MAPPING QTLs FOR NH_4^+ AND NO_3^- USE EFFICIENCY UNDER WATER STRESS AND NON-STRESS CONDITIONS AND EXPRESSION ANALYSIS OF GLUTAMINE SYNTHETASE AND NITRATE REDUCTASE IN RICE (*Oryza Sativa* L.)

Ph.D. Thesis

by

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Thesis

Submitted to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur

by

Rashmi Upadhyay

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

in

Plant Molecular Biology and Biotechnology

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SEPTEMBER, 2016

CERTIFICATE-I

This is to certify that the thesis entitled **"Mapping QTLs for NH_4^+ and NO_3^- use efficiency under water stress and non-stress conditions and expression analysis of Glutamine Synthetase and Nitrate Reductase in rice *(Oryza sativa* L.)"** submitted in partial fulfillment of the requirements for the degree of **Doctor of philosophy in Agriculture** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Rashmi Upadhyay** under my guidance and supervision. The subject of the thesis has been approved by Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by her.

6/9/16

Chairman

Date: 06/09/2016

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

Chairman	(Dr. S.B. Verulkar)	
Member	(Dr. Shubha Banerjee)	6/9/16
Member	(Dr. R.K. Bajpai)	6/9/16
Member	(Dr. R. R. Saxena)	6/9/16
Member	(Head of the Department)	6/9/16

CERTIFICATE – II

This is to certify that the thesis entitled **"Mapping QTLs for NH_4^+ and NO_3^- use efficiency under water stress and non-stress conditions and expression analysis of Glutamine Synthetase and Nitrate Reductase in rice *(Oryza sativa* L.)"** submitted by **Rashmi Upadhyay** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Doctor of philosophy in Agriculture** in the Department of Plant Molecular Biology and Biotechnology has been approved by the external examiner and Student's Advisory Committee after oral examination.

27/3/2017

Signature External Examiner
(Name: Padmini Swain)

Date: 27/03/2017

Major Advisor _____ 27/3/17

Head of the Department _____ 27/3/17

Faculty Dean _____

Approved /Not approved

Director of Instructions _____

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good friend and understanding me well in more ways than one. Their support has been unconditional all these years; they have given up many things for me to be at IGKV; they have cherished with me every great moment and supported me whenever I needed it. And I need to thank them in spanish now... ¡GRACIAS a toda la familia!

Finally, I would like to dedicate this work to my all relatives, who encouraged me to work forward with immense confidence and having faith upon myself. I hope that this work makes them proud.

At the end I would like express appreciation to my beloved husband Sanjay Joshi who spent sleepless nights with and was always my support in the moments when there was no one to answer my queries.

Whenever I will open my thesis, each page of this manuscript will tell story of contribution of my friends, batch mates and well-wishers. It will be untold story and can never be expressed in words. I frankly admit that it is not possible to remember all the faces that stood behind the facade at this juncture and omission of any names does not mean lack of gratitude. Now as I carry this thesis in my hand. I carry memories with me that will enrich my nostalgia. I have taken literary license but any factorial errors are my responsibility alone.

Rashmi

(Rashmi Upadhyay)

Date: 6/9/2016

Department of Plant Molecular Biology & Biotechnology

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%	percent
&	and
°C	degree celsius
@	at the rate of
#	Chromosome
ρ	Correlation coefficient
σ	Variance
N	total sample size
n	Sample size
F	F-distribution
σ_x	Variance of random variable X
SEM	standard error of the mean

LIST OF ABBREVIATIONS

µ	micron
µl	microlitre
Approx	Approximately
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
APS	Ammonium per sulphate
bp	Base pair
BY	biological yield
C/Chr	chromosome
CIM	Composite Interval Mapping
cm	centimeter
CEC	Cation exchange capacity
cM	centimorgan
d.f.	degree of freedom
dATP	deoxy adenosine 5’ triphosphate
dCTP	deoxy cytidine 5’ triphosphate
dGTP	deoxy guanosine 5’ triphosphate
DNA	deoxyribo nucleic acid
dNTPs	deoxynucleotide triphosphates
dTTP	deoxy thymidine 5’ triphosphate
DEPC	Diethyl pyrocarbonate
EDTA	ethylene diamine tetra acetic acid
et al.	and others
EtBr	ethidium bromide
EtOH	ethenol
Fdx	Ferredoxin
g	gram
gms	grams
GOGAT	Glutamate synthase
GS	Glutamine synthetase
µg	micrograms
GY	grain yield
H_2O	water
ha	hectare
HCl	hydrochloric acid
HI	harvest index
Hrs	hours
HvSSR	highly variable simple sequence repeat
HATS	High-affinity transport system

i.e.	that is
KCl	potassium chloride
LATS	Low-affinity transport system
m	meter
M	molar
MAS	marker assisted selection
mb	mega base pairs (10^6 bp)
Mg	Magnesium
$MgCl_2$	magnesium chloride
min	minute
ml	Milliliter
mm	Millimeter
MC	Moisture content
NaCl	sodium chloride
ng	nanogram
nm	nanometer
NILs	near isogenic lines
N	Nitrogen
NH_4^+	Ammonical Nitrogen
NUE	Nitrogen use efficiency
NUpE	Nitrogen Uptake Efficiency trait
NUtE	Nitrogen Utilisation Efficiency trait
NO_3^-	nitrate Nitrogen
NR	Nitrate reductase
NiR	Nitrite reductase
PCR	polymerase chain reaction
ppm	Parts per million
PO4, Pi	Phosphate ion (inorganic phosphate)
pH	logarithm of the reciprocal of the H^+ ion activity
QTL	quantitative trait loci
RIL	recombinant inbred line
RT-PCR	reverse transcription polymerase chain reaction
rpm	rotations per minute
SDS	sodium dodecyl sulphate
SNP	single nucleotide polymorphism
spp.	species
SSR	simple sequence repeats
S. No.	serial number
SO_4	sulphate
SA	Special activity
TBE	tris boric acid EDTA buffer
TEMED	N,N,N',N'-tetramethylethylenediamine

TSD	terminal stage drought
U	Units
viz.	for example

THESIS ABSTRACT

a)	Title of Thesis:	Mapping QTLs for NH_4^+ and NO_3^- use efficiency under water stress and non-stress conditions and expression analysis of Glutamine Synthetase and Nitrate Reductase in rice *(Oryza sativa* L.)
b)	Full name of student:	Rashmi Upadhyay
c)	Major Subject:	Plant Molecular Biology and Biotechnology
d)	Name and Address of the Major Advisor :	Dr. S. B. Verulkar (Proff. & Head) Dptt. of Plant Molecular Biology and Biotechnology, IGKV, Raipur.
e)	Degree to be awarded:	Ph.D. (Ag.) Plant Molecular Biology and Biotechnology

Rashmi.
Signature of the Student

6/9/11
Signature of the Major Advisor

Date: 06/09/2011

6/9/11
Signature of the Head of the Department

Rice is the most important staple crop in Asia, where it provides 35–60% of total calorie intake. Faced with scarcity of water resources, water deficit condition/drought depresses the rice production by reducing water and nutrient availability to the plant. Nitrogen (N) is one of the most critical inputs and the current average nitrogen use efficiency (NUE) in the rice field is approximately 33%, poorest among cereals. Predominant form of N in aerobic soils is nitrate (NO_3^-) while ammonium (NH_4^+) exists in anaerobic soils. Rice prefers to utilize NH_4^+ over NO_3^- as rice is pertained to waterlogged conditions. Development of cultivars with improved NH_4^+ or NO_3^- use efficiency by harnessing inherent significant variability for NUE can be an important approach. Considering these facts, the present study was undertaken with one hundred twenty two and selected thirty two recombinant inbred lines (RILs) of two indica genotypes, Danteshwari × Dagad deshi which were evaluated in factorial RCBD design with two replications each under three nitrogen forms and three environments along with root studies under soil filled glass rhizotrons at research cum instructional farm of COA, IGKV, Raipur, for generation of phenotypic data. The previously generated genotypic data of 162 SSR and HvSSR based markers were used for identifying

QTLs for agronomically complex trait *i.e.* NUE. The study of NH_4^+ & NO_3^- dynamics in soil, enzyme assay and expression analysis of key known genes was accomplished at Molecular Marker Laboratory, Department of Genetics and Plant Breeding, IGKV, Raipur.

The trend analysis of NH_4^+-N & NO_3^--N dynamics revealed that NH_4^+-N concentration persisted more under anaerobic condition and NO_3^--N concentration under aerobic conditions. Three way-ANOVA showed high level of significance for variance components (G, N, E) and their interactions effects (GXN, GXE, NXE, EXNXG) for yield & NUE and their component traits. Mean performance of genotypes depicted higher values for agronomically important traits *i.e.* yield and NUE under NH_4^+ treatment as compared to NO_3^--N and N^0 treatment under all environments. Genotype G-31 manifested the highest NUE values under irrigated condition in all treatments (17.2 $gg^{-1}N$, 12.72 $gg^{-1}N$, 16.98 $gg^{-1}N$) while Genotype G-27 has the maximum NUE values under rainfed condition in all treatments (8.4 $gg^{-1}N$, 11.6 $gg^{-1}N$, 6.2 $gg^{-1}N$). Grain yield showed significant and positive association with NUE indices under all sets of environments. Furthermore, significant and positive correlation existed between total root length and NUE parameters irrespective of NH_4^+, NO_3^- and N^0 treatment.

The phenotypic and genotypic data was statistically analyzed for QTLs identification for yield & NUE traits using QTL Cartalographer and results were projected graphically using Graphical Genotypes (GGT). At logarithmic odds of 2.5, a total of 58 QTLs conferring the corresponding five traits were detected under three N forms and two environments; that as a matter of fact included 14, 15 & 11 QTLs under NH_4^+, NO_3^- and N^0 level of irrigated condition and 5, 10 & 1 QTLs under NH_4^+, NO_3^- and N^0 level of rainfed conditions respectively. QTLs for GY under irrigated condition, had positive additive effect indicating allele from female parent (Danteshwari) while under rainfed condition, had negative additive effect revealing contributing allele from tolerant parent genotype (Dagaddeshi). 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. Furthermore, the graphical genotypes (GGT) of NH_4^+, NO_3^- and N^0 responsive lines under irrigated and rainfed condition with GY and GYR QTLs regions flourish us with the information of novel regions influencing the grain yield.

We also 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, Os*Gln1;1*, Os*Gln1;2,* Os*Gln1;3* , Os*Gln2,* Os*Glt1* and Os*Glt2* manifested different and reciprocal responses to nitrate and ammonium supply. Overall, Os*Gln2* isoform of Glutamine Synthetase showed strong upregulation in shoot under NH_4^+ (125.8) and NO_3^- (186.7) treatment in Genotype G-4 which falls in dark green spectrum in colour classes suggesting Os*Gln2* is major assimilatory form in green tissues. Systemic expression patterns for the AMT and NRT gene families revealed that, Os*AMT1;2* exhibited significant strong expression in shoot of genotype G-4 (133.4) also having high Os*Gln* activity thus there is strong induction by endogenous glutamine while Os*AMT1;3* exhibited strong expression in root of genotype G-9 (42.1) also having low Os*Gln* activity which signifies suppression by endogenous glutamine. With regard to NRT 7.8 gene, genotype G-10 showed significant upregulation under NH_4^+ treatment in root while NRT 2.4 gene was significantly upregulated in genotype G-3 (10.3) under NO_3^- treatment in shoot. The activity of enzymes NR, NiR, GS & GOGAT was significantly affected by NH_4^+ and NO_3^- treatment. The NR and NiR activity was highest in NO_3^- treatment in shoot (1.49 & 0.31) as compared to root (0.006 & 0.017) indicating NR & NiR is nitrate inducible enzyme while GS & GOGAT activity decreases with application of N forms with N^0 having highest value for GS and GOGAT activity in shoot (0.15 & 0.29) as well as in root (0.038 & 0.068). Among all genotypes, genotype G-4 which is high yielding and relatively nitrogen efficient showed highest activity values for GS and GOGAT under NH_4^+, NO_3^- and N^0 treatment suggesting correlation between grain yield and GS activity. These results assist us to identify NH_4^+ & NO_3^- responsive cultivars which could be used for cultivation and/or used as parent's in future breeding program to produce better nitrogen use efficiency varieties under water stress and non-stress conditions.

शोध ग्रंथ सारांश

(अ) शोध ग्रंथ का शीर्षक : पानी के प्रतिबल एवं सामान्य स्थिति में नाइट्रोजन एवं अमोनियम प्रयोग कुषलता हेतु QTLs का परिसीमन तथा धान में ग्लूटामिन सिंथेटेज एवं नाइट्रेट रिडक्टेज का अभिव्यक्ति विश्लेषण

(ब) छात्रा का पूर्ण नाम : रश्मि उपाध्याय

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(इ) प्रदान की जाने वाली उपाधि : पी. एच. डी. (कृषि) पादप आणविकी विज्ञान एवं जैव प्रौद्योगिकी

मुख्य सलाहकार के हस्ताक्षर

Rashmi.
छात्रा के हस्ताक्षर

दिनांक 6/9/15

6/9/15
विभागाध्यक्ष के हस्ताक्षर

एशिया की अत्यंत महत्वपूर्ण फसल धान है, जिससे कुल कैलोरी की 35–60 प्रतिशत प्राप्त होती है। जल संसाधनों की सीमितता, जल की कमी होने/सूखा पड़ने से पौधों को जल और पोषक तत्वों की उपलब्धता कम होती है तथा चांवल उत्पादन कम होता है। नत्रजन (एन) एक ऐसा जटिल आदान है और धान के खेतों में वर्तमान में अनाज में से लगभग 33 प्रतिशत औसतन नत्रजन उपयोग दक्षता (एन यू इ) पायी गई। एरोविक मिटटी में नत्रजन नाइट्रेट (NO_3^-)के रूप में पायी जाती है, जबकि गैर एरोविक मिटटी में यह अमोनियम (NH_4^+) के रूप में पायी जाती है। जल रिसाव की स्थिति में धान में NO_3^- की अपेक्षा (NH_4^+) की उपयोगिता को प्राथमिकता दी जाती है। नत्रजन उपयोग दक्षता के माध्यम से (NH_4^+) या No_3^- की उपयोगिता बढ़ाकर प्रजातियों का विकास किया जाता है। इन तथ्यों को ध्यान में रखकर वर्तमान अध्ययन में 122 प्रभेदों में दो इंडिका के 32 प्रजनक कतारों का चयन किया गया । दंतेश्वरी X दगाद देशी का फेक्टोरियल RCBD डिजाइन में दो पुनरावृत्तियों में मूल्यांकन किया गया। इं.गां.कृ.वि.वि. के अनुसंधान शैक्षणिक प्रक्षेत्र में प्रत्येक परीक्षण में नत्रजन के तीन रूपों और तीन पर्यावरणीय स्थितियों में कांच के राइजोट्रांस में मिटटी भरकर जड़ों का अध्ययन किया गया। इसमें फीनोटाइप आंकड़ों के आधार

पर विश्लेषण किया गया। पहले जीनोटाइप आंकड़ों से 162 SSR और HvSSR आधारित मार्कर के उपयोग से QTLs की पहचान की गई तथा नत्रजन उपयोग दक्षता सस्य विज्ञान की दृष्टि से परिलक्षित की गई। इं.गां.कृ.वि.वि. रायपुर के आनुवंषिकी एवं पादप प्रजनन् विभाग की आण्विक मार्कर प्रयोगशाला में मिटटी, एंजाइम की उपलब्धता के विष्लेषण से जीन ज्ञात करने परीक्षण किया।

NH_4^+N और NO_3^-N के डायनामिक्स के प्रवृत्ति विश्लेषण से ज्ञात हुआ है कि गैर एरोविक परिस्थिति में NH_4^+N सांद्रण अधिक प्रभावी रहा, जबकि एरोविक परिस्थिति में NO_3^-N का सांद्रण असरकारी रहा। तीन खानों वाली ANOVA से ज्ञात होता है कि विभिन्नता के घटक (G,N,E) और उपज और नत्रजन उपयोग दक्षता तथा उसके घटकों के परीक्षणों में (GXN, GXE, NXE, EXNXG को) प्रभावित करता है। सस्य विज्ञान आधारित परीक्षणों में उपज और नत्रजन उपयोग दक्षता NH_4^+ में सर्वाधिक जीनोटाइप का औसत आंकलन मिला है, जिसकी तुलना में सभी परीक्षणों में NO_3^- और N^0 परीक्षण का कम आंकलन किया गया है। सभी परीक्षणों (17.2gg -1N, 12.72 gg-1N, 16.98gg-1N) में सिंचाई में जीनोटाईप G-3 की सर्वाधिक नत्रजन उपयोग दक्षता आंकी गई, जबकि G-27 जीनोटाईप में वर्षा आधारित परिस्थिति में नत्रजन उपयोग दक्षता सभी परीक्षणों (8.4gg $^{-1}$N, 11.6 gg^{-1}N, 6.2gg^{-1}N) सर्वाधिक पायी गई। सभी प्रकार के वातावरण में नत्रजन उपयोग दक्षता के साथ दानों की उपज का धनात्मक प्रभाव पाया गया। NH_4^+, NO_3^- और N परीक्षणों के नत्रजन उपयोग दक्षता के मापकों में जड़ों की कुल लम्बाई का धनात्मक संबंध पाया गया।

उपज और नत्रजन उपयोग दक्षता के लिए QTL का उपयोग कर फीनोटाईप और जीनोटाईप आंकड़ों का सांख्यिकी विश्लेषण QTLs पहचान हेतु किया गया। ग्राफीकल जीनोंटाईप के उपयोग से कारतलोग्राफर और निष्कर्षों को ग्राफ में प्रस्तुत किया गया। लाग आड़ 2.5 के अनुसार कुल 58 QTLs पांच परीक्षणों के सुनिष्चित किये, जिसमें तीन N के रूप में और दोनों परिस्थितियों में NH_4^+, NO_3^- और N^o वर्षा आधारित परिस्थितियों में 14 , 15 और 11 QTLs शामिल किये गये। सिंचित परिस्थिति (दंतेष्वरी) का धनात्मक प्रभाव देखा गया, जबकि वर्षा आधारित परिस्थिति में सहनषील पैतृक जीनोटाईप (दगद देषी) में ऋणात्मक प्रभाव देखा गया। विभिन्न परिस्थितियों में क्लस्टर HvSSR 1-87 (38.9 cM) से HvSSR 1-89 (40.6 cM) के बीच में क्रमोसोम 6, RM 449 (81.9 cM) से RM (9.4 cM) में क्रोमोसोम 5, HVSSR 9-25 (14.6 cM) से HVSSR 9-27 (15.5 cM) में क्रोमोसोम 9 और RM 434 (57.7 cM) से RM 410 (64.4cM) से क्रोमोसोम 5 का महत्वपूर्ण जेनोमिक क्षेत्रों में परीक्षण किये गये तथा धान में नत्रजन उपयोग दक्षता के लिए उपयोगी मार्कर सहायतित प्रजनन किया गया। उसके बाद सिंचित और वर्षा आधारित

परिस्थिति में GY और GYR QTLs क्षेत्रों में NH_4^+ , NO_3^- और N^0 कतारों में ग्राफिकल जीनोटाइप (GGT) से उपयुक्तता जानने तथा दानों की उपज पर प्रभाव देखा गया।

हमने अमोनियम ट्रांसपोर्टर, नाइट्रेट ट्रांसपोर्टर, ग्लूटामिन सिन्थटेज, ग्लूटामेट सिन्थेज वंषाणु के विभिन्न सदस्यों की जांच की जो कि नाइट्रोजन उपयोग कुषलता में कार्यारत हैं तथा वंषाणु विषिष्ट प्राइमर की सहाचयता से मात्रामन – पी सी आर प्रक्रिया द्वारा युवा धान की पौध में प्रत्येक वंषाणु की अभिव्यक्ति का विष्लेषण किया। सामूहिक रूप से OS*Gln1;1*, Os*Gln1;2*, Os*Gln1;3*, Os*Glt1*, Os*Glt2* वंषाणु में विभिन्न पारस्परिक परतसद, अमोनियम तथा नाइट्रेट के अंतर्गत गहरे हरे रंग वाली जीन प्रारूप –4 (186. 7) तना कोषिका में Os*Gln2* का मजबूत उपक्रियान्वयन दिखाती है। मुख्यतः Os*Gln2* हरी कोषिकाओं में ग्लूटामिन सिन्थेटेज का प्रमुख स्वरूप है। अमोनियम तथा नाइट्रेट ट्रांसपोर्टर वषाणु के परिवारों की क्रमबद्वतः अभिव्यक्ति प्रतिमानों से पता चला है कि Os*AMT1;2* की जीन प्रारूप –4(133.4) सषक्त अभिव्यक्ति है जो Os*Gln2* की भी उच्च गतिविधि दिखाती है, इस प्रकार ग्लूटामिन इन वंषाणु के मजबूत प्रवेरक के लिये कार्यरत है। जबकि Os*AMT1;3* ने जीन प्रारूप –9 (42.1) की जड़ों में मजबूत अभिव्यक्ति का प्रदर्षन किया है। जिसमें Os*Gln* की गतिविधि कम थी जो अंतर्जात ग्लूटामिन के दमन का प्रतीक है।

नाइट्रेट ट्रांसपोर्टर वंषाणु के सम्बन्ध में Os*NRT7.8* ने अमोनियम उपचार के अंतर्गत जीन प्रारूप 10 की जड़ों में महत्वपूर्ण उपक्रियान्वयन दिखाया है। जबकि नाइट्रेट उपचार के अंतर्गत Os*NRT 2.4* ने जीन प्रारूप –3 की शूट कोषिका में (10.3) महत्वपूर्ण उपक्रियान्वयन दिखाया है। इसके अतिरिक्त नाइट्रेट तथा अमोनियम उपचार ने एन्जाइम्स GS, GOGAT, NR, NiR की गतिविधियों को क्रियान्वित किया है। नाइट्रेट उपचार में NR तथा NiR की गतिविधि जड़ों की तुलना में तना कोषिकाओं में सबसे अधिक थी। ये NR तथा NiR की नाइट्रेट इन्डयूसिबिलिटी को दर्शाता है जबकि GS एवं GOGAT का कार्यकलाप नाईट्रोजन के अनुपयोग से घटता जाता है। सभी प्रारूपों में अधिक उपज वाली एवं अति नाइट्रोजन उपयोग कुषलता वाली जीन प्रारूप–4, ने GS एवं GOGAT की उच्चतम गतिविधि दिखायी। यह उपज तथा GS स्वरूपों के कार्यकलापों के बीच महत्वपूर्ण रिश्ता दर्शाता है। यह परिणाम हमें धान की फसल में नाइट्रेट और अमोनियम उत्तरदायी कल्टिवार का आंकलन करने में मदद करता है जिससे भविष्य में खेती एवं प्रजनन द्वारा कम पानी में नाइट्रोजन उपयोग कुशलता वाली किस्म को उत्पादित करने के लिए प्रयोग में लाया जा सकता है।

CHAPTER - I

INTRODUCTION

Rice is first mentioned as *vrihi* in the Yajur Veda (1000-3000 BC) and then is frequently referred in Sanskrit texts. This entails its importance as one of the most important crops from the ancient time. Rice is a member of family Poaceae and finds its home in South-East Asia and Asia, where more than 90% of world's rice is produced and consumed (Li and Xu, 2007). Rice commended recognition, as a supreme commodity to mankind, because rice is truly a life, culture, a tradition, staple diet and a means of livelihood to millions (Barah and Pandey, 2005). Considering its position, the United Nation assembly, in a resolution declared the year of 2004 as the "International Year of Rice".

Rice is globally grown in about 154 million hectares annually with total production of 600 million tons (FAOSTAT, 2016). India contributes the largest area (44.1 million hectares) followed by China (30.31 million hectares), Indonesia (13.3 million hectares), Bangladesh (12 million hectares) and Myanmar (8 million hectares) (INDIASTAT, 2016). Among the rice growing states in India, Chhattisgarh the central eastern state marks the prominent place and often referred as the "Rice bowl of India. It occupies an area of around 3.71 million ha, production is 6.62 million tonnes and productivity is 1780 kg/ ha, in 2013-14 (INDIASTAT, 2016).

Despite of the large acreage, global food security is at stake since demand of rice is exceeding production. Having surpassed the seven billion mark in late 2011; population is expected to reach 9.3 billion by 2050 (Tester, 2010). Evans (1998), argued that world population expansion would outstrip growth in food supply, resulting in starvation. In the intervening period these gloomy predictions have reappeared from time to time, only to be banished again by arable land expansion. Thus, increase in rice production has to be obtained by increasing productivity, i.e. an increased yield per unit land by identifying more nutrient efficient genotypes (Vinod *et al*., 2012).

Rice farming is .practiced in several agro-ecological zones in India. Widespread cultivation has led to occurrence of four distinct types of ecosystems:

irrigated, rainfed upland, rainfed lowland and flood prone. More than half of the rice area (55 %) is rainfed and distribution wise 80 % of the rainfed rice areas are in eastern India, because of this rice yield is low and uncertain and vulnerable to vagaries of nature. Rice is a wetland crop adapted and evolved under anaerobic conditions. Faced with scarcity of water resources, drought is the single most critical threat to rice production thus affecting world food security (Farooq *et al*., 2008).

Drought is the composite stress condition that includes soil water deficit and reduced nutrient availability to the plant (Oliver *et al*., 2011). Plant species and genotypes of a species may vary in their response to mineral uptake under water stress. Haefele *et al*. (2008) strengthen the conception of water and nutrient stress as two major constraints in most rice-based rainfed system in Asia. Both stresses interact and contribute to the low productivity in cultivated areas worldwide. However, bottlenecks of water scarcity have been chased and breeders even developed a new plant type ‘‘aerobic rice’’ targeted at water limited rice environment (Atlin *et al*., 2006). Area under aerobic 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).

Of all the nutrients, Nitrogen (N) is fundamental to crop development (Lea and Miflin, 2011). Nitrogen (N) is one of the most critical inputs that define crop productivity and yield under field conditions and must be supplemented to meet the food production demands of an ever-increasing population (Pathak *et al*., 2008). It is also one of the most expensive as the commercial N fertilizers represent the major cost in plant production (Singh, 2005). Furthermore, the statistics reveals that the doubling of agricultural food production worldwide over the past 4 decades has been associated with a 7-fold increase in the use of nitrogen fertilizers (Hirel *et al*., 2007). The current average nitrogen use efficiency (NUE) in the rice field is approximately 33%, poorest among cereals and a substantial proportion of the remaining 67% is lost into the environment N reducing economic efficiency of applied N (Hakeem *et al*., 2012a). This calls for immediate development of comprehensive approach to optimize N management in every sphere of life. Therefore, the form and amount of N available to the plant can be improved by managing fertilizer–soil–water–air interactions and by

harnessing the innate efficiency of genotypes/species to utilize the available N and grow well and yield better.

Nitrogen use efficiency (NUE) in plants is a complex quantitative trait that involves many genes and depends on a number of internal and external factors in addition to soil nitrogen availability (Gupta *et al.*, 2012). NUE at the plant level includes nitrogen uptake and assimilatory processes, redistribution within the cell and balance between storage and current use at the cellular and whole plant level. Rice genotypes shows significant variability for N uptake (external efficiency) and N utilization (internal efficiency) with yield being predominantly determined by the uptake process, particularly under low-N conditions (Witcombe *et al.*, 2008).

Predominant forms of N changes with change in water availability. Plant-useable N is consumed as nitrate (NO_3^-) from aerobic soils and as ammonium (NH_4^+) from flooded wetland, anaerobic soils (Huang *et al.*, 2000). Field drainage has profound effect on N dynamics in soil. When the field is drained and the soil becomes aerobic, ammonium is oxidized through microbial processes (known as nitrification) into nitrate (NO_3^-).

Rice roots are exposed to a mixed N forms in rhizosphere (Briones *et al.*, 2003 and Li *et al.*, 2003) but it prefers to utilize ammonium (NH_4^+) over nitrate (NO_3^-) as rice is pertained to waterlogged growth conditions (Li *et al.*, 2009). Rice root and whole metabolic system has evolved and adopted for efficient utilization of NH_4^+ as compared to NO_3^-. It is therefore not surprising that NH_4^+ nutrition, as opposed to NO_3^- nutrition, has received almost exclusive attention in rice (Wang *et al.*, 1993). However, kinetic and comparative analysis of ammonium and nitrate acquisition by Kronzucker *et al.* (2000) has opened new insight for NO_3^- nutrition studies.

Furthermore, root architecture and the activities of ammonium and nitrate transporters that are regulated by N form and concentration affects the N acquisition by roots (Garnett *et al.*, 2009; Gifford *et al.*, 2008). Intrinsic aspects of N utilization includes many gene families, including NO_3^- and NH_4^+ transporters genes and primary assimilatory genes that have been identified by different approaches. To cope with varying NO_3^- & NH_4^+ concentrations in soils, N uptake in roots is mainly regulated by a high affinity transport system (HATs) that regulates uptake at N levels <1mM, and a

low affinity transport system (LATs) that functions at high N concentrations >1mM (Glass *et al*., 2001). Nitrate transporter system along with nitrate reductase (NR) and nitrite reductase (NiR) enzymes subsequently facilitate reduction of NO_3^- in to NH_4^+. Expression of the NRTs is highly regulated (Feng *et al*., 2011; Krouk *et al*., 2010). NH_4^+ from both the nitrate reduction pathway and direct absorption are subsequently incorporated into amino acids through the synthesis of glutamine and glutamate (Meyer and Stitt, 2001; Campbell, 2002) primarily in the chloroplasts and plastids via the glutamine synthetase (GS)/glutamate synthase (GOGAT) cycle (Tobin and Yamaya, 2001; Andrews *et al*., 2004).

Rice genotypes have shown wide variability and considerable potential for NO_3^- assimilation. Rice plant is able to assimilate NO_3^- at early seedling stage owing to high nitrate reductase activity at this stage (Ouko, 2003). Genotypic differences in nitrate transporter system and nitrate assimilatory enzymes *i.e*. NR & GS among rice genotypes is accomplished well by Hakeem *et al*., 2012b. Moreover, differential expression of ammonium transporter and glutamine synthetase genes in rice genotypes under influence of different N inputs has been earlier studied (Gaur *et al*., 2012). Studies have shown nitrogen assimilation to be critical for plant acclimation to water stress conditions. It has been demonstrated that GS isoforms are regulated both at transcript and protein levels in response to plant status as well as environmental cues (Swarbreck *et al*. 2011). However, the regulation of these transporters and enzymes under different forms of N i.e. NO_3^- and NH_4^+ during water stress has not been investigated in detail. The role of GS isoforms, GOGAT gene and transporter system in controlling metabolism during water stress and aerobic condition can be understood by studying their regulation in differentially drought tolerant rice genotypes.

In rice, the first attempt to use marker based QTL analysis to map the QTL associated with associative nitrogen fixation, nitrogen uptake and NUE was started at the International Rice Research Institute involving a population from Palawan X IR 4. As of now, several genomic regions associated with N use and response have been mapped in rice (Fang and Wu, 2001; Ishimaru *et al*., 2001; Obara *et al*., 2001). Hirel *et al*. (2001) used a combined quantitative genetic and physiological approach to investigate the relationship between yield, nitrate content, and NR and

GS activities under low and high nitrogen conditions. Yield and its components were found to show positive correlations with GS activity and nitrate content, and negative correlations with NR activity. Moreover, coincidences of QTLs for yield and components, with genes encoding cytosolic GS and corresponding enzyme activity were also detected. These QTLs were found to co-localise, suggesting that increased nitrate accumulation and the GS activity are important factors for determining yield, and providing a strong candidate gene.

The highly complex objective requires comprehensive knowledge and deep understanding of the physiological, biochemical and molecular responses of rice genotypes under different nitrogen regimes. In the present investigation, attempts has been made to identify efficient lines in mapping population derived from *Indica* parents that shows differential expression of transporter systems and key assimilatory enzymes along with biochemical characterization of these enzymes & morphological characterization of their root system. Keeping in view all the above facts the present study was planned with the following objectives.

The objectives of the present study are:

1. Evaluation of mapping population for root traits in mini-rhizotron with ammonical form of Nitrogen
2. Evaluation of mapping population for root traits in mini-rhizotron with nitrate form of Nitrogen
3. Identification of root traits associated with response to nitrate and ammonium in a RIL mapping population.
4. Genotyping of mapping population with SSR markers.
5. Identification of QTLs for response to nitrate and ammonium.
6. Expression analysis of Glutamine Synthetase and Nitrate Reductase.

CHAPTER - II

REVIEW OF LITERATURE

2.1 The model plant, Rice (*Oryza sativa* L.)

A Chinese proverb says, "Most precious things are not jade and pearl but rice grains". Over 90 per cent of world's rice is produced and consumed in Asia. Rice is the most important staple crop in Asia, where it provides 35–60% of total calorie intake (IRRI, 1997). More than 75% of the rice supply comes from 79 million ha of irrigated land. Thus, Asia's present and future food security depends largely on the irrigated rice production system. However, the water-use efficiency of rice is low, and growing rice requires large amounts of water. In Asia, irrigated agriculture accounts for 90% of total diverted freshwater, and more than 50% of this is used to irrigate rice. Until recently, this amount of water has been taken for granted, but now the global "water crisis" threatens the sustainability of irrigated rice production (Amudha *et al*., 2009). Furthermore, the rainfed lowland system in Asia has its own limitation. It covers about 46 million ha, and most of the area in this ecosystem faces various biophysical constraints to rice production (Maclean *et al*., 2002). Nutrient availability might be further reduced by the often alternating soil water regime and its effects on soil chemistry (O'Toole and Baldia, 1982; Bacon *et al*., 1986 and Singh *et al*., 1995). Therefore, researchers are looking for ways and means to improve water use and nutrient use efficiency in rice production.

2.2 Water deficit and nutrient relations

Water is one of the most critical inputs in plant growth and development. Water deficit condition is one of the major constraints depressing rice production. Food security becomes critical as drought intensifies. The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands and moisture storing capacity of soils (Wery *et al*., 1994). The effects of drought range from morphological to molecular levels and are evident at all phonological stages of plant growth at whatever stage the water deficit takes place. Most profound effect of drought is on nutrient relations.

Nutrient uptake of crop plants is generally decreased under water stress conditions owing to substantial decrease in transpiration rates and impaired active transport and membrane permeability and results in reducing root power of absorbing nutrient of crop plants. Plant species and genotypes of a species may vary in their response to mineral uptake under water stress (Garg, 2003). Nutrient and water requirements are closely related. There is significant interaction between soil moisture deficits and nutrient acquisition. It was shown that N and K uptake was hampered under drought stress in cotton (McWilliams, 2003). Likewise, P and PO_4^{3-}contents in the plant tissues diminished under drought, possibly because of lowered PO_4^{3-} mobility as a result of low moisture availability (Peuke and Rennenberg, 2004). In drought-treated sunflower, the degree of stomatal opening of K^+-applied plants initially indicated quicker decline. However, at equally low soil water potential, diff usive resistance in the leaves of K^+- applied plants remained lower than those receiving no K^+ (Lindhauer *et al*., 2007). In summary, drought stress reduces the availability, uptake, translocation and metabolism of nutrients. A reduced transpiration rate due to water deficit reduces the nutrient absorption and efficiency of their utilization.

2.2.1 Water deficit and nitrogen relations

Nitrogen transformation processes closely depend on water and its mobility in the soil (Keller, 2005). The size of soil NO_3^- and NH_4^+ pools reflects the balance between various fluxes which result from several soil processes (Fig. 2.1): plant N uptake, mineralization, immobilization, nitrification and losses to the atmosphere following denitrification and volatilization (Lemaire *et al*., 2004). Mineralization, immobilization and nitrification are affected by soil water content (Gorissen *et al*., 2004; Lemaire *et al*., 2004). Westerman and Tucker (1978) showed that during a wet period, the soil mineral N content decreased because of the increase in immobilization by microorganisms and losses to the atmosphere. Rain pulses can thus enhance microbial activity without exerting any significant effect on plant growth and hence on N demand by plants (White *et al*., 2004a). During dry periods, root death increases (Huang and Gao, 2000); the soil organic labile N pool may do so as well (White *et al.*, 2004b). Austin *et al*. (2004) demonstrated that mineralization was activated by a water pulse after a drought

period. However, following a dry period, during which root and bacterial death increased the amount of soil organic matter, both the immobilization rate and nitrification increased after soil rewetting (Smolander *et al*., 2005). No general effect of drought on long-term soil mineral N availability can thus be expected from such a complex network.

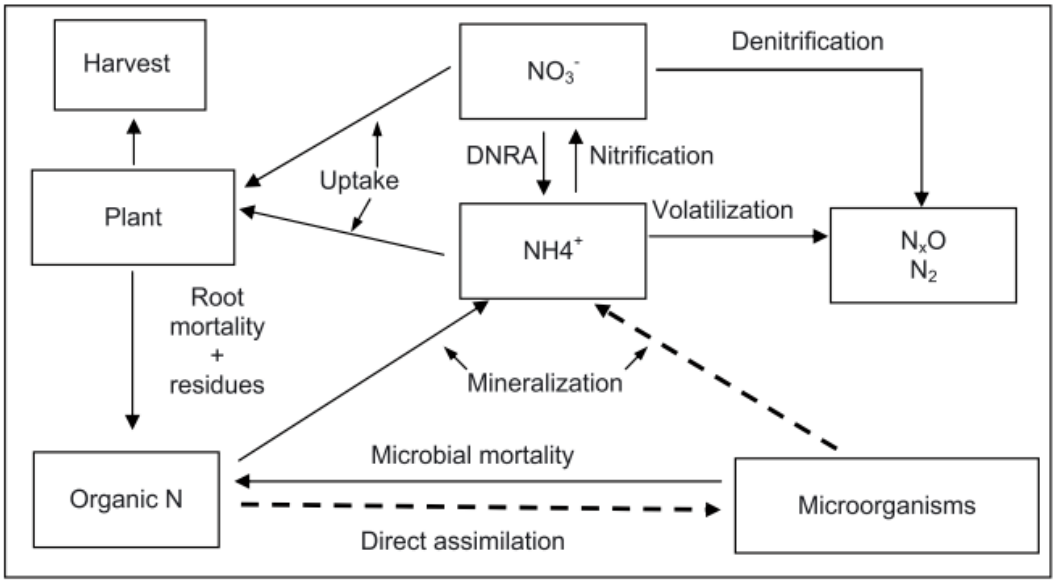


Fig 2.1: N cycle in the soil-plant-atmosphere system. Each process can be altered by the soil water regime. (Source: Lemaire *et al*., 2004)

2.2.2 Water deficit and nitrogen nutrition

Among all the plant nutrients effect of water deficit on N nutrition has been the subject of considerable research in past at both plant (Morgan, 1984; Nicolas *et al*., 1985; Larsson, 1992; Matzner and Richards, 1996) and canopy (Lemaire and Denoix, 1987; Cantero-Martinez *et al*., 1995; Onillon *et al*., 1995; Gonzalez-Dugo *et al*., 2005; Mistele and Schmidhalter, 2008) levels. Jury and Vaux, 2005 asserted that water and N have been overused in agriculture for decades, but this is no longer sustainable, considering the economic and environmental costs of these practices. More than 55% of the increase in crop production, especially in emerging countries, comes from the use of chemical fertilizers, with N fertilizers being dominant (Li *et al*., 2009). However, N is also a significant pollutant that has a great impact on ecosystem deterioration (Galloway *et al*., 2008) and biodiversity (Stevens *et al*., 2004).

During its life cycle, a plant may be subjected to a water deficit, an N deficit or a combination of both, thus co limiting (Sadras, 2005) its productivity. The effects of water and N deficit on plant production therefore depend on their timing and intensity (Bradford and Hsiao, 1982; Nielsen and Halvorson, 1991). Under water scarcity, N demand by plants is reduced, as growth rate is diminished. If the effect on N supply is greater than that on plant growth, the result will be an N deficiency. The implications of this demand/supply duality can be observed when different species exhibiting contrasted growth rates are compared under similar conditions (Gonzalez-Dugo *et al*., 2005a). This series of direct and indirect (via N limitation) affects of water shortage on plant growth makes any quantification of the effect of water deficit on N nutrition a complex task.

There is some evidence in the literature that shows how N dynamics is altered by water deficit, both in the shorter and the longer term. Using the tabulated data generated by Alvarez de Toro, (1987) during an irrigation experiment on sunflower, the relationship between shoot growth and N content during the pre-anthesis period was established. Li (2001) found that N-use efficiency was increased by water deficit and diminished by the dose of N applied. Primoradian *et al*.(2004) reached the same conclusion in rice but the effect was only significant at particular level of N. Gonzalez-Dugo *et al*. (2005b) showed the variability in resulting N status in two forage species displaying contrasting growth rates and root architecture when water and N are both limited. The effect of water deficit on plant N recovery capacity and on N-use efficiency was reviewed by Aulakh and Malhi (2005).

It has been well established that water deficit reduces plant growth, primarily due to a reduction of the stomatal conductance that inhibits the C assimilation (Bradford and Hsiao, 1982). Concerning yield, the stage at which drought occurs is critical; its effects are most pronounced if it takes place at an early stage. The first process affected by water deficit is foliar development and expansion (Fischer and Hagan, 1965). As leaves are the vegetative organs that display higher N content during early developmental stages, N demand is also drastically reduced in early water deficits. Furthermore, during exponential growth, when the soil is not fully covered, drought restricts the active

photosynthetic surface area, which causes a reduction in carbon assimilation and transpiration (Durand *et al.*, 1989). When the leaf area index is below a value of approximately 3, the percentage of PAR (Photosynthetic active radiation) absorbed to irradiate is below its potential maximum (Durand *et al*., 1991; Akmal and Janssens, 2004). This also diminishes N needs (Nielsen and Halvorson, 1991), and may lead to a somewhat paradoxical situation where, for the same N uptake, an irrigated plant might experience N deficiency while a rainfed (and smaller) plant will maintain optimum nutrition status (Gonzalez-Dugo *et al.*, 2005c).

The root/shoot ratio tends to increase with drought, largely due to a stronger effect of water deficit on shoot growth than on root growth (Sharp *et al*., 1988; Durand *et al*., 1989). Root mass changes under water deficit may be overestimated because of the restriction of root fragmentation and destruction by soil organisms, which depend on soil water content. However, some authors have found that the root growth of rice increased under water deficit (Ingram *et al*., 1994). An increase in root depth penetration has also been reported in rice (Mambani and Lal, 1983). By contrast, Yamauchi *et al.* (1996) observed a reduction in root growth as a result of drought. Engels *et al.* (1994) found that drought reduced root growth near the soil surface but enhanced it at lower levels. Onillon (1993) stated that the effect of water deficit on root dry weight was dependent on N fertilizer application.

2.3 Nitrogen use efficiency

Owing to inherently inefficient use of N of around 33% in cereal crops, nitrogen use efficiency becomes economically and environmentally highly desirable trait. There are several definitions for NUE as indicated in table 2.1, depending on whether authors are dealing with agronomic, genetic, or physiological studies (Good *et al*. 2004). As a function of multiple interacting genetic and environmental factors, NUE is inherently complex. N use, grain yield and N accumulation, N in aboveground plant biomass, N harvest index, and grain N accumulation are the key indicators of N use efficiency (Huggins and Pan, 2003). The definition of NUE itself is also complex, and the term can mean different things in different contexts, including N use efficiency (NUE), N uptake efficiency (NUpE), N utilization (assimilation) efficiency (NUtE), apparent N

recovery rate (ANR), agronomy efficiency of fertilizer N (AE), N physiological use efficiency (NpUE), N transport efficiency (NTE), and N remobilization efficiency(NRE). In general, two plant physiological components—NUpE and NUtE— contribute to plant NUE (Xu *et al*., 2012a).

NUE in plants is a complex trait that in addition to soil nitrogen availability can also depend on a number of internal and external factors (Lewis *et al*., 2000).

Table 2.1: Definitions and methods of calculating nitrogen use efficiency

Nutrient efficiencies	Definitions and formulas for calculation
➢ Nitrogen uptake efficiency (NUpE)	N uptake at harvest/kg available N from soil and fertilizer)
➢ Nitrogen use efficiency (NUE)	Total biomass or grain yield / available N from soil and fertilizer
➢ Nitrogen utilization efficiency (NUtE)	Grain yield/ N uptake at harvest
➢ Apparent nitrogen recovery rate (ANR)	N uptake by the plant with N application - N uptake without fertilization/N application
➢ Physiological N-use efficiency (NUpE)	Grain yield with N application-grain yield without N application / N application
➢ N transport efficiency (NTE)	N transported into the above g round parts / N in the whole plants
➢ Nitrogen remobilization efficiency (NRE)	Grain N uptake / total plant N uptake.

Source: Tayefe *et al*. (2011)

2.3.1 Soil N heterogeneity and plants

Soil N exists as a mixture of organic and inorganic forms, and is heterogeneously distributed. Inorganic soil N exists in two forms: reduced ammonium (NH_4^+) or oxidized nitrate (NO_3^-). Both forms exist in many terrestrial ecosystems over wide concentration ranges (Miller and Cramer, 2004). Factors which directly control the relative abundance of NH_4^+ vs. NO_3^- , in soil, are soil type, and environmental factors such as aeration, soil moisture, temperature, pH and light. Poor soil aeration, high soil moisture, low temperature, and pH results in

a reducing soil environment, and potentially, high soil NH_4^+ concentrations. The converse is true for well-aerated, dry, warm, basic soils, where NO_3^- may become the predominant N form in soil (Pearson and Stewart, 1993).

In some agricultural soils, NH_4^+ can be higher, ranging from 2 to 20 mM (Britto and Kronzucker, 2002). High NH_4^+ in soil may be due to the application of large quantities of N fertilizers in intensive agriculture, and therefore high levels of NH_4^+ accumulation are becoming more common in many natural and agricultural soils. Consequently, NH_4^+ toxicity has been linked to plant species extinction in recent years (Britto *et al*., 2001). On the other hand, many species occupying such habitats have become specialists, absorbing NH_4^+ or amino acids in preference to NH_4^+ (Kielland, 1994; Kronzucker *et al*., 1997). One such example is the cereal grass rice, which is grown in the paddies of Asia, where NH_4^+ is the predominant N source. By contrast, under ideal nitrification conditions, NO_3^- can be the predominant inorganic N source, with soil concentrations varying between 1 to 5 mM (Miller and Cramer, 2004). Many species occupying these habitats are NH_4^+-susceptible, showing toxicity under relatively low NH_4^+ soil concentrations, and as a consequence have adapted to prefer NO_3^- as an N source for growth and development. One example is the cereal grass barley, grown in North American soils, where NO_3^- is the predominant N source. Plants have evolved numerous mechanisms based on physiological, biochemical, developmental and life history strategies that enable them to optimize N acquisition, and assimilation while coping with variability in both N form and supply. Physiological adaptations include the 'up-regulation' of N uptake under N-limiting conditions, but also the restriction of N uptake under conditions of N excess; serving to minimize potentially harmful osmotic or specific ion effects (Glass *et al.,* 2002).

2.3.2 Ammonium vs. Nitrate Nutrition in rice

Nitrogen is one of the essential macronutrients for rice growth and one of the main factors to be considered for developing a high yielding rice cultivar. The majority of plant-useable N is consumed as NO_3^- from aerobic soils and as NH_4^+ from anaerobic soils (Huang *et al*. 2000). In a paddy field, ammonium (NH_4^+) tends to be considered the main source of nitrogen rather than nitrate

(NO_3^-) for rice (Wang *et al*., 1993) as discussed above. It is established in past research that rice roots can release O_2 via aerenchyma at rates sufficient to support non-specific aerobic microbial processes (Armstrong *et al*., 1990; Bedford *et al*., 1991; Begg *et al*., 1994; Kirk & Du, 1997). Also, customization of water management practice to include intermittent drainage of rice paddies during vegetative stages of rice growth, allows nitrification to occur (Arth *et al*., 1998). Rice variety and water management affects the activity of nitrifies in the rice soils. The in-depth study of rhizosphere nitrification and nitrogen nutrition of rice plants as affected by water management is provided by Dandeniya and Thies (2012). Thus, an environment where both NH_4^+ and NO_3^- coexist is created in such conditions. Therefore, it forces us to focus on acquisition potential of NO_3^- of a given species.

Kronzuker *et al*. (1998) proved that rice is capable of assimilating both forms of N thus adapted to aerobic as well as anaerobic growth conditions. Also, Kronzuker *et al*. (1999) propagated the idea of increased nitrogen acquisition by increase in influx of NH_4^+ is accredited to NO_3^-. Kirk, (2001) reported that substantial quantities of NO_3^- were produced in the rhizosphere of rice plants through nitrification, and microbial nitrification. Under cultivation, the proportion of available nitrate in soils increases, and NO_3^- becomes the dominant form instead of NH_4^+, which is traditionally assumed to be the preferred N source for paddy rice (Ying, 2002). Further, Qian *et al*. 2004 in their solution culture experiment fed rice plants with a mixture of NH_4^+ and NO_3^- and compared with either of the nitrogen sources applied alone at the same concentration, yield increase of 40-70% were observed. Using model calculations and experiments, Kirk and Kronzucker (2005) concluded that NO_3^- uptake by lowland rice might be far more important than was previously thought, its uptake rate could be comparable with that of NH_4^+ and it could amount to one third of the total N absorbed by rice. Furthermore, growth and the nitrogen acquisition of rice were significantly improved by the addition of NO_3^- to nutrition solution with NH_4^+ alone (Duan *et al*., 2005). However, in recent years, researchers have paid more and more attention to the partial nutrition (PNN) of rice crops, and their results have shown

that lowland rice was exceptionally efficient in absorbing NO_3^- formed by nitrification in the rhizosphere (Duan *et al*., 2006).

Ammonium (NH_4^+) is beneficial for plant growth under many circumstances and indeed serves as a ubiquitous intermediate in plant metabolism (Glass *et al*., 1992). Its assimilation is simple and has been shown to act as an inducer of resist further more entails lower energy costs compared to NO_3^- (Mehrer and Mohar, 1989). Additionally, studies have shown that NH_4^+ can improve the capacity to tolerate water stress in rice along with NO_3^- (Guo *et al*., 2007). Nevertheless, NH_4^+ frequently reaches levels in soils that affect plant growth negatively. These negative affects manifest in stunted root growth, yield depression and chorosis of leaves (Balkos *et al*., 2010a; Li *et al*., 2011). Despite its reputation as NH_4^+ tolerant species, rice can be affected negatively by high NH_4^+, particularly at low K^+ (Balkos *et al*., 2010b) which in turn may be relieved by elevated K^+ similar to conclusions reached in Arabidopsis (Li *et al*., 2010).

2.4 Nitrogen uptake and metabolism: genes and pathways

The molecular responses to N are complex in rice, as shown by rapid induction/repression of many stress responsible genes and transcription factors coupled with repression of photosynthetic and energy metabolism genes in seedlings subjected (Lian *et al*., 2006). However, N utilization are well studied in model plants such as Arabidopsis .Many gene families, including NO_3^- and NH_4^+ transporters and primary assimilation genes, amino acid transporters, as well as transcription factors and other regulatory genes, have been identified by different approaches. With the identification of orthologous genes from rice, opportunities are now emerging for utilizing these genes in marker-assisted breeding for N efficiency (Li *et al*., 2009; Kant *et al*., 2011).

N uptake prior to panicle initiation is crucial in building up the internal N reservoir. External efficiency declines as the crop progresses to maturity, with a reduction in the daily uptake of N towards terminal stages due to increasingly inefficient roots (Sheehy *et al*., 1998) and internal N recycling from senescing tissues to the developing panicle (Mae and Ohira, 1981). Under low N supply, internal recycling accounts for 70 – 90 % of the total panicle N (Tabuchi *et al*., 2007). On the other hand, the N concentration in the straw at crop maturity is not

significantly affected by changes in N supply at terminal stages (Tirol-Padre *et al*. 1996). Masclaux-Daubresse *et al*. (2010) overall examined the physiological, metabolic, and genetic aspects of nitrogen uptake, assimilation and remobilization in their review. The detail aspects of enzymes and regulatory processes manipulated to improve NUE components were presented. Results obtained from natural variation and quantitative trait loci studies were also discussed. Their review presented the complexity of NUE and supports the idea that the integration of the numerous data coming from transcriptome studies, functional genomics, quantitative genetics, and ecophysiology and soil science into explanatory models of whole-plant behaviour will be promising.

2.4.1 Root characteristics

Among the morphological traits associated with the adaptation to N-depleted soils, the qualitative and quantitative importance of the root system in taking up N under N-limiting conditions has been pointed out in several studies (Guingo *et al.*, 1998; Kamara *et al.*, 2003; Coque and Gallais, 2005). One of the main difficulties in evaluating the influence of the size, the volume, and the root system on NUpE and traits related to yield or grain N content is to remove the entire intact root system from soil when plants are grown under agronomic conditions (Kondo *et al*., 2003a). To solve this problem, alternative techniques have been developed under controlled environmental conditions using either ‘rhizotrons’ (Devienne-Barret *et al*., 2006; Laperche *et al*., 2007), artificial soil (Wang *et al*., 2004), or hydroponic culture systems (Tuberosa *et al.*, 2003). Consequently, there are only a limited number of reports describing the response of the root morphology of cereals to different levels of N fertilization (Kondo *et al*., 2003b).

In rice plants, particularly during the vegetative stage, roots play a significant role in N absorption with root density and distribution in the soil being the major determinants (Youngdahl *et al*., 1982). Although there are many studies related to variation in N uptake in rice, there seems to be little information on differences in root morphology that may contribute to this variation. Root characteristics such as root length density and root weight density have been identified as important factors because N uptake is determined by root mass and N uptake per unit root volume (Shimono and Bunce, 2009).

Hamaoka *et. al.* (2013) recently evaluated genetic variations in dry matter production, nitrogen uptake, N and water use efficiencies (NUE and WUE), bleeding sap rate (BR), and root morphological traits at vegetative stage in 6 cultivars and 4 strains of 6 species (*O. sativa, O. glaberrima, O. barthii, O. nivara, O. meridionalis, and O. rufipogon*) grown under different nitrogen conditions. Their results suggested that a high NUE is associated with the development of a root system, increased BR, and probably increased capacity of NO_3^--N uptake.

2.4.2 Physiology of ammonium and nitrate uptake

Dynamic variations of nitrate and ammonium concentrations exist in soil solutions (Miller *et al*., 2001). To acquire sufficient N for growth, plants have to cope with temporal and spatial fluctuation in the availability of N sources in the soil; plant roots have uptake systems for both NO_3^- and NH_4^+ with different affinities. These transport systems are generally abbreviated as HATS and LATS, respectively (Forde, 2000). High-affinity transport system regulates uptake at N levels <1 mM and a low-affinity transport system that functions at higher N concentrations >1mM (Glass *et al*., 2001; Williams and Miller, 2001). Similarly to Arabidopsis, high-affinity rice NH_4^+ transporters are encoded by members of the AMT1 and AMT2 gene families (Gazzarini *et al*., 1999; Howitt and Udvardi, 2000). The activity of AMT members in the ammonium-preferring rice may play a more important role in nitrogen physiological use efficiency (NUpE) than in nitrate utilizing crops. 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*., 2007a; Lie *et al*., 2009a). So far, studies on expressions and regulations of AMT genes in rice have been focused on the three genes of Os*AMT1* family, which displayed different spatio-temporal expression patterns in response to changes in N levels. Os*AMT1;1,* Os*AMT1;2,* Os*AMT1;3* (Fig 2.2) have been identified as members of AMT1, each showing a distinct expression pattern: Os*AMT1;1* shows constitutive expression in both shoots and roots (Ding *et al*., 2011); Os*AMT1;2* shows root-specific and ammonium-inducible expression and Os*AMT1;3* shows root-specific and nitrogen-suppressible expression (Sonoda *et al*., 2003a).Os*AMT1;1* expression was promoted by low ammonium in rice

roots and regulated by endogenous glutamine rather than by endogenous ammonium (Kumar *et al*., 2003; Sonoda *et al*., 2003b). Ding *et al*., 2011a reported that artificial selection from wild progenitors to cultivated rice has dramatically decreased the genetic diversity of theOs*AMT1;* 1 gene, demonstrating a selective sweep caused by strong selection within or nearby the gene during the domestication process. As the Os*AMT1;1* alleles are fixed in cultivated rice, it is possible to discover novel alleles in wild relatives to broaden the genetic variation for improving NUpE. The expression of AMTs is affected by nitrogen conditions, and strictly regulates the influx of ammonium in plants including rice (Loqué and Wirén, 2004) .Ammonium transporters in rice are studied well as compared to nitrate transporters. The molecular mechanism of how rice takes up nitrate is not yet fully understood.

Since rice roots are exposed to NO_3^- nutrition under aerobic or water deficit condition and the importance of NO_3^- nutrition has been already dictated in above mentioned researches, the regulation and function of nitrate transporter genes in rice are worthy of investigation. Most of our knowledge about nitrate uptake and translocation is from the study of Arabidopsis, and little is known about these processes in rice (Wang *et al*., 2012a). Therefore, an understanding of the molecular mechanisms of nitrate uptake and translocation in rice is very important for improving the efficiency of nitrogen use in rice. The uptake of NO_3^- is an active process driven by H^+/NO_3^- co-transporters (Zhou *et al*., 2000). Moreover, each high and low-affinity nitrate transport system is composed of constitutive and nitrate-inducible (Miller, 2007). Numerous membrane proteins function in nitrate uptake, compartmentation, translocation and remobilization (Dechorgnat, 2011). Several families of membrane proteins are involved in uptake, allocation, and storage of NO_3^- in plants: the NO_3^- peptide transporter family (NRT1/PTR), NO_3^- transporter 2 family (NRT2), chloride channel (CLC) family, and slow anion channel-associated homologues (SLAC/SLAH) (Wang *et al*., 2012b; Krapp *et al*., 2014). However, in the rice genome Os*NRT1.1/PTR* renamed as Os*NRT1.1a* was identified as a low-affinity NO_3^- transporter gene that is constitutively expressed in roots (Lin *et al*., 2000). Os*NRT1.1b*, another mRNA splicing product, plays a key role in accumulation of more N in rice under low N

regime (Fan *et al*., 2015). Four NRT2 and two NAR2 genes (Fig.2) encoding HATS components have been identified (Araki and Hasegawa, 2006 a; Cai *et al*., 2008). One gene Os*NRT2.3* was mRNA spliced into Os*NRT2.3a* and Os*NRT2.3b*. Os*NAR2.1* was mainly expressed in roots epidermal cells and induced by nitrate and suppressed by ammonium. Os*NAR2.1* interacts with Os*NRT2.1/2.2* and Os*NRT2.3a* at both the messenger RNA (mRNA) and protein levels and plays an important role in nitrate uptake over both high and low concentration ranges (Yan *et al*., 2011). Furthermore, it was shown that most transporter genes are up-regulated by NO_3^- and suppressed by NH_4^+, with the exception of Os*NRT2.3b*, which is insensitive to NH_4^+ (Feng *et al*., 2011).From NPF family, Os*NPF8.9*/Os*NRT1* (Lin *et al*., 2000) and OsNPF2.4 (Xia *et al*., 2015) have been functionally demonstrated to transport nitrate. Recently, Li *et al*. (2015) characterized the vascular specific transporter Os*NPF2.2*. Their findings demonstrated that Os*NPF2.2* can unload nitrate from the xylem to affect the root-to-shoot nitrate transport and plant development. Mechanisms and gene candidates identified as responsible for influx and efflux of NH_4^+ and NO_3^- is depicted in table 2.2.

Plant NO_3^- uptake is regulated by both N and C status (Krouk *et al*., 2010). Paddy rice has NH_4^+ as a primary N source, the regulation of Os*NAR2.1* /Os*NRT2* gene expression by N sources and light /dark diurnal and sugar supply as in Arabidopsis (Girin *et al*. , 2007) shows that there are common feedback regulatory pathways for C/N balance in both species.

2.4.2.1 Regulation of NO_3^- LATS and NH_4^+ LATS

Plants respond to changing N availability in the LATS concentration range, with differences in flux modulation is observed between NO_3^- and NH_4^+ LATS as shown in fig. 2.2. With regard to NO_3^- the highest LATS fluxes at a given external ion concentration are found in plants having had no recent exposure to NO_3^-, with NO_3^-, with NO_3^- pre-treatment down regulating this influx within

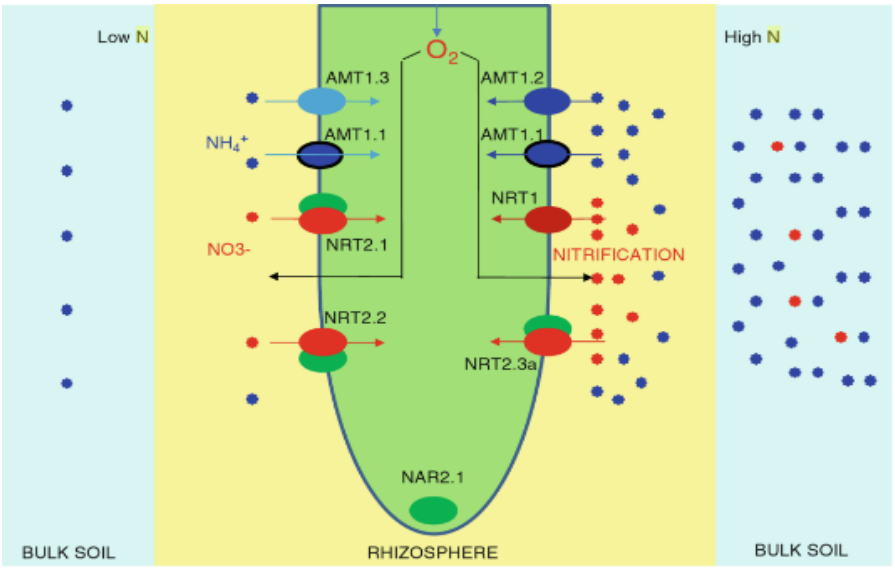


Fig 2.2: The Schematic representation of predicted functions for the rice NAR2-NRT2 two components nitrate transporters and three AMT 1 ammonium transporters.

a few days. The extent of this downregulation is dependent on the nitrate external [NO_3^-] ext. Conversely, with NH_4^+ nutrition, the highest LATS fluxes observed are with plants adapted to the highest ammonium external [NH_4^+]ext (Min *et al*., 2000; Cerezo *et al*., 2001). Within the context of NH_4^+ nutrition, a contrast between the HATS and LATS for NH_4^+ emerges. The HATS for NH_4^+ is sensitive to N root status, while the LATS for NH_4^+ is insensitive to N root status. Failure to regulate the LATS for NH_4^+ may indeed be a cause for the massive NH_4^+ accumulation occurring in plant cells exposed to high (mM) [NH_4^+]ext, leading to NH_4^+ toxicity symptoms in many plant families (Britto and Kronzucker, 2002).

2.4.3 Key assimilatory enzymes

Upon absorption, reduction of NO_3^- is facilitated by nitrate reductase (NR)/ nitrite reductase (NiR). In higher plants, NR seems to be generally located in cytoplasmic compartment, while NiR is in chloroplasts or other plastids. Primary NO_3^- assimilation takes predominantly in roots of the plants, being strongly dependent on the age and limitation of space for root growth. NO_3^- taken up by plants is reduced to nitrite by nitrate reductase (Cao *et al*., 2008). Since NO_3^- is

highly reactive, plant cells immediately transport the nitrite from the cytosol in to chloroplast in leaves and plastid in roots.

Table 2.2: Mechanisms and gene candidates identified as responsible for influx and efflux of NH_4^+ and NO_3^-

Ion	Flux	Mechanism	Gene Candidates
NH_4^+	Influx (HATS) Influx (LATS)	Carrier Channel	AMT1, 2, 3 AMT1, 2, AQP, KIRC,NSCC, NH_4^+-Specific channel
	Efflux	Proton Anti-port (NH_4^+/H^+) /Channel (NH_3)	CHX, AQP
NO_3^-	Influx (HATS)	Carrier-mediated proton symport (NO_3^-:$2H^+$)	NRT1, 2
	Influx (LATS)	Channel mediated proton symport (NO3-:$2H^+$)	NRT1.1, 1.2, 1.4
AQP AlAAC,	Efflux	Channel mediated	ARAC,

Source: Britto and Kronzucker (2006)

In this organelle nitrate is further reduced to NH_4^+ by nitrite reductase (Rose *et al*., 2011). The ammonium taken up by plant root or produced by reduction of nitrate is first assimilated by glutamine synthetase (GS GS, EC 6.3.1.2; Ireland and Lea, 1999a) to yield the amino group of glutamine (Gln) and then transferred to the position of 2-oxoglutarate, yielding two molecules of Glu by GOGAT either reduced ferredoxin (FdGOGAT, EC 1.4.7.1) or NADH (NADH-GOGAT, EC 1.4.1.14; Ireland and Lea, 1999b) (Yamaya *et al.,* 1996). In higher plants, GS/GOGAT cycle (Fig 2.3) in the chloroplast is the first and foremost step of incorporation of inorganic nitrogen into organic nitrogenous compounds

(Andrews *et al*., 2004), which is a major checkpoint for controlling nitrogen assimilation. Although roots have high constitutive levels of GS and GOGAT, both enzymes are inducible by NH_4^+ (Ishiyama *et al*., 2003).

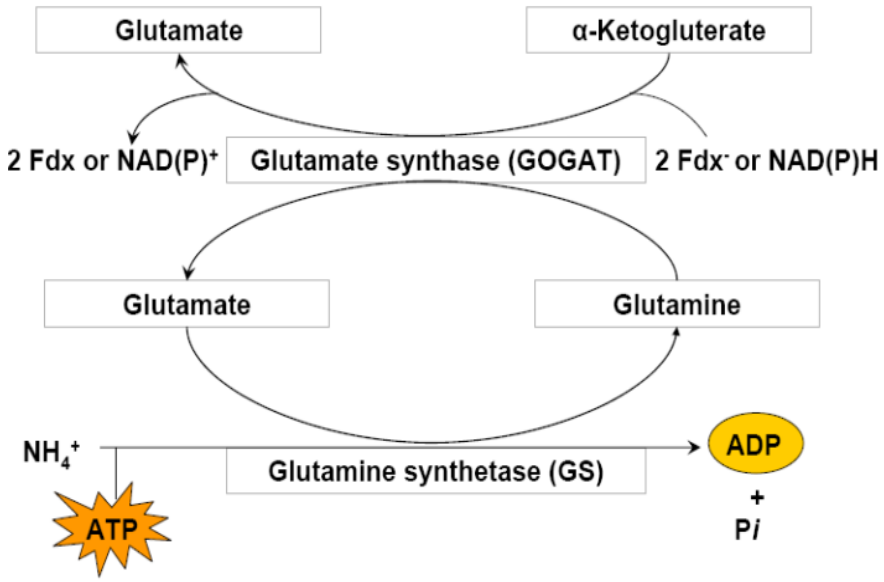


Fig 2.3: The Glutamine Synthetase-Glutamate Synthase (GS-GOGAT) cycle

GS have two major isoforms cytosolic (GS1) and plasidic (GS2), as discussed earlier. The GS1 functions primarily in assimilating ammonia generated from the various processes involved during the remobilization of assimilate, is encoded by multiple genes in plants: three in rice and five in maize and Arabidopsis (Bernard *et al*., 2009; Lam *et al*., 1996; Martin *et al*., 2006b). The GS1 genes from rice, Os*GS1;1*, Os*GS1;2*, are expressed in all organs, but with higher expression in leaf blades and roots, respectively. Os*GS1; 3* is found specifically in the spikelet (Tabuchi *et al*., 2005a) and OsGS2 is mainly expressed in leaves & used to recycle assimilated ammonia, derived from photorespiration (Bernad *et al*., 2008) and is encoded by single gene in rice and Arabidopsis (Segonzac *et al*., 2007; Sentoku *et al*., 2007). These isoforms have been shown to be regulated by developmentally controlled manner as well as by light and nitrogen nutrition (Tabuchi *et al*., 2007a). Among three genes for cytosolic glutamine synthetase (Os*GS1;1*, Os*GS1;2* and Os*GS1;3*) in rice plants, the Os*GS1;2* gene is known to be mainly expressed in surface cells of roots, but its function was not clearly understood. Funayama *et al*. (2013) recently revealed that

GS1;2 is responsible for the primary assimilation of ammonium in rice roots and with GS1;1 in the roots unable to compensate for GS1;2 functions**.**

In addition, several studies highlighted the importance of cytosolic GS1 genes in determining grain filling in cereal crops. Positive correlations were shown between grain number/size and cytosolic GS protein content/GS activity in rice (Tabuchi *et al*., 2005b; Obara *et al*., 2004), maize (Martin *et al*., 2006; Hirel *et al*., 2007), wheat (Habash *et al*., 2010). Bao *et al*., (2014) reported negative correlation between the cytosolic GS1 gene expression level and the grain filling in GS1; 1, GS1; 2-overexpressing plants under altered carbon-nitrogen metabolism. Compared to the wild type plants, the yields of GS1; 1, GS1; 2-overexpressing plants were significantly declined. Thus, the unbalanced carbon-nitrogen metabolic status and poor ability of nitrogen transportation from stem to leaf in GS1;1-, GS1;2-overexpressing plants may explain the poor growth and yield.

Remobilization of internal N during grain filling is another key process in N metabolism. Among the primary N assimilation genes, physiological and biochemical evidence indicates that GS1 plays a major role in the synthesis of glutamine in older leaves, which is then transported to panicles (Habash *et al*., 2001; Masclaux *et al*., 2001), a process positively related to yield and N-use efficiency. These genes are not regulated in a similar manner, and GS1 isoenzymes are located in various plant tissues and have different kinetic properties, suggesting that each plays important roles in N assimilation (Ishiyama *et al*., 2004; Martin *et al*., 2006a). In rice plants, NADH-GOGAT is coded by two genes, Os*NADH-GOGAT1* andOs*NADH-GOGAT2*, while one gene codes for Fd-GOGAT (Tabuchi *et al*., 2007b). Occurrence of a pseudo-gene for rice Fd-GOGAT has been reported by Zhao and Shi (2006). The major function of GS2 and FdGOGAT is in photorespiratory nitrogen metabolisms (Lea and Miflin, 2003) Overexpression of NADH-GOGAT increased the panicle weight in rice, in agreement with its important role in transporting glutamate to major sink tissues during grain filling (Yamaya *et al*., 1992b). This enzyme may therefore play a key role in N utilization and grain filling in rice (Andrews *et al.*, 2004b). The rice Os*NADH-GOGAT1* gene is mainly expressed in growing

tissues such as root tips, young spikelet's and developing leaf blades, and is important for N remobilization, whereas the second rice gene, Os*NADH-GOGAT2*, is mainly expressed in mature leaves and leaf sheaths (Tabuchi *et al.*, 2007c). A recent study on non-functional Os*NADH-GOGAT2* mutants has shown a significant decrease in spikelet number per panicle associated with a reduction in yield and plant biomass, as well as total N content in senescing leaves. This implies that NADH-GOGAT2 is also important for remobilization of N and glutamine generation in senescing leaves (Tamura *et al*., 2011). Fig 2.4 shows the cellular compartmentation of AMT1;2 & NH_4^+-assimilatory isoenzymes and fig 2.5 shows the systemic route for N uptake.

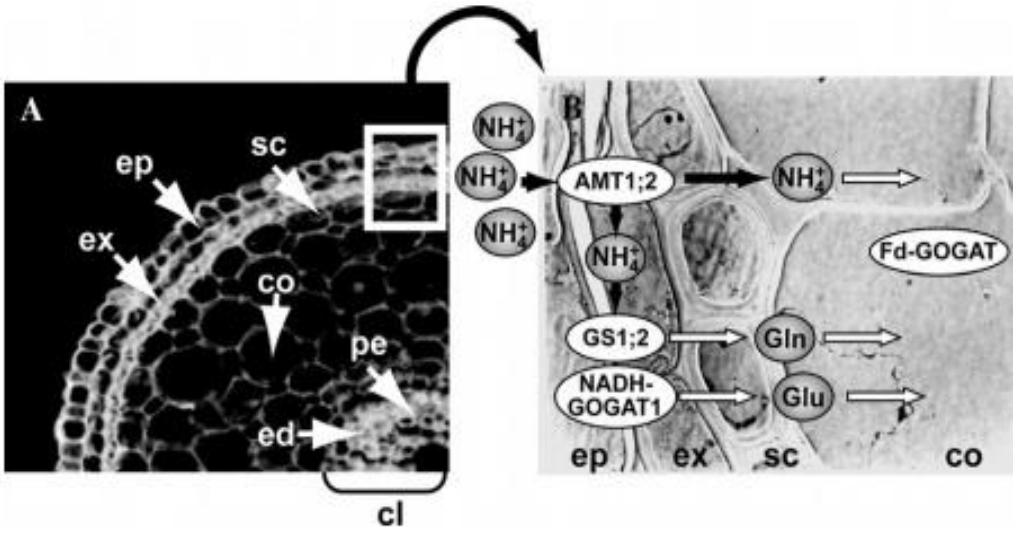


Fig. 2.4: (A) Cross-section of seminal roots and (B) schematic cellular compartmentation of AMT1;2 and NH_4^+-assimilatory isoenzymes showing nitrogen flow in rice roots. Abbreviations: co, cortex; cl, central cylinder; ed, endodermis; ep, epidermis; ex, exodermis, pe, pericycle; and sc, sclerenchyma.

Source: Tabuchi *et al*. (2007c)

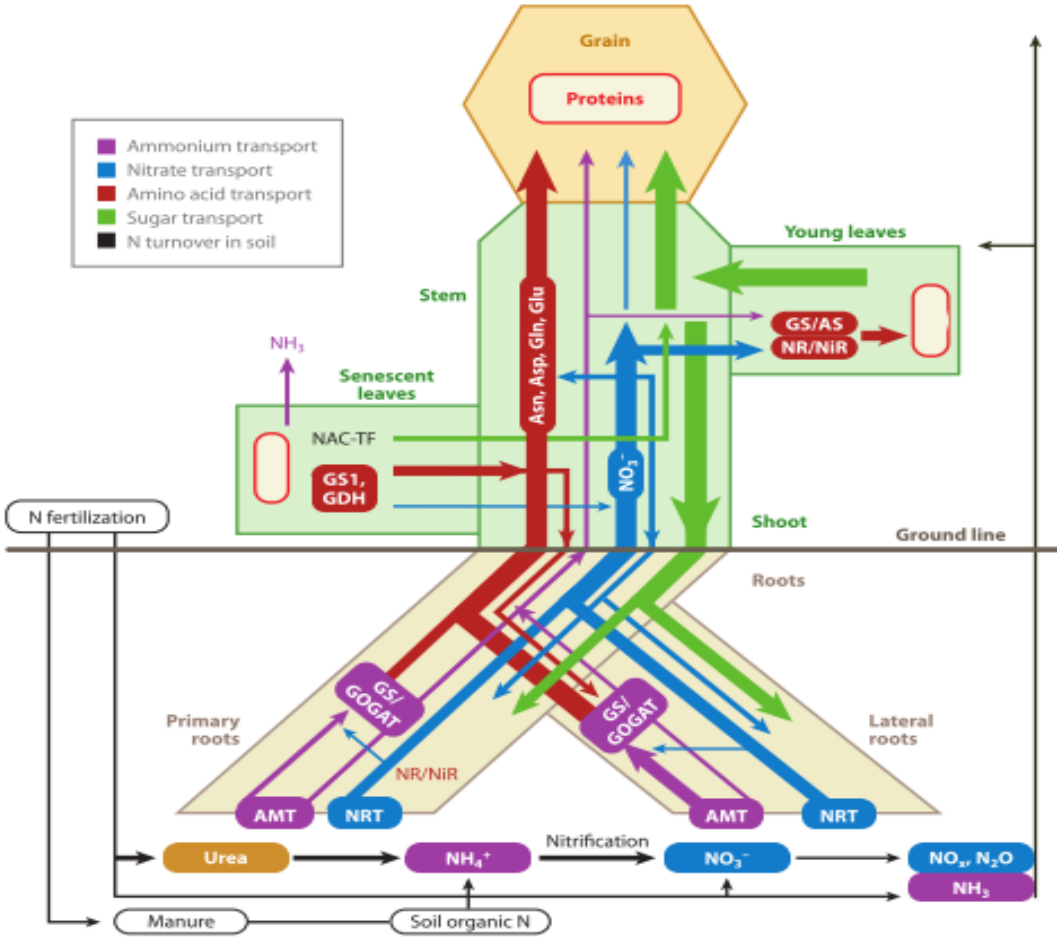


Fig 2.5: Schematic routes of N uptake from the rhizosphere including the source of N to be acquired, mainly in the form of ammonium and nitrate by roots, transportation and assimilation, and remobilization inside the plant. The thicknesses of the arrows schematically represent the relative amounts of nitrogen and sugar inside the plant. Abbreviations: AMT, ammonium transporter; AS, asparagine synthetase; Asn, asparagine; Asp, aspartate; GDH, g lutamate dehydrogenase; Gln, glutamine; Glu, glutamate; GOGAT, glutamine-2-oxoglutarate aminotransferase; GS glutamine synthetase; NAC-, certain transcription factors belonging to the NAC family; NiR, nitrite reductase; NR, nitrate reductase; NRT, nitrate. Source: Xu *et al.* (2012b)

2.4.3.1 Structural and catalytic properties of NR, NiR, GS and GOGAT

(A) Nitrate Reductase (NR)

Structure:

Partial proteolysis of the enzyme produces discrete fragments that display partial enzymatic activity. One fragment binds FAD and can use NADH to reduce ferricyanide, an artificial electron donor. A second fragment contains MoCo and heme-Fe and can reduce nitrate in the presence of methy viologen, an artificial electron donor. The partial activities associated with NR fragments are consistent with the structural evidence for discrete functional regions (Campbell, 1999). The spatial arrangement of function region is shown in proposed model for the structure of NR showing each functional region in fig 2.6.

