RICE NITROGEN USE EFFICIENCY: GENETIC DISSECTION

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ABSTRACT

A better understanding of genomic region might provide a genetic basic for the improvement of nitrogen use efficiency (NUE). The objective of this study was to identify the genetic regions affecting NUE in rice through the study of contrast cultivars and recombinant inbred lines (RILs) for QTLs analysis. A total of 169 RILs and their parents IR64 and Azucena were cultivated in the same conditions under different nitrogen conditions in two separated experiments. The WinQTL Cartographer version 2.5 was used to analyze joint QTL for multiple traits of each experiment. The first mapping experiment showed a total of 44 QTLs for all 15 observed parameters including number of leaves (NL), number of tillers (NT), plant height (PH), total fresh matter (FM), dry weight of roots (DWR), dry weight of leaf sheaths plus stems (DWS), dry weight of leaf blades (DWL), total dry matter (DM), chlorophyll content index (CCI), N concentration in roots (%NR), N concentration in leaf sheaths plus stems (%NS), N concentration in leaf blades (%NL), absorption NUE (aNUE), physiological NUE (pNUE) and agronomical NUE (agNUE) on chromosome 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12. The second experiment detected 44 QTLs for NL, NT, PH, FM, DWR, DWS, DWL, DM, CCI, %NR, %NL, aNUE and agNUE on chromosome 1, 2, 3, 5, 6, 7, 8 and 12.

Key words: nitrogen use efficiency (NUE), recombinant inbred lines (RILs), quantitative trait loci (QTL)

Phân tích thông tin di truyền liên quan đến hiệu suất sử dụng đạm ở lúa

TÓM TẮT

Những thông tin đầy đủ hơn về các vùng di truyền trong hệ gen sẽ là cơ sở cho việc nâng cao hiệu suất sử dụng đạm ở cây trồng. Mục đích của nghiên cứu này nhằm xác định các vùng di truyền trong hệ gen của lúa có liên quan đến hiệu suất sử dụng đạm thông qua việc phân tích QTL đối với các dòng thuần tái tổ hợp (RILs) từ hai dòng bố mẹ Azucena và IR64. 169 RILs và hai dòng bố mẹ được trồng trong cùng điều kiện môi trường trong phytotron với các mức bón đạm khác nhau. Thí nhiệm được lặpp lại hai lần riêng biệt. Phần mềm WinQTL Cartographer version 2.5 được sử dụng trong việc phân tích QTL với từng thí nghiệm riêng biệt. Thí nghiệm thứ nhất xác định được 44 QTL cho 15 tính trạng theo dõi bao gồm: số lá (NL), số nhánh (NT), chiều cao cây (PH), tổng khối lượng chất tươi (FM), khối lượng rễ khô (DWR), khối lượng thân và cuống lá khô (DWS), khối lượng phiến lá khô (DWL), tổng khối lượng chất khô (DM), hàm lượng chlorophyll (CCI), hàm lượng N trong rễ (%NR), hàm lượng N trong thân và cuống lá (%NS), hàm lượng N trong phiến lá (%NL), hiệu suất sử dụng đạm hấp thụ (aNUE), hiệu suất sử dụng đạm sinh lý (pNUE), hiệu suất sử dụng đạm nông học (agNUE). Các QTL này nằm trên các nhiễm sắc thể 1, 2, 3, 4, 5, 6, 7, 8,10 và12. Thí nghiệm lặp lại thứ 2 xác định được 44 QTL cho các tính trạng: NL, NT, PH, FM, DWR, DWS, DWL, DM,CCI, %NR, %NL, aNUE và agNUE trên các nhiễm sắc thể 1, 2, 3, 5, 6, 7, 8 và 12.

Từ khóa: Dòng thuần tái tổ hợp (RILs), hiệu suất sử dụng đạm (NUE), QTL.

1. INTRODUCTION

Nitrogen (N) is a crucial macro nutrient needed in the greatest amount of all mineral elements required by plants. Rice plant takes up nitrogen directly or indirectly from different external sources such as nitrate, nitrites, ammonia in soil (inorganic nitrogen); amino acids in soil (organic form) and fertilizers. Application of N is one of the major reasons that crop production has kept pace with human population growth. In general, crop plants are able to utilize only 30- 40% of the applied N (Raun and Johnson, 1999). Thus, more than 60% of the soil N is lost through a combination of leaching, surface run-off, denitrification, volatilization, and microbial consumption.

The excessive use of fertilizer not only resulted in lower nitrogen use efficiency (NUE) of plants but also wastes money and cause adverse effects to our environment as well as to human health. Overuse of N fertilization often leads to a reduction in net returns and groundwater contamination due to NO_3-N leaching (Hashimoto et al., 2007). These concerns led the World Health Organization to set limits on the amount of nitrates in drinking water. The incomplete capture or poor conversion or excessive usage of N fertilizer also plays a large role in stratospheric ozone depletion and global warming through nitrous oxide emissions (Wuebbles, 2009). The overuse of N fertilizer is a reason of air pollution of the wider environment by ammonia emissions (Misselbrook et al., 2000). These are causing serious N pollution and become a threat to global ecosystems (Giles, 2005).

