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RESEARCH ARTICLE

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Meta-QTLs linked to nitrogen use efficiency are randomly distributed in Indian rice germplasm

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Abstract

Nitrogen (N) recognized as a critical element for plant growth plays a fundamental role in rice cultivation. The N use efficiency (NUE) hovers around 30-35% in rice, suggesting a significant loss of N from the rice fields. Improving the NUE therefore would require genetic interventions and breeding. The cultivar improvement for N uptake and utilization is required to elevate NUE to further heights. Several quantitative trait loci (QTLs) for NUE under varying conditions and genetic backgrounds have been reported in rice. Consolidation of this distributed and unorganized information is necessary to identify critical genomic regions to be used for crop improvement. Therefore, a Meta-analysis from an assembly of 506 QTLs reported from 18 different studies was performed to identify the most significant genomic regions associated with NUE in rice. A total of 12 meta-QTLs (mQTLs) related to the traits such as NUE and grain yield per plant under N deficit conditions have been identified over four rice chromosomes namely 1, 3, 4, and 8. Evaluation of these mQTLs in a set of Indian rice germplasm revealed a significant association of the meta loci with N use parameters and showed wide distribution in the germplasm. Identification of mQTLs on different chromosomes together with their respective markers will help recruit them in marker-assisted selection (MAS) to develop N use efficient genotypes.

Keywords: Crop production, marker-assisted selection, meta-QTLs, nitrogen use efficiency, rice

Introduction

Rice (*Oryza sativa* L.), the principal cereal crop, requires more nitrogen (N) than any other crop of the world. As a plant nutrient element, availability of N is naturally limited in the soil due to its high mobility and volatility. Therefore, intervention through fertilization is essential in agriculture to maintain the soil N status. Although under-use of N can jeopardize crop production, excess use can end up in environmental degradation. Owing to several factors, exogenous and endogenous, plants are unable to utilize applied N efficiently. The exogenous factors include soil denitrification and rapid N loss through leaching, volatilization, and run-off. The average level of N use efficiency among the field crops is about 33% (Abrol et al. 1999), with crop utilization ranging between 30 - 40 % of the applied N (Raun and Johnson 1999). A sizeable portion of the unutilized N is lost into the environment and becomes unavailable to the plants (Abrol 1 et al. 2007). A sustainable solution for this impasse is to develop nitrogen-efficient varieties that can give optimum or high yield at reduced N input. The plant use of applied N in agriculture is measured in terms of the parameter, overall N use efficiency (NUE) which is the ratio of grain yield to the applied N (Pathak et al. 2008). NUE has two components, N uptake efficiency (NUpE) and N utilization efficiency (NUtE), the product of which gives NUE.

Improving NUE is possible through breeding because the trait is genetically regulated, but the progress is slow due to the low heritability of the trait and its complex regulatory nature (Gallais and Hirel 2004). Quantitative

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genetic variation in responses to low N has already been reported in rice and several QTLs have been identified. These QTLs are predominantly minor and numerous (Vinod and Heuer 2012) and are associated with several traits related to N nutrition and efficiency. Since many of the QTLs fall specific to the population from which they are mapped, the utility of such QTLs is limited in breeding. Therefore, it is essential to identify QTLs that are consistent across different backgrounds. Especially for complicated traits like N response, where there is a large number of reported QTLs but with low effects, identification of meta-QTLs (mQTLs) would be a great boon in utilizing them for crop improvement programs (Goffinet and Gerber 2000). A Meta-analysis is a computational procedure for identifying consensus QTLs that are located at the same locus with statistical confidence. The procedure involves comparison and identification of consensus QTLs using model-based strategies. Although several N related QTLs are reported, a meta-analysis of QTLs has not been done so far. Once identified mQTLs can be utilized for marker-assisted improvement of target varieties. The purpose of this study is to identify mQTLs for N use and related traits in rice from the reports of the last two decades and to investigate their distribution in Indian rice germplasm.

Materials and methods

Plant materials

An assembly of 65 rice varieties, recommended for cultivation at different parts of India was collected from the Division of Genetics, ICAR-Indian Agricultural Research Initiate at New Delhi. The genotypes consisted of cultivars, landraces, Basmati lines out of which some are known identified as N efficient verities (Supplementary Table S1). The genotypes were field grown and the genomic DNA was extracted using standard protocols (Murray and Thomson 1980).

