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**Identification of meta-QTLs (mQTL) for nitrogen
efficiency and profiling of the mQTL diversity in
Indian rice germplasm**

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Identification of meta-QTLs (mQTL) for nitrogen efficiency and profiling of the mQTL diversity in Indian rice germplasm

By

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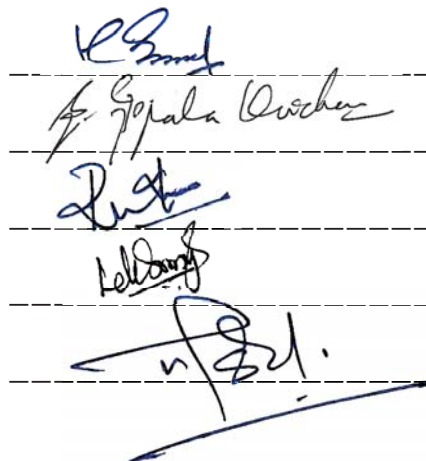
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CERTIFICATE

This is to certify that the thesis entitled **Identification of meta-QTLs (mQTL) for nitrogen efficiency and profiling of the mQTL diversity in Indian rice germplasm** submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of requirement of degree of **Master of Science in Genetics**, embodies the result of a *bona-fide* research work carried out by **Mr. Rahul Kumar** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or source of information, as has been availed of in this work, has been duly acknowledged.

Date: 28 June 2018

Place: New Delhi



(K. K. Vinod)

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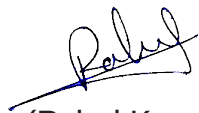
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Introduction

Rice, *Oryza sativa* L., belonging to the family Poaceae is a semi-aquatic annual grass cultivated as an important cereal crop globally. Mostly cultivated in the tropical world, 90% of which falls in the Asian region, rice is the one of staple cereals in human diet. Asians consume 90% of the rice produced in the world. In 2017-18, over 161.1 million hectares of rice were planted all over the world and production of milled rice was about 483.9 million metric ton (www.statista.com). In India, rice contributes to about 40.9% of total food grain production in the country, from a total area of 44.0 million ha and production of 111.0 million tonnes during 2017-18. In the modern era, where science based agriculture is being practiced, rice is also popular as a model monocot plant species for genetic investigations. Rice genome is relatively small spanning about 430 Mb (million base pairs) and its full genome constitution is publicly available (Feng et al. 2002; Sasaki et al. 2002).

Rice cultivation in India is an age-old tradition sustaining human generations over four millennia (Chang 1976). Rice had served as sources of food, income and employment, thereby providing a wholistic support to the human societies. Continuous cultivation of rice and increasing food demand from the growing population has given impetus to improvement of yield and soil fertility. Nutrient depletion was a major consequence of chain cultivation, which has to be supported by addition of manures. Currently, the mineral fertilization is the major method for soil nutrient repletion. Nitrogen (N) is one of the major nutrient elements for rice which is often limited naturally due to its high mobility in soil systems. Among the primary nutrients, N is highly mobile in both plant and soil systems and is highly volatile from the soil environment. Therefore, under-use of N can jeopardize crop production, while excess use can culminate in environmental degradation. Further, over application of N can

directly results in increased cost of production by means of increased fertilizer cost; and indirectly through increased susceptibility to pest and diseases.

Contemporary data indicate that more than 90% of the applied nitrogen fertilizers is consumed by three prime cereals, rice, wheat, and maize. As per the estimated annual growth in global fertilizer consumption, there would be an annual increase of about 1.4% in demand for N fertilizers amounting to 7.3 million tons, of which Asia's share will be about 69% (FAO 2008). A sizable portion of this increased N demand in Asia would be from the rice production systems (Witt et al. 2009). In irrigated rice agro-systems of Asia and Africa, where N fertilizers are applied in three splits at different rice growth stages, a mere enhancement of N efficiency by 10% or more can result in substantial savings in fertilizer use.

Owing to several factors, the applied fraction of N fertilizers in soil are not utilized efficiently by the crops. The factors include poor ability of the plants to uptake N, loss of N through leaching, volatilization and surface run-off and the soil denitrification process. The average level of N use efficiency among the field crops is about 33% (Abrol et al. 1999), with crop utilization ranging between 30 - 40 % of the applied N (Raun and Johnson 1999). A sizeable portion of the unutilized N is lost into the environment and become unavailable to the plants (Abrol et al. 2007). Under such circumstances, supplementation of N through inorganic fertilizers is inevitable for the enhanced crop production and productivity. Complicating the issue further, the petroleum resource, the natural resource for production of N fertilizers are getting depleted constantly (Hirel et al. 2011; Cordell et al. 2009), while there is a growing concern of indiscriminate use of inorganic fertilizer especially N fertilizers in high input production systems. Excess levels of N in the soil can deprive the availability of other nutrients such as calcium, magnesium and phosphorus. This can predispose toxic effects of minor ions on plants such as aluminium and heavy metals, because of the relative reduction in the availability of other important mineral nutrients. Over-usage of N in agricultural systems can cause N pollution in air by volatilisation, and together with other N related atmospheric pollution by nitrates from automobile and industrial emissions can result in soil acidification through acid rains. Therefore, the abatement of ill-effects of excess

N in the environment is a priority in sustaining agricultural systems (Galloway et al. 2008), especially in rice ecosystems. Optimization of the use of inorganic fertilizers such as nitrogenous fertilizer has become a contemporary need in order to reduce the cost of cultivation and to lessen the deteriorating impact on the environment. A sustainable solution for this impasse is to develop N efficient varieties that can give optimum or high yield at reduced N input.

The plant use of applied N in agriculture is measured in terms of the parameter, overall N use efficiency (NUE) which is the ratio of grain yield to the applied N (Pathak et al. 2008). NUE has two components, the uptake efficiency (NUpE) and utilization efficiency (NUtE), the product of which gives NUE. NUpE is the ratio of the increased N content in the whole plant after addition of fertilisers to the amount of N applied, while NUtE is the ratio of increased grain yield after feralization to the N content in the plant (Han et al. 2015). There are other measures of NUE, considering physiological and agronomic parameters, which are again components of either NUpE or NUtE (Mosier et al. 2004; Samborski et al. 2008). In general, NUE improvement in crops can be realized by improving NUpE or NUtE or both (Han et al. 2015).

Notwithstanding the benefits of N fertilisation in pushing the yield levels high in crop plants, the ill-effects of excess N in the environment due to poor crop NUE has urged to reduce the N inputs in agriculture to counterbalance the imminent threat. Improving the NUE in crops can buffer the reduction in N fertilizer use. Improving NUE is possible through breeding because the trait is genetically regulated, but the progress is slow due to low heritability of the trait and its complex regulatory nature (Gallais and Hirel 2004). Use of genomic approaches offers greater promise to improve the NUE, by directly manipulating the genes involved in driving and controlling the N metabolism. This involves identification of candidate genes and/or quantitative trait loci (QTL) that govern the N efficiency and mobilize these into elite genetic backgrounds by breeding techniques such as marker-assisted selection (MAS).

Quantitative genetic variation in responses to low N has already been reported in rice and several QTLs have been identified for various traits related to N nutrition and efficiency. Expression profiling of various genes under nutrient deficiency stress has also been attempted (Lian et al. 2006). Unlike QTLs for

nutrient starvation tolerance with the other major nutrient, phosphorus (P), for which a major QTL, *Pup1* is identified (Wissuwa et al. 1998, 2002), the QTLs for N use remains predominantly minor and numerous (Vinod and Heuer 2012). Therefore, it is essential to identify those QTLs that are consistent for a particular N response across different backgrounds. This will aide us to fill the wide gap by using modern breeding tools such as MAS while developing varieties with improved N efficiency. A meta-analysis is a statistical procedure for identifying consensus QTLs for a particular trait that are located at same locus and found across different genetic backgrounds. The procedure involved comparison and identification of consensus QTLs using model based strategies. Especially for complicated traits like N response, where there are large number of reported QTLs but with low effects, identification of meta-QTLs (mQTLs) would be great boon in utilising them for crop improvement programmes (Goffinet and Gerber 2000). Although several N related QTLs are reported, a meta-analysis of these QTLs have not been done so far. Once identified this QTLs can be utilized for marker assisted improvement of target varieties (Vinod and Heuer 2012). By studying the mQTL diversity in a germplasm can help us to identify right sources for various mQTLs that can be used for improving N efficiency in elite varieties those lacks specific QTLs.

The research work presented in this dissertation aims to identify mQTLs using model based meta-analysis, using inputs of several reported QTLs for NUE in rice beginning from the year 1993. Identification of mQTL diversity for NUE in a subset of Indian rice germplasm would be a sensible approach in identifying potential carriers of N efficient QTLs. Once identified, these lines can either be deployed directly or used as donors of such mQTLs in MAS programs towards breeding of N efficient rice varieties of the future.

The specific objectives are,

- 1) To identify consensus QTLs for NUE using QTL meta- analysis and report them as mQTLs.
- 2) To estimate mQTL diversity and their distribution in a sub-set of Indian rice germplasm.

Review of Literature

Rice (*Oryza sativa* L.) is one of the primary staple foods for the human population, which is grown over 8.75% of the total cultivated area on earth. 57% of the world's rice is grown under irrigated ecology contributes more than 75% of the production, while the remaining 43% of upland areas produce about 20% of the rice harvest. Being a crop of relatively shorter duration cropping intensity of rice goes as high as 300% in intensive rice production regions. Because of the intensive and extensive nature of rice cultivation, rice as a single crop consumes a significant portion of the fertilisers applied to agricultural land. Among these, demand for nitrogenous fertilisers supersedes the demand for any other primary nutrients. Further, being a semi-aquatic crop, loss of nutrients through hydraulic movements soil such as infiltration, percolation and run-off is very high in rice crop.