Hence, developing crops that are less dependent on the heavy application of N fertilizer with high nitrogen use efficiency is essential for the sustainability of agriculture. It is estimated that a 1% increase in NUE could save about $1.1 billion annually (Kant et al., 2011). Advances in molecular marker technology over the past decade have led to the development of detailed molecular linkage maps in rice (Harushima et al., 1998). QTL mapping is the most available method towards understanding the molecular genetics mechanisms of complex quantitative traits behind phenotypic complexity (Guo et al., 2004; Zhang et al., 2011). QTL mapping methods have been adopted in studying nitrogen use efficiency and related parameters in rice. Fang et al. (2001) reported 8 QTLs for plant height under nutrient solution culture and 13 QTLs under soil culture in DH population of IR64/Azucena. In the research of 239 RILs from a cross between two *indica* parents with two N levels, 12 QTLs for root weight, 14 QTLs for shoot weight, 12 QTLs for plant weight were identified by Lian et al. (2005). A total of 7 QTLs for nitrogen deficiency tolerance traits at seedling stage (relative shoot dry weight, relative plant dry weight, relative maximum root length, relative plant height) in a RIL population of two *indica* crosses were detected by Feng et al. (2010). For NUE-a complex trait, some QTLs were reported in previous studies. One QTL on chromosome 6 was detected for NUE by Shan et al. (2005) in a RIL population of Zhenshan97/Minghui63- two *indica* cultivars. Wei *et al.* (2011) when investigated 127 RILs from Zhenshan97/Minghui63 cross in the field experiment concluded a total of 4 QTLs and 6 QTLs in another trial for NUE under two N levels of N supply. Although NUE has been defined in various ways (Good et al., 2004): absorption NUE (aNUE) was calculated by dividing the total net N absorbed of plant by unit of N applied; physiological NUE (pNUE) was defined as the total net dried matter per unit of N absorbed (Mosier et al., 2004); agronomic NUE (agNUE) was computed by dividing the total net dried matter to unit of available soil N (native and applied) (Mosier et al., 2004; Samborski et al., 2008), no study has been conducted mapping for all three calculated NUEs under different N

conditions. Moreover, information of the loci or genes related to NUE in different ways is very useful for breeders in molecular marker assisted breeding.

Therefore, the objectives of this study were to identify the QTLs for aNUE, pNUE, agNUE and related parameters in rice at vegetative stage under different N conditions and to gain a better understanding that might be useful for improving NUE of rice cultivars.

2. MATERIALS AND METHODS

2.1. Plant materials

The QTL analysis was performed using the segregating population developed by the Research Institute for Development (IRD) in Montpellier, consisting of F_{9-10} recombinant inbred lines (RILs) obtained by the single-seed descent method from a cross between IR64 (*O. sativa* L. *subsp. indica*), considered insensitive to nitrogen supply under low N condition and Azucena (*O. sativa* L. *subsp. japonica*), an intermediate cultivar between sensitive and insensitive group (Namai et al., 2009; Hamaoka et al., 2013).

2.2. Nitrogen application

The standard Yoshida solution (Yoshida et al., 1976) with the nitrogen source of 1.43mM NH_4NO_3 was used as the control and considered as 1X.

For the experiment during period from February 15th to April 10th, 2011 (the first replication) two different nitrogen concentrations of Yoshida solution: 1X and X with 1.43mM and 0.358mM NH_4NO_3 were applied.

For the experiment during period from October 5th to November 30th, 2011 (the second replication) three different nitrogen concentrations of Yoshida solution: 1X, X and 1/8X with 1.43mM, 0.358mM, and 0.179mM NH_4NO_3 were used.

The choice of the N supplies in the nutritive solution of the treated plants and the duration of the treatment was based on the result obtained from our previous study on effect of different nitrogen concentration to components of NUE and related parameters in rice plants under hydroponic culture.

2.3. Growth conditions and screening of the population

The experiment was conducted under hydroponic culture in phytotron at Université Catholique de Louvain, Belgium and replicated twice in 2011. The first replication was implemented from February 15th to April 10th, 2011and the second, from October 5th to November 30th, 2011. Each replication consisted of three replicate.

The seeds of each RIL and the parent cultivars were sown in Petri dishes lined with Whatman No.1 filter paper moistened with 10 ml demineralized water for 3 days. The germination was maintained at 28°C, 12-h day length and 120 µmol m^{-2} s^{-1} light intensity.

The germinated seeds of each RIL and the parents were selected to ensure the homogeneous germination. For all three independent replicate of each experiment, two or three seeds of each RILs and the parents were placed on each hole within perforated extruded polystyrene plates. The polystyrene plates were kept floating on 26L $^{-}$ tank consisting standard rice nutrient solution (Yoshida et al., 1976) in a phytotron for 2 weeks. Each plate in each tank contained seeds of 44 RILs, Azucena and IR64 cultivar. The growth condition was maintained at 30/25°C day/night, 85-95% relative humidity and 12-h photoperiod with 360µmol $m^{-2}s^{-1}$ light intensity.

