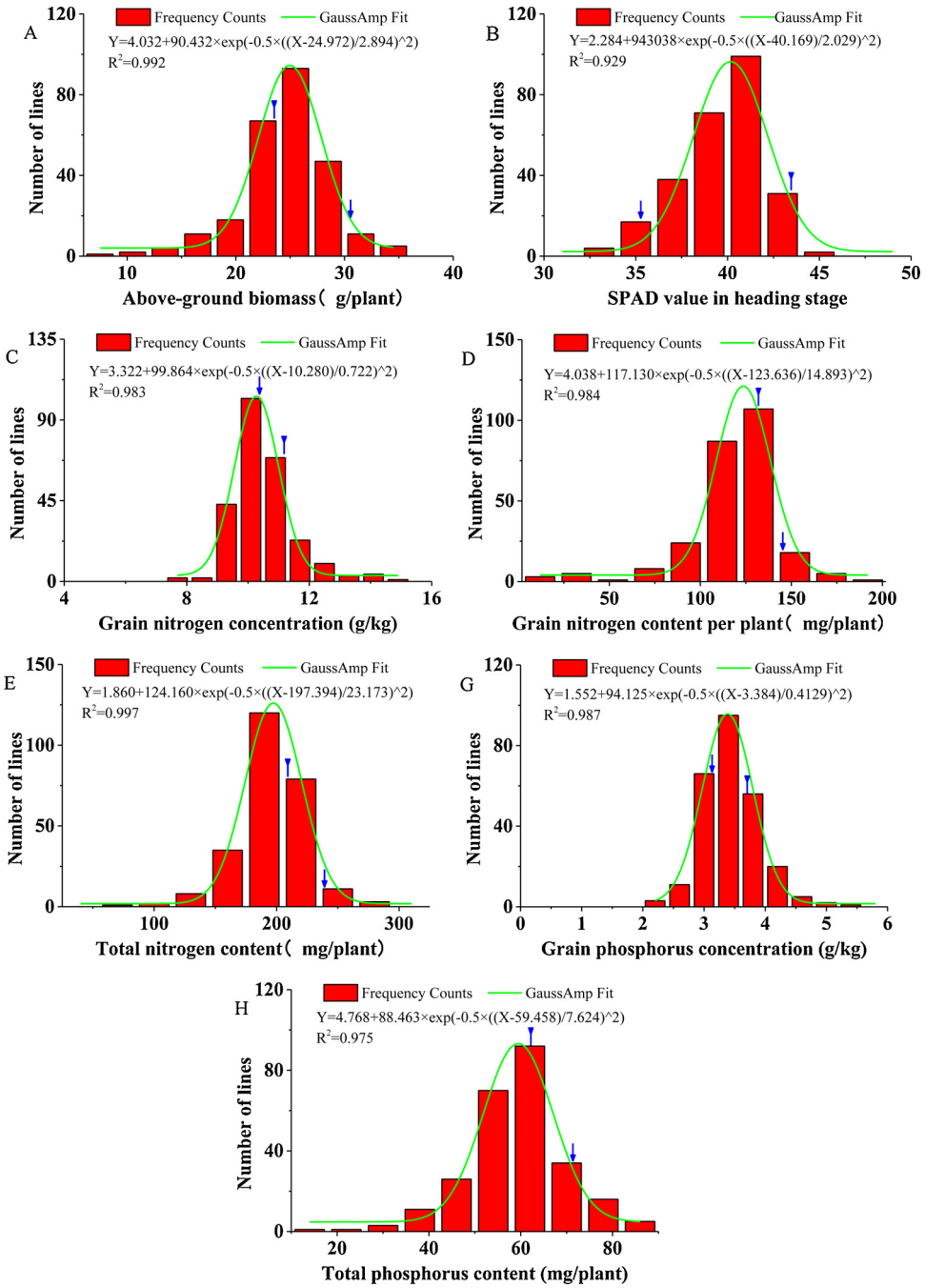

Fig. 2. Frequency distribution of phenotypes for nitrogen and phosphorus uptake related traits in the F2 populations. Arrows represent trait means of GH128, triangle indicate trait means of W6827.

