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Identification of QTLs for nitrate and ammonium use efficiency under direct seeded condition and differential expression of genes involved in their absorption and metabolic pathway

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ABSTRACT

Considering water and labor crises, area under direct seeded rice cultivation has increased. With the direct seeding during the early seedling stage the rice crop is very sensitive to water stress and nutrient deficiencies. The availability of nutrients is highly influenced by water status. Over the years, we have critically observed that under direct seeded condition at seedling stage if drought occurs the plants suffer more because of nutrient deficiency rather than water deficiency. The continued plant growth and development under changed rhizosphere depends heavily on availability of nitrogen and other nutrients (P, Fe and S) and the ability of the root system to absorb these nutrients efficiently and use in metabolism under their changed forms. Rice has evolved and adopted for efficient utilization of ammonical form of nitrogen, while nitrate from is less efficiently used at least during seedling and early vegetative phases. Most of the genotypes (except a few) we evaluated exhibited extremely low level of "Nitrate transporter" and "Nitrate Reductase" activities which are key factor and enzyme in absorption and utilization of nitrate form of nitrogen. Therefore, the identification of lines with greater nitrate use efficiency, mainly because of more efficient High Affinity Transporters, increased expression of nitrate reductase and nitrite reductase along with ammonium transporters and GS and GOGAT will certainly have key role in N efficiency of rice genotypes particularly under water stress conditions. In study 1, one hundred twenty two and selected thirty two recombinant inbred lines (RILs) of two indica genotypes, Danteshwari × Dagaddeshi were evaluated under three nitrogen forms and three environments (Irrigated, Rainfed and Terminal stage drought) along with root studies under soil filled glass rhizotrons at IGKV, Raipur, for generation of phenotypic data. The genotypic data of 162 SSR and HvSSR based markers were developed and used for identifying QTLs for agronomically complex trait i.e. NUE. The phenotypic and genotypic data was statistically analyzed for QTLs identification for yield & NUE traits using QTL Cartalographer. QTLs for nitrate and ammonical use efficiency varied significantly. A presence of QTL clusters between HvSSR 1-87(38.9cM) to HvSSR 1-89 (40.6cM) on chromosome 6, RM 449 (81.9cM) to RM5 (9.4cM) on chromosome 5, HvSSR 9-25 (14.6cM) to HvSSR 9-27 (15.5cM) on chromosome 9 and RM 434 (57.7cM) to RM 410 (64.4cM) on chromosome 5 signifies important genomic regions associated with evaluated traits under different conditions and will be useful in marker assisted breeding for NUE in rice. In study 2, we investigated the different members AMT (Ammonium transporters), NRT (Nitrate transporters), GS (Glutamine Synthetase) & GOGAT (Glutamate Synthase) genes, involved in NUE and analyzed the expression pattern of each gene using gene-specific primer in young rice seedlings by quantitative real time PCR, revealing a distinct expression pattern of these genes. Collectively, OsGln1;1, OsGln1;2, OsGln1;3 , OsGln2, OsGlt1 and OsGlt2 manifested different and reciprocal responses to nitrate and ammonium supply. The relationship of these genes / enzymes and its interaction with water stress will be presented during the conference.

Key words: *Direct seeding, nitrate use and ammonium use efficiency, gene expression*

INTRODUCTION

Rice is mainly cultivated under anaerobic conditions and primarily adapted and evolved under these sets of conditions. However, the frequency of drought has increased, and recently the scenario is changing (because of climate change and reduced water availability to agriculture), area under direct seeded rice cultivation has increased and in future it is likely to cover major area as a new way of cultivating rice that requires less water than lowland rice (Wang et al., 2002). Direct seeded rice requires less water as well as less labor, so this is the technology of near future and already as experienced during wet season 2017 at Rajnandgaon district of Chhattisgarh, all rice fields with direct seeding suffered less damage due to water stress compared to transplanted rice and also varieties adapted to direct seeding like Indira BaraniDhan-1 performed better compared to other varieties. With direct seeding the anaerobic conditions are changed to aerobic conditions, which not only change the water availability but drastically change the forms of nutrients available in predominant forms. As an example, in aerobic soils, the major form of inorganic N is nitrate while in flooded wetland the major form is ammonium (Xu et al., 2012). When the field is drained and the soil becomes aerobic, ammonium is oxidized through microbial processes (known as nitrification) into nitrate (NO_3) (Linquist et al., 2006). Nitrate-nitrogen accumulated at a rate of about 1.8 pounds per acre daily, and accumulation began about 4 days after the field was drained. During a typical drain of 10 to 14 days, about 20 pounds of nitrate-nitrogen per acre can be lost (Linquist et al., 2011). The continued plant growth and development under changed rhizosphere depends heavily on availability of nitrogen and other nutrients (P, Fe and S) and the ability of the root system to absorb these nutrients efficiently and use in metabolism under their changed forms.