2.1 Nitrogen and rice

Nitrogen (N) is required in significant quantities in plant survival, because it is a key constituent of the primary building compound, the protein (Scheible et al. 2004). Plant access N from soil, although N is aplenty in earth's atmosphere. Atmospheric N is almost inaccessible to plants, because of its free nature, therefore the only sources for accessing N is through soil or irrigation water, of which soil remains as the predominant source. Because of the significant removal of soil N through crop harvest coupled with losses through various other means, demand for N fertilisers are increasing day by day. Expected requirement of N fertilizers in 2018 is estimated to be 161 million tons, registering an increase of 1.92% from the previous year. Ironically, N fertiliser resources on earth, the petroleum, is diminishing constantly inviting a future impasse (FAO 2015).

Rice requires under optimised growth conditions, approximately 16 kg of N to produce one tonne of grain (van Keulen 1977). Approximately 63% of the N is harvested through grains, while the remaining 37% is contained in the straw. With an estimated grain production of 758.9 million tonnes (FAO 2017), N harvested through grains alone is translated to about 478.1 million tonnes of N. Share of global N fertiliser use for India and China, the world's major rice producers is 31% and 15% respectively (Lu and Tian 2017). The global N demand for these countries is estimated to increase about 18% for China and 17% for India by 2018 (FAO 2015). Majority of the N is used for rice production.

N deficiency occurs when the internal plant demand is not met by the supply from soil. Soil deficiency is a common problem in many part of the world especially in marginal lands of the developing or under developed nations. Majority of these areas fall in the tropics. Since N is a highly mobile element, deficiency can occur even in well fertilised fields if the time of application does not coincide with the crop stage demanding more N. Therefore, time of application is very critical to optimum N utilisation in rice. Most critical stages are tillering and heading phases in rice. Therefore, N application in rice is done in three split doses in all production zones, basal with 40% of the N supply, top dress at 30% each at the aforesaid two critical phases (Dobermann and Fairhurst 2000). Occurrence of N deficiency also depends on soil types, climate, water availability and water quality. It is common in soils with high salinity, alkalinity and with poor drainage. Soils having low organic matter, calcareous nature, alkaline acid-sulphates and P deficiency also experience N shortage. Other than the crop removal, N loss in soil occurs due to N leaching, weed infestation and volatilisation. N availability is low in well drained porous soils than those are rich in clay content.

When the rice plants are N starved, they produce typical symptoms such as yellowing. Yellowing generally occurs in older leaves first, and spread to whole plant. Under severe shortage, plant growth is retarded, tillering is reduced and leaf tips of the older leaves may show chlorosis and necrosis. Leaves become narrow, thin, short and erect. Yield is severely affected by the reduction of grain number.

2.2 Nitrogen uptake and assimilation

Plants access N in two forms, the oxidised form as nitrate (NO_3^-) and the reduced form as ammonium (NH_4^+). Both the forms are highly water soluble, and NH_4^+ gets easily converted to NO_3^- under aerated conditions, by nitrification process either by soil reactions or by means of microbial activity. Therefore, under aerobic conditions, NO_3^- predominates as the most abundant form. NO_3^- concentrations also increase by fertilization. The most preferred form for plant uptake is the NO_3^- (Crawford 1995). In contrary, NH_4^+ is the form available under reduced soil conditions occurring in soil that are constantly flooded and maintain anaerobic situations. Rice being a crop adapted to flooded soil, NH_4^+ is the principle type of accessible N (Sasakawa and Yamamoto 1978).

2.2.1 Nitrogen assimilation

Primary N assimilation in plants is done in two major stages, reduction of NO_3^- and assimilation of NH_4^+ (Figure 1). In the first cycle, NO_3^- is reduced to nitrite (NO_2^-) followed by the reduction of NO_2^- to NH_4^+ , and in the second process NH_4^+ is converted to glutamine and subsequently into amino acids (Mifflin and Lea 1976). Reduction of NO_3^- in cell cytoplasm of roots and shoots and is catalysed by the enzyme nitrate reductase (NR). NO_2^- formed are transported to cell organelles such as chloroplasts/ plastids where it is reduced to NH_4^+ by the enzyme nitrite reductase (NiR) (Meyer and Stitt 2001). Then the NH_4^+ ions are rapidly converted into glutamine by the secondary metabolism pathway which is governed by two enzymes, glutamine synthetase (GS) and glutamate synthase or glutamate 2-oxoglutarate aminotransferase (GOGAT). Known as the GS/GOGAT cycle this pathway quickly removes the NH_4^+ ions which are other way harmful to cell system if they are maintained for longer duration. Both GS and GOGAT are present in chloroplasts and plastids. GS catalyses conversion of a glutamate molecule into glutamine by fixing a NH_4^+ ion to it (Unno et al. 2006). During this process an ATP molecule is consumed to produce an ADP molecule and an inorganic phosphate. In the next step, the glutamine molecule is combined to one α -ketoglutarate to produce two glutamate molecules by GOGAT. In this process, one NADPH molecule is

oxidised. The GS/GOGAT cycle chooses to show a high affinity towards NH_4^+ ions (Andrews et al. 2004).

NH_4^+ ions can also be available to GS/GOGAT cycle through other processes such as direct uptake from soil, amino acid recycling and also from photorespiration (Lea and Forde 1994). NH_4^+ assimilation also takes place through other pathways such as a low affinity pathway driven by the enzyme glutamate dehydrogenase (GDH). In GDH pathway, one α -ketoglutarate is converted to glutamate by consuming one NADPH and oxidising it into NADP. No ATP is consumed in the GDH pathway (Huang et al. 2018). Other minor pathways through which N assimilation occurs in plants include asparagine biosynthesis pathway mediated by the enzyme asparagine synthetase (Coruzzi and Last 2000) and urease activity that helps to release NH_4^+ from applied urea (Gerendás et al. 1998). Asparagine synthetase drives the conversion of glutamine to asparagine and glutamate by exchanging the amido group of glutamine to an aspartate molecule in an ATP dependent pathway (Lam et al. 2003). Asparagine plays a significant role in N remobilisation process in plants, which include movement of N away from older leaves and assimilation into grains during reproductive stage (Gaufichon et al. 2016).

2.3 Genetics of nitrogen use in rice

N use in rice includes both uptake, assimilation and utilization. There are differences in the ability of the genotypes in these processes. N use process in plants are governed by complex network of pathways, and the genotypic differences are often subtle for apparent genetic manipulations (Vinod and Heuer 2012). N use is a constitutive mechanism in plants which is basic to its survival. However, the genetics of uptake and assimilation in plants seems to differently regulated but a clear-cut phenotypic distinction is not available to bifurcate both these processes for crop improvement programmes (Gallais and Hirel 2004).

2.3.1 Genes related to nitrogen uptake

To begin the N use in plants, the forms of N needs to be taken up and assimilated into the plants from soil solution. This is achieved in the plants by

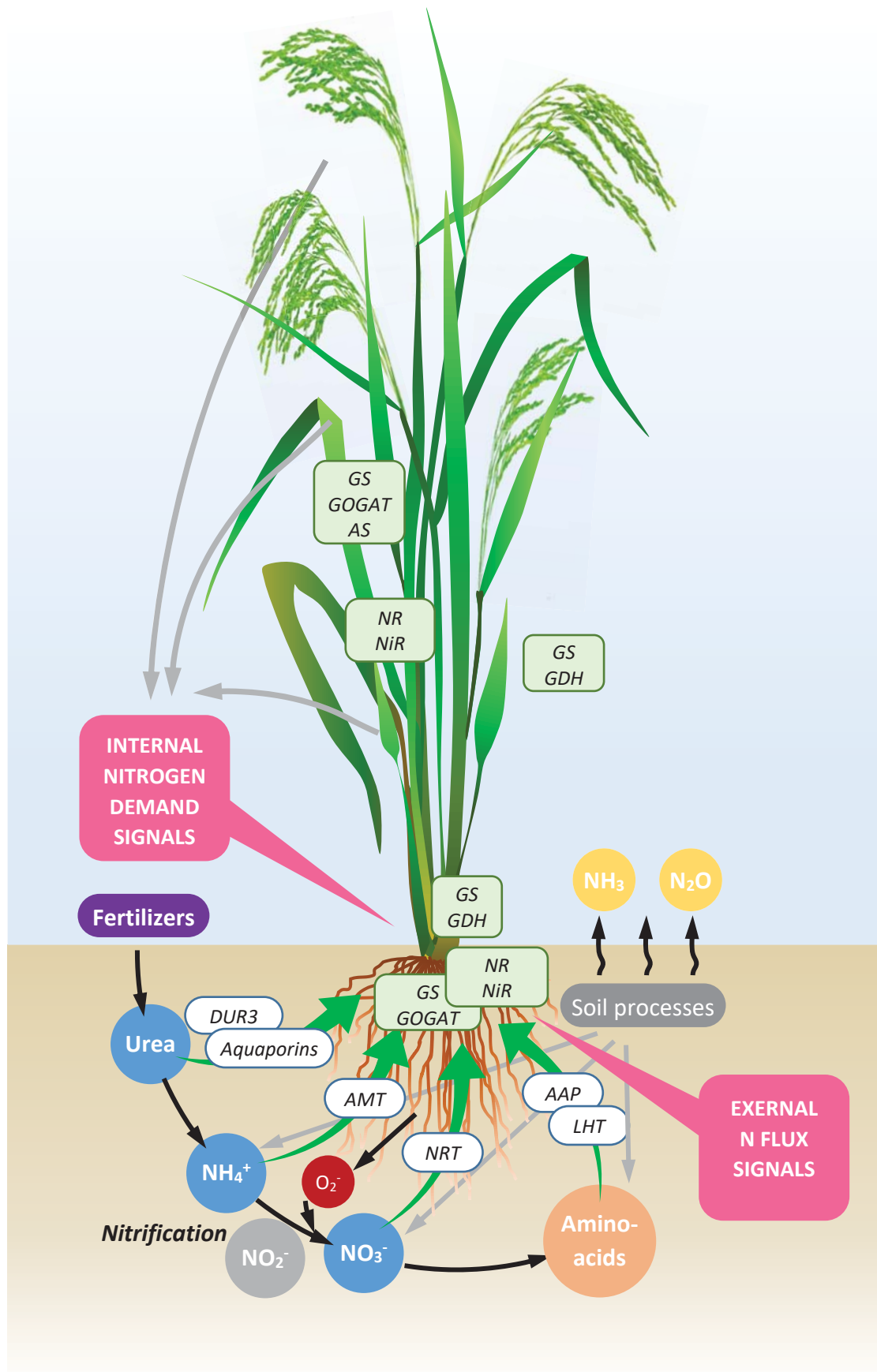


Figure 1. Nitrogen use processes in rice plant. The uptake and assimilation processes are under different genetic control and are complexly regulated.