Meta-analysis of QTLs

Initially, the published information on QTLs related to NUE in rice was surveyed for the period between 2001 to 2021. Data were collected on the reported QTLs and the linkage maps from which the QTLs are mapped in each study. Data included N use parameters and agronomic traits related to N uptake and assimilation. The meta-analysis was performed as per the algorithms built into the software, BioMercator v.4.2.3 (Sosnowski et al. 2012). BioMercator is a suite of Java-based programs integrated into a single package, that performs analyses in three steps, (a) construction of a consensus map, (b) QTL projection and (c) Meta-analysis.

QTL data compilation

The collected data were formatted as text files as prescribed in the software manual. For each study two sets of files were generated, a map file and a QTL file. The input data for the map file required details of the mapping population, size of the mapping population, marker type, number of markers used, chromosome label, marker label and map position. Supplementary information such as organism name, species, cross-type, parents used, mapping function, map name, map expansion, map quality, locus location were also incorporated in the map data. In the QTL file, details on traits used for QTL identification, QTL names, linkage group, LOD values, R^2 values, and flanking markers were included. QTL data had additional information such as experimental location, year and trait ID. The data were first compiled in a Microsoft Excel worksheet and the QTL data and the map data were individually saved in tab-delimited text files before loading into the software.

Development of a consensus map and QTL projection

The compiled data were input into the software, in the order map data first followed by QTL data. Then the final consensus map was generated through a one-step procedure using weighted least squares by accounting for the statistical properties of the estimated genetic distances. The consensus map is separately named and used subsequently for QTL projection. This process involved superimposition of the QTLs on the consensus map using an algorithm that used a dynamic procedure to identify an optimal context to place the QTL on the map. The QTL placement depended on the pair of common markers that bracketed the QTL in the input maps, provided the interval of the markers and their positions were in correspondence between the maps.

Model-based detection of mQTLs

A mQTL analyses was performed using two approaches. The first one used a QTL pooling algorithm developed by Goffinet and Gerber (2000) in which five models were built to test whether the QTLs detected from different experiments were congruent. By assuming a Gaussian (normal) distribution, the QTL models were analyzed for best fit using maximum likelihood estimates. Akaike information criterion (AIC) from the model was compared for the minimum value and the model with the lowest AIC value was taken as the best fit model. The analysis was based on the variance at the QTL position originating from different independent experiments estimated through confidence intervals (CI).

The second approach (Veyrieras et al. 2007) used QTL a clustering procedure based on the Gaussian mixed model and the parameter estimates were obtained using the expectation-maximization (EM) algorithm, which used a basic assumption that all the QTL positions followed an asymptotic Gaussian distribution and the variance were functions of their LOD values or amount of phenotypic variation explained. Therefore, a maximum likelihood estimation of QTL positions was possible under the assumptions made. Based on the best fit model, a number of mQTLs were determined and their positions were identified

Table 1. Details of mapping studies used for the identification of meta-QTLs

Cross	Population		Marker		QTLs	References
	Type	Size	Type	Number		
93-11/ Milyang 352	DH	117	KASP	240	28	Kwon et al. 2021
BPT5204/ PTB1	RIL	291	SSR	254	32	Vishnukiran et al. 2020
Zhonghui 9308/ Xieqingzao B	CSSL	75	SSR/InD	120	9	Anis et al. 2018
Xieqingzao B/ Zhonghui 9308	RIL	138	SSR	198	52	Yue et al. 2016
IR64/ Azucena	RIL	174	SSR	228	86	Nguyen et al. 2016
Xieqingzao B/ Zhonghui 9308	RIL	281	SSR	196	13	Dai et al. 2015
Zhenshan 97/ Minghui 63	RIL	127	RFLP/SSR	220	30	Wei et al. 2012
R9308 / Xieqingzao B	RIL	138	SSR	198	21	Feng et al. 2011
Zhenshan 97/ Minghui 63	RIL	127	RFLP/SSR	220	24	Wei 1 et al. 2011
R9308 / Xieqingzao B	RIL	228	SSR	198	7	Feng et al. 2010
IR64/ INRC10192	RIL	140	SSR	113	18	Srividya et al. 2010
Zhenshan 97 / HR5	RIL	138	SSR	245	55	Tong et al., 2011
IR64 / Azucena	RIL	82	RFLP/RAPD	175	16	Senthilvel et al., 2008
Dasanbyeo/ TR22183	RIL	166	SSR/STS	216	20	Cho et al., 2007
IR69093–41–3–2/ IR72	RIL	101	RFLP/SSR	182	62	Laza et al., 2006
Nipponbare/ Kasalath// Nipponbare	BC	98	RFLP	245	13	Obara et al. 2001
IR64/ Azucena	DH	123	RFLP/Isozyme	175	20	Fang and Wu 2001