After two weeks, one healthy and homogeneous seedling per each hole within perforated extruded polystyrene plates was selected. After two times of selection one for homogeneous germination, one for homogeneous seedling- 169 RILs observed for the first experiment and 158 RILs for the second experiment. Thus the total of 1,062 plants from 24 tanks for experiment in period from February

15th to April 10th, 2011and 1494 plants from to 36 tanks for experiment during period from October 5th to November 30th, 2011 were screened and individually observed.

The nutrient of the control and treated solutions was renewed once a week. The pH of the solution was daily adjusted to 4.5 (Wu et al.,1998) using 1M KOH and 1M HCl. Treatments and plants in the experiment were completely randomized towards the environmental conditions by re-arranging the tanks every two days in phytotron.

2.4. Phenotypic data

Four weeks after treatment all the plants were evaluated for chlorophyll content index (CCI), plant height (PH), number of leaves (NL), number of tillers (NT), fresh weight of leaf blades (FWL), fresh weight of leaf sheaths plus stems (FWS), fresh weight of roots (FWR), total fresh matter (FM), dry weight of leaf blades (DWL), dry weight of leaf sheaths plus stems (DWS), dry weight of roots (DWR), and total dry matter (DM) on a single plant basis from all three replicate across all RILs and the parents and different nitrogen levels. The chlorophyll content index was measured on the middle upper face of the youngest fully expanded leaf using a Chlorophyll Content Meter (CCM8200 model, Opti-Sciences, Hudson, USA).

At harvest, the plants were cut at collar, and then separated into three parts: leaf blades, leaf sheaths plus stems, and roots. The fresh weights were measured right after separating. The dried weights were determined after oven drying at 60°C to a constant weight. The total dry weight (DM) was determined as the sum of dry weight of three separated organs, i.e. dry weight of leaf blades (DWL), dry weight of leaf sheaths plus stems (DWS), dry weight of roots (DWR).

A selection procedure was applied to the RILs in order to study the remaining parameters, which were too time-consuming and costly to allow the analysis on each of the 169 RILs and their parents. The RILs were classified according to their relative variation of dry matter by comparing plant dry matter of the control and the treatments according to the formula:

Relative variation of dry matter = [(DM control plant - DM treated plant) / DM control plant)] x 100

The RILs with extreme value were chosen to analyze N concentration. Ten RILs that expressed the minimum values of relative variation and other ten RILs that had the maximum values were used in the first experiment and twenty RILs/each extreme sides were selected for second experiment. For both of experiments, parental cultivars-IR64 and Azucena/each tank were analyzed for N tissue concentrations.

2.5. Nitrogen tissue concentration

The oven-dried leaf blades, leaf sheaths plus stem and roots of selected RILs and parental cultivars at two and three different nitrogen doses of the first and the second experiment, respectively, were ground separately to obtain fine powdered samples. Six mg of each sample were used for analysis of nitrogen concentration by using FLASH NC Analyzers (Model AE1112, CE Instruments UK).

2.6. NUE calculation

The nitrogen use efficiencies (NUEs) were calculated as follows:

Physiological NUE (pNUE) = [Total dry matter (g plant^{-1})]/[Total N absorbed (g plant^{-1})] [1]

Absorption NUE (aNUE) = [Total N absorbed (g plant^{-1})]/[Total N applied (g)] [2]

Agronomical NUE (agNUE) = [Total dry matter (g plant^{-1})]/[Total N applied (g)] [3]

The N absorption in each organ was calculated by multiplying of N concentration with dry weight of organ. The total net absorbed N was determined as the sum of N accumulation in all three organs. The total applied N was calculated basing on the N supply in culture solution in 2 weeks for germination and 4 weeks for treatments.

2.7. Statistical analysis and QTL mapping

Data analysis was performed with the SAS statistical program (version 9.2, SAS Institute, North Carolina, USA). The ANOVA assumption of normality was checked for all analyzed data. The effect of lines, N deficiency treatment and repetition on the parameters measured was tested using a three-way ANOVA, mixed model with three crossed factors: two fixed factors (lines and treatments) and one random factor (repetition).

The map consists of 228 marker loci, the allelic composition for each of the 169 RILs and their parents for each marker locus was determined by Ahmadi et al. (2005). The average genetic distance between the markers was about 7cM with a maximum distance of 23cM and a minimum of 0.2cM. QTLs were analyzed jointly by composite interval mapping for multiple traits of each experiment (Dufey et al., 2009) using the Windows QTL Cartographer software package version 2.5. The walking speed chosen for all QTL analyses was 2cM. The threshold for declaring a QTL for the various traits was from 3.0 as a minimum. If the LOD score exceeded the threshold, the position with the highest LOD score on each chromosome was estimated as the most likely position of the QTL. To present a QTL on the map, the chromosome region corresponding to a LOD greater than the maximum LOD minus 1 was selected, called an LOD-1 interval (Hirel et al., 2001) and considered as position interval.

