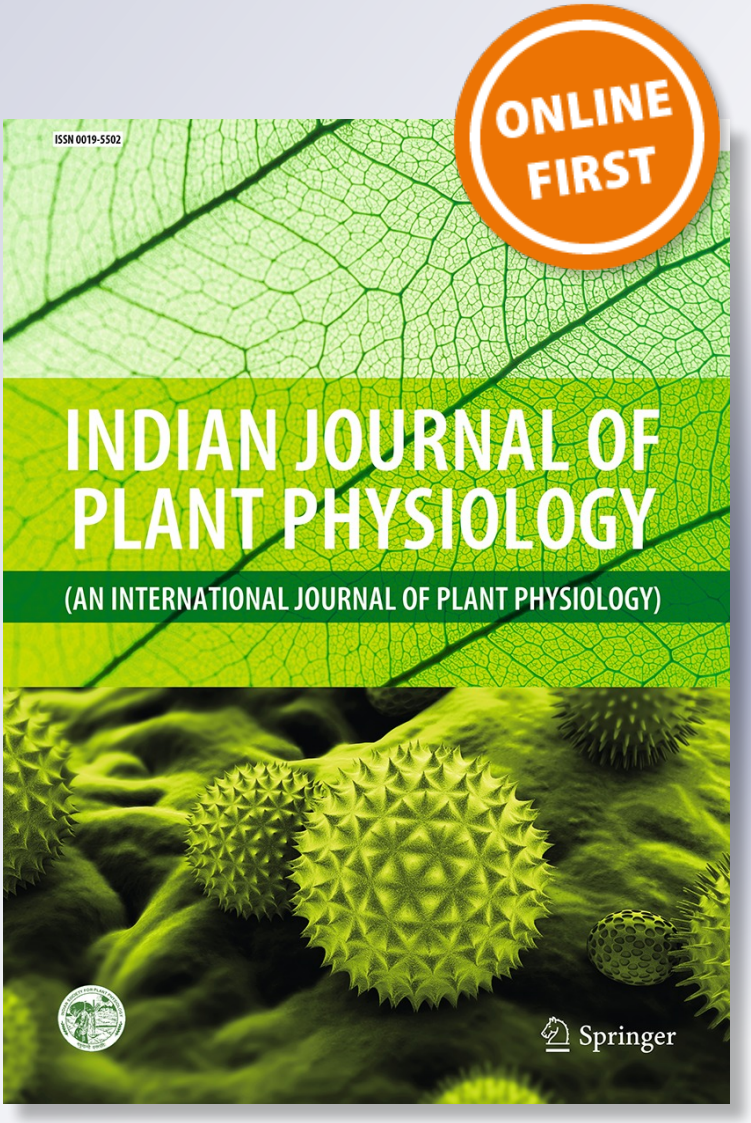
Advances in genetic basis of nitrogen use efficiency of rice

C. N. Neeraja, D. Subramanyam, K. Surekha, P. Raghuveer Rao, L. V. Subba Rao, M. B. B. Prasad Babu & S. R. Voleti

Indian Journal of Plant Physiology
An International Journal of Plant Physiology

ISSN 0019-5502

Ind J Plant Physiol.
DOI 10.1007/s40502-016-0254-z



Ind J Plant Physiol.
DOI 10.1007/s40502-016-0254-z

REVIEW ARTICLE

Advances in genetic basis of nitrogen use efficiency of rice

C. N. Neeraja[1] · D. Subramanyam[1] · K. Surekha[1] · P. Raghuveer Rao[1] · L. V. Subba Rao[1] · M. B. B. Prasad Babu[1] · S. R. Voleti[1]

Received: 18 October 2016 / Accepted: 25 October 2016

Abstract Nitrogen is one of the fundamental key element required for crop growth and development. Excessive use of N in crop production has become a major global concern as it is causing environmental degradation through soil–water and also atmosphere. Increasing N use efficiency (NUE) and a balance of crop yields to achieve food security is the need of current situation. It is also important that, judicious application of N along with developing N efficiency crops are to be targeted for reducing the global climate change impacts. Ample opportunities existing in the form of germplasm resources which are yet to be utilized, advancement of genetic tools such as QTLs, genes associated with N metabolism, proteomics, association mapping, microarray, miRNA, genetic transformation and transcriptomic strategies are yet to be fully implemented to decipher the secrets of natural efficiency of N in crop plants. Progress of N use efficient developed using some of the above tools in a multidisciplinary mode and the first set of improved N efficient rice lines are field tested so as to understand genetic basis of NUE.

Keywords NUE-QTLs · Genes · Association mapping · Field evaluation

✉ C. N. Neeraja
voletisr58@rediffmail.com

[1] Indian Institute of Rice Research, Rajendranagar, Hyderabad 500030, India

Introduction

Nutrient use efficiency of crops is being improved through temporal and spatial management of the form and amount of nutrient inputs as being demonstrated by various agronomic practices. In addition, understanding of the genetic basis of nutrient efficiency would lead to the development of nutrient use efficient varieties thus reducing the application of nutrients. Earlier studies on genetics of nutrient use efficiency were limited as increasing productivity with heavy nutrient inputs was the main focus. But in the backdrop of environmental degradation due to the excessive nutrients and its impact in climate change, nutrient use efficiency in crops is need of the hour for sustainable and eco-friendly agriculture.

Of the various essential nutrients required for crops, nitrogen (N) is fundamental to crop development because it forms the basic component of nucleic acids, proteins and many organic molecules. Till now varieties responding to nutrients with high uptake efficiency and utilization efficiency manifested in terms of yield were selected. Since uptake and utilization are interdependent, the efficiency for nutrients was not studied earlier.

Germplasm of major food crops has been screened under low inputs to identify the promising genotypes, however the mechanism of nutrient use efficiency was complex to be deciphered by explanation of single major genes and the effect of environment has confounded the studies. The genetic studies for nutrient use efficiency have been further complicated mostly by low heritability, high environmental variability, difficulty of field screening and absence of clear selection criteria. From the reported studies, it can be inferred that nutrient use efficiency should be targeted for optimum nutrient inputs rather than zero inputs as the nutrients are essential building blocks for

Published online: 26 November 2016

realizing the output. With high throughput phenotyping and genotyping approaches along with the availability of genome sequences, the genetics of nutrient use efficiency is now being interpreted. Being major nutrient, the advances in genetic basis of nitrogen use efficiency (NUE) and the significant progress made on rice briefly summarized.