Table 3
Correlation coefficients among nitrogen and phosphorus uptake related traits in the F2 populations derived from the cross of GH128 × W6827.

trait	AGB	SVHS	GNC	GNCP	TNC	GPC	TPC
AGB	1	−0.20**	−0.54**	0.82**	0.91**	−0.15*	0.84**
SVHS		1	0.28**	0.04	−0.01	0.21**	−0.02
GNC			1	−0.40**	−0.30**	0.38**	−0.35**
GNCP				1	0.87**	−0.08	0.76**
TNC					1	−0.05	0.84**
GPC						1	0.34**
TPC							1

Note: * and ** represent significant difference at the 0.05 and 0.01 level, respectively.

qAGB5.1. The phenotypic variation is explained by the individual QTLs ranged from 4.51% to 7.69%.

Six QTLs (*qSVHS2*, *qSVHS3.1*, *qSVHS3.2*, *qSVHS4*, *qSVHS5.1*, *qSVHS5.2*) for SPAD value were detected in heading stage on chromosomes 2–5, and the positive-effect alleles were from both W6827 and GH128. They explained a phenotypic variation ranged from 1.2% to 8.19%.

Two QTLs (*qGNC2.1*, *qGNC2.2*) associated with grain nitrogen concentration were detected on chromosome 2 and the explained phenotypic variation of 1.61%–1.67%. The W6827 contributed to the increase of grain nitrogen concentration at both loci.

One QTL (*qGNCP5*) for grain nitrogen content per plant was detected on chromosome 5 and the positive-effect alleles was from GH128. It explained a phenotypic variation of 5.36% in the population.

One QTL for total nitrogen content was detected. Positive-effect allele of *qTNC2* from GH128 is responsible for increasing the total nitrogen content, explaining a phenotypic variance of 1.56%.

Three QTLs (*qGPC2*, *qGPC6.1*, *qGPC6.2*) associated with grain phosphorus concentration were detected on chromosomes 2 and 6, respectively. They explained a phenotypic variance ranging from 1.67% to 2.03%. Alleles from W6827contributed positively for the grain phosphorus concentration.

Three QTLs (*qTPC2.1*, *qTPC2.2*, *qTPC7*) associated with total phosphorus content were detected on chromosomes 2 and 7, respectively. They explained a phenotypic variance ranged from 3.47% to 3.73%. Alleles from GH128 conferred a positive effect on TPC.

4. Discussion

It is reported that RAD-seq approach has high resolution and accuracy than traditional gel-based SSR markers [22,31]. High-throughput SNP genotyping has been used in a number of QTL mapping studies in rice [32–34]. Yu et al [35] revealed that increasing density markers due to construct bin map based on high quality SNPs, improve significantly the resolution and accuracy of QTL mapping. In this study, a total of 25,117 SNPs was identified in the genome between the GH128 and W6827 using RAD-seq approach. As a result, a high-density genetic map containing 1582 bin markers was generated, 95.7% of bin marker length were less than 1.0 Mb, the average distance between two bin markers across the map was 0.79 cM (Table 1, Supplementary Figure S1). It is implied that high-density genetic map constructed in this study is an ideal map for QTL mapping or map-based gene cloning.

The use efficiency of nitrogen, phosphorus, and potassium is controlled by complex gene networks [36]. Taken nitrogen use efficiency (NUE), Wei et al [13] found that some QTLs for NUE traits were detected on chromosomes 1, 2, 3, 4, 6, 7, 9, 10, and 11 [37]. Genomic regions in the RM135-RM168 interval on chromosome 3 and RM5556-RM310 interval on the chromosome 8 may be enriched with the key N metabolism genes [38]. Four genomic regions, including C86-C2340 on chromosome 1, RZ577-R1738 on chromosome 2, RZ471-C1023 on chromosome 7 and R3203-RM20a on chromosome 11, were found to contain QTLs for both NUE and GY [14]. In addition, a major rice nitrogen use efficiency QTL (*qNGR9*) on the chromosome 9 is synonymous with the previously identified gene *DEP1* (DENSE AND ERECT PANICLES 1), which complex regulates nitrogen signaling [39]. These previously reported results revealed that QTLs for nutrient use efficiency may be observed on different chromosomes due to differences in materials used and climatic conditions. Under our experimental condition, a total of 21 QTLs controlling 7 nitrogen and phosphorus uptake-related traits were detected on the chromosomes 2–7 (Table 4, Fig. 3).