active and passive transports. There are four active transport systems to transport NO_3^- (Huang et al. 2018), while at least one passive and one active systems transport NH_4^+ . Other than these, other minor forms of organic N such as amino acids are also transported, but their role in defining N use of plants may be negligible (Glass et al. 1999). NO_3^- ions play dual role, the major role as the nutrient and also as signals for activating the N signalling cascade (Krouk et al. 2010; Undurraga et al. 2017). NO_3^- uptake primarily occurs within roots and is carried out by two nitrate transporter systems viz., high affinity transporter (HAT) and low affinity transporter (LAT). HATs mediate N transport under low N conditions (<1.0 mM) while LATs are active when N pool concentration is >1.0 mM. Both these systems are encoded by multigene families (Glass et al. 2002; Orsel et al. 2002), broadly falling into two gene clusters, *NRT1* and *NRT2*. *NRT1* genes are constitutively expressed and are involved in general N uptake irrespective of the N pool in soil solution. They are prominently LATs, but are also induced under low NO_3^- signals. *NRT2* genes contributes specifically to the nitrate inducible, and they belong to HATs (Crawford and Glass 1998; Krouk et al. 2010).

Among the four different NO_3^- transporters/channels identified in rice, *NRT1*/peptide transporter (NPF) and *NRT2* plays major role of uptake and translocation. NPF genes are predominantly LATs, and almost 80 genes of these are identified on rice genome (Tsai et al. 2007). Among the two *NRT1* genes characterised, *OsNRT1.1a* (*OsNPF8.9*) is primarily expressed in roots and is shown to have significant effect on yield and N use (Lin et al. 2000). *OsNRT1.1b* (*OsNPF8.9*) is believed to be a splicing product of *OsNRT1.1a*, also plays an active role in root to shoot transport of NO_3^- . This gene shows dual affinity (Hu et al. 2015). NPF genes showing peptide transport functions are also characterised. *OsNPF8.20* which promotes lateral root growth and yield is involved in NH_4^+ transport and *OsNPF4.1* (*short panicle 1*) influences panicle elongation (Li et al. 2009). Several other PTR genes also have been characterised (Huang et al. 2018). These includes *OsNPF7.3* (*OsPTR6*) which influence NH_4^+ transport, *OsNPF2.2* (*OsPTR2*) and *OsNPF2.4* involved in long distance root-shoot NO_3^- transport. Another LAT, tonoplast *OsNPF7.2*, that regulates intracellular NO_3^- exchange is also believed to be a PTR gene.

At least five NRT2 genes are characterised in rice. *NRT2.1* and *NRT2.2* show considerable homology with different UTR, and are found localised in roots. *NRT2.3* and its alternate spliced products *NRT2.3a* and *NRT2.3b*, are found to have xylem and phloem loading functions respectively and involved long distance NO_3^- transport (Cai et al. 2008; Tang et al. 2012). NRT2 genes function together with nitrate reductase (NAR) gene, *OsNAR2.1* (Feng et al. 2011a; Liu et al. 2014; Yan et al. 2011).

Uptake of NH_4^+ in rice is carried out by NH_4^+ transporter (AMT) gene family. Divided into four sub-families, *OsAMT1*-*OsAMT4*, ten putative genes have been reported (Suenaga et al. 2003), among which AMT1 family has been investigated extensively. *OsAMT1;1* is both constitutively root expressed and induced with NH_4^+ supply and involves in its transport (Ranatunge et al. 2014; Li et al. 2016). *OsAMT1;2* and *OsAMT1;3* also show root specific expression, and is fully induced by the presence of NH_4^+ ions. Expression studies reveal that the former involves in N assimilation, while the latter acts as a sensor of N (Yao et al. 2008; Bao et al. 2015). The other genes, *OsAMT2;1* shows constitutive expression while the expression of *OsAMT2;2* is induced (Li and Shi 2006). The expression of *OsAMT3;1* was reported to be weak in both roots and shoots (Suenaga et al. 2003).

2.3.2 Genes related to nitrogen assimilation

In angiosperms, GS1 is encoded by a complex multigene family comprising of three to six genes (Hirel and Lea 2002); however, GS2 is encoded by a single nuclear gene (Tingey and Coruzzi 1987; Cock et al. 1991). In maize, at least five GS1 genes are reported (Li et al. 1993). At least four different genes encode subunits of the active protein and different combinations of these produce different isozymes of GS (Howitt and Udvardi 2000). In rice, content of GS1 protein in *indica* leaves is higher than that in *japonica* leaves. However, higher content does not accompany higher activity (Obara et al. 2000).

NADH-GOGAT in rice is reported to undergo post-translational processing. It contains pre-sequences that are similar to plastidic transit peptides and believed to play an important role in plastid targeting. The diversity of the primary set of N assimilation genes Two classes of nuclear genes code for GS:

the GLN2 and GLN1 genes. GLN2 gene is present in every species considered till now and codes for the chloroplastic GS2, which is associated with the essential assimilation of ammonium originating from nitrate reduction and in the assimilation of ammonium delivered from photorespiration in C plants. The magnitude of the ammonium flux through the photorespiration pathway in the leaves of C plants was for sure evaluated to surpass that produced from nitrate reduction by five to ten times (Keys et al. 1978).

On the other hand, the GLN1 gene family codes for cytosolic GS1 isoforms, which are present in various organs, for example, roots or stems and are thought to be associated with ammonium recycling during particular developmental process such as leaf senescence, in glutamine synthesis and during transport into the phloem sap (Bernard and Habash 2009). Two unique types of glutamate synthase are available in plants: FdGOGAT and NADH-GOGAT, that utilizes ferredoxin and NADH as the electron donors, respectively (Vanoni et al. 2005). FdGOGAT is mainly located in leaf chloroplasts, while NADH-GOGAT is essentially situated in plastids of non-photosynthetic tissues, such as roots, etiolated leaf tissues and companion cells. The structural, mechanistic and regulatory properties of GOGAT enzymes and their role in amino acid metabolism have been reviewed by Suzuki and Knaff (2005). In addition to the GS/GOGAT cycle, three proteins most likely take a participation in ammonium absorption.

2.4 The need of enhancing nitrogen use efficiency

Among all the plant nutrients basic for crop development, N is the nutrient which frequently limit crop production (Mosier et al. 2001). N is very important in crop development framework due to its extensive prerequisite as the basic element in every single metabolic action of plants. Considering the heavy loss within soil-plant frameworks (Ladha et al. 2003), internationally farmers are utilizing around 120 million metric tonnes of nitrogenous fertilizer every year. Farmer needs to apply huge amount of N fertilizer to realise better harvests on account of its lower recovery (30-50%) and due to different losses from soil-plant system (Fageria 2002). Soil applied N is taken up and utilized by the crops for their development and advancement. However, in the

meantime a huge quantity of this applied N can be lost from the soil plant-system through different processes i.e. soil erosion, surface runoff, leaching, de-nitrification, ammonia volatilization etc.

N is all around lacking in all the agrarian soils and cropping system of the world so it is basic to utilize outside N inputs (N fertilizer) to produce the crops for fulfilling the ever-increasing demand of human populaces (Mohan et al. 2015). Ordinarily, to satisfy this substantial prerequisite of N, Farmers totally rely upon synthetic N. Overabundance utilization of N as crop input can disrupt its transformation and natural movement among its different pools brought about imbalance in ecosystem function and services (Hutchinson et al. 2003; Meena et al. 2014). Sustainable agriculture production requires adjusted and sensible, productive, eco-accommodating, and earth sound administration practices. To accomplish the national goal of agriculture sustainability and food security, vertical diversification of agriculture in terms of more products yield from unit amount of land through wise utilization of fertilizer inputs particularly N has great significance in present day agriculture (Fageria and Barbosa 2001; Kumar et al. 2015, 2016). Effective N fertilizer management can just be characterized as lessened different losses and expand the amount of nutrient made accessible for crop (Ladha and Reddy 2003). Under current situation, there is an important requirement for enhancing N use efficiency (NUE) and adjust utilization of natural resources which is basic for sustainable agriculture production (Shoji et al. 2001; Paul et al. 2014).

2.4.1 Genetics of nitrogen use efficiency

NUE is consist of N take-up, utilization or acquisition efficiency and expressed as a ratio of output (add up to plant N, grain N, biomass yield, grain yield) and input (add up to N, soil N or N-fertilizer applied). N use efficiency (NUE) is the integration of both N uptake efficiency (NUpE) which is a root-related attribute, and N utilization efficiency (NUtE), which is a component of canopy activity (Brauer and Shelp 2010). In simple term NUE is yield per unit of accessible N. Yield is primarily controlled by C fixation in the canopy (which is dependent on N for growth and development); grain additionally requires N

for protein, which is transported specifically from the soil or from remobilization of leaf senescence (significant contribution).

Productive agriculture needs a lot of costly nitrogenous fertilizers. Therefore, enhancing N use efficiency (NUE) of harvest plants is of key significance. For most plant species, NUE essentially relies upon how plants extract inorganic N from the soil, absorb nitrate and ammonium, and reuse natural N. Efforts have been made to examine the hereditary basis and in addition the biochemical and enzymatic components engaged with N take-up, absorption, and remobilization in harvests and model plants.