Fort traits that were measured only on 20 RILs (N tissue concentrations and derived parameters-NUEs) in the first experiment or 40 RILs in the second experiment, phenotypic values of non-measured individuals were included into the analysis as missing values in order to avoid biased estimates of QTL effects (Lander and Botstein, 1989).

3. RESULTS AND DISCUSSION

3.1. Performance of RILs and parents

Chlorophyll content index (CCI), plant height (PH), number of leaves (NL), number of tillers (NT), fresh weight of leaf blades (FWL), fresh weight of leaf sheaths plus stems (FWS), fresh weight of roots (FWR), total fresh matter (FM), dry weight of leaf blades (DWL), dry weight of leaf sheaths and stems (DWS), dry weight of roots (DWR), total dry matter (DM), N concentration in leaf blades (%NL), N concentration in leaf sheaths plus stems (%NS), N concentration in roots (%NR) and derived parameters, i.e., absorption NUE (aNUE), physiological NUE (pNUE) and agronomical NUE (agNUE) were investigated under normal and low N conditions. All traits segregated continuously and almost fitted normal distribution under all N supplied (Data not shown). The frequency distributions showed more extreme values than the parents for most of parameters suggested that both parents may carry interesting alleles for NUE and related traits.

3.2. Identifying QTLs for N-related traits

The joint QTL analysis of supplied N levels for multiple traits of each experiment was performed. The result of the first experiment revealed a total of 44 QTLs. Among of them 36 QTLs were detected for NUE-related traits (Table 1). These QTLs were located on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 (Figure 1). The result of second experiment revealed a total of 44 QTLs with 36 QTLs for NUE-related traits (Table 2). These QTLs were located on chromosomes 1, 2, 3, 5, 6, 7, 8 and 12 (Figure 2). The probable position of the QTLs (Figure 1, 2) was determined as described by Hirel et al. (2001), by LOD-1 from the maximum. When two LOD peaks fell in a common support interval, it was considered that only one QTL was present and its approximated position was given by the greatest peak. For this reason, a total of 42 QTLs are presented in Figure 1 instead of 44 QTLs for the first experiment and 35 QTLs are presented in Figure 2 instead of 44 for the second experiment.

In the present study, joint QTL for multiple traits was undertaken using a RIL population of

Table 1. Joint QTLs analysis for number of leaves (NL), number of tillers (NT), plant height (PH), total fresh matter (FM), dry weight of roots (DWR), dry weight of sheaths plus stem (DWS), dry weight of leaf blades (DWL), total dry matter (DM), chlorophyll content index (CCI), N concentration in roots (%NR), N concentration in sheaths plus stem (%NS), N concentration in leaf blades (%NL), absorbed NUE (aNUE), physiological NUE (pNUE), and agronomical NUE (agNUE) of the first experiment

No.QTL	Trait[a]	Chromosome number[b]	Marker Interval[c]	Position(cM)[d]	Joint LOD score[e]	Interval Position(cM)[f]
1	NL	2	RM250-RM166	136.20	3.36	131.5-136.3
2		7	RM214-RM2819	19.94	3.12	12.4-32.2
3		8	RM080-RM230	124.21	5.17	117.5-128.5
4		12	RM020a-RM004a	11.83	3.16	4.1-19.4
5	NT	5	RM440-RM188	88.46	3.82	76.9-95.7
6		5	RM538-RM274	110.17	4.23	106.8-118.1
7		7	RM481-RM125	5.76	3.49	4.5-11.6
8		8	RM433-RM230	124.21	3.10	118.1-129.9
9		10	RM171-RM294a	74.38	3.91	69.1-78.1
10	PH	1	RM431-RM165	155.06	3.55	148.5-155.1
11		3	RM468-RM143	163.66	3.09	154-169.9
12	FM	5	RM440-RM188	88.46	4.08	79.0-96.1
13	DWR	3	RM2334-RM426	112.21	4.88	105.7-114.6
14		3	RM468-RM143	157.66	3.54	151.6-167.5
15		3	RM514-RM442	170.59	3.29	167.7-170.6
16		5	RM440-RM188	91.46	4.48	86.0-97.7
17	DWS	3	RM293-RM468	151.19	3.23	143.8-165.5
18		5	RM440-RM188	91.46	3.45	77.9-99.5
19		7	RM481-RM125	5.76	4.13	2.0-12.5
20		8	RM433-RM230	124.21	3.10	117.9-129.6
21	DWL	3	RM468-RM143	157.66	3.15	151.6-169.9
22		5	RM440-RM188	91.46	3.16	76.9-98.7
23	DM	3	RM468-RM143	157.66	3.10	144.1-167.0
24		5	RM440-RM188	88.46	3.30	77.3-98.1
25	CCI	4	RM261-RM307	22.97	3.49	20.5-26.2
26	%NR	1	RM476a-RM084	14.09	3.09	12.2-25.4
27		2	RM279-RM423	17.15	4.24	10.5-20.4
28		5	RM289-RM509	46.60	3.54	37.2-54.6
29	%NS	1	RM443-RM403	106.58	4.25	102.9-110.2
30		3	RM016-RM135	102.17	3.54	96.7-106.0
31		6	RM275-RM030	88.83	3.23	86.5-93.7
32	%NL	1	RM265-RM315	129.78	6.33	128-131.1
33		1	RM472-RM431	137.65	11.44	134.7-141.5
34		3	RM135-RM503	105.98	5.54	99.7-115.1
35		3	RM2334-RM426	112.21	5.34	98.5-115.4
36		3	RM055-RM3199	126.82	4.01	121.6-131.1
37	aNUE	2	RM526-RM221	109.63	6.71	106.3-110.5
38		2	RM221-RM318	114.41	6.71	113.2-118.3
39		5	RM413-RM153	13.93	4.20	10.7-16.1
40		5	RM153-RM013	23.07	4.45	18.5-29.0
41	pNUE	1	RM319-RM265	122.46	4.60	120.4-140.7
42		1	RM315-RM472	131.82	5.32	128.0-137.3
43	agNUE	3	RM468-RM143	157.66	3.75	145.7-166.2
44		5	RM440-RM188	91.46	3.56	78.7-98.0