RIL = Recombinant inbred lines; CSSL = Chromosome segment substitution line; BC = Backcross population; DH= Doubled haploid; SSR = Simple sequence repeat; InD = Insertion/deletion polymorphism; RFLP = Restriction fragment length polymorphism; RAPD = Random amplified polymorphic DNA; STS = Sequence-tagged site; KASP = Kompetitive allele-specific PCR.

along with computed CI values. The identified mQTLs were graphically generated within the programme, which could be scaled to reveal the CI and the linked markers. The estimated CI values were used to determine the precision of mQTLs.

Assessing the mQTL diversity

SSR markers were mined out from the consensus map, at the mQTL peak position and used for genotyping the germplasm panel. The genotype data was then used to classify the panel based on the mQTL diversity. Diversity of genotypes was determined using simple matching coefficients as the genetic distance measure and by clustering using the unweighted neighbour joining method. Diversity analysis was carried out using DarWin v.6.0 (Perrier et al. 2003) and the dendrogram was drawn using Dendroscope v.3.7.6 (Abrol and Scornavacca 2012).

Results

A total of 506 QTLs related to N use efficiency and related agronomic traits including grain yield were compiled from 18 studies published during the period starting from 2001 to 2021. The QTLs had a distribution all over the rice genome (Table 1). The number of individual QTL per population ranged from 7 (Feng et al. 2010) to 86 (Nguyen et al. 2016). Some of these QTLs were located with larger intervals starting from 0.4 cM to 61cM with an average of 14.24 cM.

Table 2. Details of consensus map generated and the projected QTLs

Chromosome	Length (cM)	Density		Average distance (cM)	
		Markers	QTLs	Marker	QTL
1	206.0	288	55	0.7	3.7
3	204.8	134	72	1.5	2.8
4	201.5	79	35	2.6	5.8
5	139.7	194	31	0.7	4.5
6	68.6	153	21	0.4	3.3
8	130.1	148	61	0.9	2.1
9	135.7	124	18	1.1	7.5
10	109.7	96	16	1.1	6.9
11	61.3	137	18	0.4	3.4
Total	1257.3	1353	327	0.9	3.8

QTL = Quantitative trait locus; cM = centimorgan

The consensus map generated contained a total of 1353 markers distributed across a total map length of 1257.3 cM over nine chromosomes, excepting chromosomes 2, 7 and 12 (Table 2). Chromosome 1 had the highest marker density of 288 with 0.7 cM between markers on average. The highest number of QTLs was projected on chromosome 3 (72 QTLs) followed by chromosome 6 with 61 QTLs. Average QTL distance was 2.1 cM on chromosome 8, and 2.8 cM on chromosome 3. In total, the consensus map had one marker over 0.9 cM distance with one QTL over every 3.8 cM distance on average. A lower number of QTLs were projected on