2.4.2 Nitrogen remobilization

In N remobilization, leaf proteins and specifically photosynthetic proteins of plastids are widely degraded during senescence and provide enormous source of N that plants can tap to supplement the nourishment of developing organs, for example, new leaves and seeds. In *Arabidopsis* and oilseed rape, it has been demonstrated that N can be remobilized from senescing leaves to extending leaves at the vegetative stage and also from senescing leaves to seeds at the reproductive stage (Lemaitre et al. 2008). In *Arabidopsis*, sequential (at vegetative stage) and monocarpic (after flowering) senescence stages can be recognized effectively, it was demonstrated that the N remobilization rate was associated with leaf senescence seriousness at the vegetative stage only (Diaz et al. 2008). Examinations of N following at the regenerative stage demonstrated that the rate of N remobilization from the rosettes to the blossoming organs and to the seeds was similar in early and late senescing lines (Diaz et al. 2008). At the regenerative stage, NRE is principally identified with Harvest Index.

Studies utilizing N tracing in oats, oilseed rape and vegetables demonstrated that the beginning of grain filling was a basic stage since N take-up and N fixation declined during plant development and seed filling (Salon et al. 2001). N transitions and N remobilization tests performed by Cliquet et al. (1990) demonstrated that leaf sharp edges, stalks, cob, husk and shank serve progressively as N sinks and N sources. N take-up and assimilation during the grain filling period is by and large deficient for the high demand of the seeds,

and the dynamic and various remobilization steps, happening progressively in the distinctive plant organs, are expected to route N to the seeds. The contribution of leaf N remobilization to rice, wheat or maize grain N content is cultivar reliant, varying from 50 to 90 % (Masclaux et al. 2001). N remobilization is additionally condition dependent and supported under restricting nitrate supplies (Lemaitre et al. 2008).

In spite of the fact that N remobilization is a well-ordered system that includes the distinctive plant organs, evidences demonstrates that grain N content is connected with flag leaf senescence in maize, wheat and barley. Flag leaf senescence appears to be the important part in N accessibility for grain filling. The beginning and the speed of Flag leaf senescence determine NRE. Leaf senescence is not only important for N assembly but also critical for yield as demonstrated by several evidences. Delaying the leaf senescence brings in prolongation of photosynthesis that builds grain yield and carbon filling into seeds. Reproducing plants have then to adapt to the dilemma that reduced senescence could prompt higher yields yet in addition to a reduction in NRE and to a reduction in grain protein content. Then again, expanding N remobilization has the upside of reusing N from the vegetative parts and of bringing down N losses in the dry remains.

2.5 Quantitative variation in nitrogen use in rice

There is ample natural variation available in N uptake and utilization in rice. However, mineral nutrition being a life sustaining trait, there has been natural selection for optimal nutrient use efficiency. However, evolvement of nutrient responsive modern high yielding varieties has exerted a shift balance towards input responsiveness from the optimal N use. Therefore, the genes for optimal N use or low N tolerance could be available in landraces or primitive germplasm. This gives scope for identifying and transferring these N efficient genes while breeding for low N friendly varieties.

Although, nutrient deficiency tolerance in rice has low heritability and is complex (Blum 1988), there are several molecular technologies available to address this problem. Quantitative variation in responses to low N in rice has already been reported and several QTLs have been identified from several

studies for various traits related to N nutrition and efficiency. Expression profiling of various genes under nutrient deficiency stress has also been attempted (Lian et al. 2006).

2.5.1 QTLs reported for nitrogen use in rice

The first attempt to map the QTL for N uptake, NUE, N fixation, and related parameters in Rice was started at the IRRI. For this experiment they used population from Palawan/ IR 42 (Wu et al. 1994). A total of seven loci which were associated with the ability to stimulate N fixation in rice detected and they were distributed on chromosomes 1, 3, 6, and 11.

Several genomic regions associated with NUE and response are reported in rice which are related to several traits (Fang and Wu 2001; Ishimaru et al. 2001; Obara et al. 2001a,b; Srividya et al. 2010). Indications suggest the possible links between very few of these QTLs with primary N assimilation genes and transporters, especially of GS structural genes and yield components (Obara et al. 2001b; Obara et al. 2004; Senthilvel et al. 2008; Feng et al. 2010; Vinod et al. 2011). Obara et al. (2001b) reported that few of the QTLs which were associated with the enzyme content were found to be colocalized with those associated with N recycling process from senescing leaves to developing organs in a backcross inbred line population. Further coincidences of QTLs for enzyme GS1 activity and for panicle weight were confirmed by using chromosome-substituted lines in rice (Obara et al. 2004).

Among the several QTLs which are reported for N response in rice, one of the earliest reports by Fang and Wu (2001) reported QTLs for plant height in rice which were mapped using double-haploid (DH) population derived from a cross between IR64 and Azucena under low and high N levels. The QTLs on chromosomes 2, 3, 4, 5 and 6 were only detected at low N level and at high N level in the nutrient solution culture experiment the QTL on chromosome 8 was only detected. Photosynthetic N use efficiency depends on the ratio of Rubisco to total N content and the QTLs associated with this ratio were detected in rice by Ishimaru et al. (2001). Yamaya et al. (2002) further discussed that QTLs affecting the two enzymes are likely to be involved in N recycling and grain filling process, both of which are complexly regulated. Lian

et al. (2005) reported QTLs for low N tolerance at seedling stage. They identified QTLs in a 239-recombinant inbred line population from a cross between Zhenshan 97 and Minghui 63.

The three main-effect (QTLs) on chromosomes 5, 6 and 8, along with epistatic QTLs in two N conditions were identified using a double haploid population derived from a cross between a japonica Chun Jiang 06 (CJo6) and an indica Taichung Native 1 (TN1) (Hu et al. 2012). Attempts also made to map loci for associative rhizosphere N fixation in rice and independent QTLs linked to the activity of different N fixing bacterial strains were identified on chromosome 2 (Ji et al. 2005). In independent studies (Lian et al. 2005; Cho et al. 2007), many small-effect epistatic QTLs have been mapped which accounts for a large cumulative proportion of variation for traits under low and normal N conditions, many of which also show significant QTL x environment interaction that emphasize the importance of validating QTLs in multiple environments and genetic backgrounds before using them in selection.

Senthilvel et al. (2008) reported QTLs for NUE using field and pot culture studies, and reported sixteen QTLs out of which eleven QTLs showed significant QTL x environment interactions. On chromosome 3, seven QTLs were detected associated with N use, plant yield, and associated traits. They used a total of 169 RILs under different N conditions, to map a total of 44 QTLs for all 15 observed parameters including N concentration, absorption NUE (aNUE), physiological NUE (pNUE) and agronomical NUE (agNUE) (Nguyễn et al. 2013).

Feng et al. (2010) identified 7 QTLs for N deficiency tolerance traits (relative shoot dry weight, relative plant dry weight, relative maximum root length, and relative plant height) at the seedling stage in hydroponics with two N applications. They used 238 RILs (F12) derived from a cross between the indica rice and the super hybrid rice. In a field experiment with 127 RILs from the cross between two indicas with two N levels, Wei et al. (2012) reported a total of 4 and 6 QTLs for NUE in 2 separated experiments. However, the QTLs which were reported for NUE in these studies mostly were for physiological NUE.

In a recent study, stably expressed QTLs for yield and associated traits at different N levels, especially those expressed at low N, were reported in recombinant inbred line populations (Tong et al. 2011). One of these QTLs, for number of grains per panicle under low N level, is located in the same region as the Pup1 locus on chromosome 12, providing an opportunity for the use of Pup1 materials for testing low-N tolerance. Recently, Feng et al. (2015) used a recombinant inbred line population to detect QTLs for rice yield traits under the low and normal N (N) levels. They identified a total of 52 QTLs for yield traits distributed in 27 regions on 9 chromosomes. Nguyen et al. (2016) mapped QTLs for NUE parameters in vegetative phase using hydroponics.

Having identified a plethora of putative QTLs that contribute to N efficiency, the logical next step is to design appropriate selection strategies for improving genotype efficiency against low nutrient stress. However, not-so-significant effects of the identified QTLs are a serious concern in designing robust MAS strategies, since QTL-by-environment interaction may jeopardize the selection process. Further, the arcane effects of the identified QTLs confound the scientific soundness of the selection programs (Vinod and Heuer 2012). It is therefore essential that consensus QTLs are to be identified for further use of these enormous resources.

2.6 QTL meta-analysis

It reveals most significant genomic regions involved in the regulation of NUE of crop. A meta-analysis is a statistical procedure for identifying consensus QTLs for a particular trait, especially complicated traits like N response, from large number of reported QTLs. The procedure involved comparison and identification of consensus QTLs using model based strategies. Since the last decade, the significant use of molecular markers has increased the pace of discovering the loci which are involved in quantitative trait variation. Quantitative Trait Loci (QTL) mapping usually started with the collection of genotypic and phenotypic data from a segregating mapping population. First, from the genotypic data order and positioning of marker is done on a genetic map using standard linkage mapping approaches. Further refinement of analytical methods has made it possible to detect one or several QTL on each

chromosome. Nevertheless, due to limiting number of individuals and generations in usual experiment this approach generally leads to QTL locations with a large confidence interval (CI) around 10 cM (Kearsey and Farquhar 1998) which in plant generally corresponds to a thousand of genes or more.

Because of its simplicity and precision for detecting QTL it has been widely used and more and more QTL detection results are now available in public databases. One of the main objective of these databases is to assist the comparison of different QTL detection results by providing both standard description of these results and ontologies. Significance of comparative analysis of QTL studies have been demonstrated by several authors (Khavkin and Coe 1997; Lin et al. 1995). However, these studies often depend on simple descriptive statistics. QTL congruency study was improved by Goffinet and Gerber (2000). They proposed a meta-analysis based approach in order to integrate QTL results from various experiments. Their method makes it possible to determine "actual" QTL locations underlie the distribution of the observed QTL on the genome.

This approach has been implemented in BioMercator software developed by Arcade et al. (2004). This software works under Java environment and integrates several routines through a graphical use interface. Using this software, it is possible to merge both markers and QTL onto a consensus map by means of an iterative projection procedure. Then the algorithm devised by Goffinet and Gerber (2000) can be used to evaluate the likelihood of clustering the observed QTL in 1, 2, 3 or 4 groups. After that, the optimal number of clusters is selected by using a Akaike like criterion. Based on recent methodological developments, MetaQTL implements a series of Java programs to carry out whole-genome QTL meta-analysis. All the programs in MetaQTL are command line programs. Each program does a small job and the user can easily combine the programs as a group for doing a complete analysis. Once identified this QTLs can be utilized for marker assisted improvement of target varieties for traits like N efficiency. By studying the mQTL diversity in a germplasm can help us to identify right sources for various QTLs that can be used for improving N efficiency in elite varieties those lacks specific QTLs.