[a] *Parameter analyzed;* [b] *Chromosome number where the QTL were detected.;* [c] *Marker interval in which is located the most probable position of the QTL (LOD score maximum);* [d] *Most probable position of the QTL (in cM);* [e] *Likelihood ratio;* [f] *Position interval in which is located the probable position of the QTL (by LOD-1 support interval).*

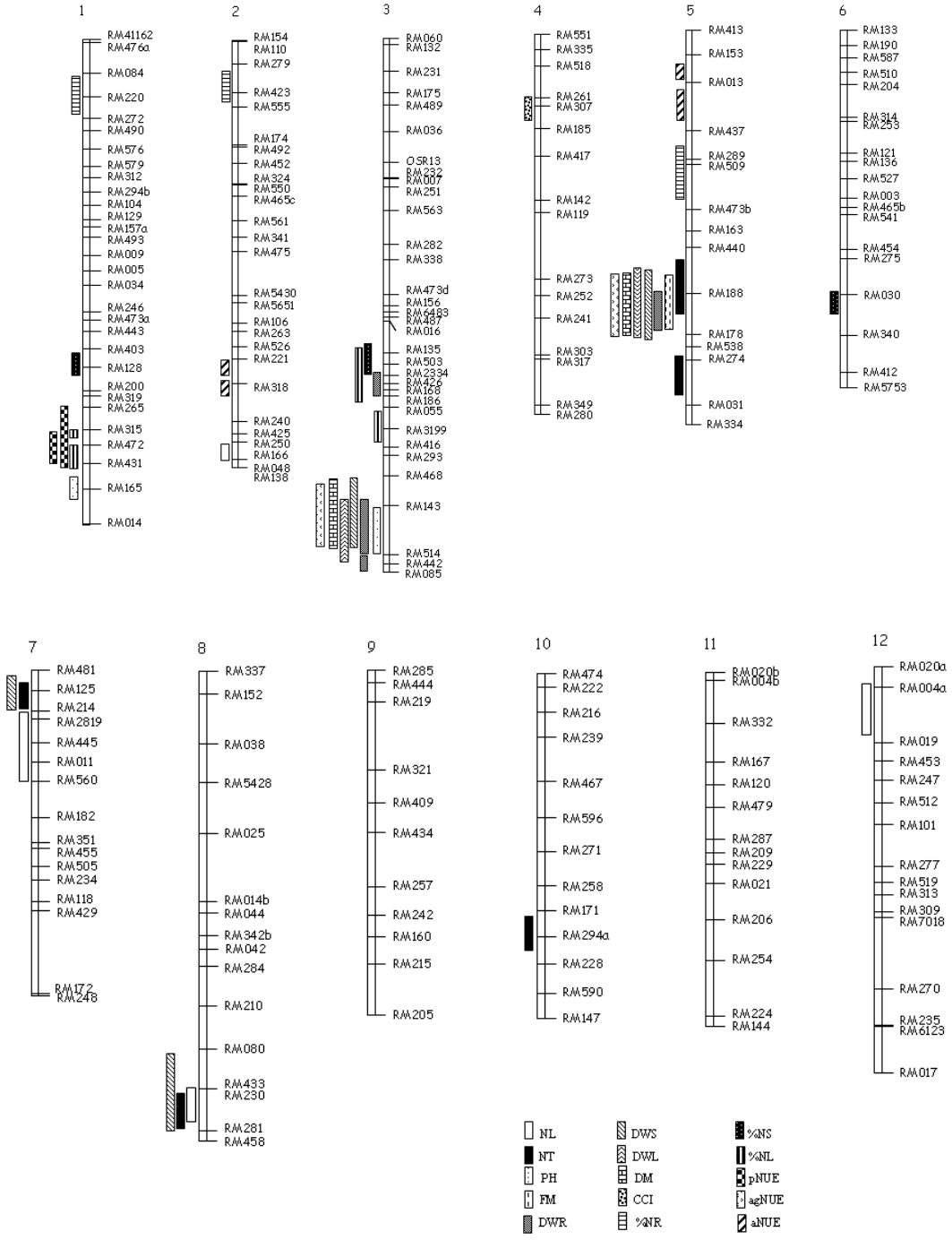

Figure1. Location of joint QTLs for number of leaves (NL), number of tillers (NT), plant height (PH), total fresh matter (FM), dry weight of roots (DWR), dry weight of sheaths plus stem (DWS), dry weight of leaf blades (DWL), total dry matter (DM), chlorophyll content index (CCI), N concentration in roots (%NR), N concentration in sheaths plus stem (%NS), N concentration in leaf blades (%NL), absorbed NUE (aNUE), physiological NUE (pNUE), and agronomical NUE (agNUE) of the first experiment