Ammonium (NH_4^+) is the preferred form of N over nitrate (NO_3^-) in rice due to its waterlogged growth environment (Li et al., 2008), when a rice field is flooded, the fertilizer largely remains as ammonium (Linquist et al., 2006) and is taken up as ammonium by the rice plant. The rice root and whole metabolic system has evolved and adopted for efficient utilization of ammonical form of nitrogen, while nitrate from is less efficiently used at least during seedling and early vegetative phases. Most of the genotypes (except a few) we evaluated exhibited extremely low level of "Nitrate transporter" and "Nitrate Reductase" activities which are key factor and enzyme in absorption and utilization of nitrate form of nitrogen. DRR has also reported similar variation for Nitrate Reductase activity in different genotypes. This lead to a situation where nitrogen or other nutrients are available (in different forms), but rice root is not able to absorb and use it. Using model calculations and experiments, Kirk and Kronzucke (2005) and Kronzucker et al. (1999, 2000) concluded that NO_3^- uptake by lowland rice might be far more important than was previously thought. Although the predominant species of mineral N in bulk soil for paddy rice fields is likely to be NH4+, rice roots are actually exposed to a mixed N supply in the rhizosphere (Briones et al., 2003;Li et al., 2006) and more so under aerobic soils. Therefore, the identification of lines with greater nitrate use efficiency, mainly because of more efficient High Affinity Transporters, increased expression of nitrate reductase and nitrite reductase along with ammonium transporters and GS and GOGAT will certainly have key role in N efficiency of rice genotypes particularly under water stress and aerobic conditions.

Keeping in view all the above facts, the present investigation was undertaken to identify efficient lines in mapping population derived from Indica parents that shows differential expression of transporter systems and key assimilatory enzymes along with identification important genomic regions controlling effect of more than one trait that will be useful in marker assisted breeding for NUE in rice.

MATERIALS AND METHODS

Plant material and Experiment detail

The plant material used in present study includes two parent *viz.*, Danteshwari and Dagaddeshi and their 122 recombinant inbred lines (RILs) population. This mapping population along with parents and check varieties were evaluated under field condition on Vertisol of Research cum Instructional farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur. Molecular Marker Laboratory, Department of Plant Molecular Biology and Biotechnology, IGKV, Raipur was used as platform to generate the genotypic data for identifying QTLs for agronomically complex

The treatments employed were:

Levels of first factor	Fertilizer	Treatment
N1	Ammonium Sulphate	$NH4_{+}$-N
N2	Calcium Nitrate	NO_3^--N
N3	Control	N^0-N

trait *i.e.,* NUEand carry out expression analysis of key known genes.

Phenotypic evaluation of RILs

The 122 RIL lines derived from a cross between Danteshwari × Dagaddeshi were evaluated in the field during wet season 2014 at research cum instructional farm of College of Agriculture, IGKV, Raipur. The direct seeded field selected for the study was upland in topology with good drainage and percolation rate. The standard package of practices was followed in experiment. Each plot consisted of 2 rows of 2 m length plants with spacing of 15 cm between rows and 15 cm between plants in factorial randomized block design with two replications. The observations were recorded on five randomly selected plants from central row of each genotype replication-1 for N content in straw and grain, grain yield and biological yield and their derivative trait grain yield response (GYR) and biological yield response (BYR), Harvest index. The observations for these traits were averaged over five plants.

The data obtained under various characters were tabulated and statistically analyzed using Proc Means of Statistical Analysis System (SAS) v.9.1. Data was analyzed by analysis of variance, and F-test was used to determine treatment significance. The mean data of each replication was used for analysis of variance using factorial design (two-way ANOVA) due to presence of multiple variables in experiments.