Table 3. mQTLs detected by Goffinet and Gerber method

mQTL	Model	AIC	Weight	Chromosome	Position	Range	Average	
							Interval	Distance
1	2	99.61	0.67	1	81.6	80.2-83.0	3.7	15.0
2	-	-	-	1	96.6	94.8-99.4	-	-
3	4	154.1	0.5	3	63.5	60.3-66.8	4.7	16.2
4	-	-	-	3	93.2	91.4-94.9	-	-
5	-	-	-	3	104.7	102.8-106.6	-	-
6	-	-	-	3	112.1	109.6-114.7	-	-
7	4	118.2	0.66	4	65.1	56.7-73.6	6.6	13.8
8	-	-	-	4	83.5	82.1-84.9	-	-
9	-	-	-	4	90.2	89.0-91.4	-	-
10	-	-	-	4	106.4	104.2-108.5	-	-
11	4	65.7		5	56.8	55.4-58.4	4.7	14.0
12	-	-	-	5	77.1	76.4-77.8	-	-
13	-	-	-	5	84.2	80.7-87.7	-	-
14	-	-	-	5	98.8	95.1-102.5	-	-
15	2	12.5	1	6	30.6	29.2-32.0	7.9	14.2
16	-	-	-	6	44.8	38.4-51.3	-	-
17	4	190.9	0.66	8	16.7	13.4-20.3	5.0	19.6
18	-	-	-	8	47.6	46.4-48.9	-	-
19	-	-	-	8	58	55.1-60.9	-	-
20	-	-	-	8	75.4	73.0-77.8	-	-
21	4	80.6	0.77	9	46.7	42.8-50.5	4.3	10.1
22	-	-	-	9	62.1	60.0-64.3	-	-
23	-	-	-	9	68.5	66.9-70.1	-	-
24	-	-	-	9	77	76.1-78.0	-	-
25	4	84.9	0.51	10	27.2	25.2-29.2	4.3	13.1
26	-	-	-	10	41.5	39.9-43.2	-	-
27	-	-	-	10	52.7	51.5-54.0	-	-
28	-	-	-	10	66.4	61.8-69.2	-	-
29	3	88.8	0.48	11	11	8.8-13.2	4.0	11.1
30	-	-	-	11	27	24.4-29.7	-	-
31	-	-	-	11	33.1	31.9-34.3	-	-

AIC= Akaike information criterion

chromosomes 9, 10, 11 and 6.

In the initial meta-analysis carried out using the method of Goffinet and Gerber (2000), a total of 31 mQTLs were identified distributed over nine chromosomes (Table 3). QTLs for traits such as NUE, N content in the shoot (SN), root (RN), grain (GN), total plant (TN), grain yield (GY), biomass (BM) and glutamine synthetase (GS) activity were used for the analysis. The optimal models for mQTLs were determined using the lowest Akaike information criterion (AIC) and highest Akaike weight. Chromosomes 3, 4, 5, 8, 9 and 10 had 4-QTL model optimized while chromosome 1 and 6 had 2-QTL model optimized. Chromosome 11 showed an optimum model with 3-QTLs. The mQTL locations on chromosome 1 had an average interval of 3.7 cM, which was the lowest among all the mQTLs identified, followed by chromosome 11 with a 4.0 cM interval. Other chromosomes with lower mQTL intervals were chromosomes 9 and 10 with 4.3 cM followed by chromosomes 3 and 5 with an average interval of 4.7 cM. The widest interval was observed on chromosome 6 (7.9 cM) followed by 6.6 cM on chromosome 4. Similarly, the distribution of mQTLs over chromosomes based on the average distance between mQTLs ranged between 10.1 cM (chromosome 9) to 19.6 cM (chromosome 8). Chromosomes with wider distribution were chromosome 3 with 16.2 cM average distance followed by chromosome 1 having a distance of 15 cM between mQTLs. The remaining

chromosomes were found to have mQTLs distributed at an average distance of 14.2 cM for chromosome 6, 14.0 cM for chromosome 5 and 13.8 cM for chromosome 4. The lowest distance was found on chromosomes 9, 10 and 11. More elaborate mQTL detection using the QTLClust method developed by Veyrieras et al. (2007), identified 12 mQTLs distributed over four chromosomes 1, 3, 4 and 8 (Fig. 1). Chromosome 1 had two mQTLs, chromosomes 3 and 4 has three mQTLs each and chromosome 8 possessed four mQTLs. These mQTLs were also identified by the previous method (Table 4).