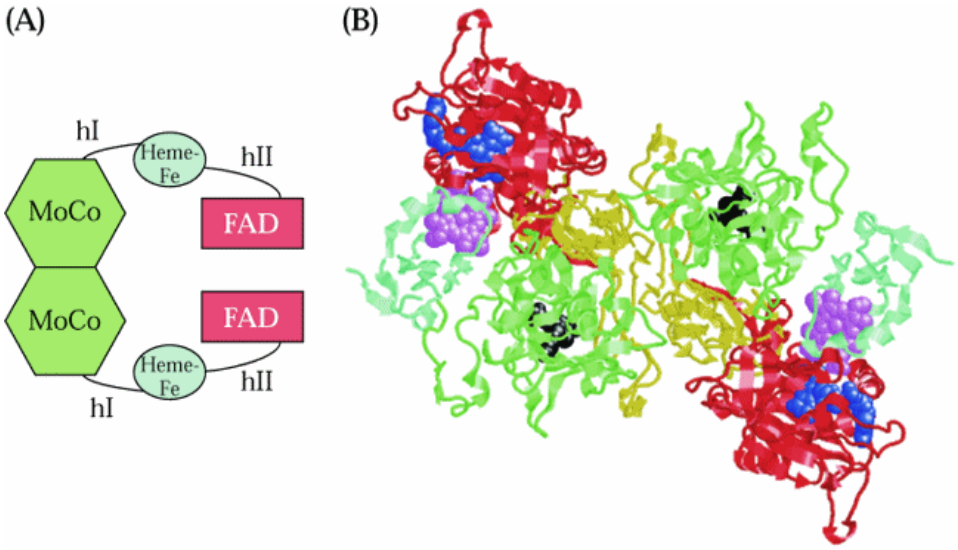


Fig. 2.6: (A) Domain structure of nitrate reductase. An NR monomer has three major domains, which binds moly-bdenum cofactor, heme, and FAD, respectively. hI and hII refer to hinge 1 and 2 which separate the functional domains.

(B) Ribbon diagram of Nitrate Reductase. The heme prosthetic group is shown in purple, FAD in blue, and MoCo in black. The interface between two monomers is shown in yellow. Source: Buchanan *et al*. (2000a)

Catalytic properties:

The first committed step in the nitrate assimilation pathway is the reduction of nitrate to nitrite.

$$NO_3^- + NAD(P)H + H^+ \longrightarrow NO_2^- + NO_2^- + NAD(P)^+ + H_2O$$

NADH or NADPH serves as the reductant, and proton is consumed in the reaction. This entire reaction is catalyzed by NR, a complex metalloenzyme that forms homodimers. NR has binding sites for NAD(P)H and for nitrate (Forde, 2000). Three cofactors-FAD, heme-Fe and MoCo provides the reaction centres that facilitate the chain of electron transfer reactions diagrammed above.

(B) Nitrite Reductase (NiR)

Structure:

NiR is nuclear encoded with N-terminal transit peptide that is cleaved from the mature enzyme. The enzyme, a monomer, has two functional domain and cofactors that shuttle electrons from Fdx_{red} to nitrite (Fig. 2.7). The N-terminal half of the enzyme binds to ferredoxin. The C-terminal half, contains binding site for nitrite, as well as two redox centers, a Fe-4S center, and a siroheme. These two prosthetic groups are in close proximity, bridge by a sulpfur ligand. Four cysteines located in two clusters provide briding ligand and sulphur ligands for the Fe-4S center.

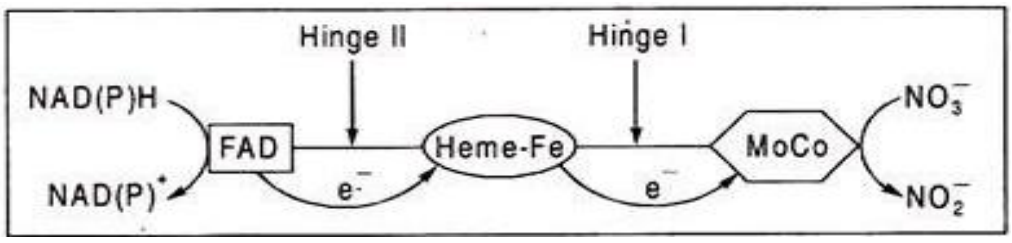


Fig 2.7: Structure of Nitrite Reductase from plants

(Buchanan *et al.*, 2000)

Catalytic properties

After nitrate reduction next step in nitrate assimilation pathway is reduction of nitrite to ammonia which is catalyzed by NiR. Six electrons are transferred in reaction1. The source of electron is reduced ferredoxin (Fdx_{red}) produced in chloroplast. In colourless plastids like roots NADH from reduces ferredoxin in a reaction catalyzed by ferredoxin-$NADP^+$ reductase reaction 2.

$$NO_2^- + 6\ Fdx_{red} + 8H^+ \longrightarrow NH_4^+ + 6\ Fdx_{oxd} + 2H_2O \quad \text{Reaction 1}$$

$$NADPH + 2\ Fdx_{oxd}\ (Fe_3^+) \longrightarrow NADP^+ + 2Fdx_{red} + Fe_2^+ + H^+ \quad \text{Reaction 2}$$

(C) Glutamine synthetase (GS)

Structure:

GS has been purified and characterized from a variety of plant species and tissues such as leaves of pea, rice (Hirel and Gadal, 1980), spinach (Ericson, 1985), jack pine (Vezina *et al.*, 1988) and rapeseed (Ochs *et al*., 1995); roots of pea (Emes and Fowler, 1979), rice (Hirel and Gadal, 1980), Arabidopsis (Ishiyama, 2004) *etc.* The atomic structure of maize cytosolic GS has recently been elucidated at 2.63-, 3.50- and 3.80-Å resolutions, indicating that plant GS polypeptides (Type II) form decamers (Unno *et al*., 2006a), which differ from the dodecameric structure of bacterial GS (Type I) (Fig. 2.8).

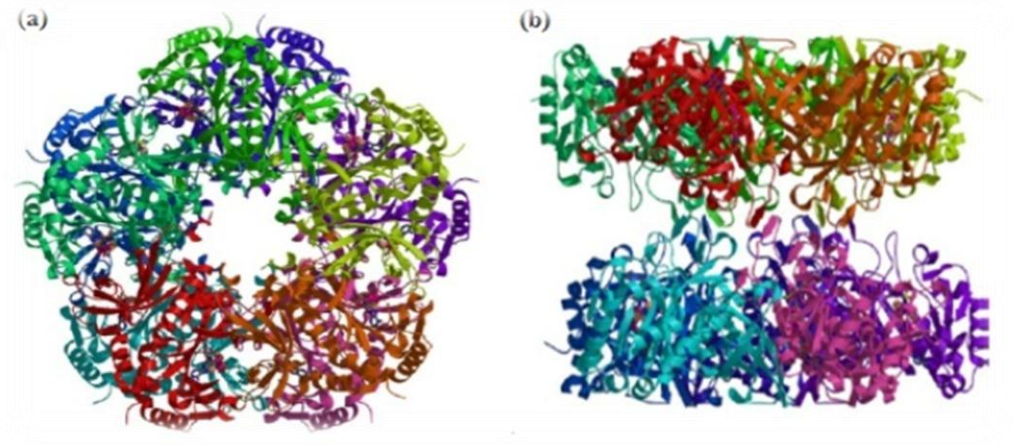


Fig. 2.8: Structure of cytosolic GS from Zea mays (Maize) composed of two face-to-face pentameric rings of subunits contain 10 active sites, each between two neighbouring subunits neighbouring subunits neighbouring subunits within each ring. Source: Unno *et al*. (2006b)

Catalytic properties:

In situ, the enzyme catalyses the following reaction which involves ATP-amination of glutamate to glutamine.

$$\text{Glutamate} + \text{ATP} + NH_4^{+} \xrightarrow{Mg_2^{+}} \text{Glutamine} + \text{ADP} + \text{Pi} \quad \text{(Reaction 1)}$$

Where, Mg_2^{+} is magnesium. The reaction has been termed the 'biosynthetic' reaction and is considered the most physiologically relevant reaction that GS catalyzes. The reaction is believed to proceed in two stages. In the first step γ-carboxylic group undergoes phosphorylation to yield γ-glutamylphosphate and in the next step phosphate group is replaced by amino group to form glutamine (Fig. 2.9). In addition of above physiological activity, isolated GS also catalyses the formation of γ-glutamylhydroxamate through either an ATP dependent semi-synthetase reaction (Reaction 2) or a transferase reaction (Reaction 3).

$$\text{Glutamate} + \text{ATP} + NH_2OH \longrightarrow \gamma\text{-glutamylhydroxamate} + \text{ADP} + \text{Pi} \quad \text{(Reaction 2)}$$

$$\text{Glutamine} + NH_2OH \xrightarrow[Mg_2^{+},\ \text{ADP}]{} \text{glutamylhydroxamate} + NH_4^{+} \quad \text{(Reaction 3)}$$

The biosynthetic reaction of the enzyme has rarely been used for determination of GS activity in plants principally because, as compared to the semi-synthetase and transferase reactions, this activity is very low. Moreover, this assay is not suitable for use with unpurified extracts because of the presence of high activities of interfering enzyme such as glutaminase, ATPase, phosphatases and nucleotidases. On the other hand, semi-synthetase and transferase reactions have been generally used for determination of GS activity, since several folds higher activity of these reactions than biosynthetic activity offers a distinct advantage of increased sensitivity of the assay procedure (Lea *et al.,* 1990).

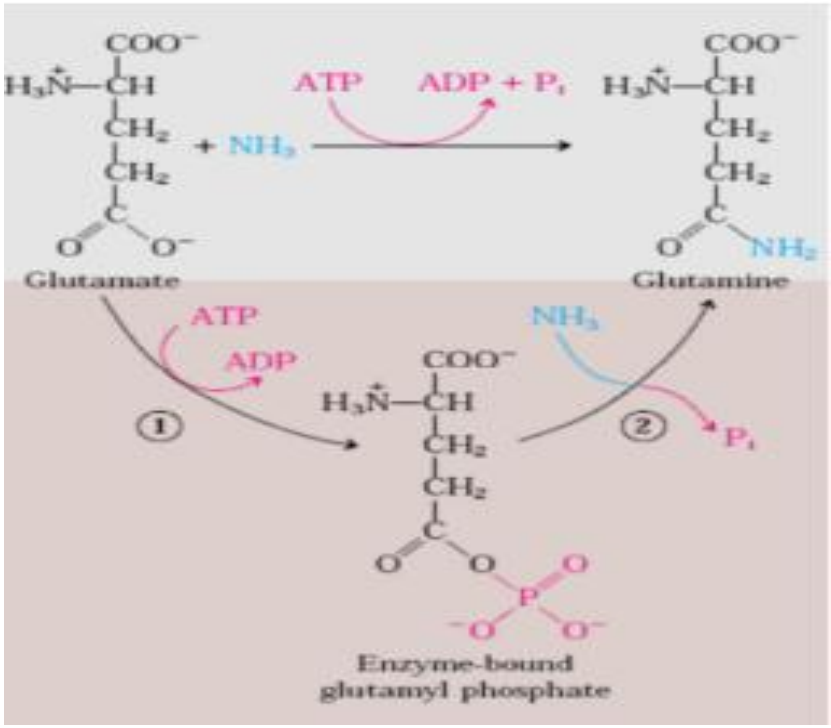


Fig. 2.9: Reaction catalysed by Glutamine Synthetase

(D) Glutamate Synthase (GOGAT/Gls)

Structure:

GltS is a ubiquitous enzyme in nature; it has been detected in prokaryotes, archae and eukaryotes. Fd-GltS from *Synechocystis sp*. (Van den Heuvel *et al*., 2003) and NADPH-GltS (Binda *et al*., 2000) from *A. brasilense* are the two best-studied GltSs. The tertiary structures of the a subunit of NADPH-GltS and Fd-GltS (Fig. 2.10), as determined by X-ray crystallography, are similar as root mean square deviation of 1.7 Å for all topological equivalent Cα atoms (Nebso *et al*., 2001). Each GltS monomer can be described in terms of four distinct domains with different function and topology. Fig. 2.9 shows the N-terminal amidotransferase domain as depicted in cornflower blue, the FMN-binding domain in yellow, the central domain in magenta and the C-terminal domain in green. The Fd molecule is depicted in red. The FMN cofactor and the iron-sulfur clusters are shown as ball-and-stick, the Fd-binding loop in red and the cavities in grey. The position of Fd relative to Fd-GltS is not based on experimental evidence (Lawrence and burke, 2000a).

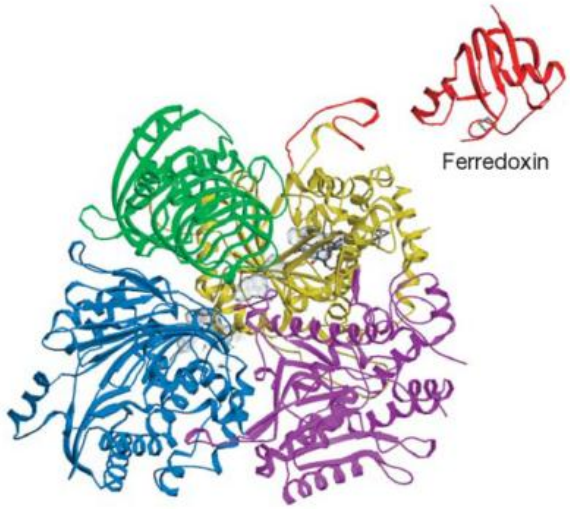


Fig. 2.10: Tertiary structures of the Fd-GltS and Fd monomers.

Source: Lawrence and burke (2000b)

Catalytic properties:

The enzyme catalyzes the reductant-dependent conversion of 2-oxoglutarate into L-glutamate in which L-glutamine serves as the nitrogen source for the reaction. The overall catalytic cycle of GltS can be described by the following equation in which the reducing equivalents originate from NADH, NADPH or reduced Fd, depending on the type of GltS:

$$\text{L-glutamine} + \text{2-oxoglutarate} + 2e^{-} \longrightarrow 2\ \text{L-glutamate}$$

This catalytic cycle (equation 1) involves distinct catalytic reactions. The ammonia produced in the amidotransferase domain is added onto 2-oxoglutarate in the FMN binding domain. The reducing equivalents originate from reduced ferredoxin (Fd-GltS) or NADPH (through the NADPH b-GltS subunit and the corresponding part of NADH-GltS) (Fig. 2.11) (Heuvel *et al*., 2004a).

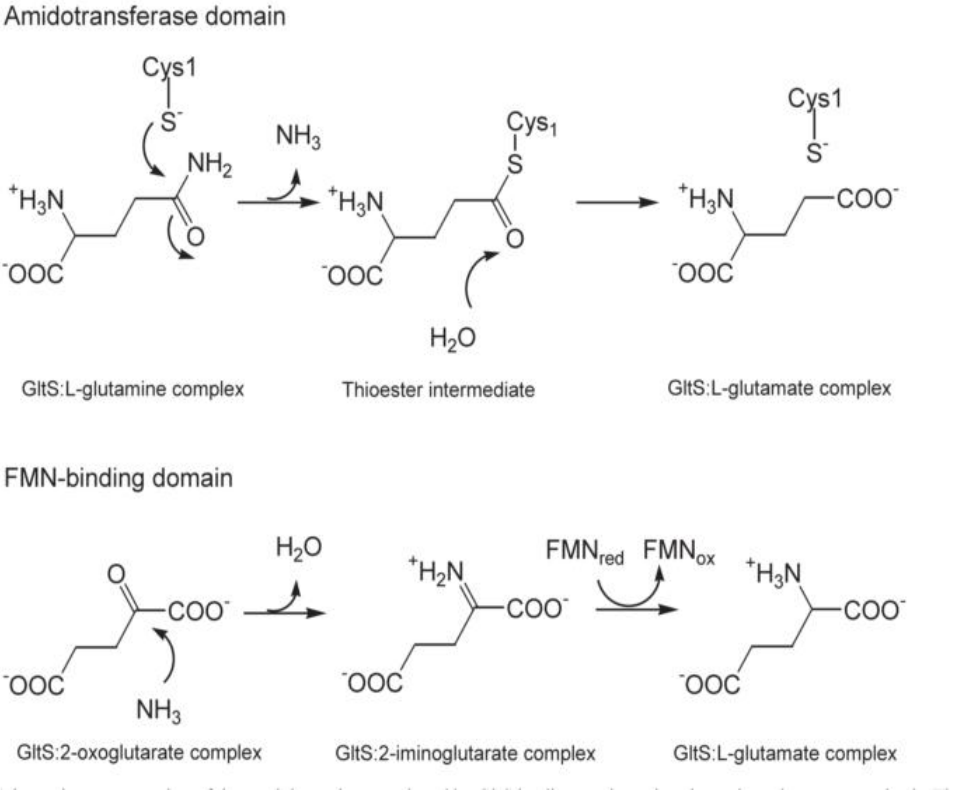


Fig. 2.11: Schematic representation of the partial reactions catalyzed by GltS leading to glutamine-dependent glutamate synthesis

Source: (Heuvel *et al*., 2004b)

Regulation of N-pathway genes by nitrogen status

Investigations have been carried out on the changes in activities and expression of GS isoforms in response to nitrogen nutrition. In barley seedlings, the specific activity of GS was found to be much higher in leaves but declined in root in presence of NH_4^+ as compared to specific activities in respective organs of NO_3^- fed or N-free grown plants. With higher concentration of NH_4^+, the specific activity of GS1 rose in leaves but fell in roots. The activity of GS2 in leaves was also elevated with increasing concentration of NH_4^+ in the nutrient medium. The alterations in activities of GS1 and GS2 were correlated with changes in the subunit composition of the active holoenzyme (Mack, 1995). NH_4^+ is supposed to substrate-induce the GS1 promotor of rice (Kozaki *et al*., 1991) and soybean (Hirel *et al*., 1987; Miao *et al*., 1991). Kozaki *et al*. (1992) showed that the GS2 promotor of rice is also activated by NH_4^+, Although some cytosolic GS gene members are up regulated by the addition of NH_4^+, some are down-regulated or do not respond

(Sakakibara *et al.* 1992; Hirel *et al*., 2005; Kusano *et al*., 2011). In Arabidopsis, GS1 mRNA and polypeptide accumulated in roots when plants were supplied with NH_4^+ however, the GS activity was maintained at a constant level. The discrepancy between the protein content and enzyme activity of GS1 was attributed to the kinetic properties and expression of four distinct isoforms encoded by *GLN1;1*, *GLN1;2, GLN1;3* and *GLN1;4* genes that function complementary to each other in Arabidopsis. *GLN1;2* was significantly up-regulated by NH_4^+ and correlated with the rapid increase in total GS1 protein. However, *GLN1;2* exhibited lower affinity to the substrates NH_4^+ and glutamate. In contrast, high affinity enzyme *GLN1;1* was abundantly expressed in surface layer of root during nitrogen limitation and down regulated by NH_4^+ excess (Ishiyama *et al*., 2004a). Similarly, in rice root the cytosolic Os*GS1;1* and Os*GS1;2* transcripts showed reciprocal response to NH_4^+ supply in the surface cell layers of roots. Os*GS1;1* accumulated in the dermatogens, epidermis and endodermis under nitrogen limited conditions. By contrast, Os*GS1;2* was abundantly expressed in the same cell layers under nitrogen sufficient conditions replenishing the loss of Os*GS1;1* following NH_4^+ treatment (Ishiyama *et al.*, 2004b). A study on quantitative comparative analyses between the metabolite profiles of a rice mutant lacking Os*GS1;1* and its background wild type (WT) was conducted. The mutant plants exhibited severe retardation of shoot growth in presence of NH_4^+ compared with the WT. Overaccumulation of free NH_4^+ in the leaf sheath and roots of the mutant indicated the importance of Os*GS1;1* for NH_4^+ assimilation in both organs. The metabolite profiles of the mutant line revealed: (i) an imbalance in levels of sugars, amino acids and metabolites of the tricarboxylic acid (TCA) cycle, and (ii) over accumulation of secondary metabolites, particularly in the roots under a continuous supply of NH_4^+. Metabolite-to-metabolite correlation analysis revealed the presence of mutant-specific networks between tryptamine and other primary metabolites in the roots. These results demonstrated a crucial function of Os*GS1;1* in coordinating the global metabolic network in rice plants grown using NH_4^+ as the nitrogen source (Kusano *et al*., 2011).

NO_3^- had little effect on appearance of GS in mustard cotyledons (Schmidt and Mohr, 1989) and scot pine seedlings, whereas in sunflower cotyledons (Haba

et al., 1992) and maize leaves (Aguera *et al*., 1987) it strongly enhanced the level of the enzyme. NO_3^- enhanced the GS2 activity approximately by 4 fold in mesophyll cells and by 1.3 fold in bundle sheath cells of maize leaves but no enhancement was detected in GS1 level (Sakakibara *et al*., 1992). Since the primary reaction of NO_3^- assimilation in C4 plants occur in mesophyll cells, the elevated activity was attributed to increased demand for assimilation of ammonia produced from nitrate reduction of these cells. An increase in the GS2 polypeptide content caused by NO3- nutrition was also found in pea roots (Vezina and Langloism, 1989) and cultured rice cells (Hayakawa *et al*., 1990). Transcript stability is another means of GS1 regulation in response to nitrogen nutrition (Ortega *et al*., 2006). However, it is not clear whether plant nitrogen status or NO_3^- molecules interact with the cis-acting element at the 3' end of the GS1 transcript.

Green tissues have much more NiR activity then NR activity, ensuring that nitrite does not accumulate to toxic amounts. Thus, NR catalyzes rate limiting step in the conversion of nitrate to ammonium (Tischner, 2000). Plants use several mechanisms to adjust the concentration and activity of NR in response to such diverse signals as nitrate abundance and nitrogen metabolite. Barley mutants with 5-24 % of NRA of NRA (compared to wild type) were able to supply sufficient N required for growth (Savidov *et al.*, 1997). The NRA is induced by nitrate (Shaner and Boyer, 1996). However, there is evidence that a depression of NR gene also occurs after depletion of N-sources such as ammonium (Samuelson *et al.*, 1995). The presence of nitrate is therefore not an absolute prerequisite for expression of NR gene. Signals influencing activity of NR is mentioned in table below.

Table 2.3: Signals that influence the activity and transcription of NR

Signal	Effect on NR
➢ Glutamine	Down-regulates transcription
➢ Nitrogen starvation	Down-regulates transcription
➢ Nitrate	Up-regulates transcription
➢ Oxygen	Down-regulates activity
➢ Anoxia	Up-regulates activity

NiR is toxic to plants so they maintain excess of NiR activity whenever NR gene expression is induced in response to nitrate.

Balotf and kavoosi (2011) investigated the effects of nitrate salts supply on nitrate accumulation, amino acid biosynthesis, total protein production, nitrate reductase activity in the roots and leaves of the plants. The results indicated that both sodium and potassium nitrate supplementation had stimulatory effects on all of the mentioned factors in a dose dependent manner. Reda (2013) further evaluated the effect of N metabolites on nitrate reductase activity in *Arabidopsis* wild type and mutants. Exogenously applied N metabolite led to increase in the total and actual activity of NR. It was due to both the increase in expression of NR genes and NR activation state. Haghighi (2015) compared the effects of applying monosodium glutamate (MG) and ammonium nitrate (NH_4NO_3) (AN) on nitrogen metabolism and growth of lettuce. The results showed that NH_4NO_3 (AN), NO_3^-, nitrite content and NR activity increased the protein content of lettuce. Moreover, Nunes (2014) evaluated biochemical responses of maize cultivars subjected to nitrate and glutamine. They observed that nitrate reductase activity was greater in leaves in relation to the roots, and glutamine does not inhibit its activity.

Increase in glutamate synthase activity in maize seedlings in response to nitrate and ammonium nitrogen was observed by Singh and Shrivastav (1987)**.** The supply of inorganic nitrogen for a short time, i.e. 3 h, to roots and leaves excised from seedlings grown without nitrogen increased the enzyme activity in these organs. This increase was more pronounced with nitrate than with ammonium nitrogen. When excised roots and leaves from NH_4NO_3-grown seedlings were incubated in a minus nitrogen medium for 24 h, the enzyme activity declined considerably. This decline was inhibited to some extent by

nitrogen, especially by nitrate. Inorganic nitrogen prevented similarly the decline in in vitro enzyme activity during 24 h storage at 25°C, more regularly for the root than for the leaf enzyme. Thus, this experiment demonstrates the role of inorganic nitrogen in the regulation of Glutamate Synthase activity.

2.4.3.2 Regulation of N-pathway genes by water stress

Teixeira and Fidalgo (2009) have suggested that nitrogen assimilation is more sensitive to water stress than CO_2^- photosynthetic assimilation. Under drought conditions the expression of gene encoding ubiquitin related proteins and various proteases were found to be induced or enhanced, consistent with the requirement of protein degradation under stress conditions. All these processes ultimately lead to either acclimation to the stress conditions or to senescence and subsequent cell death. The response of GS to drought stress hasn't been studied in much detail. During water stress the abundance of GS2 polypeptide and its activity declined, whereas GS1 tended to increase or maintain the same level in the leaves (Bauer *et al*., 1997; Santos *et al.*, 2004; Martinelli *et al*., 2007). In an experiment of water stress effect on metabolic activity of cowpea nodules was severe. The GS and NADH-GOGAT coupling was lost as the drought conditions intensified (Figueiredo *et al*., 2001). Becana *et al.* (1984) determined protein content and enzymatic activities GS, NADH-GOGAT under water stress in root nodules of alfaalfa. Patterns of glutamine synthetase and NADH-GOGAT reflected changes in ammonia content of nodules and/or availability of carbon substrates, and indicate that nodules maintain sufficient enzyme activity for ammonia assimilation throughout water stress.

Lobato (2008) evaluated consequences of the progressive water deficit and rehydration on NR activity and nitrogen compounds in soybean. The results showed significant reductions in the leaf relative water content, plant water content, NR enzyme activity, free ammonium and total soluble proteins. The leaf relative water content, Nitrate Reductase enzyme activity and total soluble proteins were recovered after the rehydration.

Pandey *et al.* (2012) evaluated cellular membrane stability index and nitrate reductase activity in the leaves of Avena species grown under well watered (control), extreme stress (by withholding water) and re-watered condition both at

vegetative and flowering stage of the crop in pot culture.

Furthermore, Bhaswatee and Nirmal (2014) observed the biochemical changes in black gram and green gram genotypes after imposition of drought stress. Drought was imposed at three different growth stages namely vegetative, early reproductive and pod filling stages and various biochemical parameters were recorded. Nitrate reductase one of the parameter were found to be positively correlated with seed yield while negative correlation was obtained with total soluble sugar content.

Abiotic stresses such as drought, salinity down regulate key genes involved in nitrogen uptake and assimilation. Goel and Singh (2015) observed the differential expression of NR, NiR, GS, and GOGAT under abiotic stress conditions in *Brassica juncea L.* Their observations indicated that expression of most of the genes is adversely affected under abiotic stress, particularly under prolonged stress exposure (24h), which may be one of the reasons of reduction in plant growth and development under abiotic stresses.

2.4.3.3 Regulation of N metabolism and abiotic stress response by N-pathway genes

Research works have also provided a new framework for understanding the role of glutamine synthetase in regulation of N metabolism and abiotic stress responses. Suan *et al*., (2005) observed that highly effective expression of glutamine synthetase genes GS1 and GS2 in transgenic rice plants increases nitrogen-deficiency tolerance. GS1-GS2 transformants obtained was able to grow well in an MS medium in which the nitrogen source was replaced and fresh weight of the transformants was significantly higher than the control rice plants. The result suggested that expression of p2GS makes the transgenic rice plants tolerant to nitrogen-deficiency. Moreover, Cai *et al*. (2009) proposed that over expressed glutamine synthetase gene modifies nitrogen metabolism and abiotic stress responses in rice. *GS1;1*, *GS1;2*-overexpressing plants showed significantly decreased plant height and root length, shoot dry weight and root dry weight at both the tillering stage and heading stage when compared to the wild type plants.

Sun *et al.* (2009) established relationship of nitrogen utilization and activities of key enzymes involved in nitrogen metabolism in rice under water–

nitrogen interaction. The activities of nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), and endopeptidase (EP) were measured at tillering, elongation, heading, and maturity stages to analyze the correlations between activities of these enzymes in functional leaves and the N absorption and utilization in plant or grain yield. There was an obvious interaction between irrigation method and N application level. Excessive N application (N270) resulted in negative influences on water-nitrogen interaction, such as slow increase of NR, GS, and GOGAT activities, reductions of N agronomy efficiency (NAE) and N recovery efficiency (NRE), and decrease of yield. The enzyme activities were positively correlated with the indices for N uptake and utilization at various stages and the grain yield at maturity. The GS activity in functional leaf was proposed as a candidate indicator for N uptake and accumulation at various growth stages, and the activities of NR, GS, GOGAT, and EP in flag leaf at heading stage as candidate indicators for rice yield and NPE, NAE, and NRE.

Moreover, very little is known about Os*AMT1;1* involvement in NH_4^+ uptake in rice roots and subsequent effects on NH_4^+ assimilation. Ranathunge *et al*. (2014) study on transgenic rice plants showed that Os*AMT1; 1* is a constitutively expressed, nitrogen-responsive gene, and its protein product is localized in the plasma membrane. Their results suggested that Os*AMT1;1* has the potential for improving nitrogen use efficiency, plant growth, and grain yield under both suboptimal and optimal nitrogen fertilizer conditions among rice genotypes.

In their recent findings, Singh and Ghosh (2013) revealed the effect of WD on regulation of GS isoforms in drought-sensitive (cv. IR-64) and drought-tolerant (cv. Khitish) rice cultivars. Their results depicted that under WD, total GS activity in root and leaf decreased significantly in IR-64 seedlings in comparison to Khitish seedlings. The reduced GS activity in IR-64 leaf was mainly due to decrease in GS2 activity, which correlated with decrease in corresponding transcript and polypeptide contents. GS1 transcript and polypeptide accumulated in leaf during WD, however, GS1 activity was maintained at a constant level. Among GS1 genes, Os*GS1; 1* expression was differently regulated by WD in the two rice varieties. Its transcript accumulated more abundantly in IR-64 leaf than in Khitish leaf. Following WD, Os*GS1;1* mRNA level in stem and root tissues

declined in IR-64 and enhanced in Khitish. A steady Os*GS1; 2* expression patterns were noted in leaf, stem and root of both the cultivars. Results further suggest that Os*GS2* and Os*GS1; 1* expression may contribute to drought tolerance of Khitish cultivar under WD conditions.

2.5 Exploring intra-specific genetic variability for improving NUE

Since NUE is a complex agronomic trait, this has prompted a number of groups to exploit its intra-specific genetic variability in a more targeted way. The most N-efficient rice genotypes are those capable of accumulating N in the first 35 days of transplanting (Peng *et al*. 1994). Sattelmacher *et al.* (1994) pointed out that genotypes might differ in nitrogen use efficiency and uptake efficiency: the two factors that are attributed to genetic variation in nutrient efficiency in crops. Rice breeders have historically not included nitrogen utilization efficiency (NUtE) as a selection criterion in breeding for cultivars. Genotypic differences in response to nitrogen fertilizers and nitrogen utilization have been previously reported (Broadbent *et al*., 1987; Cho and Koh, 2005; De Datta and Broadbent, 1990; Park and Mok, 1975; Seo *et al*., 2005). Some traits related to efficient N uptake and metabolism such as the N uptake characteristics of seedlings (Teyker *et al*., 1989) and plant nitrate content (Mollaretti *et al.,* 1987) have been suggested as selection parameters. Inthapanya (2000) studied the genotype differences in nutrient uptake and utilization for grain yield production of rain-fed lowland rice and reported the presence of significant genotypic variation in nutrient-use efficiency.

Swain *et al.* (2006) assessed variability in N uptake and utilization of medium and late duration rice varieties. They observed that difference in optimum yield of the medium and late duration varieties was due to the differences in the amount of N uptake and its use efficiency by the plant for grain production. Also, Hassan *et al.* (2009) reported the genotypic variation in traditional rice varieties for chlorophyll content, SPAD value and nitrogen use efficiency. Nitrogen % in both straw and grain, nitrogen uptake by straw and grain, total nitrogen uptake and NpUE showed significant differences due to variable nutrient levels at maturity in traditional rice varieties. Significantly higher NpUE without addition of nitrogen (Zero N) indicated the ability of utilization of the native nitrogen by traditional varieties. The higher NpUE in zero nitrogen treatment confirmed the ability of

producing similar yield level as compared to added nitrogen treatment. Moreover, Namai *et al*. (2009) investigated varietal differences in dry matter production and NUpE using a wide range of rice varieties at an early growth stage**.** Overall, this study found that there are wide variations in NUpE among varieties with similar levels of relative dry weight (RDW) under varying nitrogen conditions. Variations in NpUE were greater under lower nitrogen conditions; in contrast, variations in RDW were greater under higher nitrogen conditions. These data, along with those of other studies, indicated differences in nitrogen use efficiency between Indica- and Japonica types, upland and lowland varieties, and landraces and improved types. By the same token, Zhang *et al.* (2009) studied grain yield and physiological N use efficiency in relation to the accumulation and redistribution of biomass and N in rice. Results depicted differences in biomass, N accumulation and N redistribution at the post-heading of rice cultivars.

Information regarding low N-tolerance and NUE among genotypes of various species is rare. To unveil such information, Wu *et al*. (2011) evaluated low-nitrogen stress tolerance and nitrogen agronomic efficiency among maize inbreds. Low-N agronomic efficiency (LNAE), absolute grain yield (GY) at low-N conditions, and the ratio between GY at low-N and optimum-N conditions were taken into account to represent low-N tolerance. Additionally, N-agronomic efficiency (NAE) along with other agronomic traits was also analyzed.

Chen *et al*. (2012) in their study concluded that the larger degree of seedling growth inhibition in low- vs. high-NUE rice genotypes is associated with significantly enhanced NH_4^+ cycling and tissue accumulation in the elongation zone of the root. Additionally, Singh and Verma (2013) characterized and screened high N efficient genotypes. The genotypes were screened on the basis of growth dynamics, physiological and agronomic responses to nitrogen rates. Results showed, traits associated with growth, physiological area index, photosynthetic rate, SPAD value, leaf N content and NR activity and agronomical traits enhanced significantly with increased N rates. Experiment concluded that among all genotypes, few are more proficient to assimilate N in their tissues. Therefore, more N efficient genotypes can be suggested to farmers for yield enhancement and reducing environmental pollution. With the objective of

identifying inbred line with superior N-use. Mundin *et al.* (2013) carried genetic diversity and path analysis for NUE of popcorn inbred lines. They suggested the formation of a selection index involving the total root length (TRL), daily growth and NUpE traits to improve the accuracy of selection of NUE. Abe *et al*. (2013) evaluated genetic variation for NUE among tropical maize hybrids selected for contrasting responses to N. Their results documented significant genetic variation for grain yield (GY) and measured NUE component traits among the hybrids, as well as significant interactions between hybrid and N level for all traits except nitrogen harvest index.

Hukum and Singh (2013) screened and characterized high N-efficient genotypes on the basis of growth dynamic, physiological and agronomical responses to nitrogen rates. . Their experiment concluded that hybrid genotype has more capacity to uptake and extract applied nitrogen from the soil and proficient to assimilate in their tissues. Therefore, most N efficient genotypes can be strongly suggested to rice growers. Furthermore, Hukum *et al*. (2014) overall study on NUE and related traits concluded that there are wide variations in physiological as well as growth dynamics, biomass partitioning, chaffy grain number and weight as well as nitrogen use efficiency among rice genotypes under different nitrogen treatment conditions.

Ospina *et al*. (2014) observed the diversity of crop development traits and nitrogen use efficiency among potato cultivars grown under contrasting nitrogen regimes. Results revealed that cultivars performing well under high N also performed well under low N.

2.6 Morpho-physiological response of genotypes to different nitrogen and water levels

Nitrogen concentration in the vegetative organs at later developmental stages is commonly much lower than it is in the seeds, relatively lower protein content (low seed N concentration) represents a higher NpUE. Single-seed dry weight and N concentration are robust traits, highly heritable, whereas GHI (grain harvest index) and NHI (nitrogen harvest index) are highly correlated and affected largely by N supply level and availability, particularly at the seed-filling stage

(Masclaux-Daubresse, 2011). Therefore, lowering total N concentration in high-yield seeds has the advantage of improving NpUE if adequate essential protein components. Additionally, GHI is useful index in evaluating treatment effects on partitioning photoassimilates to grain during reproductive growth stages and varies for different cultivars (Fageria, 2007). Higher NUE has been observed in cultivars with higher GHI (Bufogele *et al.*, 1997).

Selection of the most appropriate level of nitrogen fertilization is a major concern of economic viability of crop production. Manzoor *et al.* (2000) studied the effect of different N levels on yield and yield components of basmati. According to results plant height, no. of productive tillers, panicle length, no. of grains per panicle, thousand grain weight and yield showed different trend with different N level. Similarly, Chaturvedi (2005) conducted field trial to determine the effect of different nitrogenous (N) fertilizers on growth, yield and quality of hybrid rice of different rice cultivars. The sulphur containing N fertilizers showed significant results.

Jiang *et al*. (2005) further studied the photosynthetic efficiency and nitrogen distribution under different nitrogen management and its relationship with physiological N-use efficiency in rice. An experiment conducted by Islam *et al*. (2009) showed that root, stem and hill^{-1}, TDM (total dry matter) hill^{-1} and chlorophyll content in leaves, varies with varying rates of N applications. Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency is important. On farm trials led by Bradley and Kindred (2009) on barley made them suggest that, in order to elicit faster improvement in NUE on farms, breeding and variety testing should be conducted at some sites with more than one level of applied N, and that grain N%, N harvest index, and perhaps canopy N ratio (kg N ha^{-1} green area) should be measured more widely. They also suggested that, instead of using empirical functions, N responses might be analysed more effectively using functions based on explanations of yield determination for which the parameters have some physiological meaning.

Tari *et al.* (2009) evaluated flag leaf characteristics such as flag leaf area, flag leaf angle and flag leaf chlorophyll under different N treatments. They found

that flag leaf morph physiological characteristics were influenced by nitrogen fertilization levels.

Konnerup and Brix (2010) demonstrated the effects of inorganic nitrogen (N) source (NH_4^+, NO_3^- or both) on growth, biomass allocation, photosynthesis, N uptake rate and mineral composition of *Canna indica*. NH_4^+ fed plants had higher concentrations of N in the tissues, lower concentrations of mineral cations and higher contents of chlorophylls in the leaves compared to NO_3^- fed plants suggesting a slight advantage of NH_4^+ nutrition.

Kamiji *et al*. (2011) determined the effect of nitrogen (N) top-dressing on the number of total spikelet (fertile plus sterile) production and evaluated the effect among rice cultivars. They observed that varietal variation in spikelet production efficiency is explained by CGR (crop growth rate) during the 14-day period. They eventually concluded that N applications that increase plant N 14 days before heading is highly effective in maximizing spikelet production among cultivars.

Vasileya *et al.* (2011) investigated the influence of ammonium and nitrate form of mineral nitrogen in chemical composition, nitrate reductase activity and plastid pigment contents of lucerne in conditions of optimum moisture and water deficiency stress. Crude protein content under water deficiency stress increased by 4–21% for ammonium, and by 3–12% for the nitrate form of nitrogen. Under water deficiency stress, content of calcium and phosphorus decreased, but there were no differences for two forms of mineral nitrogen. At the optimum moisture and water deficiency stress, there was a similar tendency to reduce nitrate reductase activity in leaves, when applied mineral nitrogen at a dose of 70 mg N kg^{-1} soil in both forms and to increase at the doses of 140 and 210 mg N kg^{-1} soil.

Over the past 3 decades, the study of various mechanisms of grain yield (GY) formation and its relationship with nitrogen (N) uptake dynamics has been increasingly acknowledged in the scientific literature. However, few studies have combined investigations of GY response to N fertilizer with detailed physiologically based analyses of plant N dynamic and the complex interactions of those with specific genotypes (G) and production environments (E). This need was catered by Ciampitti and Vyn (2012a). They undertook a comprehensive review to

discern trends in physiological aspects of maize response to changing plant densities and fertilizer N rates under the umbrella of evolving G × E interactions. Additionally, improved phenotyping tools for simultaneously characterizing genotypes with superior grain yield (GY) and N use efficiency (NUE) would be beneficial for breeding progress. Similarly, Ciampitti and Vyn (2012b) evaluated possible phenotypic predictors of the crowding intensity and N availability effects on maize plant N uptake, GY, and for different genotypes in two environments and also assessed correlations between predictive traits (principal component analyses identified 21 traits).

The water and N plays a pivotal role in rice production as it greatly affects the several physiological parameters of crop. This fact is proved by Sun *et al.* (2009) and Sun *et al*. (2012) who investigated the relationship between yield or NUE and ammonia assimilation enzyme activities, photosynthetic rate and root activity. Their data revealed that there were obvious interacting effects of irrigation regime and N application strategies on grain yield and NUE as well. The correlation analysis showed that the grain yield or NUE was significantly related to ammonium assimilation enzyme activities, photosynthetic rate, and root activity, respectively, at several rice growth stages. Correlation analysis also revealed that the grain yield was significantly related to the N uptake and utilization, and that the root activity was significantly related to the ammonium assimilation enzymes and photosynthetic rate. Furthermore, they found that the ammonium assimilation enzyme activities and the photosynthetic rate in flag leaves, or root activity at heading stage was able to be used as indicators for grain yield, total N accumulation, and NUE as well. These results suggest that the N application strategy should be adjusted according to different irrigation regimes in order to obtain the highest yield and the best NUE.

Zhang *et al.,* (2012) investigated ameliorating effects of N on growth, water status and N metabolism of maize cultivars. Dry matter, grain yield, relative water content, nitrate reductase activity, soluble protein concentration, and concentrations of free proline and endogenous glycine betaine were parameters under study. Correlations were more evident among all parameters under drought stress (DS) than those under control. Thus, moderate N plays an evident

physiological role in alleviation of effects of DS on plant growth by improving water status and N metabolism, especially for drought-sensitive cultivars.

Lopes *et al*. (2012) evaluated rice growth and yield at different nitrate-ammonium ratios N supply in the exclusive form of nitrate, or ammonium at higher proportions than nitrate, decreased dry matter, especially during panicle emission, affecting the yield. The maximum dry matter production of rice cultivars shoots occurred at nitrate rates between 58 and 68 %. The maximum grain yield was obtained at nitrate ratios between 75 and 78 %. The excessive accumulation of nitrate in plant tissues due to low activity of Nitrate Reductase in the initial growth phase, and excess of ammonium were the main causes of decline in rice growth and yield, when nitrate was the only N form or when ammonium was used at higher proportions than nitrate in the nutrient solution.

Ványin *et al*. (2012) studied effect of different nitrogen doses on the chlorophyll concentration, yield and protein content of different genotype maize hybrids. Rao *et al*. (2014) demonstrated effect of different doses of nitrogen on performance of promising varieties of rice in high altitude areas of Andhra Pradesh. Tiller production, days to 50 per cent flowering, dry matter production at harvest, yield attributes, yields and harvest index, gross returns, net returns and rupee per rupee invested, protein content of grain, soil organic carbon and available nitrogen were progressively augmented by incremental levels of N. Nutrient response in terms of partial factor productivity was progressively decreased with incremental levels of N from 60 kg to the highest dose tried. Post soil fertility status revealed that the status was progressively increased with incremental levels of N up to the highest dose tried that increased significantly by elevated levels of N. Kumar *et al*. (2014) carried physiological evaluation of NUE and yield attributes in rice genotypes under different N levels. Traits under study were plant height, no. of effective tillers, leaf area index, vegetative plant growth and grain yield. Owing to the performance of genotypes they suggested that these traits can be utilized as physiological markers for the selection of rice genotypes efficient in N-use. Also, Malik *et al*. (2014) examined effect of different levels of nitrogen on growth and yield attributes of different varieties of Basmati Rice. The parameters measured included plant height, number of tillers/hill , dry weight ,

length of panicle , number of filled grains / panicle , straw yield , biological yield , harvest index, benefit cost ratio and grain yield. The varieties were evaluated under three nitrogen levels. The varietal trial indicated that Pusa Basmati- 1 at one nitrogen level was significantly different for all the parameters undertaken.. Similarly, in recent study, koffi *et al.* (2016) evaluated yield and nitrogen use efficiency of aromatic rice varieties in response to nitrogen fertilizer.

Haque and Haque (2016) evaluated growth, yield and nitrogen use efficiency of new rice variety under variable nitrogen rates. From the results it was concluded that growth, yield and nitrogen use efficiency of the new rice variety were significantly influenced by different levels of nitrogen fertilizer. Although growth of the variety increased with increased nitrogen levels, assimilate mobilization towards grain was higher at 60 kg N ha^{-1}. Consecutively, the variety produced the highest yield with 60 kg N ha^{-1} with the highest nitrogen use efficiency.

Matching N supply with crop demand is prerequisite for achieving optimum utilization of N and higher NUE. Among all the different tools available to measure the leaf greenness, the non-destructive measurement of leaf green color intensity using leaf color charts (LCC) and soil & plant analysis diagnostic meter (SPAD) is gaining importance. In general, chlorophyll content in a leaf is closely correlated with leaf N concentration (Felix *et al.,* 2002). Gholizadeh *et al.* (2009a) established relationship between N content and SPAD values using geostatistical tools. Analysis of variance, variogram and kriging were conducted to determine the variability of the measured parameters and also their relationship. Tari *et al*. (2013) investigated nitrogen chlorophyll condition at different nitrogen fertilization methods in rice by applied mathematics relations with use of chlorophyll meter, at different growing stages of rice. There results showed that the SPAD reading at all stages was positively correlated with rice yield that illustrated the importance of chlorophyll content and its related to grain yield. Ahamad *et al*. (2016) used LCC management practice for all N treatments and obtained maximum tillers/hill, plant height, straw, and paddy yield..

2.7 Exploring intra-specific genetic variability for transporter system and assimilatory enzymes for improving NUE

Rice genotypes showed wide variability in NRA (Nitrate Reductase activity) (Shen, 1972). These differences were related to the nitrogen source and seedling age. NRA activity is generally used as a tool to distinguish between genotypes of crop plants (Caba *et al*., 1995; Bussi *et al*., 1997; Marwaha, 1998). Dependant on the nitrogen source, upland adapted rice genotypes showed larger variability in NRA than lowland genotypes. Andrews *et al*. (2005) reviewed literature on the relations between plant nitrogen (N) assimilation enzymes and plant/crop N assimilation, growth and yield is to assess if genetic manipulation of the activities of N assimilation enzymes can result in increased yield and/or increased N use efficiency. They stated that root or shoot nitrate assimilation can have advantages under specific environmental conditions; NADH-glutamate synthase (NADH-GOGAT) is important in the utilization of N in grain filling and its activity in developing grains is positively related to yield. In their opinion, selection of plants, with expression of nitrate reductase/nitrite reductase primarily in the root or shoot should increase plant/crop growth and hence yield under specific environmental conditions. Additionally, for cereals the selection of plants with high GS1 in senescing leaves and in some cases high NADH-GOGAT in developing grains could help maximize the retrieval of plant N in seeds.

Cao *et al*. (2008) results suggested that the increase in GS activity might be a result of the complicated regulation of the various GS genes. In addition, the NO^{-}_{3}-induced increase of biomass, NR activity, GS activity, and the transcript levels of NR and GS genes were proportionally higher in NG (Nanguang) than in YJ (Yunjing), indicating a stronger response of NG to NO^{-}_{3} nutrition than YJ. Dos *et al*. (2009) evaluated GS and NR activity of two rice cultivars. The evaluated varieties replay in different manner about accumulation and translocation of nitrate.

Xia *et al*. (2011) evaluated N adaptation of 70 selected hybrid combinations. On the whole physiologically, the rice materials with good adaption of N were due to higher nitrate reductase activity (NRA) at low nitrogen conditions, more chlorophyll content and soluble protein in high nitrogen conditions, and the Rubisco mobilized in N-free conditions as well.

Hakeem *et al.* (2012) evaluated ten genotypes of rice were grown for 30 d in complete nutrient solution with 1 mmol/L (N-insufficient), 4 mmol/L (N-moderate) and 10 mmol/L (N-high) nitrogen levels, and nitrogen efficiency (NE) was analyzed. Growth performance, measured in terms of fresh weight, dry weight and lengths of root and shoot, was higher in N-efficient than in N-inefficient rice genotypes at low N level. Of these 10 genotypes, Suraksha was identified as the most N-efficient, while Vivek Dhan the most N-inefficient. To find out the physiological basis of this difference, the nitrate uptake rate of root and the activities of nitrate assimilatory enzymes in leaves of N-efficient and N-inefficient rice genotypes were studied. Uptake experiments revealed the presence of two separate nitrate transporter systems mediating high- and low-affinity nitrate uptake. Interestingly, the nitrate uptake by the roots of Suraksha is mediated by both high- and low-affinity nitrate transporter systems, while that of Vivek Dhan by only low-affinity nitrate transporter system. Study of the activities and expression levels of nitrate assimilatory enzymes in N-efficient and N-inefficient rice genotypes showed that nitrate reductase (NR) and glutamine synthetase (GS) play important roles in N assimilation under low-nitrogen conditions.

Gaur and Singh (2012) reported genotypic differences on the members of ammonium transporter and glutamine synthetase genes of selected rice genotypes. Genotypes selected were PB1 and KN3119. It was grown under differential nitrogen inputs and showed the differences in seed/panicle, 1,000 seed weight, % nitrogen in the biomass and protein content in the seeds. All these parameters in PB1 were found to be in the increasing order in contrast to KN3119 which showed declined response on increasing nitrogen dose exceeding the normal dose indicating that both the genotypes respond differentially to the nitrogen inputs. Gene expression analysis of members of ammonium transporter gene family in flag leaves during active grain filling stage revealed that all the three members of Os*AMT3* family genes (Os*AMT1;1–3*), only one member of Os*AMT2* family *i.e*. Os*AMT2;3* and the high affinity Os*AMT1;1* were differentially expressed and were affected by different doses of nitrogen. In similar experiment, Gaur and Singh (2012) develop understanding of differential nitrogen sensing mechanism

in rice genotypes through expression analysis of high and low affinity ammonium transporter genes. Their finding revealed that *AMT1;3* is involved not only in ammonium uptake but may also in ammonium sensing. Therefore, it could be used as a biomarker for nitrogen responsiveness. There was expression of the only high affinity AMT gene, *AMT1;1*, along with six low affinity AMT genes in the shoots, which suggests that low affinity AMTs in the shoots leaves are involved in supporting *AMT1;1* to carry out its activities/function efficiently.

Khalid *et al.* (2012) studied physiological basis of this difference, the nitrate uptake rate of root and the activities of nitrate assimilatory enzymes in leaves of N-efficient and N-inefficient rice genotypes. Uptake experiments revealed the presence of two separate nitrate transporter systems mediating high- and low affinity nitrate uptake. Interestingly, the nitrate uptake by the roots of one genotype is mediated by both high- and low-affinity nitrate transporter systems, while that of another genotype by only low-affinity nitrate transporter system. Study of the activities and expression levels of nitrate assimilatory enzymes in N-efficient and N-inefficient rice genotypes showed that NR and GS play important roles in N assimilation under low-nitrogen conditions.

Nitrogen responsiveness of three-finger millet genotype of different coat colour, PRM-1(brown), PRM-701(golden), and PRM-801 (white) grown under different nitrogen doses was determined by analyzing the growth, yield parameters and activities of Nitrate Reductase (NR), Glutamine Synthetase (GS), Glutamate Synthase; GOGAT, and Glutamate Dehydrogenase (GDH) at different developmental stages (Gupta *et al.*, 2012). The study indicates that PRM-1 is high nitrogen responsive and has high nitrogen use efficiency, whereas golden PRM-701 and white PRM-801 are low nitrogen responsive genotypes and have low nitrogen use efficiency. However, the crude grain protein content was higher in PRM-801 genotype followed by PRM-701 and PRM-1, indicating negative correlation of nitrogen use efficiency with source to sink relationship in terms of seed protein content.

Tang *et al*. (2013) reported genotypic differences in nitrate uptake, translocation and assimilation of two Chinese cabbage cultivars. Their results suggested that nitrate accumulation differences were due to differential capacities

to uptake, mechanisms for nitrate transport in leaves and assimilate nitrate. Comparing contribution of three aspects in nitrate accumulation, translocation and assimilation were contributed more in low nitrate concentration in the leaf blade.

Yin *et al.* (2014) evaluated two rice cultivars one with high NUE and another with low NUE for nitrate remobilization and measured the uptake of NO_3^-, expression of nitrate reductase (NR), NO_3^- transporter genes (NRTs), and NR activity. Genotype with high NUE showed significantly higher leaf and root maximum NR activity (NRA_{max}) and actual NR activity (NRA_{act}) as well as stronger root expression of the two NR genes. 3-fold higher expression of

Kaur *et al*. (2015) observed the differential activity pattern of nitrogen assimilating enzymes viz. nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in relation to protein, amino acid and nitrogen content with respect to source-sink relationship (flag leaf and grains) under different N inputs at different developmental stages of six wheat genotypes.

Understanding how crops respond to limited nitrogen supply is essential to develop new ways of manipulating genes for breeding new crop cultivars or lines with high nitrogen use efficiency (NUE). However, little is known about the differences among genotypes in their responses to N starvation and subsequent N re-supply. Xu *et al*. (2016) observed the difference in responses to nitrogen deprivation and re-supply at seedling stage between two barley genotypes. Nitrate Reductase and Glutamine Synthetase activity was significantly different in these two genotypes.

2.8 Correlation studies

Grain yield of rice is the final product of the combination of number of panicles per unit area, spikelet density, percentage of filled spikelets and grain weight (Gravoid and Helms, 1992). For achieving higher yield use of nitrogen efficient genotypes is an important complementary strategy in improving rice yield and reducing cost of production in subsistence farming (Kim *et al*., 1993). Results of several studies have indicated that application of N fertilizer increases grain yield of rice by increasing the magnitude of its yield attributes (Panda *et al.*, 1995).

Rice breeders have historically not included nitrogen utilization efficiency (NUE) as a selection criterion in breeding for cultivar. Samonte *et al*. (2006) examined the significance and magnitude of variation in N content, NUE, N translocation ratio (NTR), and grain protein concentration among diverse rice genotypes. Nitrogen content and NUE were not significantly correlated with each other, and they had significant positive direct effects on grain yield. Grain yield was positively correlated with NUE, N content, and NTR, whereas NTR was correlated with grain protein concentration. Plant breeders could use these significant correlations to their advantage in breeding for rice cultivars that not only produce high yield but also utilize N efficiently and produce grain with a higher protein concentration.

In order to avoid N losses and to use soil-and fertilizer-nitrogen efficiently, it is necessary to develop better ways of predicting the optimum amount of N needed by the rice plants. Prudente *et al.* (2009) evaluated yield and nutrient uptake potentials of *japonica* and *indica* rice varieties with nitrogen fertilization. The yield and yield components were found to be positively correlated to the amount of N applied. Results also showed an increasing trend in the N uptake, rice yield, panicle number, tiller number and dry matter production, with increased amount of applied N fertilizer. Moreover, Frageria *et al*. (2011) conducted two greenhouse experiments to evaluate influence of ammonium sulfate and urea on growth, yield and yield components of lowland rice. Shoot dry weight and grain yield significantly ($P< 0.01$) increased in a quadratic fashion when N rate increased by ammonium sulphate as well as urea fertilization. Maximum grain yield at average N rate was 22% higher with the application of ammonium sulphate compared to urea, indicating superiority of ammonium sulphate compared to urea. Plant height, shoot dry weight, grain harvest index, 1000 grain weight and N uptake and use efficiency in shoot and grain had significant positive association with grain yield. However, spikelet sterility was negatively associated with grain yield.

Kang *et al.* (2013) conducted study on high yielding japonica rice under three nitrogen levels. Rough rice yield showed linear relationship with total nitrogen uptake within the range of nitrogen treatments. Rice varieties showed different

nitrogen uptake ability and NUE at different nitrogen level. Significant variations and relationships among grain yield, NUpE, and NUE confers an advantage to breeder and agronomist. The little information on NUE variability and its relationships with yield and yield related traits in rice has led to investigation of genetic variability in N use efficiency among upland rice genotypes, identifying genotypes with best N use efficiency and also assessing nature of association among N use efficiency and yield and yield related traits.

Singh *et al*. (2014) carried morphological and biochemical characterization of eighteen rainfed upland rice genotypes under drought stress situation. They observed that NUE related traits like chlorophyll a, Nitrate Reductase activity, carbohydrate at flowering, starch at maturity, leaf's carbohydrate, leaf's starch and lower root's starch showed positively high (desired) genotypic correlation as well as direct effect on grain yield.

Grain yield showed significant and positive correlation with grain nitrogen yield, and nitrogen harvest index. Grain nitrogen concentration had significant and positive correlation with nitrogen uptake efficiency and nitrogen use efficiency (Lakew, 2015). Zhu *et al*. (2015) study suggested that genetic improvements in rice grain yield are associated with increased NUE. Significant improvement in grain yield was obtained from increase in the harvest index and biomass, and spikelet per panicle. It can be concluded that concomitant relationship exist between grain yield and NUE. Xu *et al.* (2016) quantified yield gap and NUE of irrigated rice. Results obtained using the Pearson' correlation analysis showed significantly negative coefficients for yield response (YR) to soil nutrient contents and soil organic matter. The large variations in YR were attributed to differences in climatic conditions and soil indigenous nutrient supplies. They concluded that in order to narrow the YG and increase NUE, effective soil, plant, nutrient management measures, advances in knowledge and technologies would be required to sustain higher crop production.

2.9 Deciphering the genetic basis of nitrate and ammonium use efficiency

Nitrogen utilization within rice plants followed by NH_4^+ uptake and assimilation in the roots is a complex process that depends on many factors during the growth and development of plants. Of course, a number of activity genes and enzymes are involved in the nitrogen utilization process, in many cases, the expression of each gene is regulated in a cell type, but current knowledge is limited. Reverse genetics is a powerful approach to obtain conclusive evidence on the function of the corresponding gene products, also enzymes involved in metabolic of nitrogen assimilation pathway can be characterized by reverse genetics. But now it is very difficult to identify target genes that are involved in regulation, unlike enzymes in metabolic pathway, so an approach of quantitative trait loci (QTLs) analysis could be one way to isolate regulatory genes (Yuanyuan *et al*., 2011).

Many gene families, including NO_3^- and NH_4^+ transporters and primary assimilation genes, amino acid transporters, as well as transcription factors and other regulatory genes, have been identified by different approaches. With the identification of orthologous genes from rice, opportunities are now emerging for utilizing these genes in marker-assisted breeding for N efficiency (Li *et al*. 2009; Kant *et al*. 2011). So in rice, the effect of QTLs would be potential research to confirmed target genes controlling uptake, assimilation, and metabolism of nitrogen, as well as nitrogen use efficiency.

Studies of QTLs affecting characters related to nitrogen-use efficiency have been reported in maize (Agrama *et al*., 1999; Hirel *et al*., 2001) and Arabidopsis (Loudet *et al*., 2003). However, only limited such studies have been reported in rice. There have been a few reports of QTL analysis for low nitrogen tolerance (Lian *et al.*, 2005) and the effect of nitrogen fertilizer levels on plant height (Fang and Wu, 2001). Epistatic effects are found to be very important for complex traits in rice by several researchers (Cao *et al*., 2001; Eta-Ndu and Openshaw, 1999; Hu *et al*., 2001; Wang *et al*., 1999; Wu *et al*., 2000).

The hypothesis that GS is one of the key steps in the control of cereals productivity was strengthened by the study performed on rice, in which a co-localization of a QTL for *GS 1;1* locus and a QTL for one-spikelet weight was identified (Obara *et al*., 2001). Obara *et al*. (2004) characterized quantitative trait locus (QTL) associated with the protein content of cytosolic glutamine synthetase in senescing leaves, panicle number, and panicle weight in rice. Their data indicated the importance of target QTL (Pnn1; panicle number 1) in the development of tillers and panicles in rice. This confirms hypotheses that GS play important role in plant productivity, the idea which arose from either whole plant physiological studies, or genetic manipulations (Andrews *et al*., 2004; Good *et al*., 2004). GS activity in N remobilization, growth rate, yield, and grain filling has been emphasized by functional genomics and quantitative trait loci (QTL) approaches and by using cultivars exhibiting contrasting NUE (Bernad and Habash, 2009).

Lian *et al*. (2005) mapped QTLs for low nitrogen tolerance using recombinant inbred lines using seedling traits, and found that only few QTLs were detected in both low and normal nitrogen conditions, indicating very few commonality of genetic control of these traits under both the conditions. Epistatic interactions collectively accounted for larger amount of variation for individual traits, while QTL-by-environment interactions were trivial. Shan *et al*., (2005) detected 12 QTLs for N concentration/accumulation and N-use efficiency for biomass production in rice plants. Tong (2006) identified 31 QTLs that controls plant height, the number of panicles per plant, chlorophyll content, shoot dry weight and grain yield per plant, using a population of chromosome segment substitution lines at the late developmental stage under two N conditions. Furthermore, Laza *et al*. (2006) reported co-localization of QTLs affecting leaf nitrogen content at mid-growth with that of nitrogen content in the shoot at maturity on chromosome 2 and chromosome 9. In same year, Han-Hua (2006) identified QTLs underlying related traits at the late developmental stage under two different nitrogen levels were investigated in rice using a population of chromosome segment substitution lines (CSSL) derived from a cross between Teqing and Lemont. A total of 31 QTLs referring 5 traits, that is, plant height (PH), panicle number per plant (PN), chlorophyll content (CC), shoot dry weight

(SDW) and grain yield per plant (YD), were detected. Under normal N level, 3 QTLs were detected for each trait, while under low N level, 5, 4, 5 and 2 QTLs were detected for PH, PN, CC and SDW respectively. Most of the QTLs were located on chromosome 2, 3, 7, 11 and 12. QTLs controlling different traits or the same trait under different N levels were mapped on the same or adjacent intervals, forming several clusters in rice chromosomes. More than two traits were controlled by QTLs on one of four intervals (RM30-RM439, RM18-RM478, RM309-RM270, and RM235-RM17), suggesting that there were some pleiotropic effects.

QTLs only detected at low N level might be associated with the ability to tolerate the low N stress in rice. Vinod (2007) reported a low nitrogen sensitive locus on chromosome 3 which was found to harbour QTLs corresponding to shoot biomass and flag leaf length under low nitrogen conditions, which was lying in close proximity of a locus for GS activity. Cho *et al*. (2007) conducted study to identify QTLs for traits associated with physiological nitrogen use efficiency (PNUE). PNUE was positively correlated with the harvest index and grain yield under ordinary low-nitrogen and high-nitrogen conditions. Twenty single QTLs (S-QTLs) and 58 pairs of epistatic loci (E-QTLs) were identified for the nitrogen concentration of grain, nitrogen concentration of straw, nitrogen content of shoot, harvest index, grain yield, straw yield and PNUE in both conditions.

Continuous variation and transgressive segregation for LCC were observed in the BIL population screened by Dong *et al* (2007), indicating that LCC was a quantitatively inherited trait. Seven QTL for LCC were identified and mapped to chromosomes 1 (two QTL), 2, 3, 4, 6, and 8, which individually accounted for 5.1 to 14.8% of the total phenotypic variation. Three QTL (qLCC-1-1, qLCC-1-2 and qLCC-4) were common between the tillering and heading stages. The alleles at four QTL (qLCC-1-1, qLCC-1-2, qLCC-2, and qLCC-8) from Koshihikari and the alleles at the other three QTL (qLCC-3, qLCC-4 and qLCC-6) from Kasalath increased LCC.

Senthilvel *et al*. (2008) attempted quantification of genotype x nitrogen level interaction and mapping of quantitative trait loci (QTLs) associated with nitrogen use efficiency (NUE) and other associated agronomic traits. Twelve parameters were observed across a set of 82 double haploid (DH) lines derived

from IR64/Azucena across three nitrogen regimes. The parents and DH lines were significantly varying for all traits under different nitrogen regimes. All traits except plant height recorded significant genotype x environment interaction. Individual plant yield was positively correlated with nitrogen use efficiency and nitrogen uptake. Sixteen QTLs were detected by composite interval mapping. Eleven QTLs showed significant QTL x environment interactions. On chromosome 3, seven QTLs were detected associated with nitrogen use, plant yield and associated traits. A QTL region between markers RZ678, RZ574 and RZ284 was associated with nitrogen use and yield. This chromosomal region was enriched with expressed gene sequences of known key nitrogen assimilation genes. Wang *et al*. (2009) identified several QILs related to panicle number and grain yield in field experiments under normal N fertilization and low N-treatments.

Quraishi *et al*. (2011) suggested that cross-genome map based dissection of a nitrogen use efficiency ortho-meta QTL in bread wheat unravels concerted cereal genome evolution. NUE QTL and GOGAT genes are conserved at orthologous loci in the cereal genomes of wheat, rice, sorghum, and maize, which diverged from a common ancestor some 50–70 million years ago, suggesting that some traits underlying NUE have been conserved during evolution, at least in cereals. Mapping quantitative trait loci (QTLs) for nitrogen deficiency trait and nitrogen use efficiency traits, offers a new perspective in genetic analysis of N-related traits.

Tong *et al.* (2011) identified 57 main-effect QTLs, and 33 digenic interactions for grain yield and its components under three N levels. Furthermore, Wei *et al.* (2011) detected QTLs for rice NUE and grain yield traits, and studied the genetic relationship between the two traits. They identified 68 QTLs for grain yield and its components under the low and normal N applications. Recently, qNGR9, a major rice QTL for N-use efficiency, was cloned and was found to be synonyms with the previously identified DEP1 (DENSE AND ERECT PANICLE 1) gene (Sun *et al*., 2014). Yue *et al*. (2015) identified QTLs for rice yield traits under two N levels. A total of 52 QTLs for yield traits distributed in 27 regions on 9 chromosomes were detected, with each QTL explaining 4.93%–26.73% of the phenotypic variation. Eleven QTLs were simultaneously detected under the two levels, and 30 different QTLs were detected under the two N levels,

thereby suggesting that the genetic bases controlling rice growth under the low and normal N levels were different. QTLs for number of panicles per plant, number of spikelets per panicle, number of filled grains per panicle, and grain density per panicle under the two N levels were detected in the RM135–RM168 interval on chromosome 3. QTLs for number of spikelets per panicle and number of filled grains per panicle under the two N levels, as well as number of panicles per plant and grain density per panicle, under the low N level, were detected in the RM5556–RM310 interval on chromosome. Also, Zhang *et al*. (2015) identified major QTL locus on chromosome 12, *Tolerance of Nitrogen Deficiency 1* (TOND1) that confers tolerance to N deficiency in the indica cultivar Tequing. They elucidated that overexpression of TOND1 increased the tolerance to the N deficiency in the TOND-1 deficient rice cultivars. The identification of TOND1 provides a molecular basis for breeding rice varieties with improved grain yield despite decreased input of N fertilizers. Recently, in rice, genetic studies have identified several quantitative trait loci (QTLs) including DEP1 (DENSE AND ERECT PANICLE 1) and NRT1.1B (nitrate-transporter gene), associated with NUE through linkage analysis (Sun *et al*., 2014; Hu *et al*., 2015)

At present, there were a few studies for QTL researches of Nitrogen content (NC) in rice, and the previous researches mainly focused on the NC of flag leaf in rice. Ishimaru *et al.* (2001) reported that there was one QTL on Chromosome 2, controlling NC of flag leaf in rice. Yang *et al.* (2005) chose IR24 (*indica*) and Asominori (*japonica*) and their chromosome segment substitution lines (CSSLs) as the material to detect NC of flag leaf at five different growth stages and detected seven QTLs, which were located on chromosome 2, 3, 8 and 11, respectively. However, there was no report available about QTL analysis of NC of plant shoot in rice. To investigate the genetic mechanism of N absorption and utilization efficiency and to improve N utilization efficiency in rice a QTL analysis was done by Hu *et al.* (2012). The main-effect quantitative trait loci (QTLs), epistatic QTLs and QTL-by-environment interactions (QEs) for nitrogen content (NC) of plant shoot at heading and mature stages in two N conditions were identified using a double haploid population. A total of three NC QTLs were detected on chromosomes 5, 6 and 8 in two N conditions. Two pairs of digenic

epistatic loci were associated with NC. The QTL qNc8-4 was detected in both high nitrogen (HN) and low nitrogen (LN) conditions. The analysis revealed that the additive effect of qNc8-4 came from TN1. Then the qNc8-4 was considered as the main effect loci affecting NC of rice shoot. We suggest that QTL markers of qNc8-4, RM4085 and RM1111, expressing in LN conditions, may be used to improve the N absorption and utilization efficiency

Root system development is an important target for improving yield in cereal crops. Active root systems that can take up nutrients more efficiently are essential for enhancing grain yield. To understand genetic adaptation of rice to environmental nitrogen status QTLs for root system development have been identified. In Arabidopsis, all QTL for root length were detected at different chromosomal regions depending on the nitrogen source, *i.e.*, NH_4^+,NO_3^- , NH_4 NO_3 (Rauh *et al.*, 2002). It has been reported that reductions in nutrient concentration are generally observed in hydroponic nutrient solutions, and unstable nutrient concentrations can affect gene expression or accumulation of gene products associated with root elongation. In maize, QTLs for axial root length were not detected only under low NO_3^- concentrations but also under high NO_3^- concentrations (Liu *et al.*, 2008). Thus, regulatory genes associated with root elongation are likely to be different in rice depending on the nitrogen concentration. Despite the suggestion that different genetic controls of root elongation may be related to nitrogen source or nitrogen concentration, QTL for rice seedling root length have been identified only under conditions where plants were supplied with a combination of the NH_4^+-form and the NO_3^--form at a single nitrogen concentration (Xu *et al.*, 2004).

Obara *et al*. (2010) made an attempt to identify QTL for root system development by measuring root length of rice seedlings grown in hydroponic culture. NH_4^+ was used as a single reliable N source. Thirty-eight chromosome segment substitution lines derived from cross between Koshihikari and Kasalath were used to detect QTL for seminal root length of seedlings. Eight chromosomal regions were found to be involved in root elongation. Among them, the ‘Kasalath’ allele QTL, *qRL6.1,* localized on long-arm of chromosome 6 greatly promoted root elongation under all NH_4^+ concentrations. Moreover, Obara *et al*. (2015)

mapped and verified the quantitative trait locus/loci for root elongation in response to exogenous NH_4^+ concentration. Five quantitative trait locus/loci were detected: two on chromosome 1 and three singles on chromosomes 2, 3 and 6. Comparative mapping with genes for NH_4^+ uptake and nitrogen metabolism showed the apparent location of the aspartate amino transferase gene (OsAAT2) within the candidate region of *qRL1.1*. Together, these results suggested that *qRL1.1* is an adaptive quantitative trait locus/loci for rice root elongation in response to sufficient supply of external NH_4^+ and OsAAT2 may be a candidate gene responsible for *qRL1.1*. .Thus, regulatory genes associated with plant growth are likely to be different between the two forms of nitrogen in rice.

CHAPTER-III
MATERIALS AND METHODS

This chapter provides precise and necessary details of experiment conducted to substantiate objectives marking the balance between brevity and completeness. The present study entitled "Mapping QTLs for NH_4^+ and NO_3^- use efficiency under water stress and non-stress conditions and expression analysis of Glutamine Synthetase and Nitrate Reductase in rice (*Oryza sativa L.*)" was accomplished with the aim of understanding mechanisms associated with NUE and the nitrogen uptake and assimilatory enzymes under different forms of Nitrogen *viz*., NH_4^+ and NO_3^- . The material used, techniques adopted and tools that have helped in interpreting the data and concluding the final results during the course of investigation are briefly presented in this chapter.

3.1 Experimental site

Phenotypic data was generated on Vertisol of Research cum Instructional farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur during wet season 2014, 2015 and summer 2014-15. 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 trait *i.e.* NUE, study NH_4^+ and NO_3^- dynamics in soil and carry out enzyme assay and expression analysis of key known genes.

3.2 Geographical situation

The Research Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur is situated on National Highway no. 53 in eastern part of Raipur city and situated in mid-eastern part of Chhattisgarh state and lies at 21^0 16' N latitude and 81^o 36' E longitudes with an altitude of 298.56 meter above the mean sea level**.**

3.3 Climatic and weather conditions

The region comes under dry and sub-humid climate. Weekly rainfall pattern, maximum and minimum temperature for the wet cropping season 2014, 15

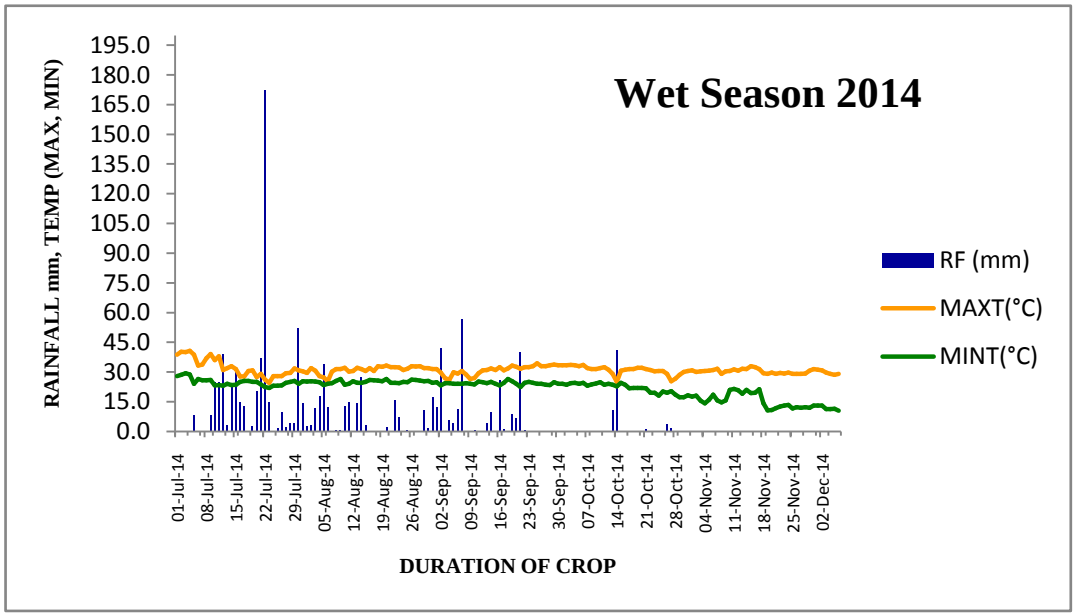
and summer 2015 along with soil moisture content (gravimetric method) at the depth of 0-15 cm, were recorded at regular intervals throughout the growing season. The total rainfall during crop cycle wet season 2014, 15 was 1101.4 and 980.6 mm and summer 2015 was 415.3 mm as shown in fig.3.1.

3.4 Physcio-chemical characteristics of soil of the experimental field

The experimental soil (Vertisol) is fine montmorillonite, hyperthermic, udic chromustert, locally called as Kanhar and is identified as Arang II series. It is usually deep, heavy, clayey, dark brown to black in colour and neutral. Soil from experimental fields was analyzed for its initial characteristics and some important physico-chemical properties of the soil are given in table 3.1.

Table 3.1: Physico-chemical properties of the soil from the experimental site

Physio-chemical properties	Values			
	2014		2015	
	I	RF	I	RF
➢ pH(1:2.5)	7.72	7.1	7.3	7.1
➢ EC(dsm^{-1})	0.20	0.18	0.21	0.16
➢ Organic carbon (%)	0.62	0.26	0.59	0.34
➢ N (kg/ha)	211.8	206.9	210.5	184.9
➢ P (kg/ha)	16.00	13.23	18.73	14.2
➢ K (kg/ha)	422	310	516	342
➢ Ammonical N (mg/kg)	8.87	8.80	9.01	8.26
➢ Nitrate N (mg/kg)	9.93	9.12	9.66	8.55



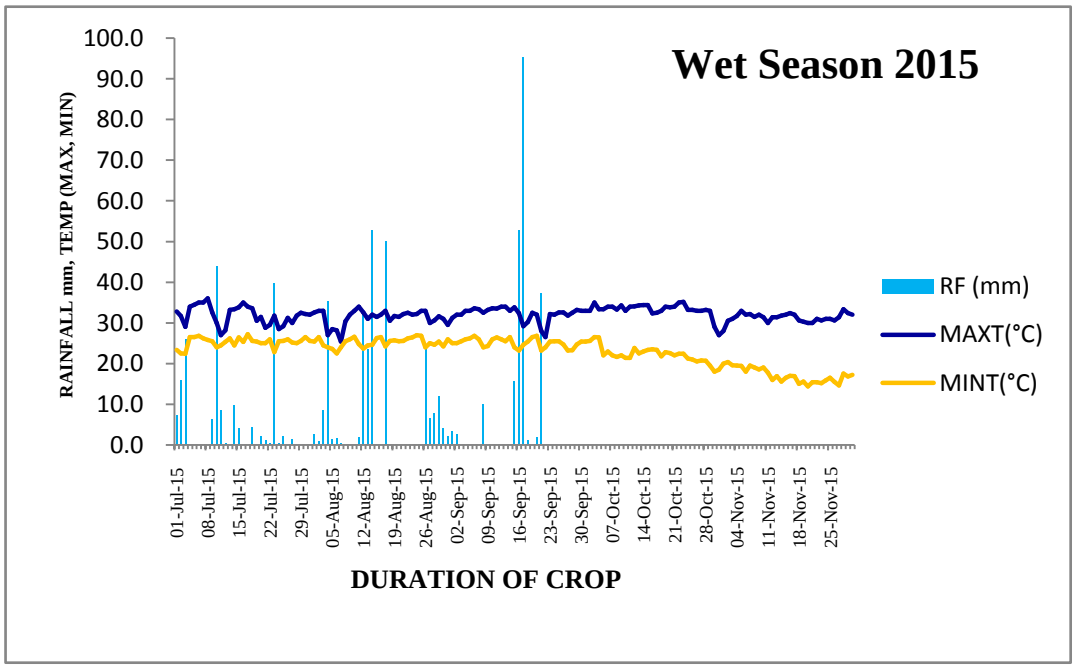


Fig. 3.1: Weekly meteorological data depicting maximum, minimum temperature (horizontal bars) and rainfall pattern (vertical bars) during crop season kharif 2014 and 2015.