A set of 122 RILs along with the parent were used for molecular studies and identification of QTL for NUE and yield components. DNA was isolated from single tagged plant of each line during wet season 2014, by MiniPrep method (Doyle and Doyle, 1987). Genotypic data was developed using SSR and HvSSR primers. A set of 830 primers were used in this study for amplification of genomic DNA of mapping population. Out of 830 SSR and HvSSR primers, 162 primers showed parental polymorphism and were used to generate genotypic data. 19.52 % of primers exhibited parental polymorphism. The markers were taken from previously published rice genetic and sequence maps (Singh et al., 2009; IRGSP, 2005; McCouchet al., 2002 and Temnykhet al., 2001).

Genetic linkage map used for QTL analysis comprised of 162 markers that were distributed over 12 linkage groups. The partial separation and genome coverage of the map was suitable for QTL mapping. The linkage map consisting of vertical bars embraced with map positions and names of loci was constructed by MapChart version 2.3. A genotypic data matrix was generated based on the scoring pattern observed in the RILs, which consisted of 162 polymorphic loci (as stated above) including simple sequence repeat (SSR) and highly variable simple sequence repeat (HvSSR) markers. Composite interval mapping (CIM) was applied to marker data and trait averages under differential nitrogen and water regimes to identify precisely the location of QTL. The CIM analysis was established in the software Windows QTL Cartographer V 2.5 using forward and backward regression method with a walk speed of 2 cM and a window size of 10 cM was used to select the co-factor for controlling background effect. Logarithm of the odds (LOD) for each trait was estimated from 1000 permutations. As a result, a locus with a LOD threshold value higher than 2.5 was used for declaring putative QTL in a given genomic region. In addition, the additive effect and percentage of variation explained by an individual QTL were estimated at the maximum-likelihood QTL position.

Gene expression analysis

Based on the data of 122 RILs 10 rice genotypes with highest and lowest NUEs and falling in different group of colour classeswere selected (Table 1). Root and shoot tissue samples were harvested separately from all the replication of respective treatments. In total there were 120 samples obtained from three treatments, two tissues and two biological replicate of 10 genotypes. After collection, samples were snap frozen under ice cold conditions and stored immediately in -80 0C for total RNA extraction using Trizol reagent (Protocol developed at IOWA state university, Iowa) with slight modification.

Quantitative qPCR assay

In the present investigation qPCR was performed with selected gene specific primers with actin and tubulin

Table 1. Lines selected for expression analysis

Sample	Sample ID (Leaf/shoot type)
1	DxD-4-Y
2	DxD-21-DG
3	DxD-30-Y
4	DxD-46-DG
5	DxD-75-Y
6	DxD-121-DG
7	Danteshwari-G
8	Dagaddeshi-LG
9	Swarna-DG
10	Indira SugandhitDhan-Y

Y= yellow, DG= dark green, G= green and LG= light green

as internal controls or housekeeping genes. qPCR was accomplished on Mx3000P® QPCR System (Stratagene, USA) using gene specific primers . qPCR was performed using SYBR green qPCR mix (Applied biosystems and Thermo Fisher make) using approximately 700-1000 ng total cDNA in a 20 ul reaction mixture containing final composition of 1X qPCR mix and 0.5-0.8 uM of each forward and reverse primers. Reaction was set as per manufacturer's instructions. Blank was always set for each primer during every PCR setup. The relative quantification of expression was done by using the mathematical model given by Livak and Schmittgen (2001).

RESULTS AND DISCUSSION

The statistical analysis of trait performance of parents and their RIL population revealed significant differences under differential N forms. In addition, significant effect of environment and nitrogen was observed for five traits *i.e.,* grain yield, biological yield, harvest index, chlorophyll content, grain yield response and biological yield response under study. Significant interaction between RILs and nitrogen and environment was speculated for all traits at 1% level of significance and at 5% level of significance.