For the above-ground biomass, five QTLs (namely *qAGB2.1*、*qAGB2.2*、*qAGB5.1*、*qAGB5.2*、*qAGB5.2*) were detected on chromosomes 2 and 5. Among them, *qAGB2.1*、*qAGB2.2* on the chromosome 2 were in the similar location with the reported *qSDW2* (AQEX016, 31497147-34652502) and *qTDW2* (AQEX029, 31497147-34652502) [40]. The *qAGB5.1*、*qAGB5.2*、*qAGB5.2* on the chromosome 5 were not reported before (http://archive.gramene.org, the same below), which were defined as new QTLs, explained 13.55% of phenotypic variance.

Table 4
QTLs detected and genetic effects on nitrogen and phosphorus uptake in the GH128 × W6827 F2 populations.

QTL	Chr.	LOD score	Peak marker	Physical position (bp)	Marker length (bp)	Additive effects	Percentage of variance explained (%)
qAGB2.1	2	9.50	bin140	31512510-31624623	112114	2.11	7.69
qAGB2.2	2	8.90	bin146	32004023-32335219	331197	2.03	5.95
qAGB5.1	5	2.76	bin82	18594041-18627492	33452	1.06	4.58
qAGB5.2	5	3.11	bin92	19092875-19115460	22586	1.15	4.46
qAGB5.3	5	3.09	bin101	19567050-19607064	40015	1.18	4.51
qSVHS2	2	5.63	bin141	31624624-31650031	25408	−0.79	1.20
qSVHS3.1	3	4.12	bin3	1689335-1830842	141508	0.72	3.19
qSVHS3.2	3	3.42	bin12	3095743-3295118	199376	0.67	2.92
qSVHS4	4	3.18	bin75	23305817-23330133	24317	−0.59	1.85
qSVHS5.1	5	3.96	bin103	19651282-19689449	38168	−0.70	7.75
qSVHS5.2	5	4.48	bin113	21600062-21634677	34616	−0.77	8.19
qGNC2.1	2	5.81	bin140	31512510-31624623	112114	−0.40	1.61
qGNC2.2	2	5.08	bin146	32004023-32335219	331197	−0.38	1.67
qGNCP5	5	3.03	bin3	249702-277783	28082	7.69	5.36
qTNC2	2	2.89	bin140	31512510-31624623	112114	8.78	1.56
qGPC2	2	2.53	bin139	23596772-31512509	7915738	−0.13	1.67
qGPC6.1	6	3.56	bin15	1262704-1339252	76549	−0.21	2.03
qGPC6.2	6	3.61	bin24	1794316-1892269	97954	−0.22	1.70
qTPC2.1	2	3.59	bin140	31512510-31624623	112114	3.80	3.73
qTPC2.2	2	3.38	bin146	32004023-32335219	331197	3.73	3.47
qTPC7	7	2.97	bin34	6698865-6911906	213042	0.68	3.55