3.5 Materials

The material in present study includes two parent viz., Danteshwari and Dagad deshi and their recombinant inbred lines (RILs) population. A study was conducted with 122 RIL lines along with parents during wet and dry growing period of 2014 and 2015 under two forms of N *viz*., NH_4^+ and NO_3^- and different soil moisture regimes *viz*., Irrigated, Rainfed and Terminal stage drought (TSD). Frequency distribution of leaf colour was recorded to categorize RILs into distinct classe's *viz.,* dark green, green, light green, yellow. Leaf colour accompanied by evaluation of NUE, root traits, yield and yield related parameters led us to the subsequent selection of 32 and 10 lines for further study.

3.5.1 Characteristic features of parent

The characteristic features of parents included in present study are presented in table 3.2.

Table 3.2: Characteristic features of parent

S. No.	Parent	Pedigree	Reaction to water stress under field	Salient Features
1	Danteshwari	Shamridhi × IR 8608-298	Moderately susceptible	High yielding, Dwarf, Early and high tillering, Resistant to gall midge, Early maturity 105 days, Long slender grain
2	Dagad deshi	Land race	Tolerance	Strong culm, Tall, Shy tillering, Broad leaves, Bold seeded, Early maturity 100 days

The mapping population of recombinant inbred lines (RILs) was developed by using modified single seed descent method (SSD) to F12, F13 generation as shown in fig. 3.1.

3.6 Experimental details

The mapping population was evaluated under field conditions and soil filled glass rhizotrons. The treatments employed for all sets of environments are depicted in table 3.3.

Table 3.3: Treatments for studying NH_4^+ and NO_3^- use efficiency

Levels of first factor	**Fertilizer**	**Treatment**
N1	Ammonium Sulphate	NH_4^+-N
N2	Calcium Nitrate	NO_3^--N
N3	Control	N^0-N
Levels of second factor	**Environment/ field conditions**	**Soil moisture regime**
E1	Irrigated/Anaerobic	Non-stress
E2	Rainfed/Aerobic	Stress
E3	(TSD) Terminal stage drought	High level of stress

Nitrogen (80 kg ha^{-1}), phosphorus (60 kg ha^{-1}) and potassium (40 kg ha^{-1}) was applied in the form of ammonium sulphate/ calcium nitrate, single super phosphate and murate of potash, respectively under all environments.

3.7 Evaluation of mapping population under different levels of Nitrogen and soil moisture regime

3.7.1 Field studies

The RIL lines derived from a cross between Danteshwari × Dagad deshi were evaluated in the field during wet season 2014, 2015 and summer 2015-16 at research cum instructional farm of College of Agriculture, IGKV, Raipur. The rainfed and TSD fields selected for the study were upland in topology with good drainage and percolation rate. In case of terminal drought trials to reduce the chance of rainfall interfering with drought development in the wet season, trials were planted later and water was drained from the field so that the crop has a better chance of being exposed to drought. Good bunds were a prerequisite for this experiment to limit seepage and treatment flow. The entire experiment was laid out

in factorial randomized block design with two replication of different forms of nitrogen under varied sets of conditions. Few important details and field view of experiment is presented in table 3.4 and fig 3.2.

Table 3.4: General information about different environments

Conditions	Date of sowing	Date of Trans-planting	Spacing (cm)		No. of row/ line	Length of row (m)	Number of replication
			Row to row	Plant to plant			
Wet season -2014							
I T	18 June	12 July	15	15	2	2.10	2
RF Ds	26 June	-	15	15	2	2.00	2
Summer season 2014-15							
IT	19 Jan	3 Feb	15	15	2	2.40	2
IDs	24 Jan	-	15	15	2	2.40	2
Wet season -15							
I T	26 June	11 July	15	15	4	2.40	2
RF Ds	30 June	-	15	15	2	3.00	2
TSD T	18 July	18 Aug	20	20	4	2.4	2

Where, I= Irrigated, RF= Rainfed, TSD= Terminal Stage Drought, Ds=Direct seeded, T= Transplanted

3.7.1.1 Cultural practices

3.7.1.1.1 Raising the seedling

Well pulverized raised nursery beds were prepared. The size of each nursery beds were 1 x 25 cm drainage channel of 30 cm width was provided between the beds. The dose of fertilizers was @ 80 kg N_2, 60 kg P_2O_5 and 40 kg K_2O ha^{-1} in the form of urea, SSP and MOP. The seeds were sown in the raised nursery bed by hand drilling methods in rows. Light and frequent irrigation were given until the seedlings were transplanted.

3.7.1.1.2 Field preparation

The final preparation of the field was done by two criss-cross tractor ploughing followed by harrowing. The soil surface was labelled and the field was divided for the respective treatments and replication.

3.7.1.1.3 Bund preparation and management

To limit seepage losses and movement of fertilizer, a well compacted bund was prepared and any cracks or rat holes was plastered with mud before application of treatments. Bunds were high enough to avoid over bund flow during heavy rainfall**.**

3.7.1.1.4 Fertilizer application

The fertilizers were applied as per the recommended package of practice. The ratio of 80:60:40 kg ha^{-1} N: P: K was employed in the form of ammonium sulphate/ calcium nitrate, single super phosphate and murate of potash, respectively. Nitrogen was applied in 3 splits, viz. 50% of the total N as basal dose, 25% at panicle initiation and the remaining 25% at flowering. The whole amount of phosphorus and potash was applied as basal during transplanting.

3.7.1.1.5 Transplanting

Twenty three days old seedlings and single seedling per hill was transplanted in the field. Flexible rope marked at specific interval with the help of coloured cloth strips for maintaining the distance between plant and rows.

3.7.1.1.6 Weed management

The weeds were removed from the plot manually as per requirement; four hand weeding was done at 15 days interval.

3.7.1.1.7 Water management

After transplanting the soil was kept saturated until seedlings got established in transplanted field. After establishment of seedling 5±2cm standing water was maintained throughout the growing period. In rainfed condition irrigation was not provided and standing water was drained in TSD field after 50% flowering.

3.7.1.1.8 Plant protection

Plant protection measures were adopted when required during the crop growth period.

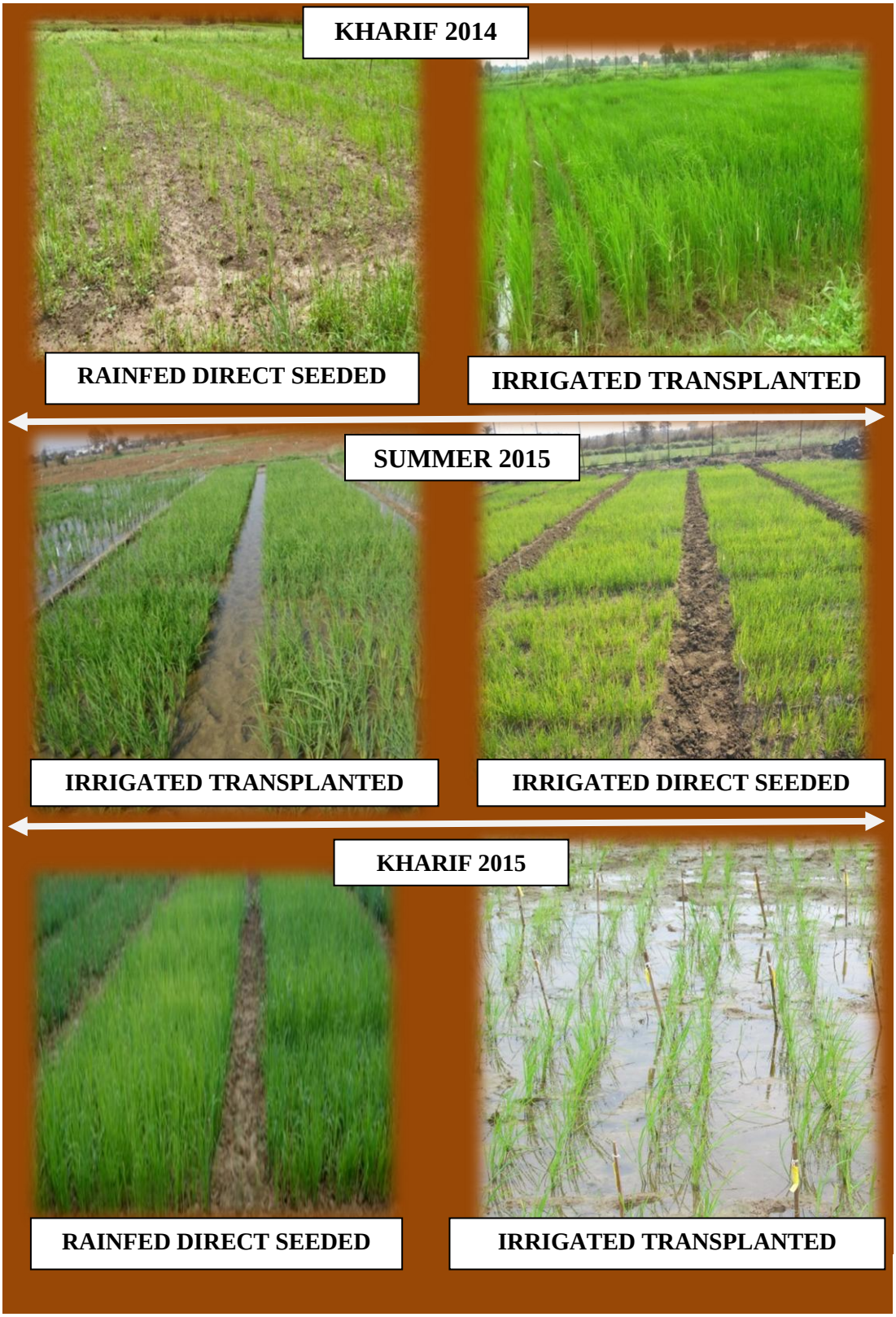


Fig 3.2: A view of experimental field

3.7.1.2 Observations recorded under field conditions

To assess the effect of treatments on growth and development of crop plants, observations were recorded periodically at all the phonological stages of crop. In order to investigate population with absolute accuracy five representative samples were selected. Standard procedures as mentioned in IRRI manual for standard evaluation system (SES), physiological studies and root drought studies in rice were amalgamated for recording the precise data on various growth, NUE, root, yield and yield components.

3.7.1.2.1 Morphological and growth parameters

- **Seedling height (cm)**

Seedling height was measured in centimetre from soil surface to tip of the upper leaf at vegetative stage.

- **Total number of tillers (tillers /m^2)**

Total number of tillers present in each line was counted and later converted to tillers/ m^2 for further analysis.

- **Days to 50 % flowering**

Days from sowing to flowering period of half of the plants were recorded on plot basis by visual means.

- **Plant height (cm)**

Plant height was measured in centimetre (cm) from soil surface to tip of the tallest panicle at maturity.

- **Panicle length (cm)**

Panicle length of 5 randomly selected plants was measured in centimetres from base to tip of panicle and averaged it.

- **Flag leaf length (cm)**

Flag leaf length of 5 randomly selected plants was recorded by measuring the length (cm) from node to tip of flag leaf and averaged it.

- **Flag leaf width (cm)**

Flag leaf width (cm) was recorded by measuring the leaf width at the central part of the leaf.

- **Flag leaf length: width ratio**

It was worked out by dividing flag leaf length to width.

- **Flag leaf area**

The upper most fully expanded leaf of the mother tiller was selected for the estimation of flag leaf area at flowering stage. The maximum length and maximum width of flag leaf were recorded at flowering stage and a factor of 0.75 was used to calculate the flag leaf area. It was expressed as cm^2.

Flag leaf area = Length x Width x k (Factor 0.75)

- **Penultimate leaf length (cm)**

Second leaf (after flag leaf) length of 5 randomly selected plants were recorded by measuring the length (cm) from nod to tip of second leaf and averaged it.

- **Penultimate width (cm)**

Second leaf (after flag leaf) length of 5 randomly selected plants were recorded by measuring the length (cm) from nod to tip of second leaf and averaged it.

- **Penultimate leaf length: width ratio**

It was worked out by dividing Penultimate leaf length to width.

- **Biological yield (g/m^2)**

The biological yield was recorded as actual weight in gram of total biomass of shoot portion per plot and later converted to g/m^2 for further analysis.

- **Grain yield** (g/m^2)

The actual yield of grain in gram per plot was recorded and later converted to g/ m^2 for further analysis.

- **Grain yield per plant (g/m^2)**

The weight of grains (filled) of five representative plants from each line was recorded.

- **Straw yield (g/m^2)**

The actual yield of straw was recorded and later converted to g/m^2 for further analysis. It was also worked out by subtracting biological yield to grain yield.

- **Harvest index (%)**

Harvest index was worked out by using the formula as given below:

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \text{ X } 100$$

- **Number of filled spikelet's per panicle**

It is the number of filled grains in single panicle. Five panicles were used than averaged it.

- **Number of unfilled spikelet's per panicle**

It is the number of chaffy grains in single panicle. Five panicles were used than averaged it.

- **Total number of spikelet's per panicle**

It is the sum of chaffy and filled grains.

- **Spikelet fertility (%)**

Spikelet fertility was worked out by using the formula as given below:

$$\text{Spikelet fertility} = \frac{\text{Total number of filled spikelets}}{\text{Total number of spikelets}} \text{ X } 100$$

- **Spikelet sterility (%)**

Spikelet sterility was worked out by using the formula as given below:

$$\text{Spikelet fertility} = \frac{\text{Total number of filled spikelets}}{\text{Total number of spikelets}} \text{ X } 100$$

- **Seed length (mm)**

Seed length was measured by arranging 10 seeds in length wise order, after that with the help of a measuring scale it was recorded in millimetre (mm) and average length of single seed was calculated as

Seed length = Total length of 10 seeds / 10

- **Seed breadth (mm)**

Seed breadth was measured in millimetre as the distance across the fertile lemma and the palea at the widest point of 10 seeds, and average width of single seed was calculated as

Seed width = Total width of 10 seeds / 10

- **`Seed L: B ratio**

L: B ratio was obtained by dividing length of seed to breadth of seed.

- **Test weight (100 seeds weight) (g)**

Test weight was recorded for 100 seeds of each line in grams.

3.7.1.2.2 Physiological and biochemical parameter

The observation of physiological trait under study was recorded between 11:00 AM to 14:30 PM in the bright sunny day, since the atmospheric condition during this period was relatively stable.

- **Leaf rolling**

Leaf rolling was scored visually with scale from "0" to "9" at afternoon when the difference among the lines becomes most obvious, following Standard Evaluation System for Rice, IRRI. A score of "0" indicated no rolling and the score "9" indicated full rolling.

- **LCC values**

The colour of the five representative leafs were measured by holding the leaf colour chart (LCC) vertically and placing the middle part of leaf in front of colour strip for comparison, as prescribed by IRRI, Philippines in 1996. Readings were taken during seedling and flowering stages and by in vitro procedure using acetone as extraction solvent.

- **Chlorophyll content**

Leaf chlorophyll content was measured by *in vivo* procedure using Soil Plant Analysis Diagnostic Meter (SPAD-502, 1989 Minolta Co. Ltd.)

***In vivo* assay**

SPAD-502 was used to measure chlorophyll content of leaves in SPAD units. Leaf chlorophyll content was measured by light absorbance in the range of red and infrared with the chlorophyll meter in the middle region of fully open flag leaf and penultimate leaf of five representative plants. Readings were measured during seedling and flowering stages. Mean SPAD reading was recorded. SPAD reading is equivalent to chlorophyll content in g/cm^2.

- ***In vitro* assay**

To obtain relationship between SPAD value and chlorophyll content of leaves, leaves were harvested just after taking the SPAD value and were stored at -20^0 C for further in vitro analysis. All steps were accomplished under dim light to prevent any degradation of photosynthetic pigments. Leaf samples was segmented, 20mg sample accurately weighted and then pulverize in a porcelain mortar and pestle containing 20ml 80% acetone. Leaf homogenate obtained was filtered through filter paper and extract obtained was used for chlorophyll measurement by a spectrophotometer following equation published by D.I. Arnon (1949).

Calculations: Use Arnon's equation (below) to convert absorbance measurements to mg Chl g^{-1} leaf tissue

Chl a (mg g-1) = [(12.7 × A663) - (2.6 × A645)] × ml acetone / mg leaf tissue

Chl b (mg g-1) = [(22.9 × A645) - (4.68 × A663)] × ml acetone / mg leaf tissue

Total Chl = Chl a + Chl b.

Where, A663, A645 = Absorbance at 663nm and 645nm, respectively

3.7.2 Assessing effect of NH_4^+-N and NO_3^--N treatment on root growth dynamics under soil-filled glass rhizotrons

To assess the degree of variation for root traits under different forms of Nitrogen, a set of selected lines were grown in thin rhizotrons with glass sides that are filled with soil. Dynamics of shoot and root characteristics were measured after 45 days. To obtain information on fine root structure, instead of drying, the roots

were preserved in 25-50% ethanol before a sample is scanned for analysis using WinRhizo software.

3.7.2.1 Materials used

- Sheets of 4 mm thick clear glass cut to 450 × 300 mm.
- Soil sieved using a coarse sieve (approximately 5 mm mesh) to remove stones and large clumps.
- Supplies including duct tape, two straight 15 mm thick and 400 mm long wood, 15 mm thick plastic ring, and chemicals for nutrient solution.

Procedure

- Two clean glass sheets were taken. One was placed on a work surface with two of the four edges slightly overhanging.
- Two thick woods of 15mm lengths were placed on top of the first sheet, a 15 mm thick plastic ring was placed at the top and the bottom of the glass as spacers and then the second sheet of glass was placed over the top.
- Brown duct tapes were used to join the two sheets of glass together at the overhanging edges (Fig 3.3). The sheets were turned so that the remaining long edge was overhanging, and that was then sealed with duct tape. Three of the four sides were therefore completely sealed with duct tape.
- The empty rhizotrons was set vertical, a single strip of duct tape was wound right around the rhizotrons at the top and bottom, and the two lengths of wood were removed. The two plastic ring prevented glass from sticking together and empty rhizotrons was stacked.
- One at the top ring was removed at the time of soil-filling process. The plastic ring at the bottom remained in the rhizotron. The empty rhizotrons was balanced straight upon a soft support *i.e.* expanded polystyrene sheet and sieved soil was then encouraged into the rhizotrons. When the rhizotrons was nearly full, the upper ring was removed and gently tapped on the support. This caused the soil level drop by 10-15 cm due to packing of the soil. The rhizotrons was refilled, gently tapped once more and finally refilled to 5 mm at the top.

- During watering, there is a tendency for the soil to move down. This is minimal if the rhizotron is well packed with soil (not loose) and if water is applied slowly. Practice packing and watering before deciding on the amount of soil to use.
- A small drainage hole was made at each side at the bottom using a sharpened pencil.
- Rhizotrons were placed in stacks of eight and was leaned at an angle of 15^0 to encourage roots to grow on the lower face (Fig 3.3). The exposed face of the first stack was backed with an insulation sheet to reduce heat exchange and prevent light penetration.
- Three seeds were sown in each rhizotron and thinned to one when they have emerged completely.
- Watering is typically done two times a week with 250 ml of nutrient solution for the first three weeks, moving to larger volumes of nutrient and water as plant grows. Weeding was also done on regular intervals. Proper application of nutrient solution was followed up to 45 days.
- Rhizotrons was photographed with a high-resolution (12 mega pixel) digital camera Fig 3.3 on the final day of experiment.
- At the end of the experiment, plants were removed to assess various parameters under study.
- Roots were subjected to washing in an area under continuous supply of water to remove all loose soil.

3.7.2.2 Acquiring washed roots

i) Materials required

Plastic containers, 50 ml tarson tube, metal forceps, sharp cutter, markers for labelling and 25/50% ethanol

ii) Root washing procedure

This can be the most difficult and laborious step in the experiment if plants are grown in a solid medium. Before washing roots, tarson tubes were marked with the sample name. Subsequently, brown tape was removed with a sharp cutter and one plate above another was separated carefully. Roots were washed properly with

immense care to avoid supplementary root damage and losses. Its length was recorded and preserved in eppendorf tubes dipped in 25-50% ethanol solution for further studies. Debris and dead roots were removed from vital roots.

3.7.2.3 Preparing Roots for Scanning

Roots were floated in water in acrylic trays on the scanner. This allows the roots to be arranged to reduce overlap and crossing of roots to provide accurate measurement of length and area. Plastic forceps were used as tools (Fig 3.4). This is a delicate work; good lighting and steady hands are helpful.

3.7.2.4 Root scanning

1. Material required

Plastic forceps, Water, Plexiglas trays (Acrylic trays) for WinRhizoReg 2009 scanner (clean with no scratches), computer, WinRhizoReg 2009 USB key.

2. Root scanning procedure

Roots can be measured automatically by WinRHIZO when they are extracted from the soil and washed of debris and soil particles. Before analysis, the roots must first be digitized with a scanner or camera. WinRHIZO standard systems use a desktop optical scanner as the image acquisition device. For best results, optical scanner machine Epson Perfection V700/ V750, 3.81 Version, WinRhizoReg 2009 well adapted to image acquisition of macroscopic objects like roots was used. Positioning system allows the trays to be consistently placed, thus obviating the need to preview each scan. Optimum scanning resolution depends on the type of samples. Lower resolution can speed up scanning significantly, especially if the samples require the use of large trays. The preferred resolution is generally 600 dpi. Root length analyses are carried out with grayscale images; saving images in grayscale reduces the image file size substantially. WinRHIZO provides with an overlap compensation algorithm conferring it of greater advantage.

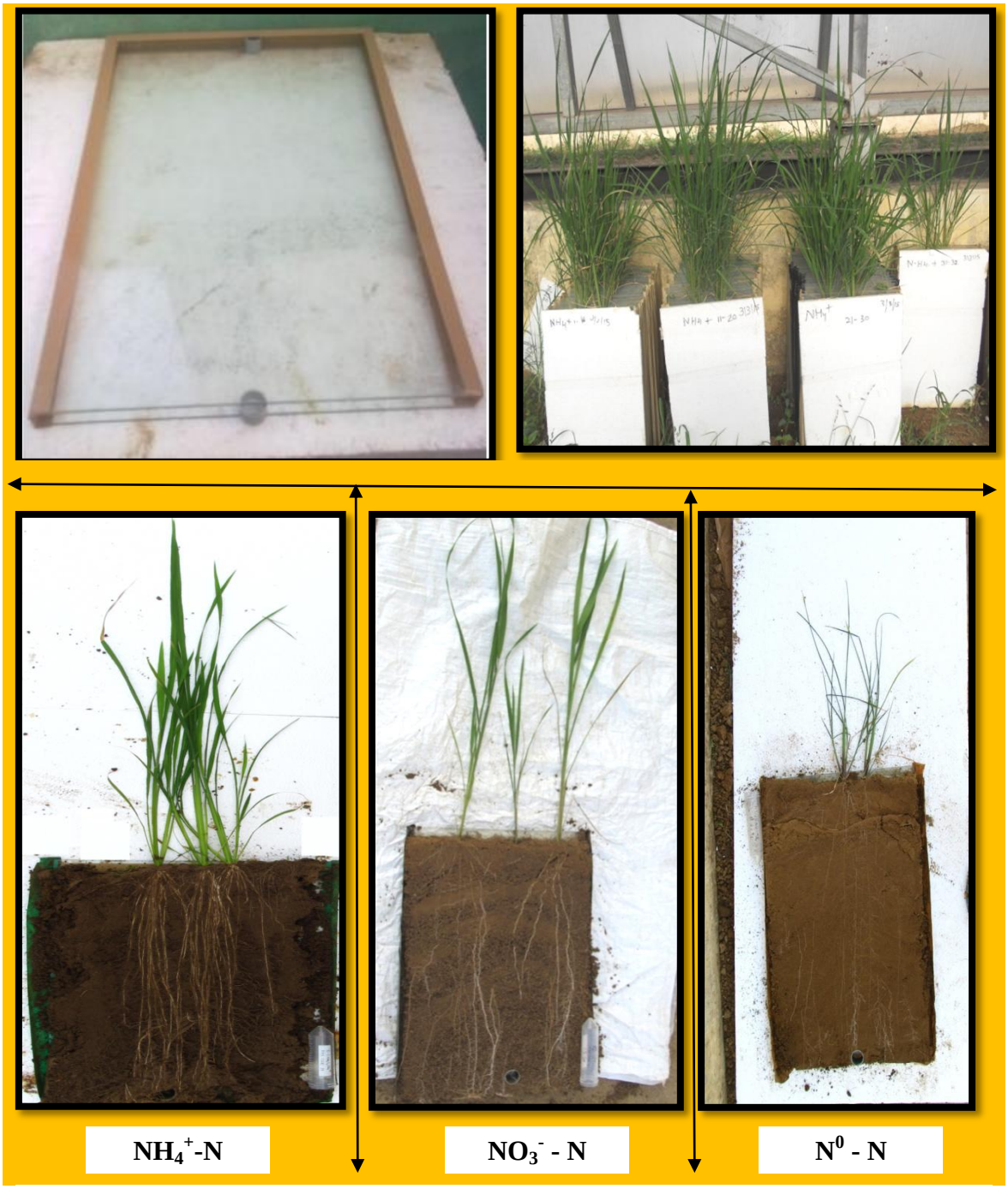


Fig 3.3: Effect of NH_4^+-N and NO_3^--N treatment on root development under soil-filled glass rhizotrons

3.7.2.5 Fixed Threshold Parameters

Analysis results can be sensitive to the threshold parameters used. WinRhizo can automatically set these, but can be manually reoriented.

3.7.2.6 Analyzing Scanned Images

To analyze the image, region(s) of interest was selected and subsequently analyzed. When scanned images are analyzed, the software uses threshold values to determine what is root and what is not root (each pixel is classified as either root or not root based on its grayscale value; this is why shadows in images are problematic). Portions of the image were excluded from analysis that were not necessary, and basic editing tools provides editing platforms if minor image editing is required.

3.7.2.7 Saving measured data

The last step of the analysis is data saving. WinRHIZO knows when data are ready to be saved and does this automatically. Data files are in ASCII (text) format easily readable by many programs including spreadsheet style like *Excel*. Images and their analyses are saved to files for later validations, reanalyzes or for visualization in other software programs.

3.7.2.8 Observations under glass rhizotrons

1. Total shoot length (cm)

For measuring total shoot length, plants in rhizotrons were washed off carefully then the total shoot length was measured from collar region to the tip of longest shoot with the help of a meter scale.

2. Total root length (cm)

For measuring total root length, plants in rhizotrons were washed off carefully then the total shoot length was measured from collar region to the tip of longest shoot with the help of a meter scale.

3. Root volume (cm^3)

Root volume is measured by volumetric flask (700ml). First the volumetric flask bottle was filled with water up to fixed mark on the neck bottle. The lower

meniscus was fixed carefully with the help of micropipette. Thereafter, free water adhering over the roots was whipped out with filter paper and such clean and dry roots are inserted inside the empty volumetric flask. After that measured amount of water was filled in the volumetric flask up to the previous fixed mark on of bottle .The net volume was determined using relation:

$$Vr = Vwp – Vwpr$$

Where,

Vr = Net root volume (cm^3)

Vwp = Volume of water required to fill the empty volumetric flask

Vwpr = Volume of water required to fill the volumetric flask with fresh root

4. Shoot and root fresh weight (g)

Shoot and root fresh weight was measured by removing plants from rhizotron and any loose soil was washed off. Plants were blot gently with soft paper towel to remove any free surface moisture and were weighed immediately in electronic balance.

5. Shoot and root dry weight (g)

Shoot and root dry weight was determined by removing plants from rhizotron and wash off any loose soil. To remove any free surface moisture, plants were blotted immediately and dried in an oven set to low heat (100° F) overnight. Then plants were allowed to cool in a dry environment. Once the plants have cooled, it was immediately weigh on a scale.

6. Average roots diameter (mm)

For measuring average root diameter software WinRhizo Reg 2009 with a root scanner machine Epson Perfection V700/V750, 3.81 Version, was used for root scanning, which allows the roots to be scanned from above and below both side. Sample was scanned and scanned file was saved with exact name labelled at the time of sampling. From the saved excel file average root diameter was noted in millimetre (mm).

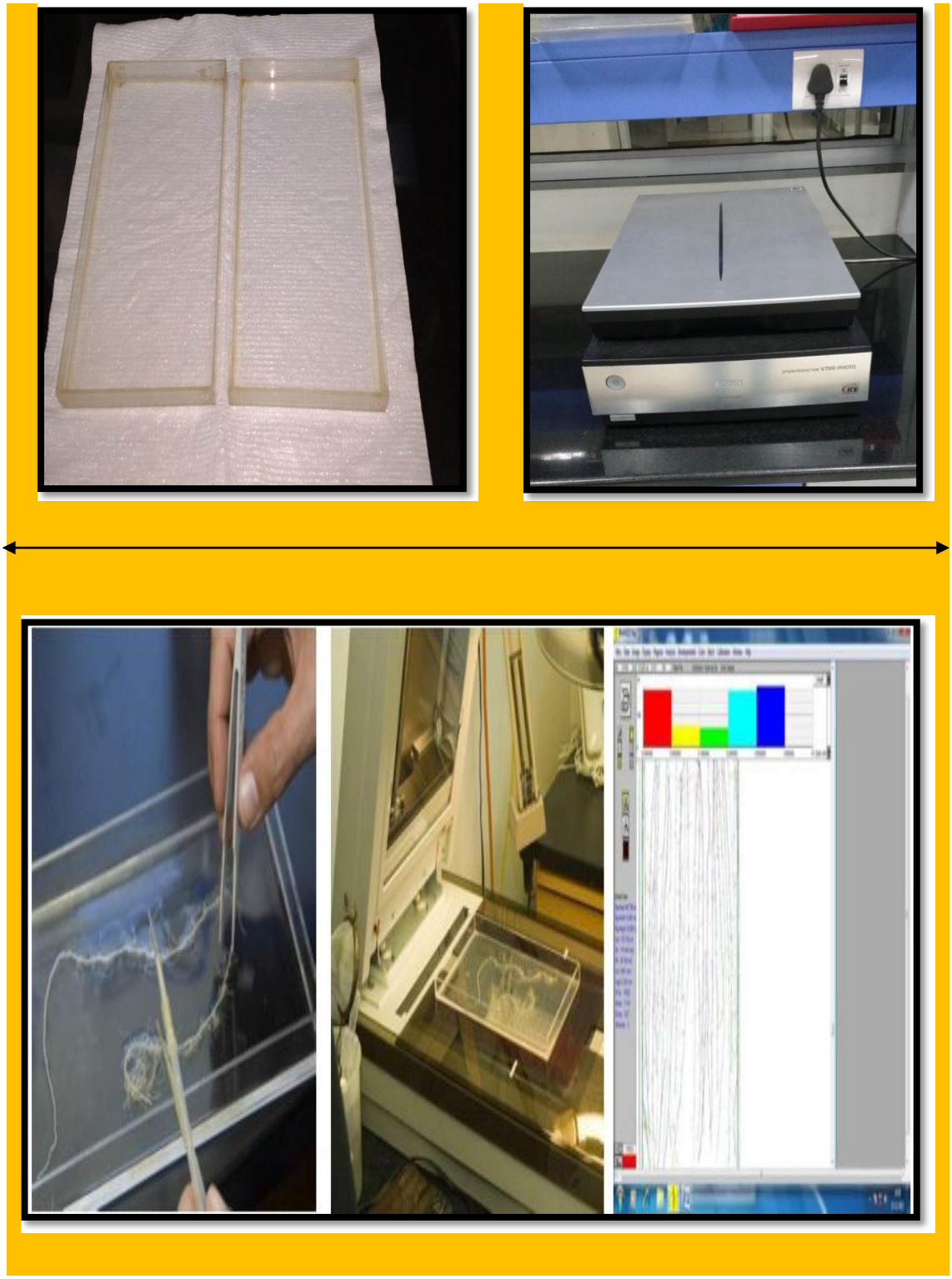


Fig. 3.4: Root scanning through WinRhizo software

3.8 Evaluating NH_4^+-N and NO_3^--N dynamics in soil

On-farm experiment conducted during wet season of 2014, 2015 was subjected to analysis of soil nitrogen fraction and available nitrogen in each treatment under all environments. Steps followed in soil testing are enumerated below.

3.8.1 Sampling time

Soil was sampled after harvest of previous crop, at the time of field preparation of respective trials, then before fertilization and after fertilization with onset of monsoon. During crop growing period soil was sampled at 4 days interval after treatment application and then after panicle initiation it was procured at 7 days interval.

3.8.2 Collection of a representative soil samples

3.8.2.1 Materials needed

Polythene bags, marker, instructions on soil sampling, ice box, soil sample probe or auger, fawda or spade

3.8.2.2 Procedure

The most critical step in soil testing is collecting a soil sample. Soil sampling was well planned and preformed. Samples were collected from well pulverized and homogenized soil. Sampling depth opted was from 0-15 cm. Coarse fragments and crop residues were discarded. From standing crop samples were drawn in between the rows. For sampling of soft and moist soil, a spade or khurpi was used as appropriate tool. Composite sampling being the most common and economical method of sampling was employed in present study. Sub-samples were collected from randomly selected locations from each treatment & replication, and were composited separately for analysis. Not less than 5 sub-samples were taken from the sampled area. Further, sub-samples were placed in a clean plastic bucket and mixed thoroughly. The desired sample amount was then removed from the bucket and the remainder discarded. Sample obtained finally was packed in polythene bags and labelled with marker according to respective treatment and

replication. To avoid any transformation of nitrogen to different forms, samples were immediately sent to laboratory for soil testing.

3.8.3 Laboratory analyses of the soil sample

Nitrogen fractions changes very quickly and immediate test is required to obtain accurate determinations of soil-available levels. Soil analysis must be done precisely after heavy rains or after a prolonged period of water-logging to accurately measure nitrogen fractions. The samples collected were stored in freezer for further analysis. Initial sample of soil, were kept at field capacity in laboratory for 48 hours before analysis for different nitrogen fractions.

3.8.4 Analysis of factor affecting NO_3^- -N and NH_4^+ -N fraction in soil

Methods employed for soil sample analysis is depicted in table 3.5.

3.8.4.1 Soil pH

The salt concentration of a soil may vary with the season or with fertilizer/ treatment application**.** Fluctuations in soil salt levels may result in change in pH. In 10 g of soil 25ml distilled water was added. Sample was shaken and after 30 min glass electrode was dipped in soil suspension and pH of the soil was noted from the scale. Soil pH was determined by glass electrode pH meter method (Piper, 1967) in soil water suspensions as described by Jackson, (1973).

3.8.4.2 Electrical Conductivity (EC)

Electrical conductivity (EC) of a soil extract is used to estimate the level of soluble salts. The soil sample used for pH determination was allowed to settle down for 24 hours. The electrical conductivity of the supernatant liquid was determined by conductivity meter as described by Black *et al*. (1965).

3.8.4.3 Organic Carbon

Organic carbon was determined by Walkley and Black’s rapid titration method (1934) as described by Piper (1966).

3.8.4.4 Available N

The available nitrogen in soil was determined by alkaline potassium permanganate method as described by Subbiah and Asija (1965).

3.8.4.5 Total nitrogen

Determination of total nitrogen content in soil sample was done by taking 1.0 g uniform prepared sample in digestion tube along with 1 g salt mixture i.e., K_2SO_4 and $CuSO_4$.5H2O in the ratio of 10:1 and 7 ml. of concentrated H_2SO_4 was added and material was digested at 350^0C in digestion block till the material becomes colourless. Then the nitrogen in digested material was distilled by automatic KEL plus system and titrated with 0.02N Sulphamic acid.

Table 3.5: Methods employed for the analysis of soil

Physico-chemical properties	Method employed	References
pH (1:2.5)	Glass electrode pH meter method	Jackson, (1973)
Electrical conductivity (dS m^{-1})	Soil-bridge Conductivity meter method	Black *et al*.(1965)
Organic carbon (%)	Rapid titration method	Walkley and Black, (1934)
Available Nitrogen (kg ha^{-1})	Alkaline potassium permanganate method	Subbiah and Asija (1956)
Total Nitrogen (kg ha^{-1})	Steam distillation method	Page and Bremme (1965)
Ammonical Nitrogen (kg ha^{-1})	2M KCL extractant Steam distillation method	Keeney and Nelson (1982)
Nitrate Nitrogen (kg ha^{-1})	2M KCL extractant Steam distillation method	Keeney and Nelson (1982)

3.8.5 Nitrogen inorganic fractions

The fractionation of soil nitrogen was carried out by the procedure given by Bremner and Keeney (1965), described by Keeney and Nelson (1982).Steps involved in the process are elaborated as such:

3.8.5.1 Extraction of NO_3^--N and NH_4^--N with 2.0 M KCI

3.8.5.1.1 Material

1. Analytical balance, 250.0 g capacity, resolution ± 0.01 g
2. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (OPM)
3. Polyethylene bottle of 250 ml capacity
4. Filter funnels
5. Whatman® No. 42 filter papers
6. Erlenmeyer flasks, 125 ml.
7. Aluminium tin.

3.8.5.1.2 Procedure

A. Soil moisture determination

1. Clean and dry tin + lid was weigh to 0.01 g (W_1). 30g of representative moist soil was selected and sample was placed in the weighing tin and lid was replaced. Tin and contents was weigh to 0.01g (W_2).
2. Lid was removed and tin was placed with contents in the oven and dry to constant weight between 105 °C and 110 °C
3. Tin with contents was removed from oven and placed as whole in the desiccator to cool.
4. Tin and content was weigh finally

The soil moisture was calculated in percent by formula:

$$\text{SMC}\ (\%) = \frac{\text{W2} - \text{W3}}{\text{W3} - \text{W1}}$$

Where:

SMC = Soil moisture content (%)

W1 = Weight of tin (g)

W2 = Weight of moist soil + tin (g)

W3 = Weight of dried soil + tin (g)

B. Preparation of equilibrium extract

1. **Principle:** Ammonium in held in exchangeable form in soils in the same manner as exchangeable metal cations. Fixed or non exchangeable NH_4^+ can make up a significant portion of soil N; however fixed NH_4^+ is defined as the NH_4^+ in soil that cannot be replaced by the neutral K salt solution. Exchangeable NH_4^+ is extracted by shaking with 2.0 M KCL. Nitrite is water soluble and hence can also be extracted by the 2.0 M KCL.

2. Steps involved:

- 30 g of soil sample was taken in a polyethylene bottle. 150 ml of 2.0 M KCI solution was added (If the sample is limited, it can be reduced to a minimum of 1.0 g and 5 ml 2.0 M KCI to keep 1 : 5 ratio which is mandatory).
- Bottle was capped and kept in mechanical shaker for 30 min.
- Soil-KCl suspension was allowed to settle until the supernatant liquid is clear (usually about 30 minutes). This supernatant is filtered into Erlenmeyer flasks gravimetrically through filter paper fixed in funnels.
- Analyses of NO_3^--N and NH_4^+-N elements was performed on aliquots of this liquid (If the KC1 extract cannot be analyzed within 24 hours after its preparation store the filtrate in a refrigerator until analyses can be performed).

C. Determination of NH_4^+-N and NO_3^--N in 2.0 M KCL extract using KEL plus distillation apparatus

1. Material

- Distillation apparatus consisting of:
- 500 or 800 mL Kjeldahl flasks
- Connecting bulbs
- Vertical condenser
- Hot plates
- 300 mL receiving beakers or Erlenmeyer flasks
- Microburette

2. Procedure

- 10 ml of boric acid-indicator solution was added to 125 ml Erlenmeyer flask marked to indicate a volume of 75 ml and flask was placed under the condenser of the steam-distillation apparatus so that the end of the condenser is in the boric acid.
- An aliquot (usually 20-50 ml) of the soil extract was transferred into a distillation flask and add 0.4 g of MgO was added.
- Commence distillation by placing plug in the steam bypass tube of the distillation apparatus and 75 ml of distillate was collected.
- End of the condenser was rinsed and ammonium-N in the distillate was determined by titration with .005N sulphamic acid using a microburette graduated at 0.02-ml intervals. The colour change at the endpoint is from green or blue to a permanent pink.
- Blank titration value is obtained before running representative samples.
- After removal of NH_4^+-N from the sample as described above. The sample in the distillation flask was treated with 1 ml of (1%) Sulphamic acid solution and the flask was swirled for a few seconds to destroy nitrite (NO_2^-). 0.2-0.4 g of Devardas alloy was added to the distillation flask and the distillation was continued. The amount of NO_3^- -N was determined same as described above for exchangeable ammonical nitrogen omitting the 2 step. Brief view is given in Fig 3.5.

D. Analyses of soil testing results

1. Calculation

$$NH_4^+\text{-N} / NO_3^-\text{-N (ppm)} = \frac{(V\text{-}B') \text{ X } N \text{ X } R \text{ X } 14.01 \text{ X } 1000}{W - \Theta}$$

Where,

V= Volume of sulphamic acid required for titrating sample (ml)

B'= Blank titration value (ml)

N = Normality of sulphamic acid

R = ratio between volume of extract obtained and volume used for titration

W = Weight of oven-dried sample

Θ = Weight of water (g) per 30g oven-dried sample

SOIL SAMPLE S IN ORBITAL SHAKER

FILTERATION OF SOIL SAMPLE

END POINT AFTER TITRATION

Fig 3.5: A view of soil nitrogen estimation procedure

2. Soil test interpretation software

SMART interprets for the entire variety of extraction methods used by laboratories, so it can easily decipher any soil test report. This software takes in to consideration multiple soil parameters such as EC, pH, organic carbon etc. Optional are customized for importing multiple soil test reports. Based on the soil test report, this software is used to design fertilizer program of any crop (Fig. 3.6). In current investigation this software is used for developing precise and clear bar charts depicting the range of various soil parameters under study.

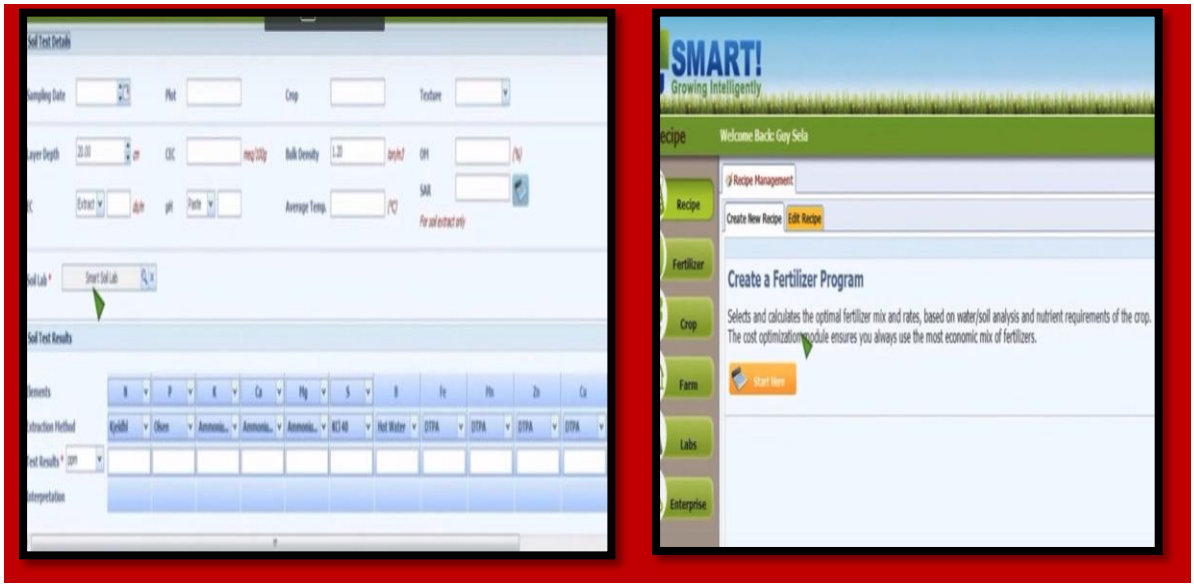


Fig.3.6: SMART software for interpretation of soil test results

3.9 Plant chemical analysis

At maturity, representative samples of grain and straw were obtained from the central net plot area of each plot. The samples were oven dried at 70 ^{0}C to a constant weight. The Steps are enumerated below:

3.9.1 Equipments required

Polyurethane coated hand dehusker

Weighing balance

KEL plus digestion and distillation apparatus

Kjeldahl flasks

Micro Burette

3.9.2 Principle

The nitrogen in any organic material is converted to ammonium sulphate by H_2SO_4 during digestion. This salt, on steam-distillation, liberates ammonia which is collected in boric acid solution and titrated against standard acid. Since 1ml of 0.1 N acid is equivalent to 1.40 mg N, calculation is made to arrive at the nitrogen content of the sample.

3.9.3 Procedure

1. Processing of rice straw and grains

Before analyzing the rice samples for total grain protein the rice grains were subjected to dehusking and oven dry straw samples were grounded using rotor mill and allowed to pass through a 0.5 mm sieve to prepare a sample for digestion.

Dehusking

Around 500 seeds of each sample were hand dehulled using polyurethane coated hand dehusker unit to avoid metal contamination (Fig. 3.3).

1. Estimation of protein

Total protein content of brown rice grains of all samples was estimated by modified micro-Kjeldahl method (Johri *et al*., 2000). The details of the procedure are as under.

i) Digestion process

About 0.5 gm of rice grain was transferred into the digestion tube and 5-7 gm of K_2SO_4 and $CuSO_4$ mixture was added (sample and K_2SO_4 & $CuSO_4.5H_2O$ in the ratio of 1:10). 10 ml of concentrated Sulphuric acid was added and digestion tubes were placed on the digestion block with temperature set at 400 ºC (Fig. 3.7). After 2 to 3 hours when the samples colour turned light green, the digestion tubes were taken out of digestion block. The tubes were allowed to cool at room temperature.

ii) Distillation process

Digested samples were subjected to Pelican make distillation unit (Fig. 3.7) and distillation of samples was carried using 4% Boric acid and 40% Sodium hydroxide. 10 ml of Boric acid was then taken in conical flask, to which 2-4 drops of mixed indicator dye was added. The flask was beneath the condenser with the delivery tip immersed in the solution. The digested samples were transferred to distillation apparatus and 8-10 ml of 40% sodium hydroxide was added to it. Around 20 ml of distillate was collected in a conical flask. A blank was always run

containing the same quantities of the entire reagent but without the sample for every set of nitrogen determination.

iii) Titration process

The distilled samples were titrated against the 0.05 N Sulfamic acid until the first appearance of violate colour as the end point (Fig. 3.7). The titer value was used to calculate percent Nitrogen, which is then used to estimate total protein content by using conversion factor 5.95 (Julliano, 1993).

$$\% \text{ Nitrogen} = \frac{(\text{Vol. of Sulfamic acid} - \text{Vol. of blank}) \times \text{Normality} \times 14 \times 100)}{\text{Sample weight (gm)} \times 1000}$$

$$\text{Protein \%} = \% \text{ N X } 5.95$$

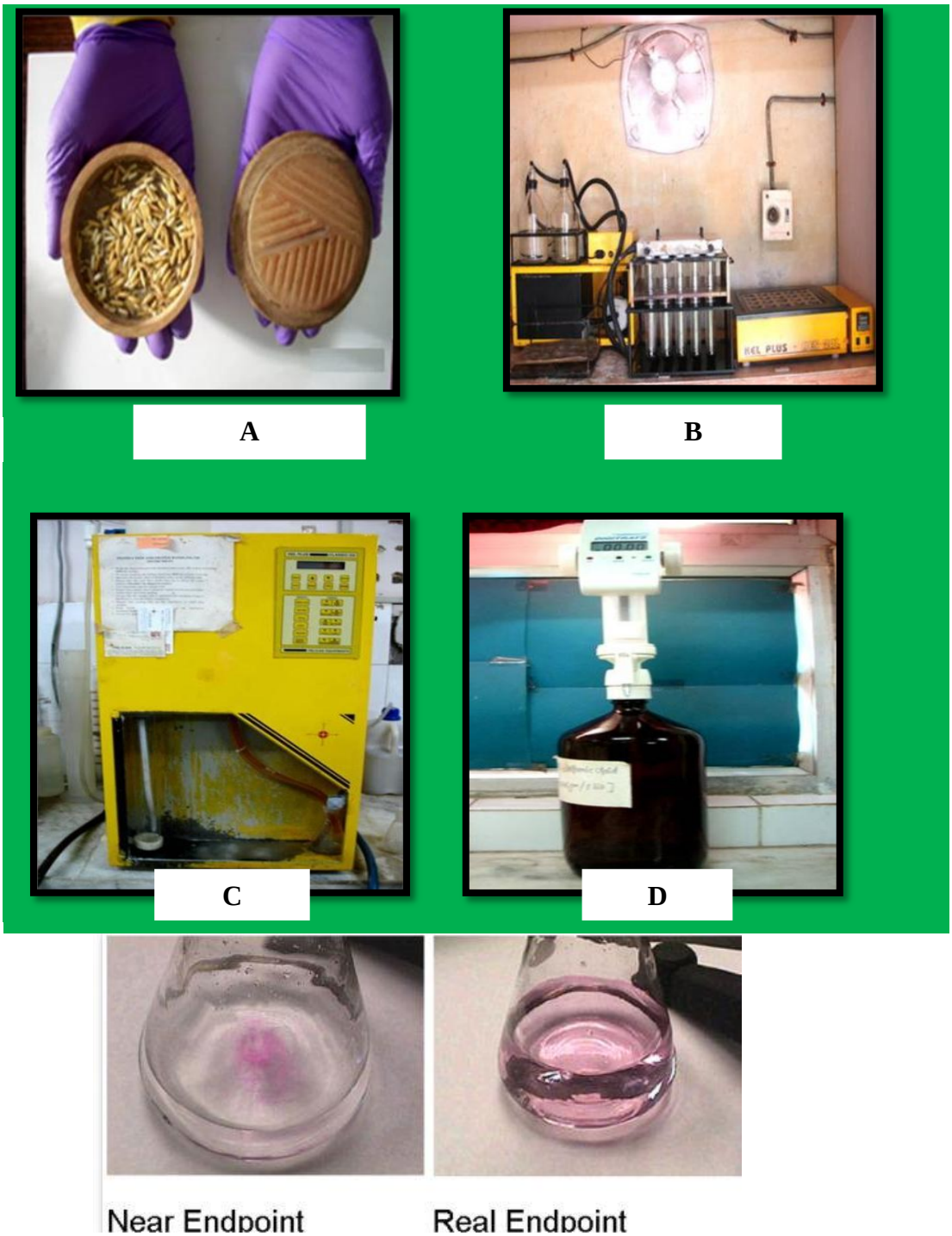
4. Determination of Nitrogen uptake and nitrogen efficiencies

Nitrogen uptake in seed and straw yields were computed by multiplying their respective nutrient contents with yields using of following formula:

Nutrient uptake (kg ha-1) in seed and straw = Seed and straw yield × Nitrogen content

- **Nitrogen use efficiency**: - It was calculated by using the following formula

Trait	Abbreviations	Units	Trait measurements
Grain yield	GY	$g\ m^{-2}$	–
Nitrogen use efficiency	NUE	g/g	Grain yield/total amount of nitrogen supply
Nitrogen uptake	Nup	$g\ m^{-2}$	GY x GNC + SY x SNC
Nitrogen uptake efficiency	NupE	g/g	Total N uptake/total amount of nitrogen supply
Nitrogen utilization efficiency	NutE	g/g	Grain yield/total nitrogen content
Stover yield	SY	$g\ m^{-2}$	–
Harvest index	HI	g/g	GY/(GY + SY)
Grain nitrogen concentration	GNC	%	Kjeldahl method
Stover nitrogen concentration	SNC	%	Kjeldahl method
Nitrogen harvest index	NHI	$g\ m^{-2}/g\ m^{-2}$	GY x GNC /(GY x GNC + SY x SNC)



A: Polyurethane dehusker **B: Digestion unit**

C: Distillation unit **D: Titration unit**

Fig. 3.7: Apparatus required for modified Micro-Kjeldahl method

3.10. Extraction and assay of nitrogen uptake and ammonium assimilatory enzymes

The four enzymes, namely, NR, NiR, GS and GOGAT were assayed in freshly harvested leaf of selected 10 rice genotypes at seedling stage (Fig.3.8). The protein was determined from all of the enzyme extracts. All the enzyme extraction process was performed as mentioned in Sadasivam and Manickam (1996). Each assay experiment was repeated thrice and the mean data was plotted as relative specific activity (%) along with standard errors. Specific activity of an enzyme has been defined as µmol of product formed per mg protein.

3.10.1 Plant material and growth conditions

Seeds of genotypes under study was washed thoroughly, surface sterilized for 10 minutes in 5 % v/v sodium hypochlorite, washed several times with tap water and soaked in distilled water. Imbibed seeds were planted in a wooden tray. The seedlings were watered daily with distilled water for 10-12 days (Fig.3.8).

3.10.2 Treatments

To study the involvement of the NH_4^+-N and NO_3^-N in the regulation of enzymes, 12 days old seedlings were treated with respective calcium nitrate and ammonium sulphate treatment in the recommended ratio as stated earlier along with control without treatment. After 2 days plant tissue was excised, washed, blotted on tissue paper, wrapped in foil, frozen in liquid N_2 and used immediately or stored at 20° C to determine enzyme activities and protein content.

Fig 3.8: Growth conditions of rice genotypes for enzyme assay

3.10.3 Material used

- Weighing balance
- Tarsons (50 ml)
- Epperndorf centrifuge 5417 R
- Borosil black capped glass bottle
- Spectrophotometer (Biomate 5, Thermo Electron Corporation)
- Quartz cuvette
- Mortar pestle

3.10.4 *In vitro* nitrate reductase assay (NR)

1. Principle

Nitrate Reductase (NR) is capable of utilizing the reduced form of pyridine nucleotide, flavins or benzyl viologen as electron donars for reduction of nitrate to nitrite. NADH-dependent nitrate reductase is most prevalent in plants. Hence, NR activity in plants can be measured by following the oxidation of NAD (P) H at 340 nm.

2. Preparation of standard graph

- 0.750 g of sodium nitrite was dissolved in distilled water and diluted to 1000 ml (500 µg nitrite/ml)
- 10 ml of this stock solution was diluted to 100 ml with distilled water (50 µg nitrite/ml)
- Finally 10 ml of this preparation was diluted to 1000 ml with distilled water (0.5 µg nitrite/ml)
- Series of aliquot of 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of nitrite standard (corresponding to 0, 2, 5, 10 and 25 µg of nitrite) was pipette out in volumetric flask and volume make up in each tube was done to 2 ml by adding distill water.
- Reaction was terminated subsequently by rapid addition of 1ml sulphanilamide followed by 1 ml napthyl ethyelenediamine reagent (NED).
- Absorbance was measured at 540 nm by placing cuvette in spectrophotometer (Biomate 5, Thermo Electron Corporation) and a good linear standard graph was obtained for further study (Fig. 3.9).

3. Crude extract preparation

Extraction of NR was done by the method of Hageman and Flesher (1960). Steps involved are:

- A weighted quantity of plant material (root or shoot tissue) was homogenized with mortar pestle in a known volume of medium (6ml for 1g fresh tissue) containing 1mM EDTA, 25mM cysteine and 25 mM potassium phosphate adjusted to final pH of 8.8 with KOH.
- All the contents were transferred to eppendorfs tubes and centrifugated at 15,000 rpm for 15 min, 4^0C to obtain supernatant.
- Supernatant was filtered through 4 layers of cheese cloth and decant through glass wool in black capped glass borosil bottle of 15 ml and used for assays.
- Entire extraction process was accomplished under ice-cold conditions.

4. Procedure for assay

- NR activity was measured by modification of the method described by Evans and Nason (1953). Steps involved are:
- 0.5ml phosphate buffer (pH 7.5) was pipette out in a borosil glass bottle
- 0.2 ml potassium nitrate solution, 0.4 ml NADH solution and .7 ml water was added in bottles
- Reaction was initiated by addition of 0.2ml enzyme extract and control was set up in the same way but with water instead of enzyme extract
- Incubation was done at 30^0C for 15 min
- Reaction terminated by rapid addition of 1 ml sulphanilamide followed by 1 ml NED.
- The pink colour developed was measured at 540nm spectrophotometer
- The amount of nitrite formed was calculated from a standard curve plotted using the A_{540} values obtained from known amounts of nitrite.

5. Calculations

$$\text{Units/ml enzyme} = \frac{(\mu\text{mole Nitrite formed})(\text{df})}{(\text{T})(0.2)}$$

df = Dilution factor

T= Time of assay (in minutes)

0.2= Volume of enzyme (in millilitre) used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

Unit definition:

Activity is expressed as µmole of nitrite produced per min per mg protein.

3.10.5 *In vitro* nitrite reductase assay (NiR)

1. Principle

The disappearance of nitrite is measured in the reaction. Reduced methyl viologen is used as electron donor.

2. Preparation of standard graph

Standard graph prepared for NiR assay is same as NR assay (Fig. 3.9)

3. Crude extract preparation

For nitrite reductase (NiR) enzyme extraction, crude homogenates were prepared according to Gupta and Beevers (1984). Steps involved:

- 1g of root or shoot tissue was pulverized with mortar pestle in a known volume of 0.5 M Tris HCL buffer (10ml for 1g fresh tissue) and adjusted to final pH of 7.5 with NaOH.
- All the contents were transferred to tarson tubes and centrifugated at 15,000 rpm for 15 min, 4^0C to obtain supernatant.
- Supernatant was filtered through 5 layers of cheese cloth and decant through glass wool in black capped glass borosil bottle of 15 ml and used for assays.
- Entire extraction process was accomplished under ice-cold conditions

4. Procedure for assay

Nitrite reductase (NiR) activity was assayed as described by Wray and Fido (1990) by using dithionite reduced methyl viologen as an artificial electron donor**.** Steps involved:

- Assay mixture was prepared by adding 6.25ml of 0.5 M Tris HCL, 2ml of 2.5 mM sodium nitrite, 2ml of 3 mM methyl viologen in a volumetric flask and making up final volume of 20 ml by adding 14.75ml distill water.

- The reaction was started by the addition of sodium dithionite-bicarbonate solution. The blanks contained all the assay components except methyl viologen
- The reactions was carried out at Room Temperature (25° C) and stopped after 10 minutes by the addition of 1.9 ml of reaction stopping (sulphanilamide) and colour developing reagent (NED).
- The reaction was incubated for a further 15 min at RT and the pink colour developed was measured at 540 nm in spectrophotometer (Biomate 5, Thermo Electron Corporation)
- The amount of nitrite formed was calculated from a standard curve plotted using the A_{540} values obtained from known amounts of nitrite.

5. Calculations

$$\text{Units/ml enzyme} = \frac{(\mu\text{mole Nitrite disappeared})(\text{df})}{(\text{T})(0.2)}$$

df = Dilution factor

T= Time of assay (in minutes)

0.2= Volume of enzyme (in milliliter) used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

Unit definition:

Activity is expressed as µmole of nitrite disappeared per min per mg protein

3. 10.6 *In vitro* glutamine synthetase assay (GS)

1. Principle

The activity of enzyme is measured by estimating the production of inorganic phosphate. GS also catalyzes the gamma glutamyl transferase reaction.

2. Preparation of standard graph

- Standard curve was constructed using different aliquot of L-glutamic γ-monohydroxamate to obtain an accurate quantification of GS activity.

- Series of aliquot of 0, 0.2, 0.4, 0.6, 0.8 and 1 ml was pipette out in volumetric flask and volume make up in each tube was done to 3 ml by adding distill water (Fig. 3.9)
- Absorbance was measured at 540nm by placing cuvette in spectrophotometer and a good linear standard graph was obtained for further study.

3. Crude extract preparation

Extracts used of GS was prepared according to Cooper and Beevers (1969). Steps involved:

- Extract was prepared at 0-4^{0}C.
- 1 g of respective tissue was homogenized with mortar in a 4ml medium of 50 mM Tris HCL (pH=7.8) containing 15% v/v glycerol, 14mM ß-mercaptoethanol, 1 mM EDTA and 0.1 % Triton X-100.
- Homogenate was squeezed with 3 layers of cheese cloth and centrifugated at 15,000 rpm for 10 min. Further, supernatant was used as enzyme extract.

4. Procedure for assay

For determination of GS activity, method of Lillo *et al.* (1984) was followed. Steps involved:

- Standard reaction mixture containing L-glutamate (500 mM), hydroxylamine hydrochloride (200mM), magnesium sulphate (300 mM), ATP (100 mM) in Tris-HCl (200 mM) in a total volume of 2 ml was prepared.
- Final pH was maintained 8.0
- 0.1 ml of enzyme extract was added to start the reaction giving assay a total volume of 2ml
- After incubation at 270C for 30 min. reaction was terminated by adding 2.5 % $FeCl_3$
- And 5 % trichloroacetic acid in 1.5 M HCL
- After centrifugation absorbance of supernatant was recorded at 540nm.

5. Calculations

Activity is expressed as µmole of L- glutamate converted to L-glutamine per min per mg protein.

3.10.7 *In vitro* glutamate synthase assay (GOGAT)

1. Principle

Glutamate synthase is assayed spectrophotometrically by recording the rate of oxidation NADPH or NADH, as indicated by a change in absorbance at 340nm following the addition of extract.

2. Preparation of standard graph

- 10 ml of 1 mM NADH was prepared in 5mM Tris HCL (pH= 7.5)
- Series of aliquot of 0, 0.2, 0.4, 0.6, 0.8 and 1 ml was pipette out in volumetric flask and volume make up in each tube was done to 3 ml by adding Tris HCL (pH= 7.5)
- Absorbance was measured at 340nm and standard graph was prepared accordingly (Fig. 3.9)

3. Crude extract preparation

- For crude extract preparation method employed by Singh and Shrivastava (1986) was employed. Steps involved:
- Tissue was extracted in a mortar in a medium containing 100 mM phosphate buffer (pH=7.5), 1mM EDTA, 1mM dierythritol and 1 % PVP. Ratio of tissue and medium was kept 1:5 (w/v).
- The homogenate was centrifuged for 15,000 rpm for 15 min, 4^0C to remove unbroken cells and cell fragments.
- The supernatant was filtered through cheese cloth and clear solution obtained was used for further assay.

4. Procedure for assay

The GOGAT activity was determined by the method Singh and Shrivastava (1986b). Steps involved:

- Assay mixture contained 1.8 ml of 50 mM Tris-HCL buffer (pH=7.6), 1ml of 5mM L-glutamine, 1 ml of 5 mM 2-oxo glutarate, 1 ml of 0.25 mM (NADH) and 0.2 ml of enzyme preparation in a final volume of 5 ml.
- At these concentrations maximum level of activity was obtained with the extract of tissue studied.
- 2- oxoglutarate was omitted in blank sample

- Sample was incubated at room temperature for 15-30 min. at 37^0C
- Change in absorbance was recorded at 340 nm.

5. Calculations

Activity is expressed as µmole of NAD(P)H disappeared oxidized per min per mg protein.

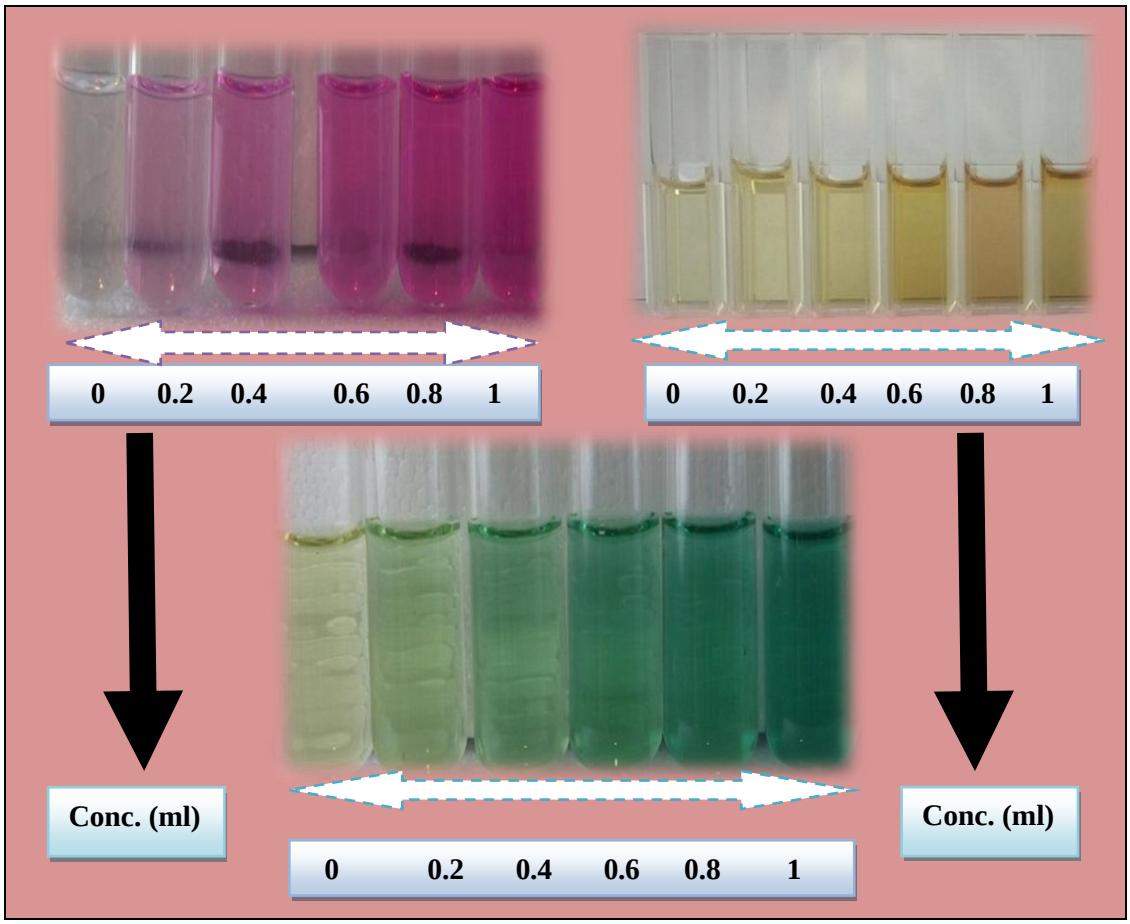


Fig. 3.9: Preparation of standard for NR, NiR, GS and GOGAT assay

3.11 Temporal expression analysis of transporter system and assimilatory enzymes

The RT-PCR based expression analysis of transporter and assimilatory enzyme genes involved in N metabolism were carried out on 10 selected rice genotypes at seedling stage. Details of lines subjected to expression analysis are mentioned in table 3.6. and the sequence of gene specific primer used for RT-PCR

analysis is listed in table 3.7. The entire experimental set up was established under controlled environment of light, CO_2 and humidity in miniature form of field which was a true representative of larger or actual field with NH_4^+-N and NO_3^-N & control treatment along with replication (Fig.3.10). Stepwise procedure for expression analysis is elaborated below:

Table 3.6: Lines selected for expression analysis

S.No.	(K-2014)	Colour
1	4	Y
2	21	DG
3	30	Y
4	46	DG
5	75	Y
6	121	DG
7	Danteshwari	G
8	Dagad deshi	LG
9	Swarna	DG
10	Indra Sugandhit Dhan 1	Y

Fig. 3.10: An experimental set up for expression analysis

Table 3.7: List of gene specific primers used for the study of expression profiles

Primer	Primer sequence forward (5'- 3')	Primer sequence reverse (3'- 5')	Length
	Glutamine Synthetase gene		
OsGln1;1	CAAGTCCGCCATTGAGAAGC	CTTGCCGTTCTGCTCCGTCT	20
OsGln1;2	GGTTGGAGGATCGGGCATAG	TCACCTTGTGGCGTGTAGCA	20
OsGln1;3	AGCCGATTCCGACGAACAAC	GTAGCGTGCCACCCAGACAT	20
OsGln2	ACCAAGAGTATGCGTGAAGA	AACCTGTCAACCTCCTTTCA	20
	Glutamate Synthase gene		
OsGlt1	GGAGGGAAATCTAATACAGG	AGTTCATCAGCGTTAGTCAG	20
OsGlt2	AGACAAACAATTTCCCTGAG	TAAAGGGTCACTTCCAACAT	20
	Ammonium Transporter gene		
OsAMT1.1	GTCGTTCACCACCATCCTCAAGACGTA	TCCTTCGCTGTGACGTCGTTCGTTC	27
OsAMT1.2	GATCTACGGCGAGTCGGGCACGAT	TTCCATCTCTGTCGAGGTCGAGACG	24
OsAMT1.3	TCAAATCCTACGGCCCGCCCGGTAG	GCCGAAGATCTGGTCCACGTACTCCTT	25
	Nitrate Transporter gene		
OsNRT2.4	GAATTGTACAGTACTTCCCC	TTCTGAGAAGAGACTGGATCTGTCC	20
OsNRT7.8	CCGGATCCATGGACTCCTCATACC	CCTCTAGAGCAACACAATTGTCC	24
	Actin gene- house keeping		
Act-Rice	ACGACATGGAGAAGATATGG	GTTGAAGGTCTCGAACATGA	20
	Tubulin gene- house keeping		
Tub-Rice	TGAGGTTTGATGGTGCTCTG	GTAGTTGATGCCGCACTTGA	20

3.11.1 Collection of tissue samples

Root and shoot tissue samples were harvested separately from all the replication of respective treatments. In total there were 120 samples obtained from three treatment, two tissues and two biological replicate of 10 genotypes. After collection, samples were snap frozen under ice cold conditions and stored immediately in -80 ^{0}C for total RNA extraction using Trizol reagent (Protocol developed at IOWA state university, Iowa) with slight modification.

3.11.2 Arrangements to be made prior to RNA isolation

3.11.2.1 Preparation of utensils for RNA Extraction:

- Soaked mortar pestles and other glassware in 30% Hydrogen Peroxide for 15 minutes were used for RNA extraction. Further, they were rinsed with DEPC water and baked overnight in hot air oven.
- RNase free 1.5 ml microcentrifuge tubes and RNase free filter tips was used every time.

3.11.3. RNA Extraction Procedure

1. The collected tissues were cute in to fine pieces using properly sterilized scissor. Tissue was then transferred to 2 ml tubes up to 500 µl mark.
2. 700 µl of Trizol reagent was added and samples were crushed in tissue lyser (Molbio).
3. Further, 300 µl of more Trizol was added and samples were vortex well
4. 700 µl of chloroform: isoamyl alcohol mixture was added and samples were vortexed again. Then, samples were allowed to mix in rotor for 5 min
5. Samples were centrifuged at 13000 rpm for 10 minutes under 4^{0}C on a bench top microcentrifuge.
6. 600 µl of top aqueous phase was carefully pipette out the into clean 1.5 ml microcentrifuge tubes
7. 600 µl of isopropanol was added and samples were transferred to -20^0 C for 10 min.
8. Pellet of RNA was allowed to form by centrifuging at 13000 rpm for 10 minutes under 4^{0}C.

9. Supernatant was discarded carefully and 500 µl of 70% ethanol (DEPC treated) was added and pellet was re-suspended by gentle tapping. Centrifugation as in step 5 for at least 3 minutes was repeated.
10. Decant the ethanol and tubes were inverted on paper towel and pellet was air dry for 10- 15 minutes until no droplets can be seen inside the tube.
11. Pellet re-suspended in ~20 µl of RNAase free DEPC water (Actual volume will depend upon the size of the pellet).

3.11.4 Quantification and purity of RNA

The concentration of RNA is assessed at 260 nm using NANODROP spectrophotometer (ND 1000). 2 µl of isolated RNA was placed over tip of nanodrop to record absorbance. The absorbance ratio (A260/A280) and (A260/A280) was recorded for each sample to estimate samples to find out the purity of RNA. The acceptable absorbance ratio (A260/A280) for pure RNA was 1.9-2.1.

3.11.5 cDNA synthesis

RNA isolated and quantified was used for cDNA synthesis using Thermo Scientific VersoTMcDNA Synthesis Kit as per manufacturer's instructions. The kit consisted of following components and is suitable for 1pg to 1µg of RNA.

1. Verso Enzyme Mix
2. 5X cDNA Synthesis Buffer
3. Anchored OligodT (500 ng/µl)
4. Random Hexamer (400 ng/µl)
5. dNTP mix (5 mM each)
6. RT Enhancer

The stepwise procedure as described in Thermo Scientific VersoTM cDNA Synthesis Kit was followed. Before start thaw the reagents provided in the kit by bringing them to room temperature and mix well by vortexing gently. Spin down to recover the maximum amount. Do not vortex and thaw the Verso Enzyme Mix.

1. Thaw the template RNA samples on ice. Workout the amount/ volume of RNA required reaching 1 µg concentration and adjusting the volume of water

accordingly. Dispense the required quantities of RNA and water in each tube (0.2 ml capacity).

2. Prepare the master mix on ice by mixing the mentioned amounts of 5x cDNA synthesis buffer, dNTP, primer solution and RT enhancer (except Verso Enzyme Mix and template RNA).
3. Mix the contents by vortexing gently for not more than 5 seconds.
4. Add Verso Enzyme Mix to master mix and mix by pipetting.
5. Dispense 10 µl of master mix into each tube containing RNA samples and water. Mix by gentle tapping and spin down the contents.
6. Incubate the tubes in PCR machine on reverse transcription cycling program described in table 3.8.

Table 3.8: cDNA synthesis (reverse-transcription) Reaction Components

Component	Volume/reaction	Final concentration
Master mix		
5x cDNA synthesis Buffer	4µl	
dNTP Mix	2µl	1x
Anchored Oligo-dT primer	1µl	500 µ each
Random Hexamer	1µl	
RT Enhancer	1µl	
Verso enzyme Mix	1µl	
Water (PCR grade)	variable	
Template RNA	1-5 µl	1 µg
	20 µl	

Reverse transcription cycling program

Step	Temperature	Time	No. of cycles
cDNA synthesis	42^0C	30 min	1 cycle
Inactivation	95^0C	2 min	1 cycle

3.11.6 Semi quantitative RT- PCR

Semi quantitative reverse transcriptase PCR was carried out to study the expression of nitrate and ammonium transporters and enzymes involved in their metabolism. The cDNAs generated from the total RNA isolated from tissues of 10

rice genotypes were subjected to semi-quantitative expression profiling in 20 µl reaction using gene specific primer. The resultant PCR product was then resolved on 1.5 % Agarose gel at 100V. The presence of amplicons and their respective intensity were recorded under gel documentation system. The expression was analyzed by comparing the relative fluorescent intensities of cDN Aamplicons under gel documentation system. Actin and Alpha tubulin was used as an internal control for normalization of the results. The semi quantitative RT-PCR amplicons were digitized using GelQuant.NET Analyzer software (www.biochemlabssolutiond.com) and the relative expression of genes were expressed in terms of fold change for each plant sample with respect to corresponding control sample. PCR components and temperature profile is depicted in table 3.9 and 3.10.

Table 3.9: PCR components and their quantity used for semi-quantitative RT-PCR

Components	Concentration	Quantity
cDNA	1,000 ng/µl	2 µl
PCR buffer	(10X)	2 µl
dNTP mix	(2 mM)	2 µl
Primer Forward	(10 µM)	1 µl
Primer Reverse	(10 µM)	1µl
Taq polymerase	(1 U/ µl)	1 µl
Nanopure water		11 µl
Total		20.0 µl

Table 3.10: Temperature profiles used for Semi quantitative RT-PCR

Steps	Temperature (^{0}C)	Duration	Cycles	Activity
1	94	2 min	1	Initial Denaturation
2	94	30 sec		Denaturation
3	Variable (55-60ºC)	30 sec	35	Annealing
4	72	1 min		Extension
5	72	7 min	1	Final extension
6	4	For ever	1	Store

3.11.7 Quantitative Real Time -PCR analysis (qPCR)

In quantitative qPCR, specific or non-specific detection chemistry allows the quantification of the amplified product. The amount detected at a certain point of the run is directly related to the initial amount of target in the sample. Relative quantification of a target against an internal standard is particularly useful for gene expression measurements. Relative quantification is the most widely used technique. Gene expression levels are calculated by the ratio between the amount of target gene and an endogenous reference gene, which is present in all samples.

In the present investigation qPCR was performed with selected gene specific primers with actin and tubulin as internal controls or housekeeping genes. qPCR was accomplished on Mx3000P® QPCR System (Stratagene, USA) using gene specific primers (Fig. 3.11). 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. PCR cocktail and thermal profile set up for qPCR are described in (Table 3.10) and (Table 3.11).

The Passive Reference (ROX™ dye) is a dye molecule included in the Power SYBR® Green PCR Master Mix that does not participate in the PCR amplification. On Applied Biosystems real-time PCR systems, the Passive Reference provides an internal reference to which the SYBR® Green dye/dsDNA complex signal can be normalized during data analysis. Normalization is necessary to correct for well-to-well fluorescent fluctuations.