QTL analysis

QTL mapping in the present experiment was carried out by calculating threshold logarithm of odds (LOD) for each trait by performing test with 1000 permutations. The experimental threshold LOD mean were 2.96 and 3.69 at 5% and 1% level of significance respectively. A total of 16 QTLs conferring the corresponding five traits were detected under three N forms under direct seeded environment (Table 2); that as a matter of fact included 5, 10 & 1 QTLs under NH_4^+, NO_3^- and N0 level of rainfed conditions respectively. These QTLs were mapped to different genomic regions of all rice chromosomes and most of them were on chromosomes 1 and 9 as shown in Fig. 1. Results with respect to trait wise QTLs identified are presented below.

QTLs for grain yield (GY)

Seven QTLs for grain yield were identified under differential N regimes and are depicted in Fig. 2. Within rainfed condition, under NH_4^+ level, three QTLs were revealed on chromosome 1, 1 and 3, explaining 12.42, 3.68 and 6.41 of total grain yield variation. Under NO_3^- level, four QTLs totally explained about 34.45 % phenotypic variation. These QTLs were embraced on chromosome 1, 9, 9 and11. The allele for al l the QTLs was carried by Dagaddeshi genotype.

QTLs for grain yield response (GYR)

Under NO_3^- level of direct seeded condition, three QTLs were located on chromosome 1 and with additive value of -23.71, -34.98 and -35.8, respectively and phenotypic variation of 30.12 % collectively.

QTLs for biological yield (BY)

Nine QTLs for biological yield were located on chromosome 1, 6, 7 ,9 and 12 under the two nitrogen level. Of these, individual QTLs exhibited phenotypic variation under NO_3^- and N^0 levels are 8.02 %, 11.86 % and 9.15 % with additive value of -58.03, -67.75 and 31.22, respectively.

QTLs for biological yield response (BYR)

Five QTLs for biological yield response were detected under NO_3^- level. Single QTL was mapped on chromosome 6 exhibiting phenotypic variation of 18.51%. The positive additive value for two QTLs indicates allele from Danteshwari.

QTLs for harvest index (HI)

A total of three QTLs were detected for harvest index. Under NH_4^+ level, two QTLs were located on chromosome 1 & 12. Under NO_3^- level, one QTL was located on chromosome 1. Additive affect was positive for all the QTLs except one indicating direction of parent effect towards Danteshwari genotype. The phenotypic variation ranged from minimum value of 7.91% and

Table 2. Results of QTLs for four traits under three N levels

Trait	N/E	Chr.	Left marker	Right marker	LOD	Add.	R2 (%)
Grain Yield (GY)							
	NH_4^+ R	1	RM449	RM5	3.42	-32.73	12.42
		1	RM572	RM24	2.91	-38.10	14.31
	NO_3^- R	1	HvSSR1-34	HvSSR1-49	2.56	-28.92	3.68
		9	HvSSR9-25	HvSSR9-27	5.54	-35.88	11.70
		9	RM434	RM410	3.97	-35.63	11.75
		11	RM21	RM26334	3.38	-38.13	7.32
Biological Yield (BY)							
	NO_3^- R	9	HvSSR9-25	HvSSR9-27	2.89	-58.03	8.02
		9	HvSSR9-37	HvSSR9-57	3.88	-67.75	11.86
	N^0 R	7	RM2	RM11	2.57	31.22	9.15
Harvest index (HI)							
	NH_4^+ R	1	RM449	RM5	4.68	-3.57	19.83
		12	RM270	RM17	2.53	-2.57	9.3
	NO_3^- R	1	RM449	RM5	3.43	-2.89	12.85
Grain yield response (GYR)							
	NO_3^- R	1	HvSSR1-34	HvSSR1-49	2.64	-23.71	5.07
		9	HvSSR9-25	HvSSR9-27	7.35	-34.98	16.34
	NH_4^+ R	6	HvSSR6-35	HvSSR6-44	3.41	-91.51	18.51

maximum value of 31.50% for a QTL detected.

NUE and yield in plants is a complex quantitative trait and is highly influenced by environmental levels (as stated above). At present, a number of QTLs associated with NUE have been identified in plants, and the way by which QTLs can be used for genetic improvement of crop NUE has become a research focus. By integrating the QTLs for NUE in previous studies and excavating QTLs detected in different populations through comparative analysis, the accuracy and reliability of QTL mapping can be improved.