Among the major fertilizer inputs, nitrogen (N) is the key nutrient element required in large quantities by rice. Nitrogen is a primary constituent of the nucleotides, amino acids, proteins, chlorophyll and several plant hormones and is a crucial macronutrient essential and rate-limiting for the growth and development of plants. It comprises 1.5–2% of plant dry matter and approximately 16% of total plant protein (Frink et al. 1999). As nitrogen is crucial for cell/tissue expansion and multiplication, limitation of N would impose constraint in total biomass and therefore yield (Hirel et al. 2007, 2009). Reduced growth, gradual chlorosis of older leaves followed by abscissions, altered root architecture, an increased root to shoot ratio and increased root surface have been reported for N deficient plants (Swamy et al. 2015a). Plant height is the most affected by N limitation in rice as observed by 20–30% height reduction under low N. The number of tillers and productive tillers were also relatively less in low N situation owing the less meristametic activity. The panicle weight also been reported to be reduced by approximately 40%. Under low N situation, there is a decrease in the panicle weight owing to the decrease in the number of filled grains per panicle (Vijayalakshmi et al. 2015a, b). Several genotypes have been identified with promising performance for most of the parameters under low N and the performance of these genotypes needs to be validated at 50% of recommended dose of N (Rao et al. 2014). The medium doses of nitrogen application N medium application have a positive effect on the activities of enzymes of physiological importance, thereby increasing the grain size and promoting grain filling by remobilizing assimilates towards panicles to increase grain yield by accelerating the endosperm cell number, grain length and grain width.

QTLs for nitrogen use efficiency

QTL analysis based on molecular linkage maps is now proven methodology for identification of genomic regions associated with complex traits. The initial studies for QTL identification for nitrogen metabolism and use efficiency were based on physiological parameters of nitrogen metabolism. Using backcross inbred lines (BILs) between Nipponbare and Kasalath, seven QTL for GS1 protein content and six QTL for NADH-GOGAT protein content were detected. Some of these QTLs were co-located in QTL regions for various biochemical and physiological traits affected by nitrogen recycling. A structural gene for GS1 was also mapped on chromosome 2 co-located in the QTL region for spikelet weight and a QTL region for NADH-GOGAT protein content was also coincided with the physical position of NADH-GOGAT gene on chromosome 1 (Obara et al. 2001; Yamaya et al. 2002). QTL controlling the ratio of Rubisco to total leaf N has been identified (Ishimaru et al. 2001). Two QTL associated with uptake of nitrogen from ammonium source have been identified on chromosomes 2 and 5 and two QTL associated with uptake of N from nitrate source have been identified on chromosomes 5 and 6. In the same study, a QTL for N use efficiency has also been identified on chromosome 12 (Fang et al. 2001). QTL for plant height under high and low N levels in nutrient solution and soil solution culture experiments (Fang and Wu 2001). A QTL on chromosome 2 associated with the protein content of cytosolic glutamine synthetase (GS1; EC 6.3.1.2) in senescing leaves, panicle number and panicle was characterized and substitution line with this QTL showed more active tillers during vegetative stage and more panicle number and total panicle weight (Obara et al. 2004). Using 239 recombinant inbred lines (RILs) screened under low and normal N solutions, several QTLs for low N tolerance in seedling stage have been identified, however very few QTLs were found to be common for low and normal N conditions (Lian et al. 2005). Under two nitrogen levels, QTL have been identified for plant height, panicle number per plant, chlorophyll content, shoot dry weight at late developmental stage (Tong et al. 2006). Screening of DH population under three nitrogen regimes and mapping of QTLs led to the identification of seven QTL on chromosome 3 associated with nitrogen use, plant yield and associated traits (Senthilvel et al. 2008). Using 166 RIL population, 22 single QTL and 58 pairs of epistatic QTL associated with physiological nitrogen use efficiency in rice have been identified (Cho et al. 2007). With the same mapping population, 28 main effect QTL and 23 pairs of epistatic QTL were detected (Piao et al. 2009). Several QTL for yield components were reported in chromosomal segment substitution lines of Nipponbare and 9311 grown under nitrogen and phosphorus deficiency conditions (Wang et al. 2009). A set of RILs grown in four different seasons in two locations with three nitrogen fertilizations were analyzed for QTL for grain yield components and two main effect QTL were detected viz., grain yield per panicle on chromosome 4 and grain number per panicle on chromosome 12 under N zero level (Tong et al. 2011). Four QTLs for trait differences of plant height and heading date between two N levels have been mapped on chromosomes 2 and 8 co-locating with reported QTLs for NUE (Feng et al. 2011). In response to low nitrogen application for two years, 33 QTL have been identified in RIL population, out

of which only ten QTL were consistent under low N (Wei et al. 2012a). QTL mapping for NUE and nitrogen deficiency tolerance traits in RIL population for two years resulted in four common QTL on chromosomes 1, 3, 4 and 7 (Wei et al. 2012b).

Genes for the N metabolism and NUE

Though the candidate genes involved in nitrogen metabolism in rice are well characterized (Hirel and Gadal 1980; Wang et al. 1993, Kronzucker et al. 2000; Von Wiren et al. 2000; Tabuchi et al. 2007; Yuan et al. 2007), the molecular response to N cannot be exactly attributed to candidate genes as shown by rapid induction/repression of genes and transcription factors (Lian et al. 2006). The sources of nitrogen for rice in field, either ammonium or nitrate are absorbed through transporters (Srikanth et al. 2015a, b; Srikanth et al. 2016). These transporters are divided into high-affinity transporter system (HATS) and low-affinity transport system (LATS). Under low nitrogen concentration (<1 mM), HATS mediate most of the N uptake while under high concentration of N (>1 mM), LATS play role in N uptake (Forde and Clarkson 1999; Glass et al. 2001; Williams and Miller 2001). Both root architecture and the activities of ammonium and nitrate transporters regulated by N form and concentration, diurnal fluctuations, and temperature fluctuations after N acquisition by roots (Garnett et al. 2009).

For the uptake of ammonia, initially three ammonium transporter (AMT) genes have been identified in rice (Sonoda et al. 2003). Now ~12 putative rice AMT proteins have been identified and grouped into five sub-families (AMT1–AMT5) with one to three gene members (Suenaga et al. 2003; Deng et al. 2007; Li et al. 2009). OsAMT1;1 is constitutively expressed in shoots and roots (Ding et al. 2011), OsAMT1;2 show root specific expression and induced by ammonium and OsAMT1;3 is root specific and show ammonium de repressed expression (Sonoda et al. 2003). Recently, a high affinity urea transporter (OsDUR3) has been identified in rice roots is up regulated under N deficiency (Wang et al. 2012). For nitrate transporters, low affinity nitrate transporter OsNRT1 has shown to contribute to N uptake in the root epidermis and root hairs (Lin et al. 2000) and high affinity nitrate transporter OsNRT2 is nitrate inducible (Cai et al. 2008). Recently it was found that rice OsNAR2.1 interacts with OsNRT2.1, OsNRT2.2 and OsNRT2.3a nitrate transporters to provide uptake over high and low concentration ranges of N (Yan et al. 2011).