Quantitative PCR software uses the exponential phase of PCR for quantification. PCR is initially an exponential process but eventually reaches a plateau phase, when one of the reagents becomes limited. Reactions can plateau at different levels even if they have the same starting concentration of target. During the exponential phase, the amount of target is assumed to be doubling every cycle and no bias is expected due to limiting reagents. Analysis takes the Ct (cycle number) value, at the point when the signal is detected above the background and the amplification is in exponential phase. The more abundant the template sample,

the quicker this point is reached, thus giving earlier Ct values. Delta delta Ct (ΔΔCt) method is the simplest one for quantitative estimation, as it is a direct comparison of Ct values between the target gene and the reference gene. Thus the relative quantification of expression was done by using the mathematical model given by Livak and Schmittgen (2001).

$$R = 2^{-\Delta\Delta Ct}$$

$$\Delta\Delta Ct = [\Delta Ct \text{ treatment} - \Delta Ct \text{ control}]$$

ΔCt treatment= Ct with primer-Ct with endogenous control (treatment)

ΔCt control= Ct with primer-Ct with endogenous control (Control)

Table 3.10: PCR components and their quantity used for qPCR

Components	Concentration	Quantity
cDNA	1,500 ng/µl	2.0 µl
Brilliant SYBR Master mix (Stratagene, USA)	2 X	10.5 µl
Primer Forward	10 pM	1.0 µl
Primer Reverse	10 pM	1.0 µl
Distilled water	-	8.5 µl
Total		**25.0 µl**

Table 3.11: Temperature profiles used for qPCR

Steps	Temperature (^{0}C)	Duration	Cycles	Activity
1	94	2 min	1	Initial Denaturation
2	94	15sec		Denaturation
3	60	1 min	40	Annealing
4	72	30 sec		Extension
5	95	15sec	1	
6	55	30 sec	1	Dissociation curve analysis
7	95	30 sec	1	

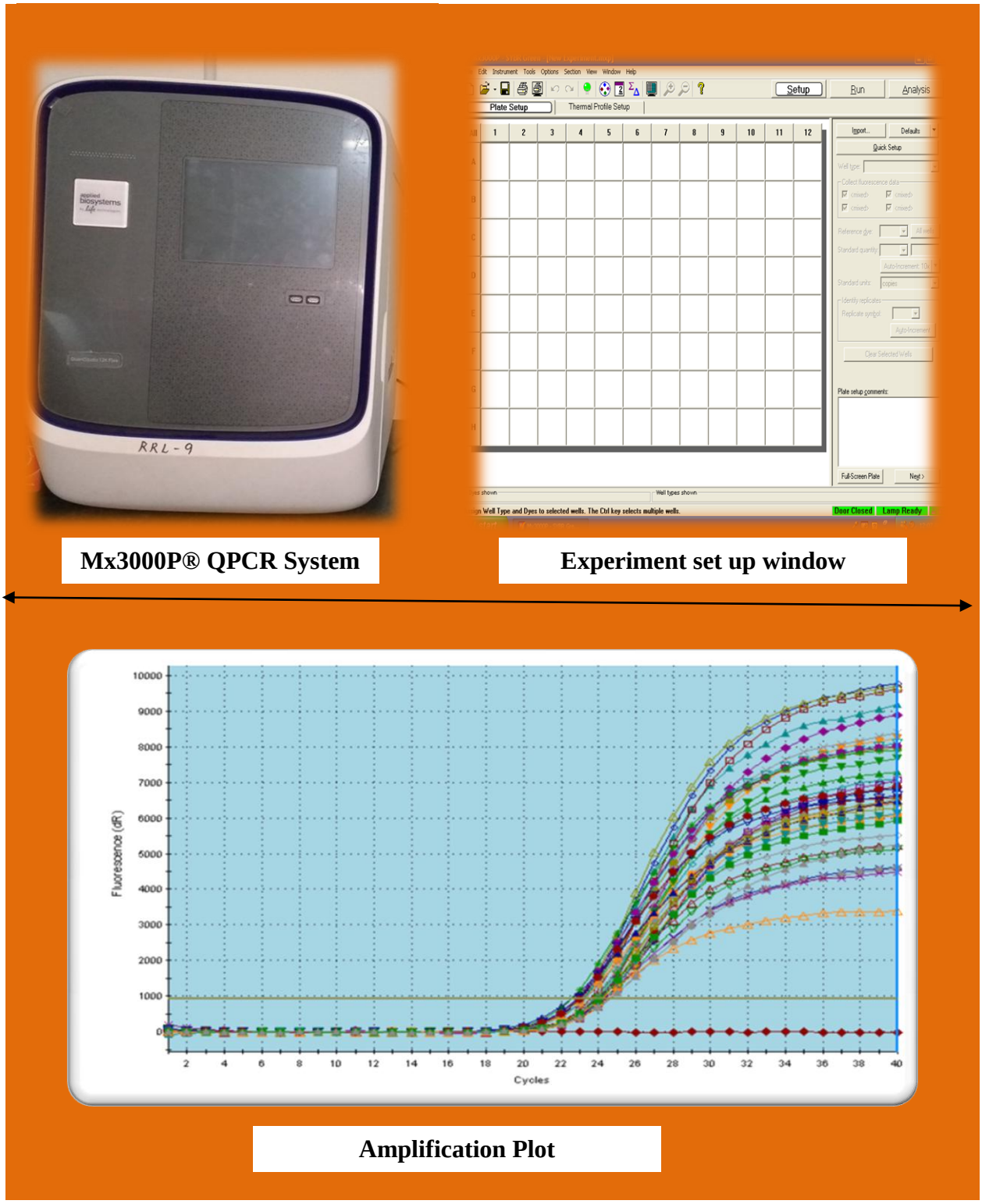


Fig. 3.11: A view of Mx3000P® QPCR System and experiment set up window

3.12. Molecular studies

A set of 122 RILs along with the parent were used for molecular studies and identification of QTL for NUE, root traits and yield components.

3.12.1 Genomic DNA isolation

- DNA was isolated from single tagged plant of each line during wet season 2011, by MiniPrep method (Doyle and Doyle, 1987). Its procedure was as followed.
- Around 0.1 g of leaf sample was grinded in a 2 ml eppendorf tube contained 0.4 ml of extraction buffer with the help of MoBIO tissue lyzer.
- 0.4 ml of chioroform-isoamyl alcohol (24:1) mixture was added. Mixed well by vortexing. Centrifuged at 13000 rpm for 30 sec. Supernatant was collected and transferred to a new eppendorf tube.
- 0.8 ml of absolute ethanol was added and mixed properly by tube inversion.
- Centrifugation was done at 13000 rpm for 2 min. Supernatant was discarded and pellets were washed with 70 % ethanol.
- Dried the pellets for 15-20 minutes.
- Pellets were dissolved in 50-100 µl (based on the size of pellet) TE buffer.

3.12.2 Quantification of DNA

The DNA samples were quantified on Nano Drop Spectrophotoscopy (NANODROP 2000c). After quantification, the DNA was diluted with nuclease, protease free water at the final concentration of approx.40 ηg/µl. This was used for PCR analysis.

3.12.3 PCR amplification using HvSSR and SSR primers

2 µl of diluted template DNA of each genotype was dispensed in the bottom of PCR plates. Separately cocktail was prepared in an eppendoff tube as described in table 3.12. 18µl of cocktail was added in each tube. The mixture was carried out for 34 cycles in Applied Biosystems thermal cycler. Temperature profile for PCR is presented in table 3.13.

Table 3.12: PCR mix for one reaction (Volume 20 µl)

Reagent	Stock concentration	Volume (µl)
Sterile and nanopure H_2O	-	13.9
PCR buffer	10x	2.0
dNTPs (Mix)	1 mM	1.0
Primer (forward)	5 pmol.	0.5
Primer (Reverse)	5 pmol.	0.5
Taq polymerase	1 unit/µl	0.5
DNA template	40-50 ng/ µl	2.0
Total		**20**

Table 3.13: Temperature profile used for PCR amplification using microsatellite markers

Steps	Temperature (^{0}C)	Duration	Cycles	Activity
1	95	5	1	Denaturation
2	94	1	↑	Denaturation
3	55	1	34	Annealing
4	72	1	↓	Extension
5	72	7	1	Final extension
6	4	24 hrs	1	Storage

After the PCR reaction was completed, 5 µl of 6 X loading dye was added to 20 µl PCR amplicons and 7 µl (PCR product with dye) was loaded on 5 % PAGE in a mini vertical electrophoresis system (CBS scientific, model MGV202-33) along with 50 bp ladder. Electrophoresis was done for 1 hour at 180 volts. Gels were stained using EtBr solution then visualized and photographed by using Gel Doc Unit, detailed below.

3.12.4 Gel electrophoresis

Five per cent polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified microsatellite products, since polyacrylamide gels have better resolution for amplified products. Gels were casted in CBS-SCIENTIFIC electrophoresis unit. Glass plates were prepared before making the gel solution. Both glass plates (outer and inner notched glass plates) were cleaned thoroughly with warm water, detergent and then with deionised water.

3.12.5 Assembling and pouring the gel

- Gasket was fixed to the three sides of the outer plate (without notches). Spacers of 1.5 mm thickness were placed along the sides by just attaching the gasket of outer plate.
- Later, notch plate was kept on the outer plate so that spacers were between the two plates. Clamps were put on the three sides of plates leaving notch side of unit. It was checked with water to found any leakages.
- For casting, gel was prepared just prior to pouring. For preparation 65 ml of 5 % PAGE solution was taken, 600 µl of 10 % ammonium per sulphate and 60 µl of TEMED (N,N,N',N'-Tetra methyl ethylenediamine) was added to initiate the polymerization process.
- The contents were mixed gently by swirling, but bubbles were avoided. Before pouring, assembly was kept on the bench top so that it made 45 degree angle with bench top.
- Then gel solution was poured from notch side and avoided air bubbles. Comb of 1.5 mm thickness (60 wells) was inserted with tooth side in the gel.
- Later assembly was kept for polymerization for 20-30 min.

3.12.6 Electrophoresis

- After polymerization process, gasket and clamps were removed and assembly was kept in the electrophoresis unit with electrophoresis unit clamps where notch side was faced inner side of the unit.

- TBE (1 X) was poured in upper tank in the unit and the rest was poured in bottom chamber.
    - Comb was removed with care so that it did not disturb the wells formed.
    - 5µl loading dye (6 X) was added to 20 µl PCR products.
- Finally, 7 µl of each sample were loaded into the wells for facilitating the sizing of the various alleles. Ladder (50 and 100bp) was loaded in the first well.
- Electrophoresis was done at 180 volts till the dye reached bottom of the gel.

3.12.7 Visualization of bands

- After electrophoresis, clamps were removed and glass plates were separated without damaging the gel.
- Gel was taken out from plate into staining box with care by flipping the gel with help of spatula. Little amount of water was poured for easy removal.
- Ethidium bromide solution was poured into the staining box.
- It was agitated for about five minutes to stain the gel.
- Gel stained with ethidium bromide was washed two times with double distilled water to have clear images.
- The gels were scanned with the help of BIO-RAD gel doc XR^{+}.

3.12.8 Development of genotypic data of population

The primer exhibited polymorphism on parent was further used for PCR amplification on all of the 122 selected lines of rice. Genotypic data were generated with a set of 162 polymorphic primers.

3.12.9 Scoring of data

The banding pattern of population developed by each set of SSR and HvSSR primers were scored separately as described in table 3.14.

Table 3.14: Scoring of data

S.No.	Code	Types of band
1	A	Danteshwari like allele (female parent)
2	B	Dagad deshi like allele (male parent)
3	H	Both alleles
4	O	Other types

3.13. Buffers and chemicals

3.13.1 Estimation of chlorophyll content

- 80% acetone
- Add 20ml of milli Q water to 80 ml of acetone. Store in -20 ^{0}C.

3.13.2 Estimation of extractable ammonium and nitrate nitrogen

a. 2M KCL: Dissolve 150 g of of KC1 reagent in 800 ml of milli Q water and dilute the solution to 1L.

b. MgO

c. Boric acid-indicator solution: Dissolve 0.5 g bromocresol green and 0.1 g methyl red in 100 ml of 95% ethanol. Add 5 ml of this indicator and 0.1 g P-nitrophenol to 1 liter at 4% boric acid indicator solution and adjust to pH 4.6.

d. Devardas alloy

e. .005 N Sulphamic acid: Dissolve .48g of sulphamic acid in 1L milli Q water

f. 1% Sulphamic acid: Dissolve 1 g sulphamic acid in 100 ml milli Q water

3.13.3 Estimation of plant protein

a. 15% NaOH: Dissolve 150 g of of NaOH reagent in 800 ml of milli Q water and dilute the solution to 1L.

b. 4 % Boric acid-indicator solution: Dissolve 40 g of boric acid in 800 ml of milli Q water. Add 5ml mixed indicator and dilute the solution to 1 L.

c. 40% NaOH: Dissolve 400 g of NaOH reagent in 800 ml of milli Q water and dilute the solution to 1L.

d. .05 N sulphamic acid: Dissolve 4.854 of sulphamic acid in 800 ml of milli Q water and dilute the solution to 1L.

3.13.4 Estimation of nitrate reductase activity:

a. Potassium phosphate buffer 0.1 M (pH=7.5)

b. Potassium Nitrate 0.1 M: Dissolve 1.01 g potassium nitrate in 100 ml milli Q water.

c. 2mM NADH: Dissolve 14 mg NADH disodium salt in 10 ml milli Q water.

d. .02 % NED (N-(1-naphthyl) ethyelenediamine dichloride: Dissolve 20 mg in 100ml water.

e. .01 M sodium nitrite standard solution

f. 1mM EDTA

g. 25 mM cysteine

h. 25 mM potassium phosphate

f. KOH

3.13.5 Estimation of nitrite reductase activity:

a. 0.5 M Tris-HCL buffer (pH 7.5)

b. Sodium nitrite solution: Dissolve 43.2 mg sodium nitrite in 20 ml milli Q water

c. Methyl viologen solution: Dissolve 60.1 mg methyl viologen in 20 ml milli Q water.

d. Sodium diothinite-bicarbonate solution: Dissolve 250 mg each of $Na_2S_2O_4$ and $NaHCO_3$ in 10 ml milli Q water.

3.13.6 Estimation of glutamine synthetase activity:

a. 50mM Tris-HCL buffer(pH=7.8)

b. 15% Glycerol:

c. 14mM βmercaptoethanol: Dissolve .978 ml in 700 ml milli Q water and volume make up to 1 L.

d. 1mM EDTA:

e. 0.1 % Titon X-100

f. 500m M L-Glutamate:

g. 100mM ATP

f. 200 mM Tris-HCL

g. Ferric chloride reagent: Dissolve 10 g trichloroacetic acid and 8 g ferric chloride in 250 ml of 0.5 N HCL.

3.13.7 Estimation of glutamate synthase activity:

a. 50mM Tris HCL buffer (pH=7.6)

b. Prepare the following reagents in Tris-HCL buffer 50mM (pH =7.6)

- Glutamine, 5mM (36.5mg/10ml)
- 2-oxoglutarate, 5mM (36.5mg/10ml)
- NADPH. 0.25 mM (10mg/10ml)

c. 1mM EDTA

d. 1mM Dierythritol

e. 1% PVP

3.13.8 Reagents for PCR

a. Primers: Highly variable microsatellite markers from ILS, USA

b. dNTPs: (dATP/dCTP/dGTP/dTTP)

1 mM stock of dNTP (GeNei) was used.

c. PCR buffer (10 X)

d. Taq polymerase (5 unit/µl, 500 U *taq* (GeNei) was used for PCR).

3.13.9 Stock solutions

a. extraction buffer

Tris (1 M, pH 8.0) 5 ml.

SDS (20 %) 5 ml.

EDTA (1 M, pH 8.0) 5 ml.

NaCl (4 M) 7.5 ml.

Double-distilled water 67.5 ml.

Total 100 ml.

b. TE buffer

1 M Tris-HCl (pH-8) 1 ml

0.5 M EDTA 0.2 ml

Final volume was adjusted to 100 ml and autoclaved

c. EDTA (0.5M, pH-8)

186.12 g of EDTA was dissolved in 700 ml of distilled water. NaOH pallets were added for proper dissolving of EDTA. The pH was set to 8 using NaOH/HCl. Final volume was adjusted to 1000 ml with distilled water and sterilized by autoclaving.

d. 4 M NaCl

35.04 g of NaCl was dissolved in 100 ml of distilled water. Final volume was adjusted to 150 ml and sterilized by autoclaving.

e. 1 M Tris HCl (pH 8.0 at 25°C)

31.52 g of Tris Cl/Trizma base was dissolved in 100 ml of distilled water. The pH was set to 8.0 using concentrated HCl. The final volume was adjusted to 200 ml with distilled water and sterilized by autoclaving.

f. RNase (10mg/ml)

Dissolved 10 mg RNase powder (Sigma) in 1 ml distilled water.

g. 1 M KCl.

18.64 g of Potassium Chloride was dissolved in 200 ml of distilled water and the final volume was made to 250 ml with distilled water and sterilized by autoclaving.

h. 15 mM $MgCl_2$

1.43 g of Magnesium Chloride was dissolved in 80 ml of distilled water. Final volume was adjusted to 100 ml with distilled water and sterilized by autoclaving.

i. Absolute alcohol (pre chilled)

j.70 % Ethanol

70 ml of absolute ethanol was taken and volume makeup 100 ml with distilled water.

3.13.10 Solutions for electrophoresis

a. 10 X TBE buffer

Tris base 104 g

Boric Acid 55 g

EDTA (0.5 M) 40 ml

Distilled water 500 ml

Final volume was adjusted to 1 litre with distilled water.

b. Tank buffer (1 X TBE)

100 ml of 10 X TBE + 900 ml of distilled water.

c. 5 % Acrylamide gel solution (1000 ml)

Acrylamide 47.5 g

Bis-Acrylamide 2.5 g

10 X TBE 100 ml

Final volume was adjusted to 1 liter with distilled water.

Acrylamide and bis-acrylamide were weighed and dissolved one by one in 500 ml distilled water then added to 100 ml of 10 X TBE and the volume was made up to 1000 ml by adding autoclaved double distilled water. The solution was sterilized by passing through 0.22 micron and stored in amber colour bottle at 40 ^{0}C.

d. 10 % Ammonium persulphate (APS)

Ammonium persulphate 1.0 g

Distilled water 10 ml

e. Ethidium bromide

Ethidium bromide powder 10 mg

Water 1 ml

f. Gel staining solution

Ethidium bromide 10 µl

Distilled water 200 ml

c. 6 X loading dye

Bromophenol blue 0.25 g

Glycerol 40 ml

Final volume was adjusted to 100 ml with distilled water.

d. 50 bp DNA ladder (GeNei)

Step up 50 bp ladder (500 µg/ml) 0.1 ml

Gel loading buffer (6 X) 0.2 ml

Water (Nuclease free) 0.4 ml

1.5 µl are used at the time of sample loading on gel

3.13.11 Isolation of RNA

a. Trizol reagent

b. Chloroform:Isoamyl alcohol (24:1): Mix 24 ml of chloroform to 1ml of isoamyl alcohol to make 25 ml of chloroform.

c. Isoamyl alcohol

d. Pre-chilled isopropanol

e. DEPC H_2O: 0.1 % DEPC treated water was prepared by adding 1 ml od diethyl pyrocarbonate to 1000 ml of Milli Q water. This solution was allowed to stir for at least 2 hrs on magnetic stirrer. The solution as then autoclaved twice to eliminate residual DEPC.

f. 70 % ethanol (with DEPC treated water)

Add 70 ml of ethanol to 30 ml of DEPC treated water

3.14 Statistical analysis

The data obtained under various characters were tabulated and statistically analyzed. 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 as depicted in table 3.15.

Table 3.15: Skeleton of ANOVA table for factorial design

Source of variation	Sum of Squares	Degrees of freedom	mean squares	F statistic
Factor A	SSA	a-1	MSA=SSA/(a-1)	MSA/MSE
Factor B	SSB	b-1	MSB=SSB/(b-1)	MSB/MSE
AB (Interaction)	SSAB	(a-1)(b-1)	MSAB=SSAB/[(a-1)(b-1)]	MSAB/MSE
Error	SSE	N-ab	MSE=SSE/(N-ab)	
Total	SST	N-1		

3.14.1 Correlation analysis

To determine the degree of association between traits, the correlation co-efficient were calculated using the following formula:

$$r\,(xy) = \frac{\mathrm{Cov}(xy)}{\sqrt{\mathrm{Var}\,(x) * \mathrm{Var}\,(y)}}$$

Where,

$r_{(xy)}$ = Correlation co-efficient between variable x and y

$Var_{(x)}$ = Variance of x variable

$Var_{(y)}$ = Variance of y variable

$Cov_{(xy)}$ = Covariance between variable x and y

3.14.2 Mean

The mean of the recorded traits was calculated as follows;

$$\text{Mean} = {\sum X}/{N}$$

Where , $\sum X$ = Summation of all observed values

N = Number of observations

3.14.3 Range

A lower and higher value of a trait determines the range which is expressed as follows;

Range = Highest value – lowest value

3.14.4 Heritability

Heritability in broad sense was estimated using the formula suggested by Robinson *et al*., (1949):

$$\text{Broad sense heritability (H)} = \frac{Vg}{Vp}$$

Where V_g = genotypic variance

V_p = Phenotypic variance

3.14.5 Coefficient of variation (CV)

Coefficient of variation in percentage was calculated by the formula

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

3.14.6 Frequency graphs (Histograms)

The frequency distributions of all physiological and morphological traits were estimated under all treatments using MS Excel.

3.14.7 Stability analysis

Analyzed the data of two years over all environments (locations) and treatments by using stability model proposed by Eberhart and Russell (1966). The model involves the estimation of mean, regression coefficient and deviation from regression, which are defined by a mathematical formula.

$Yij = \mu i + \beta i\ Ij + Sij$

Where,

Yij = Mean of the ith genotype at the jth environment

μi = Mean of ith genotype over all environments

βi = Regression coefficient that measures the response of ith genotype to varying environments

Ij = Environmental index obtained by subtracting the grand mean from the mean of all genotypes at the jth environment

Sij = Deviation from regression of the ith genotype at the jth environment

Table 3.16: Mean, yield and Regression Co-efficient (b) values.

Regression Coefficient	Stability	Mean yield	Remarks
b=1	Average	High	Well adopted to all environments
b=1	Average	Low	Poorly adapted to all environments
b>1	below average	High	Specially adopted to favourable environments
b<1	below average	High	Specially adopted to unfavourable environments

3.14.8 QTL analysis

The linkage map was constructed with QTL Cartographer 2.5. Graphical genotyping of these molecular data was done using GGT 2.0 (Van Berloo, 1999). The phenotypic and genotypic data was analyzed using QTL cartographer 2.5 (Composite Interval Mapping) with a threshold value of 3.0 LOD (Wang *et al*., 2005) and QTL IciMapping 3.2 where a LOD score of 2.5 was used for declaring the presence of a suggestive QTL (Li *et al.*, 2007).

CHAPTER IV
RESULT AND DISCUSSION

In the present investigation entitled "Mapping QTLs for NH_4^+ and NO_3^- use efficiency under water-stress and non-stress conditions and expression analysis of Glutamine Synthetase and Nitrate Reductase in rice (*Oryza sativa L.*)" efforts were made to examine the genetic basis as well as the biochemical and enzymatic mechanisms involved in nitrogen uptake, assimilation and remobilization processes in rice. The numerous data obtained from soil analysis, QTL studies, gene expression and enzyme assays of the important proteins involved in the pathway are integrated in to explanatory models to study whole plant/genotype behaviour. The results of the present study are put through statistical analysis to make necessary inferences and are further validated with experimental findings by establishing a cause and effect relationship on the basis of available data & literature evidence and are presented in this chapter.

4.1 Influence of NO_3^--N and NH_4^+-N sources on nitrogen mineralization in aerobic and anaerobic soils

Nitrogen fractions were estimated in representative soil samples collected (0-15 cm) from irrigated (anaerobic) and rainfed (aerobic) rice fields during wet season 2014 and 2015.

4.1.1. Nitrogen Mineralization

Soil nitrogen supplied through organic fertilizers *i.e.* ammonium sulphate are transformed by hydrolysis to ammonium while calcium nitrate is directly transformed to nitrate. The ammonium released is further oxidized to nitrate *via*. Nitrification governed by the microbial activity during mineralization process. Thus, the majority of plant available nitrogen is in the inorganic form *i.e.* NO_3^--N or NH_4^+-N. Nitrogen mineralization is a relatively slow microbial process that is affected by factors such as aeration and moisture. Irrigated paddy fields are periodically flooded and thus temporarily under anaerobic conditions while rainfed paddy fields generally have dry soils, thus are continuously aerobic. These contrasting environments are greatly influenced by differential irrigation and seasonal variation in rainfall leading to variation in soil moisture regime, which in

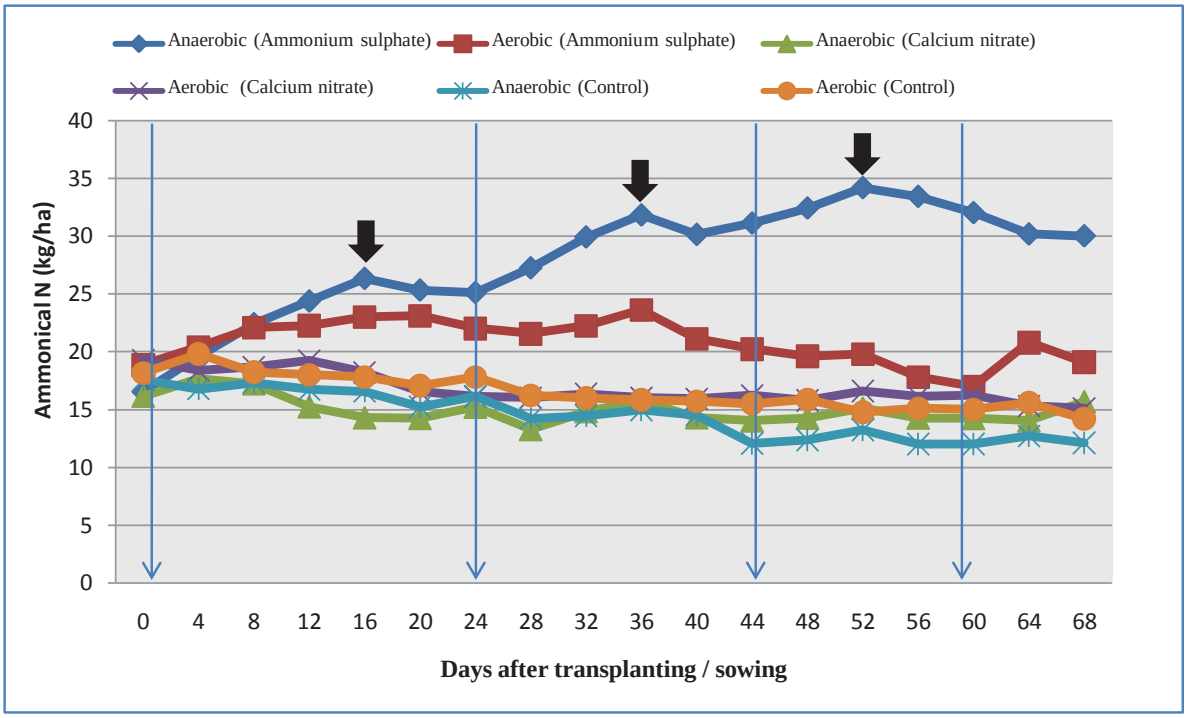
turn alters the NO_3^--N, NH_4^+-N and water dynamics of soil. Thus, in order to maximize the water and nitrogen use efficiency in paddy field, present objective of studying soil N dynamics was framed and results of variation in mineral nitrogen pool along the rice growing period are presented below:

4.1.1.1 Seasonal NH_4^+-N dynamics in soil (kg ha^{-1})

NH_4^+-N dynamics during *kharif* 2014 and 2015 under different sets of environment/condition were evaluated and results are depicted in table 4.1. The data of *kharif* 2014 of ammonium sulphate treatment reveals that NH_4^+-N persisted more under anaerobic condition as compared to aerobic condition. Maximum drop of NH_4^+-N was observed under aerobic trial. The NH_4^+-N concentration although was comparatively higher under aerobic condition initially but it could not eliminate the nitrification of the NH_4^+-N, therefore, NH_4^+-N subsequently dropped with passage of time due to its conversion to nitrate.

Meanwhile NH_4^+-N concentration in aerobic and anaerobic soil had different days for maximum concentration and gradual increase was observed after 0, 24 & 44 DAT and 0, 25 and 60 DAS which might be due to application of fertilizer on same day as well as mineralization of the respective treatment further. In calcium nitrate and control treatments of different sets of condition, dynamics of NH_4^+ release varied along the crop growing period depending upon seasonal rainfall regulating soil moisture regime and crop demand & uptake. The trend analysis can be seen in fig. 4.1.

The data of *kharif* 2015 shows the dynamics of NH_4^+-N under aerobic and anaerobic conditions under all treatment. There were no significant differences in NH_4^+ release pattern under all treatments and conditions which might be due to spatial variability & temporal heterogeneity in rainfall and longer dry period during crop growing period. Maximum values of NH_4^+-N concentration was obtained under anaerobic environment as compared to aerobic during both season trials. The ammonium ion is in the reduced state & gets trapped in the clayey particles of soil and therefore is stable in anaerobic conditions accounting for its higher concentration in flooded fields under all treatments. The trend analysis can be seen in fig. 4.1.



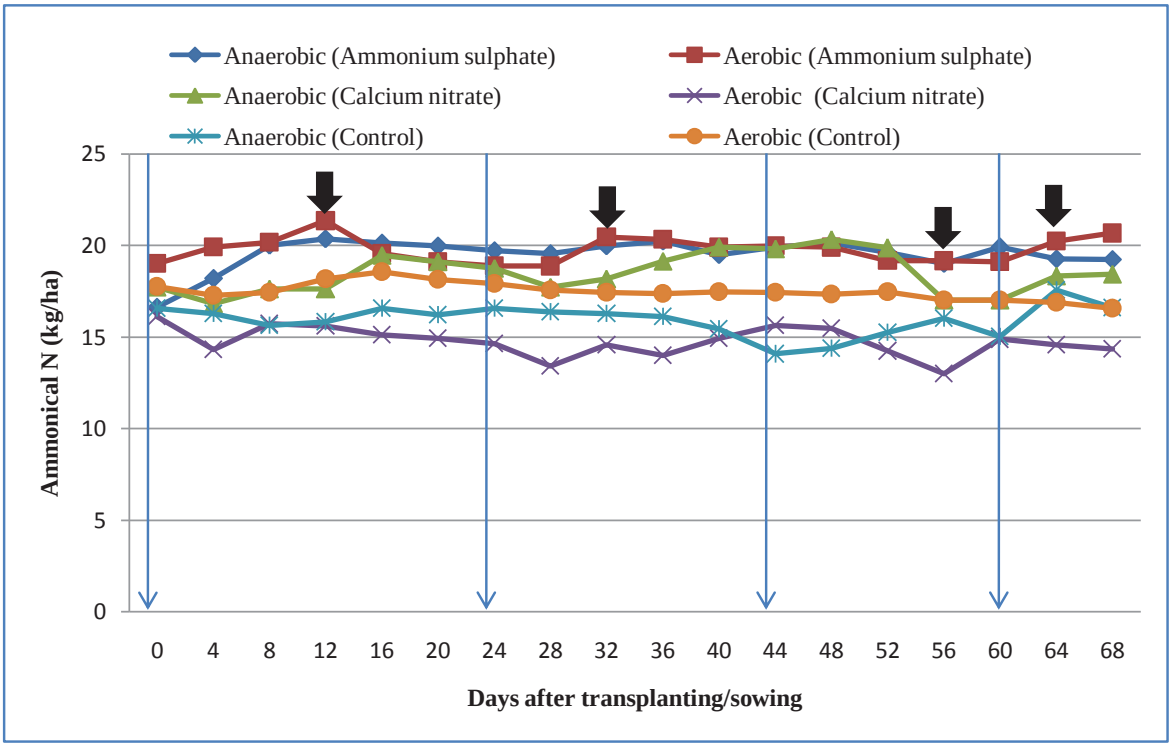


Fig 4.1: Periodic changes in ammonical nitrogen status under different levels of nitrogenous fertilizer and soil moisture regime during *kharif* 2014 and 2015

Table 4.1: Periodic changes in ammonium nitrogen (NH_4^+-N) status under different levels of nitrogenous fertilizer and soil moisture regime

Season	Treatment	Soil conditions	Days after transplanting/sowing																
			0	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**36**	**40**	**44**	**48**	**52**	**56**	**60**	**64**	**68**
Kharif-2014	Ammonium Sulphate	Anaerobic	16.52	19.56	22.4	24.36	26.32	25.31	25.1	27.23	29.9	31.8	30.12	31.1	32.4	34.16	33.41	32.00	30.16	30.00
		Aerobic	18.86	20.42	22.1	22.26	23	23.1	22.01	21.56	22.24	23.61	21.12	20.23	19.61	19.8	17.8	17.00	20.8	19.10
	Calcium Nitrate	Anaerobic	16.16	17.68	17.23	15.23	14.28	14.23	15.2	13.26	14.82	15.78	14.28	14.01	14.26	15.12	14.26	14.22	14.01	15.67
		Aerobic	19.18	18.35	18.66	19.23	18.23	16.52	16.12	16	16.34	16.03	15.91	16.23	15.78	16.6	16.1	16.21	15.3	15.10
	Control	Anaerobic	17.56	16.77	17.33	16.72	16.56	15.22	16.12	14.22	14.45	14.96	14.53	12.07	12.36	13.23	12.01	12.02	12.70	12.11
		Aerobic	18.16	19.82	18.23	18.013	17.81	17.06	17.81	16.23	16.01	15.85	15.71	15.50	15.87	14.78	15.11	15.00	15.6	14.2
Kharif-2015	Ammonium Sulphate	Anaerobic	16.63	18.19	19.99	20.36	20.14	19.96	19.72	19.56	19.96	20.23	19.48	19.89	20.14	19.63	19	19.89	19.26	19.23
		Aerobic	19.00	19.9	20.16	21.34	19.54	19.1	18.89	18.86	20.45	20.32	19.89	19.96	19.89	19.17	19.15	19.11	20.24	20.66
	Calcium Nitrate	Anaerobic	17.73	16.81	17.63	17.62	19.45	19.11	18.76	17.72	18.16	19.14	19.9	19.8	20.32	19.86	17.01	17.01	18.33	18.42
		Aerobic	16.1	14.32	15.71	15.6	15.11	14.91	14.62	13.4	14.56	14.00	14.91	15.63	15.47	14.23	13.00	14.87	14.56	14.34
	Control	Anaerobic	16.56	16.27	15.63	15.82	16.56	16.22	16.56	16.38	16.28	16.12	15.43	14.07	14.36	15.23	16.01	15.021	17.56	16.6
		Aerobic	17.76	17.26	17.43	18.17	18.56	18.13	17.92	17.56	17.44	17.36	17.45	17.43	17.34	17.45	17.00	17.01	16.89	16.55

4.1.1.2 Seasonal NO_3^--N dynamics in soil (kg ha^{-1})

NO_3^--N dynamics during *kharif* 2014 and 2015 under different sets of environment/condition were evaluated and results are depicted in table 4.2. The data of *kharif* 2014 reveals that under anaerobic condition concentration of NO_3^--N was very low in soil and subsequently fluctuated along growing period of crop in ammonium sulphate and control treatment. The possible reason may be that, with the onset of monsoon and flooding of soil, NO_3^--N which is soluble in water either moved with the percolating water and leached down to ground water or is lost through dentrification.

Additionally, in calcium nitrate treatment maximum concentration of NO_3^--N was observed after 0, 24 and 44 DAT which might be due the application of fertilizer on the respective days. In between fluctuation in nitrate concentration was observed which might be due to wetting and drying of soil and the cultivation practices followed in field. Under aerobic conditions all treatments showed higher NO_3^--N concentration as compared to anaerobic condition which may be attributable to nitrification phenomenon. Among the treatments under aerobic concentration NO_3^--N showed higher release pattern as compared to calcium nitrate and control specifically after 0, 25 and 60 DAS when fertilizer/treatment was applied. Nitrogen mineralization is greatly affected by aeration of soil which greatly affects the soil N pool and dynamics of NH_4^+-N and NO_3^--N in soil.

The data of *kharif* 2015 shows the dynamics of NO_3^--N under aerobic and anaerobic conditions under all treatment. The trend analysis in fig. 4.2. shows that maximum values of NO_3^--N concentrations are observed under aerobic conditions as compared to anaerobic conditions under all treatments and soil environments. Due to less longer dry period and heterogeneity in rainfall NO_3^--N shows the fluctuating values across conditions and treatments because NO_3^- N is greatly affected by soil moisture regime and alternate wetting & drying of soils.

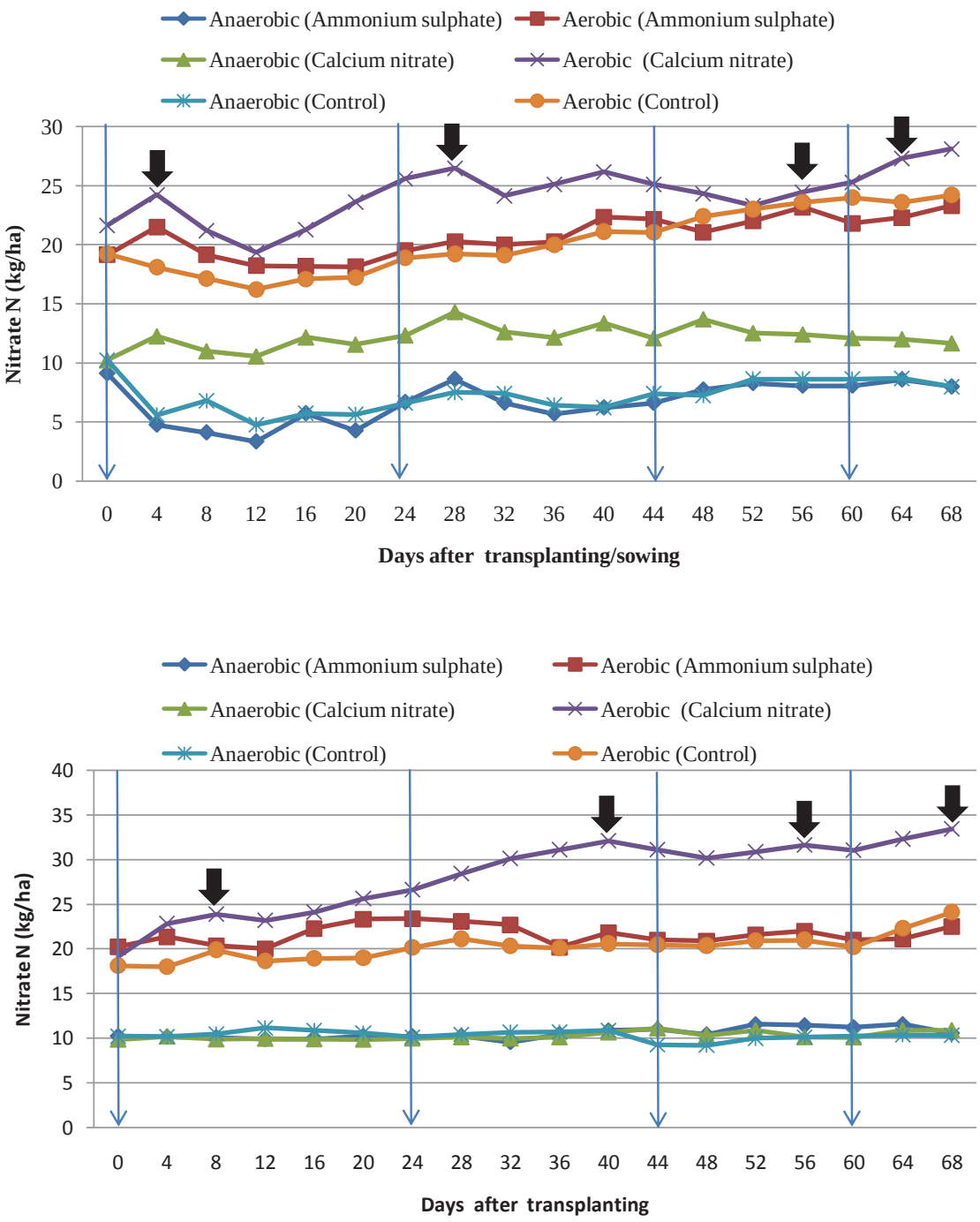


Fig. 4.2: Periodic changes in nitrate nitrogen (NO_3^--N) status under different levels of nitrogenous fertilizer and soil moisture regime during *kharif* 2014 & 2015.

Table 4.2: Periodic changes in nitrate nitrogen (NO_3^--N) status under different levels of nitrogenous fertilizer and soil moisture regime

Season	Treatment	Soil conditions	Days after transplanting/sowing																
			0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68
Kharif-2014	Ammonium Sulphate	Anaerobic	9.12	4.78	4.12	3.36	5.74	4.28	6.69	8.64	6.63	5.71	6.23	6.62	7.74	8.3	8.08	8.08	8.6	8.00
		Aerobic	19.13	21.50	19.13	18.23	18.16	18.13	19.51	20.26	20	20.23	22.32	22.15	21.06	22	23.19	21.8	22.1	23.2
	Calcium Nitrate	Anaerobic	10.23	12.26	11.00	10.56	12.17	11.56	12.34	14.32	12.61	12.16	10.37	12.1	13.71	12.53	11.42	11.11	12.01	11.67
		Aerobic	21.63	24.21	21.2	19.34	21.26	23.61	25.61	26.48	24.13	25.12	26.17	25.12	24.32	23.31	24.46	25.3	27.3	28.1
	Control	Anaerobic	10.23	5.61	6.81	4.77	5.72	5.66	6.62	7.51	7.42	6.42	6.24	7.4	7.25	8.63	8.63	8.63	8.70	8.00
		Aerobic	19.23	18.10	17.15	16.23	17.1	17.24	18.89	19.23	19.11	20	21.11	21.05	22.41	23.00	23.60	24.00	23.6	24.2
Kharif-2015	Ammonium Sulphate	Anaerobic	10.24	10.16	10.1	9.89	9.9	10.23	10.21	10.25	9.56	10.45	10.86	10.99	10.41	11.56	11.45	11.23	11.57	10.56
		Aerobic	20.21	21.36	20.36	20.00	22.26	23.36	23.39	23.12	22.71	20.16	21.79	21	20.89	21.56	21.98	21.00	21.12	22.54
	Calcium Nitrate	Anaerobic	9.86	10.21	9.89	9.96	9.9	9.86	9.99	10.12	10	10.12	10.66	11.12	10.32	10.89	10.13	10.11	10.89	10.9
		Aerobic	19.16	22.81	23.9	23.16	24.12	25.61	26.6	28.4	30.13	31.1	32.1	31.12	30.16	30.89	31.62	31.06	32.3	33.43
	Control	Anaerobic	10.23	10.16	10.48	11.16	10.89	10.56	10.12	10.42	10.66	10.67	10.89	9.23	9.20	9.99	10.12	10.24	10.36	10.31
		Aerobic	18.12	18.00	19.89	18.62	18.92	19	20.16	21.12	20.34	20.12	20.59	20.45	20.36	20.89	21.00	20.21	22.31	24.12

4.1.2 Discussion

These findings collaborate with the work of Zia *et al.* (2001) on nitrogen dynamics under aerobic and anaerobic soil conditions. They observed the maximum drop in NH_4^+ concentration under aerobic environment while high increase in NO_3^- concentration was registered under aerobic condition. These results are further in line with Magalhaes and Chalk (1987) and Saad *et al.* (1996). They illustrated that nitrification of NH_4^+ to NO_3^- was the lowest under flooded (anaerobic) condition as compared to aerobic condition since the soil was highly in recurred state. Furthermore, Angarria *et al.* (2012) studied nitrogen mineralization is flooded and dry soils. They observed that concentration of NH_4^+ in flooded soils were higher than in dry soils, meanwhile concentration of NO_3^- in flooded soils were lower than dry soils.

4.2 Climate and field hydrology during the experimental seasons

The study of seasonal dynamics in NH_4^+ and NO_3^- release across different sets of environment and treatment revealed major difference in soil condition in experimental plots. Heterogeneity existed in soil nitrate and ammonium concentration under aerobic and anaerobic condition during entire growing period of crop. Crop N efficiency which determines its yield potential depends largely on change in soil mineral nitrogen pool which in turn is influenced by rainfall amount and temporal distribution of rainfall. The total rainfall during crop cycle in wet season 2014, 15 was 1101.4 and 980.6 mm respectively. A perusal of weekly metererological data revealed that the cumulative rainfall showed, longer and more frequent dry spells manifestation during *kharif* 2015 (3 periods having 6 days without rain during vegetative stage and 2 periods having 6 rainless days during reproductive stage) as compared to *kharif* 2014 which showed single period consisting of six rainless days and one entire week without rain during vegetative stage. Henceforth, climate resulted in tremendous year to year variation in on-farm soil moisture regime making the study of interaction of soils and genotypes of utmost importance. The question of varietal differences in the use of indigenous and applied nutrients under varying water supply conditions besides system complexity on nutrient supply side offers another platform in selection for NUE traits. Keeping all this in mind, the results of morphological traits under study are

further presented in chronology which includes study with 122 RILs along with parents for NUE and yield related traits during *kharif*, 2014 and subsequently studying genotypic differences during *kharif*, 2015.

4.3 Yield and yield related traits

4.3.1. Analysis of variance

The 122 and 32 RILs along with parents were evaluated during wet season 2014 and 2015 for various phonological, agronomical and physiological traits. The data recorded for various traits under varied N forms and water regimes was subjected to 3-way analysis of variance and the mean sum of square due to various source of variation/variance components for the investigated traits are summarized in table 4.3. Analysis of variance (ANOVA) showed significant effect for all variance components genotype (G), nitrogen (N), environment (E) and their respective interactions *i.e.* genotype x nitrogen (GXN), genotype x environment (EG), environment x nitrogen (EN), environment x nitrogen x genotype (ENG) for yield and majority of yield related traits in both seasons. Of all, ANOVA revealed significant differences among the genotypes for most of the traits studied ($p<0.05$ & $p<0.01$) indicating the presence of genetic variation among genotypes and possibility of manipulating these variations for improvement purposes. This is in accordance with the previous reports on rice by Fageria and Filho (2001), Sokat (2006) and Singh *et al*. (1998). Furthermore, genotype by nitrogen (G×N) interaction and genotype by environment interaction (GXE), which was of main consideration in present research showed high level of significance for investigated traits implying the performance of genotypes are significantly influenced by N forms and water regimes. This is persistent with the work of Hafele *et al.* (2008b) who screened 19 rice genotypes adapted to different rice environments under two water and two nutrient treatments during the wet season of 2004 and 2005. They studied the variance components for grain yield traits and observed the significant values for all main factors water (W), nitrogen (N) and genotype (G) in both seasons.

Table 4.3: Mean sum of squares (MSS), variance and variance components for comparison of 124 and 32 selected RILs along with parents under NH_4^+ and NO_3^- forms of nitrogen and variable environments for yield and yield related traits during wet season, 2014 and 2015.

	Wet season 2014							Wet season 2015						
Traits	**G**	**N**	**E**	**E X G**	**N X G**	**EX N**	**E X N X G**	**G**	**N**	**E**	**E X G**	**N X G**	**EX N**	**E X N X G**
	TMSS, DF= 138	**TMSS, DF= 2**	**TMSS, DF= 1**	**TMSS, DF= 138**	**TMSS, DF= 276**	**TMSS, DF=2**	**TMSS, DF= 276**	**TMSS, DF=31**	**TMSS, DF=2**	**TMSS, DF=2**	**TMSS, DF=62**	**TMSS, DF=62**	**TMSS, DF=4**	**TMSS, DF=124**
GY	40,178**	7,54,835 **	116,52,103 **	**6,8282 ** **	**8,476 ** **	18,13,35**	8,668.6 *	1,9233 **	129821**	1202627**	**9,775 ** **	**3,911** **	10,126 **	2,528 *
BY	1,34,241**	206,70,6 **	344,27,066 **	**1,45,753 ** **	**3,7591** **	118,66,925 **	NS	97,646**	2,269,461 **	4,711,212 **	**55,232 ** **	**24,908** **	51,178 **	18,135**
HI	244**	1,993**	5,5542**	**368** **	**61** **	6,902**	64**	232**	116*	7,304**	**120** **	**45** **	85*	42**
TT/m^2	1,8471**	5,01,86**	38,60,901* *	**1,027** **	**5,797** **	NS	4774*	20,003**	812,572**	-	-	**2,993** **	-	-
TT/P	4.8**	329 **	-	-	**1.93 ** **	-	-	11**	86**	227**	**2.9** *	**NS**	NS	NS
ET/P	5.9**	91**	-	-	**1.39 ** **	-	-	12**	30**	182**	**2.8** *	**NS**	NS	NS
SB	-	-	-	-	-	-	-	4.8**	128**	30**	**2.1** **	**1.7** **	56**	1.8**
DTF	392**	4,579**	980**	**20** **	**27** **	1,212**	NS	1,192**	42**	1,005**	**44** **	**8.1** **	65**	7.5**
PH	2,330**	4,923**	2,70,392**	**325** **	**161** **	23,42**	NS	4,955**	8740**	3,773**	**152** **	**68.5** **	452**	56.3**
PL	18.4 **	578 **	5,551 **	**6.3 ** **	**3.9 ** *	374**	NS	28.5**	219.3**	156**	**9.2** **	**5.2** **	14.4 **	4.4 **
FLL	141.7 **	7,005 **	9,196 **	**21.8 ** **	**15.6 ** **	3,195 **	14.8 *	221**	1,764 **	444 **	**42 ** **	**16.2** **	534 **	10.6 **
FLW	0.2**	8.9**	7.7 **	**0.06** **	NS	1.4**	NS	0.34**	2.5**	2.7 **	**0.06** **	NS	0.3**	NS

FLA	277 **	1,7476 **	1,7026 **	**67****	**39.5****	41,189**	34**	458**	5,204**	1,678**	**119****	NS	778**	NS
PLL	364 **	1,1429 **	10,888 **	**36.3 ****	**23****	4,736**	19.1 *	453 **	1,148**	NS	**60.2****	NS	224.9**	NS
PLW	0.12 **	9.2 **	5.5 **	**0.05 ****	**NS**	1.7 **	NS	0.4**	1.5**	1.1 **	**0.1****	NS	0.26	NS
SL	2.29 **	1.2 **	5.6 **	**0.22 ***	**0.5 ****	1.4 **	0.2 *	2.69 **	1.61 **	10.04**	**0.37 ****	**0.30****	0.39 **	0.23 **
SB	0.26 **	0.8 **	NS	**0.16 ****	**0.2 ****	NS	0.14**	0.08 **	0.06 **	0.63**	**0.03****	**0.03 ****	0.07 **	0.03 **
SLB	0.79 **	0.8**	NS	**0.10 ****	**0.2 ****	NS	0.11 **	0.35 **	0.29 **	NS	**0.19 ****	**0.12 ****	0.25 **	0.09 **
100 SW								0.83 **	0.07 **	1.83 **	**0.09 ****	**0.09 ****	0.03 *	0.07 **
SF	351 **	1,938**	2,83,87 **	**409.3 ****	**173.8 ****	677**	172 **							
SS	354**	1,994**	2,8356 **	**410 ****	**176****	611**	173**							

* and ** significance at .05, .01 levels respectively, GY=grain yield, BG= biological yield, HI= harvest index, TT= total tillers, DTF= days to 50 % flowering, TT/P= total tiller per plant, ET/P= effective tiller per plant, SB= seedling biomass, PH= plant height, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, FLA=flag leaf area, PLL= penultimate leaf length, PLW= penultimate leaf width, SL= seed length, SB= seed breadth, SLB= seed length and breadth ratio, SF= spikelet fertility, SS= spikelet sterility.

4.3.2. Mean performance of genotypes

The mean phenotypic performance of RILs and their parents for 19 characters recorded during wet season 2014 and 2015 across differential N regimes and two environments is presented in table 4.4 & 4.5. The mean values of two parents showed significant difference for almost 16, out of all evaluated traits. The phenotypic values for these traits exhibited broad and continuous variation among the 122 RILs and significant transgressive segregation for both the parents, which might be attributed to the different background of the two parents and the polygenic inheritance of the trait. The coefficient of variation (CV) ranged from 6 to 48 % for most of the traits under all sets of conditions, with the only two traits seed length (averaged 3 %) and spikelet sterility (66 %) showing extreme values.

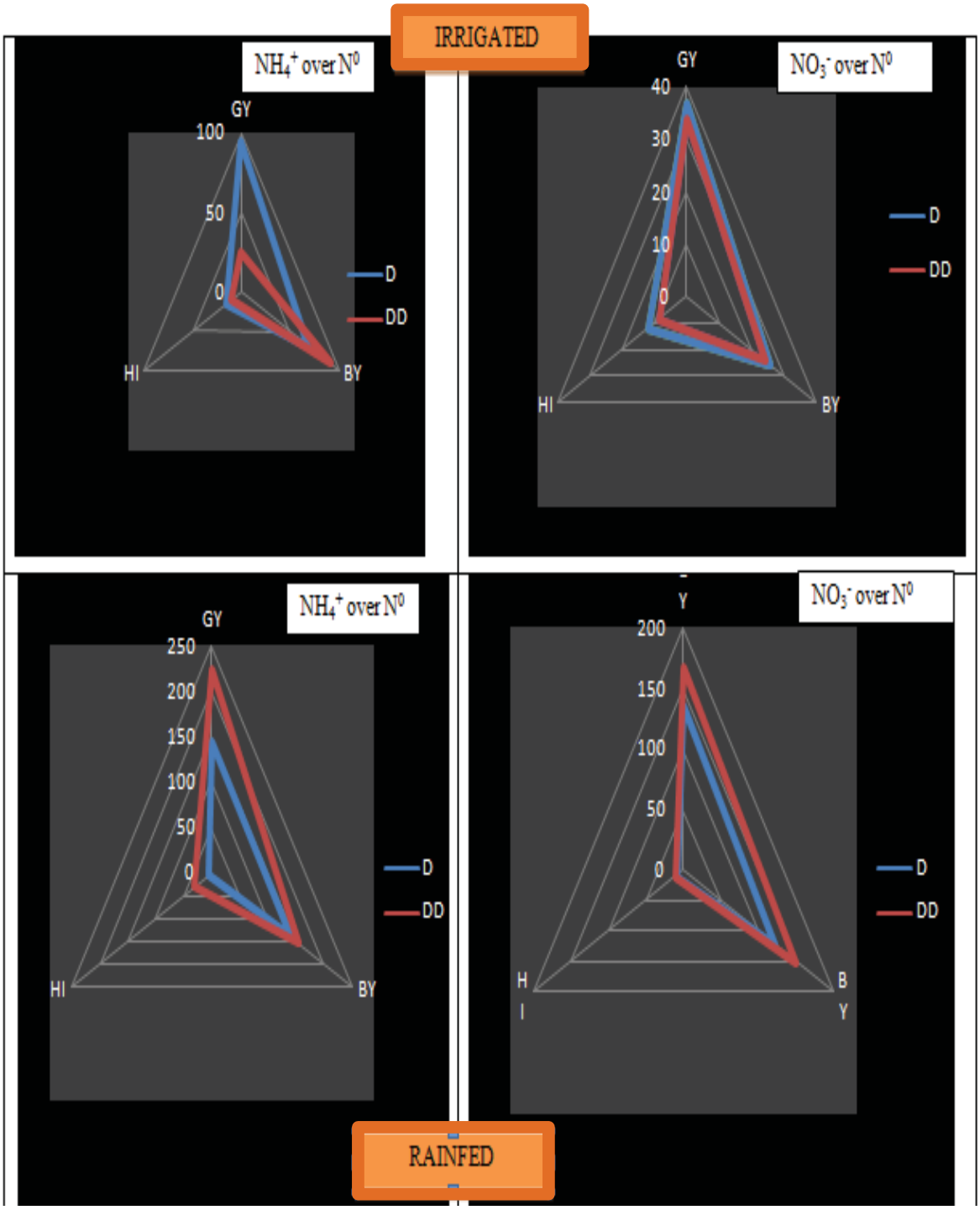
Higher values were observed for all the studied traits at the NH_4^+ treatment followed by NO_3^- and N^0 treatment across both environments. The radial graph depicting phenotypic difference between two parental lines for agronomically important traits *i.e.* grain yield, biological yield and harvest index under ammonium, nitrate treatment over control treatment across two environments during wet season 2014 is depicted in fig. 4.3. Among all the traits, grain yield, biological yield, harvest index, total tillers/m^2, plant height, days to 50 % flowering that showed significant variation across varied sets of conditions & are elaborated here. During wet season 2014, under irrigated condition in NH_4^+ treatment, grain yield ranged from 37.3 to 794 (g/m^2) with a mean value of 317.1 g/m^2. The range of variation for biological yield was 508 to 1496 (g/m^2) with an average value of 998.9 g/m^2. Harvest index of the genotypes varied from 6.4 to 64.1 (%) with mean value of 38.4%. Total tiller/m^2 values ranged from 235 to 568 (g/m^2) with a mean phenotypic performance of 361.6 g/m^2. The range of variation for plant height was 73.6-170.4 (cm) with an average value of 117.1 cm. Days to 50% flowering varied from 62.5-107 (days) with a mean value of 80.9 days. In NO_3^- treatment, grain yield ranged from 55 to 849 (g/m^2) with an average value of 311.3 g/m^2. Biological yield values ranged from 392 to 2561(g/m^2) with mean phenotypic value of 703 g/m^2. The range of variation for harvest index was 12.5 to 54.3 (%) with average value of 37.7 %. Total tiller/ m^2 values ranged from 123 to 495 (g/m^2) with a mean of 303.6 (g/m^2). Plant height varied from 72.4 to 157.9 (cm) with mean phenotypic

value of 108 cm. Days to 50 % flowering ranged from 66 to 103 (days) with average value of 82.7 days. In N^0 treatment, grain yield ranged from 30 to 673 (g/m^2) with a mean phenotypic value of 352 g/m^2 while biological yield ranged from 415 to 1490 g/m^2 showing average value of 671 g/m^2. The range of variation for harvest index was 4.9 to 64 (%) with a mean phenotypic value of 40 %. Total tillers/m^2 ranged from 104 to 271 (g/m2) with an average value of 190 g/m^2. Plant height varied from 63 to 120 (cm) with an average value of 111.3 cm. Days to 50% flowering ranged from 58 to 102 days with mean value of 75 days. Under rainfed condition, in NH_4^+ treatment, value of grain yield ranged from 47 to 486 (g/m^2) with a mean phenotypic value of 241.1 g/m^2. The range of variation for biological yield was 447 to 1755 with mean phenotypic value of 933.1 (g/m^2). Harvest index ranged from 7.5 to 56.3 (%) showing average value of 25.9 %. Total tiller/m^2 ranged from 262 to 636 (g/m^2) with mean value of 450.8 (g/m^2). Days to 50% flowering ranged from 65.1 to 104.5 days with average value of 80.5 days while plant height values ranged from 61.2 to 146.1 (days) with mean value of 102.6 days. In NO_3^- treatment, the range of variation for grain yield was from 15 to 447 (g/m^2) with exhibiting average value of 179.1 g/m^2. The range of variation for biological yield was from 185 to 1231 (g/m^2) with mean phenotypic values of 656.1 (g/m^2). The range of variation for harvest index was from 3.12 to 42.6 (%) with mean phenotypic value of 26 %. Total tiller/ m^2 ranged from 168 to 984 (g/m^2) with mean value of 393 g/m^2. Days to 50 % flowering ranged from 74.3 to 98.1 (days) with mean phenotypic value of 87 days. The range of variation for plant height was from 57.2 to 122.7 (cm) with average value of 86.2 cm. In N^0 treatment, grain yield varied from 14 to 165 (g/m^2) with an average value of 57.2 g/m^2. The range of variation for biological yield was from 118 to 515 (g/m^2) with mean value of 274.4 g/m^2. The harvest index ranged from 8.6 to 36.1 (%) with mean phenotypic value of 20.1 %. Total tiller/ m^2 ranged from 124 to 464 (g/m^2) with average value of 272 g/m^2. Days to 50 % flowering ranged from 58.1 to 104.2 (days) with mean phenotypic value of 79.6 days. The range of variation for plant height was from 47.3 to 99.7 (cm) with mean value of 71.6 cm.

During wet season 2015, under irrigated condition average phenotypic value recorded in NH_4^+ treatment for grain yield (g/m^2), biological yield (g/m^2),

harvest index (%), Days to 50% flowering (days), plant height (cm), effective tiller/plant, total tiller/ plant was 284, 849, 34, 76.4, 106, 6.9 & 8.1 respectively. Mean phenotypic performance values estimated in NO_3^- treatment for grain yield (g/m^2), biological yield (g/m^2), harvest index (%), Days to 50% flowering (days), plant height (cm), effective tiller/plant, total tiller/ plant 233, 674, 35.2, 78.4, 97.7, 6.2 & 6.7 respectively. Mean phenotypic performance values recorded in N^0 treatment for grain yield (g/m^2), biological yield (g/m^2), harvest index (%), Days to 50% flowering (days), plant height (cm), effective tiller/plant, total tiller/ plant was 221, 613, 35, 78.4, 96.1, 6.2 & 6.5 respectively. Within rainfed condition mean phenotypic values for grain yield (g/m^2), biological yield (g/m^2), harvest index (%), Days to 50% flowering (days), plant height (cm), total tiller/ m^{-2} under NH_4^+ treatment was 136, 588, 23, 76, 99 & 400 respectively. Mean phenotypic performance for grain yield (g/m^2), biological yield (g/m^2), harvest index (%), Days to 50% flowering (days), plant height (cm), total tiller/ m^{-2} under NO_3^- treatment recorded as 125, 505, 23, 76.8, 92 & 226 respectively. In N^0 treatment, mean phenotypic performance for grain yield (g/m^2), biological yield (g/m^2), harvest index (%), Days to 50% flowering (days), plant height (cm), total tiller/ m^{-2} was observed as 88, 375, 22, 78, 82 & 226 respectively. Under terminal stage drought, mean phenotypic performance values recorded in NH_4^+ treatment for grain yield (g/m^2), biological yield (g/m^2), harvest index (%), Days to 50% flowering (days), plant height (cm), effective tiller/plant, total tiller/ plant was reported as 130, 528, 24, 80, 101, 9 and 8 respectively. Mean phenotypic performance values in NO_3^- treatment for grain yield (g/m^2), biological yield (g/m^2), harvest index (%), Days to 50% flowering (days), plant height (cm), effective tiller/plant, total tiller/ plant was observed as 90, 365, 24, 81, 90, 8.6 and 7.8 respectively. Mean phenotypic performance values recorded in N^0 treatment for grain yield (g/m^2), biological yield (g/m^2), harvest index (%), Days to 50% flowering (days), plant height (cm), effective tiller/plant, total tiller/ plant was 89, 327, 27.9, 81, 89, 7.8 and 7.2, respectively.

During wet season 2014 and 2015 statistical significant differences between the three nitrogen regimes and two environments for the evaluated traits was established using the three parameters range, mean and coefficient of variation. In



D= Danteshwari, DD= Dagad deshi

Fig.4.3: The radial graph depicting phenotypic difference between two parental lines under ammonium, nitrate treatment over control treatment across two environments during *kharif* 2014

Table 4.4: Mean phenotypic performance, range, standard deviation (SD), Coefficient of variance (CV %) of investigated yield and yield related traits of 122 RILs and their parents under differential N regimes and environments during wet season 2014

Traits	N	Wet season 2014									
		IRRIGATED (I)					RAINFED (Ds)				
		RILs					RILs				
		D	DD	Mean±SEm	Range	CV %	D	DD	Mean±SEm	Range	CV%
GY	NH_4^+	625.52	499.0	**352.1± 14**	37.3-844	48	100	256	**241.1±7**	47- 486	37
	NO_3^-	375	323	338.3±10	55-842	36	96	212	179.1±8	15-447	55
	N^0	320	242	312.1±11	30-673	37	41	79	57.2±2	14-165	48
BY	NH_4^+	1266	923	**1065.6±20**	415-1644	24	543	826	**933.1±16**	447-1755	21
	NO_3^-	796	695	908.5±20	392-2561	28	500	810	651.6±15	185-1231	29
	N^0	752	560	905.6±13	492-1496	17	225	324	274.41±7	118-515	30
HI	NH_4^+	49	46	**37.4±1**	6.4-56.1	34	20	30	**27.1±0.6**	7.3-56.3	30
	NO_3^-	47	46	34.7±0.7	11.4-59.6	21	19	26	26.0±0.7	3.1-42.6	32
	N^0	42	43	32.6±0.8	4.9-54.3	26	17	24	20.1±0.4	8.6-36.1	27
TT/m^2	NH_4^+	392	242	**361.6±4**	238-568	16	483	281	**450.8±6**	262-636	17
	NO_3^-	358	206	303.6±5	123-495	20	393	393	401±7	168-984	23
	N^0	190	105	170.2±2	104-271	16	197	258	272±4	124-464	20
TT/P	NH_4^+	8.5	5.5	**7±0.1**	13.1-5.0	20					
	NO_3^-	7.8	4.3	6±0.1	11.2-4.0	21					
	N^0	5.9	3.5	5.7±0.1	8.8-2.2	22					
ET/P	NH_4^+	8	5	**6.4±0.1**	2.4-10	22					
	NO_3^-	7	3	5.3±0.1	2.4-8.2	22					
	N^0	6	4	5.7±0.1	2.2-8.2	18					
DTF	NH_4^+	79	76	80.9±0.6	62.5-107	9	83	72	80.5±0.5	65.1-104.5	8

	NO_3^-	80	76	**82.7±0.6**	66-130	9	87	88	**87±0.4**	74.3-98.1	6
	N^0	77	75	79.5±0.6	58-102	9	77	75	79.6±0.6	58.1-104.2	9
PH	NH_4^+	104	147	**117.1±1.6**	73.6-170.4	16	79	98	102.6±1	61.2-146.1	17
	NO_3^-	91	136	108.1±1.3	72.4-157.9	14	87	88	**86.2±1**	57.2-122.7	16
	N^0	92	139	111.3±1.5	63.2-220.8	17	61	77	71.6±0.9	47.3-99.7	15
PL	NH_4^+	25	26	**23.8±0.1**	17.5-27.6	7	19	26	**21.7±0.2**	16.3-30.1	10
	NO_3^-	24	26	23.2±.1	19.85-27.2	6	17	22	19.7±0.8	15.3-32.3	10
	N^0	24	25	23.4±0.1	18.5-26.5	7	18	18	18.1±0.1	14.2-26	10
FLL	NH_4^+	30	37	**30.8±.04**	21.8-44.5	15	26	28	**29.4±0.5**	20.5-55.5	18
	NO_3^-	28	38	28.6±0.3	21.2-39.5	13	22	35	26.2±0.4	17.4-43.7	19
	N^0	27	36	**28.2±0.3**	20.1-38.4	14	14	17	17.9±0.3	8.25-28.1	17
FLW	NH_4^+	1.1	1.4	1.4±0.02	0.98-2	12.9	1.3	1.5	**1.3±0.1**	1.02-2	21
	NO_3^-	1.2	1.8	1.3±.02	0.97-1.92	14.2	1.2	1.5	1.1±0.1	0.7-1.1	18
	N^0	1.2	1.2	1.2±0.01	1-2.6	16	0.8	1.1	1.00±0.02	0.7-2.3	20
FLA	NH_4^+	24	40	**32.2±0.6**	18.8-52.0	20	26	33	**29.9±0.7**	17.4-83.2	28
	NO_3^-	25	50	27.2±0.5	16.1=50.4	21	20	38	22.7±0.6	9.1-62.7	32
	N^0	23	33	26.2±0.5	17.2-52.2	20	8	14	13.4±0.3	2.6-33.7	30
PLL	NH_4^+	37	51	**44.3±0.6**	30.2-62.3	15	40	43	**42.5±0.6**	29.2-65.6	18
	NO_3^-	36	47	40.1±0.5	29.4-56.5	13	30	49	38.3±0.6	23.2-61.8	20
	N^0	36	41	40.2±0.5	28.7-56.9	13	22	29	28.4±0.4	20-40.4	17
PLW	NH_4^+	1	1.1	**1.2±0.01**	0.8-1.6	12	1.1	1.2	**1.1±0.02**	0.69-2.2	17
	NO_3^-	1	1.2	1.0±0.01	0.7-1.3	12	0.9	1.1	0.9±0.02	0.57-1.9	22
	N^0	0.9	1.2	1.0±0.01	0.7-1.3	11	0.6	0.7	0.7±0.01	0.49-1.3	18
SL	NH_4^+	9	9	9.1±.04	7.5-10.2	6	9	8	9.2±0.05	7.7-10.6	6
	NO_3^-	9	9	9.2±0.05	7.5-11.2	6	9	9	9.1±0.04	7.65-10.7	6
	N^0	10	8	9.1±0.04	7.4-10.3	6	10	8	9.1±0.04	7.4-10.3	6

SB	NH_4^+	2	3	2.5±.01	2-2.95	7	2	3	2.4±0.01	2.1-2.9	11
	NO_3^-	2	3	2.6±0.01	2.1-3.1	7	2	3	3.5±0.01	2.1-3.2	7
	N^0	2	3	2.6±0.01	2.1-3.2	7	2	3	2.6±0.01	2.1-3.2	7
SLB	NH_4^+	4	3	3.7±.02	2.6-4.4	9	4	3	3.6±0.03	2.39-4.84	11
	NO_3^-	4	3	3.6±.04	1.3-4.7	11	4	4	3.6±0.1	2.85-4.60	10
	N0	4	3	3.6±0.03	2.5-6.1	12	4	3	3.5±0.1	2.58-4.62	10
SF	NH_4^+	71	52	**74.8±1**	49-89.1	18	85	86	**80.9±0.7**	55.2-96.3	11
	NO_3^-	60	73	74.7±0.7	48.8-88.8	11	59	98	79.6±0.8	50.3-97.6	11
	N0	72	80	71.1±1	17.2-90.1	18	65	78	79.6±1.1	30.2-95.4	17
SS	NH_4^+	29	48	**31.3±1**	7.0-86.8	40	15	14	19.1±0.8	3.7-44.7	47
	NO_3^-	40	27	25.1±0.7	10.1-41.1	32	41	5	20.4±0.8	55.2-96.3	45
	N0	28	20	28.1±1.07	9.8-82.7	45	35	22	20.4±1.1	4.6-69.8	66

GY=grain yield, BG= biological yield, HI= harvest index, TT= total tillers, DTF= days to 50 % flowering, TT= total tiller, ET= effective tiller, SB= seedling biomass, PH= plant height, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, FLA=flag leaf area, PLL= penultimate leaf length, FLW=flag leaf width, FLA=flag leaf area, PLL= penultimate leaf length, PLW= penultimate leaf width, SL= seed length, SB= seed breadth, SLB= seed length and breadth ratio, SF=Spikelet fertility.

irrigated and rainfed environment during both seasons all the genotypes showed higher values for agronomically important traits i.e. grain yield, biological yield, plant height and tillers under NH_4^+ treatment followed by NO_3^- and N^0 treatment. From these results we speculate that N in varied forms substantially influences important yield parameters with NH_4^+ form recording more mean values for important yield traits as compared to NO_3^- and N^0 thus inflicting genotypes to be NH_4^+, NO_3^- and N^0 responsive. These findings collaborates with the study of Singh *et al.* (2015) who evaluated the genotypic variation among 5 rice genotypes at 4 nitrogen availability in relations to grain yield, biological yield, and panicle weight.

Similarly, Chaturvedi *et al*. (2005) reported earlier that more number of tillers in experiment might be due to the more availability of nitrogen, which played a vital role in cell division. Similarly, it was reported that the maximum number of tillers were at N_{200} level and the minimum at N_0 level by Meena *et al*. (2003). Also, Fageria and Baligar (2001) reported a quadratic relationship between rice grain yield and dry matter yield of shoot. Kamoshita *et al.* (1998) studied genotypic variation for grain yield and related traits among sorghum hybrids under different level of N fertilizer and water supply. They reported significant genotypic variation among sorghum cultivar and asserted that under rainfed condition, phenology was important for determining 40% genotypic variation in yield. Further, Fageria *et al*. (1995; 1995) reported significant yield differences among upland rice genotypes under low, medium and high fertility levels in Brazilian oxisol under field conditions. Similarly, Frageria (2008) reported that panicle number per unit area as one of the most important yield components in determing yield of upland rice genotypes. In converse, Samonte *et al*. (2006) pointed out that representative varietal variation in yield and nitrogen use efficiency was complex because rice yield was influenced by inherent factors such as the number of productive culms, grains per panicle and 1000 grain weight, in addition to plant management conditions.

Table 4.5: Mean phenotypic performance, range, standard deviation (SD), Coefficient of variance (CV %) of investigated yield and yield related traits of 32 selected RILs and their parents under differential N regimes and environments during wet season 2015

Traits	N	Wet season 2015														
		IRRIGATED (I)					RAINFED (R)					TERMINAL STAGE DROUGHT (TSD)				
		RILs					RILs					RILs				
		D	DD	Mean±SEm	Range	CV %	D	DD	Mean±SEm	Range	CV %	D	DD	Mean±SEm	Range	CV%
GY	NH_4^+	316	224	**284±17.1**	117-522	34.1	80	321	**136±8**	48-261	36	113	135	**130±8.6**	20-260	37
	NO_3^-	292	241	233.3±8.9	135-381	22	71	239	125±10	29-248	46	62	103	90±4.6	37-177	29
	N^0	223	106	221.3±12.9	90.6-383	33	52	162	88±6	20-162	39	70	115	89±4.5	50-179	32
BY	NH_4^+	640	594	**849.9±41.0**	372-1410	27.3	291	962	**588±27**	317-1042	26	439	497	**528±11**	398-665	12
	NO_3^-	631	494	674±31.3	427-1388	26	362	724	505±23	247-740	26	257	396	365±8.2	257-432	13
	N^0	514	221	613±26.1	310.4-921	24	208	518	375±15	151-546	23	271	279	327±8.3	255-424	15
HI	NH_4^+	49	37	**37±1.2**	11.4-46.7	19.7	27	33	**23±0.9**	9.4-36.3	23	26	27	**27±1.4**	4-49	34
	NO_3^-	43	40	35.2±0.8	25.2-43.3	13	19	32	22±1.0	11.9-35	23	24	26	24±1.0	9.6-40	24
	N^0	42	36	35.5±1.2	19.2-40.3	19	25	30	21±1.0	13-33	25	26	36	23±1.3	5-38	22
TT/m^2	NH_4^+						429	357	**440±13**	295-617	17					
	NO_3^-						362	724	394±7.7	139-301	19					
	N^0						488	323	226±13	275-569	20					
TT/P	NH_4^+	9	5.3	**8.1±0.28**	5.0-12.0	19						12	6	**9±0.2**	6.3-12	14
	NO_3^-	7.5	3.6	6.76±0.2	3.6-9.5	19						10	5.8	8.6±0.1	5.8-11	13
	N^0	6.7	3.4	6.5±0.2	3.4-9.4	19						8.3	5.5	7.8±0.1	5.5-10	12
ET/P	NH_4^+	8.5	5	**6.9±0.3**	3-9.9	25						12	6	**8±0.2**	5.6-11	16
	NO_3^-	7.3	3.6	6.2±0.2	3.6-9.3	19						8.5	6	7.8±0.1	5.9-9.7	13

SB	N^0	6.1	3.4	6.2±0.2	3.4-8.5	19						8	5.1	7.2±0.1	5.1-9.2	15
	NH_4^+	2.2	4.2	**3.85±0.28**	2.0-9.3	33	5.8	6.5	**5.0±0.2**	2.4-7.9	26					
	NO_3^-	1.3	3.5	2.9±0.10	1.3-3.5	28	2.9	5	3.4±0.1	2.0-6.1	31					
DTF	N^0	2.38	2.53	3.1±0.1	1.9-4.8	24	1.5	2.2	2.2±0.1	1.2-3.3	24					
	NH_4^+	80	70	76.4±1.4	66-102.5	11	79	67	76±1.6	62.5-102.5	17	75	80	80±1.5	71-102	11
	NO_3^-	79	70	78.4±1.5	67.5-104	10	79	67	76±1.6	65-104	11	77	78	81±1.4	71-103	10
	N^0	76	68	80.4±1.5	66.5-103	11	83	68	78±1.4	68-100	11	77	79	81±1.4	71-102	10
PH	NH_4^+	79	128	**106±3.7**	74-147	20	81	130	**99±3.2**	73.6-135	12	76	128	**101±3.3**	73-141	18
	NO_3^-	75	116	97.7±3.3	64.6-132	19	77	122	92±3.1	64-125	19	70	128	90±2.9	66-129	19
	N^0	81	117	96.1±2.9	64.4-132	17	72	103	82±2.6	63-116	18	65	122	89±2.8	63-123	19
PL	NH_4^+	22	23	**23.4±0.4**	20.3-32.6	10	20	25.5	**22±0.4**	18.7-27.4	10	21	26	**22±0.3**	17.5-26	8
	NO_3^-	22	24	22.5±0.3	19.1-30.3	9	18	24	21±0.4	18-26.4	10	19	21	20±0.3	18-25	9
	N^0	21	22	21.6±0.3	18.3-25.8	7	17	20	19±0.3	14-34	9	20	19	20±0.4	16-26	10
FLL	NH_4^+	26	33	**30.5±0.9**	21.1-44.2	17	24	37	**39±1.0**	19-45	19	22	31	**27±0.5**	21-35	11
	NO_3^-	24	30	26.6±0.6	20.1-35.3	14	28	45	32±1.2	22-52	20	19	25	24±0.5	19-36	14
	N^0	20	29	24.5±0.7	17.8-35.3	16	18	30	21±0.8	14.5-34	21	21	23	24±0.5	19-31	13
FLW	NH_4^+	1.1	1.5	**1.3±0.1**	1.06-1.72	13	1.4	2	**1.4±0.1**	1.2-2	14	1.2	1.7	**1.4±0.03**	0.8-1.7	13
	NO_3^-	1.2	1.4	1.2±0.1	0.9-1.5	13	1.4	1.8	1.3±0.03	22.6-51.9	12	1.1	1.5	1.3±0.03	0.9-1.9	14
	N^0	0.94	1.4	0.9±0.1	0.6-1.3	19	1	1.6	1.2±0.1	0.8-2.1	11	1	1.6	1.3±0.03	0.8-1.7	12
FLA	NH_4^+	23	40	**30.4±1.4**	18.9-54.3	26	27	55	**35.6-1.6**	22-60	27	20	39	**28.1±0.9**	17-39	19
	NO_3^-	22	33	23.8±0.8	16.9-36.7	20	31	63	34.5±1.9	21.1-67.6	31	16	28	24.9±0.8	16-39	19
	N^0	14.5	28.8	17.6±0.9	9.3-29.9	28	13	38	20.8±1.3	20.8-40.9	32	16	29	23±0.7	16-33	20

PLL	NH_4^+	33	48	**38.8±1.2**	29-60.2	18	38	58	**41-1.1**	31-58	16	32	40	**37.6±0.7**	28.9-47.9	11
	NO_3^-	32	44	36.5±1.1	26.6-51.2	17	32	52	38.3±1.2	28-56	18	30	45	36.4±0.4	29.4-47.1	10
	N^0	27	43	34.5±1.2	25.3-51.2	19	26	47	32.5±1.1	23.2-47.4	19	35	43	36±0.7	28-46	12
PLW	NH_4^+	0.8	1.4	**1.07±0.1**	0.51-1.44	16	1.1	1.6	**1.2±0.1**	0.9-3.1	17	0.9	1.3	**1.1±0.03**	0.6-1.4	17
	NO_3^-	1.07	1.1	1.0±0.1	0.7-1.3	14	1.1	1.5	1.1±0.02	0.8-1.6	14	0.7	1.3	0.9±0.03	0.4-1.5	22
	N^0	0.94	1.49	1.0±0.1	0.6-1.5	19	0.9	1.2	0.97±0.1	0.6-1.3	17	0.6	1.2	0.9±0.03	0.63-1.36	21
SL	NH_4^+	9.3	9.6	**9.1±0.1**	7.2-10	7	8.9	8.7	**8.8±0.1**	7.2-10.2	7					
	NO_3^-	8.7	9.7	9.0±0.1	7.3-9.9	6	8.8	8.9	8.7±0.01	7.8-9.2	3					
	N^1	8.1	8.6	8.9±0.1	7.4-10.1	7	9	8.7	8.5±0.1	7.4-9.8	7					
SB	NH_4^+	2.6	2.7	2.6±0.1	2.3-2.9	6	2.4	2.5	2.5±0.02	2.2-2.7	5					
	NO_3^-	2.4	2.5	2.5±0.1	2.3-2.8	5	2.3	2.4	2.5±0.02	2.2-2.6	5					
	N^2	2.5	2.7	2.6±0.1	2.3-2.8	4	2.4	2.7	2.5±0.03	2.1-2.9	7					
SLB	NH_4^+	3.6	3.5	3.4±0.1	3.1-4.1	7	3.6	3.4	3.5±0.0	3.1-4.1	7					
	NO_3^-	3.5	3.8	3.5±0.1	2.9-4.1	8	3.7	3.6	3.5±0.03	3.2-4.0	5					
	N0	3.2	3.1	3.5±0.1	3.1-4.1	7	3.7	3.1	3.4±0.05	2.5-4.0	10					
100SW	NH_4^+	2.3	2.8	2.6±0.1	1.8-3.1	11	2	2.5	2.3±0.05	1.7-3	12					
	NO_3^-	2.3	2.6	2.6±0.5	1.7-3.0	13	1.8	2.5	2.4±0.01	1.6-3	13					
	N0	2.3	2.4	2.5±0.1	1.7-3.0	12	2.1	3	2.4±0.1	1.7-3.1	14					

GY=grain yield, BG= biological yield, HI= harvest index, TT= total tillers, DTF= days to 50 % flowering, TT= total tiller, ET= effective tiller, SB= seedling biomass, CGR= crop growth rate, PH= plant height, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, FLA=flag leaf area, PLL= penultimate leaf length, , FLW=flag leaf width, FLA=flag leaf area, PLL= penultimate leaf length, , PLW= penultimate leaf width, SL= seed length, SB= seed breadth, SLB= seed length and breadth ratio, TT/P= total tiller per plant, ETP= Effective tillers per plant, SB= Seedling biomass.