2.6.1 Meta QTLs reported in rice

Several mQTLs have been identified in rice (Table 2). MQTLs for grain yield identified during agricultural drought in rice. Fifty-three yield QTL reported in 15 studies were projected on a consensus map and meta-analysis was performed. Fourteen meta-QTL were obtained on seven chromosomes. MQTL1.2, MQTL1.3, MQTL1.4, and MQTL12.1 were around 700 kb and corresponded to a reasonably small genetic distance of 1.8 to 5 cM and they are suitable for use in marker-assisted selection (Swamy et al. 2011). Quantitative trait loci (QTLs) controlling rice root morphological parameters have been detected in several mapping populations in order to improve drought resistance in rice. They extracted information from 24 published papers on QTLs controlling 29 root parameters including root number, maximum root length, root thickness, root/ shoot ratio, and root penetration index. A web-accessible database of 675 root QTLs detected in 12 populations was constructed. A total of 119 mQTLs were detected (Courtois et al. 2009)

Table 1. List of meta QTLs reported in rice

Trait	No. of reports	Total QTLs used	mQTLs identified	Reference
Blast resistance	-	435	165	Ballini et al. 2008
Root traits	24	306	119	Courtois et al. 2009
Grain yield under drought	15	53	14	Swamy et al. 2011
Grain yield	11	68	23	Swamy et al. 2011
Panicle-related traits	82	837	87	Wu et al. 2016
Drought-related QTLs	13		-	Khowaja et al. 2009
Grain size/ weight	7	64	6	Daware et al. 2017
Root development	-	14	1	Coudert et al. 2010

Meta QTL analysis was reported in several crops with respect to several traits (Table 2), that was hailed as highly useful in identifying robust and stable QTLs. There are reports of mQTLs identified for abiotic stress responses such as drought tolerance, low P tolerance; biotic stresses like fungal and viral disease resistance; phenological traits such as yield and yield components, biomass, fibre content etc. in crops like maize, rice, cotton, barley, bean, wheat apple, soybean etc. (Zhang et al. 2017; Soriano et al. 2017; Vasconcellos et al. 2017; Wang et al. 2016; Van and McHale 2017; Chen et al. 2017; Hwang et al. 2016; Said et al. 2013).

Table 2.1 List of meta QTLs reported in different crops

Crop	Trait	Total QTLs	No. of mQTLs	Reference
Wheat	Phenology, biomass and yield	477	71	Soriano et al. 2017
Wheat	Grain traits	332	23	Tyagi et al. 2015
Wheat	Drought and heat stress	854	43	Acuña-Galindo et al. 2015
Wheat	Rust resistance	144	35	Soriano and Royo 2015
Wheat	Cold stress response	-	20	Rodríguez et al. 2014
Winter wheat	Plant height	-	16	Griffiths et al. 2012
Bread wheat	Nitrogen use efficiency	85	11	Quraishi et al. 2011a
Bread wheat	Grain dietary fiber content	12	3	Quraishi et al. 2011b
Bread wheat	Earliness	79	18	Hanocq et al. 2007
Wheat	Fusarium head blight resistance	176	19	Löffler et al. 2009
Maize	Plant morphology, yield, stay-green, and virus disease resistance	637	113	Wang et al. 2016
Maize	Nitrogen use efficiency and seedling root traits	331	64	Li et al. 2015
Maize	Secondary traits associated with drought tolerance	203	14	Almeida et al. 2014
Maize	Low-P tolerance	191	22	Zhang et al. 2014
Maize	Grain yield, anthesis silking interval under drought	183	68	Semagn et al. 2013
Maize	Grain yield	351	54	Wang et al. 2013
Maize	Zinc and iron content	54	10	Jin et al. 2013
Maize	grain yield	103	6	Li et al. 2011
Maize	Flowering time	313	62	Chardon et al. 2004
Maize	Drought tolerance	239	39	Hao et al. 2010
Maize	Silage quality	209	68	Truntzler et al. 2010
Maize	Ear rot resistance	87	29	Xiang et al. 2010
Barley	Tolerance to abiotic stresses	337	79	Li et al. 2013
Barley	Abiotic stress	195	37	Zhang et al. 2017
Barley	Multiple disease resistance	-	20	Schweizer and Stein 2011
Common bean	White mould resistance	37	9	Vasconcellos et al. 2017
Soybean	Protein and oil content	649	55	Van and McHale 2017
Soybean	Lodging and related traits	104	53	Chen et al. 2017

Table 2.1. Contd.

Crop	Trait	Total QTLs	No. of mQTLs	Reference
Soybean	Delayed canopy wilting	44	9	Hwang et al. 2016
Soybean	100-seed weight	117	15	Sun et al. 2012
Soybean	Oil content	98	20	Qi et al. 2011
Soybean	Cyst nematode resistance	62	6	Guo et al. 2006
Tetraploid cotton	Fiber quality, yield, yield related and morphological traits, drought tolerance, and disease resistance	-	1223	Said et al. 2013
Cotton	Verticillium wilt resistance	306	28	Zhang et al. 2015
<i>Brassica napus</i>	Seed yield and yield related traits	144	72	Zhao et al. 2016
Apple	Fruit quality traits	56	27	Costa 2015
<i>Arabidopsis</i>	Leaf senescence	101	13	Chardon et al. 2014
<i>Brassica juncea</i>	Yield-associated traits	187	20	Yadava et al. 2012
Potato	Late blight resistance and plant maturity traits	144	24	Danan et al. 2011
Cocoa	Disease resistance	65	13	Lanaud et al. 2009

Several mQTLs associated with traits such as blast resistance, root traits, grain yield, panicle-related traits, drought-tolerance, grain size etc. have been reported in rice (Ballini et al. 2008; Courtois et al. 2009; Swamy et al. 2011; Swamy et al. 2011; Wu et al. 2016; Khowaja et al. 2009; Daware et al. 2017; Coudert et al. 2010). Although several N related QTLs are reported a meta-analysis of these QTLs have not been done so far. Therefore, in this research problem we have planned to identify mQTLs for N use efficiency and their profiling diversity in Indian rice germplasm.

Materials and methods

The research work consisted of a collection of information on all previously reported QTLs for N use to carry out the meta-analysis and the field evaluation of a set of 65 genotypes.

3.1 Plant Materials

The germplasm assembly contained 65 genotypes, out of which 10 were identified as N efficient varieties. The remaining genotypes were selected randomly and consisted of cultivars, landraces, Basmati lines whose N response is not well documented. A list of the genotypes used along with their basic information is given in Table 3.

3.2 Methods

3.2.1 Meta-analysis of QTLs

QTL Meta-analysis was performed using Bio Mercator v4.2 software. Initially, the published information on QTLs related to NUE in rice was surveyed. The data were shortlisted and formatted in BioMercator format. The analysis was performed as per the algorithms built in the software. BioMercator is a Java-based suite of programs that do different analyses integrated into a single package. The analyses are performed in three steps, (a) construction of a consensus map, (b) QTL projection and (c) meta-analysis.

3.2.2 Assembling the panel of QTL studies

QTL information was collected from 15 studies on Nitrogen use efficiency in rice published during the period starting from 2001 to 2016 (Table 4). Data included N use parameters and agronomic traits related to N uptake and assimilation.

Table 3. Particulars of germplasm included in the study

S.No	Genotype	Parentage	Year of Notification	Duration (days)	Recommended area
1	Acharamati	Pureline	NA	-	OR
2	ADT38	IR-1529-680-3-2 / IR-443252	1989	130-135	TN, PY, AP
3	ADT39	IR 8 / IR 20	1989	119-125	TN, PY
4	ADT42	AD 9246/ADT 29 2	1997	110-115	TN
5	ASD16	ADT31 / CO 39	1986	115	AI
6	ASD18	ADT 31/ IR 50	1993	Early	TN, PY
7	ASD19	Lalnakanda / IR 30	1997	120-132	TN
8	Basmati 370	Pureline	1973	150	HR
9	BPT 5204	GEB24 / T(N)1 / Mahsuri	1989	140-145	AP, KA
10	Chandrasahini	Abhaya / Phalgun	2007	120-125	CH
11	CO 37	TN1/ Co29	1978	115	PY
12	CO 51	ADT 43 / RR 272 – 1745	2005	105-110	TN
13	CRdhan 310	Pureline	NA	120-125	OR
14	CRdhan 311	HP-2/ Naveen	NA	120-125	OR
15	CSR 27	Nona Bokra/ IR565-33- 2	1999	-	HR
16	Himdhan	R575/ TN1	-	-	-
17	Improved Sabarmati	T(N)1 / Basmati 370//Basmati 370	1972	-	PB, HR, West UP
18	IR 24	IR8/ IR127-2-2	1974	-	East UP
19	IR 50	IR-2153-14-1-6-2 / IR-28 / IR-36	1989	115	AS
20	IR 64	IR-5857-33-2-1 / IR-2061-465	1991	115-120	AI
21	IR 66	IR 13240/ IR9129	1987	-	Philippines
22	IR 70	IR25604/IR9828	1988	-	Philippines

AI, all India; AP, Andhra Pradesh; AS, Assam; DL, Delhi; KA, Karnataka; KL, Kerala; OR, Odisha; MP, Madhya Pradesh; PB, Punjab; JK, Jammu & Kashmir; HR, Haryana; WB, West Bengal; CH, Chhattisgarh; PY, Pondicherry; UK, Uttarakhand; UP, Uttar Pradesh

Table 3. Contd.