After uptake into the plant, the nitrate is reduced to nitrite catalyzed by nitrate reductase (NR) and then to ammonium by nitrite reductase (NiR). The primary assimilation takes both in shoots and in roots and ammonium is incorporated into organic molecules by GS and GOGAT. Out of two major forms of GS viz., cytosolic GS1 expressed in roots and shoots, and plastidic GS2 expressed in chloroplasts and plastids. GS1 is complex gene family comprising three genes in rice and GS2 detected in mesophyll cells (Sakurai et al. 1996; Obara et al. 2000), plays the major role in the photorespiratory nitrogen metabolism (Ireland and Lea 1999). Among three genes in rice, OsGS1;1 was expression in all tissues with higher expression in the leaf blade during vegetative stage of growth (Tabuchi et al. 2005). OsGS1;2 transcripts have been found in all tissues with higher expression in root following the supply of ammonium at seedling stage and OsGS1;3 exclusively expressed in spikelet. Transcripts of OsGS1;1 accumulated in the dermatogens, epidermis and exodermis under ammonium limited conditions whereas transcripts of OsGS1;2 was abundantly expressed in the same cell layers under ammonium sufficient conditions. Cytosolic GS1;2 is was shown to be responsible for the primary assimilation of ammonium in rice roots (Funayama et al. 2013).

There are two GOGAT molecular species in rice plants. One is the ferredoxin (Fd)-dependent GOGAT and the other, NADH-dependent. Fd-GOGAT is known to be involved in photorespiration. NADH-GOGAT occurs as a single gene in rice (Goto et al. 1998). Cell type specific and age dependent expression of the NADH-GOGAT gene was confirmed by promoter analysis in transgenic rice (Kojima et al. 2000). Over expression of NADH-GOGAT in sink organs of *indica* genotype and the subsequent increase in grain weight strongly supported the hypothesis that NADH-GOGAT in spikelets at the early stage of ripening is important to reutilize glutamine. Not only that, but also the grain filling process is co-regulated by the status of the re-utilization in which the precise mechanism is largely unknown (Yamaya et al. 2002). Glutamate is a major free amino acid in the leaf blades (Kamachi et al. 1991), whereas glutamine and asparagine, which are synthesized from glutamine (Lea et al. 1990, Sechley et al. 1992, Ireland and Lea 1999), are major forms of total amino acids in phloem sap of rice plants (Hayashi and Chino 1990).

The glutamate amino group can be transferred to aminoacids by a number of different aminotransferases (Lam et al. 1996). Asparagine synthetase (AS) catalyzes the formation of asparagine and glutamate from glutamine and aspartate. Thus AS with GS plays an important role in primary N metabolism (Xu et al. 2012). Mitochondrial NADH-glutamate dehydrogenase (GDH) can also incorporate glutamate under high levels of ammonium (Masclaux-Daubresse et al. 2010). Map based cloning using rice mutant for reduced growth revealed loss of function in arginase gene (OsARG) and was shown to play crucial role in conditions of insufficient exogenous nitrogen (Ma et al. 2013). In rice,

OsIPT4, OsIPR5, OsIPT7 and OsIPT8 (adenoside phosphate-isopentenyltransferease) were upregulated in response to exogenously applied nitrate and ammonium with accumulation of cytokinins (Kamada-Nobusada et al. 2013).

Several genes and various metabolic and regulatory pathways appear to be involved in the adaptive response of low N. Thus, genome-wide investigation of gene expression by microarray represented an effective approach for analysing gene regulatory networks in rice. Expression profiles of *indica* variety were studied after low N with normal N as control using a microarray of 11,494 rice ESTs representing 10,422 genes. No significant differences were found in the leaf tissue and 471 ESTs were detected in root tissues with 115 ESTs up-regulated and 358 ESTs down-regulated. The up regulated genes comprised early response genes involved with biotic and abiotic stress and some transcriptions factors and signal transduction. The down regulated genes included photosynthesis and energy metabolism, stress response, transcription factors and signal transduction. Under microarray analysis, no differential expression was found in the genes known to be involved in N uptake and assimilation (Lian et al. 2006). Using Affymetrix Genechip rice arrays, the dynamics of rice transcriptome under N starvation situation, 3518 induced/suppressed genes belonging to cellular metabolic pathways including stress response, primary and secondary metabolism, molecular transport, regulatory process and organ development representing 10.88% genome were identified. 462 or 13.1% transcripts for N starvation expressed similarly in root and shoot (Cai et al. 2012). Differential expression indicates the potential target genes for nitrogen-use efficiency improvement of rice.

Proteomics is a high-throughput biotechnological approach being used to understand the biological function of proteins in response to different biotic or abiotic stresses (Agrawal et al. 2002). Considering the complexity of NUE trait, it is important to identify the signal transduction pathways and the elements that function to regulate genes involved in N uptake and assimilation. Since signal transduction and gene regulation are based on proteins at large, protein-expression pattern has been studied to identify and understand the role of various proteins at a given point in time. Quantitative as well as qualitative differential expression of protein spots may help in identifying the essential molecules (enzymes) responsible for N uptake and assimilation. Comparative proteome analyses of proteins isolated from leaves under N starvation and N sufficient conditions through matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry and electron spray ionization quadruopole TOF identified N starvation responsive proteins belonging to protein synthesis, metabolism and defence (Kim et al. 2011). In another study at JamiaHamdard University, New Delhi, root proteome of nitrogen efficient and nitrogen inefficient rice cultivars was analysed using two dimensional gradient electrophoresis (2-DGE) based proteomics approach. 504 protein spots were identified with a positive correlation observed between physiological parameters and the concentration of a number of root proteins. Analyses showed that glutamine synthetase, cysteine proteinase inhibitor-I, porpho-bilinogen-deaminase (fragment) and ferritin were involved in conferring N efficiency to N-efficient rice cultivars/genotypes (Hakeem et al. 2013).

Developing nitrogen use efficient rice genotypes

The current status on NUE in a multidisciplinary mode carried under NICRA is briefly summarized below.

From the year 2011 a small N depleted plot (without the application of N from any external source) is being maintained and the soil properties are being monitored on a regular basis. On the basis of the data in 2016, approximately 25–35 kg ha^{-1} of N is being added during the crop season through natural means of rains and of irrigated water (Table 1). The soil properties are, low to medium in organic content, low N content was low in all plots except N50 and N100. Phosphorus and potassium were high in N0, N50 and N100 plots with pH being slightly alkaline, EC being normal and nitrogen being low. N efficient rice lines from this large screening materials were subjected to gene expression analysis (Srikanth et al. 2015a, b) where in some of the key enzymes involved in N metabolism were found to be differentially expressed (Table 2).

Identification of genotypes for NUE and development of segregating material

A total of fourteen RIL populations have been developed and five—RIL were screened in low, 50 and 100 N plots and five RIL populations were screened under N-50 and N100 plots.

All the mapping populations were characterized for morphological, physiological, biochemical and yield parameters followed by Identification of genomic regions/genes for NUE.