4.4.3. Variability, Heritability, Genetic advance

Variability parameters of genotypic co-efficient of variation (GCV) and phenotypic co-efficient of variation (PCV) with respect to all the characters in RILs grown in wet season 2014 and 2015 are shown in table 4.6. GCV measures only the extent of genetic variability present for a character, GCV should be considered in combination with heritability and genetic advance while assessing the effect of phenotypic selection. In present investigation, genotypic coefficient of variation (GCV) was less than its corresponding estimates of phenotypic coefficient of variation (PCV) for all estimated traits under all sets of treatment and condition, indicating significant role of environment in the expression of these traits. Overall, high phenotypic coefficient of variability and genotypic coefficient of variability was obtained for grain yield, biological yield, harvest index, seedling biomass and spikelet sterility under all sets of conditions during wet season 2014 and 2015. Those characters having high phenotypic coefficient of variability and genotypic coefficient of variability indicate large scope for selection and improvement in the present set of RILs. Khan *et al.* (2009) reported on rice genotypes that PCV values were higher than GCV values for all important traits considered. Furthermore, The findings were in accordance with Vaishali *et al.* (2003) and Selveraj *et al.*(2011) who studied GCV and PCV for grain yield and other yield attributes.

In present research broad sense heritability estimates for the estimated 19 traits during wet season 2014 ranged from 8.2% to 84.1 % and during wet season 2015 ranged from 24% to 98.4 % under differential N and water regimes. High heritability coupled with genetic advance as per cent mean for the traits were grain yield, biological yield and plant height during wet season 2014 while days to 50% flowering, plant height and 100-seed weight during wet season 2015 under irrigated and rainfed environment. These results agree with the findings of Vikas Kumar *et al*. (2014), Thirumala Rao *et al*. (2014), Afzal *et al.* (2006). According to Singh (2001), high heritability of a trait (≥ 80%) makes selection for such traits fairly easy due to a close correspondence between the variety and the phenotype, due to the relative small contribution of the environment to the phenotype. In other words, if environmental variability is small in relation to genotypic differences,

Table 4.6: Broad sense heritability (h^2), genetic advancement (GA), phenotypic coefficient of variation (PCV) & genotypic coefficient of variation (GCV) for yield and yield related traits during wet season 2014 and 2015

Traits	N	Wet season 2014								Wet season 2015							
		IRRIGATED (T)				RAINFED (Ds)				IRRIGATED (I)				RAINFED (Ds)			
		h^2	GA (% mean)	GCV	PCV	h^2	GA	GCV	PCV	h^2	GA (% mean)	GCV	PCV	h^2	GA (% mean)	GCV	PCV
GY	NH_4^+	71.4	76.91	44.15	52.22	54.88	46.93	30.75	41.5	71.1	53.8	31	36.8	61.4	45	30.4	42.4
	NO_3^-	71.4	57.47	33.01	39.05	71.9	88.47	50.65	59.73	46.6	24.4	27.4	25.4	64.1	48.5	41.5	51.8
	N^0	81.5	64.75	34.82	38.57	51.05	58.62	39.83	55.74	42.3	34.3	25.6	39.3	56.8	51.5	33.2	44
BY	NH_4^+	71.79	36.91	26.14	28.95	47.34	23.88	16.84	24.48	65.8	40.5	24.4	29.9	71.1	41.9	24.1	28.6
	NO_3^-	72.74	45.45	25.86	30.32	36.7	25.49	20.42	33.71	68.4	40.4	23.7	28.6	65.2	39.5	23.7	29.4
	N^0	76.48	26.18	15.59	19.12	37.54	28.12	22.28	36.36	24	15.1	24.9	30.5	54.3	30.3	19.9	27.1
HI	NH_4^+	57.9	45.65	29.12	38.27	39.49	28.8	22.25	35.4	69.7	29.6	17.2	20.6	55.6	31	20.1	27
	NO_3^-	53.84	27.13	27.95	24.46	68.71	49.66	29.08	35.08	32.4	10.6	9.1	15.9	56.4	31.5	20.3	27.1
	N^0	65.2	38.15	22.94	28.4	47.15	31.08	21.97	31.99	66	28.5	17.1	20.9	75.5	42.5	23.7	27.3
TT/m^2	NH_4^+	58.47	21.34	13.55	17.72	42.6	17.9	13.31	20.39					62.6	17.9	13.3	20.4
	NO_3^-	52.9	25.6	17.08	23.49	28.87	17.06	15.41	28.68					60.5	26.9	16.8	21.6
	N^0	58.73	22.29	14.12	18.42	26.31	13.37	12.65	24.66					59.5	25.3	15.9	20.6
SB	NH_4^+									37	85.6	42.2	42.8	36.6	24.3	19.5	32.2
	NO_3^-									18.9	14.1	15.8	36.4	27.9	22.3	20.5	38.8
	N^0									36.9	22.5	17.9	29.6	33.3	20.9	17.6	30.6
TT/P	NH_4^+	55.8	24.7	19.5	25.1					55.5	25.3	16.5	22.1				
	NO_3^-	43.9	21.9	17.1	28.7					43.9	20.6	15.1	22.7				
	N^0	53.8	25.2	18.1	27.1					53.2	24.2	16.1	22.1				
ET/P	NH_4^+	47.91	25.78	18.08	26.12					71.9	40.5	23.2	27.3				
	NO_3^-	48.74	25.04	17.41	24.93					41.5	15.2	23.6	20.2				
	N^0	38.17	16.58	13.03	21.09					55.1	24.8	16.2	21.8				
DTF	NH_4^+	82	10.9	7.33	10.17	83.63	14.24	7.55	8.26	96.2	21.4	10.6	10.7	98.2	24.9	12.2	12.3
	NO_3^-	82.28	12.97	6.94	7.65	68.87	8.53	4.99	6.01	98.3	22.3	10.9	11	96.5	23.7	11.7	11.9
	N^0	74.16	14.28	8.05	9.35	74.32	14.36	8.08	9.37	98.4	22.4	10.9	11	97.1	21.5	10.6	10.7

PH	NH_4^+	**84.82**	**29.29**	15.44	16.76	45.04	18.34	13.26	19.77	**87.9**	**36.6**	18.9	20.2	**88.3**	**34.2**	17.6	18.8
	NO_3^-	**84.46**	**26.09**	13.78	14.99	66.4	26.09	15.54	19.07	**95.1**	**38.1**	18.9	19.4	**89.2**	**36.4**	18.7	19.8
	N^0	**50.9**	**21.03**	14.31	20.06	53.37	19.06	12.66	17.33	**88.6**	**32.1**	16.5	17.5	**91.1**	**34.9**	17.7	18.6
PL	NH_4^+	55.98	9.35	6.06	8.1	38.3	9.74	7.64	12.35	28.8	7.39	6.7	12.4	49.4	12	8.3	11.7
	NO_3^-	33.86	5.42	4.52	7.78	18.71	5.07	5.69	13.15	26.4	6.5	6.1	11.9	53.2	12.4	8.3	11.3
	N^0	59.5	9.7	6.1	7.91	16.49	4.31	5.15	12.69	23.2	10.8	6.6	8.3	50.4	11.4	7.8	10.9
FLL	NH_4^+	55.66	19.92	12.96	17.37	61.49	25.92	16.04	20.46	64.1	25.2	15.3	19	65.8	28.3	16.9	20.8
	NO_3^-	44.49	14.08	10.25	15.37	61.69	27.08	16.73	21.3	57.3	19.6	12.5	16.5	65.6	31.2	18.6	23
	N^0	63.6	19.78	12.04	15.09	45.53	17.43	12.54	18.58	73.9	26.6	15	17.4	84.4	37.4	19.7	21.5
FLW	NH_4^+	56.27	25.9	10.91	14.55	8.77	5.05	8.27	27.93	58.2	17.3	11	14.4	61.3	20.6	12.7	16.3
	NO_3^-	64.71	20.92	12.62	15.69	51.81	22.36	15.08	20.95	70.3	21.3	12.3	14.7	62.2	18.0	11.1	14.0
	N^0	41.57	16.28	12.26	19.01	38.85	18.93	14.74	23.65	83.2	34.6	18.4	20.1	9.4	5.7	9	29.3
FLA	NH_4^+	54.43	24.02	17.04	23.09	30.76	25.63	22.43	40.45	69.9	41	23.8	28.5	67.6	42.3	**24.9**	**30.3**
	NO_3^-	60.28	29.2	18.3	23.58	62.74	45.81	28.07	35.44	61.6	29.2	18.1	23	71.4	46.1	**26.5**	**31.3**
	N^0	50.37	24.39	16.68	23.5	52.04	36.95	24.86	34.46	63.3	51.4	27.3	29.9	46.8	42.1	**29.8**	**43.6**
PLL	NH_4^+	71.48	11.48	13.79	16.31	63.06	26.25	16.05	20.21	72.1	28.7	16.4	19.3	76.5	27.5	15.2	17.4
	NO_3^-	74.06	21.83	12.31	14.31	72.58	32.51	18.52	21.74	79.1	30.2	16.5	18.5	77.8	31.3	17.2	19.5
	N^0	67.24	20.23	11.97	14.6	56.86	22.79	14.67	19.46	79.2	33.8	18.4	20.7	79.3	34.3	18.7	21
PLW	NH_4^+	39.15	6.25	8.91	14.24	20.07	9.25	10.02	22.36	61.5	22.2	13.7	17.5	23.4	18.5	18.5	38.4
	NO_3^-	49.68	13.6	9.36	13.29	32.93	18.4	15.57	27.13	50.4	17.1	11.6	16.3	51.9	17.6	11.8	16.4
	N^0	44.16	11.35	8.29	12.48	37.48	17.01	13.53	22.1	37.8	29	22.9	37.2	52.2	21.9	14.7	20.4
SL	NH_4^+	44.42	5.65	4.55	6.83	56.98	9.96	6.4	8.48	70.9	11.5	6.6	7.8	96.3	14.1	6.9	7.1
	NO_3^-	43.05	8.32	6.15	9.38	59.66	8.53	5.36	6.94	96.8	14.1	6.9	7	96.1	14.4	7.1	7.6
	N^0	65.25	8.92	5.36	6.63	65.25	8.92	5.36	6.63	89.4	13.3	6.8	7.2	97.6	13.8	6.7	6.8
SB	NH_4^+	27.57	9.75	5.22	9.95	51.35	8.41	5.7	7.95	38.7	5.5	4.3	6.9	71.9	9.1	5.2	6.2
	NO_3^-	45.44	7.98	5.75	8.53	96.45	48.64	24.04	24.48	32.8	8.2	4.7	5.5	72.4	9.8	5.6	6.5
	N^1	40.45	18	13.74	21.61	32.28	5.49	4.69	8.26	35.1	3.8	3.1	5.3	72.3	11.1	6.3	7.4
SLB	NH_4^+	44.79	20.13	7.07	10.56	57.88	15.16	9.67	12.71	63.1	5.7	4.8	8.3	83.6	14.2	7.5	8.2
	NO_3^-	57.75	14.9	9.52	12.52	71.17	17.52	10.08	11.95	88.3	15.4	7.9	8.4	82.3	14.6	7.5	8.4
	N^2	60.71	14019	8.84	11.34	60.71	14.19	8.84	11.34	68.2	11.2	6.6	7.9	81	16.8	9.1	10.1

100SW	NH_4^+									**84.1**	**29.6**	10.4	11.3	**96.8**	**25.5**	12.6	12.8
	NO_3^-									**93.1**	**26.8**	13.4	13.9	**97.8**	**27.8**	13.6	13.8
	N^2									**83.7**	**21.7**	11.5	12.6	**97**	**29.1**	14.3	14.5
SF	NH_4^+	46.51	44.48	14.33	21.01	56.8	14.58	9.39	12.46								
	NO_3^-	48.82	42.64	14.32	14.54	21.65	6.57	6.86	14.74								
	N^3	48.82	22.54	15.66	22.41	90.24	32.17	16.44	17.3								
SS	NH_4^+	46.51	13.86	**31.66**	**46.42**	56.8	61.93	**39.88**	**52.92**								
	NO_3^-	46.91	43.93	**32.89**	**43.19**	22.44	26.66	**27.31**	**57.66**								
	N^4	43.37	47.79	**35.22**	**53.48**	90.24	25.59	**64**	**67.37**								

GY=grain yield, BG= biological yield, HI= harvest index, TT= total tillers, DTF= days to 50 % flowering, TT= total tiller, ET= effective tiller, SB= seedling biomass, PH= plant height, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, FLA=flag leaf area, PLL= penultimate leaf length,PLW= penultimate leaf width, SL= seed length, SB= seed breadth, SLB= seed length and breadth ratio, SF= spikelet fertlity, SS= spikelet sterility.

Table 4.7: Potential change in yield of slected 32 rice genotypes under different N regimes during wet season, 2015

G	IRRIGATED (I)								RAINFED (RF)								TERMINAL STAGE DROUGHT (TSD)							
	Grain yield (g/m^2)			Potential change (%)			Increase/decrease (%)		Grain yield (g/m^2)			Increase/decrease (%)			Increase/decrease (%)		Grain yield (g/m^2)			Potential change (%)			Increase/decrease (%)	
	NH_4^+	NO_3^-	N^0	NH_4^+	NO_3^-	N^0	NH_4^+/N^0	NO_3^-/N^0	NH_4^+	NO_3^-	N^0	NH_4^+	NO_3^-	N^0	NH_4^+/N^0	NO_3^-/N^0	NH_4^+	NO_3^-	N^0	NH_4^+	NO_3^-	N^0	NH_4^+/N^0	NO_3^-/N^0
1	449	412	384	58	8	74	17	7.4	210	168	145	-19	43	71.9	44	0.16	102	85	81	-21	-6	-9	26	4.8
2	288	223	200	1	-4	-10	43	11.6	107	90	76	-17	-23	-9.7	40	0.19	135	90	86	4	0	-4	58	5.1
3	310	215	117	9	-8	-47	163	83	104	88	56	-32	-11	-32.7	83	0.55	117	84	86	-10	-7	-4	36	-1.8
4	347	258	245	22	11	11	41	5.2	159	118	94	22.6	0.55	12.0	68	0.25	142	94	86	9	4	-4	65	9.7
5	210	199	111	-45	-15	-50	89	78	60	52	45	-54	-75	-46.6	33	0.16	95	87	76	-27	-3	-15	24	14.7
6	266	211	216	-7	-10	-2	23	-2.4	143	131	75	10.6	10.9	-11.0	91	0.74	101	65	67	-22	-28	-25	51	-3.1
7	338	310	154	18	-35	-30	119	101	137	103	64	5.84	-13	-23.5	112	0.59	98	68	76	-25	-24	-15	28	-10.6
8	290	265	239	2	14	8	21	10	97.8	81	71	-25	-31	-15.6	37	0.14	104	87	69	-20	-4	-22	50	25.1
9	294	221	155	3	-5	-30	90	42	177	151	95	36.7	28.4	12.6	86	0.59	261	94	82	101	4	-8	219	15.3
10	264	203	164	-7	-13	-26	61	24	126	48	20	-3.2	-59	-76.2	527	1.42	154	111	92	18	24	4	66	20.5
11	259	226	102	-9	-3	-54	153	120	160	145	96	11.8	36	13.9	66	0.51	103	90	97	-21	0	9	5.3	-8.0
12	336	233	144	18	0	-35	133	61	192	154	99	48.3	30.8	17.9	93	0.55	141	97	84	8	8	-6	67	15.8
13	293	250	240	3	-10	9	22	4.4	113	51	59	-13	-57	-29.5	89	-0.14	141	90	78	8	0	-12	80	15.3
14	282	260	201	-1	-6	8	40	29	118	106	83	-8.7	-9.8	-0.50	41	0.26	173	132	127	33	47	42	36	4.3
15	180	177	165	-58	-24	-26	9.4	7.4	156	125	96	-3.6	32.2	13.9	62	0.30	121	97	80	-7	-11	9	51	21.2
16	264	255	210	-7	-10	15	25	21	162	145	96	-3.6	32.2	13.9	68	0.51	169	125	124	30	39	39	36	0.8
17	265	236	211	-7	1	-5	25	12	121	107	48	-62	2.43	26.5	152	1.23	101	79	71	-22	-21	-11	42	11.2
18	253	240	271	-11	3	23	-6.5	-11	126	125	121	-3.2	6.21	43.6	3.6	0.03	119	88	77	-8	-15	-1	54	14.2
19	330	305	300	16	31	36	10	1.7	126	122	121	-3.2	3.85	43.6	3.6	0.01	192	133	120	47	48	35	60	11.3
20	250	228	292	-12	-2	32	-14	-22	184	143	110	42.3	21.8	30.4	67	0.30	158	120	102	21	13	34	54	17.6
21	265	209	183	-7	-22	-5	44	14	84.4	80	65	-35	-32	-22	29	0.24	105	79	71	-19	-13	-20	47	10
22	224	210	138	-22	-10	-37	61	52	131	84	65	-35	11.4	-22	101	0.29	70	70	51	-46	-23	-42	37	35
23	243	245	228	-15	-2	11	6.6	7.5	167	106	86	29	-9.8	2.7	92	0.22	118	87	82	-9	-3	-8	44	6
24	270	258	237	-5	2	17	14	8.9	139	75	39	7.55	-36	-53	253	0.92	176	92	80	35	3	-10	120	15

25	191	185	91	-59	-20	-59	110	104	128	92	64	-1.4	-22	-23	98	0.43	126	76	68	-3	-24	-14	84	11
26	302	254	268	-41	9	21	12	-5	80	71	64	-38	-39	-23	25	0.11	113	70	63	-13	-30	-21	79	11
27	257	233	202	-10	0	-9	27	15	239	221	163	70.5	103	93	46	0.36	135	116	103	4	15	30	30	12
28	324	307	274	14	32	24	18	12									42	51	51	-67	-43	-43	-16	1.0
29	451	432	313	58	-42	42	44	37	125	79	50	-3.6	-32	-40	147	0.57	180	112	109	38	24	23	64	2.3
30	500	450	372	75	50	68	34	21	108	74	71	-17	-37	-15	51	0.05	122	91	91	-6	2	2	34	0.5
31	523	382	234	83	64	6	123	63	261	242	127	92	122	50	105	0.90	240	180	177	85	97	102	35	1.6
32	382	279	276	-56	20	25	38	1									63	27	31	-84	-58	-29	103	-12

G = Genotypes

Dark green colour shows highest value and dark red shows lowest value

E	Genotypes	NH_4^+		Genotypes	NO_3^-		Genotypes	N^0	
		Average Yield kg/m^2	Potential change in Yield (%)		Average Yield kg/m^2	Potential change in Yield (%)		Average Yield kg/m^2	Potential change in Yield (%)
I	Parent-line 29,30,31	409	43.5	Parent-line 29,30,31	349	35.6	Parent-line 29,30,31	281	30.7
	RIL- line 1,12,7,19			RIL- line 4,8,19			RIL- line 1,4,16,19,20,23,24		
R	Parent-line 27,31	192	44.1	Parent-line 27,31	162	43.8	Parent-line 27,31	111	31.7
	RIL- line 4,9,11,12,20,23			RIL- line 1,9,11,12,16,20			RIL- line 1,9,11,12,16,19,20		
TSD	Parent-line 27,29,31	183	41.2	Parent-line 27,29,31	129	40.1	Parent-line 27,29,31	123	34.5
	RIL line 9,10,14,16,17,20,24			RIL line 10,14,16,19			RIL line 14,16,19,20		
	Mean	261.3	**42.9**		213.3	**39.8**		171.7	**32.3**

selection will be efficient because the selected character will be transmitted to its progeny. But selection may be considerably difficult or virtually impractical for traits with low heritability (≤ 40%) due to the masking affect of the environment so that the greater the proportion of the total variability is due to environment. Based on this benchmark, the traits in the present study exhibited low to high heritability estimates. Similar result was reported by Woldeyesus *et al*. (2004) on barely genotypes and by Alemayehu *et al*. (2006) on tef genotypes that broad-sense heritability estimates were higher for grain yield and NUE related traits.

4.4.4. Yield and N- responsiveness of selected rice genotypes

Yield performance, potential change in yield of a genotype with respect to all other tested genotypes and variation in yield over control in NH_4^+, NO_3^- and N^0 treatment under irrigated, rainfed and TSD condition during wet season 2015 is depicted in table 4.7. With the estimation of these parameters, genotypes can be classified in to NH_4^+ or NO_3^- efficient genotype. Genotype, G-1, 4, 29, 30 & 31 in NH_4^+ treatment under irrigated condition and G-9, 12 & 31 under rainfed showed high potential change in yield with respect to other genotypes. Genotype, G-30, 31 in NO_3^- treatment under irrigated condition and G-1,9,15,16, 27 & 31 under rainfed manifested higher values for potential change in yield. G-1, 19, 20, 29 & 31 in N^0 treatments under irrigated and G-1, 18, 19 under rainfed environment were the best among all other genotypes. These findings are collaborated by research results of Fan Jun *et al.* (2013) who reported potential increased yield and reduction in fertilizer input achievable by using high-yielding and nutrient-efficient cultivars.

4.4 NUE and its component traits

4.4.1. Analysis of variance and mean phenotypic performance

The selected 32 RIL lines along with parents during wet season, 2015 was subjected to three way analysis of variance for each character in order to ascertain existence of genotype x environment x nitrogen interaction. Analysis of variance revealed that genotypic effects and genotype x nitrogen interaction effects were significantly different for investigated N use efficiency and its component traits ($p<0.05$, $p<0.01$). In the current study, wide ranges of mean values were recorded for chlorophyll parameters, grain nitrogen content, grain protein content, straw nitrogen content, grain N yield, straw N yield, biomass N yield, N harvest index,

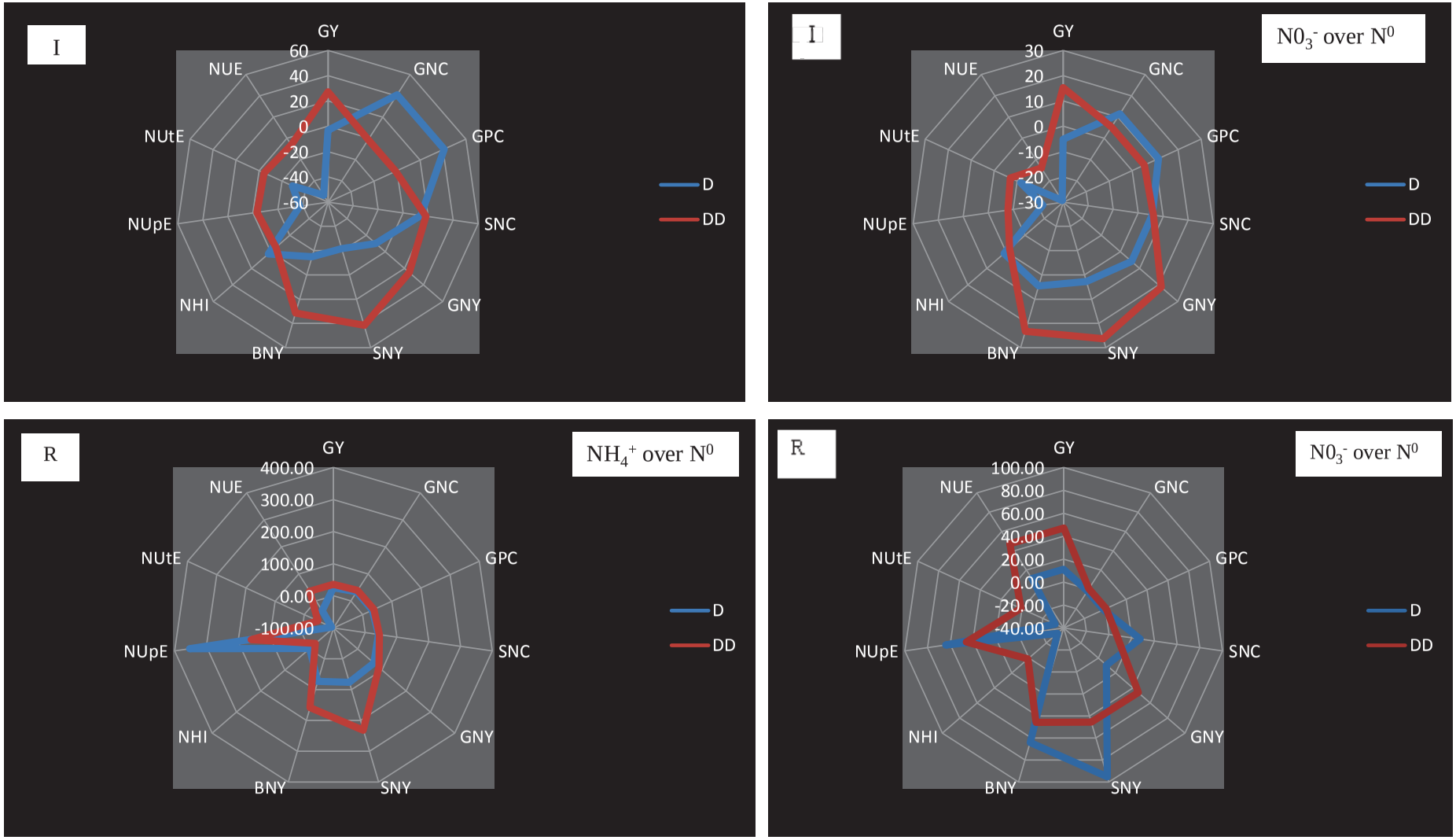


Fig 4.4: The radial graph depicting phenotypic difference between two parental lines under ammonium, nitrate treatment over control treatment across two environments The line in spokes closer to NUE or yield shows the better performance of a particular parent as compared to other parent
D=Danteshwari, DD= Dagad deshi, I=Irrigated, R=Rainfed

N-uptake efficiency, N-utilization efficiency and N-use efficiency. N-concentration traits and N-use efficiency traits varied significantly across different N and water regimes. The results of detailed statistics with ANOVA and its variance component and mean values estranged with standard error of mean are presented in table 4.8. The radar graph depicted in fig. 4.4 shows the difference in phenotypic performance of two parents *i.e.* Danteshwari and Dagad deshi for yield and NUE related traits under NH_4^+ and NO_3^- treatment over N^0 treatment across two environments.

Mean phenotypic performance of two parents showed significant differences for evaluated NUE traits. The coefficient of variation (CV) ranged from 6 % to 34 % for investigated traits. Under irrigated condition, in NH_4^+ treatment, grain nitrogen content ranged from 1.02-1.4 (%) with an average value of 1.23 (%).Mean phenotypic value for straw nitrogen content ranged from 0.3-0.6 (%) with an average value of 0.5 (%). Grain nitrogen yield values ranged from 1.49 to 6.9 (g/m^2) with a mean value of 3.5 g/m^2. The mean phenotypic values for straw nitrogen yield ranged from 1.4-4.8 (g/m^2) with an average value of 2.7 (g/m^2). Biological nitrogen yield ranged from 2.9-11.2 (g/m^2) with a mean value of 6.3 g/m^2. Nitrogen harvest index ranged from 25.8-69.1 (%) depicting mean of 55.5 (%). N-uptake efficiency values ranged from 0.1-0.3 (gg-1 N) with average value of 0.20 (g g-1 N).N-utilization efficiency recorded mean values of 45.2 ($gg^{-1}N$) and range of 18.9-59 ($gg^{-1}N$). N-use efficiency ranged from 3.8-17.1 ($gg^{-1}N$) with mean value of 9.8 ($gg^{-1}N$). In NO_3^- treatment, mean phenotypic value of grain nitrogen content was 1.14 % and values ranged from 0.98-1.32 (%). Straw nitrogen content ranged from 0.35-0.62 (%) with a mean value of 0.47 (%). Mean phenotypic value of grain N yield ranged from 1.6-4.8 (g/m^2) with an average value of 2.6 (g/m^2). Straw N yield ranged from 1.1-5.3 (g/m^2) with mean value of 2.1(g/m^2). Biological N yield ranged from 2.8-10.1 (g/m^2) with an average value of 4.7 (g/m^2). The range of variation for nitrogen harvest index was from 45.8 to 66.3 (%) with mean value of 56.1 (%). The range of variation for N-uptake efficiency was 0.1-0.3 (gg^{-1} N) with average value of 0.15 (gg^{-1} N). The range of variation for N-utilization efficiency is from 34.6 to 60.6 (gg^{-1} N) with mean value of 48.9. Mean phenotypic value for N-use efficiency is 7.77 (gg^{-1} N) with range from 4.5-12.7

(gg^{-1} N). In N^0 treatment, the range of variation for grain nitrogen content ranged from 0.7-1.3(%) with mean phenotypic value of 1.11 (%). The range of variation for straw nitrogen content is from 0.27-0.65 (%) with mean value of 0.45 (%). Phenotypic performance for grain nitrogen yield ranged from 0.91-4.1 (g/m^2) with mean value of 2.42 (g/m^2). Straw nitrogen yield ranged from 0.5-3.01 (g/m^2) with average value of 1.7 g/m^2. The range of variation for biomass N yield was from 2.0 to 6.3 g/m^2, for nitrogen harvest index was from 36.4-74.7 g/m^2, for N-uptake efficiency was from 0.1-0.3 $gg^{-1}N$, for N-utilization efficiency from 28.5-66.6 $gg^{-1}N$, for N-use efficiency ranged from 4.0-17.1 $gg^{-1}N$ and average value for these traits are 4.2 g/m^2, 56.7 %, 0.18 $gg^{-1}N$, 51.6 $gg^{-1}N$, 9.81 $gg^{-1}N$.

Under rainfed condition, in NH_4^+ treatment mean phenotypic values recorded for grain N content (%), straw N content (%), grain N yield (g/m^2), straw N yield (g/m^2), Biological N yield (g/m^2), Nitrogen harvest index (%), N-uptake efficiency ($gg^{-1}N$), N-utilization efficiency ($gg^{-1}N$), N-use efficiency ($gg^{-1}N$) are 1.5, 8.9, 0.9, 2.02, 4.05, 6.07, 32.8, 0.22, 21.8 & 5.02. In NO_3^- treatment mean phenotypic values recorded for grain N content (%), straw N content (%), grain N yield (g/m^2), straw N yield (g/m^2), Biological N yield (g/m^2), Nitrogen harvest index (%), N-uptake efficiency ($gg^{-1}N$), N-utilization efficiency ($gg^{-1}N$), N-use efficiency ($gg^{-1}N$) are 1.37, 8.21, 0.81, 1.7, 3.05, 4.76, 34.7, 0.17, 25.1 & 4.7. In N^0 treatment, mean phenotypic values recorded for grain N content (%), straw N content (%), grain N yield (g/m^2), straw N yield (g/m^2), Biological N yield (g/m^2), Nitrogen harvest index (%), N-uptake efficiency ($gg^{-1}N$), N-utilization efficiency ($gg^{-1}N$), N-use efficiency ($gg^{-1}N$) are 1.2, 6.9,0.63,1.02, 1.78, 2.82, 35.5, 0.10, 30.5 & 3.3, respectively. These results conclude that ammonium form is more readily absorbed as compared to nitrate form. Furthermore, N available from NH_4^+ treatment is more than NO_3^- treatment and still less under N^0 conditions, therefore these kinds of variation was noticed in different parameters of NUE.

These findings synchronize with the work of Beatty *et al.* (2010), who reported that nitrogen efficient genotypes could be able to produce high grain yields under both low and high N fertility conditions. Similarly, Wolde *et al.* (2004) dictated the importance of NUE varieties in future breeding program. Martin *et al.,* (2008) studied N-use efficiency of maize genotypes in contrasting

Table 4.8: Mean phenotypic performance, range, standard deviation (SD), Coefficient of variance (CV %) of investigated chlorophyll parameters, NUE and its component traits of 32 selected RILs and their parents under differential N regimes and environments during wet season, 2015

Traits	N	Source of variation							IRRIGATED (I)					RAINFED (R)				
											RILs					RILs		
		G	N	E	E X G	N X G	EX N	E X N X G	D	DD	Mean±SEm	Range	CV %	D	DD	Mean±SEm	Range	CV %
		TMSS, DF=31	TMSS, DF=2	TMSS, DF=1	TMSS, DF=31	TMSS, DF=62	TMSS, DF=2	TMSS, DF=62										
SPAD till	NH_4^+								35	32	**32±0.3**	29-38	6	35	31	**32±0.4**	29-38	6
	NO_3^-	49.2**	186**	NS	NS	5.3 *	NS	NS	33	30	31±0.4	26-37	7	33	30	31±0.4	26-36	7
	N^0								31	31	30±0.4	26-35	7	32	30	30±0.4	26-34	7
SPAD flow	NH_4^+								37	35	**36±0.4**	30-42	6	38	40	**37±0.6**	28-43	8
	NO_3^-	50.2**	190**	NS	NS	6.6*	NS	NS	36	32	35±1.3	28-41	6	34	37	35±0.1	29-41	8
	N^0								32	29	32±0.5	24-37	7	31	28	28±0.5	22-33	10
GNC	NH_4^+								1.4	1.2	**1.2±0.1**	1-1.4	10	1.7	1.6	**1.5±0.1**	1.1-2	12
	NO_3^-	0.05**	1.6**	3.1**	0.04**	0.03**	0.4**	0.29 *	1.1	1.2	1.1±0.3	0.9-1	8	1.2	1.1	1.3±0.2	1.1-2	11
	N^0								1.0	1.2	1.1±0.2	0.7-1	7	1.2	1.1	1.2±0.1	1.0-2	8
GPC	NH_4^+								8.2	6.9	**7.3±0.1**	6.1-8.7	10	10	9.6	**8.9±0.2**	6.9-11	13
	NO_3^-	1.8**	59**	114 **	1.6**	1.0**	15.4 **	1.0**	6.5	7.4	6.8± 0.1	5.8-8.1	8	7.5	6.9	8.2±0.2	6.8-10	12
	N^0								5.8	7.0	6.6±0.1	4.6-7.8	8	7.6	6.9	6.9±0.1	6.1-8	8
SNC	NH_4^+								0.6	0.5	**0.5±0.1**	0.3-0.6	18	1.1	0.7	**0.9±0.1**	0.6-2	21
	NO_3^-	0.09**	0.89**	9.3**	0.04**	0.02**	0.47**	0.01**	0.5	0.5	0.47±0.1	0.3-0.6	12	0.9	0.5	0.8±0.1	0.5-1	19
	N^0								0.5	0.5	0.45±0.0	0.2-0.6	14	0.7	0.5	0.6±0.1	0.4-1	21
GNY	NH_4^+								2.4	3.0	**3.5±0.2**	1.4-6.9	35	1.4	3.6	**2.0±0.1**	0.8-3	37
	NO_3^-	3.0**	35**	158**	2.1**	0.73**	2.73**	0.61**	2.8	2.9	2.6±0.1	1.6-4.8	23	0.9	2.7	1.7±0.1	0.5-3	44
	N^0								2.6	2.4	2.4±0.1	0.9-4.1	22	0.8	1.9	1.0±0.1	0.2-2	21

SNY	NH_4^+	1.6**	84**	53**	1.6**	0.9**	13**	0.7**	1.5	2.1	**2.7±0.2**	1.4-4.8	33	2.6	6.1	**4.0±0.1**	2.5-7	23
	NO_3^-								2.0	1.9	2.1±0.2	1.1-5.3	35	2.8	2.6	3.0±0.1	1.9-5	21
	N^0								1.9	1.5	1.7±0.1	0.5-3.0	32	1.4	1.8	1.7±0.1	0.8-3	41
BNY	NH_4^+	6.3**	22**	27**	5.6**	2.3**	17.7**	1.7**	3.9	5.1	**6.3±0.3**	2.9-11	28	4.0	9.6	**6.1±0.2**	3.9-10	23
	NO_3^-								4.7	4.8	4.7±0.1	2.8-10	26	3.7	5.4	4.7±0.2	2.6-7	25
	N^0								4.5	3.9	4.2±0.2	2.0-6.3	24	2.2	3.7	2.8±0.1	1.1-4.3	27
NHI	NH_4^+	381**	121*	45,655**	183**	84**	20**	84**	58	58	**57±1.6**	25-69	17	35	36	**35±1.2**	17-45	21
	NO_3^-								57	60	56±1.0	45-66	10	24	50	34±1.7	16-50	27
	N^0								57	61	56.7±1.6	36 ±74	11	36	49	32±1.7	20-52	24
NUpE	NH_4^+	0.25**	0.17**	0.01**	0.01**	0.003**	0.11**	0.003**	0.1	0.2	**0.20±0.1**	0.1-0.3	28	0.1	0.4	**0.22±0.1**	0.1-0.3	24
	NO_3^-								0.2	0.2	0.18±0.0	0.1-0.3	26	0.1	0.2	0.17±0.1	0.1-0.2	25
	N^0								0.2	0.2	0.15±0.0	0.1-0.3	23	0.09	0.1	0.10±0.2	0.04-0.2	28
NUtE	NH_4^+	343**	1,753**	47,295**	166**	100**	NS	104**	42.8	50.3	45±1.4	18-59	18	20	22	21.8±0.8	9.4-33	22
	NO_3^-								53.3	48.9	49±1.1	34-60	13	19	43	25.1±1.4	9.5-43	31
	N^0								60.3	52.6	**51±1.7**	28-66	12	28	42	**30.5±1.5**	18-45	27
NUE	NH_4^+	29.5**	33**	2,003**	16.5**	6.5**	99**	4.8 **	5.6	8.5	**9.8±0.5**	3.8-17	34	3.1	8.4	**5.0±0.3**	1.8-9	35
	NO_3^-								8.5	7.8	9.3±0.3	4.5-12	21	2.7	9.0	4.7±0.4	1.1-9.9	46
	N^0								12.0	9.0	8.8±0.5	4.0-17	20	2.4	6.1	3.3±0.2	0.7-6.1	39

*** and ** significance at .05, .01 levels respectively, SPADtill = SPAD at tillering , SPADflow = SPAD at flowering, GNC= grain nitrogen content (%), GPC= grain protein content (%),SNC= straw N content (SNC), GNY = grain N yield (g/m^2), SNY = straw nitrogen yield (g/m^2), BNY= biological N yield (g/m^2), NHI = nitrogen harvest index (%), NUpE= nitrogen uptake efficiency (gg^{-1} N), NUtE= nitrogen utilization efficiency ((gg^{-1} N) , NUE= nitrogen use efficiency ((gg^{-1} N)**

environments. Their results revealed that parameters like chlorophyll content, leaf nitrogen and grain yield are sensitive to N availability and are important contributors to nitrogen use efficiency. Ali (2005) asserted that productivity and resource use efficiency of rice are affected by crop establishment and nitrogen management. Inthapanya *et al.* (2000); Zhang *et al*. (2008) reported genetic variation among rice genotypes in NUE and related traits in rice. Lea-Azevado (2006) further stated that it is very important to identify or develop high NUE genotypes in rice for its production under low cost crop management practices and also to protect environment.

4.4.2. Variability, Heritability, Genetic advance

Genetic variability studies gives basic information regarding the genetic architecture of the population based on which breeding methods are formulated for further improvement of the crop. These studies are also helpful to gain knowledge about the nature and extent of variability that can be attributed to different causes, sensitivity of crop to environment, heritability of the character and genetic advance. The results of variability parameters are presented in table 4.9. In the present investigation, broad sense heritability for evaluated NUE and component traits ranged from 4.1% to 87.8% under differential N and water regimes. High heritability coupled with genetic advance as per cent mean for the traits was obtained for nitrogen use efficiency, which is very important in present study. Overall, high phenotypic coefficient of variability and genotypic coefficient of variability was obtained for grain N yield GNY), straw N yield (SNY), Biomass N yield (BNY), N-utilization efficiency (NUtE) and N-uptake efficiency (NUE). These results are in harmony with Prasad *et al*. (2001) and Paikhomba *et al*. (2014) who studied GCV, PCV and heritability in NUE and its component traits.

4.4.3. SPAD value and leaf chlorophyll profile

Results showed that there are significant differences in leaf chlorophyll profile and SPAD value under variable nutrient levels at flourishing tillering stage. Results revealed that SPAD value ranged from 32.25 to 24.23 as presented in Table 1. NH_4^+-N treatment recorded significantly higher SPAD value as compared to NO_3^--N and N^0-N treatment. This finding is in compliance with the research of

Table 4.9: Broad sense heritability (h2), genetic advancement (GA), phenotypic coefficient of variation (PCV) & genotypic coefficient of variation (GCV) for NUE and its component traits during wet season 2015

Traits	N	Wet season 2015							
		IRRIGATED (T)				RAINFED (Ds)			
		h^2	GA (% mean)	GCV	PCV	h^2	GA (% mean)	GCV	PCV
GNC	NH_4^+	87.8	18.1	9.3	9.9	**78.2**	**22.6**	12.4	14.1
	NO_3^-	85.3	14.6	7.7	8.3	**79.1**	**20.3**	11.1	12.4
	N^0	79.6	13.9	6.3	10.5	**60.8**	**11.2**	6.9	8.9
GPC	NH_4^+	85	17.3	9.1	9.9	**78.3**	**22.7**	12.4	14.6
	NO_3^-	85.3	14.7	7.6	8.2	**79.2**	**20.4**	11.2	12.8
	N^0	78.5	13.7	6.1	8.1	**61.7**	**11.4**	7	8.9
SNC	NH_4^+	**88.2**	**33.7**	17.4	18.5	**87.1**	**39.6**	**20**	**25**
	NO_3^-	**87.7**	**20.8**	12.3	14.9	**80.3**	**33.4**	**18**	**20**
	N^0	**81.2**	**32**	17.2	19.1	**88.6**	**40.5**	**20**	**22.1**
GNY	NH_4^+	**71.7**	**57.2**	**32.8**	**38.7**	54.6	47.9	**31**	**42**
	NO_3^-	**69.1**	**57.9**	**30.6**	**33.4**	65.4	65.4	**39**	**48**
	N^0	**68.2**	**52.6**	**24.3**	**38.7**	58.8	56.2	**35**	**46**
SNY	NH_4^+	**65.1**	**48.9**	**29.4**	**36.5**	43.2	24.7	**28**	**27**
	NO_3^-	**77.9**	**60.4**	**33.2**	**37.6**	54.8	30.6	**20**	**27**
	N^0	**72.3**	**56.7**	**27.6**	**34.5**	64.6	40	**24**	**30**
BNY	NH_4^+	**64.5**	**41.5**	**25**	**31.2**	58.3	32	**20**	**26**
	NO_3^-	**65.7**	**39.8**	**23.8**	**29.4**	62.2	35.8	**22**	**27**
	N^0	**60.6**	**40.8**	**26.7**	**30.8**	49.1	28.3	**22**	**28**
NHI	NH_4^+	**76.1**	**27.7**	15.4	17.6	51.5	36.2	17	24

	NO_3^-	**69.4**	**26.6**	13.7	16.8	67.9	42.2	24	30
	N^0	**56.7**	**25.6**	18.1	23.1	70.3	49.9	26.6	29.3
NUpE	NH_4^+	**65.3**	**42.4**	**25.4**	**31.5**	57.6	31.3	**20**	**26.4**
	NO_3^-	**65.8**	**40.1**	**26.7**	**30.9**	59	34.4	**21.7**	**28.3**
	N^0	**57.7**	**44.6**	**26.3**	**21.4**	47.2	27.5	**19.4**	**28.3**
NUtE	NH_4^+	**80.6**	**31.8**	17.2	19.1	**82.1**	**48.8**	**29.3**	**34.6**
	NO_3^-	**75.9**	**25.2**	14.5	15.6	**87.3**	**46.1**	**27.8**	**30.1**
	N^0	**67.8**	**20.8**	13.6	15.3	**80.2**	**48.5**	**26.2**	**29.3**
NUE	NH_4^+	**71**	**53.8**	**31**	**36.8**	**71.4**	**45.1**	**30.4**	**42.4**
	NO_3^-	**66.6**	**48.1**	**29.8**	**33.4**	**64.1**	**58.5**	**39.3**	**45.6**
	N^0	**67.5**	**52.3**	**28.8**	**32.6**	**56.7**	**51.5**	**33.1**	**44.2**
SPADtill	NH_4^+	42.2	6.4	4.8	7.4	51.9	7.5	5.1	7
	NO_3^-	41.1	10.1	3.3	3.6	68.8	12.1	7	8.5
	N^0	42.1	12.6	4.1	5.7	55.7	13.2	8.6	11.5
SPADflow	NH_4^+	51.6	8.8	5.9	8.3	53.4	10.7	7.1	9.7
	NO_3^-	42.3	12.8	4.5	8	35.7	7.3	6.9	9.9
	N^0	41.7	11.9	4.1	7.9	61.4	10.5	6.5	8.3

SPADtill = SPAD at tillering , SPADflow = SPAD at flowering, GNC= grain nitrogen content (%), GPC= grain protein content (%),SNC= straw N content (SNC), GNY = grain N yield (g/m^2), SNY = straw nitrogen yield (g/m^2), BNY= biological N yield (g/m^2), NHI = nitrogen harvest index (%), NUpE= nitrogen uptake efficiency (gg^{-1} N), NUtE= nitrogen utilization efficiency ((gg^{-1} N) , NUE= nitrogen use efficiency ((gg^{-1} N)

Jinwen *et al*. (2009) who investigated response of rice SPAD readings to different nitrogen supply rates. Gholizadeh *et al*., (2009) also evaluated SPAD chlorophyll meter readings in two different rice growth stages to establish the fact that SPAD readings indicate the plant nitrogen status and physiological nitrogen requirement of crops at different growth stages. Results also revealed that rice genotypes showed considerably low level of leaf chlorophyll a, chlorophyll b and total chlorophyll under NO_3^- -N and N^0-N treatment. As opposed to this, leaf chlorophyll a, chlorophyll b and total chlorophyll of rice genotypes were higher under NH4+-N treatment. Chlorophyll profile showed significant differences under variable nutrient conditions. These results synchronize with findings of Hassan *et al*., (2009). They confirmed genotypic variation in traditional rice varieties for chlorophyll content and SPAD values. The varieties evaluated by them showed consistent level of leaf chlorophyll profile under variable nutrient levels.

Table 4.10: Effect of variable nutrient levels on leaf chlorophyll profile and SPAD value of rice genotypes at the flourishing tillering stage under rainfed condition

	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll ((mg g^{-1})
NH_4^+-N	2.104 [a]	1.367 [a]	3.225 [a]
NO_3^- -N	1.825 [b]	1.113 [b]	3.194 [b]
N^0-N	1.690 [c]	0.742 [c]	2.423 [c]

Means followed by different letter within column indicate significant differences at $p<0.05$ by DMRT

4.4.4 Relationship between Chlorophyll profile and SPAD value under variable nutrient levels

The relationships between leaf total chlorophyll and SPAD readings of rice genotypes under NH_4^+-N, NO_3^- -N, N^0-N treatment are depicted in fig. 4.5. This study shows that leaf chlorophyll profile shows linear and positive relationship with SPAD values at the variable nutrient levels at flourishing tillering stage. The corresponding R^2 values of NH_4^+-N, NO_3^- -N, N^0-N treatment are 0.670, 0.708 and 0.705. There are studies that show high correlation between chlorophyll content and SPAD readings. A study conducted by Islam *et al.,* 2009 evaluated SPAD and LCC based N management in rice. There results indicated higher chlorophyll content in rice leaves showing higher SPAD readings. This relationship depicts the

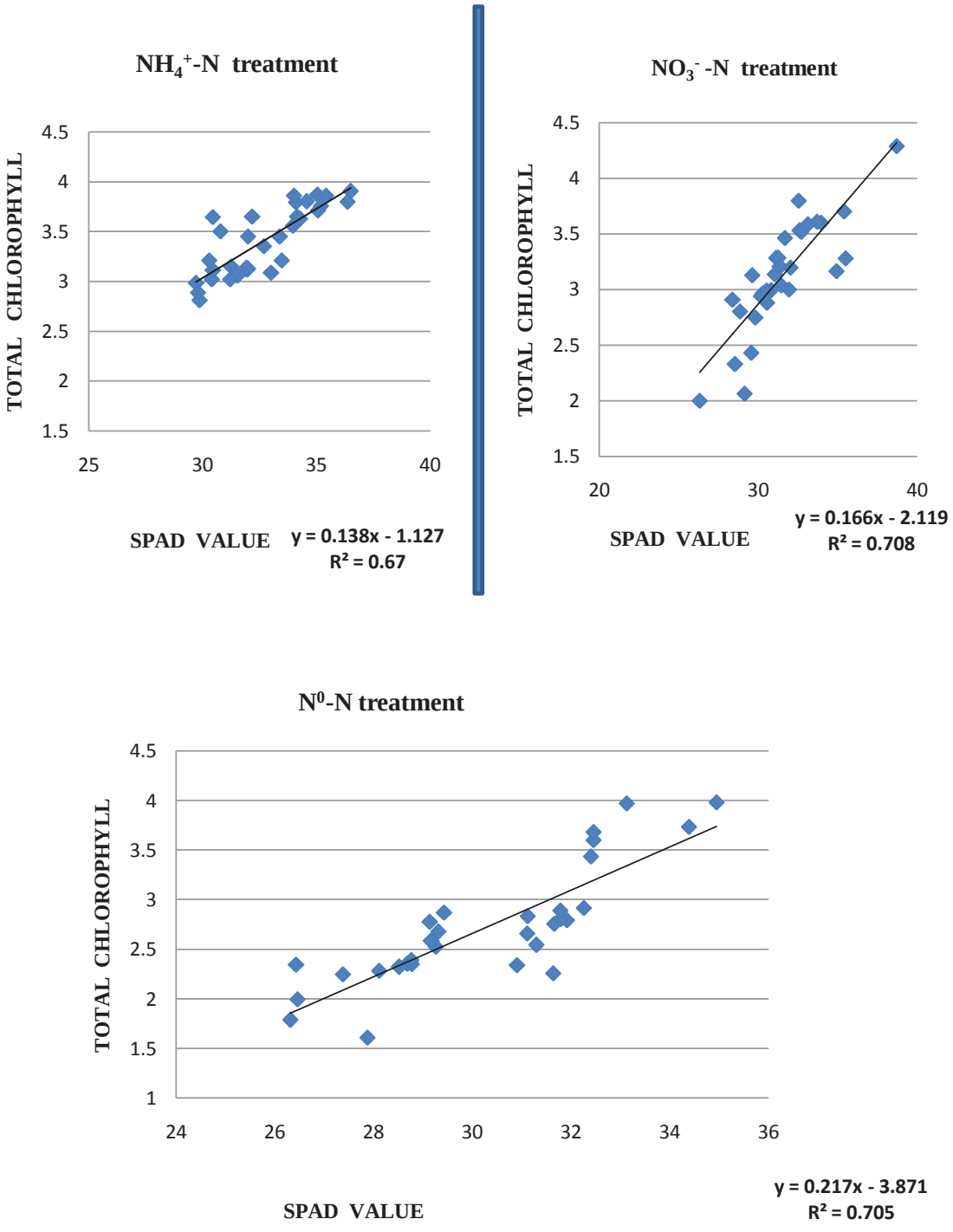


Fig. 4.5: The relationships between leaf total chlorophyll and SPAD readings of rice genotypes under NH_4^+-N, NO_3^- -N, N^0-N treatment

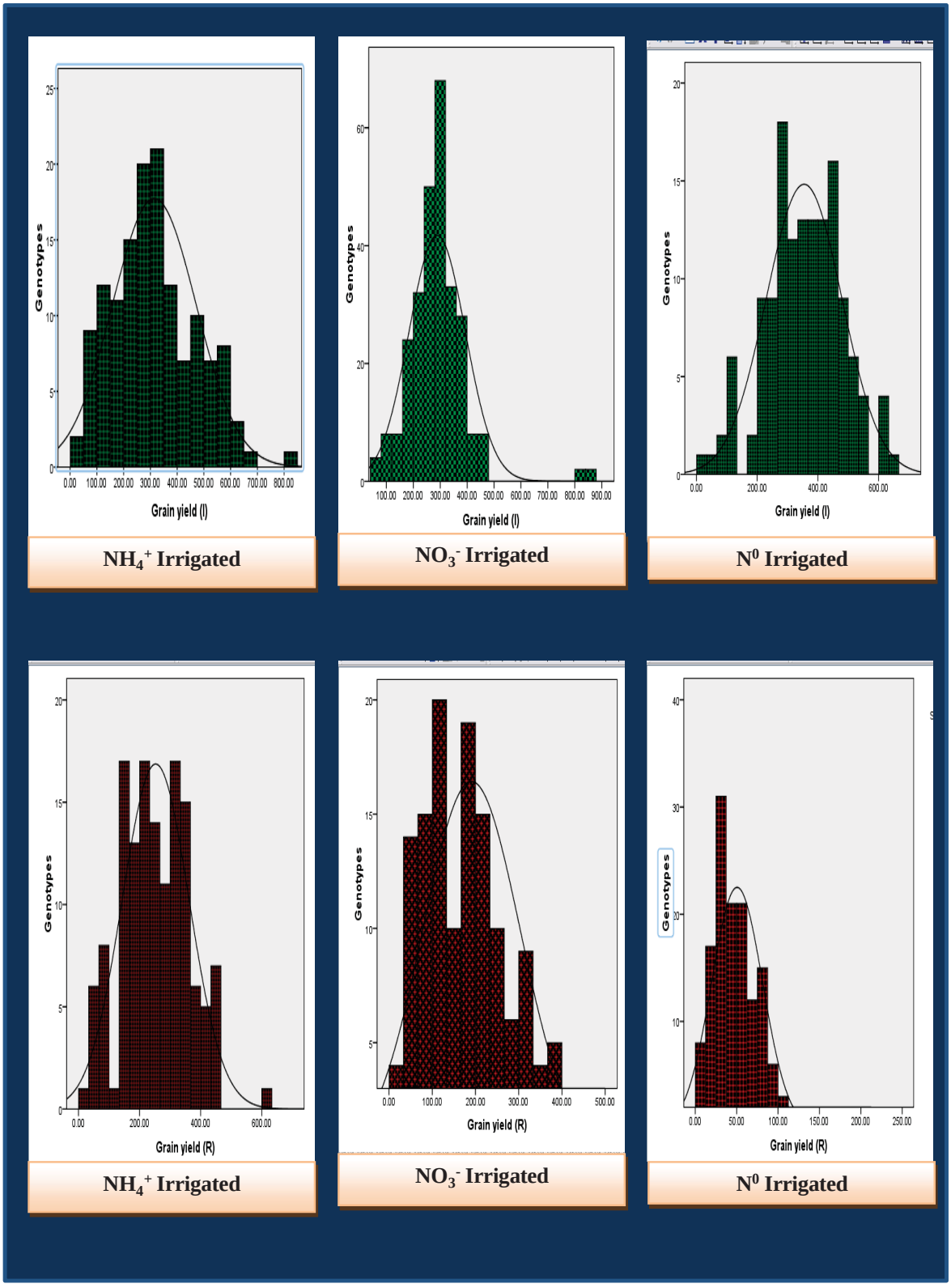


Fig 4.6: Frequency distribution of grain yield under two N forms and two environments

dependency of SPAD values on chlorophyll profile. Miami. (2003) reported a strong, linear and positive relationship between SPAD values and chlorophyll a and b contents.

4.5. Test of normality

Grain yield accompanied by acceptable NUE is targeted in present research. Genotype (G), environment (E), Nitrogen (N) and their interaction (GEIN) play a profound & crucial role in the final expression of grain yield attributes. Considering this, on grain yield basis out of 122 lines, 32 lines were selected further for assessing genotypic variability among rice genotypes for NUE and N-related traits. The distribution curves for grain yield under differential N regimes across two environments are depicted in fig 4.6.

4.6. Genotypic differences, Cluster and correlation analysis

The use of nitrogen efficient genotypes as stated before is an important complementary strategy in improving rice yield and reducing cost of production in subsistence farming. The results achieved for framed objectives of investigating genetic variability in nitrogen use efficiency (NUE) indices of selected 32 rice genotype, to identify genotypes with best nitrogen use efficiency, classifying these genotypes by using cluster analysis to disperse genotypes in to qualitative groups and to assess nature of association of nitrogen use efficiency and yield traits are presented in this section.

4.6.1. Assessing the genotypic differences in NUE and related parameters among rice genotypes

The mean results on nitrogen use efficiency indices *i.e.* grain N content, grain protein content, straw N content, grain N yield, straw N yield, biomass N yield N harvest index and nitrogen uptake efficiency, nitrogen utilization efficiency, nitrogen use efficiency, of selected 32 rice genotypes under differential nitrogen and water regimes during wet season 2015 are shown in table 4.11, 4.12 & 4.13 and fig. 4.7. The detailed explanations of findings along with support of relevant researches are presented below:

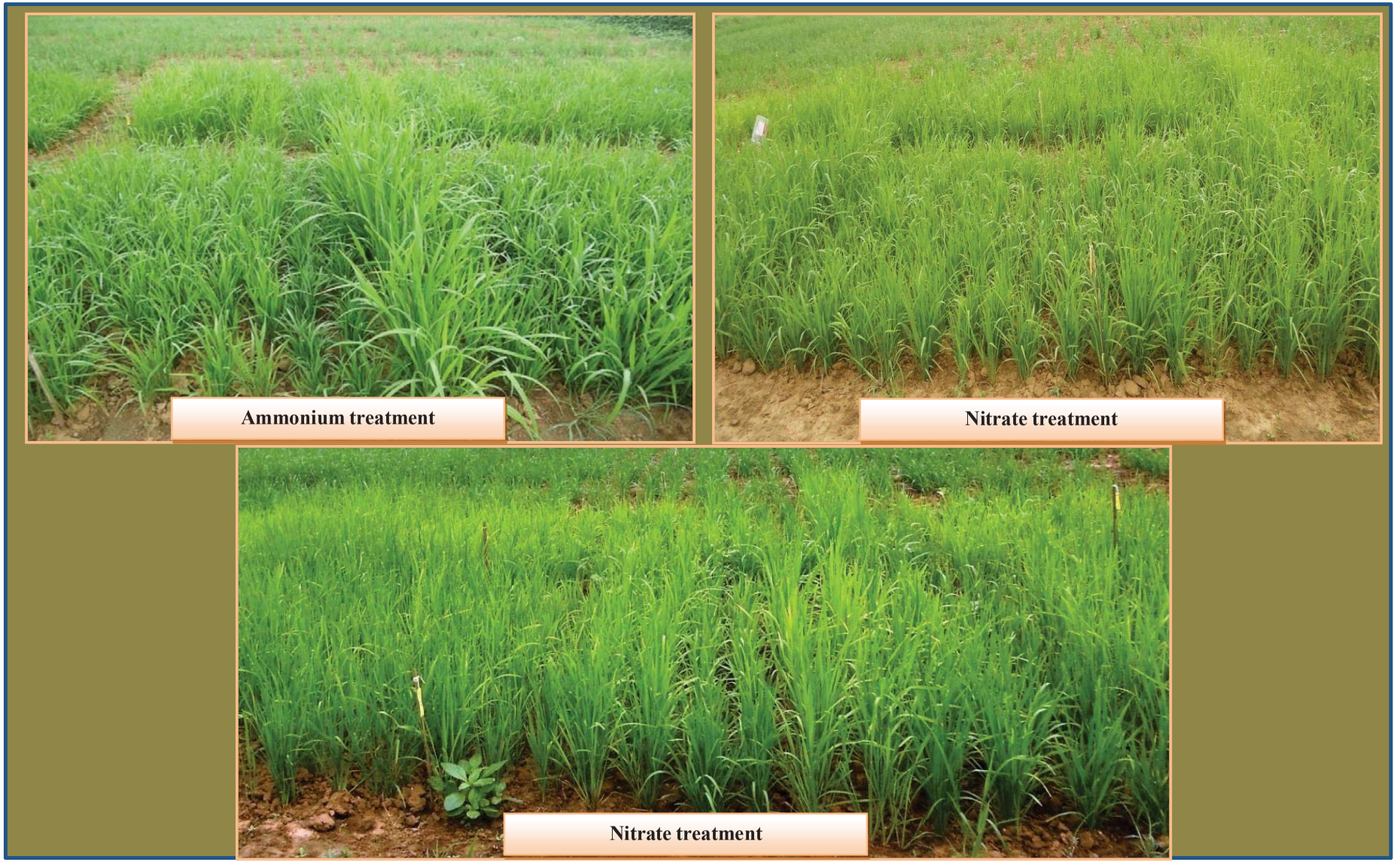


Fig 4.7: An illustration of genotypic differences in RILs evaluated under different forms of nitrogen

4.6.1.1. Grain N, protein content and grain nitrogen yield of individual genotypes in response to nitrogen and water regimes

Grain N (GNC), protein content (GPC) and grain N yield (GNY) of rice genotypes evaluated in the study showed typical response pattern to NH_4^+, NO_3^- and N^0 treatment under irrigated and rainfed condition. Result showed the wide variation in grain N, protein content and yield due to variable N forms and water regimes. Grain N % of all genotypes was considerably more than N% in straw under all sets of condition. Nitrogen and protein content of NH_4^+, NO_3^- and N^0 treatment under both set of environment were divided in different ranges for classifying studied genotypes accordingly. Within NH_4^+ treatment, under irrigated condition, mean comparison revealed that 9 genotypes *i.e.*G-2, G-3, G-9, G-20, G-24, G-26, G-29, G-30, and G-32 has significantly similar and higher GNC & GPC which ranged from 1.48-1.32 and 7.83-8.79. GNC & GPC of remaining genotypes ranged from 1.02-1.25 and 6.08-7.50. GNY was highest in G-29, G-30 and G-31 was 6.6, 6.9 and 6.1, while others genotypes showed the range of almost 1.49 to 4.70. Under rainfed condition among all genotypes, higher values of GNC & GPC was obtained for 11 genotypes *i.e.* G-5, G-6, G-7, G-13, G17, G-18, G-20, G-23, G-26, G-27 and G-28, which showed the range of 1.6 to 1.7. GNY results dictated to be highest in genotype, G-20, G-27 and G-31. Comparative results of GNC & GPC under both sets of environment of NH_4^+ treatment flourished us with the genotypes, G-3, G-9, G-13, G-18, G-20 and G-26 manifesting higher values of studied parameters as compared to other genotypes while for GNY, efficient genotypes were G-4, G-6, G-12 and G-9. Within NO_3^- treatment, under irrigated set of condition G-30, G-32, G-29, G-27, G-1, G-13, G-5, G-3 and G-2 recorded considerable values of GNC and GPC that ranged from 1.22 to 1.33 and 6.06 to 7.75 while rainfed condition recorded much higher values that ranged from 1.6 to 1.75 and 10.4 to 7.2 of G-1, 5, 11, 18, 15 and 10. GNY in G-30, 31, 32 was higher than the other genotypes possessing almost similar values for the trait under irrigated condition while G-15, 16, 17 & 27, under rainfed condition showed better performance than others for this trait value.G-5 was one of the genotypes that performed well for GNC & GPC and G-11, showed greater grain N yield under both sets of conditions. Within N^0 treatment, differences in GNC and GPC of

irrigated and rainfed condition was very subtle and minimal. Under rainfed condition, G-1 and G-13 values of grain N and protein content was 8.3 and 7.7 which was highest among all other genotypes while G-2, 3, 5, 11, 26, 29 & 31 showed almost similar and considerable values then remaining ones. Under irrigated condition, grain N and protein content of genotype, G-5, 3, 6, 11, 14, 15, 21 & 29 ranged from 1.2 to 1.3. Comparatively, G-3, 5, 26 & 29 were having similar values for GNC & GPC and relatively, GNC was higher for G-1 under both irrigated and rainfed condition.

From these results, it is very clear that grain N and protein content tends to be higher under rainfed environment as compared to irrigated environment. Grain N yield which is actually a derived parameter of grain yield and grain N was higher under irrigated condition as grain yield obtained under it was more than rainfed condition. In present study remobilization from vegetative organ (shoot) was with greater efficiency, as stated before, N content in grain was more than straw. These results collaborates with the findings of Hassan *et al*. (2009) who reported genotypic variation in traditional rice varieties for grain N and protein content and N-use efficiency. Cox *et al*. (1986) in past found the differences in the percentage of grain N between various wheat genotypes. Also, Cassman *et al*. (2002) reported N concentration in grain as one of the important quality parameter for selection of genotypes for NUE and related trait. Chandel *et al.* (2010) performed the comparative analysis of grain protein content of 32 rice genotypes under different treatments and concluded that grain nutritive traits including protein content manifest significant effect of locations/condition, interaction between genotype× location, N levels and genotype × N application and genotypic difference was found to be the most significant factor determining grain protein and other nutrient contents in rice grains. Other authors, Brian *et al*. (2007), also reported an increase in grain protein concentration with changing N levels. Genetic variation for grain protein content in which older varieties provided higher protein was also reported May (1991) & Sanford and Mackown (1991). In contrast to the findings of this study, Cooper *et al.* (2001) reported significant effect of variety x N rate interaction on wheat grain protein content. In oppose of the present research, Frageria *et al.* (2010) reported that N content in grain is not determined by N or

Table 4.11: Genotypic differences among rice genotypes for NUE component traits i.e. Grain N content, Grain protein content and Straw nitrogen content during wet season, 2015

G/P	Irrigated									Rainfed								
	NH_4^+			NO_3^-			N^0			NH_4^+			NO_3^-			N^0		
	GNC (%)	GPC (%)	SNC (%)	GNC (%)	GPC (%)	SNC (%)	GNC (%)	GPC (%)	SNC (%)	GNC (%)	GPC (%)	SNC (%)	GNC (%)	GPC (%)	SNC (%)	GNC (%)	GPC (%)	SNC (%)
1	1.0±0.03	6.2±0.2	0.4±0.01	1.1±0.03	6.6±0.2	0.5±0.01	1.2±0.1	6.4±0.2	0.3±0.2	1.4±0.10	8.3±0.6	0.7±0.1	1.6±0.0	9.5±0.1	0.7±0.1	1.4±0.0	8.3±0.2	0.6±0.0
2	1.3±0.02	8.0±0.1	0.4±0.01	1.2±0.03	7.2±0.2	0.5±0.02	1.1±0.1	6.5±0.4	0.3±0.1	1.3±0.03	8.0±0.21	0.7±0.0	1.3±0.0	7.8±0.0	0.7±0.0	1.2±0.0	7.2±0.2	0.5±0.0
3	1.3±0.04	8.0±0.2	0.4±0.01	1.3±0.04	7.5±0.2	0.4±0.03	1.3±0.2	7.8±0.2	0.2±0.2	1.5±0.05	9.1±0.2	0.7±0.0	1.4±0.0	8.6±0.2	0.6±0.1	1.2±0.0	7.2±0.1	0.5±0.0
4	1.2±0.03	6.3±0.2	0.4±0.1	1.4±0.02	6.2±0.1	0.4±0.02	1.1±0.0	6.7±0.1	0.3±0.4	1.5±0.00	9.2±0.04	0.7±0.0	1.3±0.0	8.2±0.0	0.5±0.0	1.1±0.0	6.6±0.2	0.5±0.0
5	1.2±0.1	7.4±0.4	0.6±0.01	1.2±0.05	7.2±0.2	0.5±0.01	1.3±0.1	7.8±0.2	0.5±0.0	1.7±0.06	10.6±0.3	1.1±0.0	1.7±0.0	10.2±0.2	1.1±0.0	1.2±0.0	7.5±0.0	0.9±0.0
6	1.2±0.0	7.4±0.1	0.4±0.06	1.2±0.1	6.9±0.2	0.4±0.00	1.2±0.0	7.1±0.1	0.3±0.2	1.7±0.02	10.2±0.1	0.9±0.2	1.4±0.0	8.9±0.0	0.9±0.2	1.1±0.0	6.6±0.1	0.8±0.1
7	1.1±0.2	6.6±0.1	0.4±0.02	1.1±0.02	6.7±0.1	0.3±0.03	1.1±0.3	6.6±0.2	0.3±0.1	1.1±0.02	6.9±0.16	0.7±0.0	1.1±0.0	6.8±0.0	0.7±0.0	1.0±0.1	6.0±0.5	0.5±0.0
8	1.0±0.3	6.4±0.2	0.6±0.02	1.08±0.2	6.4±0.4	0.4±0.0	1.1±0.2	6.5±0.3	0.4±0.2	1.6±0.01	9.9±0.08	1.0±0.1	1.3±0.0	8.2±0.4	1.1±0.0	1.0±0.0	6.3±0.2	1.0±0.0
9	1.3±0.3	8.0±0.2	0.3±0.02	1.0±0.02	6.2±0.1	0.4±0.01	1.1±0.2	6.5±0.1	0.4±0.1	1.3±0.05	8.2±0.04	0.8±0.1	1.1±0.0	7.0±0.1	0.9±0.0	1.1±0.0	7.0±0.1	0.5±0.1
10	1.2±0.1	7.4±0.1	0.5±0.1	1.1±0.02	6.4±0.1	0.6±0.0	1.0±0.1	6.2±0.2	0.5±0.1	1.1±0.06	6.9±0.3	1.0±0.0	1.2±0.1	7.4±0.4	0.9±0.0	1.1±0.1	6.8±0.9	0.6±0.1
11	1.1±0.7	6.6±0.4	0.4±0.01	1.2±0.03	7.1±0.2	0.4±0.04	1.2±0.2	7.1±0.2	0.4±0.3	1.3±0.29	8.0±1.7	0.8±0.1	1.7±0.0	10.4±0.2	0.8±0.1	1.2±0.1	7.5±0.5	0.6±0.0
12	1.2±0.1	7.5±0.1	0.4±0.2	1.1±0.05	6.7±0.2	0.5±0.00	1.1±0.2	6.7±0.1	0.4±0.2	1.2±0.02	7.4±0.16	0.7±0.0	1.2±0.0	7.2±0.2	0.7±0.0	1.1±0.0	6.6±0.1	0.6±0.0
13	1.3±0.3	7.7±0.2	0.5±0.03	1.2±0.04	7.4±0.2	0.5±0.01	1.1±0.2	6.7±0.1	0.4±0.1	1.7±0.02	10.2±0.1	0.9±0.2	1.3±0.1	7.9±0.8	1.0±0.0	1.3±0.0	7.7±0.2	0.5±0.0
14	1.2±0.1	7.3±0.2	0.4±0.02	1.2±0.03	7.2±0.2	0.4±0.01	1.2±0.1	7.1±0.1	0.5±0.0	1.2±0.02	7.7±0.12	0.8±0.0	1.3±0.0	7.7±0.0	0.6±0.0	1.0±0.0	6.2±0.2	0.4±0.0
15	1.2±0.0	7.4±0.1	0.5±0.01	1.1±0.05	6.7±0.3	0.5±0.06	1.2±0.1	7.1±0.1	0.6±0.1	1.6±0.01	9.6±0.08	1.0±0.0	1.5±0.1	9.0±0.4	0.8±0.0	1.1±0.0	6.7±0.1	0.6±0.0

16	1.3±0.3	7.6±0.2	0.3±0.02	1.2±0.02	6.3±0.2	0.3±0.00	1.0±0.2	6.2±0.2	0.3±0.1	1.6±0.04	10±0.25	0.9±0.0	1.5±0.0	9.4±0.2	0.9±0.0	1.1±0.0	6.5±0.2	0.6±0.0
17	1.1±0.1	7.0±0.7	0.3±0.01	1.1±0.00	6.6±0.1	0.3±0.0	1.0±0.0	5.9±0.1	0.3±0.0	1.8±0.02	10.7±0.1	0.91±0.04	1.3±0.1	8.2±0.9	0.7±0.0	1.0±0.0	6.1±0.1	0.3±0.02
18	1.3±0.0	7.8±0.0	0.4±0.00	1.1±0.00	6.7±0.0	0.4±0.0	0.9±0.0	5.6±0.1	0.3±0.0	1.8±0.0	11.1±0.2	0.74±0.00	1.6±0.1	10.0±0.5	0.7±0.0	1.2±0.0	7.1±0.1	0.5±0.0
19	1.0±0.0	6.0±0.0	0.3±0.01	0.9±0.07	5.8±0.4	0.4±0.0	1.1±0.1	6.5±0.2	0.5±0.0	1.4±0.1	8.5±0.7	0.68±0.02	1.4±0.01	8.6±0.1	0.7±0.0	1.0±0.0	6.2±0.2	0.5±0.0
20	1.4±0.01	8.3±0.1	0.6±0.05	1.0±0.00	6.2±0.0	0.4±0.1	1.0±0.0	6.4±0.0	0.5±0.0	1.7±0.01	10.1±0.1	1±0.01	1.2±0.04	7.1±0.2	0.7±0.0	1.±0.0	6.5±0.1	0.6±0.0
21	1.0±0.0	6.4±0.0	0.6±0.01	1.0±0.02	6.1±0.1	0.5±0.0	1.2±0.1	7.4±0.5	0.3±0.0	1.5±0.10	9.1±0.6	1.08±0.03	1.4±0.0	8.5±0.0	0.8±0.0	1.2±0.1	7.4±0.5	0.7±0.01
22	1.1±0.0	6.7±0.0	0.4±0.02	1.1±0.0	6.6±0.0	0.4±0.1	1.1±0.0	6.6±0.2	0.5±0.0	1.2±0.08	7.5±0.5	0.92±0.02	1.3±0.02	8.0±0.1	0.9±0.0	1.1±0.0	6.7±0.1	0.7±0.0
23	1.1±0.02	6.8±0.1	0.6±0.02	1.1±0.02	6.9±0.1	0.5±0.1	1.1±0.02	6.7±0.1	0.4±0.0	1.6±0.02	10.0±0.1	1.07±0.02	1.4±0.02	8.3±0.1	0.8±0.0	1.0±0.0	6.2±0.2	0.7±0.0
24	1.3±0.02	8.2±0.2	0.6±0.01	1.2±0.03	6.9±.2	0.6±0.0	1.0±0.1	6.2±0.6	0.5±0.0	1.2±0.06	7.7±0.3	0.96±0.05	1.3±0.02	7.9±0.1	0.7±0.0	1.0±0.0	6.4±0.2	0.7±0.0
25	1.2±0.0	7.6±0.0	0.5±0.01	1.0±0.02	6.2±0.1	0.3±0.0	1.1±0.0	6.5±0.1	0.4±0.0	1.4±0.04	8.3±0.2	1.12±0.07	1.3±0.02	8.0±0.1	0.9±0.0	1.1±0.0	6.6±0.1	0.6±0.0
26	1.3±0.03	8.1±0.2	0.5±0.03	1.0±0.03	6.4±0.2	0.5±0.0	0.9±0.1	5.7±0.9	0.4±0.0	1.7±0.05	10.2±0.3	1.08±0.02	1.2±0.00	7.5±0.0	0.9±0.0	1.2±0.0	7.6±0.1	0.7±0.0
27	1.1±0.03	6.9±0.1	0.5±0.04	1.2±0.01	7.3±0.0	0.4±0.0	1.1±0.1	6.9±0.1	0.4±0.0	1.6±0.00	9.6±0.0	0.74±0.01	1.1±0.03	6.9±0.2	0.5±0.0	1.1±0.0	6.9±0.1	0.5±0.0
28	1.1±0.01	6.9±0.0	0.5±0.01	1.0±0.01	6.0±0.1	0.4±0.03	1.1±0.1	6.9±0.2	0.6±0.0									
29	1.4±0.03	8.7±0.2	0.5±0.05	1.2±0.02	7.6±0.1	0.5±0.2	1.2±0.1	7.3±0.2	0.4±0.0	1.3±0.08	7.7±0.4	1.65±0.01	1.3±0.01	8.2±0.0	0.8±0.0	1.2±0.0	7.6±0.1	0.6±0.0
30	1.3±0.0	8.2±0.0	0.5±0.01	1.3±0.01	8.1±0.1	0.5±0.0	1.0±0.2	6.0±0.1	0.5±0.0	1.3±0.03	7.9±0.2	0.84±0.03	1.3±0.02	8.0±0.1	0.8±0.0	1.1±0.0	6.6±0.0	0.5±0.0
31	1.1±0.02	6.9±0.1	0.4±0.0	1.1±0.02	6.5±0.1	0.4±0.0	1.1±0.0	6.5±0.0	0.4±0.0	1.3±0.05	8.1±0.3	0.94±0.02	1.2±0.07	7.4±0.4	0.7±0.0	1.2±0.0	7.3±0.1	0.5±0.0
32	1.3±0.0	7.4±0.5	0.5±0.01	1.3±0.00	7.7±0.0	0.4±0.0	0.7±0.3	4.6±2.0	0.4±0.0									
CD P= (0.05)	0.08	0.57	0.06	0.07	0.04	0.08	0.22	NS	0.07	0.2	1.2	0.14	0.16	0.95	0.15	0.13	0.78	0.09

G= genotypes, P= parameters, GNC= Grain nitrogen content, GPC= Grain protein content, SNC=Straw N content

genotypic treatments. If we say in terms of yield, genotypes with high GY tend to posses low GNC which makes us rethink about using GNC as a sole parameter for evaluating NUE efficient genotypes.