In the present study, the QTLs for grain yield, biological yield, harvest index, chlorophyll content, grain yield response and biological yield response under different nitrogen and water regimes have been speculated and identified. These results are consistent with previous studies. Cho et al. (2007) found that QTLs detected under high and low N levels are widely different. However, the study of Tong et al. (2006) showed the presence of the same and specially expressed QTLs under low and high N levels. Hu et al. (2012) detected QTLs for N content and NUE in adjacent regions, respectively. Wei et al. (2012) detected QTLs for nitrogen deficiency and nitrogen use efficiency traits. For NDT and NUE traits, seven and eight QTLs were identified in 2006 and 2007, respectively. Tong et al. (2011) analyzed the QTLs for rice yield and its components under high, middle and low N levels, and detected 15, 23 and 19 QTLs at three N levels, respectively, thereby indicating the occurrence of obvious interactions between QTLs for yield traits and N levels. QTL for chlorophyll content (CC) was rarely reported previously. The genomic region RZ599-RM53 on chromosome 2, where grain yield and biological response QTL was located, was reported to have QTLs for grain yield under low nitrogen and normal nitrogen by Wei et al. (2011).

Quantitative qPCR assay results

In quantitative analysis we tried to eliminate experimental inconsistencies by adopting normalization strategies at different experimental steps.. In the present work actin was taken as housekeeping reference gene. Comparative quantification was applied to the present gene expression study where, the expression level of a gene of interest is assayed for up- or down-regulation in a calibrator (normal) sample and one or more experimental samples.

Expression of GS and GOGAT gene families under different N forms

Plant GS and GOGAT genes are very important in N assimilation, but the systematic expression patterns of the GS and GOGAT genes families have not yet been clearly established. Thus, we examined the level of expression of each member of the GS and GOGAT

gene families under NH_4^+ and $N0_3^-$ treatment using quantitative real time PCR. The relative values for gene expression in root and shoot and details are elaborated below:

Actin Vs GS gene family

GS1 and GS2 are two major isoforms of Glutamine Synthetase (GS). GS gene family comprises of three cytosolic *GS1* gene (*OsGln1; 1*, *OsGln1; 2* and *OsGln*) and a single plastidic OsGln2 gene. Distinct roles for this enzyme have been suggested by a number of studies on organ, tissue and development (Weber and Harrison, 2002).

In the present qPCR experiment for GS genes, the levels of transcripts in organs under study were calculated relative to the Actin gene. The relative expression values more than one shows the increased expression or up regulation while values less than one shows the decreased expression or down regulation as compared to control. Under both NH_4^+ and NO_3^- treatment, two of the genes, *OsGln1;1* & *OsGln1;2* showed a markedly preferential expression pattern in roots whereas *OsGln1;2* was mainly expressed in shoot. *OsGln2* showed the differential expression level both in root and shoot tissues among all selected genotypes. Furthermore, significant effect of NO_3^- and NH_4^+ treatment was observed on GS expression. Overall, in root tissue, *OsGln1*; 1gene seems to be upregulated in root under both NH_4^+ and NO_3^- treatment with line no.10 (14.82) manifesting significantly strong expression while in shoot line no. 4 (55.13) and line no. 3 (6.16) showed strong expression under NH_4^+ and NO3- treatment. *OsGln1*; 2 in root showed signifcant down-regulation by NH_4^+ treatment except for line no. 10 (8.1) and up-regulation by NO_3^- with strong expression harbouring in line no.4 (13.08) while in shoot, excluding line no. 9 (3.27) a significant down regulation and weak expression was observed by NH_4^+ treatment while NO_3^- treatment resulted in up regulation as well as down regulation in 10 selected rice genotypes. *OsGln1;3* gene in root exhibited strong expression in line no. 4 (10.12) & 5 (7.54) while in shoot most of the genes were up-regulated as a result of NH_4^+ treatment. Additionally, most of the genotypes showed significant up regulation of *OsGln1;3* by NO_3^- treatment in both root and shoot with line no. 3 (11.91) manifesting strong expression. NH_4^+ treatment revealed strong expression of *OsGln2* gene in shoot of line no. 4 (125.8) and in root of line no. 1 (30.59) while in NO_3^- treatment strong expression in shoot is visible in line no. 4 (186.7) and line number 10 (63.11) in root.