Association mapping for identification of genomic regions for NUE

Association mapping is a proven strategy for identifying genes underlying quantitative traits in plants. Using the data available for morphological, physiological and yield

Table 1 Soil properties of the experimental plot (Kharif-wet season 2014) initial N in 2011 was 238 kg ha^{-1}

Soil property	Initial			40 dat			At maturity		
	N0	N50	N100	N0	N50	N100	N0	N50	N100
pH	7.6	7.35	7.62	7.28	7.4	7.36	7.25	7.24	7.35
E.C (dS/m)	0.24	0.25	0.30	0.25	0.28	0.27	0.28	0.3	0.28
O.C (%)	0.51	0.5	0.52	0.54	0.61	0.60	0.52	0.56	0.59
N (kg ha^{-1})	216	204	210	223	226	256	224	238	240
P_2O_5 (kg ha^{-1})	62	68	64	67	64	62	68	68	66
K_2O (kg ha^{-1})	592	580	665	628	636	638	640	652	645
Urease activity (μg NH4 N g^{-1} soil/2 h)	62	57	62	65	69	74	56	60	64
KCl N (mg N kg^{-1} soil)	3.05	3.12	3.12	3.18	3.24	3.42	3.18	3.24	3.46

Simultaneously, all the 800 germplasm lines categorized into various groups were assessed for their grain and straw N contents at different applied N levels (Table 2). From this laboratory, Swamy et al. (2015a, b) reported such variation in the different rice lines. A change in germination behaviour and also the root architecture of the N sensitive and tolerant lines and some of their physiological and biochemical responses to varied N levels was reported (Vijayalakshmi et al. 2013; 2015a, b). It appears the %N level, variation was little and due to genetic variation where in the range is wide can be assessed. It is interesting to note that the %N with reference to different N levels did not vary much is due to the capacity of the organ (grain or straw) was limited. Thus, there is a need to assess N at plant level or per unit area basis is required for judging NUE

Table 2 Grain N% in different rice lines under different soil N conditions

Rice varieties	No.			N50			N100		
	Range		Mean	Range		Mean	Range		Mean
Aromatic lines	0.52	0.95	0.84	0.55	0.99	0.91	0.57	1.19	1.00
Germplasm lines	0.55	0.95	0.87	0.65	0.97	0.92	0.65	1.3	1.05
UP land rice varieties	0.65	1.21	0.91	0.67	1.17	0.97	0.68	1.21	1.12
Aerobic rice	0.66	1.15	0.92	0.63	1.25	0.99	0.67	1.27	1.07
Heat tolerant rice	0.65	1.2	0.96	0.63	1.15	0.98	0.65	1.31	1.10
IRHTN	0.67	1.27	0.95	0.63	1.25	0.99	0.66	1.02	1.08
DRR and other released varieties	0.72	1.05	0.96	0.75	1.25	0.98	0.87	1.29	1.15
R-lines	0.75	1.15	0.99	0.81	1.20	1.01	0.95	1.35	1.20
B line	0.78	1.19	1.00	0.83	1.25	1.10	0.99	1.39	1.24

parameters from earlier field screening experiments, 472 genotypes were selected and characterized with 50 rice microsatellite (RM) markers covering all the 12 chromosomes. A total of 170 phenotypic characters were studied. Genotypic and phenotypic data was subjected to TASSEL software. TASSEL analysis revealed the markers which are significantly ($P < 0.05$) associated with several traits. For the markers which are significantly associated with the traits, the alleles were estimated for their positive or negative contribution for a particular trait (Table 3). As a proof of concept, the associated genomic region was saturated with RM markers and a local linkage map was constructed and through bioinformatics, putative candidate genes were identified. Associations were found with the genomic region and its markers with yield under low N and a candidate gene viz., Alanine Amino Transferase was identified through differential expression.

Analysis of AAT gene sequence in Varadhan and BPT5204

The high quality forward and reverse reads were assembled using the DNA Baser and the sequenced data were aligned using Clustal W 2.0 (Larkin et al. 2007) at their default alignment parameters and manually corrected by MEGA 4.0 and Nippon bare for detecting SNPs and InDels. The deduced amino acid sequences of two genotypes were aligned to find amino acid substitutions by the DnaSP 5.10 software and also analysis of nucleotide polymorphism. The aligned DNA sequences were imported into the DnaSP software to calculate S (number of polymorphic or segregating sites), along with the Nippon bare sequence to find out structural alterations, if any, in the protein. The position of exons and introns were identified using gene prediction software FGENESH (www.softberry.com) and verified

Table 3 Segregation for RIL validation using different markers

No.	Marker	$c^2_{1:1}$	*P*		hmzA	hmzB	*N*	m.d.	$c^2_{1:3}$	*P*		$c^2_{3:1}$	*p*	
1	SC3776	1.06	0.30234	ns	42	52	94	0	19.4184397	0.00001	****	46.0851064	0.00000	*****
2	SC3779	0.62	0.43095	ns	36	43	79	15	17.8270042	0.00002	****	36.4936709	0.00000	*****
3	SC3780	8.34	0.00388	**	33	61	94	0	5.12056738	0.02364	*	79.787234	0.00000	*****
4	SC3781	5.50	0.01902	*	33	55	88	6	7.33333333	0.00677	**	66	0.00000	*****
5	SC3782	0.05	0.82726	ns	41	43	84	10	25.3968254	0.00000	*****	30.7301587	0.00000	*****
6	SC3784	0.58	0.44770	ns	46	39	85	9	38.4352941	0.00000	*****	19.7686275	0.00001	*****
7	SC3787	0.89	0.34545	ns	50	41	91	3	43.5201465	0.00000	*****	19.5201465	0.00001	*****
8	SC3791	8.52	0.00351	**	32	60	92	2	4.69565217	0.03024	*	79.3623188	0.00000	*****
9	SC3794	1.90	0.16820	ns	51	38	89	5	49.5318352	0.00000	*****	14.8651685	0.00012	***

$P < 0.05$: *, $P < 0.01$: **, $P < 0.001$: ***, $P < 0.0001$: ****, $P < 0.00001$: ****

manually by checking against the full length cDNA sequence of the ref gene Upon comparison of sequence of these two alleles) with reference sequence Nippon bare, in the case of Vardhan we found 127 SNPs and 9 Indels, whereas in BPT5204 we found 81 SNPs and 9 Indels. Whole gene analysis of Vardhan we found the 35 synonymous and 88 non-synonymous substitutions where as in coding region we found 9, 6 respectively and in BPT5204 we found the 27 synonymous and 52 non-synonymous substitutions where as in coding region we found 6, 20 respectively.

Association mapping with candidate gene primers

Polymorphism survey was performed in twelve genotypes viz., Basmati370, Kolazoha3, Thururbhog, Ratnasundari, IC576898, IC463254, GZ948-2-2-1, N22 (ACC19379), IR64, Narendradhan359 and Rasi for 23 candidate genes using 169 primers, out of 169 primers 61 primers shown polymorphism among genotypes. Polymorphic markers were screened in all the 155 genotypes of 11 categories. The current results revealed that 52 markers were significantly associated with different physiological and yield traits during two consecutive seasons of Kharif and Rabi 2011–2012. Association analysis identified the marker trait association ($P < 0.01$) for all the traits evaluated. Total 164 phenotypic traits were measured in different N levels during two consecutive seasons. TASSEL analysis using mixed linier model (MLM) revealed 52 markers associated with different genes viz., Gluatamine Synthetase2, Nitrate Transporters 2.5, Nitrate Transporters1.2, MADS-box protein AGL16-II, Gluatamine Synthetase1;3, Glutamate synthase, Urea active transporter, Nitrate and chloride transporter, Ferredoxin nitrite reductase, High affinity nitrate transporter, Nitrate reductase, Low affinity nitrate transporter NRT1.2, Glutamine synthetase, Glutamine synthetase shoot isozyme, phenylalanine ammonia-lyase, early nodulin 20 precursor and Ammonium transporter genes. Candidate gene based primers were associated with different traits and are being further analysed for validating gene expression analysis.