The CI of mQTLs at the 95% level ranged from 0.96 to 7.03 with an average of 4.04. Since the lowest CI determines the most accurate position of mQTLs, only one mQTL on chromosome 1 indicated better accuracy with the lowest CI value. This mQTL, *mQTL1.2* had an average percent variation explained (PVE) of 15.5% and was found associated with NUE and traits such as SN, GN, LN and BM. On chromosome 1, the *mQTL1.1* was located at 81.6 cM position and had a CI of 2.94. This locus had an average PVE of 11.0% and was found associated with traits such as GY, TN, RN, and BM, besides NUE. On chromosome 3, we could detect three meta loci, *mQTL3.1*, *mQTL3.2* and *mQTL3.3*. Among these, *mQTL3.3* was the most robust with a CI of 2.49 and explained a PVE of 26.4%, and having an influence on NUE, RN and GY. Of the remaining, *mQTL3.2* showed a CI of 4.07 cM followed by *mQTL3.1* with a CI of 6.6 cM. On the chromosome 4, three mQTLs detected had CI ranging between 2.23 to 4.63 cM. The first, *mQTL4.1* was found associated with LN and GY besides NUE with a PVE of 14.3%. The *mQTL4.2* was linked to GY but with a PVE of 6.7%. However, the third meta locus, *mQTL4.3* was having an average PVE of 4.5% and was mostly associated with NUE, BM, TN and LN. Among the mQTLs on chromosome 8, *mQTL8.1* and *mQTL8.4* had relatively higher average PVE with 30.1 and 21.4% respectively. Although the CI of *mQTL8.1* was 7.03 cM, it was located distinctly away from other meta loci with a peak position at 13.5 cM on the consensus map. The *mQTL8.2* was located at 46.3 cM, *mQTL8.3* was at 54.9 cM and *mQTL8.4* was at 75.5 cM. These

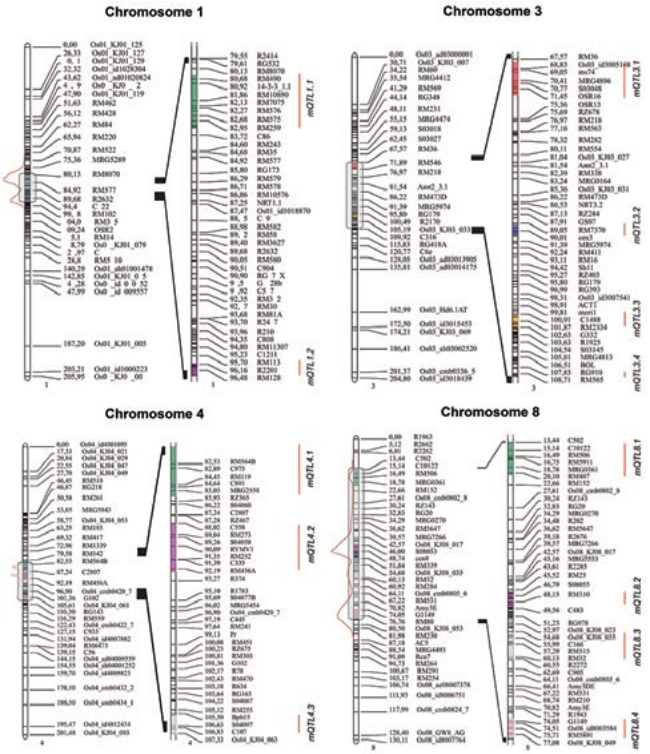

Fig. 1. Genomic locations of Meta QTL detected for nitrogen use efficiency and associated traits in rice

Table 4. Meta QTLs for nitrogen use efficiency and associated traits detected by Veyrieras' method.

mQTL ID	Chr.	mQTL location				95% CI (cM)	Associated traits*	PVE (%)
		Start	End	Peak	SSR marker within mQTL			
mQTL 1.1	1	80.19	83.01	81.6	RM490, RM10890, RM7075, RM576, RM10916, RM575, RM259, RM243	2.94	GY, TN, RN, BM	11.0
mQTL 1.2	1	94.8	98.4	96.6	RM562, RM595, RM157B, RM294B, RM128, RM129, RM403	0.96	SN, GN, LN, BM	15.5
mQTL 3.1	3	60.3	66.8	64.0	RM545	6.60	GY, TN	12.4
mQTL 3.2	3	91.4	94.9	91.9	RM156, RM6483, RM487, RM411, RM16	4.07	BM	10.0
mQTL 3.3	3	104.7	114.7	110.8	RM85	2.49	RN, GY	26.4
mQTL 4.1	4	80.9	85.6	83.3	RM564B, RM119	4.63	LN, GY	14.3
mQTL 4.2	4	88.0	92.5	90.3	RM273, RM252, RM456A	4.43	GY	6.7
mQTL 4.3	4	105.6	107.8	106.7	-	2.23	BM, TN, LN	4.5
mQTL 8.1	8	13.5	20.5	17.0	RM506, RM5911, RM337, RM408, RM407	7.03	LN, RN	30.1
mQTL 8.2	8	46.3	49.0	47.7	RM556, RM310, RM544, RM547, RM126	2.62	LN, GN, SN, GY, BM	14.7
mQTL 8.3	8	54.9	61.0	58.0	RM5767, RM42, RM38, RM223, RM515, RM342A, RM32, RM284	6.09	GN, BM	18.2
mQTL 8.4	8	73.3	77.7	75.5	RM256, RM5891, RM80	4.44	GS, BM, SN	21.4