This study suggest that *OsGln1; 1*, *OsGln1;2* and *OsGln1;3* shows reciprocal responses to NH_4^+ and NO3- supply as oppose to proposed mechanism. Collectively, among all isoforms, *OsGln2* which predominantly functions in green tissue and is indispensable for assimilation of photo respiratory ammonia in high NUE genotypes revealed shoot preferential expression pattern. *OsGln2* was highly upregulated in line number 4 among all isoforms under both the N supply. This line is high yielding and falls in dark green spectrum of colour classes which denotes the relationship of GS in green tissues with grain yield. Rice plant have developed intrinsic mechanism for uneven N distribution thus assimilate N efficiently in roots by this kind of flexible and reciprocal regulatory mechanism, and control rice growth and development. Similar results for *OsGln1; 1*, *OsGln1;2*, *OsGln1;3* and *OsGln2* was earlier reported by Ishimayaet al. (2004). Also Zhao and Shi (2006) revealed reciprocal responses for GS isoforms.

Actin Vs GOGAT gene family

GOGAT catalyzes reductive transfer of the amide group of Glnto 2-oxogluarate to form two Glu molecules. In rice plants, *NADH-GOGAT* is coded by two genes, *OsNADH-GOGAT1* (*OsGlt1*) and *OsNADH-GOGAT2* (*OsGlt2*) (Tabuchi et al., 2007b).

In the present qPCR experiment for GS genes, the levels of transcripts in organs under study were calculated relative to the Actin gene. The relative expression values more than one shows the increased expression or up regulation while values less than one shows the decreased expression or down regulation as compared to control. Expression of *OsGlt1* and OsGlt2 genes was strongly repressed by NH_4^+ and NO_3^- treatment for most of the genotypes. Exceptionally, *OsGlt1* gene in root tissue of line no. 7 (7.31) of green colour and line no. 10 (1.17) of yellow colour was upregulated by NH_4^+ treatment while line no.4 (2.74) and line no.5 (2.50) which falls in dark green and yellow colour in colour class was upregulated by NO_3^- treatment. *OsGlt1* gene in shoot tissue of line no. 4 (1.46) showed increased expression in NH_4^+ treatment

and line no. 9(2.71) of dark green colour class and line no. 10 (2.35) of yellow colour class in NO_3^- treatment. *OsGlt2* gene in root tissue of line no. 10 (3.81) and in shoot tissue of line no. 4 (8.63) was upregulated by NH_4^+ treatment whereas line no. 3 (5.83) showed significant upregulation in shoot tissue and line no. 9 (4.12) showed significant upregulation in root tissue by NO_3^- treatment.

The expression of these genes encoding NADH-GOGAT remarkably reduced by external N forms and conditions. Our results also showed that transcription of *OsGlt1* isdecreased in root and shoot by N concentration while transcription of *OsGlt2* is relatively increased in root and shoot by N concentration. When N level is relatively high in soil, expression of *OsGlt1* and *OsGlt2* can be decreased in order to limit N acquisition but in the opposite side, expression of *OsGlt1* and *OsGlt2* can be enhanced in order to increase N acquisition. This may be kind of buffering effects in higher plants. Previous studies by Watanabe et al. (1996) and Ishiyama et al. (1998) show similar responses and contradict previous report showing ammonium inducibility (Tobin et al., 2001).