Sequencing of Osmir156 target motif region in all the ten genotypes

Genomic primers were designed for *Os SPL14* gene, and the primer (forward primer: CCTCTACAGA-GACCAATCCA, reverse primer: TAGCTCCTCATGGT-CACTCT) which covers miR156 target motif was identified. PCR was performed for all the ten genotypes, and the PCR products were sequenced using ABI 3730xl DNA Analyzer (Applied Biosystems, USA) (Srikanth et al. 2015a, b).

Validation of genes identified by transcriptome analysis

Fifty key genes related to NUE were shortlisted; primers designed for shortlisted genes and validated by Real-time PCR analysis in four panicle samples collected from both low and recommended N conditions. In the identified N efficient lines from the large scale screening, information on few key genes such as Nitrate reductase, Phenyl alanine ammonia lyase, Ammonium transporter genes and Alanine ammonia lyase was validated (Srikanth et al. 2015b).

Field evaluation

Improving NUE will ensure lower level of N fertilizer usage thus reduce environmental contamination. Under National Innovative climate resilient project (NICRA) one of the major activities undertaken was developing nitrogen use efficient rice varieties. Facilities to screen large number of rice genotypes were created at IIRR field and promising donors have been identified and crosses were made (12

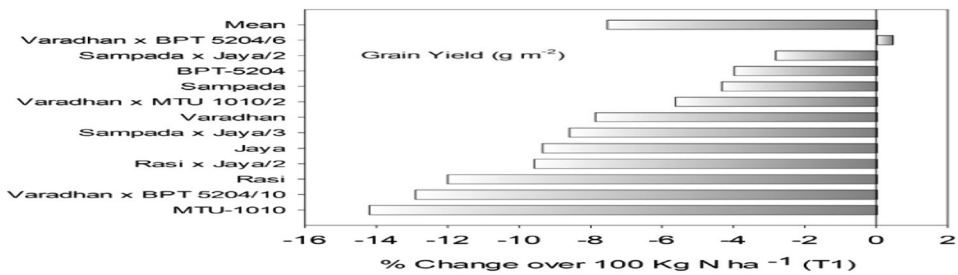


Fig. 1 Genotypic differences of grain yield in response to N level

entries with various cross combinations and High yielding varieties) and promising lines were identified and evaluated along with high yielding lines during 2015–2016 at 9 locations. The trial was conducted with 2 N treatments (100 kg ha^{-1} and 50 kg N^{-1}) and recommended P and K fertilizer (45 P_2O_5, 60$K_2$0 kg ha^{-1} applied as basal dose and the N was applied in 3 splits were used.

Significant differences were noticed between genotypes under both T1 and T2. The mean days to maturity for all locations varied between 114 days (Rasi) to 141 days(G6) under T1 and under T2 it varied between 115 days (G1) to 142 (G6). The interaction between location × treatment × genotype was also found to highly significant ($P < 0.01$) indicating that the genotypes behaved differently at different locations and under different treatments (Table 2). The flag leaf length was measured at flowering stage. Nitrogen treatments showed significant ($P < 0.01$) effect on flag leaf length. Under 50 kg N ha^{-1} treatment (T2) flag leaf length was 31.9 cm as against 29.9 cm under T1. The interaction between location × treatment and location × genotype was found to be highly significant ($P < 0.01$). The mean flag leaf length for all genotypes varied between 21.2 to (REWA) to 45 (MTU) under T1 and 22.7 (REWA) to 44.9 (MTU) under T2. No significant effects were observed between nitrogen treatments for leaf width.

No significant differences in mean tillers number were noticed between T1 and T2. Significant differences were observed amongst the genotypes. Grain number per panicle an important yield contributing factor showed a non-significant difference between nitrogen treatments. The differences amongst the genotypes was highly significant ($P < 0.01$). The mean grain number per panicle for all centres varied between 87 (G1) to a maximum of 125 (BPT 5104) under T1 and under 50 kg ha^{-1} N treatment (T2) the mean grain number per panicle varied between a maximum of 139 (Sampada) to a minimum of 91 (G1). Similarly, the mean of all tested genotypes varied significantly amongst the locations under both the treatments 7. Analysis of variance revealed significant interaction between location × treatment. Similarly, the interaction between location × genotype was found to be highly significant. However, the interaction between genotype × treatment was non-significant.

The data on the influence of nitrogen levels on grain yield (g m^{-2}) recorded at different centres. The analysis of variation indicated that, the nitrogen treatments had influenced the mean grain yield (Fig. 1). The reduction in the grain yield under T2 (50 kg N ha^{-1}) was >7%. The interaction between location × treatment was significant ($P < 0.01$) implying that the response of nitrogen level different at different locations. With the exception of Varadhan × BPT 5204/6 (G1) all the entries included in this trial showed reduction in grain yield under 50 kg N ha^{-1} (T2). Maximum reduction was observed in MTU-1010 (>14%) followed by Varadhan × BPT-5204/10 (G2) and Rasi in which the reduction in grain yield >10%. All other entries recorded marginal reduction. In Sampada × Jaya/2 (G3), BPT-5204, Sampada and Varadhan × MTU1010/2 (G5) the reduction is <5% over recommended N level. These genotypes which show <5% reduction in grain yield have higher N use efficiency.

In order to identify genotypes which performed well across locations and produced higher yield and stability "Shukla's stability variance and Kang's" statistic was performed (Table 4).

Based on the YSi value the genotypes Varadhan × BPT 5204/10 (G2), Varadhan × BPT-5204/6 (G2), Sampada × Jaya/3 (G4), Varadhan and Jaya performed well across locations and produced higher grain yield under 50 kg N ha^{-1} and can be recommended as they showed high YSi value and non-significant stability variance (σi^2).