BM = Biomass; SN = Shoot nitrogen; GN = Grain nitrogen; LN = Leaf nitrogen; RN = Root, nitrogen; TN = Total plant nitrogen; GY = Grain yield; GS = Glutamine synthetase; CI = Confidence interval; PVE = Average percentage of phenotypic variation explained; *Associated traits are in addition to nitrogen use efficiency-related QTLs

Table 5. SSR markers identified polymorphic among the germplasm panel

Marker	mQTL	Alleles	Frequency			HWE probability
			Allele1	Allele2	Allele3	
RM243	*mQTL1.1*	2	0.38	0.62	-	0.02
RM252	*mQTL4.2*	2	0.29	0.54	-	0.00
RM273	*mQTL4.2*	2	0.37	0.40	-	0.02
RM407	*mQTL8.1*	2	0.45	0.55	-	0.28
RM310	*mQTL8.2*	3	0.29	0.31	0.38	0.48
RM80	*mQTL8.4*	2	0.31	0.40	-	0.00

HWE = Hardy Weinberg equilibrium

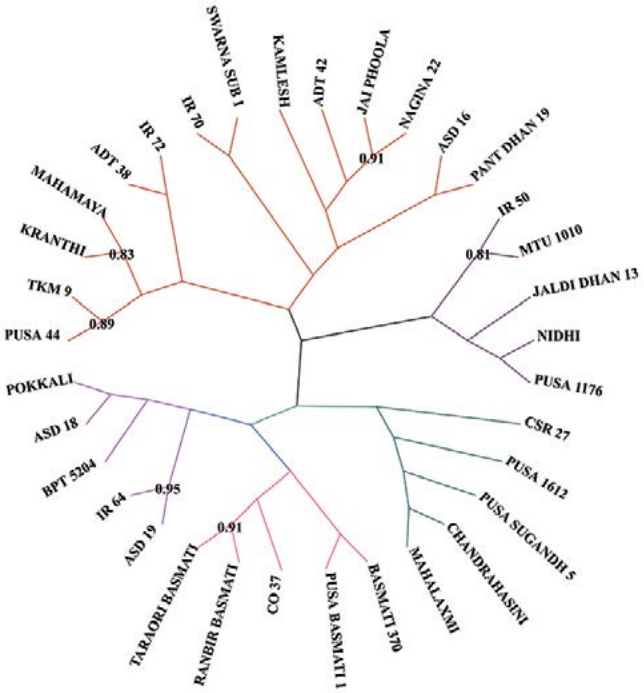

Fig. 2. The pattern of grouping of germplasm lines based on the mQTL linked markers. Significant bootstrap values are indicated in the respective nodes.

QTLs were associated with LN, GN, SN, GY and BM. Besides *mQTL8.4* has reported an association with GS activity.

Diversity of few mQTLs in the germplasm panel

Microsatellite markers were mined out from the mQTL intervals across the chromosomes. We could identify 47 SSR markers falling within the QTL regions from the consensus map (Table 4), except for *mQTL4.3*, which did not contain any SSR marker. Out of these, we have selected a maximum of two representative SSRs per mQTL, wherever possible for genotyping. Twenty selected markers were run across 65 genotypes used for constituting the germplasm assembly and found that only six markers could generate sufficient genotype data for further analysis. These markers belonged to five mQTLs, *mQTL1.1*, *mQTL4.2*, *mQTL8.1*, *mQTL8.2* and *mQTL8.4*. Data for markers that generated monomorphic bands, poor and ambiguous amplification etc. were dropped from the analysis. Genotyping revealed that all the five mQTLs showed significant variation among the genotypes, based on the marker allele distribution, which was predominantly biallelic, except for one triallelic marker, RM310 (Table 5). The frequency of alleles indicated random distribution for only two markers RM407 and RM310 associated with *mQTL8.1* and *mQTL8.2*. For the remaining markers, however, the allele distribution was near random. The genotypes were further grouped based on the allele pattern into three major groups, of which one group contained 14 genotypes comprising mostly of semidwarf *indica* rice lines and some of the tall landraces (Fig. 2). The second group was relatively small with five genotypes but contained high N use efficient genotypes such as Nidhi, IR 50 and MTU 1010. The third group was the largest with 15 genotypes, which majorly included Basmati and aromatic rice and mega varieties such as IR 64 and BPT 5204.