S.No	Genotype	Parentage	Year of Notification	Duration (days)	Recommended area
23	IR 72	IR32445/IR9129	1988	-	Philippines
24	Jaiphoola	Landrace	-	-	-
25	Jaldi Dhan 13	Mutant	2003	Extra early	WB
26	Kamlesh	NA	-	-	-
27	Kasturi	Basmati 370/ CR88-17-1-5	-	-	-
28	Kavya	WGA-27120/ WGL-17672/ Mahsuri/ Surekha	1993	135	AP
29	Kranthi	Cross16/ IR8	1982	120	-
30	Mahalaxmi	Pankaj/ Mahsuri	1992	-	OR
31	Mahamaya	Asha/ Kranthi	1994	125	MP, TR
32	MTU 1010	Krishnaveni/ IR-64	2000	120	AP
33	MTU 5249	Sowbhagya x ARC 6650	1987	150	AP
34	Nagina 22	A selection from Rajbhog	1978	85-102	UP
35	Nidhi	Sona/ ARC-14529	1997	120-125	TN, PY, WB
36	Pant Dhan 19	BG132/ UPR195-141	2007	-	AI
37	Pant Sugandh Dhan 15	Basmati370/Sadri/Baharai/Muskan 41	2006	140-145	AI
38	Pant Sugandh Dhan 17	Pusa Basmati1/UPRM 500	2006	135-140	AI
39	Panvel	IR8/Bhura rata 4-10	1985	125-130	MH
40	PB 1121	Pusa 614-1-2/ Pusa 614-2-4-3	2003	140-145	PB, HR, West UP, UK
41	PB 1509	Pusa 1301/ Pusa1121	2013	115	West UP, DL
42	PNR 162	Jaya mutant (induced)/ Basmati-370	1994	90-120	UP, WB
43	Pokkali	Landrace	-	-	KL
44	Pusa 1176	BM34/ACC16136 (Asamia Basmati)	-	-	-

AI, all India; AP, Andhra Pradesh; AS, Assam; DL, Delhi; KA, Karnataka; KL, Kerala; OR, Odisha; MP, Madhya Pradesh; PB, Punjab; JK, Jammu & Kashmir; HR, Haryana; WB, West Bengal; CH, Chhattisgarh; PY, Pondicherry; UP, Uttar Pradesh

Table 3. Contd.

S.No	Genotype	Parentage	Year of Notification	Duration (days)	Recommended area
45	Pusa 1592	Isogenic line of Pusa Sugandh 5		120-125	AI
46	Pusa 1612	Pusa Sugandh 5 / C101A51 // Pusa Sugandh 5*2 / Pusa Sugandh 5 / Tetep // Pusa Sugandh 5*2	2013	120	PB, HR, DL, JK
47	Pusa 44	IARI5901-2 / IR8	1994	115-125	KA, KL
48	Pusa 677	Pusa 312 / TKM-9	1997	77-85	TN, KA, AP, MP
49	Pusa Basmati 1	Pusa 150 / Karnal Local	1989	130-135	PB, HR, West UP, UK
50	Pusa Sugandh 5	Pusa 3A/ Haryana Basmati 1	2004	125-130	AI
51	Ranbir Basmati	Selection from Basmati 370-90-95	1996	120-125	JK
52	Rasi	T(N)1 / Co29	1978-82	120-125	AP, TN, OR, MP, MH
53	Sharbati	Landrace	-	-	-
54	Sitwadhan	Landrace	-	-	-
55	Sonasal	Landrace	-	-	-
56	Super Basmati	Selection	2004	145	PB
57	Swarna Sub1	Swarna 3/IR 49830-7-1-2-3	2009	140	AI
58	Taipei 309	NA	-	-	-
59	Taraori Basmati	Pure line selection from local Basmati	1996	155	HR
60	Thai Rice	NA	-	-	-
61	TKM 6	Pureline	1952	-	TN
62	TKM 9	TKM 7 / IR-8	1982	107-110	TN
63	Vandana	C 22 / Kalakeri	2002	Early	OR
64	WGL 23985	Kavya / AC20	2009	-	AP
65	White Ponni	Pureline	-	-	TN

AI, all India; AP, Andhra Pradesh; AS, Assam; DL, Delhi; KA, Karnataka; KL, Kerala; OR, Odisha; MP, Madhya Pradesh; PB, Punjab; JK, Jammu & Kashmir; HR, Haryana; WB, West Bengal; CH, Chhattisgarh; PY, Pondicherry; UK, Uttarakhand; UP, Uttar Pradesh

3.2.3 QTL data compilation

The input data for the BioMercator required details of the mapping population, size of the mapping population, type of marker type, number of markers used, traits used for QTL identification, QTLs, linkage group, confidence interval (CI) of each QTL, flanking markers of QTL, peak marker etc. For every QTL, details such as LOD value, R^2 values and map position were integrated into the analysis.

Further, the entire map data over which the QTLs are mapped were also required as a separate input for the analysis. If any of these variables were missing the map or QTLs were discarded. Supplementary information such as organism name, species, cross type, parents used, mapping function, map name, map expansion, map quality, locus location was also incorporated in the map data, while QTL data had additional information such as experimental location, year and trait ID.

The data were first compiled in Microsoft Excel worksheet and the QTL data and the map data were individually saved in a text file before loading into the software.

3.2.4 Development of a consensus map

In the first step of the analysis, map data were integrated from initial 15 maps, into a final consensus map through a one-step procedure using weighted least squares that accounts for the statistical properties of estimated genetic distances (Veyrieras et al. 2007). The basic principle of the procedure is that estimated recombination fractions between two adjacent markers would be distributed around the true recombination fraction, and hence a maximum likelihood function can estimate the most likely positions of the markers based on n number of independent experiments involving same linkage group. The assumptions are, (a) independency of the population samples, (b) no interference among the recombination events at same marker interval, (c) marker order and recombination rates are common for all populations and (d) all the genetic maps are connected. The estimated fraction will have a variance that depends on the information provided such as cross design, sample number etc.

Table 4. Details of mapping studies undertaken for meta-QTL analysis

Cross	Population		Marker		No. of QTLs	References
	Type	Size	Number	Type		
Xieqingzao B/Zhonghui 9308	RIL	138	198	SSR	52	Feng et al. 2015
IR64/Azucena	DHL	174	228	SSR	86	Nguyen et al. 2016
XieqingzaoB(XieB)/ Zhonghui9308 (9308)	RIL	281	196	SSR	13	Dai et al. 2015
Zhenshan97 (ZS97) and Minghui63 (MH63)	RIL	127	220	RFLP/SSR	30	Wei et al. 2012
R9308 and XQZB	RIL	138	198	SSR	21	Feng et al. 2011b
Zhen Shan 97 (ZS97)/ Minghui 63 (MH63)	RIL	127	220	RFLP/SSR	24	Wei et al. 2011
R9308 / XieqingzaoB	RIL	228	198	SSR	7	Feng et al. 2010
IR64/INRC10192	RIL	140	113	SSR	18	Srividya et al. 2010
Zhen Shan 97 / HR5	RIL	138	245	SSR	55	Tong et al. 2011
IR64 / Azucena	DHL	82	175	RFLP/RAPD	16	Senthilvel et al. 2008
Dasanbyeo/TR22183	RIL	166	216	SSR/STS	20	Cho et al. 2007
IR69093-41-3-2/IR72	RIL	101	182	RFLP/SSR	62	Laza et al. 2006
Nipponbare/Kasalath//Nipponbare	BIL	98	245	RFLP	13	Obara et al. 2001a
IR64/Azucena	DHL	123	175	RFLP/Isozyme	20	Fang and Wu 2001
RIL, recombinant inbred lines; DHL, doubled haploid lines; BIL, backcross inbred lines; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeats; RAPD, random amplified polymorphic DNA; STS, sequence tagged sites;						

RIL, recombinant inbred lines; DHL, doubled haploid lines; BIL, backcross inbred lines; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeats; RAPD, random amplified polymorphic DNA; STS, sequence tagged sites;

3.2.5 QTL projection

QTL projection was carried out by superimposing the QTLs on the consensus map using an algorithm that used a dynamic procedure, that identified an optimal context to place the QTL on the map based on the pair of common markers that bracketed the QTL in the input maps, in such a way that the interval of the markers and their positions were in correspondence between the maps. Two parameters were estimated that control the precision of the QTL projection, the ratio of the interval distances and homogeneity of the interval distances between the test map and the reference map, both were to be minimum for the precise positioning of the QTL (Sosnoswki et al. 2012).

3.2.6 Meta-analysis

A meta-analysis is a combinatory approach for determining the most common element in a set of data, by taking statistical confidence into the decision-making process. The model assumes that (a) the QTL mapping experiments are independent, and (b) for any trait there are a fixed number of underlying QTLs that co-segregates in all the experiments. In the present investigation, a meta QTL analysis was performed using two approaches. The first one used a QTL pooling algorithm developed by Goffinet and Gerber (2000) in which five models were built to test whether the QTLs detected from different experiments were congruent. By assuming a Gaussian (normal) distribution, the five QTL models were analysed for best fit using maximum likelihood estimates. Akaike information criterion (AIC) from the model was compared for the minimum value and the model with the lowest AIC value was taken as the best fit model. The analysis was based on the variance at the QTL position originating from different independent experiments estimated through confidence intervals.

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

were determined and their positions were identified along with computed CI values.

3.3 Agronomic evaluation of the germplasm

3.3.1 Location of the experiment

The field experiment was conducted during *Kharif* season of 2017 at Research plot of Division of Genetics and Division of Agronomy in Indian Agricultural Research Institute, Pusa, New DL. In IARI Soil pH at experimental plot was around 7.9. Available N (Kg/ha) is estimated at 253. Soil EC (dS/m) in IARI Farm is around 0.41.

3.3.2 Experimental design and agro-management

The trial was begun on 5 July 2017 and laid out in Augmented Design with three checks namely Pusa 44, IR64, and Nagina22 with rest of germplasm. After 27 days in the nursery, the seedlings were transplanted on 2 Aug 2017 in the experimental field with a plot size of 1.02 m² per genotype with 72 plants per plot planted with a spacing of 20 x 10 cm. The entire experiment was managed with recommended agronomic inputs with three different dose of Nitrogen application such as Zero, fifty and hundred per cent of the recommended dose (120kg/ha) in three different blocks respectively. Nitrogen fertilizer was given in the form of Urea in three split doses, the first dose (50% of the total) was given at the gap filling stage. Second dose (25% of total) given at tillering stage on 5 Sept 2017. The third dose was given at flowering stage on 28 Sept 2017.