4.6.1.2. Straw N content (%) and straw N yield (g/m^2) of individual genotypes in response to nitrogen and water regimes

Results from evaluation of genotypes for shoot/straw N content and straw yield (SNC & SNY) which is actually derivative trait of straw/shoot N concentration and yield differs significantly under varied set of treatment and condition. Using yield as a perspective, shoot N concentration in wholesome rather than alone is an important and contributing trait to total N uptake. Within NH_4^+, under irrigated and rainfed environment G-29 comparatively showed the higher value for SNC. If we talk differentially or separately, then under irrigated condition G-24, 23 & 5 SNC was having the highest value of 0.62, 0.63 & 0.67 while under rainfed condition G-29, 23, 21, 20, 16, 8 & 6 recorded values that ranged from 0.917-1.07, which was highest among all genotypes. SNY was of highest value for G-1, 2, 8, 21, 24, 29 & 32 under irrigated condition and for G-25, 27, 29 under rainfed condition. Genotype, G-29 inhabited highest values for SNY under both environments.

Within NO_3^- treatment, under irrigated and rainfed environment G-29 comparatively showed the higher value for SNC. If we talk differentially or separately, then under irrigated condition G-24, 23 & 5 SNC was having the highest value of .62, .63 & .67 while under rainfed condition G-29, 23, 21, 20, 16, 8 & 6 recorded values that ranged from 0.91-1.07, which was highest among all genotypes. GNY was of highest value for G-1, 2, 8, 21, 24, 29 & 32 under irrigated condition and for G-25, 27, 29 under rainfed condition. Genotype, G-29 inhabited highest values for GNY under both environments. Within N^0 treatment, under irrigated and rainfed environment G-5, 15 showed higher and comparatively similar values for SNC. Individual results flourish us with the genotypes, G-5 & G-8 with highest SNC value of 0.95 and 1.04 under rainfed environment and G-15, 20, 28, 31, and 32 possessing almost similar and higher range under irrigated environment. SNY was of highest value for G-5, 9, 15, 19, 28, 30 & 32 under irrigated condition and G-8, 30, 15, 16, 21, 22 & 25 under rainfed condition.

These results are in harmony with the findings of Kabir *et al.* (2011) who studied total N uptake by grain and straw under various treatment combinations with the motive of achieving high and sustainable yield goals which we actually aim with the present research. Barraclough *et al*. (2010) studied genotypic variation in N related parameters and N content of vegetative organs of wheat genotypes. Frageria (1998) reported genotypic differences in straw N content and reported adequate concentrations for maximum yield of about 8.7 g kg^{-1} in the shoot of upland rice under field condition at harvest. Likewise, Cormier *et al.* (2013a) reported genetic progress to a straw nitrogen concentration at physiological maturity of -0.52% $year^{-1}$ due to greater efficiency of nitrogen remobilization in modern cultivars.

4.6.1.3 Biological N yield (g/m^2) and N harvest index (%) (BNY and NHI) of individual genotypes in response to nitrogen and water regimes.

Biological N yield and N harvest index of rice genotypes further like all other parameters studied above varied across different N forms and water regimes. Within NH_4^+ treatment, under irrigated condition, mean comparison revealed that 5 genotypes *i.e.* G-9, G-12, G-16, G-17, G-18, G-30 and G-31 manifested highest values of NHI *i.e.* 69.1, 63.9, 63.7, 67.3 & 67.7 while under rainfed condition among all genotypes, higher values of NHI was obtained for G-4, G-23 & G-31 *i.e.* 40.1, 39.7 & 45.2. Under irrigated condition, BNY was highest in G-29 & G-30 while under rainfed condition BNY was highest in G-29, 27 & 23. Comparative results of NHI of NH_4^+ treatment under both sets of environment provided us with the genotype, G-21 that showed consistent values for studied parameter (NHI) as compared to other genotypes while G-29 recorded similar and larger values of BNY.

Under irrigated environment of NO_3^- treatment, NHI was highest for G-3, G-11, G-17,G-18, G-20 & G-31 while under rainfed condition, highest values where achieved in genotypes, G-27 & G-31. BNY was highest in G-30 under irrigated condition while under rainfed condition significant differences were not achieved for this trait. Within N^0 treatment, under irrigated and rainfed environment NHI values was relatively higher in almost all genotypes. Comparatively, G-27 manifested appreciable values for NHI under both

Table 4.12: Genotypic differences among rice genotypes for NUE component traits i.e. Grain N yield (GNY), Straw nitrogen yield (SNY), Biological N yield (BNY) and Nitrogen harvest index (NHI) during wet season, 2015

G/p	GNY	Irrigated								Rainfed							
		NH4+			CONTROL			NO3-			CONTROL			NO3			NH4+	
		SNY	BNY	NHI	GNY	SNY	BNY	NHI	GNY	SNY	BNY	NHI	GNY	SNY	BNY	NHI	GNY	SNY	BNY	NHI
1	4.7±0.3	3.2±0.5	7.93±0.8	59±1.9	2.83±0.3	2.02±0.0	4.86±0.4	58±3.0	4.1±0.0	1.9±0.2	6.1±0.3	67±2.5	1.50±0.6	2.83±1.2	4.3±1.8	34±0.5	2.71±0.3	3.52±0.0	6.23±0.3	43±2.5	2.0±0.1	2.3±0.1	4.3±0.3	46±0.8
2	3.8±0.0	3.9±0.1	7.8±0.1	49±0.4	2.7±0.1	2.8±0.3	5.5±0.2	48±2.1	1.7±0.6	0.9±0.5	2.6±1.1	68.±6.2	1.4±0.2	3.5±0.4	4.9±0.2	29±5.57	1.1±0.5	2.3±0.3	3.5±0.8	32±7.4	0.9±0.0	2.0±0.4	2.9±0.4	31±3.3
3	4.2±0.6	2.4±0.1	6.6±0.7	63±1.7	2.71±0.01	1.4±0.1	4.2±0.15	64±2.0	1.56±0.67	0.5±0.1	2.0±0.85	74±1.6	1.35±0.03	3.69±0.98	5.04±1.01	27±4.9	1.4±0.3	2.18±0.07	3.67±0.29	40±6.7	0.6±0.01	1.18±0.12	1.8±0.1	36±1.9
4	3.7±0.0	3.0±0.1	6.7±0.1	55±0.5	2.6±0.1	1.9±0.2	4.6±0.1	58±4.8	2.7±0.2	1.6±0.6	4.3±0.8	64±7.1	2.4±0.1	3.6±0.2	6.1±0.3	40±0.4	1.6±0.2	2.5±0.0	4.2±0.3	38±3.3	1.0±0.1	1.7±0.1	2.7±0.2	37±0.9
5	2.0±1.0	2.0±0.1	4.0±2.1	49±0.3	2.4±0.1	2.4±0.2	4.8±0.3	49±1.6	1.4±0.1	2.5±0.6	3.9±0.7	37±4.2	1.0±0.0	3.2±0.0	4.3±0.0	24±0.4	0.5±0.0	2.5±0.1	3.0±0.1	16±0.7	0.5±0.0	1.7±0.0	2.2±0.0	25±0.5
6	3.3±0.6	1.9±0.2	5.2±0.9	62±1.4	2.4±0.0	1.7±0.0	4.2±0.0	57±0.6	2.5±0.0	1.8±0.1	4.4±0.0	57±1.6	2.4±0.0	4.4±1.5	6.9±1.4	37±8.4	1.9±0.5	3.6±0.4	5.6±0.0	34±8.9	0.8±0.1	2.0±0.1	2.8±0.0	29±5.4
7	3.7±0.1	2.5±0.7	6.2±0.9	60±6.47	1.6±0.8	1.1±0.3	2.8±1.1	57±5.9	1.7±0.6	0.9±0.2	2.6±0.9	63±4.3	1.6±0.2	3.4±0.2	5.1±0.0	31±4.3	1.1±0.2	1.9±0.1	3.1±0.3	37±3.3	0.6±0.1	1.2±0.2	1.9±0.4	33±3.1

20±2.5	35±5.8	21±3.2	38±1.5	34±1.4	41±6.5	39±8.9	32±5.5	31±4.9
3.8±0.5	3.0±0.5	1.0±0.1	3.1±0.3	3.2±0.4	1.8±0.3	2.1±0.3	3.2±0.6	3.3±0.8
3.0±0.5	1.9±0.1	0.8±0.0	1.9±0.2	2.1±0.3	1.0±0.0	1.2±0.0	2.1±0.2	2.2±0.3
0.7±0.0	1.1±0.3	0.2±0.0	1.2±0.0	1.1±0.1	0.7±0.2	0.8±0.3	1.0±0.3	1.0±0.4
23±2.1	25±8.4	22.7±2.	46.1±2.	36±0.0	16.9±1.9	39±2.	39±0.5	37±0.9
4.8±0.2	7.2±0.7	2.6±0.1	6.1±0.2	5.1±0.1	3.7±0.5	3.4±0.2	6.0±0.5	6.6±0.7
3.7±0.0	5.4±1.1	2.0±0.0	3.2±0.2	3.2±0.1	3.1±0.3	2.1±0.2	3.6±0.3	4.1±0.5
1.1±0.1	1.7±0.4	0.6±0.0	2.8±0.0	1.8±0.0	0.6±0.1	1.3±0.0	2.3±0.2	2.4±0.2
31±1.3	37±2.3	28±1.4	33±0.7	35±3.2	34±2.1	28±0.0	31±3.2	34±3.8
5.1±0.2	6.7±1.2	5.2±0.0	5.7±0.9	6.6±0.5	5.6±0.3	5.3±0.1	6.3±0.4	6.0±0.4
3.5±0.2	4.2±0.9	3.7±0.0	3.8±0.6	4.3±0.1	3.7±0.3	3.7±0.1	4.3±0.0	3.9±0.0
1.6±0.0	2.4±0.2	1.4±0.0	1.9±0.2	2.3±0.4	1.95±0.01	1.5±0.0	2.0±0.3	2.1±0.3
53±1.9	54±5.1	53±4.5	36±16.8	59±5.0	63±1.8	57±6.3	44±1.4	63±0.6
4.9±0.3	3.0±1.1	3.1±1.5	2.7±1.5	2.6±0.9	4.2±0.3	5.0±1.1	4.4±0.4	4.2±0.0
2.3±0.2	1.3±0.3	1.4±0.5	1.4±0.5	1.0±0.2	1.5±0.2	2.2±0.8	2.4±0.3	1.5±0.0
2.6±0.0	1.7±0.8	1.7±0.9	1.2±1.0	1.6±0.7	2.7±0.1	2.8±0.3	1.9±0.1	2.6±0.0
52±1.0	57±0.7	49±2.7	60±0.2	52±0.0	57±6.0	58±4.1	54±0.4	53±0.18
5.4±0.8	4.0±0.0	4.4±0.4	4.4±0.0	5.0±0.4	4.5±0.1	4.5±0.5	3.7±0.2	4.2±0.0
2.5±0.4	1.7±0.0	2.2±0.3	1.7±0.0	2.3±0.2	1.9±0.1	1.8±0.0	1.6±0.1	1.9±0.0
2.8±0.3	2.3±0.0	2.2±0.0	2.6±0.0	2.6±0.2	2.6±0.3	2.6±0.4	2.0±0.1	2.2±0.0
39±4.14	63±1.5	50±5.2	49±3.9	61±2.0	58±0.7	58±10.0	50.0±2.1	64±2.4
7.6±0.2	6.3±0.0	6.4±0.6	5.8±0.5	6.8±0.1	6.5±0.2	5.7±1.1	2.9±1.0	5.8±0.6
4.6±0.4	2.3±0.1	3.1±0.0	2.9±0.0	2.6±0.0	2.7±0.1	2.2±0.1	1.4±0.4	2.0±0.3
3.0±0.2	3.9±0.0	3.2±0.6	2.9±0.5	4.2±0.2	3.8±0.1	3.4±1.2	1.4±0.6	3.7±0.2
8	9	10	11	12	13	14	15	16

47±3.4	50±0.5	45±2.1	40±3.5	24±5.2	22.±2.8	33±2.4	21±2.3	23±2.6
2.3±0.1	2.9±0.1	2.8±0.1	2.9±0.7	3.3±0.7	3.1±0.7	2.7±0.2	2.0±0.1	3.0±0.1
1.2±0.1	1.4±0.03	1.5±0.1	1.6±0.3	2.4±0.4	2.4±0.5	1.81±0.10	1.5±0.1	2.32±0.0
1.0±0.0	1.4±0.1	1.2±0.0	1.2±0.4	0.8±0.3	0.7±0.2	0.9±0.1	0.4±0.0	0.7±0.1
32±10.6	41±3.1	42±1.4	37±0.9	30±6.9	35±0.1	30.±0.3	29±0.2	23.6±2.4
5.0±1.1	5.0±0.0	4.1±0.0	4.6±0.3	3.5±0.7	4.9±0.0	4.8±0.6	3.3±0.6	5.3±0.7
3.3±0.2	2.9±0.1	2.3±0.1	2.8±0.2	2.4±0.3	3.1±0.0	3.3±0.4	2.3±0.4	4.1±0.6
1.7±0.9	2.1±0.1	1.7±0.0	1.7±0.0	1.1±0.4	1.7±0.0	1.4±0.2	1.0±0.1	1.2±0.0
17±1.4	37±2.9	33±3.3	45±5.6	23.5±0.9	23±3.2	39.7±0.6	33±0.8	22±2.8
5.1±0.0	6.2±0.5	5.3±0.3	6.7±1.0	5.4±0.0	4.6±0.2	7.0±1.1	5.3±0.6	7.9±1.2
4.2±0.0	3.9±0.5	3.5±0.3	3.5±0.1	4.1±0.0	3.5±0.0	4.2±0.7	3.5±0.3	6.1±1.2
0.8±0.0	2.3±0.04	1.7±0.0	3.1±0.8	1.2±0.0	1.0±0.1	2.8±0.4	1.7±0.2	1.7±0.0
60±3.1	60±2.9	52±5.0	60±1.1	60±0.4	40±1.4	66.2±1.3	50±2.8	36±11.7
3.5±0.3	4.2±0.7	6.3±0.3	5.2±0.8	4.32±0.0	3.8±0.1	4.1±0.4	5.5±1.6	2.3±1.0
1.3±0.2	1.6±0.15	3.0±0.4	2.0±0.4	1.70±0.0	2.2±0.1	1.4±0.1	2.7±0.9	1.3±0.3
2.1±0.0	2.5±0.5	3.2±0.1	3.1±0.4	2.6±0.0	1.5±0.0	2.7±0.2	2.7±0.6	0.9±0.6
64±2.6	65±2.6	55±0.4	64±0.7	46.1±4.5	58±5.9	59±0.8	48±5.4	52±2.0
4.0±0.1	4.12±0.01	5.3±0.4	3.7±0.1	4.1±0.0	4.0±0.1	4.5±0.1	5.7±0.2	3.67±0.0
1.4±0.0	1.40±0.10	2.3±0.1	1.3±0.0	2.2±0.1	1.6±0.3	1.8±0.0	2.9±0.2	1.7±0.0
2.6±0.2	2.72±0.11	2.9±0.2	2.4±0.0	1.8±0.2	2.3±0.1	2.6±0.1	2.7±0.4	1.9±0.0
69±5.0	63±0.02	58±0.4	59±4.8	41.7±0.7	53±2.6	50±5.6	49±1.9	44±6.3
4.4±0.3	5.2±0.9	5.5±0.2	5.9±0.7	6.8±0.0	4.6±0.3	5.6;±0.2	7.6±1.0	3.6±1.6
1.4±0.34	1.88±0.36	2.3±0.1	2.4±0.6	3.9±0.0	2.1±0.0	2.8±0.4	3.8±0.6	2.1±1.1
3.0±0.0	3.3±0.63	3.2±0.1	3.4±0.1	2.8±0.0	2.5±0.2	2.8±0.1	3.7±0.3	1.5±0.5
17	18	19	20	21	22	23	24	25

36±0.3	49±4.1		27 ±2.8	24±0.3	52±1.5		8.9	
2.2±0.3	3.7±0.9		2.2±0.5	3.2±0.1	3.0±0.3		1.1	
1.4±0.2	1.8±0.3		1.6±0.3	2.4±0.2	1.4±0.1		0.6	
0.8 ±0.1	1.8±0.6		0.6±0.2	0.7±0.0	1.5±0.2		0.6	
24±0.3	50±2.6		27±3.7	23.±0.2	49±3.7		12.1	
3.7±1.1	5.4±1.1		4.0±0.3	4.3±0.1	6.5±0.1		1.6	
2.8±0.8	2.6±0.4		2.9±0.3	3.3±0.1	3.3±0.1		1.1	
0.9±0.2	2.7±0.7		1.1±0.1	1.0±0.0	3.2±0.3		1	
35±0.8	36±11.1		19±0.1	33±0.3	45±2.7		11.5	
3.9±0.2	9.6±0.1		8.3±0.2	4.2±0.1	7.4±0.9		2.1	
2.5±0.1	6.0±0.9		6.6±0.0	2.8±0.1	4.0±0.3		1.7	
1.3±0.0	3.5±1.1		1.6±0.0	1.4±0.2	3.3±0.6		1.1	
57±1.7	61±1.9	54±0.6	67±3.3	62±0.1	56±14	48±15	16.7	
4.5±0.7	3.8±0.4	5.8±0.1	5.6±0.4	6.0±0.1	4.1±1.8	4.3±1.	NS	
1.9±0.2	1.4±0.2	2.6±0.0	1.7±0.0	2.2±0.0	1.5±0.2	2.0±0.1	1.1	
2.6±0.4	2.3±0.2	3.2±0.1	3.8±0.4	3.7±0.0	2.5±1.6	2. ±1.2	NS	
57±3.2	60±0.1	52±2.1	45±9.4	47±0.1	66±0.6	57 ±0.8	9.6	
4.7±0.6	4.7±0.1	6.0±0.1	3.2±2.4	10±0.1	6.3 ±0.	6.2±0.1	1.6	
1.9±0.1	1.8±0.1	2.8±0.2	1.5±1.0	5.3±0.1	2.12±0.	2.6±0.1	0.7	
2.7±0.5	2.88±0.1	3.1±0.0	1.7±1.4	4.8±0.0	4.1±0.1	3.6±0.0	1.0	
58±4.2	58±1.2	54±2.3	59±2.5	67±0.5	67±0.2	25±0.2	9.8	
3.8±1.9	5.0±0.8	6.9±0.5	11±0.2	10±0.1	9.0±0.2	6.5±0.0	2.4	
1.5±0.6	2.0±0.3	3.1±0.0	4.5±0.3	3.3±0.1	2.9±0.1	4.8±0.0	1.2..2	
2.3±1.2	2.9±0.5	3.8±0.4	6.6±0.1	6.9±0.0	6.1±0.1	1.7±0.0	1.4	
26	27	28	29	30	31	32	CD	P= (0.05)

G= genotypes, P= parameters, Grain N yield (GNY), Straw nitrogen yield (SNY), Biological N yield (BNY) and Nitrogen harvest index (NHI %), Unit =g/m^2

environments. G-1 showed the highest value for BNY under irrigated as well as rainfed condition. NHI values not varied much across different N regimes while water regimes considerably influenced the NHI values. Cormier *et al.* (2013b) reported that the key factor in the progress of NUE is to achieve better partitioning of nitrogen through an increase in NHI followed by a decrease in BNC & BNY at physiological maturity. Genotypes with high NHI allow us to put together, high yields and great GPC values (Hawkesford, 2012). Frageria *et al*. (2014) asserted NHI as an important trait for determining crop yield and NUE. They stated that in cereals, retranslocation of previously assimilated N in the vegetative parts is the predominant source of N for the grain thus higher NHI values determines greater yield for a crop. On contrary, Nayak *et al*. (2015) studied yield, nitrogen uptake and nitrogen use efficiency indices of aerobic rice (*Oryza Sativa* L.) under various irrigation regimes and nitrogen levels. They reported no significant difference among the irrigation regimes and nitrogen levels with respect to harvest index. Furthermore, Non significant difference among the irrigation regimes on harvest index obtained conformity with the finding of Mandal *et al.* (2013) and among nitrogen level collaboration was done by findings of Prakash *et al*. (2013).

4.6.1.4. N uptake, N-utilization and N-use efficiency ($gg^{-1}N$) of individual genotypes in response to nitrogen and water regimes.

To aid further analysis of factor contributing to NUE variability, NUE was partitioned in to NUpE and NUtE. Within NH_4^+ treatment, under rainfed condition no significant differences for NUtE was obtained among genotypes while under rainfed condition NUtE showed lower but varied range of values from 9.14 to 26.3. NUpE values under rainfed were more as compared to irrigated condition. Overall NUE values, under irrigated environment was highest for G-1, G-3, G-29, G-30 & G-31 while under rainfed environment NUE was highest for G-27 & G-31.

Within NO_3^- treatment, under irrigated condition NUtE was highest for G-20 & G-31 while under rainfed condition it was recorded highest for genotype, G-21. Overall, NUE was highest for G-19, G-28, G-30 & G-31 under irrigated environment and under rainfed it was highest for G-27. N^0 treatment also revealed not much differences for NUtE trait while under rainfed condition, G-17, 18, 19 & 27 showed higher values for NUtE trait. Genotypes, G-17, 20 & 27 performed well

Table 4.13: Genotypic differences among rice genotypes for NUE parameters during wet season, 2015

G/P	Irrigated								Rainfed							
	NH_4^+			NO_3^-			N^0		NH_4^+			NO_3^-			N^0	
	NUpE	NUtE	NUE	NUpE	NUtE	NUE	NUpE	NUtE	NUpE	NUtE	NUE	NUpE	NUtE	NUE	NUpE	NUtE	NUE
1	0.26±0.02	56±0.3	14.76±1.5	0.16±0.01	51±1.4	8.42±0.9	0.27±0.0	48±0.7	0.16±0.1	24.96±2.2	17.12±0.8	0.23±0.01	26.92±1.1	6.37±0.6	0.23±0.0	33±0.4	7.8±0.6
2	0.2±0.00	36±0.9	9.45±0.0	0.18±0.0	40±2.8	7.44±0.2	0.11±0.0	62±10.1	0.18±0.0	21.7±3.5	6.75±2.1	0.1±0.03	24.3±5.6	3.43±1.5	0.13±0.0	26±3.5	4.1±0.0
3	0.2±0.0	46±0.1	10.1±1.1	0.1±0.0	51±3.3	7.1±0.2	0.09±0.0	56±0.57	0.19±0.0	18.0±2.6	5.23±2.1	0.13±0.0	27.9±5.6	3.9±1.0	0.1±0.0	30±1.7	3.0±0.0
4	0.22±0.02	51±1.1	11.39±0.59	0.19±0.00	56±3.5	8.58±0.2	0.15±0.0	56±5.9	0.23±0.0	25.93±0.4	10.92±0.8	0.15±0.0	27±2.1	4.48±0.8	0.15±0.0	34±0.1	5.1±0.3
5	0.13±0.0	39±1.9	5.15±2.5	0.16±0.0	41±3.0	6.62±0.0	0.17±0.0	28±2.9	0.1±0.0	13 ±0.2	4.9±0.4	0.1±0.0	29.5±0.6	1.1±0.0	0.1±0.0	19±0.4	2.4±0.0
6	0.17±0.0	50±1.4	8.74±1.7	0.19±0.0	49±1.2	7.02±0.0	0.14±0.0	481±1.1	0.26±0.0	21 ±4.6	9.64±0.0	0.21±0.0	23.2±5.9	4.9±1.2	0.15±0.1	26±4.5	4.0±0.6
7	0.20±0.0	54±4.7	11.1±0.6	0.11±0.0	50±6.2	5.04±2.6	0.09±0.0	56±2.1	0.19±0.0	26 ±3.0	6.8±2.5	0.1±0.0	32.67±2.	3.89±0.7	0.10±0.	33±0.1	3.5±0.7
8	0.2±0.0	36±2.6	9.1±0.3	0.2±0.0	48±2.4	8.8±1.8	0.18±0.0	48±0.6	0.2±0.0	18.8±0.	10.6±0.9	0.18±0.0	16.6±0.	3.0±0.2	0.20±0.0	18.8±2.	3.8±0.0

5.1±1.6	1.08±0.1	5.22±0.0	5.40±0.6	3.23±1.1	4.55±1.8	5.22±1.8	5.22±1.8	5.79±0.0
30±4.5	18±0.7	30±3.1	31±0.7	31±5.8	37.45±9.	28.48±4.	27.98±3.	45.68±2.
0.16±0.0	0.05±0.0	0.17±0.0	0.17±0.0	0.09±0.0	0.11±0.0	0.17±0.0	0.18±0.0	0.12±0.0
5.7±1.4	1.8±0.1	6.06±0.1	5.82±0.0	1.93±0.6	4.01±0.0	5.89±0.3	5.89±0.3	4.56±1.9
21±7.5	17±0.8	26±1.1	30±0.2	13±2.8	30±1.6	25±0.9	23±1.3	22±5.1
0.2±0.03	0.10±0.0	0.23±0.0	0.19±0.0	0.14±0.0	0.13±0.0	0.22±0.0	0.25±0.0	0.19±0.0
6.7±0.7	4.75±0.0	5.49±0.4	7.28±1.3	4.27±0.0	4.48±0.1	4.73±0.7	4.73±0.7	1.85±0.1
26±3.1	24±0.2	26±6.	28±3.2	19±1.5	22.2±0.3	19.4±1.8	20.6±1.7	9.49±0.7
0.2±0.0	0.19±0.0	0.22±0.0	0.25±0.0	0.21±0.0	0.20±0.0	0.24±0.0	0.22±0.0	0.19±0.0
6.9±3.0	7.3±4.0	4.56±3.6	6.43±2.7	10.7±0.3	10.6±1.2	7.3±0.5	11.3±0.2	9.40±0.4
49±3.4	50±3.9	29±12.3	52±3.5	56±2.6	48±5.0	37±0.7	60±1.0	60±2.2
0.13±0.0	0.140.0	0.12±0.0	0.11±0.0	0.15±0.0	0.15±0.0	0.16±0.0	0.14±0.0	0.13±0.0
7.37±0.12	6.7±0.3	7.53±0.1	7.75±0.3	7.01±0.7	7.33±1.1	5.89±0.2	7.0±0.0	7.88±0.6
55±0.4	45±1.6	51±1.3	46±2.0	45±3.2	47±2.27	47±1.67	49±1.1	57±2.0
0. ±0.0	0.14±0.2	0.15±0.0	0.16±0.0	0.18±0.0	0.19±0.0	0.18±0.0	0.18±0.0	0.15±0.0
9.6±0.0	8.6±1.6	8.52±1.0	11.0±0.6	9.6±0.5	9.2±3.3	3.89±1.5	9.62±0.4	8.7±1.0
46±0.1	40±4.6	44±0.8	49±1.6	44±0.5	47±8.3	39.±1.5	50±3.3	59.±2.0
0.2±0.0	0.2±0.0	0.19±0.0	0.22±0.0	0.21±0.0	0.22±0.0	0.19±0.0	0.19±0.0	0.14±0.0
9	10	11	12	13	14	15	16	17

6.5±0.3	6.58±0.3	5.9±2.1	3.53±1.3	3.53±1.3	4.71±0.9	2.14±0.0	3.50±0.6	3.50±0.6
41±0.	42±0.6	36±3.9	18±2.7	20±2.7	31±3.2	19±1.7	21±1.8	28±0.5
0.1±0.0	0.15±0.0	0.1±0.0	0.18±0.0	0.17±0.0	0.14±0.0	0.10±0.0	0.16±0.0	0.12±0.0
4.7±0.1	4.62±0.0	5.4±0.4	3.05±1.2	4.96±0.0	4.01±0.5	2.86±0.5	3.49±0.0	2.71±0.7
24±0.1	29±0.6	31±0.3	21±4.7	26±0.2	21±0.8	22±0.2	17±2.1	19±0.1
0.19±0.0	0.15±0.0	0.17±0.0	0.13±0.0	0.18±0.0	0.18±0.0	0.12±0.0	0.20±0.0	0.14±0.0
4.75±0.2	4.75±0.2	6.9±1.9	3.1±0.3	3.19±0.3	6.33±1.0	5.28±0.9	4.84±0.3	3.05±0.0
20.1±1.1	23.43±0.	26.9±3.5	15±1.6	18±1.34	23±0.10	25±1.89	16±1.52	20±1.13
0.23±0.0	0.20±0.0	0.2±0.0	0.21±0.0	0.17±0.0	0.27±0.0	0.20±0.0	0.31±0.0	0.15±0.0
12.0±2.9	13.3±0.0	13±1.9	9.34±0.8	6.1±0.0	10.9±1.0	11.5±1.6	4.0±2.7	11.9±0.2
63±4.9	47±2.4	55±1.0	48±3.5	36±1.10	58±0.22	49±7.96	33±11.08	60±8.21
0.13±0.0	0.28±0.0	0.2±0.0	0.19±0.0	0.17±0.0	0.18±0.0	0.24±0.0	0.10±0.0	0.20±0.0
8.00±0.3	10.1±0.	7.5±0.3	6.08±0.5	7.0±0.3	7.6±0.5	7.9±0.9	6.18±0.1	8.46±1.3
58±2.3	56±3.5	60±0.3	44±3.2	52±5.1	50±1.9	41±3.43	50±0.94	53±1.28
0.18±0.0	0.17±0.0	0.12±0.0	0.13±0.0	0.13±0.0	0.15±0.0	0.19±0.0	0.12±0.0	0.15±0.0
8.32±1.5	10.±0.3	8.21±0.4	8.7±0.15	7.35±0.8	7.99±0.3	8.88±1.0	3.86±1.2	5.57±2.9
48±0.02	57±0.4	42±3.0	38±0.6	47±2.08	43±4.06	35±0.6	34±4.9	42±2.2
0.17±`0.0	0.18±0.0	0.19±0.0	0.22±0.0	0.15±0.0	0.18±0.0	0.25±0.0	0.11±0.0	0.12±0.0
18	19	20	21	22	23	24	25	26

8.84±2.9		2.7±0.0	3.8±0.9	6.9±0.6		2.68
42±3.07		21±2.6	21±0.4	42±0.5		8.16
0.20±0.0		0.1±0.0	0.17±0.02	0.16±0.0		0.03
9.06±2.5		3.0±0.1	2.81±1.49	9.91±1.4		3.04
43±3.59		19±2.49	17±0.39	80±4.52		9.26
0.20±0.0		0.1±0.0	0.16±0.00	0.12±0.0		0.06
8.37±2.6		4.73±0.1	4.08±0.00	9.42±2.1		3.13
22±6.98		15±0.7	25±0.9	33±3.37		7.16
0.36±0.0		0.3±0.0	0.16±0.04	0.28±0.0		0.06
9.00±0.7	12.2±0.0	13±2.2	16±0.53	10±6.54	12.3±0.02	6.12
52±2.2	46±1.1	55±4.5	61±1.15	50±12.8	66±9.3	14.73
0.17±0.0	0.2±0.0	0.2±0.0	0.26±0.01	0.18±0.0	0.19±0.05	0.03
7.75±0.2	10.2±0.3	4.5±3.8	11±0.00	12±0.15	9.30±0.00	2.96
48±0.22	51±1.3	35±7.9	34.63±0.41	60±0.5	44±0.62	8.42
0.15±0.0	0.20±.01	0.1±0.0	0.33±0.10	0.21±0.0	0.20±0.00	0.05
8.45±1.8	10.6±1.4	14±0.03	16±0.0	17±0.7	4.11±0.00	1.31
50±2.2	46±2.5	40±0.7	48±0.3	57±0.7	18.9±0.1	2.69
0.16±0.0	0.23±0.0	0.3±0.0	0.33±0.00	0.29±0.0	0.21±0.00	0.08
27	28	29	30	31	32	CD

P= (0.05)

G= genotypes, P= parameters, NUpE= nitrogen uptake efficiency, NUtE= nitrogen utilization efficiency, NUE= nitrogen use efficiency, unit = $gg^{-1}N$

for this trait under both environment. NUE under rainfed condition was much lower as compared to irrigated condition. Comparatively, NUE values of G-27 were higher under both environments. Results obtained showed that NUtE and NUpE under control condition was comparatively higher then treated field condition which synchronize with the findings of Huggins and Pan (1993) who reported that NUtE decreases with increasing fertilizer levels. Furthermore, similar to our findings, Huggins *et al.* (2010) obtained NUpE in control plot of 70% while decreased to minimum 40% in field with highest level of N. Haile *et al*. (2012) reported that variations in nitrogen use efficiency of grain yield were explained more by the variations in N uptake efficiency. They reported that NUE significantly decreases with increasing N levels but yield were not plateau out in such condition. Witcombe *et al.* (2008) further reported the importance of NUpE as an important parameter for selecting or obtaining N-efficient cultivar. Roy *et al.* (2010) further studied NUpE and NUtE parameters of wet and dry season rice for selecting genotypes for NUE and yield traits

4.6.2 Cluster analysis

Hierarchical cluster analysis was carried out using Euclidean distance metric and UPGMA (unweighted paired group method and arithmetic averages) method which produced a dendrogram showing successive combination of individuals. Cluster analysis in the present study delivered 6 dendrograms showing mainly 3-4 clusters, classifying 32 rice genotypes as high, medium and low NUE genotypes based on yield and NUE related traits under differential nitrogen and water regimes.

Results are shown in table 4.14 and fig. 4.8. Overall, G31 and G-1 performed well under both the environment both for NUE and yield parameters. Based on this study, 10 genotypes belonging to different sub clusters are subjected further to biochemical studies.

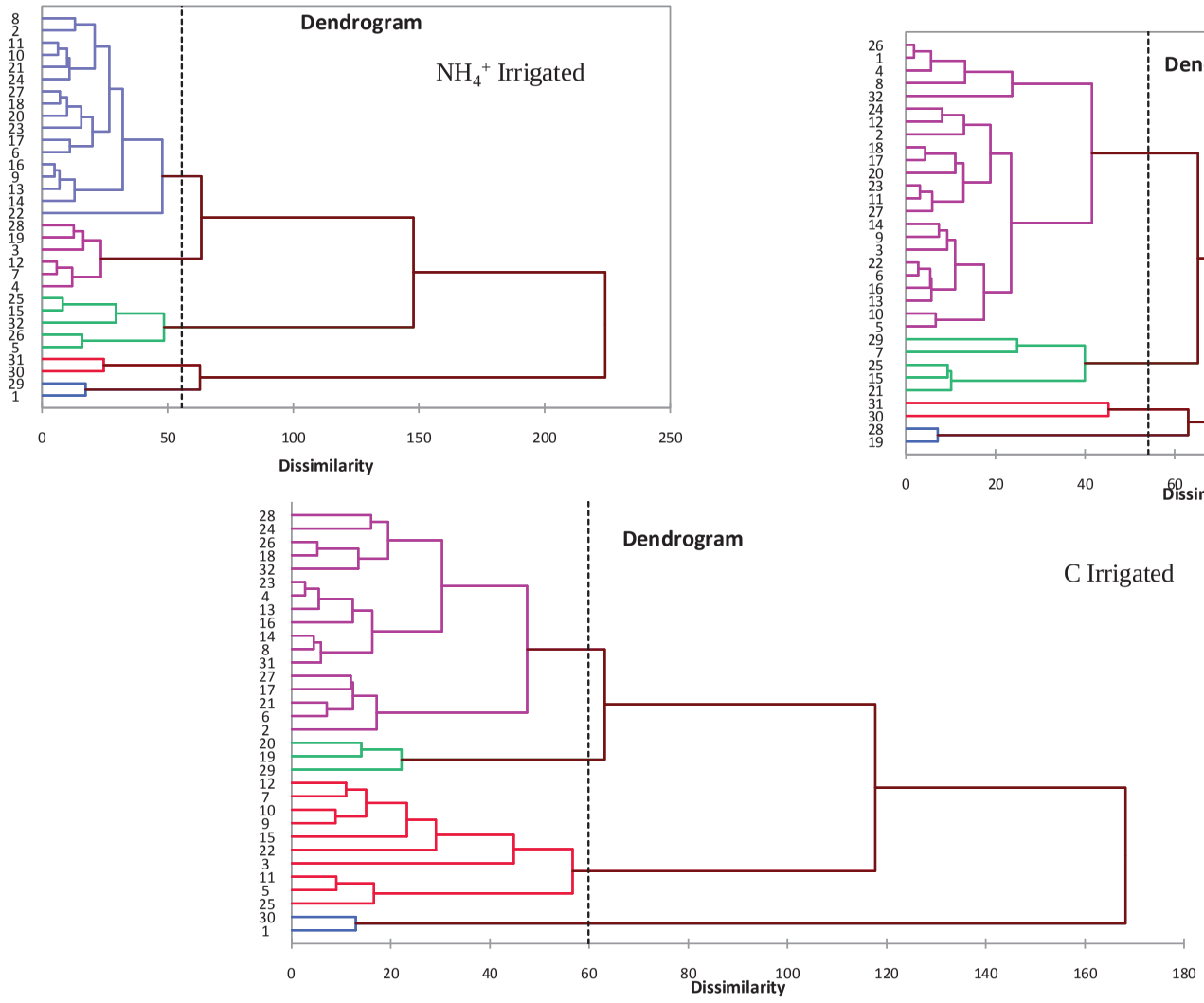
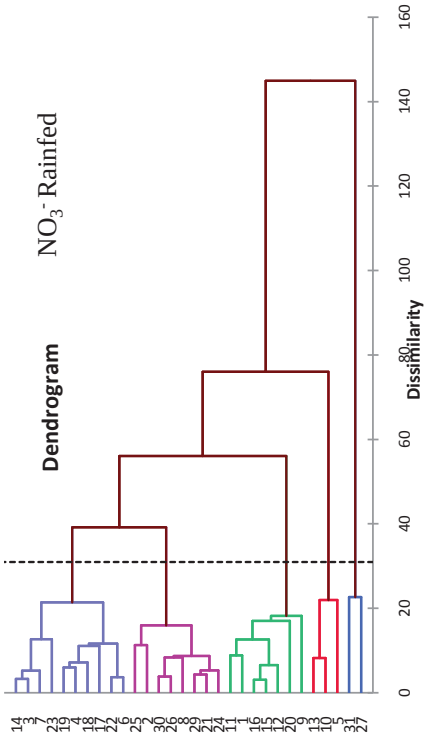
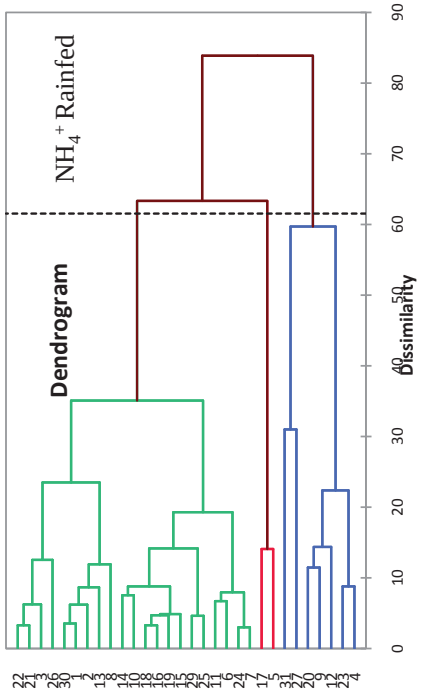


Fig. 4.8a: Hierarchal cluster analysis by UPGMA method





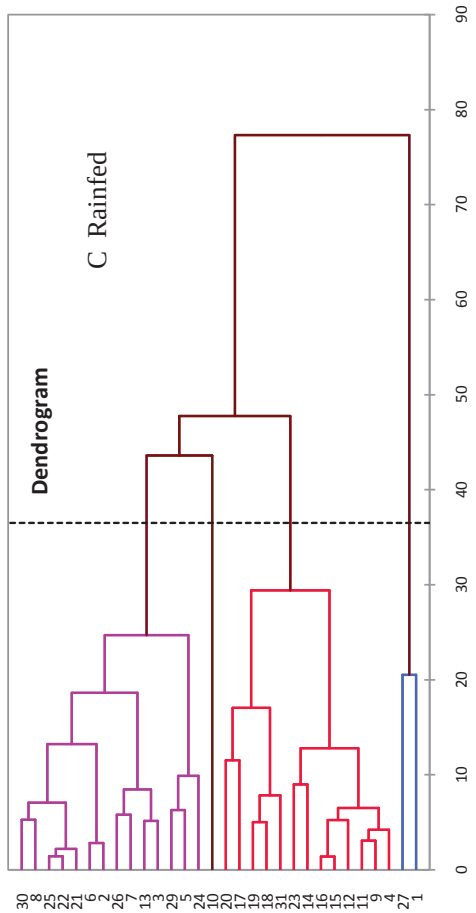


Fig. 4.8b: Hierarchal cluster analysis by UPGMA method

Table 4.14: N responsiveness in terms of yield and N responsiveness of 32 selected NO_3^- and NH_4^+ efficient cultivar

N regimes	Yield	IRRIGATED	RAINFED	I/R
NH_4^+ responsive	High	G-1, G-29, G-30, G-31	G-4, G-12, G-23, G-9, G-20, G-27, G-31	G-31
	Medium	G-2-14, 16-25, 27, 28	G-1, 2, 3,6-10, 13-19, G-21, 22, 25, 29	
	Low	G-25, G-26, G-15, G-32,G-5	G-5, 17	
NO_3^- responsive	High	G- 19, 28, 30, 31,	G- 27, 31	G-31
	Medium	G-1-6, G-8-14, G-16-20, G-23, 24, 26, 32	G-1-4, 6-9, 11, 12, 14-26,29,30	
	Low	G-29, 7, 15, 25, 21	G-5, G-10, G-13	
N^0 responsive	High	G-1, G-30, G-19, G-29, G-20	G-1, 27	G-1
	Medium	G- 2, 4,6,8, 13, 14, 16, 17, 18, 23, 24, 26, 27,28,31	G-4, 9, 11, 12, 14-21, 23	
	Low	G-3, 5, 7, 9, 10, 11, 12, 15, 22, 25, 30	G-2, 5-8, 21, 22, 24, 25,26 29 30	

I= Irrigated, R= Rainfed

This idea is supported by Yau. (1991), Ricon *et al.* (1996), Senzener *et al*. (2006) , who observed the genotypic variation in NUE indices among rice genotypes and classified biochemically & physiologically these genotypes using cluster analysis in to distinct groups based on response similarities.

4.6.3. Correlation between grain yield and NUE indices

Improvement for a trait of interest can be achieved by selection through other traits that are more heritable and easy to select. Therefore, it requires understanding of the interrelationship of the other traits among themselves and with traits of interest. In this study, correlation was established between grain yield and some important NUE indices (table 4.15). Under irrigated set of environment, Grain yield showed significant and positive association with Grain N yield, N-uptake efficiency, N-utilization efficiency, N-use efficiency and Nitrogen harvest index in NH_4^+, NO_3^- & N^0 treatment. Similarly, under rainfed condition and all sets of treatment, positive correlation existed between GY and NUE indices. The present study revealed that correlations between grain yield and N use efficiency traits were mostly positive, significant and strong. This strong and positive

correlation between grain yield and N use efficiency traits provides us with the platform of concurrent improvement of these traits. Similarly, Samont *et al*. (2006) reported significant and positive correlation between grain N yield and grain yield in rice. Under this study, N uptake efficiency had stronger correlation with N use efficiency as compared to the correlation between N utilization efficiency. Also, Muurinen *et al.* (2006) on rice genotypes reported that N uptake efficiency was far more important than N utilization efficiency in determining N use efficiency. Furthermore, Lakew's (2015) results showed that grain yield manifest significant and positive correlation with grain nitrogen yield and nitrogen harvest index which is very much similar to present findings.

Table 4.15: Pearson's correlation coefficients between important grain yield and NUE component traits under differential N regimes under irrigated and rainfed condition during wet season, 2015

	IRRIGATED			RAINFED		
Traits	**NH_4^+**	**NO_3^-**	**N^0**	**NH_4^+**	**NO_3^-**	**N^0**
	GY	**GY**	**GY**	**GY**	**GY**	**GY**
GNY	0.95**	0.92**	0.95**	0.94**	0.95**	0.98**
NUpE	0.82**	0.80**	0.81 **	0.75**	0.56**	0.64**
NUtE	0.57**	0.57 **	0.66 **	0.78**	0.81**	0.81**
NUE	0.98**	0.97**	0.99**	0.89**	0.99**	0.96**
NHI	0.57**	0.36* **	0.53 **	0.75**	0.85**	0.82**

** Significance at 5 % where, GY= grain yield, GNY=grain N yield, NUpE= N uptake efficiency, NUtE= N utilization efficiency, NUE= N use efficiency

4.7. Evaluation of mapping population for root and shoot traits in mini rhizotron with NH_4^+ and NO_3^- forms of nitrogen.

4.7.1. Correlation analysis between root and NUE-related traits

To investigate the contribution of roots to plant N efficiency, the Pearson correlation between root traits *i.e.* total root length (TRL), root volume (RV), Average root diameter (ARD) and yield & NUE-related traits *i.e.* grain yield (GY), grain nitrogen yield (GNY), nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE) and nitrogen use efficiency (NUE) was determined for 32 selected RILs (table 4.16). In our experiment differences in root and shoot parameters of genotypes under variable N forms was observed (Fig. 4.9 & 4.10). Furthermore, our study also revealed that overall correlations between root and

NUE-related traits were very weak and negative. Nevertheless, within NH_4^+, NO_3^- and N^0 treatment positive and strong correlations were observed between GY/TRL, GNY/TRL, NUpE/TRL, NUtE/TRL, NUE/TRL and NHI/TRL under irrigated and rainfed condition. Furthermore, significant correlation was also determined between root diameter and yield & NUE indices under rainfed and irrigated environment under all treatments. Moreover, within NH_4^+ treatment positive correlation existed between RV-GNY/GY/NUE/NHI under rainfed conditions.

These findings are further attributed by work of Chen *et al.* (2013); Mu *et al.* (2015) who reported that the increase in root size (root length, root volume and root density) improve N uptake ability and yield formation in maize. Besides the morphology, Trachsel *et al.* (2013) revealed that the architecture of roots also plays an important role in N acquisition; for example, deeper root more efficiently absorbs N in deep soil layers. Lawler (2002) concluded that the increase of the volume increases nitrogen uptake**.** Mazinani *et al.* (2013) studied morphologic characteristic of the root using image analysis method implemented in WinRHIZOPro, and their results showed significant differences in root length, surface area, volume and the number of forks among the studied rice cultivars. Also, Fan *et al.* (2010) reported that accumulation of nitrogen in plants has a close relationship with root characteristic, for which the main reason is the penetrance of the plants with larger roots in rice cultivars with higher nitrogen consumption rates. The differences in N-uptake, N utilization efficiency and NUE of the genotypes studied may be due to several factors. According to Jackson *et al.* (1986), root morphology and extension and biochemical and physiological mechanisms functions in nitrogen assimilation and use. Kling *et al.* (1996) also suggested that cultivar traits such as maximum rooting depth and the capacity of the roots to absorb nutrients enable plants to take up N from different soil layers. Thus, the components of roots themselves and their plasticity to soil N availability deserve important consideration as potential traits for genetic improvement of NUE indices.

Fig 4.9: A view of experimental set up under minirhizotron for evaluating root traits of genotypes

Fig 4.10: Variation in root and shoot growth of genotypes evaluated under NH_4^+, NO_3^- and N^0 treatment in minirhizotron

Table 4.16: Pearson's correlation coefficients between root traits and NUE-related trait evaluated in the minirhizotron.

		NH_4^+			NO_3^-			N^0		
Traits	**E**	**TRL**	**RD**	**RV**	**TRL**	**ARD**	**RV**	**TRL**	**RD**	**RV**
GY	I	0.700**	0.361*	0.151ns	0.612**	0.411*	0.016	0.613**	0.423*	-0.211
	R	0.715**	0.250*	0.533**	0.516**	0.420*	0.040	0.622**	0.561*	-0.124
GNY	I	0.723**	0.347*	0.173	0.543**	0.362*	0.022	0.586**	0.456*	-0.202
	R	0.735**	0.459*	0.555**	0.673**	0.323*	0.195	0.591**	0.479*	-0.071
NUpE	I	0.697**	0.337*	-0.117	0.567**	0.426*	-0.019	0.712**	0.418*	-0.146
	R	0.689**	0.317*	0.567**	0.654**	0.326*	-0.013	0.745**	0.427*	0.095
NUtE	I	0.728**	0.333*	0.270	0.540**	0.412*	0.132	0.633**	0.433*	-0.214
	R	0.712**	0.495*	0.555**	0.631**	0.482*	-0.189	0.678**	0.495*	-0.232
NUE	I	0.679**	0.315*	-0.080	0.726**	0.461*	0.016	0.762**	0.458*	-0.178
	R	0.643**	0.346*	0.511**	0.710**	0.446*	0.400	0.745**	0.461*	-0.124
NHI	I	0.561**	0.362*	0.179	0.641**	0.471*	0.185	0.623**	0.423*	-0.178
	R	0.601**	0.378*	0.561**	0.512**	0.458*	0.132	0.615**	0.418*	-0.178

E= Environment, Total root length (TRL), Root volume (RV), Average root diameter (ARD), Grain yield (GY), Grain nitrogen yield (GNY), Nitrogen uptake efficiency (NUpE), Nitrogen utilization efficiency (NUtE) and Nitrogen use efficiency (NUE).

4.7.1. Genotypic differences for root and shoot traits among selected rice genotypes grown under minirhizotron

The identification of root traits offers the potential to increase the grain yield of not only crops growing under limited soil resources but also crops growing under variable water and nutrient supply by revealing important physiological traits (Herrera *et al.*, 2012). Root-related traits playing important role in increasing grain yields has not been fully explored. Here, we studied genotypic variation in root traits, focusing on traits that may increase N-uptake ability and yield formation. Phenotypic variations in root and shoot traits among selected 10 rice genotypes under NH_4^+, NO_3^- and N^0 conditions is depicted in table 4.17. These genotypes are acquired from 32 RILs previously studied on the basis of contrasting NUE and root-related traits. In the present investigation, we found differences in average values of seedling height (SH), root fresh weight (RFW) and maximum root volume (MRV) and significant differences among genotypes in mean values of maximum root length (MRL) in NH_4^+ (23±2.0-56±2.7), NO_3^- 20±4.5-54±4.0)

Fig 4.11: Genotypic differences in root length and shoot fresh weight of G-8 and G-6 genotype under NH_4^+, NO_3^- and N^0 treatment in minirhizotron

Table 4.17: Genotypic differences for root and shoot traits among selected rice genotypes grown under minirhizotron (10 genotypes)

G	NH_4^+						NO_3^-						N^0					
	SH	**MRL**	**TT**	**SB**	**RFW**	**TRV**	**SH**	**MRL**	**TT**	**SB**	**RFW**	**TRV**	**SH**	**MRL**	**TT**	**SB**	**RFW**	**TRV**
1	37±6.0	23±2.0	7±0.4	3±0.1	4±0.3	4±0.1	46±6.1	51±2.0	11±0.6	5±0.2	6±0.2	5±0.4	45±6.1	25±1.5	8±0.5	3±0.4	4±0.8	6±0.6
2	43±6.5	36±4.5	12±0.5	5±0.7	5±1.1	7±1.9	27±6.2	20±4.5	9±0.4	2±0.3	4±0.6	7±1.6	36±6.3	49±1.0	8±0.3	3±0.5	6±0.6	8±1.7
3	42±1.0	43±3.5	7±0.7	4±1.4	6±0.3	8±0.4	46±1.4	45±4.0	9±1.0	5±0.7	6±0.8	6±2.3	43±4.1	47±1.5	6±0.4	4±0.2	5±0.5	4±0.5
4	46±3.5	18±2.0	2±1.0	2±0.1	1±0.02	2±0.3	37±6.0	26±9.0	4±0.5	2±0.1	3±0.7	4±1.7	27±3.1	46±1.5	5±0.5	4±0.8	6±0.3	7±1.2
5	53±6.1	53±3.0	11±4.0	5±2.1	6±0.2	6±0.7	51±0.8	36±17.1	8±0.4	5±0.2	4±0.4	6±0.8	56±1.0	38±9.0	7.5±0.6	3±0.1	5±0.4	8±0.9
6	46±9.5	28±4.5	15±3.5	**10±2.2**	7±0.9	9±0.2	31±3.4	20±4.5	10±1.5	**6±0.1**	4.4±0.5	7±1.6	35±2.5	43±1.5	7±0.5	**5.3±0.1**	6±0.1	9±0.1
7	41±7.5	26±2.0	11±0.7	8±0.3	5±0.6	8±1.7	34±0.5	23±0.5	7±1.5	2±1.3	3±0.1	5±1.8	30±0.5	25±1.0	6±2.0	2±0.5	4±0.6	6±0.7
8	53±6.2	**56±2.7**	9±0.2	4±2.6	5±0.1	4±1.7	51±0.7	**54±4.0**	5±0.5	3±0.2	6±1.9	10±1.5	51±3.5	**72±4.0**	5±0.5	4±0.1	6±1.7	8±2.6
9	35±3.0	54±2.1	8±0.6	6±2.9	6±0.1	8±0.2	22±1.0	22±5.1	4±0.6	5±0.1	6.8±0.1	4±3.2	28±0.5	34±4.5	11±1.5	3±1.1	5±1.4	7±1.0
10	36±7.5	26±2.7	9±1.5	5±0.6	3±0.1	4±0.4	36±2.0	34±9.1	7±0.7	2±0.4	4±1.0	7±1.8	34±1.0	38±4.1	8±0.5	3±0.9	6±0.8	7.2±1.4
C.V.	24.81	10.38	26.6	45.1	16.1	23.5	13.4	33.4	28.1	43.3	28.2	38.8	6.5	13.2	16.8	27.6	23.8	27.5
SEd	4.51	3.9	2.5	2.3	0.7	1.5	2.8	10.8	3.7	4.3	1.1	2.4	2.6	5.5	1.2	0.8	1.2	1.7

G=Genotypes, SH=Seedling height (cm), MRL= Maximum root length (cm), TT=Total tillers (no.), SB=Shoot biomass (g), RFW=Root fresh weight (g), TRV=Total root volume (cm^3).

and N^0 (25±1.5-72±4.0) treatment and shoot fresh weight in NH_4^+ (2±0.1- 10±2.2), NO_3^- 2±0.2- 6±0.1) and N^0 (2±0.5- 5.3±0.1). On the other hand, significant higher values for SH, TT, SFW was obtained under NH_4^+ and $N0_3^-$ treatment while on contrary significantly more values of RL, TRV and RFW was obtained under N^0 treatment. The higher values for root morphological traits under N^0 condition may confer plant an ability to tolerate abiotic stress such as low nitrogen. The low N tolerant cultivars are superior in the utilization of available N, either due to enhanced uptake capacity or because of more efficient use of absorbed N in grain production enhanced uptake capacity or because of more efficient use of absorbed N in grain production (Laffiete and Edmeades, 1994). Similar results were obtained by Sen *et al.*, (2013) who actually analyzed the root traits of maize under low nitrogen and obtained higher values for RL and RFW under low nitrogen as compared to high nitrogen condition.

Root plasticity, a trait that can respond to different soil and nitrogen environments, may assist plants to scavenge the nutrients in heterogeneous soils. In our results (Fig. 4.11), among all the contrasting genotypes studied, NH_4^+, NO_3^- and N^0 responsive genotype with maximum root length was G-8 (56±2.7, 54±4.0 , 72±4.0) while maximum shoot fresh weight was prevalent in G-6 (10±2.2, 6±0.1, 5.3±0.1). Rooting depth was used as selection criteria for identifying genotypes responsible for nitrogen-deficiency tolerance in rice by Ogawa *et al*. (2014). Chun *et al*. (2005) reported that under varied level of N, root of maize varies in its length. So, it can be concluded that rooting length would be helpful to enhance the nitrogen-acquisition efficiency under N-deficit conditions in rice. Also, selecting genotypes for better growth under varied N levels and improving the root traits for more efficient uptake of the nutrients present in the soil would open the door for the path of food-sufficiency and sustainability of agriculture.

4.8. Stability analysis

Rice cultivation is now foraying in to less traditional rainfed with mounting pressure of climate change coupled with less soil moisture and nutrient availability making cultivation in these delicate ecosystems rather intricate. Therefore the development of genotypes that consistently perform under these altered soil moisture and nutrient regime is a viable option. Consistent and quality

performance of genotypes is always desired as it increases the longevity of the genotypes. In breeding exercises, stable and high performance of genotypes in targeted growth environments or across different environment and seasons is an important attribute. Stability of the line is measured as a non-significant deviation from its regression coefficient and stated with reference to its mean. Lines with high means and average stability can be identified to suit in most environments.

Grain yield is the most important trait in the selection of superior rice genotypes. Identification of genotypes with high grain yield, stability and average response of immense value. The stability analysis was done according to Eberhart and Russell (1966) model using data of 124 RIL population under ten conditions. The genotypes were subjected to this analysis taking single character grain yield and stability parameters namely regression value (bi), mean (x) and deviation from regression (S^2di) were investigated further and depicted in table 4.18.

Table 4.18: Analysis of Variance for Stability parameters for grain yield (Eberhert and Russell Model)

Source of Variation	d.f.	Mean sum of Squares
Genotype	123	18.32**
Environment	5	1268.74**
Genotype X Environment	615	8.48**
Environment + Genotype X Environment	620	18.65
Environment (Linear)	1	6343.74**
Environment X Genotype (Linear)	123	15.36**
Pooled Deviation	496	6.71
Pooled Error	738	3.89
Total	743	

4.8.1. Analysis of variance for stability parameters

The results of this study for grain yield are presented in table 4.19. Mean squares due to environment (linear) was found significant for most grain yield, indicating differences between environments and their influence on genotypes for expression of these characters. This is in accordance with previous reports on rice by Sawant *et al.* (2005) and Panwar *et al.* (2008). Furthermore, the environment + (genotype x environment) was significant indicating distinct nature of environments and genotype x environment interactions in phenotypic expression. The genotype x environment (linear) interaction component showed significance for grain yield. This indicated significant differences among the genotypes for linear response to environments (bi) behaviour of the genotypes could be predicted

over environments more precisely and G X E interaction was outcome of the linear function of environmental components. Hence, prediction of performance of genotypes based on stability parameters would be feasible and reliable. Gouri-Shankar *et al.* (2008) and Parry *et al.* (2008) also noticed significant linear component of G X E and non linear components of G x E interaction for the characters studied.

4.8.2. Stability parameters for grain yield

The mean performance and stability parameters for grain yield are given in table 4.19. The overall mean grain yield pooled over six environments *i.e.* NH_4^+, NO_3^-, N^0 irrigated environment and NH_4^+, NO_3^-, N^0 rainfed environment varied from 45.5 g/m^2 to 431.3 g/m^2. High value of mean grain yield and bi>1 were recorded for line number 8, 17, 18, 36, 38, 39, 58, 62, 84 and 88. Line number 13, 61 and 39 had high value of mean grain yield and bi<1. The line number 32, 34, 56, 110 and 66 had high value of mean grain yield, unit regression (bi=1) and deviation was non-significant from zero (S^2di).

Table 4.19: Results of stability analysis

RILs	Mean GY+	bi	S^2_{di}
4	259.15	1.643	0.36
7	280.77	0.856	0.78
8	270.07	0.581	0.93*
9	255.83	0.917	0.43
10	270.17	1.08	0.51
13	247.46	1.147	0.29
17	268.41	0.039	2.38**
18	292.71	2.054	1.17**
27	245.28	0.869	0.12
28	232.54	1.211	0.19
31	290.69	1.032	0.052
32	**256.55**	**0.881**	**-0.05**
33	258.86	0.586	1.89
34	**253.95**	**1.213**	**-0.05**
35	241.57	1.468	0.31
36	289.64	1.931	0.85*
38	255.28	0.354	1.69**
39	271.82	0.352	1.69**
44	257.52	1.281	0.45
50	**256.92**	**1.294**	**-0.11**
51	228.79	1.18	-0.13
53	301.53	1.007	0.45
54	276.69	0.659	0.18
56	**305.82**	**1.365**	**-0.03**
58	240.25	1.521	0.69*
59	244.07	1.108	0.18
60	384.43	1.63	0.48
62	267.90	1.486	1.09**

RILs	Mean GY+	bi	S^2_{di}
64	330.59	1.706	0.077
65	264.12	1.842	1.86**
66	**288.28**	**0.989**	**-0.08**
67	282.05	1.667	0.43
68	318.32	1.623	0.23
69	278.81	1.233	0.038
71	243.94	1.357	0.37
72	337.61	1.599	0.061
76	257.75	0.563	0.59
77	313.74	2.285	1.60
78	431.29	2.335	2.26
83	304.45	1.233	0.30
84	283.88	1.32	0.81*
85	243.30	1.181	0.12
86	260.46	0.607	0.56
87	247.21	0.784	0.38
88	258.45	1.82	0.89*
89	265.15	0.718	0.52
100	238.54	1.705	1.35
105	281.04	1.916	1.92
106	261.69	1.041	0.12
109	282.59	1.88	0.28
110	**371.65**	**1.468**	**-0.04**
112	238.55	1.414	0.16
113	354.70	1.826	0.18
114	285.85	1.71	0.52
115	287.91	1.847	1.14
116	272.08	1.647	0.18
117	316.03	1.269	0.02
118	301.89	1.584	0.32
120	291.42	1.774	0.45
121	306.42	1.064	0.17

+ grain yield in g/m2, Grand mean = 236 g/m^2 * and ** = significant at 0.05 and 0.01 probability level, respectively

4.9 Enzymatic activities and its relationship with Nitrogen use efficiency

Standard analysis of variance techniques were used to assess the significance of treatment means (NH_4^+, NO_3^- and N^0) on the activities of nitrogen uptake and ammonium assimilatory enzymes. The data are represented as mean values ± SEm. A correlation analysis is also established between enzymatic activities and NUE indices.

4.9.1 Enzymatic activities

In a set of 122 RILs, 32 selected genotypes were evaluated for NUE & related traits and cluster analysis was further performed to classify these genotypes as high NUE, medium NUE and low NUE. Frequency distribution of leaf colour was also recorded to categorize RILs into four different classes' *viz.,* dark green, green, light green, yellow. Investigation of leaf colour trait accompanied by evaluation of NUE, yield and yield related parameters provided us with the 10 distinct genotypes. The differences observed in the leaf colour and NUE parameters under different N forms in these genotypes possibly may be due to genotypic variation in the activity of enzymes involved in assimilation of nitrogen. Earlier reports by Tabuchi *et al.,* 2007b; Martin *et al.,* 2006b; Hirel *et al.,* 2007b etc illustrates variation in enzyme activity in genotypes and their role in enhancing NUE of a genotypes. Our experiment aimed to investigate the activity of key assimilatory enzymes namely, Glutamine Synthetase (GS), Glutamate Synthase (GOGAT), Nitrate Reductase (NR) and Nitrite Reductase (NiR) at seedling stage in 10 selected rice genotypes. The results showed that N forms greatly influenced the measured traits and substantial differences among rice genotypes were recorded in the activities of the enzymes. Results are shown in table 4.21, and fig 4.12 and fig 4.13. The mean values of NR, NiR, GS and GOGAT activity is depicted in table 4.21. The perusal of this table provided us with genotype G-121 having higher NR, NiR, GS, GOGAT activity values. Results are elaborated below.

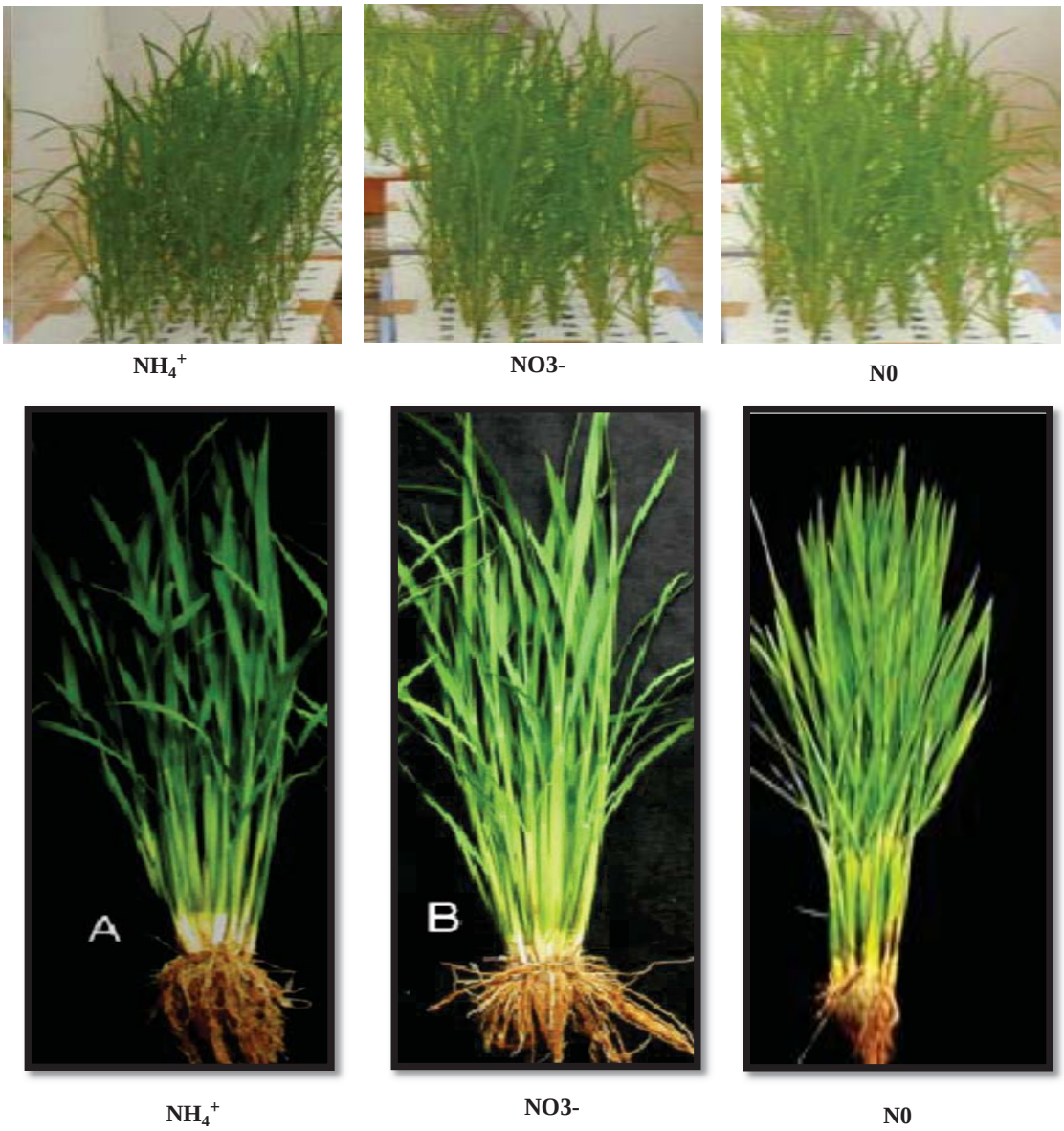


Fig 4.12: Rice genotypes grown under NH_4^+, NO_3^- and N^0 treatment

Table 4.20: Leaf colour and SPAD value of selected rice genotypes

S.No.	(K-2014)	N forms	Colour	SPAD
1		NH_4^+		29.8
	4	NO_3^-	Y	29.6
		N^0		29.2
2		NH_4^+		34.1
	21	NO_3^-	DG	34.9
		N^0		31.1
3		NH_4^+		30.4
	30	NO_3^-	Y	31.3
		N^0		28.1
4		NH_4^+		36.4
	46	NO_3^-	DG	32.6
		N^0		31.1
5		NH_4^+		29.9
	75	NO_3^-	Y	28.4
		N^0		26.46
6		NH_4^+		33.9
	121	NO_3^-	DG	33.2
		N^0		27.38
7		NH_4^+		35.1
	Danteshwari	NO_3^-	G	33.7
		N^0		31.78
8		NH_4^+		26.9
	Dagad deshi	NO_3^-	LG	30.5
		N^0		31.3
9		NH_4^+		35.4
	Swarna	NO_3^-	DG	35.4
		N^0		34.95
10		NH_4^+		30.2
	Indra Sugandhit Dhan 1	NO_3^-	Y	28.4
		N^0		29.43

4.9.1.1 NR/NiR activity

The first step in NO_3^- assimilation is the reduction of nitrate to nitrite, which is catalyzed by NR. In our experiment, N forms significantly affected the NR activity of rice genotypes in leaves rather than roots in all treatments. The highest values in leaves were recorded for NO_3^- treatment followed by NH_4^+ and N^0 treatment. The range of variation for NO_3^- treatment was from 2.19 to 0.84 µmol mg^{-1} min^{-1}, for NH_4^+ treatment was from 0.004 to 0.033 µmol $mg^{-1}min^{-1}$and for N^0 treatment was from 0.014 to 0.028 µmol $mg^{-1}min^{-1}$. Moreover, mean activity values in roots for NO_3^-, NH_4^+ and N^0 treatment was 0.006, 0.001 & 0.002 µmol mg^{-1} min^{-1}, which was very minimal and significantly not different among rice genotypes. In our experiment the rise in NR activity is leaves may be due to the process cited by Vincentz *et al.* (1993) who reported that NR is a substrate inducible enzyme and its stimulation is closely reliant on the availability of nitrate, and is known to be positively regulated by nitrate availability. Additionally, the present research also showed large variation in NR activity among three forms of nitrogen. NR activity, under NO_3^- treatment, was 2–3 times higher than that of other two treatments, which might be due to less preferable form of nitrogen under NH_4^+ and N^0 conditions as compared to NO_3^- treatment (Souza and Fernandes 2006a). The response of NiR to the N forms resembled that of NR. The enzymatic activity both in roots and leaves were more in NO_3^- treatment as compared to NH_4^+ and N^0 treatment. The mean activity values was 0.031 µmol mg^{-1} min^{-1} under NO_3^- treatment in leaves and was 0.017 µmol mg^{-1} min^{-1} in roots. In case of NH_4^+ and N^0 treatment mean value for activity was 0.023 and 0.016 (µmol mg^{-1} min^{-1}) in leaves and 0.001 & 0.001 (µmol mg^{-1} min^{-1}) in roots. Souza and Fernandes (2006b) reported higher activity of NR and NiR in leaves as compared to roots.

The ultimate goal is to improve nitrate assimilation in plants which is major form of N under aerobic/ drought environment. Rice genotypes could be classified as high, moderate and low NR and NiR categories. The high NR and NiR activity of a cultivar signifies nitrate assimilatory power of a genotype. Genotype G-21, G-30, G-75 & G121 expresses high NR activity under $N0_3^-$, NH_4^+ and N^0 treatment as compared to other genotypes. These results show efficiency of these genotypes to

utilize nitrate form of nitrogen over other forms. Furthermore, NiR activity was found to be highest for G-10 followed by G-9, 8, 7, 6 respectively denoting their capability in utilizing nitrate form of nitrogen. Barlaan and Ichhi (1996) identified cultivar with high NR and NiR activity with aim of improving nitrate assimilation rice which is actually need of an hour.

Additionally, earlier we characterized genotypes in high and medium/low NUE genotypes based on NUE and yield indices. With regard to genotypes a necessary conclusion was made that G-1, 3, 5, 7 &10 (Out of 32 - G-4, 7, 17 & 26) showed higher NR/NiR activity actually falls under medium/low NUE genotypes. These results show the opposite relationship exist between NiR activity and overall NUE indices. These findings collaborate with the research of Vijayalakshmi *et al*. (2015b) who reported that high NUE genotypes manifest the low NR/NiR activity. Anjana *et al*., 2007; Chandna *et al.,* 2012b established the fact that high NUE genotypes have low threshold level for NR activity and hence there was no change in NR activity with increase in N levels. Furthermore, the variation in NR/NiR activity between these genotypes may be related to the difference in regulation of N transporter genes or N fluxes in roots (Britto and Kronzucker, 2001b). On contrary with these findings, Hakeem *et al.* (2012b) reported that high NUE genotypes showed more NR activity and consistent even with increase in N levels where as NR activity was increased with increase in N levels in low NUE genotypes.

4.9.1.2 GS/GOGAT activity

The principal NH_4^+ pathway is the Glutamine Synthetase (GS)/Glutamate Synthase (GOGAT) cycle. With respect to the enzyme activity of GS, NH_4^+, NO_3^- and N^0 treatment had significant effect both in root and leaves of genotypes. The highest activity was recorded in N^0 treatment followed by NH_4^+, NO_3^- treatment both in roots and leaves. In NH_4^+ treatment, the range of variation in leaves was from 0.084 to 0.134 µmol mg^{-1} proteins min^{-1} with average value of 0.099 µmol mg^{-1} proteins min^{-1} and in roots it ranged from 0.002 to 0.046 µmol mg^{-1} proteins min^{-1} with mean value of 0.023 µmol mg^{-1} proteins min^{-1} in roots. In NO_3^- treatment, the range of variation in leaves was from 0.083 to 0.147 µmol mg^{-1}

proteins min^{-1} with mean value of 0.123 µmol mg^{-1} proteins min^{-1} while in roots it ranged from 0.002 to 0.055 µmol mg^{-1} proteins min^{-1} with mean activity value of 0.034 µmol mg^{-1} proteins min^{-1}. In N^0 treatment, the activity values ranged from 0.085 to 0.184 µmol mg^{-1} proteins min^{-1} with average value of 0.155 mg^{-1} proteins min^{-1} in leaves while in roots it ranged from 0.026 to 0.06 µmol mg^{-1} proteins min^{-1} with mean activity value of 0.038 µmol mg^{-1} proteins min^{-1}.

The GOGAT activity followed a similar pattern as that of GS. The highest activity was recorded again for N^0 treatment over NH_4^+ and $N0_3^-$ treatment. In NH_4^+ treatment, the range of variation in leaves was from 0.143 to 0.298 µmol mg^{-1} proteins min^{-1} with average value of 0.196 µmol mg^{-1} proteins min^{-1} and in roots it ranged from 0.038 to 0.068 µmol mg^{-1} proteins min^{-1} with mean value of 0.051 µmol mg^{-1} proteins min^{-1} in roots. In NO_3^- treatment, the range of variation in leaves was from 0.175 to 0.463 µmol mg^{-1} proteins min^{-1} with mean value of 0.281 µmol mg^{-1} proteins min^{-1} while in roots it ranged from 0.032 to 0.065 µmol mg^{-1} proteins min^{-1} with mean activity value of µmol mg^{-1} proteins min^{-1}. In N^0 treatment, the activity values ranged from 0.126 to 0.710 µmol mg^{-1} proteins min^{-1} with average value of 0.291 µmol mg^{-1} proteins min^{-1} in leaves while in roots it ranged from 0.056 to 0.081 µmol mg^{-1} proteins min^{-1} with mean activity value of 0.069 µmol mg^{-1} proteins min^{-1}. The highest GOGAT activity was achieved for G-9 (Swarna) in N^0 treatment, G-2 in $N0_3^-$ treatment and G-2 in NH_4^+ treatment while highest GS activity was observed for G-9 in NH_4^+ treatment, G-7and G-9 in NO_3^- treatment and N^0 treatment. According to cluster classification G-9 falls in high NUE genotypes which also possess high GS & GOGAT activity.

Being involved in all aspects of nitrogen metabolism, GS is a key component of nitrogen use efficiency (NUE) and plant yield, justifying the extensive amount of studies that have been dedicated to understand how GS is regulated and how it regulates nitrogen metabolism in plants (Bernard and Habash, 2009; Lea and Miflin, 2010; Thomsen *et al*., 2014). GS activity is also one of the selection criteria to identify nitrogen use efficient cultivars and their use in developing mapping population for high NUE is in progress (Vijaylakshmi *et al*.,

2013). With the ammonium and nitrate nutrition, GS and GOGAT activity in shoot and root was recorded to be highest for genotypes G-2, G-4, G-6 & G-9. These genotypes actually correspond to dark green color in color classes and possess higher SPAD values as shown in table 4.12. In green leaves, the chloroplastic/plastidic form (GS2) functions in the reassimilation of ammonium (Blackwell *et al.* 1987, Wallsgrove *et al.* 1987). Additionally, Mifflin and Habasch (2002) viewed the role of GOGAT and GS in maintaining C-N balance in plants.

In case of GS activity, the trend was contrasting to that of NR/NiR in recent findings. Hirel *et al.* (2001) and Reed *et al.* (1980) reported negative relationship between NR and GS/GOGAT activity suggesting that, when the rate of nitrate reduction was too high, GS activity was limited to cope with the stronger flux of reduced nitrogen. The present results also synchronize with Cao *et al.* (2008) who reported that GS activity was two times more in plants growing in low N conditions than that in high N levels. Under low N condition, the availability of N is limited and plant tends to take nitrogen source from other metabolic pro- cess like photorespiration, in which ammonia was released. Hence, GS and GOGAT activity may be increased to utilize this ammonia as substrate. Hirel *et al.* (2007b) and Su *et al.* (1995) reported that leaf GS activity was positively correlated with grain yield and kernel number under low N-input. In low N, GS activity was increased mainly due to higher accumulation of cytosolic glutamine synthetase (GS1) (Thomas *et al.,* 2008). The present results also in tune with these observations as GS activity in G-1 under was more in N0 than in NH_4^+ and NO_3^- conditions. Thus it may be inferred that genotypes can utilize more ammonia as alternate N source with help of more GS activity in N stress conditions.