an IR64/Azucena cross in two separated experiments under normal and N deficiency conditions. Several common regions, on which some QTLs for several traits were located, were found within each experiment. The commonalities between two experiments also were detected.

In the first experiment the common regions were found on chromosome 1 (from 119cM to 137cM flanked by RM265-RM431); on chromosome 3 (91-116cM and 142-170cM positioned from RM016 to RM186 and from RM468 to RM442); on chromosome 5 (70-102cM presented for RM440-RM538) and on chromosome 8 (106-129cM, RM080-RM281) (Figure 1). The common region on chromosome 1 contained the QTLs of %NL and pNUE. The common regions on chromosome 3 included the QTLs of %NS, %NL, PH, DWR, DWS, DWL, DM. The QTLs of NT, FM, DWR, DWS, DWL, DM were detected on the common region of chromosome 5 and the common one on chromosome 8 were the locations of QTLs of NLNT, DWS. In the second experiment the common regions were detected on chromosome 3 (126-151cM, RM3199-RM143) and chromosome 8 (106-129cM, RM080-RM281) (Figure 2). The common region on chromosome 3 included the QTLs of NL, PH, FM, DWR, DWS, DWL and DM. The QTLs of NL, FM, DWR, DWS, DWL, DM were detected on the common region of chromosome 8. The common regions for several traits highlight the linkage between parameters analyzed (Dufey et al., 2009) and suggested that these regions should be highly involved in expression of N effect and NUE traits.

The analysis of the first and second experiment showed that the QTLs for the traits detected separately in two experiments were mostly different, although several QTLs were found to have the confidence interval overlapped such as DWS, DWL, DM on chromosome 3; NL, DWS on chromosome 8 or on very close regions, i.e., PH on chromosome 1, 3; DWR, DWL on chromosome 3 (Figure 1, 2). Although it is not possible to rule out the possibility of two QTLs in close linkage, it is more likely that it is the same QTL with pleiotropic effects on these two traits. Besides that, the commonalities on chromosome 1 (119-137cM), on chromosome 3 (142-170cM) and on chromosome 8 (106-129 cM) were also identified. The certain commonalities existed within each experiment and between experiments as reflected by the QTL hotspots (Lian et al., 2005).

In this study the hotspot flanked by RM3199- RM514 on chromosome 3 containing several QTLs of PH, FM, DWR, DWS, DWL, DM has been reported for QTL of DWR, DWS by Dufey et al. (2009) using the same RIL population of an IR64/Azucena cross with the same marker map. Wei *et al.* (2012b) found that this region was associated with grain filling ratio, 1000-grain weight in the study of RILs derived from two *indica* Zhenshan 97 x Minghui 63. The region on chromosome 1 within interval RM319-RM165 containing QTL for PH has also been identified by Fang and Wu (2001) in the research of DH population from across between IR64 and Azucena. The genomic region RM174-RM324 on chromosome 2 that was found to contain the QTL for NT in the first experiment has been reported to have QTL for PH by Liang et al. (2011) in RILs of two *indica* Xieqingzao B/Zhonghui 9308 cross. The region flanked by RM475-RM5430 on chromosome 2 found to contain the QTL for CCI in the second experiment has been identified for QTLs of grain yield simultaneously under low and normal N by Wei et al. (2012b).

3.3. Identifying QTLs for NUE traits

A total of 8 QTLs were detected for pNUE, aNUE and agNUE on chromosome 1, 2, 3 and 5 in the first experiment (Table 1 and Figure 1). Two QTLs for pNUE with LOD peaks fell in a common support interval, therefore only one QTL with the greatest peak was present. Four QTLs for aNUE were located on chromosome 2 and 5; two QTLs for agNUE were positioned on chromosome 3 and 5. In the second experiment, a total of 8 QTLs were identified for aNUE and agNUE on chromosome 3, 6, 7 and 8 (Table 2 and Figure 2). Among these QTLs, two QTLs for aNUE and agNUE were detected at the same genomic region RM3199-RM143 on chromosome 8. This region was

Table 2. Joint QTLs analysis for number of leaves (NL), number of tillers (NT), plant height (PH), total fresh matter (FM), dry weight of roots (DWR), dry weight of sheaths plus stem (DWS), dry weight of leaf blades (DWL), total dry matter (DM), chlorophyll content index (CCI), N concentration in roots (%NR), N concentration in sheaths plus stem (%NS), N concentration in leaf blades (%NL), absorbed NUE (aNUE), physiological NUE (pNUE), and agronomical NUE (agNUE) of the second experiment