Conclusions and future prospects

Development of nutrient use efficient varieties is inevitable for sustainability of environmental friendly and economical agricultural practices. While several management practices are being studied for increasing efficiency of spatial and temporal inputs of nutrients, several attempts being made to identify genotypes with differential nutrient use efficiency for Indian situation. An attempt should also be made to evaluate reported exotic germplasm for nutrient use efficiency and use the sources to develop nutrient use efficient varieties with multidisciplinary approach. As a major nutrient role of nitrogen as building blocks of

Table 4 Stability analysis for simultaneous selection for stability and higher yield under low N level

Treat.	Code	Genotypes	Mean yield	Yield rank (Y^n)	Adjusted[$] ($Y^n$)	Adjusted Y	Stability varience (σi^2)	Stability rating (S)	$YS_i = (Y + S)$
50 kg N ha^{-1} (T1)	G1	Varadhan × BPT 5204/6	453.9	4	−1	3	11,994.13 ns	0	3[#]
	G2	Varadhan × BPT 5204/10	472.1	7	−1	6	16,405.56 ns	0	6[#]
	G3	Sampada × Jaya/2	582.2	12	3	15	28,782.73**	−8	7[#]
	G4	Sampada × Jaya/3	488.4	9	1	10	14,282.91 ns	0	10[#]
	G5	Varadhan × MTU 1010/2	387.1	1	−3	−2	48,198.96**	−8	−10
	G6	Rasi × Jaya/2	470.8	6	−1	5	18,419.08 ns	−4	1
	G7	Varadhan	455.6	5	−1	4	10,012.93 ns	0	4[#]
	G8	BPT-5204	503.6	10	1	11	31,199.83**	−8	3[#]
	G9	Sampada	529.3	11	2	13	22,259.36*	−8	5[#]
	G10	Jaya	484.3	8	1	9	10,124.58 ns	0	9[#]
	G11	MTU-1010	452.8	3	−1	2	29,263.26**	−8	−6
	G12	Rasi	409.9	2	−2	0	21,472.71**	−4	−4
		Yield Mean (T1)		474.2					
		YS Mean (T1)		2.3					
		LSD (0.05)		41.57					

*, ** Denote significance level at $P < 0.05$ and $P < 0.01$ respectively and also indicate the genotypes adjusted to be unstable. [$] Adjustment of +1 for mean yield >overall mean yield (OMY), +2 for mean yield ≥1LSD above OMY, +3 for 2LSD above OMY, −1 for mean yield ≤OMY, −2 for mean yield <1LSD, [#] genotypes selected on the basis of YS_i

biomass, an optimum quantity is required for realizing the yield. So, the strategy should be maximum uptake, maximum utilization and maximum remobilization of the optimum nutrient inputs to give maximum possible yield. From the observations of the reported studies across the world, the genotypes do exist in major food crops with differential ability for maximum uptake, utilization and remobilization. However, all the three traits are not usually observed in a single genotype. Therefore using multidisciplinary approach, pyramiding of possible mechanisms for nutrient use efficiency should be a possible strategy. With advent of genome sequencing and next generation sequencing, the identification of allelic variation for nutrient use efficiency appears to be a promising strategy. Earlier, several efforts were made to develop nutrient use efficient varieties using transgenic technology. Several candidate genes associated nitrogen and phosphorus metabolism were targeted for transgenic development and the proof of concept of enhancement of nutrient use efficiency and yield were shown. However, the transgenic experiments could not be taken to field level considering the constraints for the adoption of the technology. With the resources of information of candidate genes associated with nitrogen metabolism from genome sequencing studies, their expression pattern using transcritptomics and proteomics, the genomic regions and the alleles of candidate genes associated with nitrogen are being identified at several national and international research institutes and the information generated is being deployed to develop breeding lines with nutrient use efficiency.

Acknowledgements This work was supported by the National Innovations in Climate Resilient Agriculture (NICRA), Indian Council of Agricultural Research (ICAR), Ministry of Agriculture, Govt. of India [F. No. Phy/NICRA/2011–2012].

References

Agrawal, G. K., Rakwal, R., & Iwahashi, H. (2002). Isolation of novel rice (*Oryza sativa* L.) multiple stress responsive MAP kinase gene, OsMSRMK2, whose mRNA accumulates rapidly in response to environmental cues. *Biochemical and Biophysical Research Communications, 294*, 1009–1016.

Cai, H., Lu, Y., Xie, W., Zhu, T., & Lian, X. (2012). Transcriptome response to nitrogen starvation in rice. *Journal of Biosciences, 37*, 731–747. doi:10.1007/s12038-012-9242-2.

Cai, C., Wang, J. Y., Zhu, Y. G., Shen, Q. R., Li, B., Tong, Y. P., et al. (2008). Gene structure and expression of the high-affinity nitrate transport system in rice roots. *Journal of Integrative Plant Biology, 50*, 443–451.

Cho, Y. I., Jiang, W. Z., Chin, J. H., Piao, Z. Z., Cho, Y. G., McCouch, S. R., et al. (2007). Identification of QTLs associated with physiological nitrogen use efficiency in rice. *Molecules and Cells*, *23*, 72–79.

Deng, R. L., Gu, J. T., Lu, W. J., Xu, H. R., Cao, Y. F., & Xiao, K. (2007). Characterization, function and expression analysis of ammonium transporter gene OsAMT1.4 and OsAMT5 in rice (*Oryza sativa*). *Scientia Agricultura Sinica*, *40*, 2395–2402.

Ding, C., You, J., & Liu, Z. (2011). Proteomic analysis of low nitrogen stress-responsive proteins in roots of rice. *Plant Molecular Biology Reporter, 2011*, 618–625.

Fang, P., Tao, Q. N., & Wu, P. (2001). QTLs underlying rice root to uptake NH_4–N and NO_3–N and rice N use efficiency at seedling stage. *Plant Nutrition and Fertilizer, Science, 7*(2), 159–165.

Fang, P., & Wu, P. (2001). QTL × N-level interaction for plant height in rice (*Oryza sativa* L.). *Plant and Soil, 236*, 237–242.

Feng, C., Liu, G., & Gong, Y. (2011). Rice DUR3 mediates high-affinity urea transport and plays an effective role in improvement of urea acquisition and utilization when expressed in arabidopsis. *New Phytologist*, *193*, 254–265.

Forde, B. G., & Clarkson, D. T. (1999). Nitrate and ammonium nutrition of plants: Physiological and molecular perspectives. *Advances in Botanical Research, 30*, 1–90.

Frink, C. R., Waggoner, P. E., & Ausubel, S. H. (1999). Nitrogen fertilizer: Retrospect and prospect. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 1175–1180.

Funayama, K., Kojima, S., Tabuchi-Kobayashi, M., Sawa, Y., Nakayama, Y., & Hayakawa, T. (2013). Cytosolic glutamine synthetase1;2 is responsible for the primary assimilation of ammonium in rice roots. *Plant and cell physiology, 54*, 934–943.

Garnett, T., Conn, V., & Kaiser, B. K. (2009). Root based approaches to improving nitrogen use efficiency in plants. *Plant, Cell and Environment, 32*, 1272–1283.

Glass, A., Brito, D., Kaiser, B., Kronzucker, H., Okamoto, M., Rawat, S., et al. (2001). Nitro-gen transport in plants, with an emphasis on the regulation of fluxes to match plant demand. *Journal of Plant Nutrition and Soil Sciences, 164*, 199–207.

Goto, S., Akagawa, T., Kojima, S., Hayakawa, T., & Yamaya, T. (1998). Organization and structure of NADH-dependent glutamate synthase gene from rice plants. *Biochimica et Biophysica Acta, 1387*, 298–308.

Hakeem, K. R., Mir, B. A., Qureshi, M. I., Ahmad, A., & Iqbal, M. (2013). Physiological studies and proteomic analysis for differentially expressed proteins and their possible role in the root of N-efficient rice (*Oryza sativa* L.). *Molecular Breeding*, *32*, 785–798.