Table 4.21: Effect of NH_4^+, NO_3^- & N^0 treatments on activities of NR, NiR, GS and GOGAT

Treatment	NR [b]	NiR[c]	GS[d]	GOGAT[e]
Leaves				
NH_4^+	0.053 ± 0.004	0.023 ± 0.001	0.099 ± 0.006	0.196 ± 0.014
NO_3^-	1.49 ± 0.197	0.031± 0.002	0.123 ± 0.007	0.280± 0.032
N^0	0.019 ± 0.001	0.016 ± 0.001	0.155 ± 0.012	0.290 ± 0.050
Roots				
NH_4^+	0.002 ± 0.001	0.001 ± 0.000	0.023 ± 0.001	0.051± 0.003
NO_3^-	0.006 ± 0.001	0.017 ± 0.001	0.034 ± 0.001	0.056± 0.003
N^0	0.001 ± 0.008	0.001 ± 0.000	0.038 ± 0.003	0.068 ± 0.002

[b] NR activity expressed in µmol NO_2^- formed mg^{-1} proteins min^{-1}.

[c] NiR activity expressed in µmol NO_2^- reduced mg^{-1} proteins min^{-1}.

[d] GS activity expressed in µmol Pi formed mg^{-1} proteins min^{-1}.

[e] GOGAT activity expressed in µmol NADH oxidized mg^{-1} proteins min^{-1}

Table 4.22: Mean value of NR, NiR, GS and GOGAT activity in root and shoot

S.N	Genotype	N forms	NR[b]		NiR[c]		GS[d]		GOGAT[e]	
			Root±SEm	Shoot±SEm	Root±SEm	Shoot±SEm	Root±SEm	Shoot±SEm	Root±SEm	Shoot±SEm
1		NH_4^+	0.0008±0.0001	0.072±0.01	0.0017±0.001	0.014±0.001	0.0240±0.002	0.117±0.01	0.046±0.006	0.176±0.0033
	4 (HGY)	NO_3^-	0.0031±0.0003	1.29±0.03	0.0018±0.002	0.0136±0.002	0.037±0.003	0.140±0.05	0.059±0.003	0.317±0.0056
		N^0	0.00059±0.002	0.0148±0.02	0.0007±0.0001	0.016±0.001	0.037±0.002	0.156±0.06	0.043±0.020	0.126±0.012
2		NH_4^+	0.0001±.001	0.0258±	0.0004±0.000	0.018±0.003	0.046±0.007	0.123 ±0.01	0.071± 0.0041	0.298 ±0.001
	21(LGY)	NO_3^-	**0.0048[h]±0.0002**	**2.00[h]±0.1**	0.0015±0.0001	0.012±0.002	0.046 ±0.0032	0.114 ±0.05	0.052 ±0.0044	0.343 ±0.012
		N^0	0.00092±0.001	0.017±0.01	0.0007±0.0002	0.017±0.004	0.034 ±0.0011	0.169± 0.04	0.055 ±0.0081	0.454 ±0.071
3		NH_4^+	0.0007±0.0001	0.0198±0.02	0.0001±0.000	0.017±0.001	0.021±0.003	0.078±0.001	0.051±0.009	0.244±0.022
	30(LGY)	NO_3^-	**0.012[h]±0.002**	**2.19[h]±0.31**	0.001±0.0001	0.019±0.002	0.024±0.008	0.097±0.006	0.052±0.007	0.379±0.031
		N^0	0.00059±0.001	0.018±0.012	0.0006±0.000	0.016±0.002	0.027±0.002	0.141±0.01	0.038±0.004	0.131±0.007
4		NH_4^+	0.0002±0.003	0.0222±0.01	0.0002±0.000	0.006±0.001	**0.026 [h]±0.005**	**0.134 [h]±0.06**	**0.059 [h]±0.0012**	**0.234 [h]±0.061**
	46 (HGY)	NO_3^-	0.0016±0.0001	0.97±0.21	0.0003±0.000	0.013±0.002	**0.038 [h]±0.003**	**0.147 [h]±0.04**	**0.060 [h]±0.021**	**0.554 [h]±0.021**
		N^0	0.00052±0.0001	0.029±0.014	0.001±0.0001	0.014±0.007	**0.051±0.002**	**0.185 [h]±0.07**	**0.061 [h]±0.034**	**0.863 [h]±0.011**
5		NH_4^+	0.0012±0.004	0.027±0.012	0.0001±0.000	0.002±0.005	0.026±0.0011	0.076±0.005	0.051±0.0012	0.211±0.011
	75(LGY)	NO_3^-	**0.0033[h]±0.002**	**2.19[h]±0.11**	0.003±0.0001	0.01±0.006	0.021±0.001	0.109±0.004	0.065±0.0051±	0.175±0.012
		N^0	0.00039±0.001	0.015±0.006	0.0009±0.000	0.012±0.002	0.028±0.0021	0.113±0.021	0.068±0.0011±	0.136±0.012
6		NH_4^+	0.04±0.01	0.078±0.013	0.0002±0.000	0.026±0.001	**0.026 [h]±0.0011**	**0.115 [h]±0.012**	**0.066 [h]±0.0001**	**0.295 [h]±0.002**
	121 (HGY)	NO_3^-	0.016[h]±0.16	0.402[h]±0.17	**0.013 [h] ±0.002**	**0.028[h]±0.003**	**0.027 [h]±0.0021**	**0.175 [h]±0.031**	**0.061 [h]±0.0001**	**0.363 [h]±0.004**
		N^0	0.022±0.012	0.0144±0.003	0.0006±0.0001	0.026±0.0021	**0.036 [h]±0.0012**	**0.214 [h]±0.012**	**0.067 [h]±0.0021**	**0.575 [h]±0.0012**
7		NH_4^+	0.003±0.002	0.0039±0.002	0.0001±0.000	0.018±0.0012	0.024±0.0011	0.084±0.0021	0.057±0.00023	0.148±0.0034
	Danteshwari	NO_3^-	0.0044±0.0014	1.97±0.12	0.0003±0.000	0.013±0.0011	0.029±0.0010	0.111±0.018	0.058±0.0006	0.173±0.031
		N^0	0.00032±0.002	0.025±0.009	0.0004±0.000	0.014±0.0011	0.050±0.0021	0.137±0.003	0.052±0.0032	0.183±0.0032

8		NH_4^+	0.0012±0.002	0.014±0.004	0.0001±0.000	0.02±0.001	0.016±0.0002	0.089±0.0022	0.065±0.0032	0.161±0.003
	Dagad deshi	NO_3^-	0.008±0.0018	1.3±0.14	0.003±0.000	0.021±0.006	0.028±0.0016	0.083±0.0013	0.061±0.0003	0.143±0.006
		N^0	0.00072±0.0014	0.019±0.003	0.001±0.000	0.011±0.008	0.026±0.0031	0.112±0.0021	0.049±0.0002	0.292±0.0021
9		NH_4^+	0.0019±0.0012	0.0066±0.001	0.0001±0.000	0.032±0.0011	**0.026^h±0.0041**	**0.176 ^{h}±0.011**	**0.068 ^{h} ± 0.0021**	**0.143 ^{h}±0.010**
	Swarna	NO_3^-	0.0044±0.0012	0.84±0.021	**0.0018^h±0.0001**	**0.033^h±0.0021**	**0.055^h±0.0061**	**0.147 ^{h}±0.020**	**0.064 ^{h}±0.0001**	**0.184 ^{h}±0.012**
		N^0	0.00052±0.0011	0.02±0.004	0.0003±0.000	0.016±0.0022	**0.037 ^{h}±0.0045**	**0.221 ^{h}±0.011**	**0.069 ^{h}±0.0002**	**0.710 ^{h}±0.18**
10		NH_4^+	0.005±0.002	0.336±0.012	0.0002±0.000	0.011±0.0001	0.024±0.007	0.097±0.0021	0.053±0.0012	0.154±0.0012
	ISD-1	NO_3^-	0.0008±0.0001	1.78±0.11	0.0012 ±0.0001	0.14 ±0.011	0.027±0.0018	0.114±0.010	0.056±0.004	0.167±0.0021
		N^0	0.00046±0.0001	0.016±0.002	0.0009±0.0001	0.013±0.0001	0.026±0.0014	0.085±0.0021	0.058±0.0021	0.346±0.0031

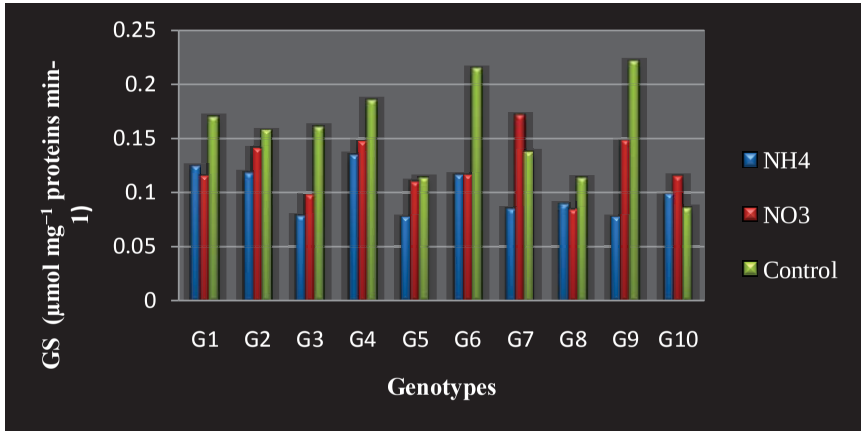
[b] NR activity expressed in µmol NO_2^- formed mg^{-1} proteins min^{-1}.

h= high value recorded for activity of enzyme

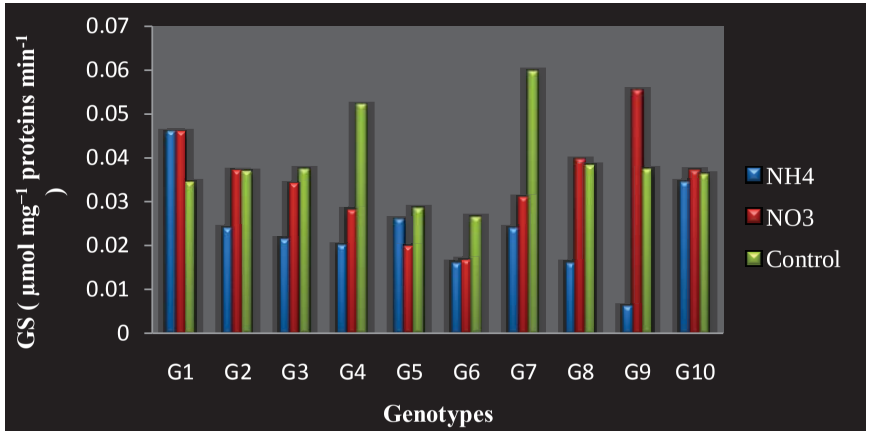
[c] NiR activity expressed in µmol NO_2^- reduced mg^{-1} proteins min^{-1}.

[d] GS activity expressed in µmol Pi formed mg^{-1} proteins min^{-1}.

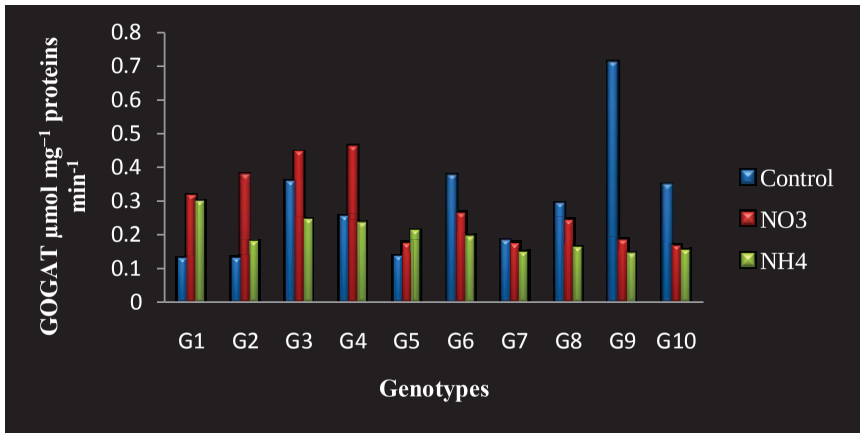
[e] GOGAT activity expressed in µmol NADH oxidized mg^{-1} proteins min^{-1}.



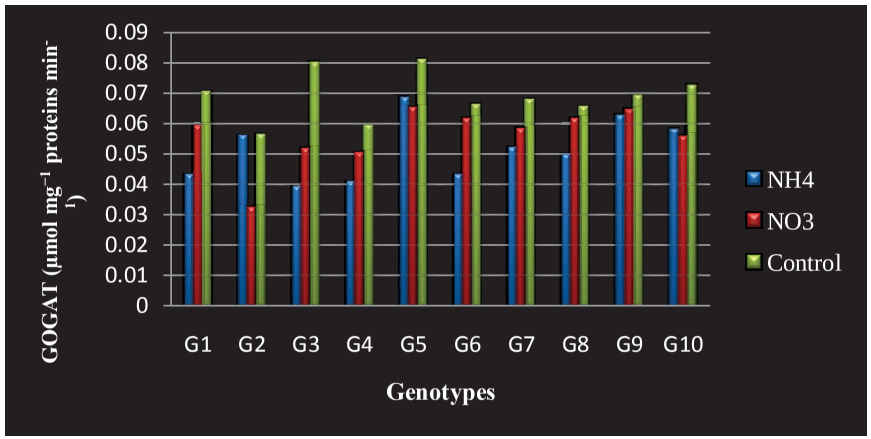
Glutamine Synthetase activity in shoot



Glutamine Synthetase activity in root

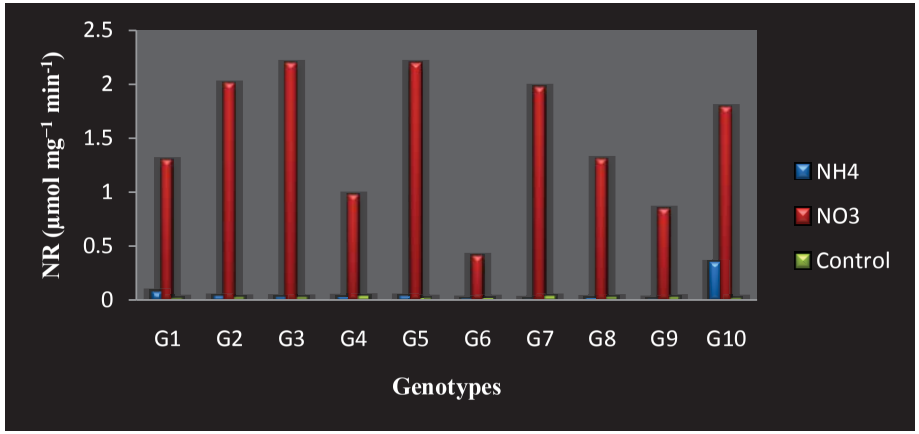


Glutamine Synthetase activity in shoot

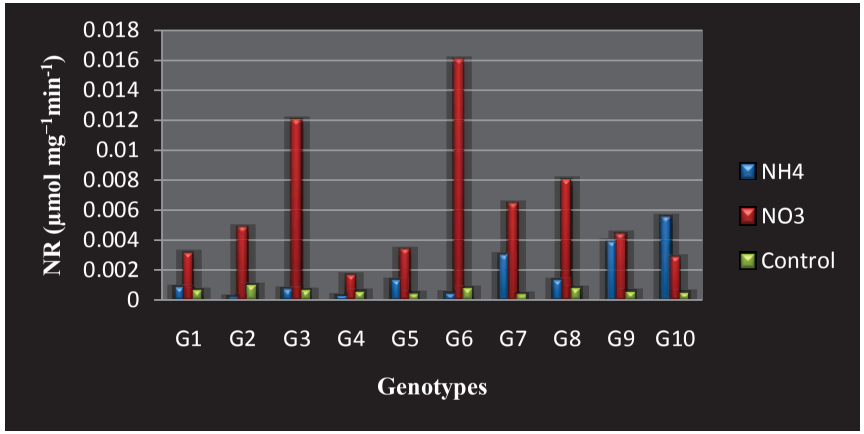


Glutamine Synthetase activity in root

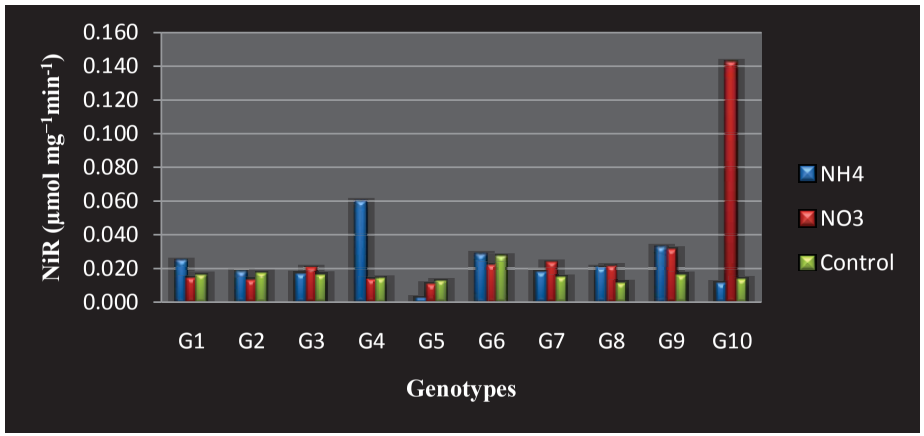
Fig 4.13a: Bar diagram for comparing NR, NiR, GS and GOGAT activity in rice genotypes



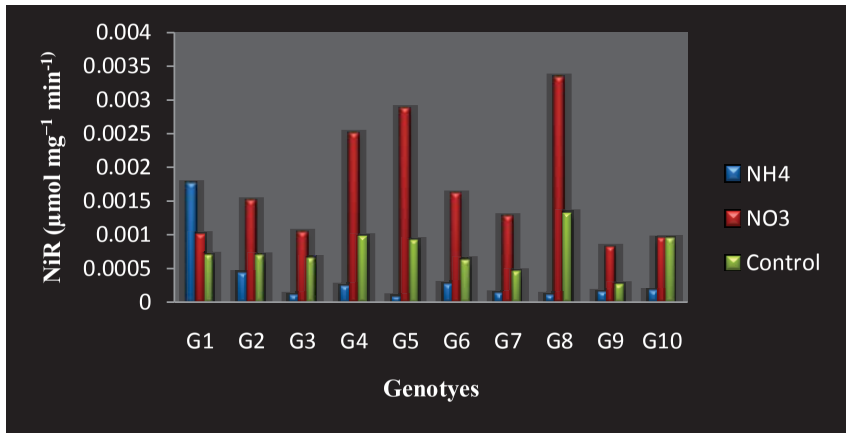
Nitrate Reductase activity in shoot



Nitrate Reductase activity in root



Nitrate Reductase activity in shoot



Nitrate Reductase activity in root

Fig 4.13b: Bar diagram for comparing NR, NiR, GS and GOGAT activity in rice genotypes

4.9.3 Relationship of NUE indices with the activities of enzymes involved in nitrogen uptake and assimilation

The correlation studies (table 4.23) revealed that there is a positive correlation between grain yield and GS activity under all treatments in leaf tissue. Moreover, shoot NR has positive correlation with N-uptake efficiency in NH_4^+ and NO_3^- treatment while negative correlation exists in N^0 treatment whereas in roots negative correlation exists between NR/NUpE in NH_4^+ and NO_3^- while positive relationship exists between NR/NUpE in NO_3^-. In case of NiR activity in leaves NR/NUpE shows positive values while in root negative relationship is obtained between NR/NUpE under all conditions. GS shows positive relationship with NUpE in both leaves and roots under all conditions. GOGAT exhibit positive values in leaves under NH_4^+ and NO_3^- conditions while in roots positive coefficient is obtained only in NO_3^- treatment. The association between GOGAT and NUtE is positive only in leaves of NH_4^+ treated tissues. In roots NUtE/GS shares positive values under NH_4^+ and NO_3^- conditions while NR, NiR/NUtE show positive values under NO_3^- treatment and NR/NUtE have positive correlation in roots treated with N^0 condition. In leaves NUE/GS-GOGAT have positive values under NH_4^+ condition while NiR-GS/NUE shows positive correlation under NO_3^- condition. In roots, NUE/GS-NiR shows positive indices in NH_4^+ condition, GS/GOGAT with NUE in NO_3^- and N^0 condition. These variable results in relationship of enzyme activity and NUE indices compel us to speculate about the regulation and modulation in enzymatic activity under different sets of applied treatments which finally exhibit variation in phenotypic and biochemical trait. Only few scientist, have made an effort to understand these complex relationships. Gupta *et al.* (2012b) is one among them who studied relationship of nitrogen use efficiency with the activities of enzymes involved in nitrogen uptake and assimilation of finger millet genotypes grown under different nitrogen inputs. Also, Gallais and Hirel (2004) studied correlation between grain yield and NUE indices.

Table 4.23: Relationship between NUE indices and enzymatic activities

Traits	T	NH_4^+				NO_3^-				N^0			
		NR	NiR	GS	GOGAT	NR	NiR	GS	GOGAT	NR	NiR	GS	GOGAT
GY	**L**	-0.231	0.321	**0.321***	**0.361***	-0.231	0.341*	**0.342***	**0.412***	-0.121	0.222	**0.322***	**0.561****
	R	-0.021	0.212	**0.223***	**0.212***	0.033	0.121	0.121	0.011	-0.111	-0.061	**0.211***	**0.312***
NUpE	L	**0.359***	**0.318***	0.133	-0.451	0.159	**0.593****	0.171	-0.519	-0.174	**0.366***	0.111	0.135
	R	-0.251	-0.492	**0.331***	-0.019	**0.646****	-0.233	-0.301	0.072	- 0.24	-0.28	0.169	-0.212
NUtE	L	-0.681	-0.035	-0.227	**0.539****	0.087	-0.359	-0.097	**0.422****	0.194	-0.651	-0.533	**0.558****
	R	-0.54	-0.268	0.004	**0.371***	0.148	0.143	-0.218	**0.771****	-0.79	0.151	**0.303***	**0.571****
NUE	L	-0.281	-0.223	**0.521****	**0.684****	-0.141	-0.123	**0.322***	-0.724	0.16	-0.489	-0.15	-0.025
	R	-0.254	**0.568****	**0.224****	**0.239***	-0.604	-0.196	**0.469***	**0.446***	-0.57	-0.07	**0.296***	**0.281***

T=tissue, L=leaves, R=root, NR=Nitrate Reductase, NiR=Nitrite Reductase, GS=Glutamine Synthetase, GOGAT= Glutamate Synthase

4. 10 Identification of QTLs for response to nitrate and ammonium

Most of the agronomically important traits *i.e*. yield and NUE related traits are complex and follow quantitative inheritance. Information on the number and chromosomal locations of the genetic loci influencing expression of a trait, their relative contribution to the trait expression and their sensitivity to variations in environments are very important for the utilization of these loci for crop improvement. A key development in the field of complex trait analysis was the discovery of DNA based genetic markers, physical establishment of high density genetic maps and development of QTL mapping methods. The disappearance of QTLs detected in one environment in another factor that has been considered as a manifestation of G×E interaction and the detection of QTLs with consistent expression across environments is considered as stability indicator for the utilization of these QTLs in breeding programs. There are number of reports of mapping and introgression of QTLs governing various traits under variable nitrogen conditions in rice and other crops (Agrama *et al.* 1999; Bertin and Gallais, 2000; Hirel *et al*. 2001; Gallais and Hirel, 2004). However, the study of Bertin and Gallais (2001) collaborates with the idea of present work that actually showed that QTLs detected at normal N input were different from those detected under low N stress conditions. It was also reported that different N sources, such as nitrate, ammonium, ammonium and nitrate, or low N treatments, by which the studies were conducted, also lead to different results of QTL mapping in *Arabidopsis* (Rauh *et al.*, 2002). Based on the suffice previous knowledge, the present study is one of the first attempt in rice of mapping novel genomic regions influencing

NUE, yield and yield related traits under variable forms of nitrogen *i.e.* NH_4^+, NO_3^- and N^0 using multi-location phenotyping data from a recombinant inbred population. The details of experiment are elaborated below.

4.10.1 Development of phenotypic and genotypic data for QTL identification

The mapping population used in the present study for generation of phenotypic data consisted of $122F_{16}$ recombinant inbred lines (RILs) derived from a cross between two *indica* genotypes Danteshwari and Dagad deshi parents, which showed a low level of difference at DNA levels. However, it has been observed that most of the populations used in QTLs analysis are derivatives of *indica* × *japonica* crosses because of high level of polymorphism that contributes to large range of phenotypic variation which is a prerequisite in the construction of linkage maps and QTLs mapping. Many authors however emphasized the necessity of QTLs identification based on variation from the crosses between two related varieties belonging to same subspecies so as to make rice breeding fruitful (Yano and Sasaki, 1997; Redona and Mackill, 1996).

The population was phenotyped for yield and NUE related traits under differential N and water regimes. These trait measurements were used for identification of QTLs. The statistical analysis of trait performance of parents and their RIL population revealed significant differences under differential N forms across two environments. 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. The genotypic data used in present study was kindly provided by Dr. S.B. Verulkar, Proff. and Head, Dptt. of Plant Molecular Biology and Biotechnology. 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; McCouch *et al*., 2002 and Temnykh *et al*., 2001).

4.10.2 Molecular linkage construction and QTL mapping

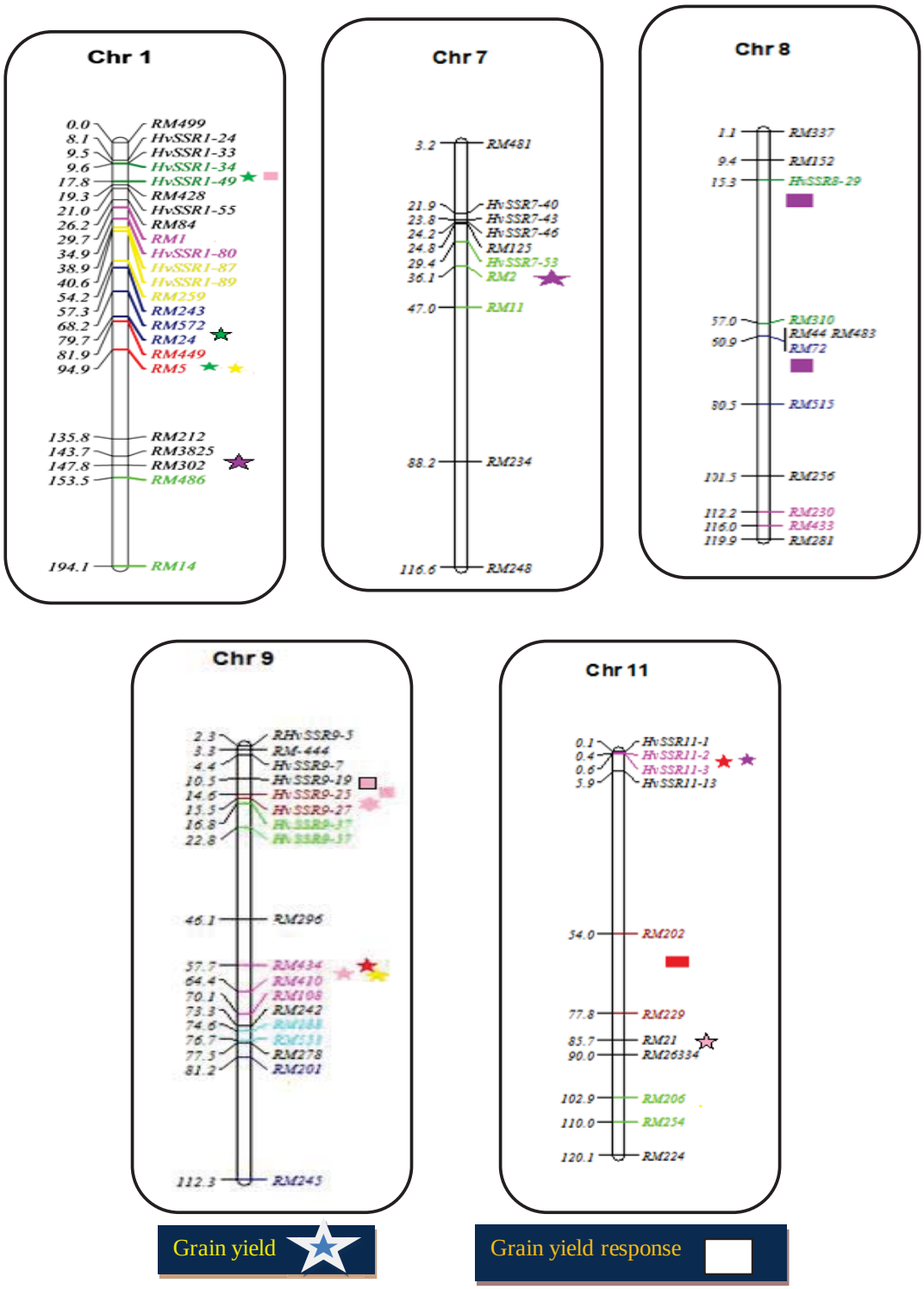
Genetic linkage map used for QTL analysis comprised of 162 markers that were distributed over 12 linkage groups. The partial separation and genome coverage of the map was suitable for QTL mapping. The linkage map consisting of vertical bars embraced with map positions and names of loci was constructed 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.

4.10.3 QTL analysis

A total of 58 QTLs conferring the corresponding five traits were detected under three N forms and two environments (table 4.24); that as a matter of fact included 14, 15 & 11 QTLs under NH_4^+, NO_3^- and N^0 level of irrigated condition and 5, 10 & 1 QTLs under NH_4^+, NO_3^- and N^0 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 4.18.

4.10.3.1 QTLs for grain yield (GY)

Fifteen putative QTLs for grain yield were identified under differential N and water regimes and are depicted in fig. 4.14. Within irrigated condition, under



NH_4^+ I= Purple, NO_3^- I= Red, N^0I= Yellow
NH_4^+ R= Green, NO_3^- R= Pink, N^0R= Light blue

Fig. 4.14: Location of QTLs of grain yield (GY) and grain yield response (GYR) in rice chromosome

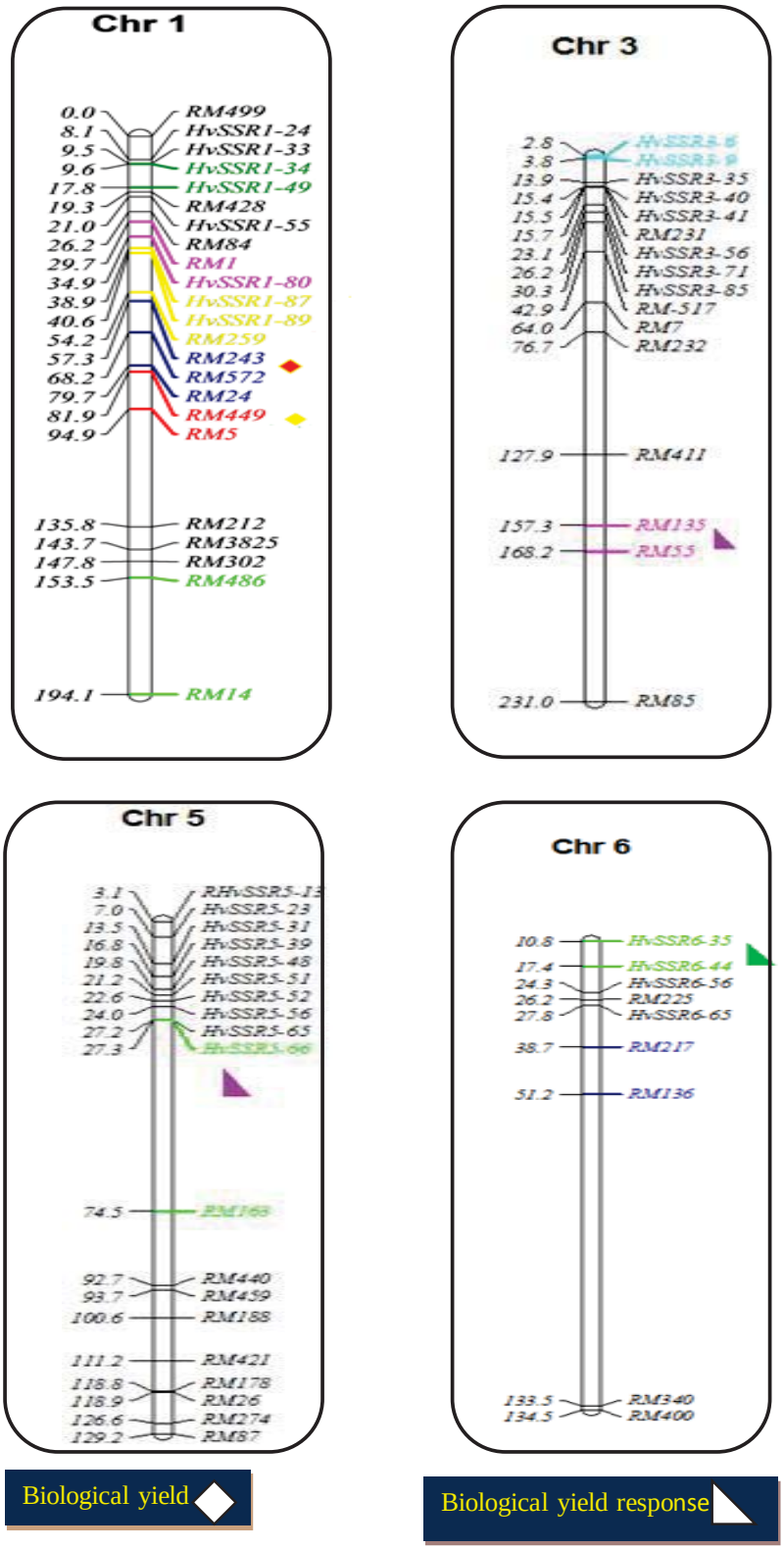
NH_4^+ level three QTLs were mapped on chromosomes 1, 7, 11 & 12 individual QTL explained 9.8%, 18.28% and 8.81% of the total phenotypic variation, respectively. Collectively, QTLs explained 34.70% of the phenotypic variation. Under NO_3^- level, three QTLs were located on chromosomes 9 and 11, explaining the phenotypic variances of 10.97%, 9.17% & 9.47%. Under N^0 level, two QTLs were identified on chromosome 1&9 accounting for 7.21% and 8.48% of the total variation in grain yield. The alleles in the direction of increasing GY for all QTLs except for one came from Danteshwari genotype. Within rainfed condition, under NH_4^+ level, three QTLs were revealed on chromosome 1, 1 &3, explaining 12.42, 3.68 & 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 &11. The allele for all the QTLs was carried by Dagad deshi genotype.

4.10.3.2 QTLs for grain yield response (GYR)

Under different N forms and two environments six QTLs were identified for grain yield response and are depicted in fig. 4.14. These QTLS were mapped on chromosome 8, 11, 1 & 9, respectively. Within irrigated condition, under NH_4^+ level two QTLs were detected on chromosome 8 with additive value of -41.28 & -44.14, respectively and together explaining phenotypic variation of 34.28 %. Under NO_3^- level, a QTL was mapped on chromosome 11 with phenotypic variation of 13.19 % and additive value of -31.05. Under NO_3^- level of rainfed condition, three QTLs were located on chromosome 1 & 9 with additive value of -23.71, -34.98 & -35.8, respectively and phenotypic variation of 30.12 % collectively.

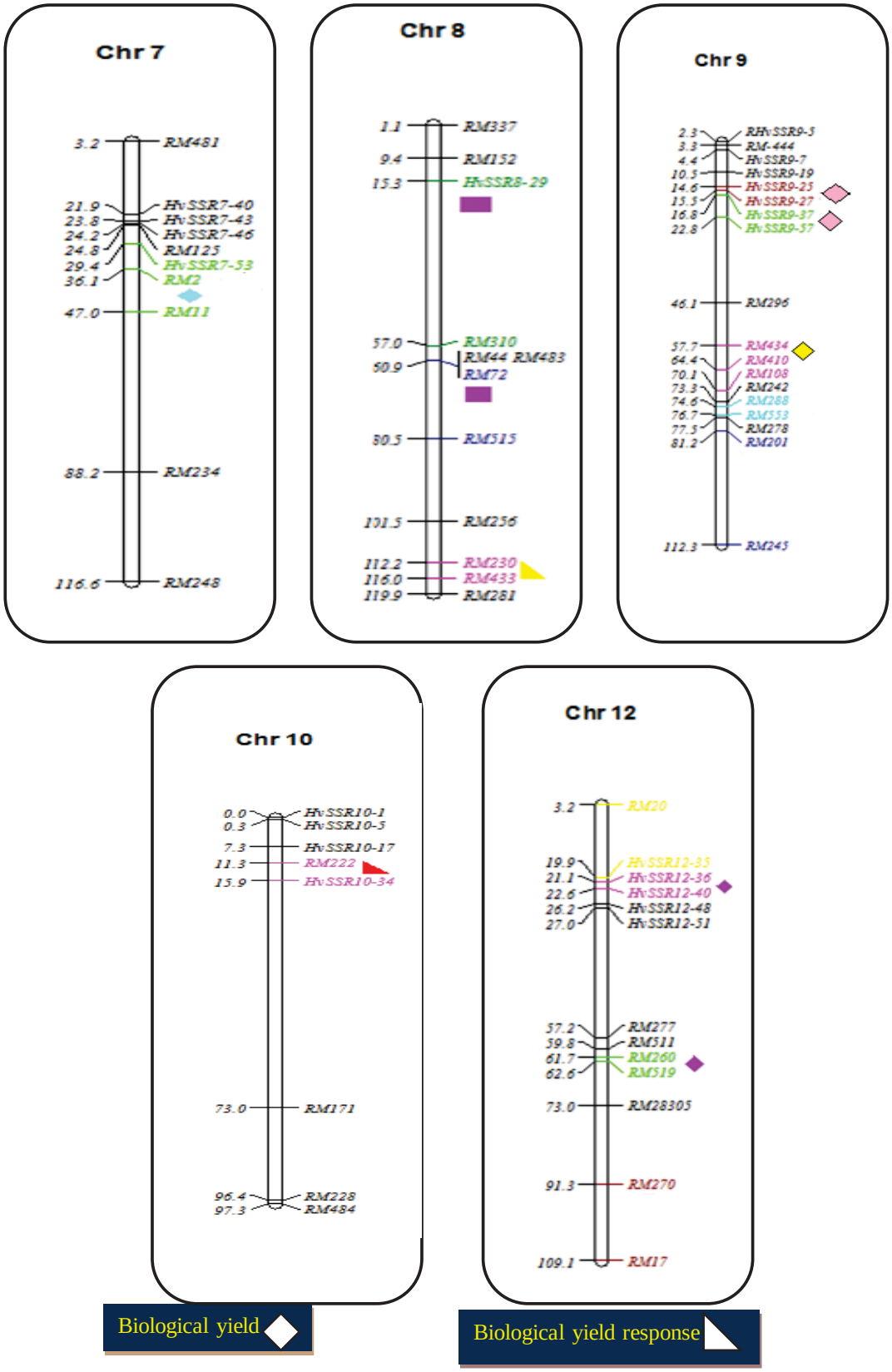
4.10.3.3 QTLs for biological yield (BY)

Nine QTLs for biological yield were located on chromosome 1,6,7,9 & 12 under the two nitrogen level & two environments and results are presented in fig.4.15, including six and three QTLs under irrigated and rainfed conditions, respectively. Of these, individual QTLs explained total phenotypic variation of 12.31% and 17.20% with additive value of -103.52 & 132.91 under NH_4^+ level, phenotypic variation of 15.65% with additive value of 65.14 under NO_3^- level and the phenotypic variation of 9.63, 13.29% & 7.64 % with additive value of 51.86, -



NH_4^+ I= Purple, NO_3^- I= Red, N^0I= Yellow
NH_4^+ R= Green, NO_3^- R= Pink, N^0R= Light blue

Fig. 4.15a: Location of QTLs of Biological yield (BY) and Biological yield response (BYR) in rice chromosome



NH_4^+ I= Purple, NO_3^- I= Red, N^0I= Yellow
NH_4^+ R= Green, NO_3^- R= Pink, N^0R= Light blue

Fig. 4.15b: Location of QTLs of Biological yield (BY) and Biological yield response (BYR) in rice chromosome

65.42 & 46.21 under N^0 level of irrigated environment. Within rainfed condition, phenotypic variation exhibited under NO_3^- and N^0 levels are 8.02%, 11.86% & 9.15 % with additive value of -58.03, -67.75 and 31.22, respectively.

4.10.3.4 QTLs for biological yield response (BYR)

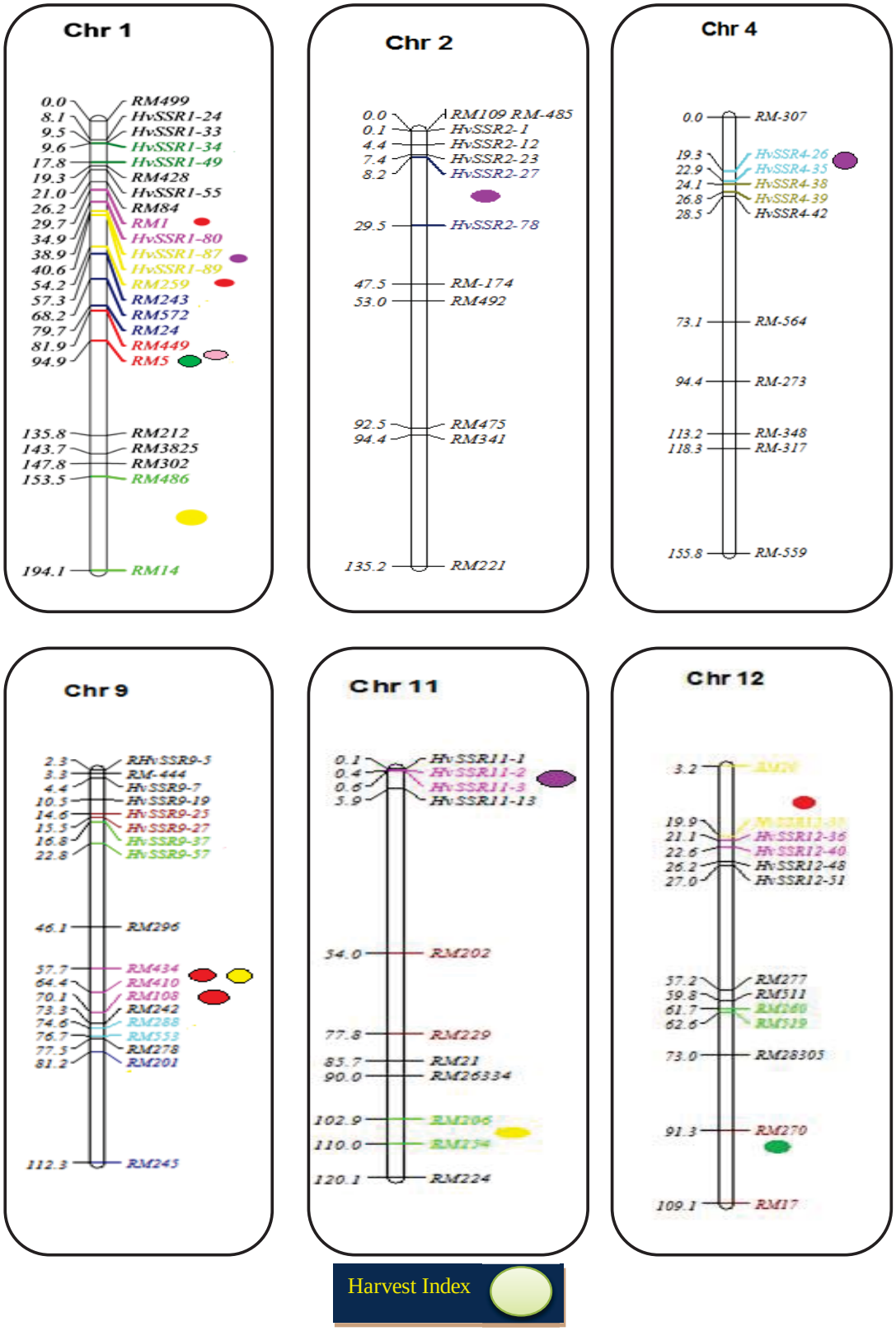
Five QTLs for biological yield response were detected only under NH_4^+ & NO_3^- level of irrigated condition and NO_3^- level of rainfed condition and results are presented in fig. 4.15. Within irrigated condition, under NH_4^+ level two QTLs were located on chromosome 3 & 5 with phenotypic variation of 10.99 % & 35.61 %, respectively. Within NO_3^- level, two QTLs were identified on chromosome 8 & 9 with phenotypic variation of 7.97 & 9.46, respectively. Under rainfed condition, single QTL was mapped on chromosome 6 exhibiting phenotypic variation of 18.51%. The positive additive value for two QTLs indicates allele from Danteshwari while negative additive value for remaining QTLs indicates alleles from Dagad deshi.

4.10.3.5 QTLs for harvest index (HI)

A total of sixteen QTLs were detected for harvest index and shown in fig. 4.16. These QTLs were located on chromosome 1, 2,3,4,9, 11 & 12 under differential nitrogen and water regimes, including thirteen and three QTLs under irrigated & rainfed sets of environment. Under NH_4^+ level, five QTLs were mapped on chromosome 1, 2 & 4, respectively under irrigated condition and two QTLs were located on chromosome 1 & 12 under rainfed condition. Under NO_3^- level, six QTLs were embraced on chromosome 1, 9, 11&12, respectively under irrigated condition and one QTL was located on chromosome 1 under rainfed condition. Under N^0 level, three QTLs were revealed on chromosome 1 & 12, respectively under irrigated condition. The 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 maximum value of 31.50% for a QTL detected.

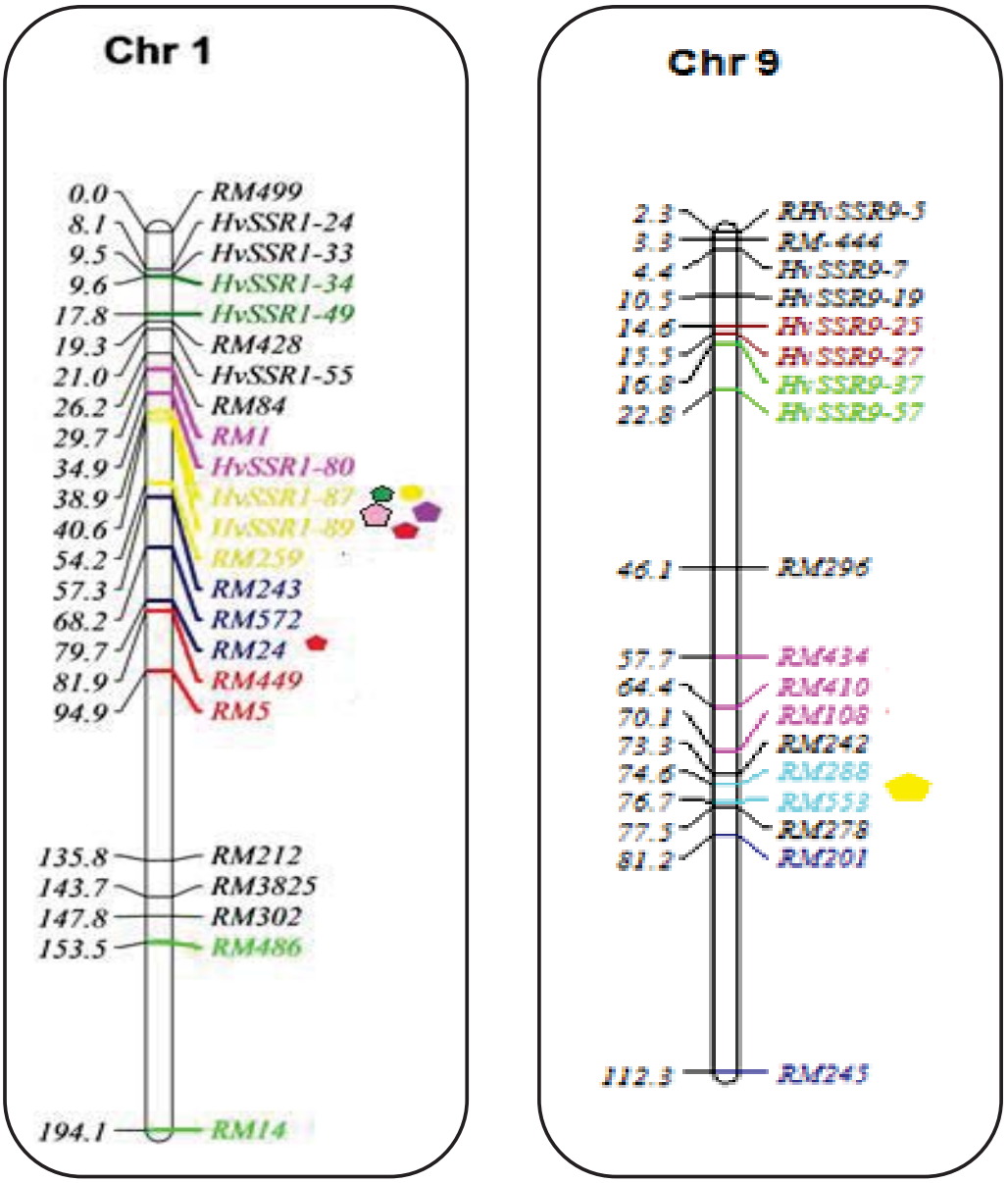
4.10.3.4 QTLs for leaf chlorophyll content (LCC)

Five QTLs controlling LCC were identified under differential N regimes of irrigated condition and were mapped to chromosomes 1 & 9, collectively. Results are shown in fig. 4.17. Interestingly, four QTLs obtained under variable N regimes



NH_4^+ I= Purple, NO_3^- I= Red, N^0I= Yellow
NH_4^+ R= Green, NO_3^- R= Pink, N^0R= Light blue

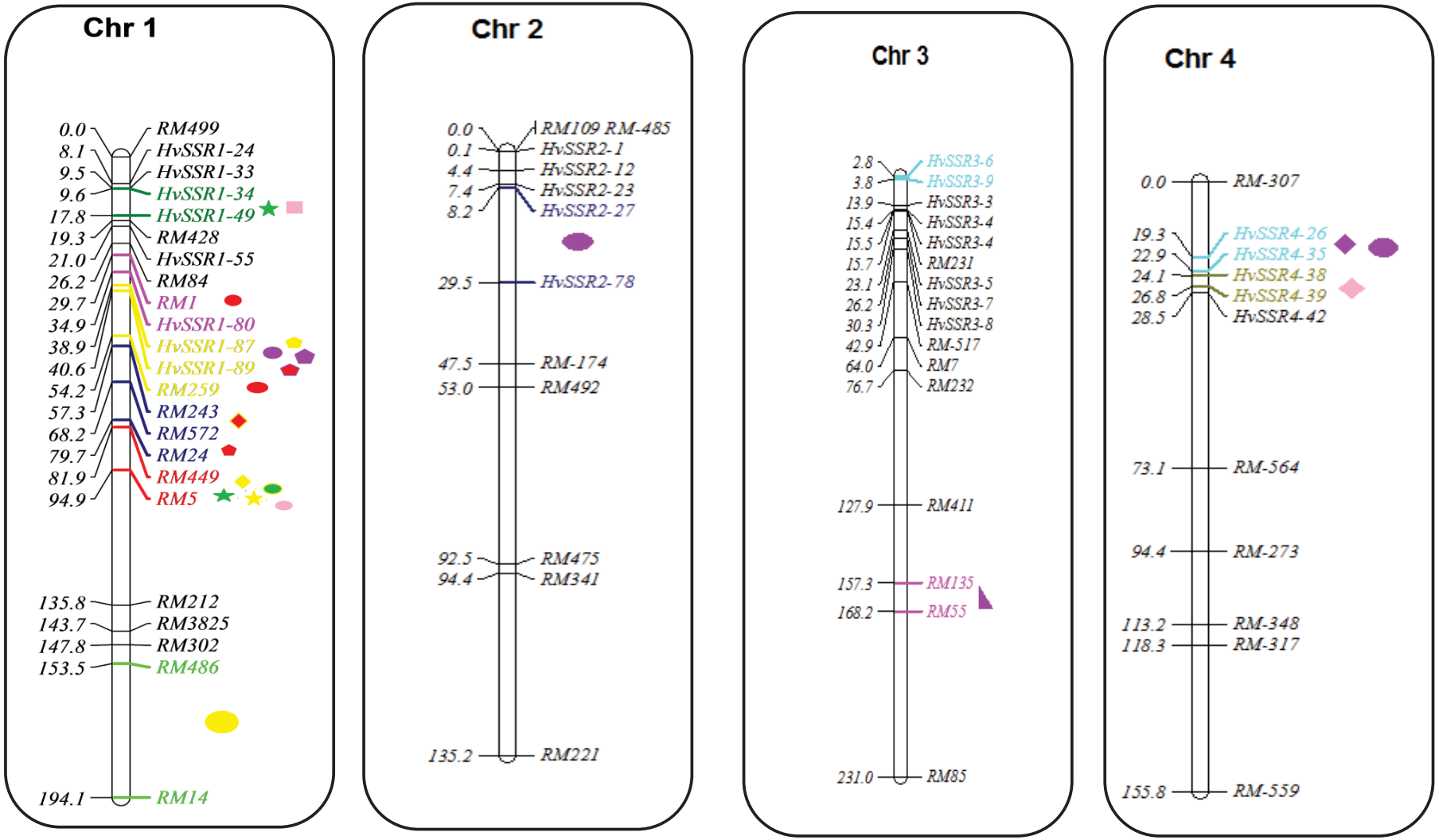
Fig. 4.16: Location of QTLs of Harvest index (H.I.) in rice chromosome



NH_4^+ I= Purple, NO_3^- I= Red, N^0I= Yellow
NH_4^+ R= Green, NO_3^- R= Pink, N^0R= Light blue

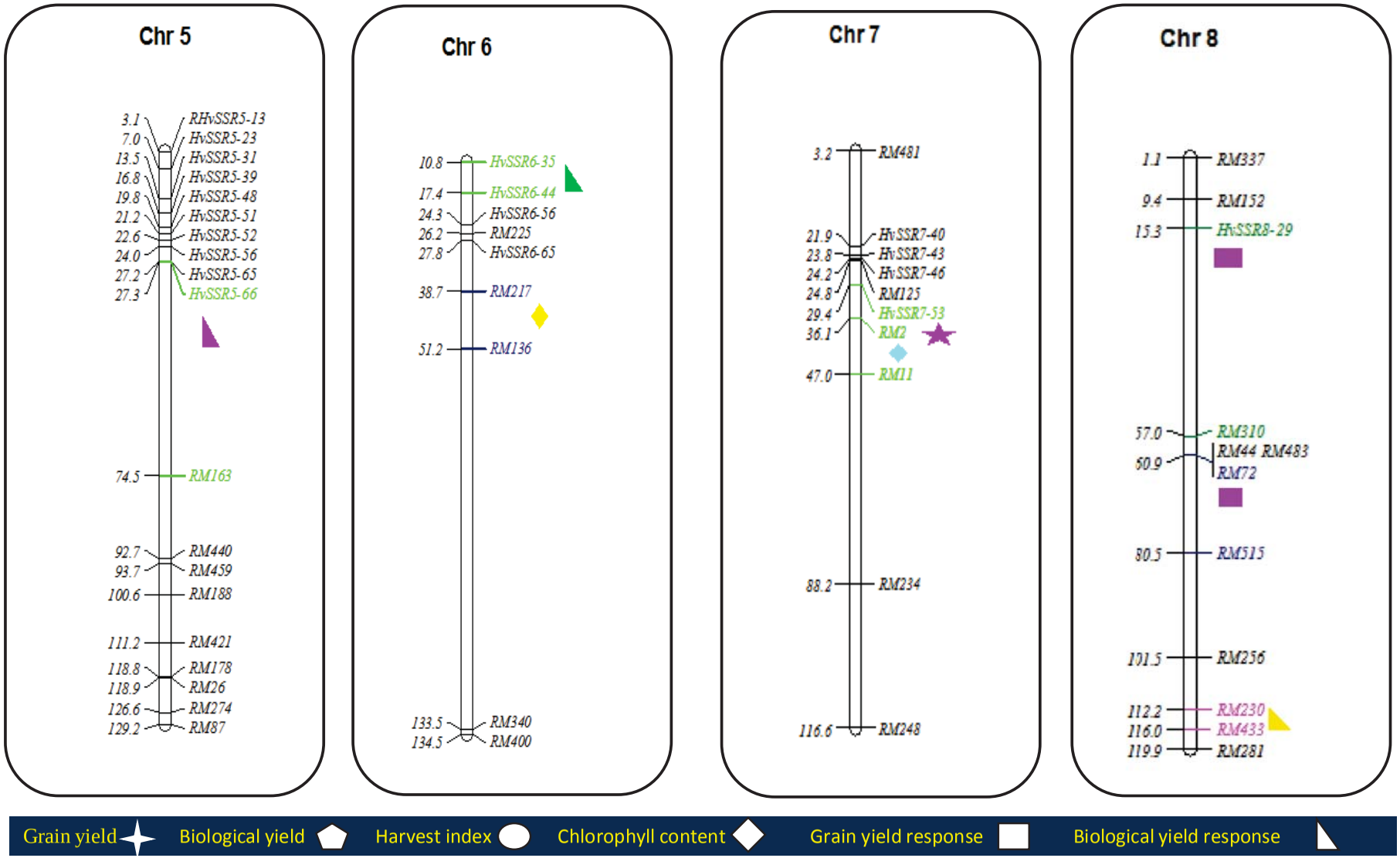
Fig. 4.17: Location of QTLs of Chlorophyll content (C.I.) in rice chromosome

were located on same marker interval of HvSSR1-87 & HvSSR1-89. These QTLs explained phenotypic variation of 25.56 %, 8.27%, 15.3% and 27.34 %, respectively. Under N0 level, a QTL was located on chromosome 9 explaining 6.73 % of total phenotypic variation. The positive allele for four QTLs was from Danteshwari while Dagad deshi carried allele for single QTL.



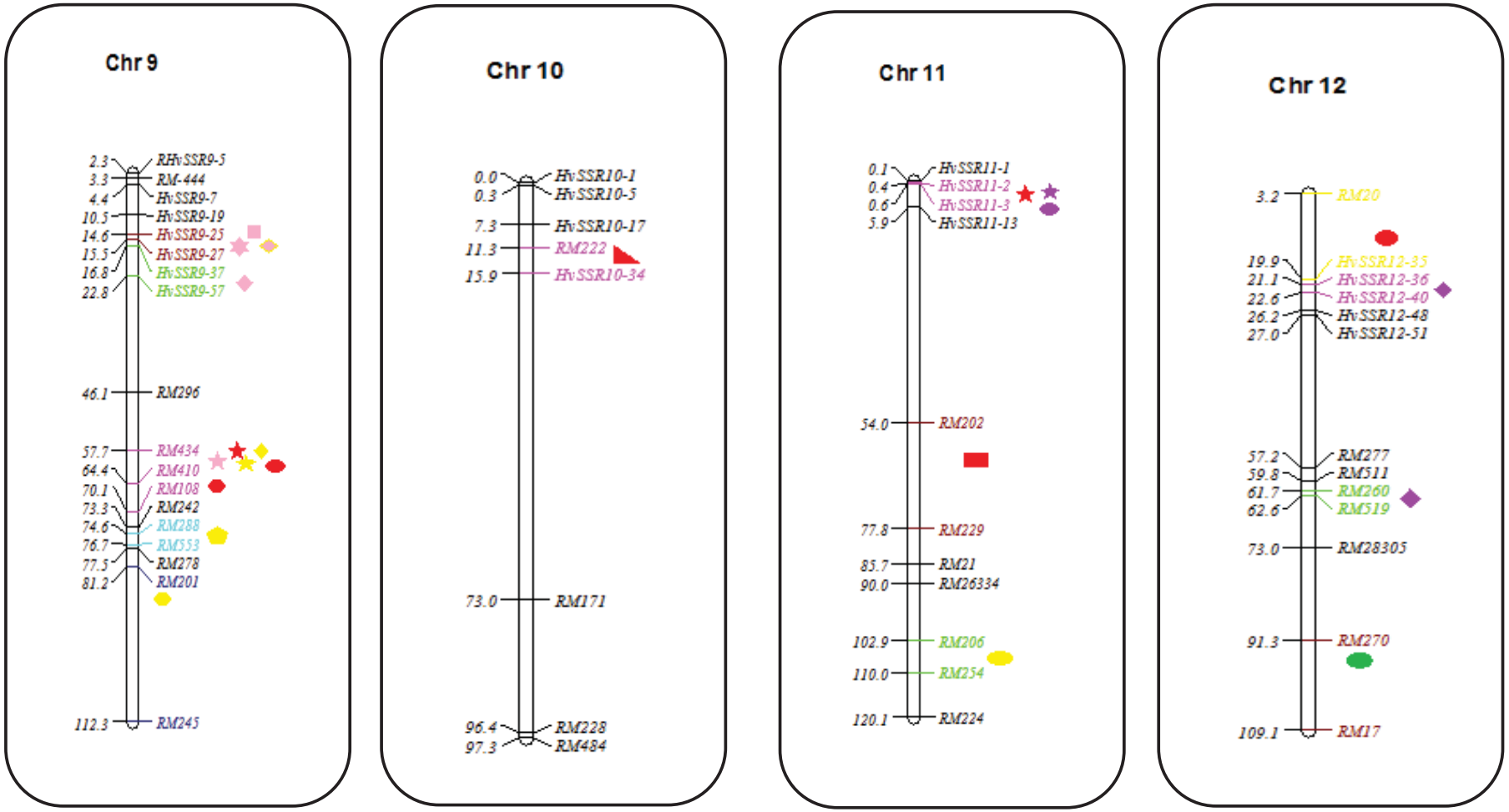
NH_4^+ I= Purple, NO_3^- = Red, N^0I= Yellow, NH_4^+ R= Green, NO_3^- R= Pink, N^0R= Light blue

Fig 4.18a: Location of QTLs pooled in the genetic map



NH_4^+ I= Purple, NO_3^- = Red, N^0I= Yellow, NH_4^+ R= Green, NO_3^- R= Pink, N^0R= Light blue

Fig 4.18b: Location of QTLs pooled in the genetic map



NH_4^+ I= Purple, NO_3^- = Red, N^0I= Yellow, NH_4^+ R= Green, NO_3^- R= Pink, N^0R= Light blue

Fig 4.18c: Location of QTLs pooled in the genetic map

Table 4.24: Results of QTLs for five traits under three N levels under two environments

Trait	N/E	Chr.	Left marker	Right marker	*LOD*	Add.	R^2 (%)
Grain Yield (GY)							
	NH_4^+ I	1	RM3825	RM302	2.8	43.6	9.8
		7	HvSSR7-53	RM-2	3.05	-72.12	18.28
		11	HvSSR11-2	HvSSR11-3	2.82	46.53	8.81
		12	RM277	RM260	3.13	29.31	8.10
	NO_3^- I	9	RM434	RM410	3.11	31.84	10.97
		9	RM410	RM108	3.03	33.75	9.17
		11	HvSSR11-2	HvSSR11-3	3.19	30.51	9.47
	N^0 I	1	RM449	RM-5	2.51	35.4	7.20
		9	RM434	RM410	2.55	38.77	8.48
	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)							
	NH_4^+ I	12	HvSSR12-36	HvSSR12-40	3.85	-103.52	12.3
		12	RM260	RM519	4.89	132.91	17.92
	NO_3^- I	1	RM243	RM572	3.34	65.14	15.65
	N^0 I	1	RM449	RM5	3.31	51.86	9.63
		6	RM136	RM340	3.78	-65.42	13.29
		9	RM434	RM410	2.54	46.20	7.64
	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^+ I	1	HvSSR1-87	HvSSR1-89	2.7	3.39	8.02
		2	HvSSR2-27	HvSSR2-78	2.59	-3.77	7.91
		4	HvSSR4-26	HvSSR4-35	3.12	3.64	9.1
	NO_3^- I	1	RM1	HvSSR1-80	2.58	3.42	14.53

		1	HvSSR1-89	RM259	3.21	3.23	11.52
		9	RM434	RM410	2.73	3.03	12.1
		9	RM410	RM108	2.81	2.72	9.98
		11	HvSSR11-2	HvSSR11-3	3.93	3.27	13.44
		12	RM20	HvSSR11-35	3.56	3.89	19.02
	N^0 I	1	RM486	RM14	5.13	5.71	31.5
		3	HvSSR3-6	HvSSR3-9	3.92	4.46	17.05
		9	RM434	RM410	3.22	2.94	8.68
		11	RM206	RM254	2.57	3.54	10.49
	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
Chlorophyll content (CC)							
	NH_4^+ I	1	HvSSR1-87	HvSSR1-89	8.48	2.14	25.56
	NO_3^- I	1	HvSSR1-87	HvSSR1-89	2.83	1.3	8.27
		1	RM572	RM24	4.27	-1.37	15.31
	N^0 I	1	HvSSR1-87	HvSSR1-89	8.8	1.89	27.34
		9	RM288	RM253	2.58	0.98	6.73
Grain yield response (GYR)							
	NH_4^+ I	8	HvSSR8-29	RM310	3.05	-41.28	15.57
		8	RM72	RM515	3.27	-44.14	18.71
	NO_3^- I	11	RM202	RM229	2.89	-31.05	13.19
	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
Biological yield response (BYR)							
	NH_4^+ I	3	RM135	RM55	2.83	66.06	10.99
		5	HvSSR5-66	RM163	2.51	-116.23	35.61
	NO_3^- I	8	RM230	RM433	2.85	41.26	7.97
		10	RM222	HvSSR10-34	2.76	-46.01	9.46
	NH_4^+ R	6	HvSSR6-35	HvSSR6-44	3.41	-91.51	18.51

N= Nitrogen forms, I= Irrigated, R=Rainfed, E=Environments

Add. = Additive effect of substituting Danteshwari allele for a Dagad deshi allele, R^2= Percentage of explained phenotypic variation

4.10.4 DISCUSSION

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). Wu *et al*. (1996) detected three QTLs affecting CC in rice leaves on chromosomes 2, 4 and 7 using a set of F2 population under N stress. Wang *et al.* (2003) identified three QTLs controlling chlorophyll a and b on chromosomes 1, 4 and 11.Teng *et al.* (2004) reported three QTL for LCC at flourishing tillering stage of rice located on chromosome 1, 3 and 8. In addition, six QTL for LCC at 5 d after heading, located on chromosomes 1, 3 (two QTL), 5, 8, and 12, and the other four QTL for

decreased LCC at 5 to 25 d after heading, located on chromosomes 4, 6, 8, and 9, were detected further using the BIL population**.**

4.11 Graphical genotypes of selected lines using data

4.11.1 Selection of genotypes

A field experiment was conducted during wet season 2014 & 2015 to study the responses of rice genotypes under variable nitrogen and water regimes. Variance studies showed the significant differences among the genotypes for yield, NUE and related traits. Furthermore, heritability, genetic advance and correlation studies revealed various characters that an ideal plant type of genotype should manifest for being NO_3^- and NH_4^+ efficient. Based on a field evaluation, two extreme groups were framed *viz.* High-yielding cultivar and low yielding cultivar under differential nitrogen and water inputs. Group of high yielding cultivar consisted of genotypes G-10, G-53, G-60, G-110 & G-121 while group of low yielding cultivar comprised of genotypes G-21, G-40, G-43, G-93 & G-111. Genomic regions harbouring 50 QTLs (earlier detected via QTL mapping) for yield and nitrogen related traits under different N forms studied across two independent environments were speculated and identified. These extreme groups along with the genomic regions of interest were chosen further for graphical representation of site specific introgression of chromosomal fragments governing yield and nitrogen efficiency among genotypes.

4.11.2 Marker selection and development of graphical genotypes

A graphical representation of molecular marker data can be an important tool in the process of selection and evaluation of plant material. A computer program was developed that enables representation of molecular marker data by simple chromosome drawings in several ways. Commonly used marker file types that contain marker information serve as input for this program, which is named ‘GGT’ (an acronym of Graphical Geno Types) (GGT user manual 2007). Graphical outputs of genotyping data in his study were generated using GGT version 2.0 tools.

4.11.2.1 Marker selection

Out of 162 polymorphic markers spanning 12 chromosomes, 56 marker loci detected the presence of putative QTLs, which were embraced in almost all

rice chromosomes. Clusters of QTLs were detected exclusively on chromosome 1&9 at marker interval HvSSR 1-87 - HvSSR 1-89 and RM 434 - RM 410, conferring it as a genomic region of extreme interest. Based on the QTL presence in vicinity of markers, efforts were made to generate the graphical genotypes of single chromosome (true representative of all chromosomes with actual marker loci) introgressed with 56 markers for 10 selected lines of extreme groups.

4.10.2.2. Introgressed segment analysis

The allelic contribution of marker alleles A and B is presented in table 4.25 for 10 lines of extreme groups selected in the study. Marker alleles for each locus are marked in different colours and incorporated in ascending order of markers count across 12 rice chromosomes (Figure 4.19). Graphical Geno Types (GGT) analysis results of high yielding RIL variants showed an average recovery of 50.00% of the Danteshwari genome while that of Dagad deshi genome was 49.62% whereas low yielding RIL variants showed an average recovery of 64.7% of the Danteshwari genome while that of Dagad deshi genome was 17.6%. Furthermore, marker statistics for high yielding genotypes revealed that the two alleles contributed more or less in case of most of the markers while in case of marker HvSSR 9-57, RM245, HvSSR12-35, RM 260 & RM519, allele B made all the contribution to the genomic constitution. On contrary, in case of RM20 marker the genomic constitution was contributed majorly by allele A with no contribution of allele B. Marker statistics for low yielding genotypes revealed that the majority of contribution to the genomic constitution is flourished by allele A of most of the markers. Detailed statistics is shown in fig. 4.20.

Table 4.25: Summary of results for introgressed segment analysis

Group I	A (%)	B (%)	Group II	A (%)	B (%)
G-10	40	60	G-21	54.5	23.6
G-53	73.6	24.5	G-40	70.9	12.7
G-60	40.9	59.1	G-43	70.9	10.9
G-110	46.4	53.6	G-93	70	12.7
G-121	49.1	50.9	G-111	57.3	28.2
Average	50	49.62	Average	64.7	17.6

Group I= High yielding genotypes, Group II= Low yielding genotypes

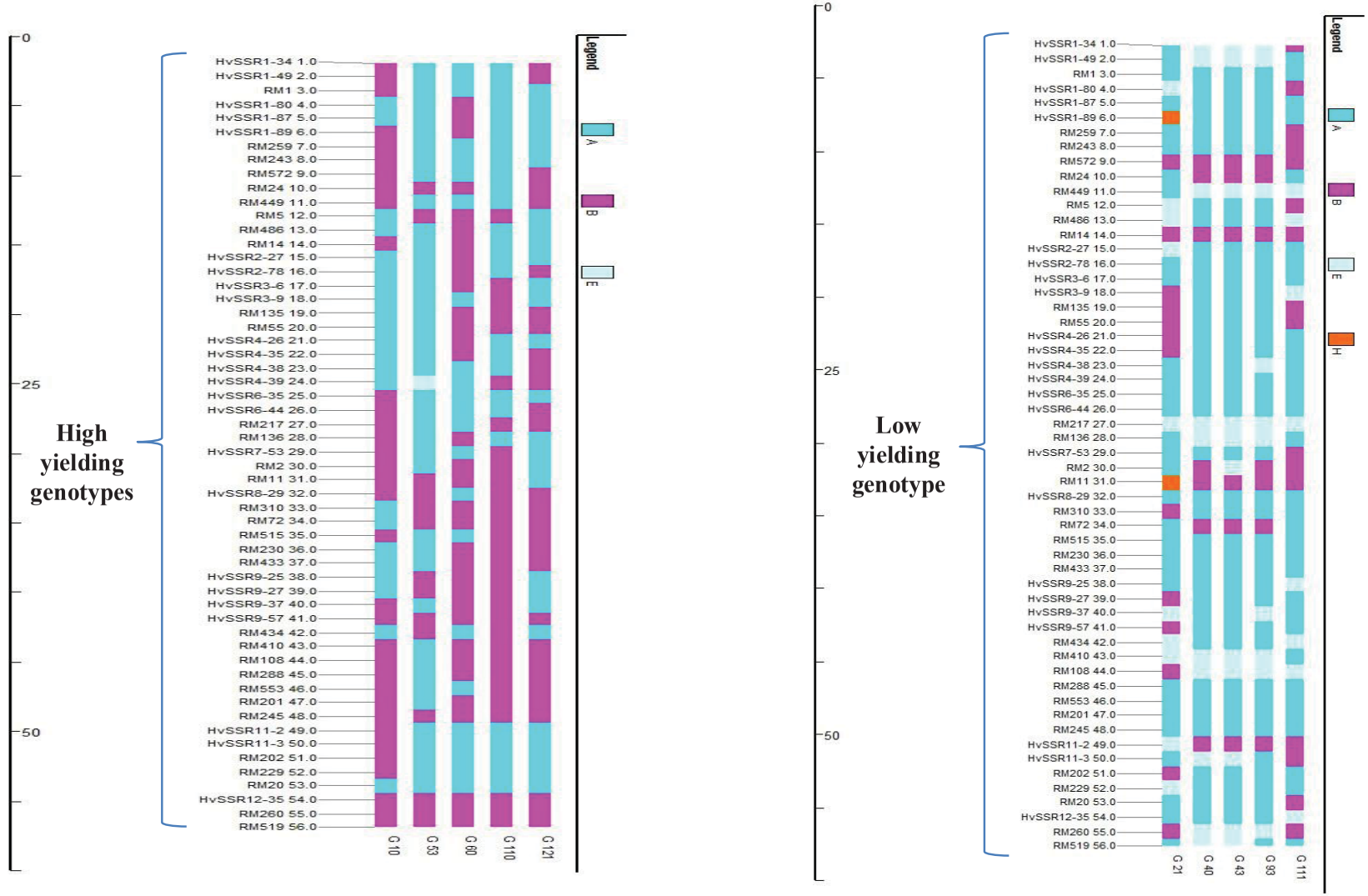


Fig. 4.19: **Graphical genotypes of the 5 introgression lines of group I & II showing single rice chromosome. Blue squares represent the recurrent parent (Danteshwari) proportion of the genome. Pink squares represent the donar parent (Dagad deshi) homozygous introgressions.**

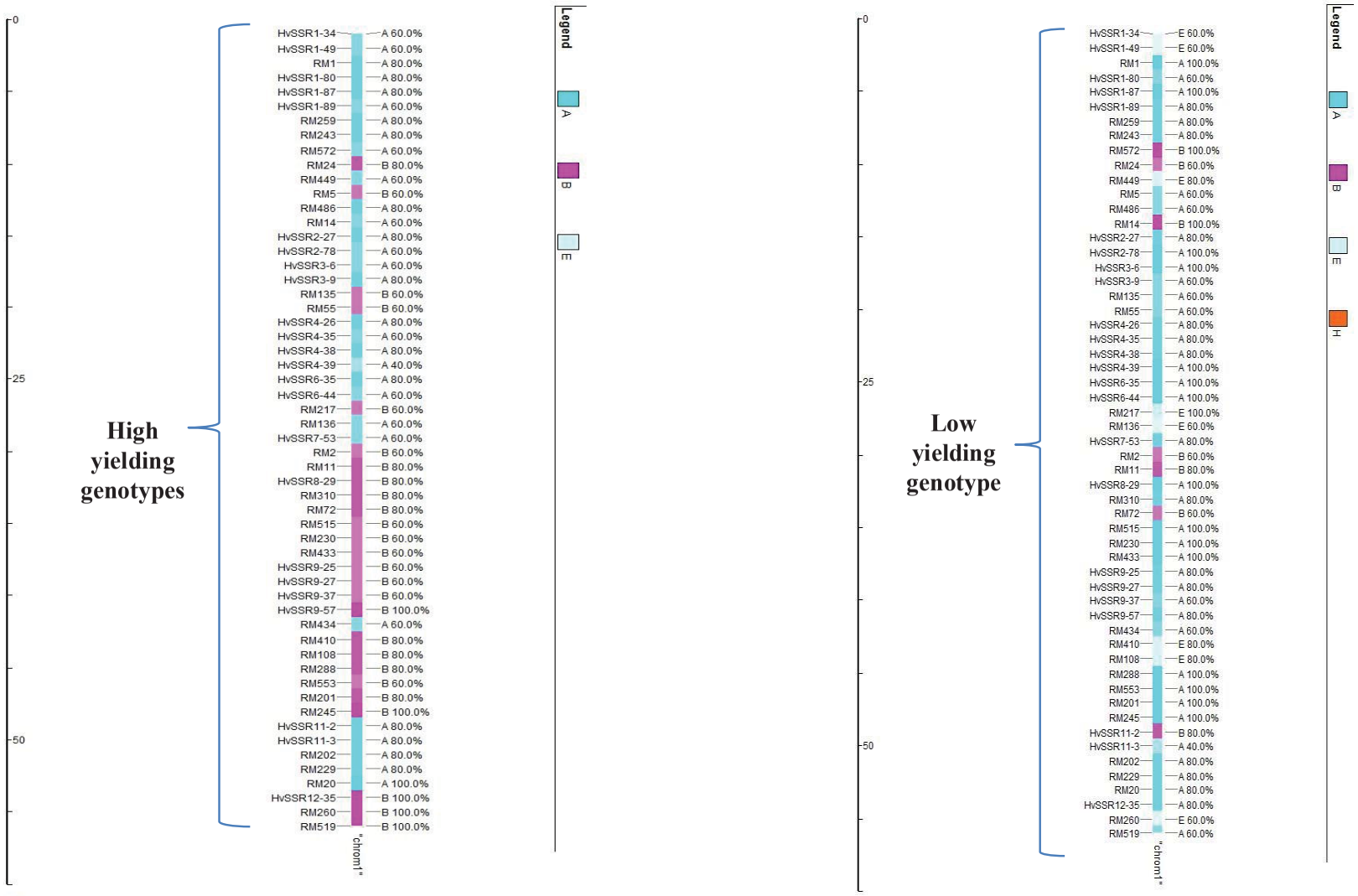
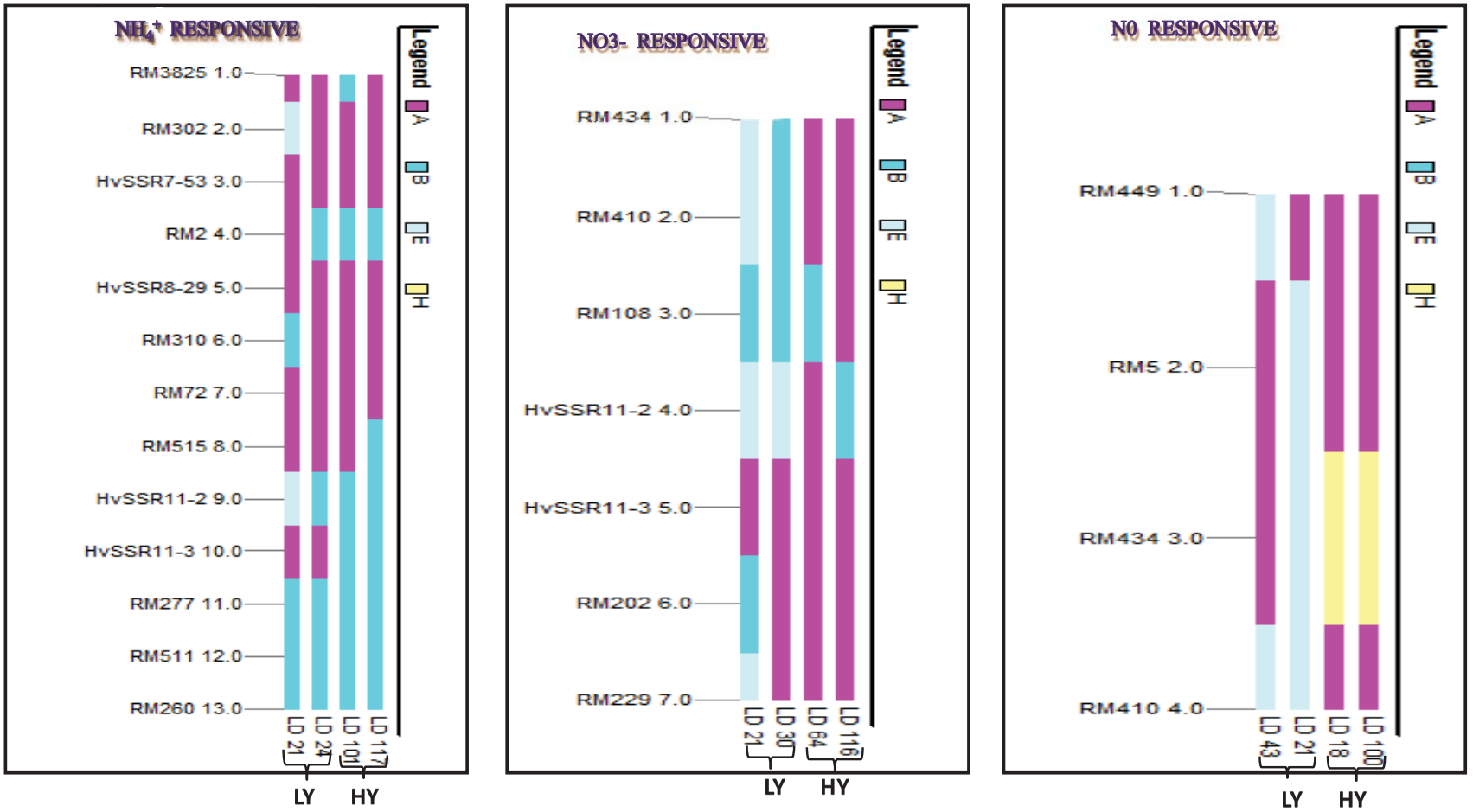


Fig. 4.20: **Marker statistics of the 5 introgression lines of group I & II showing single rice chromosome. Blue squares represent the recurrent parent (Danteshwari) proportion of the genome. Pink squares represent the donar parent (Dagad deshi) homozygous introgressions.**

Moreover, individual graphical genotypes (GGT) of NH_4^+, NO_3^- and N^0 responsive lines under irrigated and rainfed condition with grain yield (GY) and grain yield response (GYR) QTLs regions is shown in fig. 4.21 and fig. 4.22. A perusal of these results flourish us with information of novel regions influencing the grain yield trait and furthermore, utilizing these regions by introgression for improvement of recurrent or elite parent.