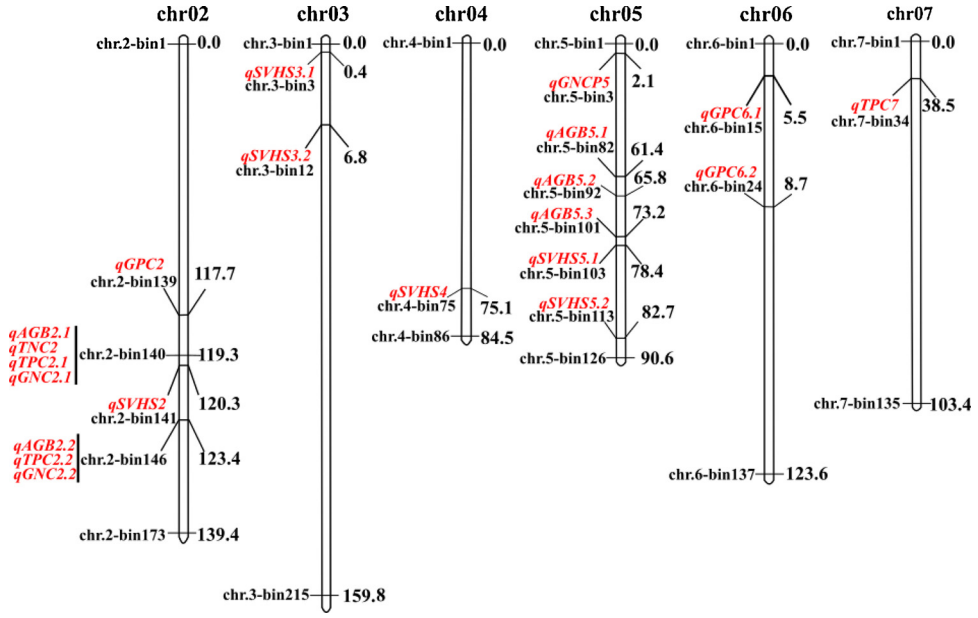

Fig. 3. QTLs for nitrogen and phosphorus uptake-related traits on the bin map.

For SPAD value of heading stage, six QTLs (*qSVHS2*、*qSVHS3.1*、*qSVHS3.2*、*qSVHS4*、*qSVHS5.1*、*qSVHS5.2*) explained total phenotypic variance of 21.0% were detected on chromosomes 2, 3, 4, 5. The *qSVHS2* on chromosome 2 was located in the same genetic interval of AQCW012 (32883159-33626591) as Yang et al [41] reported. q*SVHS3.1*、*qSVHS3.2*、*qSVHS4*、*qSVHS5.1*、*qSVHS5.2* are new QTLs.

For nitrogen uptake, two QTLs (*qGNC2.1* and *qGNC2.2)* and *qTNC2*, new QTLs, mainly detected on chromosome 2 were associated with grain nitrogen concentration and total nitrogen content per plant, respectively. The *qGNCP5* on chromosome 5 associated with grain nitrogen content per plant was similar to the previously reported CQF7 (179187-3344572) [42].

For phosphorus uptake, three QTLs (*qGPC2*, *qGPC6.1*, *qGPC6.2*) associated with grain phosphorus concentration were detected on chromosomes 2, 6. Four QTLs on chromosomes 2 and 7 associated with total phosphorus content were detected. Among them, *qTPC2.1*, *qTPC2.2*, *qTPC2.3* were similar to CQAA17 (3479656-32774553) and CQAA16 (3479656-32774553) reported by Ming et al [43], while *qTPC7* on the chromosome 7 was not found in the documents, which is defined as a new QTL.

Senthivel et al [44] detected several QTLs for nitrogen use, yield traits and associated traits on chromosome 3, and QTLs for soluble protein content related to rice N recycling were also detected in similar regions in rice [42]. Feng et al [45] found that two QTLs for heading date and plant height in the intervals RM5916-RM166 on chromosome 2 and RM2366-RM5767 on chromosome 8, improved obviously nitrogen use efficiency in rice. These results implied that QTLs of different traits may be clustered together. Gu et al [7] showed that P uptake efficiency (PUPE) was more closely correlated with PUE in maize, and root system architecture (RSA) in hydroponics was significantly related to PUPE but not to P utilization efficiency (PutE). Two QTLs for PUE, three for PUPE and three for RSA in a chromosome region were assigned into two QTL clusters, which provided a successful study case of developing P-efficient crops through QTL-based selection in maize.

In our study, two QTL clusters, including seven QTLs for above-ground biomass, total nitrogen content, total phosphorus content and grain nitrogen concentration, in the region bin 140 and 146 on chromosome 2 were identified. It is suggested that the nitrogen and phosphorus uptake-related genes are enriched in the region (Fig. 3). The alleles from GH128 for above-ground biomass, total nitrogen content, total phosphorus content are positive-effect ones which increase phenotypic values of all the three traits. the positive-effect alleles for grain nitrogen concentration were from W6827. These results implied that multiple alleles existed in these genomic regions on chromosome 2 (Table 4). Remarkably, these two clusters are closely located with the distance of only 4.1 cM. Additionally, QTLs located at each cluster had the same allele contributor and the same additive effect direction (Table 4). The correlation analysis showed that above-ground biomass, total nitrogen content, total phosphorus content was positively correlated, but negatively correlated with grain nitrogen concentration (Table 3). The above results suggested the possibility of simultaneous improvement in the nitrogen and phosphorus uptake-related traits in rice. Thus, the two genomic regions on chromosome 2 could be used as targets for improving nitrogen and phosphorus uptake traits. Further fine mapping is needed to identify the underlying functional gene, and further dissecting the genetic and molecular mechanisms of P and N uptake in rice.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2019.110209.

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