No.QTL	Trait[a]	Chromosome number[b]	Marker Interval[c]	Position(cM)[d]	Joint LOD score[e]	Interval Position (cM)[f]
1	NL	3	RM489-RM036	36.40	3.70	31-41.9
2		3	RM416-RM293	135.63	4.22	130-141.1
3		7	RM125-RM214	11.57	3.28	9.6-13.5
4		8	RM210-RM080	115.93	4.97	109.2-130.2
5		8	RM433-RM230	124.21	5.84	119-128.9
6		12	RM453-RM247	32.10	3.13	29.2-31.7
7		12	RM512-RM101	53.75	3.77	44.6-60.3
8		12	RM7018-RM270	91.20	3.06	78.8-97.2
9	NT	2	RM492-RM452	40.15	3.10	34.6-43.4
10		12	RM7018-RM270	91.20	4.87	85-97.9
11	PH	1	RM319-RM265	125.46	5.16	119.4-142.1
12		1	RM315-RM472	134.82	6.16	127.7-139.5
13		3	RM293-RM468	142.19	3.02	137.5-148.9
14	FM	3	RM3199-RM416	132.81	4.25	129.3-150
15		3	RM293-RM468	142.19	5.30	137.2-146.4
16		8	RM433-RM230	121.21	3.60	111-129.6
17	DWR	3	RM3199-RM416	132.81	3.95	128.1-149.1
18		3	RM293-RM468	142.19	4.88	136.8-146.1
19		8	RM433-RM230	124.21	3.44	111.7-129.6
20	DWS	1	RM005-RM034	85.99	3.77	81.1-89.6
21		3	RM055-RM3199	129.82	4.22	127.2-148
22		3	RM055-RM3199	142.19	4.46	128.1-147.1
23		8	RM433-RM230	118.21	3.99	110.8-128.8
24		12	RM453-RM247	32.10	3.05	28.9-36.5
25	DWL	3	RM3199-RM416	132.81	3.59	128.6-151.6
26		3	RM293-RM468	142.19	4.57	136.6-147.7
27		8	RM433-RM230	118.21	4.33	111.8-129.3
28	DM	3	RM3199-RM416	132.81	4.12	127.9-148.9
29		3	RM293-RM468	142.19	4.80	129.5-146.8
30		8	RM433-RM230	121.21	3.99	110.8-130
31	CCI	2	RM561-RM341	64.15	3.42	60.9-68.4
32		2	RM341-RM475	77.55	3.97	70.7-82.4
33		3	RM055-RM3199	129.82	5.37	125.6-132
34		3	RM416-RM293	135.63	4.62	123.8-140.7
35	%NR	3	RM143-RM514	167.70	3.40	157.8-170.5
36	%NL	5	RM473b-RM163	65.18	4.05	57.4-75.4
37	aNUE	3	RM055-RM3199	126.82	3.57	123.6-131.5
38		6	RM527-RM003	54.56	3.66	50.8-56.1
39		6	RM465b-RM541	65.90	3.39	58.9-78.1
40		7	RM118-RM429	77.12	3.80	72.5-83.6
41		8	RM210-RM080	115.93	7.23	110.8-122.6
42	agNUE	3	RM3199-RM416	132.81	4.13	127.9-149.1
43		3	RM293-RM468	142.19	4.82	129.7-146.6
44		8	RM433-RM230	121.21	3.99	111-130

[a] Parameter analyzed; [b] Chromosome number where the QTL were detected; [c] Marker interval in which is located the most probable position of the QTL (LOD score maximum); [d] Most probable position of the QTL (in cM); [e] Likelihood ratio
[f] Position interval in which is located the probable position of the QTL (by LOD-1 support interval).