Discussion

There are several genes identified in the rice genome that regulate N uptakes and assimilation (Baligar et al. 2001). Primarily, uptake is regulated by nitrate transporters (NRT) and ammonium transporters (AMT) which carry the N from the soil into the plant system and undergo primary and secondary assimilation processes (Vinod and Heuer 2012). Key enzymes involved in the uptake processes are *OsNRT1* (Lin et al. 2000) and *OsAMT1* (Sonoda et al. 2003), while the primary N assimilation takes place with the help of nitrite reductase (NR) and nitrite reductase (NiR), followed by GS and glutamate synthase (GOGAT) (Obara et al. 2001a). Other than these, there are minor enzymatic systems involved in the N metabolism. Although a constitutive trait, the efficiency of N use in plant vary widely due to the presence of several associated genes that are continuously being mapped as QTLs. Since most of these QTLs are reported to have a low effect and are specific to certain backgrounds, use of them in crop improvement programmes remains limited. Taking into account an impressive number of studies for N use efficiency and yield-related parameters in rice over the past 20 years and a large number of initial QTLs predicted, this mQTL study could assemble significant regions on an integrated map. The meta-analysis in this study used an initial set of 506 QTLs, which was reduced by 35.4% in the

initial projection. From these, 9.48% was initially identified as mQTLs using the model-based approach (Goffinet and Gerber 2000), which was further reduced to 3.7% using the clustering approach by Veyrieras et al. (2007). In the former approach, the QTLs are checked to see whether they fall into any one of the five models, namely, 1-, 2-, 3-, 4- or N-QTL models when they are colocalised. One QTL model identifies a single location where QTLs can be congregated. The optimal models are determined using the AIC criterion, wherein the lowest AIC is taken as the best model. Since it is assumed that each QTL has a Gaussian distribution from its peak position, maximum likelihood estimates are used to determine the QTL position by considering the mean QTL distribution that maximizes the likelihood. The reason why only four QTL models are used other than the N-QTL model comes from the assumption of a 200 cM chromosome, that can be divided into a maximum of four linkage segments of 50cM each. Therefore, this method is useful when the QTLs are few to many. Ideally, each chromosome of 200 cM must contain 10 to 40 QTLs for performing the analysis (Arcade et al. 2004). Unlike this method, the second approach looks for more realistic mQTLs through a clustering procedure. In this case, also a perfect Gaussian distribution is assumed around the QTL position, and the unbiased approximation of the positions is made through the maximum likelihood method. This method reports fewer QTLs than the previous method and is useful when QTLs per locus is more than ten.