Expression of AMT and NRT transporters under different N forms

Actin Vs AMT gene family

Although functionally not well characterized, twelve putative AMT proteins have been identified located on different chromosome and grouped in to five subfamilies (AMT1-AMT5) with one to three gene members (Deng et al., 2007b; Lie et al., 2009). So far, studies on expressions and regulations of AMT genes in rice have been focused on the three genes of *OsAMT1* family, which displayed different spatio-temporal expression patterns in response to changes in N levels. *OsAMT1;1*, *OsAMT1;2*, *OsAMT1;3* have been identified as members of AMT1, each showing a distinct expression pattern: *OsAMT1;1* shows constitutive expression in both shoots and roots (Ding et al., 2011b). In the present study, similar results were obtained influx of NH_4^+ ion resulted in up regulation and significant strong expression of *OsAMT 1.1* in both root and shoot tissue of line no. 5 (1.11, 10.52), 7 (1.82, 6.36) and line no. 9 (3.95, 4.00) whereas NO_3^- influx resulted in strong expression in shoot tissue of line no. 3 (28.54). *OsAMT1; 2* shows root-specific and ammonium-inducible expression (Ding et al., 2011). This is in contrary with present results in which up regulation of *OsAMT1;2* was obtained in both root and shoot by NH_4^+ and NO_3^- treatment of some genotypes with exceptionally high and strong expression by NH_4^+ in shoot of line no. 4 (133.43) and in shoot of line no. 3 (99.04) & 5 (86.82) by NO_3^-. Furthermore, NH4+ and NO_3^- treatment resulted in up regulation of *OsAMT1*; 3 in both root and shoot of genotypes under study with comparatively strongest expression in root and shoot tissue of line no. 9 (42.23) and line no. 4 (11.39) by NH_4^+ treatment and root & shoot tissues of line no. 8 (11.63) and line no. 3 (11.55) by NO_3^- treatment. In, contrary, Sonodaet al. (2003b) reported that *OsAMT1; 3* shows root-specific and nitrogen-suppressible expression.

Our results dictates that *OsAMT1;2* shows maximum mRNA accumulation in line no. 4 which also pertains high GS2 activity thus *OsAMT1;2* expression increases with increase in endogenous glutamine. It also seems that *OsAMT1;2* functions in ammonium uptake in ammonium enriched soils as its expression was highest in NH_4^+ treatment. These findings collaborate with Sonoda et al. (2003) who studied feedback regulation AMT1 gene family by glutamine in rice.

Actin Vs NRT gene family

When breeding crops that utilize nitrogen efficiently, it is important to reveal the regulation of nitrate uptake at molecular level. We focussed on expression of two nitrate uptake related genes, *OsNRT2.4* and *OsNRT7.8*, who are nitrate inducible and is mainly expressed in parenchyma cells around the xylem. *OsNRT2.4* and *OsNRT7.8* mainly participate in unloading nitrate from the xylem and maintains root-to-shoot nitrate transport and vascular development (Xia et al., 2015). In the present study, significant upregulation of *OsNRT2.4* was observed in shoot tissue of line no. 4 (6.10) and root tissue of line no. 5 (1.39) by NH_4^+ treatment while in shoot tissue of line no. 3 (10.26) and root tissue of line no. 5 (3.4) by NO_3^- treatment. *OsNRT7.8* revealed significant strong upregulation in root tissues of line no. 10 under NH_4^+ (19.35) and NO_3^- (6.96) treatment.

Since rice roots are exposed to NO_3^- nutrition under aerobic or water deficit condition and the importance of NO_3^- nutrition can be dictated and the regulation & function of nitrate transporter genes in

rice are worthy of investigation. Strong upregulation of *OsNRT7.8* in line no. 10 which is actually of pale-yellow colour allow us to select this line for breeding and production purposes for efficient nitrate uptake under drought and water limited condition where major form of available nitrogen is NO_3^-. Krick and Kronzucker (2005) and Aaraki and Hasegawa (2006) revealed the importance of nitrate uptake and nitrate transporters studies in rice. Systemic expression pattern for nitrate transporters is not studied in past thus we open new insight for nitrate-nitrogen studies.

CONCLUSION

A total of 16 QTLs conferring the corresponding four traits were detected under three N forms; that as a matter of fact included 5, 10 & 1 QTLs under NH_4^+, NO_3^- and N^0 level. These QTLs were mapped to different genomic regions of all rice chromosomes and most of them were on chromosomes 1 and 9. QTL clusters were obtained in chromosome 1 between HvSSR1-87 and HvSSR 1-89 & RM449 and RM5. In chromosome 9 between RM434 and RM10. These regions are important genomic regions controlling effect of more than one trait. These regions will be useful in marker assisted breeding for NUE in rice

Most of the genotypes (except a few) we evaluated exhibited extremely low level of "Nitrate transporter" and "Nitrate Reductase" activities which are key factor and enzyme in absorption and utilization of nitrate form of nitrogen. Therefore, the identification of lines with greater nitrate use efficiency, mainly because of more efficient High Affinity Transporters, increased expression of nitrate reductase and nitrite reductase along with ammonium transporters and GS and GOGAT will certainly have key role in N efficiency of rice genotypes particularly under water stress conditions.

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