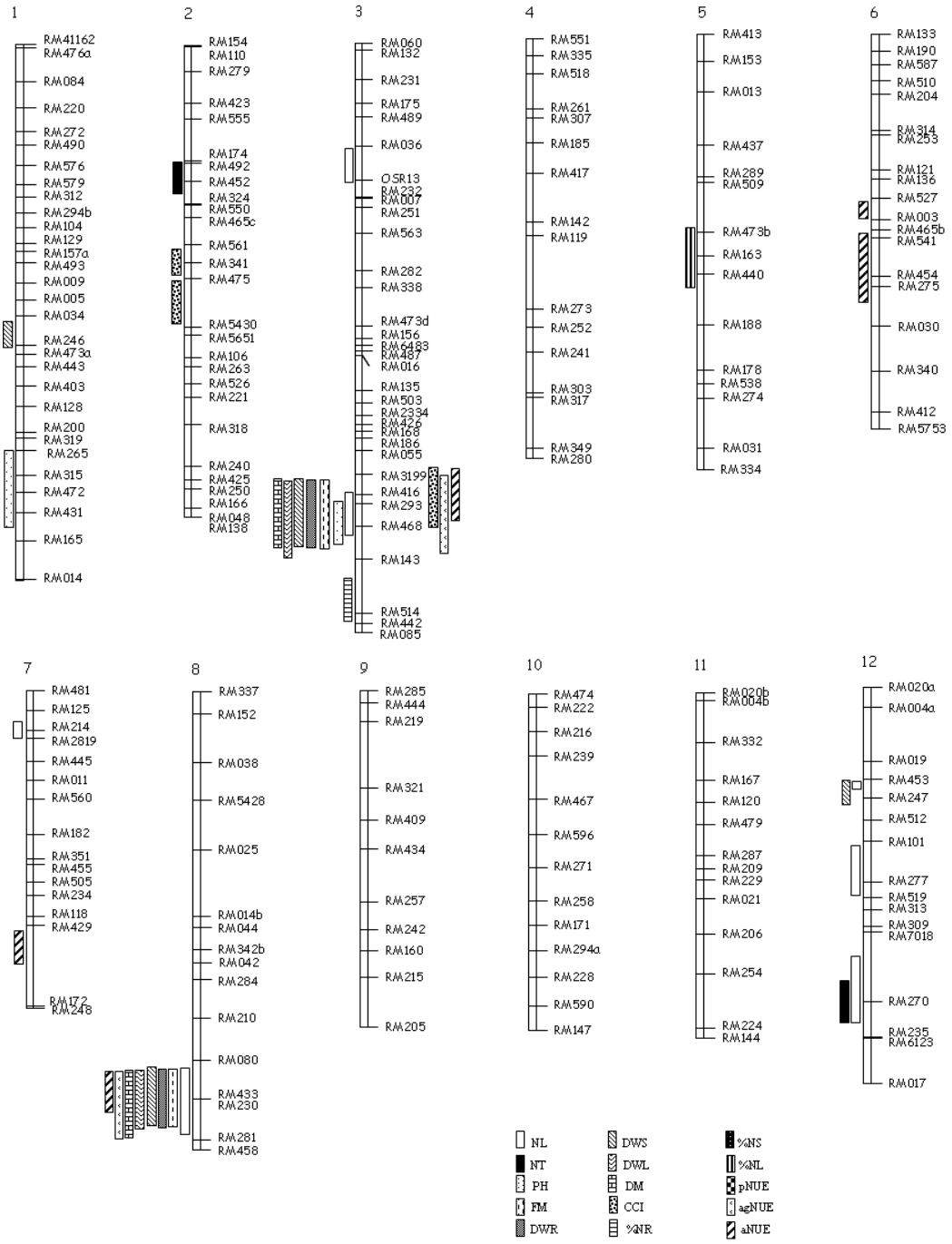

Figure 2. Location of joint QTLs for number of leaves (NL), number of tillers (NT), plant height (PH), total fresh matter (FM), dry weight of roots (DWR), dry weight of sheaths plus stem (DWS), dry weight of leaf blades (DWL), total dry matter (DM), chlorophyll content index (CCI), N concentration in roots (%NR), N concentration in sheaths plus stem (%NS), N concentration in leaf blades (%NL), absorbed NUE (aNUE), physiological NUE (pNUE), and agronomical NUE (agNUE) of the second experiment

identified as a hotspot containing QTLs of N-related traits. The presence of common QTLs for several traits suggested that they can be improved simultaneously. Two QTLs for agNUE on chromosome 3 had LOD peaks fell in a common support interval, so only one QTL was presented.

In these QTLs, some QTLs were new ones and some QTLs were matched with the QTLs of NUE in the previous reports. The genomic region flanked by RM3199 and RM143 on chromosome 3 was detected for QTLs of aNUE, agNUE and some N-related traits (NL, PH, FM, DWR, DWS, DWL and DM). Senthilvel et al. (2008) found that this region was associated with NUE in their research of DH population derived from IR64/Azucena cross. Although it was difficult to say whether the chromosomal locations of QTLs are the same due to the lack of common markers, Wei et al. (2012a) detected a QTL for NUE on chromosome 3 which is very close to QTL of agNUE in the first experiment by using RILs cross from two indica. Wei et al. (2012a) also identified a QTL for NUE at overlapped genomic region of aNUE on chromosome 7 in the second experiment. In the genomic regions of RM 527-RM003 and RM 465b-RM030 on chromosome 6, where aNUE QTLs was detected in the present sudy, two QTLs for PH was positioned by Liang et al. (2011).

4. CONCLUSION

Among 44 QTLs in the first experiment and 44 QTLs in the second experiment for aNUE, pNUE, agNUE and other N-related traits under normal-N and low-N conditions, the QTLs for agNUE, DWS, DM on chromosome 3 and the QTLs for NL, DWS on chromosome 8 were identified in both experiments at the same or overlapped genomic regions. Several hotspots flanked by RM265- RM165 on chromosome 1, by RM3199- RM514 on chromosome 3, by RM080- RM281 on chromosome 8 containing QTLs for aNUE, pNUE, agNUE and some other traits were identified. This suggested that these genomic regions could be used as targets for a better understanding of NUE and for improving NUE traits.

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