The CI of the detected mQTLs ranged from a narrow distance to wide in the present study. In the meta-analysis, CIs are determined based on the reported QTLs and their location accuracy. While preparing the consensus map, the accuracy of the initial QTLs and the critical points were considered. To measure the locations and CI on mapping of initial QTLs, it is recognized that interval mapping (IM) and composite interval mapping (CIM) are more reliable than the ANOVA-based methods. Therefore, to guard the accuracy, we have taken note to include only those studies in which IM and CIM were used for QTL mapping. Identification of several consensus QTLs further reaffirmed that N use in rice is governed by complex genetic regulation. The PVE of the mQTLs were found relatively stronger and ranged between 6.7% to 30.1% indicating significant association to NUE and its associated traits. Therefore, one of the possible ways to improve N use efficiency is to use the QTLs individually or to pyramid them, in marker-assisted breeding programmes. The mQTL distribution showed that chromosomes 1, 3, 4 and 8 harboured the most frequently detected QTLs for NUE and associated traits from the previous studies. Chromosome 1, despite having the largest number of markers, showed only two mQTLs, but with shorter confidence intervals and good PVE values, indicating that these QTLs can be of greater significance in crop improvement. It was interesting to find that the *mQTL1.1* linked marker, RM243 had shown significant association with GN, particularly under high N conditions, indicating that this QTL location may be important in the N assimilation process. On chromosome 3, three mQTLs were found which were mostly associated with GY under N nutrition. Of the meta loci detected on chromosome 4, the *mQTL4.2* was also associated with GY. Confirming this, the associated marker RM252 showed significant association with agronomic and physiological NUE, shoot N content and chlorophyll content on the flag leaf. Other mQTLs on chromosome 4, were predominantly associated with N content in the plant system, indicating the plausible role in N reservoir activity. On chromosome 8, all the four mQTLs showed prominence, based on the PVE, out of which three were tested in this study among the germplasm. We found that *mQTL8.1* could be of particular interest concerning low N tolerance, because of the significant association shown by its linked marker RM407 on traits such as grain N, N assimilation efficiency and physiological NUE. Similarly, RM310 linked to *mQTL8.2* and RM80 linked to *mQTL8.4* also indicated significant relation to N content in shoot and grain as well as with flag leaf chlorophyll content. RM310 was found consistently associated with several N use parameters such as N harvest index, N content in grain, NUE, N assimilation efficiency and grain yield efficiency index. The trait associations on chromosome 8 revealed that there may be several genes associated with N assimilation found distributed on this chromosome.

These observations on meta-loci further lead to studies on exploring candidate genes involved in N use processes in rice. Although we have tested the validity of five mQTLs out of 12 identified, the results from the linked markers have been very encouraging because all the markers showed significant association with one or the other trait linked to N use either under low, medium, or high N conditions. Additional studies by including all the loci need to be undertaken, particularly using a larger germplasm set. Altogether, the twelve mQTLs found on chromosomes 1, 3, 4 and 8 constituted about 17.1% of the total QTLs projected on the consensus map implying QTL Meta-analysis as an effective tool to integrate and evaluate QTLs from several studies. Based on the results, we could assimilate some general conclusions related to the genetic architecture of N use efficiency in rice. The NUE in rice is determined by a large number of QTLs which are distributed on all chromosomes and therefore show broad diversity. This is particularly relevant because the sources of the QTLs analysed herein were mostly independent genotypes that were originated in different regions across the world. Some of these genotypes were developed in China such as Xieqingzao B, Zhonghui 9308, Zhenshan 97 and Minghui 63 and some others were developed at IRRI (IR64, IR72 and IR690093). Some genotypes were originated from India, such as BPT5204, PTB1 and Kasalath. Besides, there were

several *japonica* lines involved such as Azucena, Niponbare, and Milyang 352. Notwithstanding with a limited search with five mQTLs in a relatively small set of Indian rice lines, we could find that the linked markers had a near-random distribution, indicating that most of the major loci related to N use are well distributed in local germplasm. Therefore, careful integration of these QTLs into target backgrounds can help in improving N use. However, the QTLs diversity for this trait also implies the complexity of breeding for NUE and forewarns on the judicious selection of founder parent in an introgressive breeding programme. This partly explains the slow progress in breeding for NUE where a random selection of parents might not provide any significant advantage. Also, this partly explains the slow response to selection for NUE-related traits.

The mQTL analysis provided a wonderful opportunity for consolidating a large set of discovered QTL to a handful of mQTLs with accurate CI. Having marked effect on the NUE, these QTLs can be used for the production of N use efficient genotypes by Marker-assisted selection. The following criteria can be taken into account for choosing mQTL for selection, such as the one with a small CI, and having a high mean additive effects. Since these mQTLs were consolidated from different genetic backgrounds, they may likely work across a large set of breeding lines and will aid in identifying the donors in the local germplasm. These mQTLs may be attempted into marker-assisted breeding programmes aimed at developing N use efficient rice genotypes.

Authors' contribution

Conceptualization of research (KKV, SGK); Designing of the experiments (RK, SGK, KKV); Contribution of experimental materials (AKS, PKB, SGK, MN); Execution of field/lab experiments and data collection (DK, SM, LS, HB, PKM); Analysis of data and interpretation (RK, KKV, RKE); Preparation of the manuscript (RK, KKV).

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