3.3.3 Phenotyping

The phenotyping of plants for yield and yield-related traits were done from every germplasm, for this purpose five random uniform plants were selected and tagged. The selection of plants was done from the centre of the plot. All the data were recorded as per the standard evaluation system for rice (IRRI 2013).

3.3.3.1 Flowering duration (Days to 50% flowering, DFF)

The number of days from sowing to the day when 50% of the plants were flowered on the primary tiller in each of the genotypes was recorded. The data were recorded plot-wise from different N treatments. Data obtained from different replications/ blocks were averaged to obtain the days to 50% flowering.

3.3.3.2 Plant height (PHT)

Plant height measurement was done in cm on sampled plants from the ground level to the tip of the main panicle and average value was taken. Plant height was measured when panicle was fully exerted.

3.3.3.3 Tiller number (NT)

At maturity number of total tillers were counted and recorded.

3.3.3.4 Panicle Length (PL)

After harvesting the primary panicle was separated from the plant and its length was measured in centimetre.

3.3.3.5 Number of grains per panicle (GPP)

Primary panicle was harvested after that they were threshed separately and the number of filled grains were separated from chaffy grains and counted.

3.3.3.6 Spikelet fertility (SF)

In the primary panicle, the total number of spikelets was counted and the ratio of filled grains to the total number of grains was calculated and expressed in percentage.

3.3.3.7 Grain yield per plant (GYPP)

After harvesting, all the panicles from a plant were threshed and the filled grains were separated from the chaffy grains and weighing was done on an electronic weight machine and expressed in grams.

3.3.3.8 Upper ground biomass (UGB)

All the plants in a plot were harvested closer to the ground individually and washed free of all soil debris. Ten random plants were blot dried and kept for hot air oven drying at 38°C for 24 hours. The samples after drying were weighed using an electronic precision balance and the dry weight is recorded in grams.

3.3.3.9 SPAD Chlorophyll reading

For the measurement of chlorophyll content in plants, a hand-held chlorophyll meter (atLEAF® CHL PLUS) was used at the beginning of flowering. The measurement was taken for the SPAD values, which was converted into

chlorophyll content. Reading was taken from five plants in each genotype and its average value was taken.

3.3.3.10 Flag leaf nitrogen (FLN)

At the time of flowering Flag leaf was collected from the plants in each genotype and their protein estimation was done with the Bradford method and presented in mg/g. Flag leaf N was calculated by the formula,

$$\text{FLN} = \frac{\text{Protein content}}{6.25}$$

3.3.3.11 Total Nitrogen estimation by Kjeldahl method

Total Nitrogen (N) in grain and straw is determined separately as the major parameters for determining genotypic N uptake by Kjeldhal method (Winkler 1913; Bradstreet 1954). 0.5g of finely ground plant samples were taken and passed through a 40-mesh sieve in a piece of butter paper. The samples were transferred to a 500 ml Kjeldahl flask after proper wrapping. 25ml of concentrated H₂SO₄ containing 3.5% salicylic acid was added to the flask and allowed to stand for 30 minutes. After the initial acid digestion, 5g of sodium thiosulphate was added to the sample and allowed to stand for 30 minutes (Bradstreet 1940). To this, catalyst mixture (digestion accelerator) was added. The catalyst mixture per sample contained 10g of anhydrous sodium sulphate (Na₂SO₄) and 1.0 g of pure dry copper sulphate pentahydrate (CuSO₄.5H₂O). The flask was initially heated slowly to prevent frothing and then by accelerated heating for half an hour and until the digest became clear. The digest was cooled, and 150 ml of distilled water was added slowly and cooled again. 120 ml of 40% NaOH was poured into the flask along the sides together with few glass beads and a drop of mineral oil. Flask was connected to a Kjeldahl trap in the distillation unit immediately.

On distillation, the ammonia evolved is collected in 25 ml of 4% Boric acid solution to which mixed indicator has already been added. The mixed indicator was prepared by dissolving 1g of bromo-cresol green and 70 mg methyl red indicator in 100 ml of 95% ethyl alcohol. 5 ml of the mixed indicator was added to 1 litre of 4% boric acid before using in distillation. On collection of ammonia, the indicator will change the colour from bluish purple to bluish green.

Distillation was carried out until the collection of 150 ml distillate. The ammonia captured in the distillate is titrated against 0.01 N H₂SO₄ until the blue colour disappears. Run a blank simultaneously with a piece of paper (same size and type as used for wrapping of samples) and other reagents.

Concentrated H₂SO₄ oxidises the organic carbon in the plant samples to CO₂ which is liberated during the digestion process. The salicylic acid prevents the loss of nitrate on heating by getting nitrified. Later, sodium thiosulphate reduces the nitrified salicylic acid to ammonium, thereby assuring the complete conversion of nitrate into ammonia. In the catalyst mixture, copper sulphate acts as the catalyst for oxidation of organic carbon and accelerates the process. Anhydrous sodium sulphate raises the boiling point of sulphuric acid so that the digestion occurs completely and quickly at a higher temperature. Acid digestion converts all the nitrogen in the plant sample to Ammonium sulphate. By distilling it with NaOH, liberates ammonia from (NH₄)₂SO₄, converting it to sodium sulphate. The entire liberated NH₃ is trapped by Boric Acid in the distillate and is quantified through titrimetry using standard dilute sulphuric acid. The indicators help to determine the end point of the reaction.

Amount (g) in the sample (s) = (ml of acid used for sample – ml of acid used for blank) x normality of acid x 14 x 10⁻³

N in sample (%) = (s x 100) / sample weight in g

A. Total grain N (TGrN)

All the plants from a plot were harvested, threshed and the grain and straw were separated and sundried for 96 hours. After the proper sun drying, the grains were oven dried for 24 hours at 38°C and was powdered with the help of a Grinder. 0.5g of the sample was taken and Nitrogen estimation. The total grain N was computed by the formula and expressed in g per plant.

$$\text{TGrN} = \frac{\% \text{ Grain N} \times \text{GYPP}}{100}$$

B. Total shoot N (TShN)

The shoot samples from the plot harvest were oven dried after the proper sun drying of the shoot, chopped into very small pieces and pulverized using a tissue

pulveriser. 0.5 g of the pulverized shoot was taken and N estimation. The total shoot N was computed by the formula and expressed in g per plant.

$$\text{TShN} = \frac{\% \text{ shoot N} \times \text{UGB}}{100}$$

C. Total plant N (TPIN)

The total N content of the upper ground biomass, which is the sum of grain and straw N at maturity was computed total plant N and expressed in g per plant.

$$\text{TPIN} = \text{TGrN} + \text{TShN}$$

3.3.4 Estimation of Nitrogen use efficiency

Seven parameters related to NUE were estimated targeting both N uptake efficiency and N utilization efficiency (Xu et al. 2012; Fageria et al. 2015) from the agronomic and physiological traits measured.

3.3.4.1 Agronomic N use efficiency (AgNUE)

This is the net increase in grain or biomass yield when unit N was applied. AgNUE is the product of physiological NUE (pNUE) and N recovery efficiency (NRE). AgNUE is calculated as,

$$\text{AgNUE} = \frac{(\text{GYPP}_{N_x} - \text{GYPP}_{N_0})}{x}$$

where GYPP_{N_x} was the grain yield per plant when x gram of N was applied, GYPP_{N_0} is the grain yield per plant when no N was applied.

3.3.4.2 Physiological N use efficiency (pNUE)

pNUE is the ratio of increased grain yield to increased N accumulated in plants when N was applied over N unapplied condition.

$$\text{pNUE} = \frac{(\text{GYPP}_{N_x} - \text{GYPP}_{N_0})}{(\text{TPIN}_{N_x} - \text{TPIN}_{N_0})}$$

where TPIN_{N_x} is the total plant N when x gram of N was applied and TPIN_{N_0} is the total plant N when no N was applied. The TPIN_{N_0} is the amount of N utilised by the plant that was exclusively sourced from soil N reserves. This may contain N residues from previous crop as well as natural N released from soil processes.

3.3.4.3 Nitrogen recovery efficiency (NRE)

NRE is the quantum of N recovered when unit N was applied. This parameter is also known as absorption NUE (aNUE) or N uptake efficiency (NUpE)

$$NRE = \frac{(TPIN_{Nx} - TPIN_{No})}{x}$$

3.3.4.4 Nitrogen harvest index (NHI)

NHI was calculated as the ratio of grain N to the total plant N at maturity and expressed in percentage. The total plant N was confined to the N content of the upper ground biomass.

$$NHI = \frac{TGrN \times 100}{TPIN}$$

3.3.4.5 Nitrogen assimilation efficiency (NAE)

N assimilation efficiency is the ratio of plant biomass to the TPIN

$$NAE = \frac{Biomass}{TPIN}$$

3.3.4.6 Grain yield efficiency index (GYEI)

GYEI is the product of the ratios between grain yield of a genotype to the average grain yield under N fertilised and unfertilised conditions (Fageria 2009).

$$GYEI = \frac{GYPP_{No}}{GYPP_{No}} \times \frac{GYPP_{Nx}}{GYPP_{Nx}}$$

GYEI is used to classify the genotypes based on their efficiency. Genotypes with GYEI of 0.0 to 0.5 are recognised as inefficient, those genotypes with GYEI of 0.5 to 1.0 are recognised as moderately efficient and genotypes having GYEI of greater than 1.0 are identified as efficient genotypes. This is an arbitrary index that takes into account low and high N response for classification, which separates the stable and efficient genotypes from the inefficient ones effectively.

3.3.4.7 Chlorophyll sufficiency index (CSI)

CSI is the ratio of chlorophyll content in any plot to the chlorophyll content in a sufficiently N fertilised plot (Hussain et al. 2000).

$$CSI = \frac{SPAD_{N?}}{SPAD_{N100}} \times 100$$

where $SPAD_{N?}$ is the SPAD chlorophyll reading in any plot, and $SPAD_{N100}$ is the SPAD chlorophyll reading in a plot 100% of N is applied (sufficient N is applied).