Hayashi, H., & Chino, M. (1990). Chemical composition of phloem sap from the upper most internode of the rice plant. *Plant and Cell Physiology, 31*, 247–251.

Hirel, B., & Gadal, P. (1980). Glutamine synthetase in rice. *Plant Physiology, 66*, 619–623.

Hirel, B., Le Gouis, J., Ney, B., & Gallais, A. (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany, 58*, 2369–2387.

Hirel, B., Chardon, F., & Durand, J. (2009). The contribution of molecular physiology to the improvement of nitrogen use efficiency in crops. *Journal of Crop Science and Biotechnolgy*, *10*, 123–132.

Ireland, R. J., & Lea, P. J. (1999). The enzymes of glutamine, glutamate, asparagine and aspartate metabolisms. In B. K. Singh (Ed.), *Plant amino acids. Biochemistry and biotechnology* (pp. 49–109). New York: Marcel Dekker.

Ishimaru, K., Kobayashi, N., Ono, K., Yano, M., & Ohsugi, R. (2001). Are contents of rubisco, soluble protein and nitrogen in flag leaves of rice controlled by the same genetics? *Journal of Experimental Botany*, *52*, 1827–1833.

Kamachi, K., Yamaya, T., Mae, T., & Ojima, K. (1991). A role for glutamine synthetase in the remobilization of leaf nitrogen during natural senescence in rice leaves. *Plant Physiology, 96*, 411–417.

Kamada-Nobusada, T., Makita, N., Kojima, M., & Sakakibara, H. (2013). Nitrogen-dependent regulation of de novo cytokinin biosynthesis in rice: The role of glutamine metabolism as an additional signal. *Plant Cell Physiology, 54*, 1181–1893.

Kim, S. G., Wang, Y., Wu, J., Kang, K. Y., & Kim, S. T. (2011). Physiological and proteomic analysis of young rice leaves grown under nitrogen-starvation conditions. *Plant Biotechnology Reports*, *5*, 309–314.

Kojima, S., Kimura, M., Nozaki, Y., & Yamaya, T. (2000). Analysis of a promoter for NADH-glutamate synthase gene in rice (*Oryza sativa*): Cell-type specific expression in developing organs of transgenic rice plants. *Australian Journal of Plant Physiology, 27*, 787–793.

Kronzucker, H. J., Glass, A. D. M., Siddiqi, M. Y., & Kirk, G. J. D. (2000). Comparative kinetic analysis of ammonium and nitrate acquisition by tropical lowland rice: Implications for rice cultivation and yield potential. *New Phytologist, 145*, 471–476.

Lam, H.-M., Koschigano, K. T., Oliveira, I. C., Melo-Oliveira, R., & Coruzzi, G. M. (1996). The molecular-genetics of nitrogen assimilation into amino acids in higher plants. *Annual Review Plant Physiology and Plant Molecular Biology, 47*, 569–593.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., et al. (2007). Clustal W and clustal X version 2.0. *Bioinformatics*, *23*, 2947–2948.

Lea, P. J., Robinson, S. A., & Stewart, G. R. (1990). The enzymology and metabolism of glutamine, glutamate and aspergine. In B. J. Mifflin & P. J. Lea (Eds.), *The biochemistry of plants, vol 16. Intermediary nitrogen metabolism* (pp. 121–157). San Diego: Academic Press.

Li, B. Z., Merrick, M., Li, S. M., Li, H. Y., Zhu, S. W., Shi, W. M., et al. (2009). Molecular basis and regulation of ammonium transporter in rice. *Rice Science*, *16*, 314–322.

Lin, C. M., Koh, S., Stacey, G., Yu, S. M., Lin, T. Y., & Tsay, Y. F. (2000). Cloning and functional characterization of a constitutively expressed nitrate transporter gene, OsNRT1, from rice. *Plant Physiology*, *122*, 379–388.

Lian, X., Wang, S., Zhang, J., Feng, Q., Zhang, L., Fan, D., et al. (2006). Expression profiles of 10, 422 genes at early stage of low nitrogen stress in rice assayed using a cDNA microarray. *Plant Molecular Biology, 60*, 617–631. doi:10.1007/s11103-005-5441-7.

Lian, X., Xing, Y., & Yan, H. (2005). QTLs for low nitrogen tolerance at seedling stage identified using a recombinant inbred line population derived from an elite rice hybrid. *Theoretical and Applied Genetics*, *112*, 85–96.

Ma, X., Cheng, Z., Qin, R., Qiu, Y., Heng, Y., Yang, H., et al. (2013). OsARG encodes an arginase that plays critical roles in panicle development and grain production in rice. *The Plant Journal*, *73*, 190–200.

Masclaux-Daubresse, C., Daniel-Vedele, F., & Dechorgnat, J. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany, 2010*, 1141–1157.

Obara, M., Kajiura, M., Fukuta, Y., Yano, M., Hayashi, M., Yamaya, T., et al. (2001). Mapping of QTLs associated with cytosolic glutamine synthetase and NADH-glutamate synthase in rice (*Oryza sativa* L.). *Journal of Experimental Botany, 52*, 1209–1217.

Obara, M., Sato, T., Sasaki, S., Kashiba, K., Nagano, A., & Nakamura, I. (2004). Identification and characterization of a QTL on chromosome 2 for cytosolic glutamine synthetase content and panicle number in rice. *Theoretical Applied Genetics, 110*, 1–11.

Obara, M., Sato, T., & Yamaya, T. (2000). High content of cytosolic glutamine synthetase does not accompany with a high activity of

the enzyme in rice (*Oryza sativa* L.) leaves of indica cultivars during the life span. *Physiologia Plantrum, 108*, 11–18.

Piao, Z., Li, M., & Li, P. (2009). Bayesian dissection for genetic architecture of traits associated with nitrogen utilization efficiency in rice. *Journal of Biotechnology*, *8*, 6834–6839.

Rao, P. R., Neeraja, C. N., Vishnukiran, T., Srikanth, B., Vijayalakshmi, P., Rao, I. S., Swamy, K. N., Kondamudi, R., Sailaja, N., Subrahmanyam, D., Surekha, K., Prasadbabu, M. B. B., Subbarao, L. V., & Voleti, S. R. (2014). Nitrogen use efficiency in irrigated rice for climate change—A case study. Technical Bulletin no.82/2014, ISBN 978-81-928249-6-3. p. 57. Directorate of Rice Research, Rajendranagar, Hyderabad 500030, AP, India.

Sakurai, N., Hayakawa, T., Nakamura, T., & Yamaya, T. (1996). Changes in the cellular localization of cytosolic glutamine synthetase protein in vascular bundles of rice leaves at various stages of development. *Planta, 200*, 306–311.

Sechley, K. A., Yamaya, T., & Oaks, A. (1992). Compartmentation of nitrogen assimilation in higher plants. *International Review of Cytology, 134*, 85–163.

Senthilvel, S., Vinod, K. K., Malarvizhi, P., & Maheswaran, M. (2008). QTL and QTL × environment effects on agronomic and nitrogen acquisition traits in rice. *Journal of Integrative Plant Biology, 50*, 1108–1117.