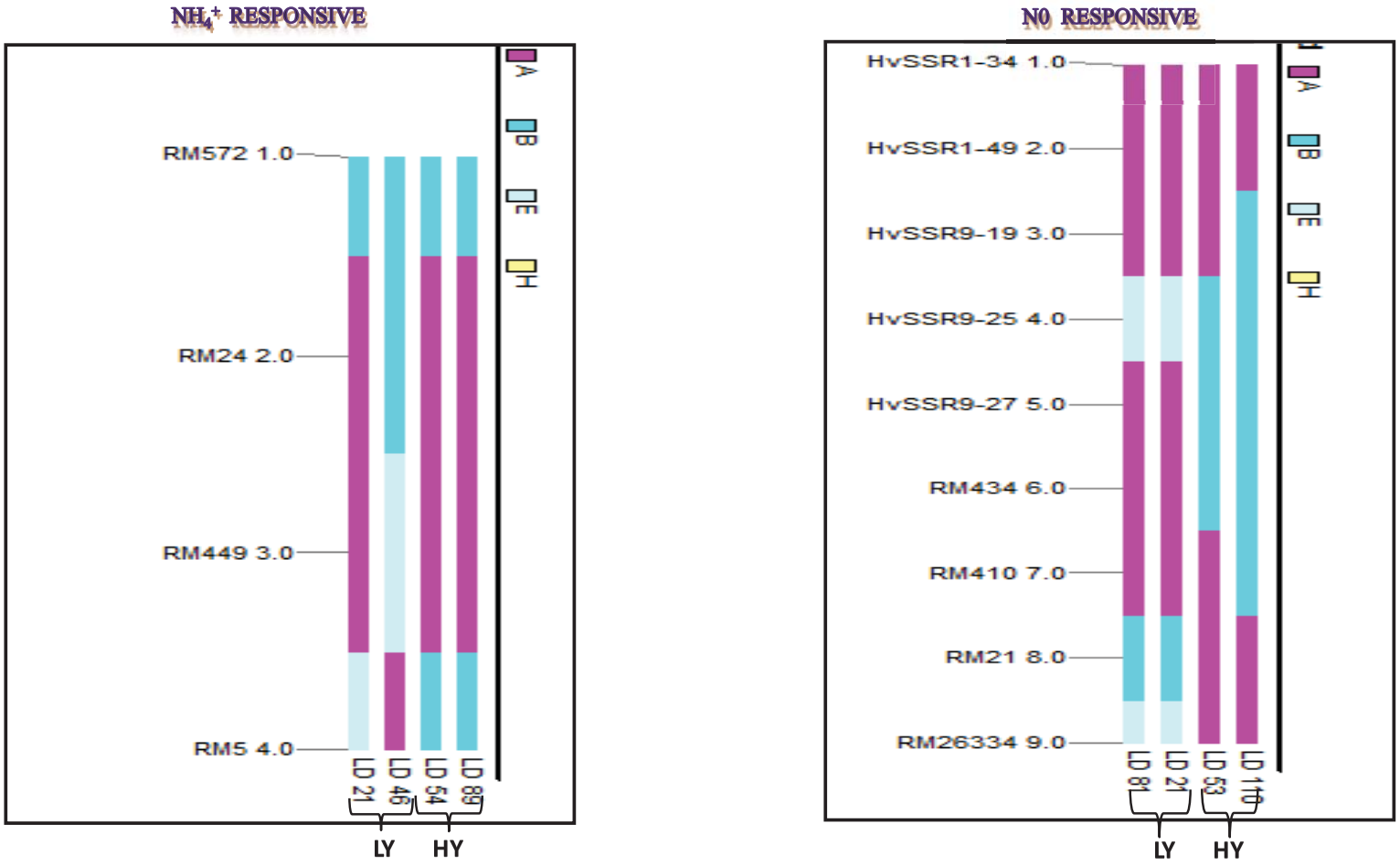
4.11.3 Discussion

Selection of parental lines having the desirable traits that will face the objective of the breeder is the first step in crop improvement. This is followed by making crosses between the parents to produce a segregating population. Selection of progeny indicating the desirable traits then initiates and continues as the population is advanced from one generation to the next. Introgression of one or more genes from a donor into the background of an elite variety (recurrent or recipient parent) and to improve the recurrent parent genome as quickly as possible is one of the main objectives of plant breeding.These results collaborate with many of the research findings. Septiningsih *et al.* (2003) detected one QTL for brown rice in the interval RM474-RM239 in an *O. sativa* IR64 x *O. rufipogon* backcross progeny. Graphical genotyping software programs, such as GGT (Van Berloo, 2008), was used as a tool for selecting preferable backcross progeny on the basis of their genotypic content. Zheng *et al.* (2008) identified 32 putative QTLs in double haploid rice lines of *indica* and japonica cross for four phenotypic traits with 208 RFLP and 76 SSR markers. Marker associated with economically important traits were subjected to GGT for monitoring effective transformation of such QTLs to elite lines. Kanbe *et al*. (2008) identified QTLs for chlorophyll content, plant height, panicle no. and culm length using CSSLs lines and back cross progeny. These QTLs were further graphically projected to identify the important genomic regions. Pawar *et al.* (2012) studied response of selected RILs for yield and yield attributing traits under aerobic conditions and established the marker-assisted graphical representation of superior rice genotypes. Patil *et al.* (2014) identified five high protein germplasm lines and carried out GGT analysis of these using 25 polymorphic SSR markers. Graphical genotyping analysis revealed that these markers differed significantly for the genomic constitution contributed by marker alleles A and B.



LY = Low yielding genotypes, HY = High yielding genotypes

Fig. 4.21: Graphical genotypes of selected NH_4^+, NO_3^- and N^0 responsive genotypes under irrigated condition



LY = Low yielding genotypes, HY = High yielding genotypes

Fig. 4.22: Graphical genotypes of selected NH_4^+ and NO_3^- responsive genotypes under rainfed condition

4.12 Gene expression analysis

The mapping population comprising of 122 RILs derived from a cross of Danteshwari X Dagad deshi was screened to obtain 10 rice genotypes with disparate NUEs and falling in different group of colour classes (table 4.26). Considering the important fact of applying great amount of basal N fertilizer but lower uptake ability at young rice seedling stage, it is essential for us to explore the molecular basis of N efficiency of young rice seedlings in order to improve NUE of basal N fertilizer and reduce N pollution (Zhao and Shi, 2006a). Therefore, the expression patterns of transporters and assimilatory gene families were studied at young rice seedling stage in the present study. For genome-wide analysis of gene expression both root and leaf materials were harvested from two biological replication under NH_4^+, NO_3^- & N^0 conditions at 27-days-old seedling stage. Total RNA was extracted by using commercial TRIZOL and cDNA was subsequently synthesized by using Thermo Scientific Verso^{TM} cDNA Synthesis Kit. The cDNA samples were used as templates to quantify target gene expression levels. The integrity of the extracted RNA was also tested by denaturing formaldehyde agarose gel and carrying out electrophoresis followed by ethidium bromide staining, of the RNA samples 28S and 18S ribosomal bands appeared as sharp bands with 28S band having approximately double the fluorescence intensity than 18S band showing the integrity of the extracted RNA samples. Furthermore, in brief to better understand and to systematically clarify the role and regulation of NUE genes in young rice seedlings, we investigated different members of the transporters and assimilatory genes, and analyzed the pattern of expression of each gene in different plant organ and in plants grown under different forms of nitrogen.

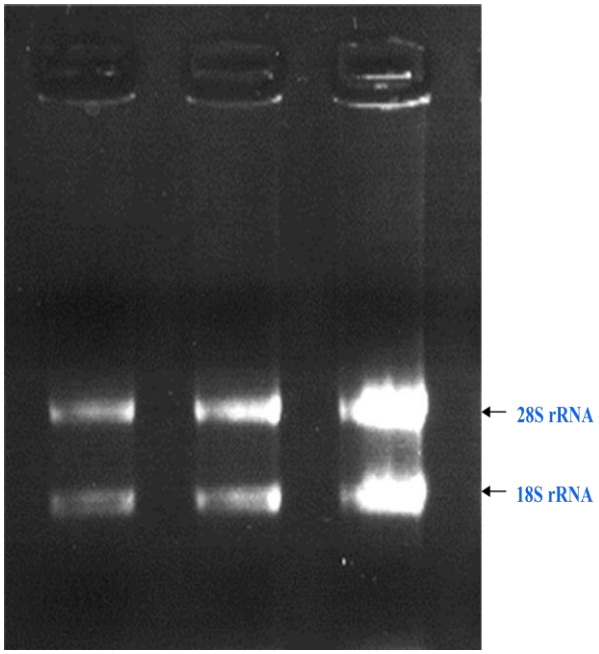
Table 4.26: Lines selected for expression analysis

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	Dagad deshi-LG
9	Swarna-DG
10	Indira Sugandhit Dhan-Y

Y= yellow; DG= dark green; G= green; LG= light green

Table 4.27: Proposed mechanism and tissue localization of gene candidates

Gene Candidates	Tissue	Induction/Mechanism	References
OsAMT1;1	shoots and roots	low ammonium	Ding *et al.*, 2011
OsAMT1;2	root-specific	ammonium inducible	Sonoda *et al*., 2003
OsAMT1;3	root-specific	nitrogen suppressible	Sonoda *et al*., 2003
OsNRT 2.4	root- specific	nitrate inducible	Xia *et al*., 2015
OsNRT 7.8	root- specific	nitrate inducible	Xia *et al*., 2015
OsGln1.1	All organs but leaf blades and root- preferentially	stimulated by N	Tabuchi *et al*., 2005
OsGln1.2	All organs but leaf blades and root- preferentially	stimulated by N	Tabuchi *et al*., 2005
OsGln1.3	Root and shoot	stimulated by N	Tabuchi *et al*., 2005
Gln2	leaves preferentially	stimulated by N	Bernad *et al*., 2008
OsGlt1	root and shoot	stimulated by N	Tabuchi *et al*., 2007
OsGlt2	root and shoot	stimulated by N	Tabuchi *et al*., 2007



Determination of RNA integrity by FA gel electrophoresis

4.12.1. Semi-quantitative RT-PCR

When breeding crops that utilize the nitrogen efficiently, it is important to reveal the regulation of NH_4^+ & NO_3^- uptake and assimilation, at molecular level. Thus we investigated the different members AMT (Ammonium transporters), NRT (Nitrate transporters), GS (Glutamine Synthetase) & GOGAT (Glutamate Synthase) genes, and analyzed the expression pattern of each gene to identify the network of genes involve in NUE in plant by semi-quantitative RT-PCR and later on by quantitative real time PCR, which actually revealed the distinct expression pattern of these genes. GS, GOGAT, AMT and NRT gene family taken for study were expressed in all condition (Control, ammonium and nitrate), while some genes showed differential expression level on selected 10 rice genotypes (fig.4.23a,b) that belonged to different colour classes in colour spectrum. Earlier, Ishiyama *et al.* (2004) studied ammonium dependent regulation of GS isoform and Hayakawa *et al.*, (1998) observed changes in content of NADH-GOGAT in response to differential N supply. AMT gene family regulation in tomato due to ammonium ion influx is earlier studied by Becker *et al.* (2002) representing regulatory pattern of AMT transporters. Aaraki (2005) studied regulation of NRT transporters in rice under varied N concentration.

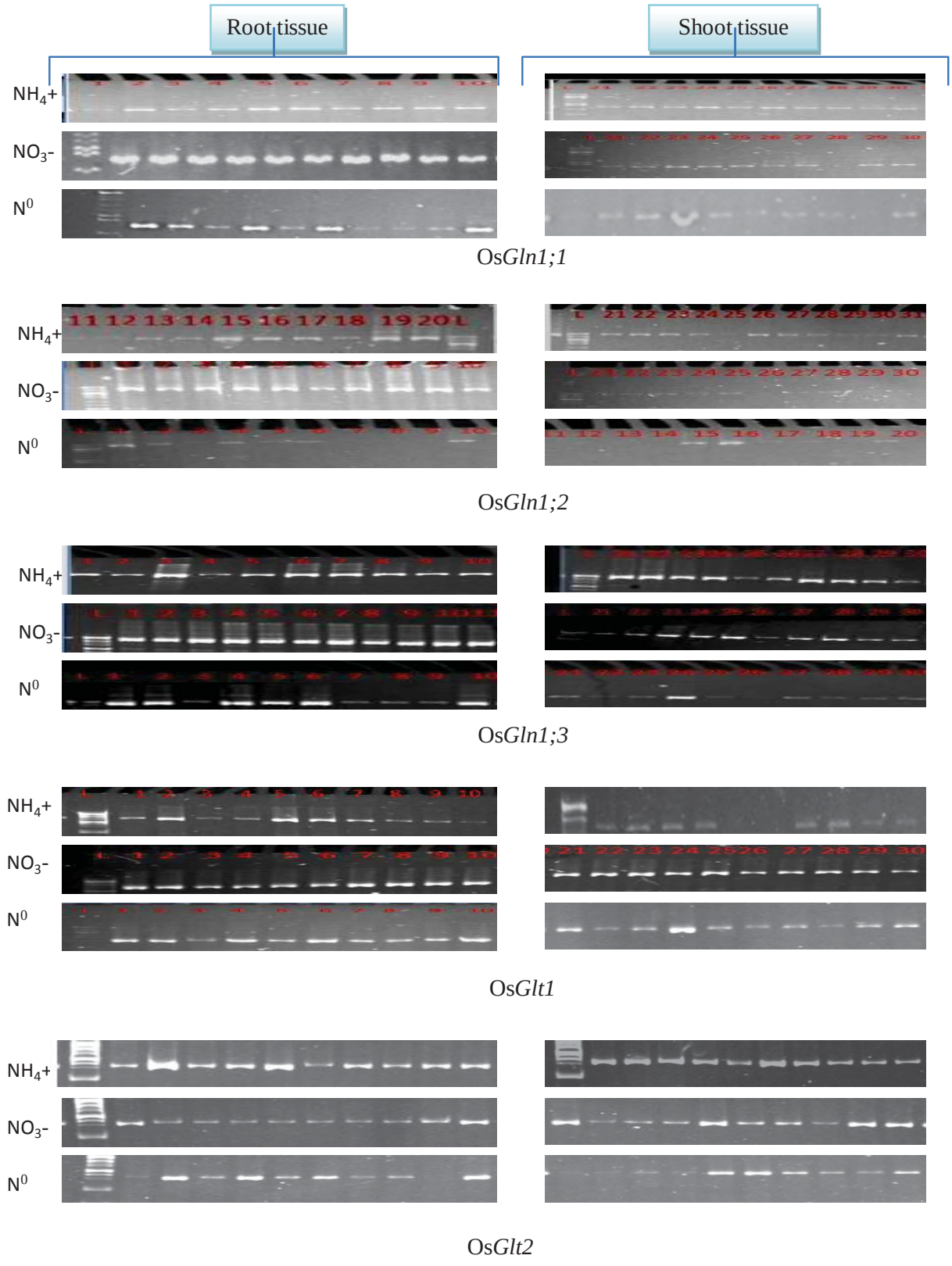


Fig. 4.23a: Expression profiles of GS and GOGAT gene family under ammonium, nitrate and control treatment in rice.

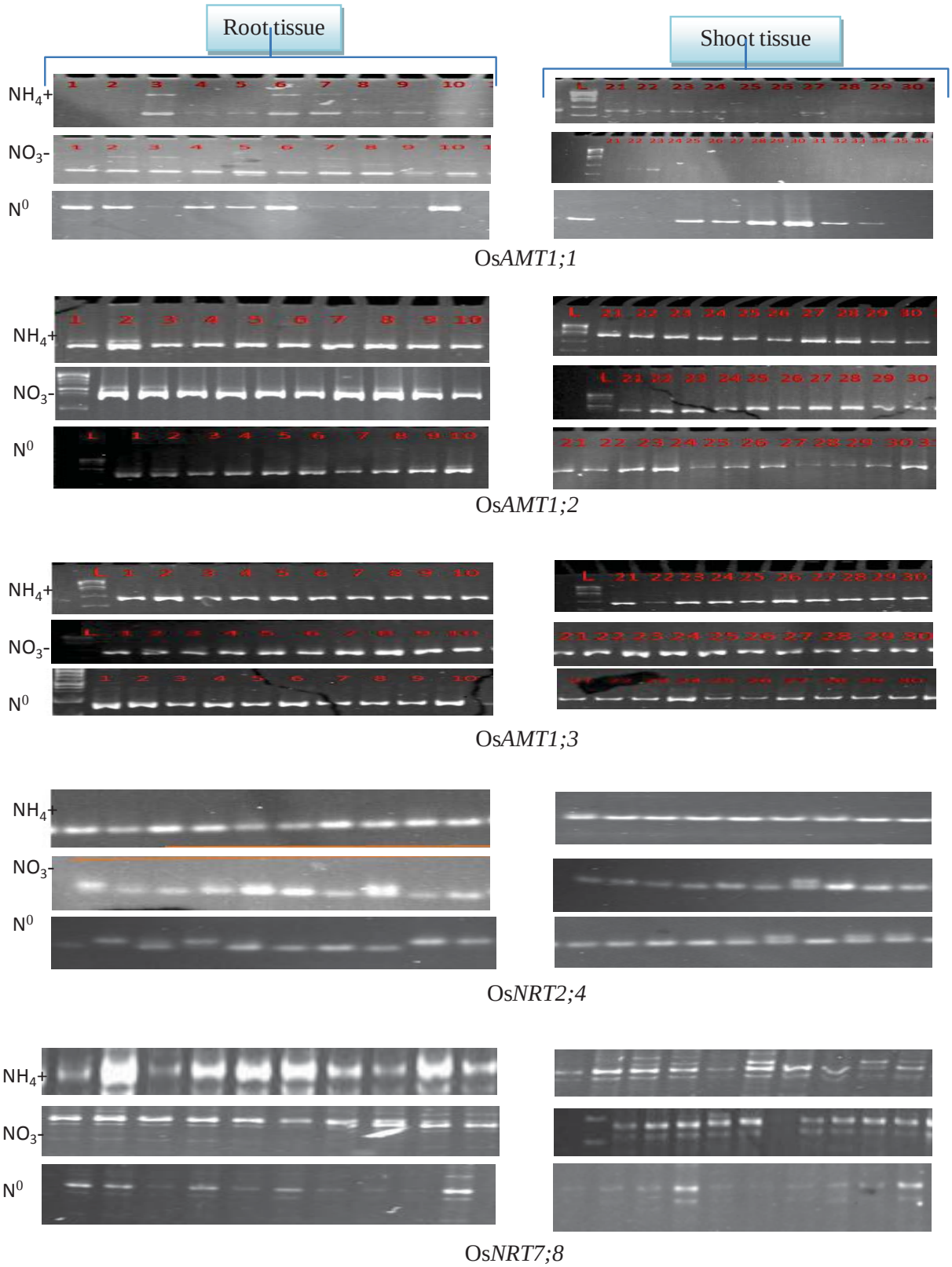


Fig. 4.23b: Expression profiles of AMT and NRT gene family under ammonium, nitrate and control treatment in rice.

4.12.2 Quantitative qPCR assay

In quantitative analysis we tried to eliminate experimental inconsistencies by adopting normalization strategies at different experimental steps. Normalization is an essential process of neutralizing the effects of experimental variability such as the nature and amount of starting sample, the RNA isolation process, reverse transcription, and, of course, real-time PCR amplification. Normalizing to sample quantity was performed by initiating the RNA isolation with a similar amount of sample (100 mg root tissue). Normalizing to RNA quantity: Precise quantification and quality assessment of the RNA samples are necessary and therefore almost equal concentration (~700 ng) of RNA samples were taken for cDNA preparation. Later for qPCR set up equal quantity of cDNA (1 to 2 µl) was taken for preparing reaction mixture (assuming equal concentration of cDNA for all the samples). Pipetting error was minimized by careful handling and avoiding bubbles. Normalizing to a reference gene: The use of a normaliser gene (also called a reference gene or endogenous control) is the most thorough method of addressing almost every source of variability in real-time PCR. Relative expression levels of the different genes (root development and drought related) in each cDNA sample were obtained by normalization to either of the two endogenous reference genes ("housekeeping" genes) actin or tubulin genes. 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.

The high organ specificity of genes under does not allow the easy detection of each gene transcript and moreover, not sensitive enough, to unravel gene expression at very low levels. RT-PCR technique is highly reproducible technique which detect expression at very low levels so results were distinct from semi-quantitative- PCR results**.**

4.12.2.1 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 is shown in table 4.27 and details are elaborated below:

4.12.1.1 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 (Os*Gln1; 1*, Os*Gln1;2* and Os*Gln1;3*) and a single plastidic Os*Gln2* gene. Distinct roles for this enzyme have been suggested by a number of studies on organ, tissue and development (Harrison *et al*., 2000; 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 (Fig.4.24 & table 4.28). Under both NH_4^+ and NO_3^- treatment, two of the genes, Os*Gln1;1* & Os*Gln1;2* showed a markedly preferential expression pattern in roots whereas Os*Gln1;2* was mainly expressed in shoot. Os*Gln2* 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, Os*Gln1;1* gene 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 NO_3^- treatment. Os*Gln1;2* in root showed significant 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. Os*Gln1;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 Os*Gln1;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

Fig. 4.24a: Expression profile of Glutamine Synthetase genes in root and shoot under ammonium (A) & nitrate (N) treatment

Figure 4.24b: Expression profile of Glutamine Synthetase genes in root and shoot under ammonium (A) & nitrate (N) treatment

of Os*Gln2* 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 no. 10 (63.11) in root.

This study suggest that Os*Gln1; 1,* Os*Gln1;2* and Os*Gln1;3* shows reciprocal responses to NH_4^+ and NO_3^- supply as oppose to proposed mechanism. Collectively, among all isoforms, Os*Gln2* which predominantly functions in green tissue and is indispensable for assimilation of photo respiratory ammonia in high NUE genotypes revealed shoot preferential expression pattern. Os*Gln2* was highly upregulated in line no. 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 Os*Gln1; 1*, Os*Gln1;2,* Os*Gln1;3* and Os*Gln2* was earlier reported by Ishimaya *et al.* (2004b). Also Zhao and Shi (2006b) revealed reciprocal responses for GS isoforms.

4.12.1.2 Actin Vs GOGAT gene family

GOGAT catalyzes reductive transfer of the amide group of Gln to 2-oxogluarate to form two Glu molecules. In rice plants, *NADH-GOGAT* is coded by two genes, Os*NADH-GOGAT1* (OsGlt1) and Os*NADH-GOGAT2* (Os*Glt2*) (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 (Fig.4.25 & table 4.28). Expression of Os*Glt1* and Os*Glt2* genes was strongly repressed by NH_4^+ and NO_3^- treatment for most of the genotypes. Exceptionally, Os*Glt1* 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. Os*Glt1* gene in shoot tissue of line no. 4 (1.46) showed increased expression in NH_4^+ treatment and line

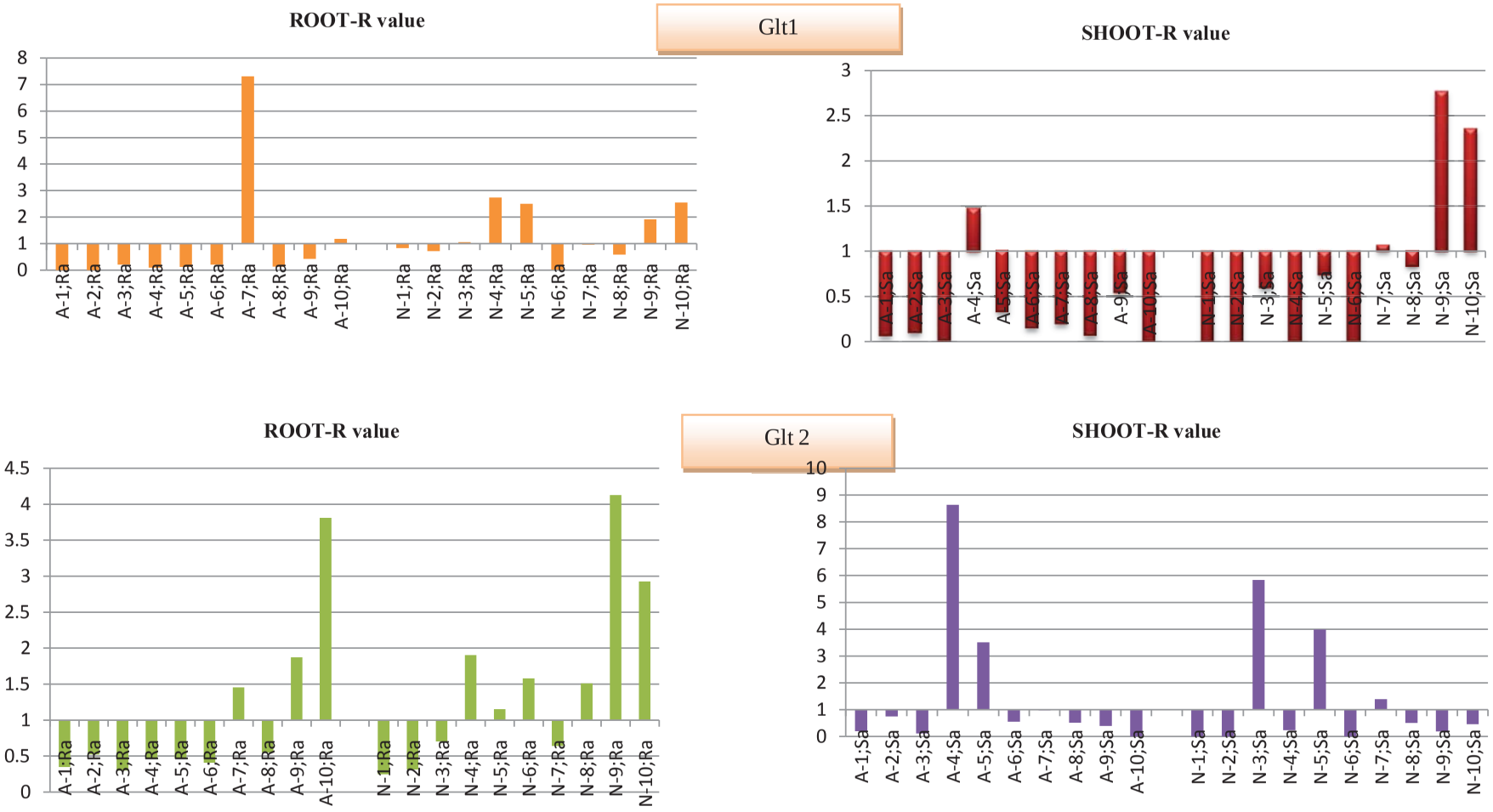


Fig.4.25: Expression profile of Glutamate Synthase gene in root and shoot under ammonium (A) and nitrate (N) treatment

Table 4.28: Comparative R values of shoot and root for Glutamine Synthetase and Glutamate Synthase under different treatments with respect to control

Line no.	Sample ID	Genes	Gln 1.1		Gln1.2		Gln1.3		Gln 2		Glt 1		Glt 2	
		Tissue	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
1	DxD-4	Root	7.1354	0.6854	0.5377	0.7047	0.1719	0.0016	30.5903	0.2882	0.0027	0.8322	0.3475	0.2474
		Shoot	1.8025	0.0161	0.0127	0.0011	1.6935	0.5645	0.0059	0.0029	0.0643	0.0022	0.1982	0.0035
2	DxD-21	Root	5.8767	1.9252	0.3909	1.4340	0.7145	0.0001	0.7927	5.3332	0.0003	0.7145	0.5212	0.3143
		Shoot	2.9690	0.0000	0.1138	0.0001	3.0420	2.4794	0.0977	0.0005	0.0984	0.0000	0.7448	0.0001
3	DxD-30	Root	3.5554	1.3996	0.1830	2.0069	2.1810	11.9588	3.7321	1.6644	0.2045	1.0570	0.2983	0.7047
		Shoot	0.3356	6.1688	0.1019	5.9587	3.1492	3.4822	0.7096	61.6060	0.0060	0.5946	0.1115	5.8361
4	DxD-46	Root	4.2575	5.7557	0.5606	13.0864	10.1261	0.1678	17.3276	45.2548	0.0928	2.7416	0.4665	1.9053
		Shoot	55.1390	0.1888	1.6529	0.4585	1.7231	3.7974	125.8010	186.7546	1.4692	0.0034	8.6338	0.2325
5	DxD-75	Root	1.0140	1.3851	0.3937	0.9234	7.5423	4.9588	8.5445	2.4708	0.1207	2.5053	0.4698	1.1527
		Shoot	7.0616	2.2736	1.3613	9.9866	2.2579	2.4284	1.0570	21.9325	0.3242	0.7346	3.5064	3.9862
6	DxD-121	Root	1.0210	2.4801	0.3816	2.9088	1.1134	0.0095	0.1416	0.0142	0.2052	0.0229	0.4075	1.5805
		Shoot	1.2702	0.0005	0.1934	0.0083	3.5801	1.3617	11.2746	2.5669	0.1487	0.0028	0.5510	0.0028
7	Danteshwari	Root	2.8879	0.5987	0.5529	0.1349	0.5285	0.5453	0.0224	1.0461	7.3107	0.9593	1.4540	0.6417
		Shoot	0.5017	0.3882	0.1774	0.6713	0.9395	0.2679	21.4068	30.9100	0.1928	1.0681	0.9828	1.3899
8	Dagad deshi	Root	0.7195	1.0317	0.0629	0.3711	0.2415	0.4429	5.4076	14.2215	0.1281	0.5885	0.5490	1.5105
		Shoot	0.29833	0.3882	0.0794	0.0845	1.5369	1.3803	0.3356	0.0677	0.0679	0.8351	0.5141	0.5035
9	Swarna	Root	1.9862	2.6027	1.3519	2.2815	1.7053	0.5303	0.0170	0.1069	0.4293	1.9185	1.8725	4.1267
		Shoot	10.2674	0.7220	3.2716	0.0783	2.6945	3.9177	5.1337	0.1555	0.5415	2.7702	0.3937	0.1888
10	ISD-1	Root	14.8254	3.1821	8.1117	1.1173	0.0001	2.2423	16.3929	63.1189	1.1769	2.5491	3.8106	2.9282
		Shoot	0.0001	1.5856	0.0010	3.7451	2.6482	1.4389	0.0014	0.3597	0.0000	2.3538	0.0000	0.4585

no. 9(2.71) of dark green colour class and line no. 10 (2.35) of yellow colour class in NO_3^- treatment. Os*Glt2* 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 Os*Glt1* is decreased in root and shoot by N concentration while transcription of Os*Glt2* is relatively increased in root and shoot by N concentration. When N level is relatively high in soil, expression of Os*Glt1* and Os*Glt2* can be decreased in order to limit N acquisition but in the opposite side, expression of Os*Glt1* and Os*Glt2* 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).

4.12.1.3 Expression of AMT and NRT transporters under different N forms

4.12.1.3.1 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*., 2009b). So far, studies on expressions and regulations of AMT genes in rice have been focused on the three genes of Os*AMT1* family, which displayed different spatio-temporal expression patterns in response to changes in N levels. Os*AMT1;1*, Os*AMT1;2*, Os*AMT1;3* have been identified as members of AMT1, each showing a distinct expression pattern: Os*AMT1;1* shows constitutive expression in both shoots and roots (Ding *et al*., 2011b). In the present study, similar results (Fig.4.26 & table 4.29) were obtained influx of NH_4^+ ion resulted in up regulation and significant strong expression of Os*AMT 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). Os*AMT1; 2* shows root-specific and ammonium-inducible expression (Ding *et al*., 2011c). This is in contrary with present results in which up regulation

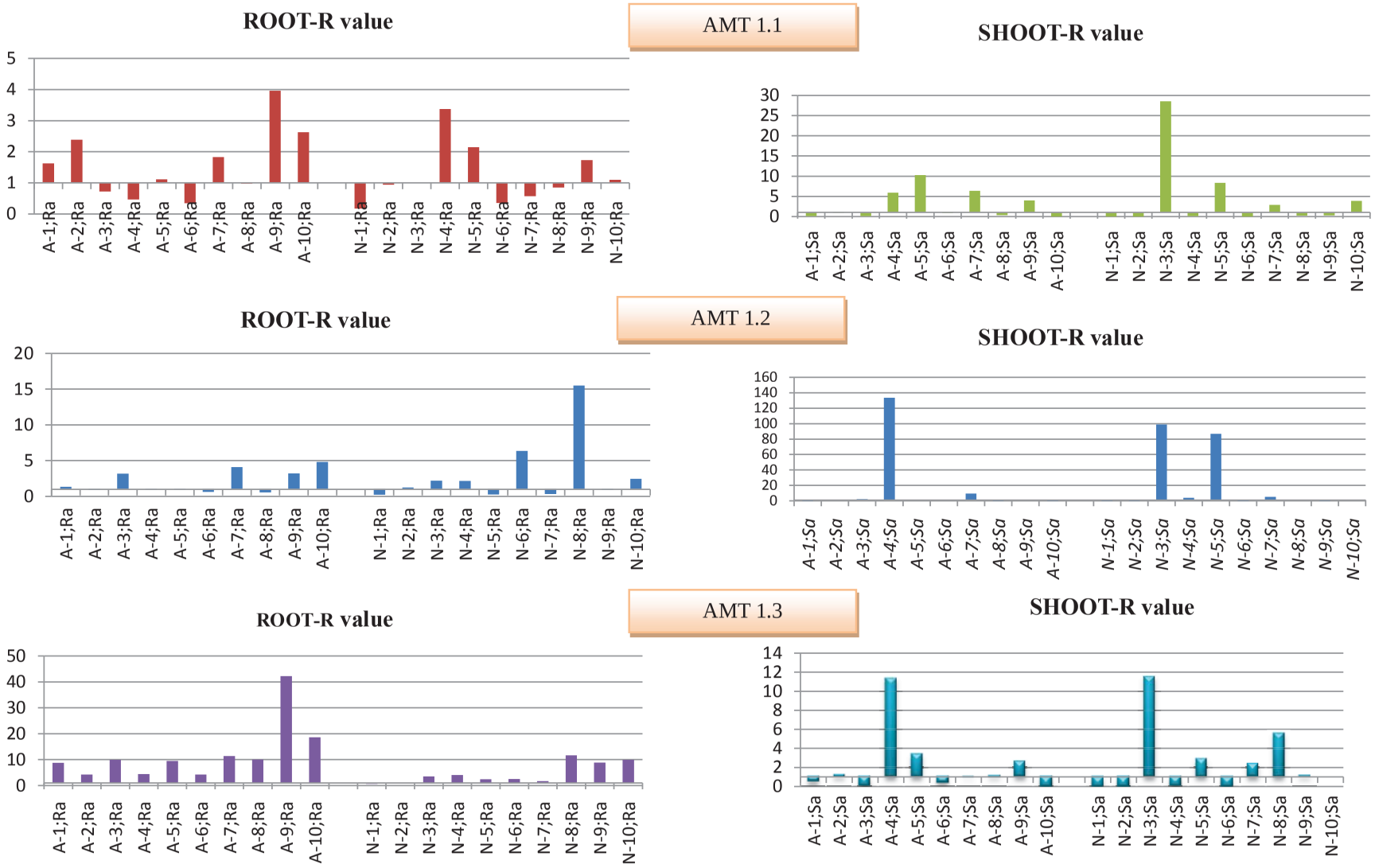


Fig. 4.26: Expression profile of ammonium transporter genes in root and shoot under ammonium (A) & nitrate (N) treatment

of Os*AMT1;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, NH_4^+ and NO_3^- treatment resulted in up regulation of Os*AMT1; 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, Sonoda *et al*. (2003b) reported that Os*AMT1; 3* shows root-specific and nitrogen-suppressible expression.

Our results dictates that Os*AMT1;2* shows maximum mRNA accumulation in line no. 4 which also pertains high GS2 activity thus Os*AMT1;2* expression increases with increase in endogenous glutamine. It also seems that Os*AMT1;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*. (2003c) who studied feedback regulation AMT1 gene family by glutamine in rice.

4.12.1.3.2 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, Os*NRT2.4* and Os*NRT7.8,* who are nitrate inducible and is mainly expressed in parenchyma cells around the xylem. Os*NRT2.4* and Os*NRT7.8* 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, (Fig.4.27 & table 4.29) significant upregulation of Os*NRT2.4* 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. Os*NRT7.8* 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 Os*NRT7.8* in line no. 10 which is actually of pale-yellow colour

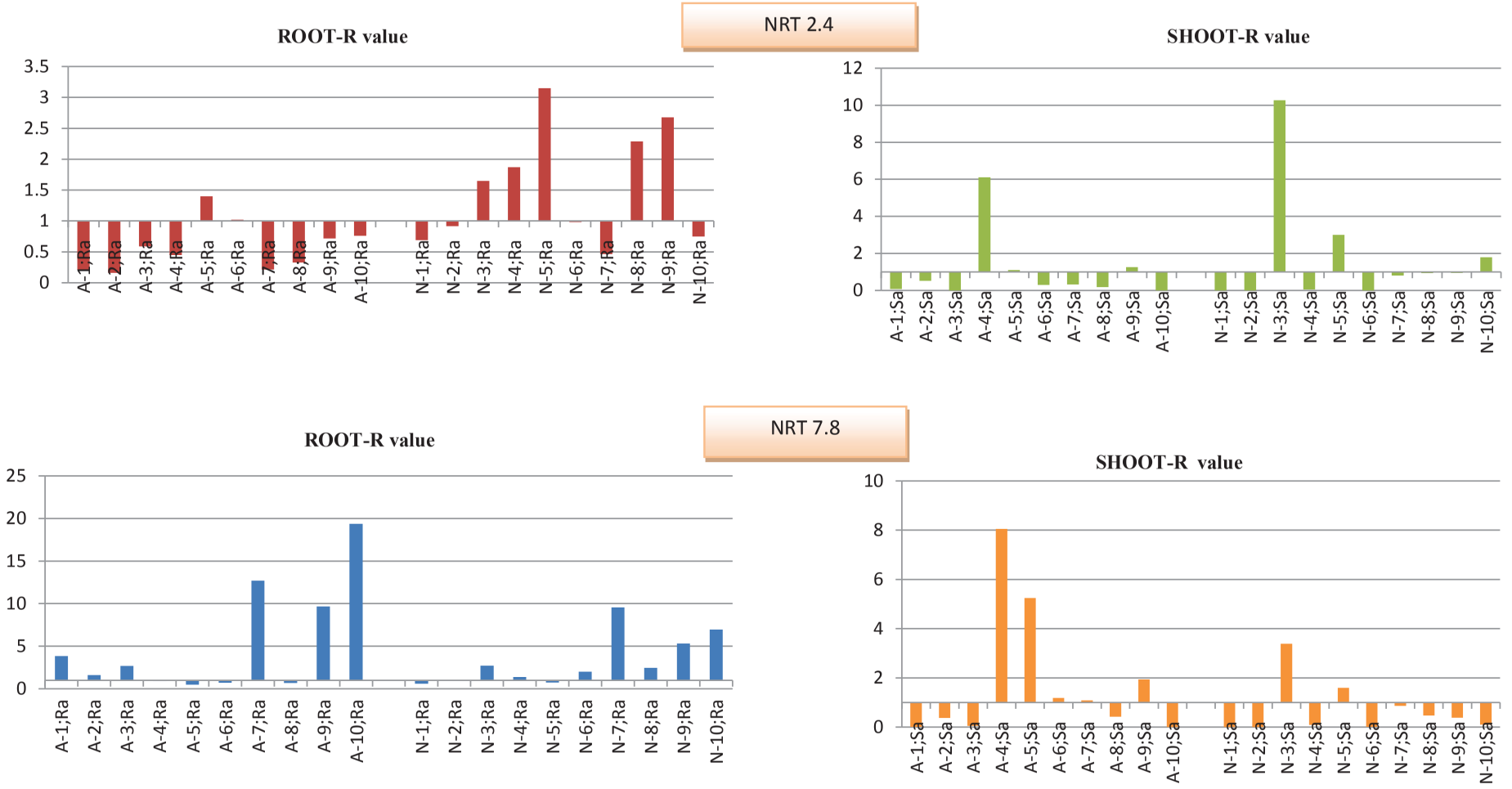


Fig. 4.27: Expression profile of nitrate transporter genes in root and shoot under ammonium (A) & nitrate (N) treatment

Table 4.29: Comparative R values for shoot and root for ammonium and nitrate transporters in different treatments with respect to control

	Sample ID	Genes	AMT 1.1		AMT 1.2		AMT 1.3		NRT2.4		NRT7.8	
		Tissue	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
1	DxD-4	Root	1.6301	0.1768	1.3519	0.2285	8.7847	0.6620	0.2095	0.6902	3.8371	0.5987
		Shoot	0.0984	0.0031	0.0693	0.0098	0.4730	0.0048	0.0842	0.0019	0.0115	0.0034
2	DxD-21	Root	2.3867	0.9428	0.8796	1.2483	4.2281	0.8094	0.1593	0.9170	1.6245	0.9234
		Shoot	1.1251	0.0002	0.6092	0.0033	1.2527	0.0000	0.5194	0.0000	0.3686	0.0000
3	DxD-30	Root	0.7245	1.0070	3.1932	2.1886	9.9177	3.4822	0.5885	1.6472	2.6851	2.7132
		Shoot	0.1392	28.5417	2.0069	99.0442	0.0330	11.5514	0.0064	10.2674	0.0451	3.3870
4	DxD-46	Root	0.4649	3.3753	0.9693	2.1510	4.4537	4.0840	0.4491	1.8725	0.9107	1.3803
		Shoot	5.9176	0.1088	133.435	4.0278	11.3924	0.0571	6.1050	0.0464	8.0556	0.0878
5	DxD-75	Root	1.1134	2.1435	0.9428	0.2483	9.4807	2.4033	1.3996	3.1492	0.4931	0.7371
		Shoot	10.2674	8.3687	1.6472	86.8227	3.3753	2.8879	1.1096	3.0001	5.2416	1.5966
6	DxD-121	Root	0.3463	0.3561	0.6263	6.3662	4.2871	2.5676	1.0210	0.9831	0.7120	2.0215
		Shoot	0.8706	0.0066	0.6854	0.0365	0.3816	0.0029	0.2942	0.0018	1.1851	0.0083
7	Danteshwari	Root	1.8277	0.5724	4.0840	0.3403	11.3530	1.7351	0.2154	0.4633	12.6846	9.5467
		Shoot	6.3643	2.8779	9.4479	5.1694	0.9461	2.3702	0.3253	0.8123	1.0867	0.8615
8	Dagad deshi	Root	0.9794	0.8497	0.5415	15.5086	10.0213	11.6318	0.3276	2.2894	0.6854	2.4708
		Shoot	0.3220	0.2245	0.0736	1.1251	1.1688	5.6178	0.1869	0.9298	0.4204	0.4714
9	Swarna	Root	3.9586	1.7351	3.2043	0.8888	42.2243	8.8766	0.7170	2.6759	9.6800	5.3147
		Shoot	4.0000	0.2652	0.8066	0.0944	2.5847	1.1647	1.2658	0.9428	1.9386	0.3789
10	ISD-1	Root	2.6299	1.0981	4.8065	2.4453	18.6357	9.9521	0.7605	0.7500	19.3599	6.9644
		Shoot	0.0002	3.9041	0.0002	1.2142	0.0001	1.0000	0.0000	1.7901	0.0000	0.0941

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 (2005b) & 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.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Drought is the composite stress condition that includes soil water deficit and reduced nutrient availability to the plant. Plant species and genotypes of a species may vary in their response to mineral uptake under water stress. Both stresses interact and contribute to the low productivity in cultivated areas worldwide. Of all the nutrients, Nitrogen (N) is one of the most critical inputs that define crop productivity and yield under field conditions and must be supplemented to meet the food production demands of an ever-increasing population. Nitrogen use efficiency (NUE) in plants is a complex quantitative trait that involves many genes and depends on a number of internal and external factors in addition to soil nitrogen availability and it is also highly influenced by the changing environmental conditions. The current average nitrogen use efficiency (NUE) in the rice field is approximately 33%, poorest among cereals and a substantial proportion of the remaining 67% is lost into the environment N reducing economic efficiency of applied N. The calls for immediate development of comprehensive approach to optimize N management in every sphere of life *i.e.* biochemical, physiological and molecular level. Therefore, the present study was undertaken to establish for mapping QTLs for NH_4^+ and NO_3^- use efficiency under water stress and non-stress conditions and expression analysis of Glutamine Synthetase and Nitrate reductase in rice (*Oryza sativa* L.)

CONCLUSIONS

- The trend analysis of NH_4^+-N dynamics during *kharif* 2014 and *kharif* 2015 revealed that NH_4^+-N concentration persisted more under anaerobic condition as compared to aerobic condition while trend analysis of NO_3^--N dynamics during *kharif* 2014 and *kharif* 2015 showed that maximum values of NO_3^--N concentrations under aerobic conditions as compared to anaerobic conditions under all treatments and soil environments.

- Yield and yield related traits were evaluated in 122 and 32 RILs during wet season 2014 and 2015. Three way-ANOVA showed high level of significance for variance components (G, N, E) and their interactions effects (GXN, GXE, NXE, EXNXG). Nitrogen and environment was the main consideration of present study and they significantly affected investigated traits which allow us to conclude that it is possible to manipulate these plant parameters in favour of higher grain yield by using appropriate nitrogen form and environment. During wet season 2014, mean performance of genotypes depicted higher values for agronomically important traits *i.e.* grain yield, biological yield, plant height and total tillers under NH_4^+ treatment (317.1 g/m^2, 998.6 g/m^2, 117.1 cm & 361.6 g/m^2) followed by NO_3^- (311.3 g/m^2, 827.7 g/m^2, 117.1 cm & 303.6 g/m^2) and N^0 (352 g/m^2 , 850 g/m^2 , 170 cm, 108 g/m^2) treatment under irrigated condition . Furthermore, under rainfed condition high mean phenotypic values were observed under grain yield, biological yield, plant height and total tillers in NH_4^+ treatment (241.1 g/m^2, 933 g/m^2, 86 cm, 450 g/m^2) followed by NO_3^- treatment (179 g/m^2, 651 g/m^2, 86 cm, 401 g/m^2) and N^0 treatment (57 g/m^2, 274 g/m^2, 71 cm, 272 g/m^2). Moreover, during wet season 2015, NH_4^+ form showed highest values for all evaluated traits then NO_3^- and N^0 forms. High phenotypic coefficient of variability and genotypic coefficient of variability was obtained for grain yield (>25.6%), biological yield (>19.9%), harvest index (>20.1%), seedling biomass (>17.9%) and spikelet sterility (>27.3%) under all sets of conditions during wet season 2014 and 2015 and broad sense heritability estimates for the estimated 19 traits during wet season 2014 ranged from 8.2% to 84.1 % and during wet season 2015 ranged from 24% to 98.4 % under differential N and water regimes.
- NUE and NUE component traits ANOVA revealed that genotypic effects and genotype x nitrogen interaction effects were significantly different for investigated N use efficiency and its component traits ($p<0.05$, $p<0.01$). During wet season 2015, mean performance of genotypes depicted higher values for agronomically complex traits *i.e.* straw nitrogen content (SNC), grain protein content (GPC), grain nitrogen content (GNC), straw nitrogen yield (SNY), grain nitrogen yield (GNY), biological nitrogen yield (BNY), nitrogen harvest

index (NHI), N uptake (NUE), N-utilization (NUtE) and N-use efficiency (NUE) was highest in NH_4^+ treatment (1.23%, 7.3%, 0.5%, 3.5 g/m^2, 2.7 g/m^2, 6.3 g/m^2, 57%, 0.20 gg^{-1}, 45.2 gg^{-1}, 9.8 gg^{-1}) followed by NO_3^- treatment (1.1%, 6.8%, 0.47%, 2.6 g/m^2, 2.1 g/m^2, 4.7 g/m^2, 56%, 0.18 gg^{-1}, 49.4 gg^{-1}, 9.3 gg^{-1}) and N^0 treatment (1.1%, 6.6%, 0.4%, 2.4 g/m^2, 1.7 g/m^2, 4.2 g/m^2, 56.7%, 0.15 gg^{-1}, 51.6 gg^{-1}, 8.8 gg^{-1}). Similarly, under rainfed condition, maximum values of mean performance of genotypes was obtained in NH_4^+ treatment (1.5%, 8.9%, 0.9%, 2.0 g/m^2, 4.1 g/m^2, 6.1 g/m^2, 35.5%, 0.22 gg^{-1}, 21.8 gg^{-1}, 5.0 gg^{-1}) followed by NO_3^- treatment (1.3%, 8.2%, 0.8%, 1.7 g/m^2, 3.1 g/m^2, 4.7 g/m^2, 34.7%, 0.17 gg^{-1}, 25.1 gg^{-1}, 4.7 gg^{-1}) and N^0 treatment (1.2%, 6.9%, 0.6%, 1.1 g/m^2, 1.7 g/m^2, 2.8 g/m^2, 32.5%, 0.10 gg^{-1}, 30.5 gg^{-1}, 3.3 gg^{-1}). Broad sense heritability estimates was highest for NUE under differential N and water regimes.

- Genotypic differences existed in genotypes for NUE parameters with yield predominantly determined by N-uptake efficiency. Genotype G-31 manifested the highest NUE values under irrigated condition in all treatments (17.2 gg^{-1}N, 12.72 gg^{-1}N, 16.98 gg^{-1}N) while Genotype G-27 has the maximum NUE values under rainfed condition in all treatments (8.4 gg^{-1}N, 11.6 gg^{-1}N, 6.2 gg^{-1}N). Grain yield showed significant and positive association with NUE indices under all sets of environments.
- Cluster analysis showed G31 and G-1 performed well under both the environment both for NUE and yield parameters. Correlation between Grain yield showed significant and positive association with Grain N yield (GNY), N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), N-use efficiency (NUE) and Nitrogen harvest index (NHI) in NH_4^+ (0.95**, 0.82**, 0.57**, 0.98**, 0.57**), NO_3^- (0.92**, 0.80**, 0.57 **, 0.97**, 0.36 **) & N^0 (0.95**, 0.81 **, 0.66 **, 0.99**, 0.53 **) treatment under irrigated condition. Similarly, under rainfed condition and all sets of treatment, positive correlation existed between GY and NUE indices.
- Morphological characteristic of the root in rice can affect more efficient nitrogen uptake. With the identification of the better rice genotypes based on the morphological attributes of the root system which are important in more

and faster nitrogen uptake, in addition to the maintenance of the high yield, the nitrogenous fertilizers can be consumed more economically. Rooting depth can be used as selection criteria for identifying genotypes with enhanced nitrogen uptake efficiency. In the present investigation, total root length of selected 32 genotypes varied from 213 cm to 874 cm under NH_4^+ treatment, 200 to 852 cm under NO_3^- treatment while 342 to 1143 under N^0 treatment.

- Correlation analysis between root traits *i.e.* total root length (TRL), root volume (RV), Average root diameter (ARD) and yield & NUE-related traits *i.e.* grain yield (GY), grain nitrogen yield (GNY), nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE) and nitrogen use efficiency (NUE) revealed positive and strong correlations between GY/TRL, GNY/TRL, NUpE/TRL, NUtE/TRL, NUE/TRL and NHI/TRL under irrigated condition in NH_4^+ (0.56 **-0.700**), NO_3^- (0.516**-0.726**) and N^0 treatment (0.586**-0.762**) and under rainfed condition in NH_4^+ (0.601**-0.735**), NO_3^- (0.512**-0.710**) and N^0 treatment (0.591**-0.745**) and under rainfed condition. Positive correlation also existed in root diameter and yield & NUE indices under NH_4^+ (0.259*-0.495*), NO_3^- (0.323*-0.482*) and N^0 (0.418*-0.456*) of irrigated condition while NH_4^+ (0.315*-0.362*), NO_3^- (0.362*-0.461*) and N^0 (0.418*-0.561*) of rainfed condition. Within NH_4^+ treatment positive correlation existed between RV-GNY/GY/NUE/NHI (0.511**-0.561**) under rainfed conditions.
- Genotypic differences for root and shoot traits among selected 10 rice genotypes grown under minirhizotron revealed significant variation in maximum root length (MRL) in mean values of NH_4^+ (23±2.0-56±2.7), NO_3^- 20±4.5-54±4.0) and N^0 (25±1.5-72±4.0) treatment and shoot fresh weight (SFW) in NH_4^+ (2±0.1- 10±2.2), NO_3^- 2±0.2- 6±0.1) and N^0 (2±0.5- 5.3±0.1) and variation in seedling height (SH), root fresh weight (RFW) and maximum root volume (MRV). Among all the contrasting genotypes studied, NH_4^+, NO_3^- and N^0 responsive genotype with maximum root length was G-8 (56±2.7, 54±4.0 , 72±4.0) while maximum shoot fresh weight was prevalent in G-6 (10±2.2, 6±0.1 , 5.3±0.1).

- Stability analysis revealed line number 8, 17, 18, 36, 38, 39, 58, 62, 84 and 88 and line number 13, 61 and 39 stable under favourable and unfavourable environment.
- Genotype G-21, G-30, G-75 & G121 expresses high NR activity (mg^{-1} proteins min^{-1}) in both root (0.009, 0.012, 0.003, 0.016) and shoot (2.0, 2.1, 2.1, 0.4). NiR activity (mg^{-1} proteins min^{-1}) was found to be highest for G-10 (0.14) followed by G-9, 8, 7, 6. GS (µmol Pi formed mg^{-1} proteins min^{-1}) and GOGAT activity (µmol NADH oxidized mg^{-1} proteins min^{-1}) in shoot and root was recorded to be highest for genotypes G-2 , G-4, G-6 & G-9. Furthermore, significant and positive correlation of enzymatic activity and NUE existed in leaves NR, NiR, GOGAT/NUpE (0.359*, 0.318*, 0.451*), GOGAT/NUtE (0.539**), NUE/GOGAT (0.684**) and NiR/NUE (0.568**) under NH_4^+ treatment. In NO_3^- treatment, between NiR/NUpE (0.593**) in leaves and GS/NUtE (0.601**) in roots while in N^0 treatment NiR/NUpE (0.366*). This relationship depicts role of enzymes in nitrogen assimilation.
- A total of 58 QTLs conferring the corresponding five traits were detected under three N forms and two environments; that as a matter of fact included 14, 15 & 11 QTLs under NH_4^+, NO_3^- and N^0 level of irrigated condition and 5, 10 & 1 QTLs under NH_4^+, NO_3^- and N^0 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. 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 and projected further graphically *via* GGT.
- qPCR results revealed that Os*Gln1;1* and Os*Gln1;2* exhibited strong root preferential expression pattern while Os*Gln1;3* and Os*Gln2* showed preference for leaves. The Os*Gln1;1*, Os*Gln1;2*, Os*Gln1;3* and Os*Gln2* also manifested different and reciprocal responses to nitrate and ammonium supply. Overall, Os*Gln2* isoform of Glutamine Synthetase showed strong upregulation in shoot under NH_4^+ (125.8) and NO_3^- (186.7) treatment in Genotype G-4 which falls in

dark green spectrum in colour classes suggesting Os*Gln2* is major assimilatory form in green tissues. With regard to Os*Glt1* and Os*Glt2* genes, they showed strong repression by NH_4^+ and NO_3^- treatment in both root and shoot. Therefore, the expression of gene coding NADH-GOGAT possibly changes with external N forms concentration. Systemic expression patterns for the AMT and NRT gene families is not yet been clearly established. Our results revealed that, Os*AMT1;2* exhibited significant strong expression in shoot of genotype G-4 (133.4) also having high Os*Gln* activity thus there is strong induction by endogenous glutamine while Os*AMT1;3* exhibited strong expression in root of genotype G-9 (42.1) also having low Os*Gln* activity which signifies suppression by endogenous glutamine. With regard to NRT 7.8 gene, genotype G-10 showed significant upregulation under NH_4^+ treatment in root while NRT 2.4 gene was significantly upregulated in genotype G-3 (10.3) under NO_3^- treatment in shoot.

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APPENDIX-A: Weekly meteorological data during *kharif* 2014 and 2015Weekly Meteorological Data: 2014

Wk No.	Date	Max. Temp. (°C)	Min. Temp. (°C)	Rain-fall (mm)	Rainy days	Relative Humidity (%)		Vapour Pressure (mm of Hg)		Wind Velocity (Kmph)	Evapo-ration (mm)	Sun Shine (hours)
						I	II	I	II			
1	Jan 01-07	28.6	13.6	0.0	0	90	40	11.1	11.0	0.7	2.6	6.9
2	08-14	27.8	14.1	0.0	0	90	47	11.5	12.5	1.6	2.5	5.4
3	15-21	29.0	16.1	0.0	0	89	46	13.0	13.0	2.5	2.8	4.6
4	22-28	28.2	13.7	0.0	0	87	38	11.0	10.0	2.3	3.5	7.0
5	29-04	28.8	10.1	0.0	0	86	28	8.5	7.9	2.0	3.7	9.5
6	Feb 05-11	31.7	14.8	0.0	0	85	33	11.3	10.8	3.2	4.3	8.7
7	12-18	27.9	15.4	20.4	2	83	39	11.8	9.9	4.1	4.3	6.6
8	19-25	28.9	14.6	18.6	1	86	41	11.7	12.0	2.9	4.1	8.7
9	26-04	27.9	17.7	45.8	3	91	61	14.5	14.4	4.1	4.1	6.6
10	Mar 05-11	27.5	17.5	2.4	0	88	44	14.5	12.3	2.7	2.9	4.9
11	12-18	33.3	19.5	2	0	89	38	16.5	14.3	1.4	4.6	7.5
12	19-25	36.4	19.4	0	0	74	21	14.4	9.1	2.7	6.9	9.0
13	26-01	38.4	22.2	0.0	0	67	24	15.5	11.8	3.1	7.0	7.7
14	Apr 02-08	38.5	22.4	2.8	1	59	17	13.4	8.9	3.5	8.5	8.6
15	09-15	38.5	22.4	2.8	1	58	23	14.0	11.4	5.1	8.4	6.8
16	16-22	37.8	23.4	18	1	67	28	16.6	13.1	5.1	8.2	9.1
17	23-29	41.1	25.1	0.2	0	58	18	16.1	10.1	4.5	9.5	9.7
18	30-06	40.1	25.6	2.4	0	53	23	15.5	11.9	8.7	11.3	9.4
19	May 07-13	38.8	26.0	0.0	0	59	27	17.3	13.2	7.8	10.0	8.3
20	14-20	42.1	27.6	0.0	0	43	16	13.8	9.5	5.2	11.3	9.2
21	21-27	40.8	27.1	32.2	2	54	30	16.8	13.1	6.2	11.2	7.5
22	28-03	41.7	28.7	0.0	0	56	26	18.6	14.9	6.6	10.8	9.1
23	Jun 04-10	44.1	30.9	0.0	0	54	23	20.6	14.8	7.8	13.0	8.0
24	11-17	40.4	28.4	14.0	2	56	36	18.2	16.7	9.7	10.6	5.0
25	18-24	33.6	26.0	30.0	3	79	60	21.5	21.1	9.5	5.7	3.0
26	25-01	35.7	26.0	27.6	2	78	50	21.7	20.2	8.1	6.4	3.3
27	Jul 02-08	37.7	27.0	9.0	1	72	44	21.6	19.7	9.0	8.5	5.3
28	09-15	34.3	23.8	152.8	7	92	72	23.6	24.8	8.4	6.6	4.1
29	16-22	28.5	24.6	260.2	6	95	88	22.8	22.6	12.1	2.8	0.5
30	23-29	28.7	23.8	37.2	4	95	82	22.1	23.0	9.4	2.7	1.6
31	30-05	29.8	24.8	136.0	7	95	86	23.2	24.3	9.7	4.0	1.9
32	Aug 06-12	30.2	24.8	42.1	3	91	71	22.7	22.5	9.1	3.6	2.8
33	13-19	31.8	25.3	45.0	3	91	70	23.7	22.6	7.0	4.7	5.5
34	20-26	32.3	25.1	25.8	2	92	73	24.0	23.7	4.0	3.7	3.4
35	27-02	31.8	25.0	84.8	4	91	76	23.1	23.6	5.8	4.1	3.6
36	Sep 03-09	28.3	24.2	79.5	4	94	83	22.5	22.6	6.2	1.7	0.5
37	10-16	30.5	24.3	41.0	3	95	79	23.0	24.0	5.8	3.3	3.4
38	17-23	32.1	24.6	57.6	3	94	68	23.6	23.3	3.6	3.7	4.4
39	24-30	33.4	24.0	0.0	0	93	57	22.5	20.6	2.1	4.1	8.3
40	Oct 01-07	33.2	24.0	0.0	0	91	57	22.0	20.6	2.5	3.9	8.3
41	08-14	30.4	23.6	52.2	2	89	66	20.8	20.4	6.9	3.6	4.9
42	15-21	31.5	22.5	1.2	0	91	56	20.4	18.8	2.6	3.4	8.4
43	22-28	29.1	19.4	5.4	1	92	52	17.0	14.9	2.0	2.8	5.9
44	29-04	30.1	16.9	0.0	0	94	37	15.1	11.5	1.9	3.0	8.0
45	Nov 05-11	30.7	17.6	0.0	0	88	44	14.5	13.7	3.0	3.4	7.8
46	12-18	31.4	19.3	0.0	0	84	35	15.0	11.5	2.8	3.6	6.8
47	19-25	29.3	11.9	0.0	0	91	28	10.6	8.3	1.9	2.9	8.5
48	26-02	30.2	12.5	0.0	0	90	26	10.8	8.4	1.9	3.2	8.6
49	Dec 03-09	28.9	10.8	0.0	0	90	28	9.6	8.0	2.2	3.4	9.0
50	10-16	28.6	15.8	0.0	0	89	49	12.8	12.7	2.3	2.2	3.0
51	17-23	25.0	8.3	0.0	0	89	31	8.0	7.1	2.2	2.8	7.8
52	24-31	26.0	9.9	0.0	0	86	34	8.7	8.3	2.2	2.9	8.3

Weekly Meteorological Data : 2015

Wk No.	Date	Max. Temp. (°C)	Min. Temp. (°C)	Rain-fall (mm)	Rainy days	Relative Humidity (%)		Vapour Pressure (mm of Hg)		Wind Velocity (Kmph)	Evapo-ration (mm)	Sun Shine (hours)
						I	II	I	II			
1	Jan 01-07	25.0	14.8	9.4	2	95	52	13.2	11.9	4.4	2.6	4.5
2	08-14	25.8	8.0	0.0	0	90	29	7.7	6.6	2.2	2.8	9.2
3	15-21	26.0	8.3	0.0	0	90	29	7.9	6.9	2.2	2.7	8.3
4	22-28	28.9	13.5	0.0	0	88	37	10.9	10.8	2.1	2.9	7.4
5	29-04	28.3	11.7	0.0	0	88	29	10.1	7.8	2.6	3.5	7.6
6	Feb 05-11	29.9	14.0	0.0	0	83	36	10.5	10.2	2.5	3.5	5.7
7	12-18	29.3	13.6	2.2	0	88	39	11.2	11.2	3.2	3.6	8.2
8	19-25	33.5	15.7	0.0	0	84	28	12.1	10.2	2.7	4.6	9.8
9	26-04	30.8	18.5	19.2	2	83	49	14.0	13.8	4.2	3.8	6.7
10	Mar 05-11	31.3	16.6	0.0	0	81	34	12.6	11.7	3.0	4.5	8.1
11	12-18	34.1	19.9	0.1	0	76	33	14.1	12.3	4.1	5.8	7.6
12	19-25	35.1	18.7	0.0	0	66	22	12.4	9.4	3.4	6.7	9.0
13	26-01	37.3	21.5	0.0	0	66	31	14.6	13.1	4.7	7.5	7.2
14	Apr 02-08	39.1	22.8	0.0	0	65	26	15.4	12.9	6.6	10.0	8.2
15	09-15	33.7	20.8	21.6	3	83	49	16.7	16.4	6.7	6.0	5.4
16	16-22	38.0	24.6	14.2	2	71	33	18.5	16.6	7.0	8.9	9.4
17	23-29	38.1	24.1	15.6	1	74	34	18.3	15.9	9.0	10.2	9.0
18	30-06	39.6	25.4	12.4	1	73	36	19.7	17.9	6.8	8.7	7.7
19	May 07-13	40.2	26.2	0.0	0	65	36	18.4	18.2	7.8	10.2	7.3
20	14-20	39.8	27.2	1.0	0	63	31	18.9	16.1	7.7	9.5	7.8
21	21-27	45.7	29.5	0.0	0	40	14	14.1	10.0	6.8	13.6	9.3
22	28-03	42.9	27.7	5.8	1	57	25	17.7	13.7	7.2	11.2	7.7
23	Jun 04-10	39.2	27.4	34.8	2	70	38	21.1	18.7	8.3	9.3	6.4
24	11-17	37.0	26.1	51.4	2	80	53	21.9	20.9	7.8	7.3	5.4
25	18-24	33.8	25.8	201.8	3	85	72	23.3	22.4	7.9	5.3	4.9
26	25-01	33.5	25.0	25.8	4	87	59	22.8	21.6	9.3	6.4	4.3
27	Jul 02-08	33.6	25.2	41.8	2	79	64	21.7	22.0	9.1	6.4	5.9
28	09-15	31.2	25.2	72.8	5	89	80	23.2	24.1	7.9	3.3	1.7
29	16-22	31.8	25.6	7.8	1	91	71	23.4	23.6	8.0	4.7	2.4
30	23-29	30.7	25.1	43.6	1	90	70	22.3	21.6	7.9	4.1	3.4
31	30-05	31.2	25.2	48.7	3	86	69	21.3	21.2	10.4	4.6	4.6
32	Aug 06-12	30.8	24.7	36.6	1	94	73	23.2	23.7	4.8	3.1	2.5
33	13-19	31.7	25.3	126.4	3	94	73	24.0	24.2	7.5	4.7	4.1
34	20-26	32.3	25.9	23.6	1	87	65	22.6	22.0	8.1	5.0	6.5
35	27-02	30.8	25.0	37.9	6	94	80	23.5	24.7	4.9	2.5	1.2
36	Sep 03-09	33.0	25.5	10.0	1	93	64	23.7	21.5	4.7	3.9	6.9
37	10-16	33.5	25.4	68.4	2	93	62	23.9	22.0	3.8	4.7	6.8
38	17-23	30.1	25.1	135.4	2	94	78	23.6	24.0	5.8	2.6	3.1
39	24-30	32.5	24.6	0.0	0	92	57	22.3	20.2	3.0	3.8	7.2
40	Oct 01-07	33.7	24.4	0.0	0	92	51	22.7	19.3	2.4	4.4	7.7
41	08-14	33.9	22.2	0.0	0	89	47	19.5	17.9	3.0	4.3	8.7
42	15-21	33.4	22.8	0.0	0	91	45	20.2	16.7	2.4	3.8	8.7
43	22-28	33.7	21.3	0.0	0	90	37	18.5	13.8	2.1	3.6	8.2
44	29-04	30.0	19.4	0.0	0	90	55	16.6	16.4	4.1	3.2	6.7
45	Nov 05-11	31.7	18.8	0.0	0	91	37	15.8	12.4	2.6	3.5	7.8
46	12-18	31.7	16.3	0.0	0	89	33	13.3	11.0	2.4	3.3	7.5
47	19-25	30.6	15.5	0.0	0	88	36	12.6	11.3	2.8	3.3	8.3
48	26-02	31.9	16.7	0.0	0	87	34	13.4	11.6	2.4	3.3	7.5
49	Dec 03-09	31.2	14.8	0.0	0	88	31	12.0	10.0	2.3	3.0	8.0
50	10-16	30.1	17.3	4.4	1	77	46	12.2	13.6	2.9	2.7	4.4
51	17-23	27.7	16.6	9.4	1	85	52	13.1	13.3	3.1	2.3	2.0
52	24-31	26.9	10.8	0.0	0	87	29	9.1	7.4	2.4	2.6	6.2

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P.O:Kusumkhera, Village: Harinag
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PIN:2631

EDUCATION DETAILS

Qualification	College/ University	Year of passing	% of marks/CGPA
Ph.D. (Agriculture)	Indira Gandhi Krishi Viswavidyalaya, Raipur	Pursuing	
M.Sc (Agriculture)	Indira Gandhi Krishi Viswavidyalaya, Raipur	2012	8.33/10
B.Sc (Agriculture)	Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand	2010	7.4/10
XII	DAV Centenary Public School, Haldwani	2005	74.8%
X	St Theresa's school, kathgodam	2003	74.8%

- **DBT scholarship Provided for entire post graduation by Ministry of Science and Technology**
- **Secured First Rank in M.Sc at Plant Biotechnology Department at IGKV, Raipur**
- **Availing Inspire Fellowship, DST for Ph.D. programme**

RELEVANT COURSES

1) Agronomy and Crop Production
2) BioStatistics and Computers
3) Bioinformatics
4) Practical crop production and RAWE
5) Plant Tissue Culture & Genetic Transformation
6) Plant Breeding Techniques
7) Seed Technology
8) Techniques in Molecular Biology

INTERNSHIP AND PROJECT

In GBPUAT

Rural Agricultural Work Experience (RAWE) at College of Forestry and Hill Agriculture (GBPUAT), Ranichauri, uttrakhand

Period: 6 Months (July 2009 to December 2009)

- **Assessing the Pattern of Fertilizer and Farmyard Manure consumption by the farmers in Kharif and Rabi season** in Taluka: Khairna, District: Tehri in Uttarakhand
- **Transfer of Technology on Vermi composting and assistance to hilly farmers in making on farm Vermi compost unit.**
- Analysis and Assessment of Small and Medium Farmers in producing horticulture product and its sustainability at **Kanak Food Products, Haldwani, Nainital,Uttarakhand**

Practical crop Production (PCP) Period: 1 Year (May 2008 to April 2009)

- Cultivation, Management, Marketing of Paddy and Wheat Crop in an area of 1 hectare.
- Group Leader in the Practical crop production group.

In IGKV

Fieldwork: **Feasibility of *Andrographis Panniculata* as a potential botanical against major crop pests and pathogens**

Duration: 1 Year (July 2011 to June 2012)

- **Use of Bioinformatics tools to identify *Andrographis Panniculata* as a potent botanical**
- **Molecular Modeling and Molecular Docking of nine proteins (animal and plant proteins) with andrographaloide.**
- Understanding the prospects of its viability in major crop pests.
- Impact of ***Andrographis Panniculata*** as potent botanical in different geographies
- Feasibility and comparative analysis of the botanical as compared to other prevalent botanicals

Job Experiences

- Worked as technical personal on **"Public Private Paternship trial on Maize, Kharif 2012"** under Dr. Nandan Mehta, Principal Scientist, GPB, IGKV, Raipur.
- Worked as trainee in **"Biotech Industrial Training Programme 2012-13", at devleela biotec, Raipur ,** DBT, Government of India for one month.
- Working as Senior Research Fellow in KVK, Bhatapara under "NATIONAL INITIATIVE ON CLIMATE RESILENCE AGRICULTURE", a project funded by CRIDA, Hyderabad.

ACHIEVEMENTS & AWARDS

- Secured Highest Marks in M.Sc (Agriculture) at IGKV, Raipur (2011-12)
- **Paper published in National Confrence on "Demostrated Options for Improved Livelihood in Disadvantaged Areas of India" held at IGKV, Raipur in 2012**
- Secured **60th Rank at Junior Research Fellow Entrance** exam Conducted by ICAR (2010)
- Won 3rd prize **North East Zone** Sports meet held at Army Cantonment, Moradabad in Athletics
- Participated in All India 7th Agri University Sports Meet held at Rahuri, Maharastra organized by ICAR.
- Certificate of Appreciation from Vice chancellor for bringing laurels to University
- Winner of Kabaddi, Kho Kho, Badminton and Athletic competitions at University level
- Participated in **"National Symposium on Sustainable Agriculture "**at College Of Agriculture, Pantnagar
- Organizing Member of Yuva 2007 (National Level Academic Meet) organized at GBPUAT, Pantnagar
- Class Representative at GBPUAT (2007-08)
- Member of Mess committee, Agriculture society at GBPUAT
- Secured N.S.S.'B' certificate in 2008-2009 at GBPUAT
- Organizing member of **"Annual Group meet on Linseed and Safflower" held at** IGKV, Raipur
- Certificate of ICAR- NET, 2013
- Paper presented in "International Conference on Emerging Challenges and Issues in Enviornmental Protection" sponsored by Chhattisgarh Council of Science and Technology at Raipur Institute of Technology.
- Participated in "Symposium on Biosafety,Genetically Modified Crops and Public Awareness" organized by CHiPS, Chhattisgarh.
- Poster presentation in national seminar on "Non-Timber Forest produce, Medicinal, Aromatic Plants & Spices: Innovation For Livelihood Security" organized by I.G.K.V, Raipur,C.G.
- Certificate of participation from "National Seed Congress" organized by Chhattisgarh State Seed Certification Agency, Raipur and National Seed Research and Training Centre held at I.G.K.V., Raipur, C.G.

SOFTWARE PACKAGES: Microsoft Office, Visual Basics, Blast, Argus Lab, Corel Draw, statistical tools.

I, Rahmi Upadhyay, hereby declare that all the information provided by me in this resume is true and authentic to the best of my knowledge.

Rashmi

Rashmi Upadhyay

Place: Raipur

Date: 30-9-2016