Sonoda, Y., Ikeda, A., Saiki, S., von Wiren, N., Yamaya, T., & Yamaguchi, J. (2003). Distinct expression and function of three ammonium transporter genes (OsAMT1;1–1;3) in rice. *Plant and Cell Physiology, 44*, 726–734.

Srikanth, B., Rao, I. S., Moiz, S. Z., Swamy, K. N., Surekha, K., Subrahmanyam, D., et al. (2016). Physiological and molecular characterization of three rice genotypes with differential nitrogen use efficiency. *Asian Journal of BioScience*, *11*, 162–171.

Srikanth, B., Rao, I. S., Swamy, K. N., Vijayalakshmi, P., Surekha, K., Moiz, S. Z., et al. (2015a). Physiological and molecular analysis of GQ-25, a promising genotype for Nitrogen use efficiency. *Progressive Research-An International Journal, 10*, 651–656.

Srikanth, B., Subhakara Rao, I., Surekha, K., Subrahmanyam, D., Voleti, S. R., & Neeraja, C. N. (2015b). Enhanced expression of *OsSPL14* gene and its association with yield components in rice (*Oryza sativa*) under low nitrogen conditions. *Gene, 576*, 441–450.

Suenaga, A., Moriya, K., Sonoda, Y., Ikeda, A., von Wiren, N., & Hayakawa, T. (2003). Constitutive expression of a novel-type ammonium trans-porter OsAMT2 in rice plants. *Plant and Cell Physiology, 44*, 206–211.

Swamy, K. N., Kondamudi, R., Kiran, T. V., Vijayalakshmi, P., Rao, P. R., Subrahmanyam, D., et al. (2015a). Screening for nitrogen use efficiency with their root characteristics in rice (*Oryza* spp.) genotypes. *Annals of Biological Science, 3*, 8–11.

Swamy, K. N., Kondamudi, R., Vijayalakshmi, P., Jaldhani, V., Munnam, S. B., Kiran, T. V., et al. (2015b). A comparative study on nitrogen response among upland, IRHTN, DRR and other released rice groups. *African Journal of Agricultural Research, 10*, 4364–4369. doi:10.5897/AJAR2015.10323.

Tabuchi, M., Abiko, T., & Yamaya, T. (2007). Assimilation of ammonium ions and reutilization of nitrogen in rice (*Oryza sativa* L.). *Journal of Experimental Botany, 58*, 2319–2327. doi:10.1093/jxb/erm016.

Tabuchi, M., Sugiyama, T., Ishiyama, K., Inoue, E., Sato, T., Takahashi, H., et al. (2005). Severe reduction in growth and grain filling of rice mutants lacking OsGS1;1, a cytosolic glutamine synthetase 1;1. *The Plant Journal, 42*, 641–655.

Tong, H. H., Mei, H. W., Yu, X. Q., Xu, X. Y., Li, M. S., Zhang, S. Q., et al. (2006). Identification of related QTLs at late developmental stage in rice (*Oryza sativa* L.) under two nitrogen levels. *Acta Genetica Sinica*, *33*, 458–467.

Tong, H., Chen, L., Li, W., Mei, H., Xing, Y., Yu, X., et al. (2011). Identification and characterization of quantitative trait loci for grain yield and its components under different nitrogen fertilization levels in rice (*Oryza sativa* L.). *Molecular Breeding*, *28*, 495–509.

Vijayakshmi, P., VishnuKiran, T., Rao, Y. V., Srikanth, B., Rao, I. S., Sailaja, B., et al. (2013). Physiological approaches for increasing nitrogen use efficiency in rice. *Indian Journal of Plant Physiology, 18*, 208–222.

Vijayalakshmi, P., Rao, Y. V., Vishnukiran, T., Yugandhar, P., Swamy, K. N., Surekha, K., et al. (2015a). Germination behavior and chlorophyll content of aromatic rice (*Oryza sativa* L.) genotypes under varied nitrogen concentrations. *Journal of Global Biosciences., 7*, 2911–2920.

Vijayalakshmi, P., Vishnukiran, T., Kumari, B. R., Srikanth, B., Rao, I. S., Swamy, K. N., et al. (2015b). Biochemical and physiological characterization for nitrogen use efficiency in aromatic rice genotypes. *Field Crops Research, 179*, 132–143.

Von Wiren, N., Gazzarrini, S., Gojon, A., & Frommer, W. B. (2000). The molecular physiology of ammonium uptake and retrieval. *Current Opinion in Plant Biology, 3*, 254–261. doi:10.1016/S1369-5266(00)00073-X.

Wang, M. Y., Siddiqi, M. Y., Ruth, T. J., & Glass, A. D. M. (1993). Ammonium uptake by rice roots. I. Fluxes and subcellular distribution of $^{13}NH_4+$. *Plant Physiology*, *103*, 1249–1258.

Wang, X., Wang, Y., Tian, J., Lim, B. L., Yan, X., & Liao, H. (2009). Overexpressing AtPAP15 enhances phosphorus efficiency in soybean. *Plant Physiology*, *151*, 233–240.

Wang, W. H., Kohler, B., Cao, F. Q., Liu, G. W., Gong, Y. Y., Sheng, S., et al. (2012). Rice DUR3 mediates high-affinity urea transport and plays an effective role in improvement of urea acquisition and utilization when expressed in arabidopsis. *New Phytologist*, *193*, 432–444.

Wei, D., Cui, K., Pan, J., Wang, Q., Wang, K., Zhang, X., et al. (2012a). Identification of quantitative trait loci for grain yield and its components in response to low nitrogen application in rice. *AJCS., 6*(6), 986–994.

Wei, D., Cui, K., Ye, G., Pan, J., Xiang, J., Huang, J., et al. (2012b). QTL mapping for nitrogen-use efficiency and nitrogen-deficiency tolerance traits in rice. *Plant and Soil, 359*, 281–295.

Williams, L., & Miller, A. (2001). Transporters responsible for the uptake and partitioning of nitrogenous solutes. *Annual Review of Plant Physiology and Plant Molecular Biology, 52*, 659–688.

Xu, G., Fan, X., & Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*, *64*, 153–82.

Yamaya, T., Obara, M., Nakajima, H., Sasaki, S., Hayakawa, T., & Sato, T. (2002). Genetic manipulation and quantitative-trait loci mapping for nitrogen recycling in rice. *Journal of Experimental Botany, 53*, 917–925.

Yan, M., Fan, X., Feng, H., Miller, A. J., Shen, Q., & Xu, G. (2011). Rice*OsNAR2.1* interacts with *OsNRT2.1*, *OsNRT2.2* and *OsNRT2.3a* nitrate transporters to provide uptake over high and low concentration ranges. *Plant, Cell and Environment, 34*, 1360–1372.

Yuan, L., Loqué, D., Ye, F., Frommer, W. B., & von Wirén, N. (2007). Nitrogen-dependence posttranscriptional regulation of the ammonium transporter AtAMT1;1. *Plant Physiology, 143*, 732–744.