3.4 Molecular analysis of the germplasm

3.4.1 Isolation, purification and quantification of genomic DNA

For isolation of genomic DNA, fresh and young leaves were harvested in the morning from 50-60 days old plants and stored at -20°C. Leaf samples were ground in a tissue grinder in liquid nitrogen for DNA extraction following the CTAB method (Murray and Thompson 1980). The DNA extraction buffer (DEB) contained 0.5M EDTA (pH 8.0), 1M Tris-HCl (pH 8.0), 4M NaCl and 10% CTAB, mixed in the proportion of 20:50:175:100 ml and made to a volume of 500ml using double distilled water. The powdered leaf samples were taken in a 2ml micro-centrifuge tubes containing 1ml of pre-warmed (65°C) DEB and 2.0µl of 2-mercaptoethanol.

The tubes were placed in a water bath at 65°C for one hour with swirling occasionally in about 10-15 minutes. The incubated tubes were then cooled to room temperature to prevent DNA damage. The mixture in the tubes was then added with matching volume (~1 ml) of chloroform: isoamyl alcohol mix (24:1) and mixed for five minutes by flipping gently and centrifuged for 10 min at 4°C with 10,000 rpm. The clear supernatant was collected in a fresh 2 ml microcentrifuge tube to which 1/10th volume of 3M sodium acetate (pH 5.2) was added along with the equal volume of ice-cold isopropanol, mixed gently and incubated at 4°C for overnight. After incubation, the tubes were spun at 10000 rpm for 5 minutes. The pellet at the bottom of the tube was washed twice with 70% ethanol through brief centrifugation after discarding the initial supernatant solution, air dried and dissolved in TE buffer (100mM Tris and 50 mM EDTA, pH 8.0) added sufficiently depending on the DNA pellet size and stored at 4°C after complete dissolution. To remove the contamination by RNA, the extracted nucleic acid mixture was digested with DNase free RNase (10 mg/ml) incubated at 35°C for one hour and pelleted following the previous steps before final storage suspended in TE buffer.

Quantitation of DNA was done using the 1µl of extracted DNA ran in an agarose gel (0.8%) electrophoresis together with 100ng uncut λ DNA, for 30 min. The eluted DNA was ethidium bromide (EtBr) stained and visualized under UV transillumination. By comparing the intensity of fluorescence of the sample and the standard bands the DNA quantity was determined and further diluted with water in 4:1 ratio (water: DNA).

3.4.2 Markers for genotyping

A set of 16 microsatellites (SSR) markers linked to mQTLs were used for genotyping the germplasm.

3.4.3 PCR amplification and resolution of amplicons

The diluted DNA was used for PCR amplification using specific SSR primers. The PCR reaction mix was constituted by adding 1.2 µl diluted DNA, 1.0 µl of 2.0 mM dNTPs each, 1 µl of 10X buffer (4 mM Tris- HCl (pH 8.0), 20 mM KCl, 0.8 mM MgCl₂), 1.0 µl of primer dimers and 0.2 µl of Taq polymerase and made up to 10 µl by adding 5.6 µl of nuclease-free water. The PCR was carried out in the thermally sealed 96-well plates in an Eppendorf thermal cycler with a reaction profile consisting of 35 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s and extension at 72°C for 1 min, bracketed between an initial denaturation at 94°C for five minutes and a final extension at 72°C for 10 minutes before cooling to 4°C before storage.

The amplicons were resolved by running an agarose gel electrophoresis containing 3.5% (w/v) agarose prepared by dissolving 17.5 g agarose powder in 500 ml of 1X TAE in an Erlenmeyer flask. The mixture was heated to boil in a microwave oven for 10 minutes till a clear suspension was obtained, and then was allowed to cool for five minutes before adding 25µl of 1% ethidium bromide. After gentle swirling, the gel was poured slowly into casting tray without forming bubbles, combs placed and allowed to solidify at room temperature. After solidification, the gel was lowered into the buffer tank filled with 3.0 litres of 1X TAE (pH 8.0) buffer. The PCR products were added with 1 µl 6x loading dye (0.25% bromophenol blue; 0.25% xylene cyanol FF; 40% sucrose), and loaded into the wells using a micropipette. In every set of gel cast, one well was loaded with 100 bp size reference ladder. The power rating was set at 5V/cm of run and

electrophoresis was done for a period of 1.5 to 2.5 hours. After the run, the gels were removed from the gel tank and placed over the UV transilluminator and the fluorescent bands are photographed in a gel documentation system. The molecular data of the resolved amplicons were recorded in base pairs based on the size of the product in relation to the reference ladder bands. The bands with clear morphology and closer to the expected product size of the marker were only scored. Ambiguous bands were confirmed or rejected by rerunning such markers again.

3.5. Data compilation and analyses

Both the agronomic and marker data are compiled and analysed statistically. The molecular data were explored for genetic diversity pattern and allele frequency based population genetic analyses.

3.5.1 Analysis of variance

To test the statistical significance of phenotypic variation among the test genotypes under three N regimes, analysis of variance (ANOVA) was performed for all the traits. Since the treatments were characterised by different nitrogen levels, the genotypic variation apparent among the N levels could be attributed to their response with respect to the varying adaptations determined by their genotype-by-N level interaction. The null hypothesis was set as no statistical significance between the genotypes, environments and their interaction components and the alternate hypothesis as these components are significantly different. The analysis was performed using the R package ‘lme4’ which implements a restricted maximum likelihood (REML) approach. The genotypes were used as a fixed factor in the model, while N regimens were treated as a random factor. The statistical significance of the variance components was tested using appropriate tests. The fixed factor was tested based on the variance ratio, while a chi-square test was used for random factor. The mean comparison was done using Tukey’s honestly significance test. All the analyses were done using STAR v.2.0.1 (IRRI 2014).

3.5.2 Testing the genotype response similarity across N levels

The genotypic response similarity between the N levels was tested using Pearson correlation coefficients. Traits showing high correlations indicated that the

genotype performance was similar within each N level but possibly with a varying degree of magnitude. The traits which showed poor correlation indicated a high level of genotype-by-N level interaction, several of which may be showing cross-over variations.

3.5.3 Comparing the N use efficiency parameters

Correlations between N use efficiency parameters were worked out for all the genotypes across N levels. The agreement between the parameters with indicates that they are dependent on each other and hence will produce a similar ranking of the genotypes based on those parameters. Such associated parameters will be a constituent of a common N use process, either uptake or utilisation.

3.5.4 Analysing the genotype x N level interactions

Genotype x N level interactions were restricted to those traits and parameters that showed significant interaction variance. The genotype performance was tested based on GGE approach in which the combined effects of genotype (G) and genotype by environment (GE) interactions (GEI) were compared. The analysis was done using GGE biplots (Yan et al. 2000). The input data were the best linear unbiased predictors (BLUPs) of the genotype $\times$ N level for different traits. The data analysis was carried out using the R package 'GGEbiplotGUI' version 1.0-9 (Frutos et al. 2014), within the RStudio environment running the R version 3.4.4 RC.

3.5.4.1. The GEI model

The cumulative phenotypic effect of any genotype grown in different N regimes is governed by several factors such as G, E, GEI and residual. Some of the G effects were conspicuous across all the N levels and is called genotypic main effect. Similarly, some of the N levels might have a strong influence on genotypic performance, known as an environmental main effect. Besides, there may be the specific way some genotype behaves in a particular N level, which is known as genotype-by-N level interaction effect. All these effects are repeatable and can be attributed to a definite factor, say G, E and GEI. Other than these, there can be random effects that are not repeatable, and cannot be attributed to any specific factors. Such unexplained factors are known as a residual effect.

If Y_{ijk} is the phenotypic trait, μ is the overall mean of the trait, G_i is the genotypic effect, E_j is the N level effect, GE_{ij} is the interaction effect and ε_{ijk} is the residual effect for the genotype i , grown in the N level j and replication k , then the model,

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + \varepsilon_{ijk}$$

will give the relation between the different components and the resultant trait. The μ is obtained by taking the overall mean of all the genotypes, N levels and replications. G_i is obtained by subtracting μ from the average of genotype i across all N levels ($\mu_i - \mu$), E_j is obtained by subtracting μ from the average of the N level j across all genotypes ($\mu_j - \mu$) and GE_{ij} is obtained by subtracting the genotype and N level means from the performance of genotype i in the level j ($\mu_{ij} - \mu_i - \mu_j + \mu$). The residual ε_{ijk} is the difference of the phenotypic trait observed in the k^{th} replication from the mean performance ($\bar{Y}_{ij}$, averaged over replications) of genotype i in the j^{th} N level.

It is generally assumed that the residual has a mean of zero and variance of σ^2 also called error mean square. Therefore, in the analysis of variance (ANOVA), the significance of the components G, E and GE are tested against the σ^2 to estimate the probability of statistical confidence. The null hypothesis (H_0) was set as no differences between the genotype means across the environments. The alternate hypothesis (H_a) was that the genotype means always were different in different environments. The estimated probability of 0.05 is taken as a threshold, below which the significant difference is ascertained. By taking the average, the residual component is assumed to be insignificant and removed from the data. Then the model is reduced to the form, $\bar{Y}_{ij} = \mu + G_i + E_j + GE_{ij}$.

3.5.4.2 Biplot analysis

In statistical terms, a biplot is a two-dimensional graphical approximation of a matrix of rank two (Gabriel 1971). Generally, there are several environments under which several genotypes are evaluated in a crop variety evaluation. When the number of dimension of the GE matrix increases, the dimensions of the biplot also increases which makes the interpretations fairly difficult. One way to solve this problem is to reduce the dimensions of the GE matrix to a minimum

(two dimensions) by conducting a principal component (PC) analysis. Then plotting the PC1 against PC2 scores of each row and column gives the biplot. The decomposition of the data is carried out by a process called singular value decomposition (SVD). In mathematical terms, a biplot is, therefore, the graphical representation of the product of two matrices, one having two rows, and the other having two columns (Yan 2014).

GGE biplot analysis uses environment centred data for analysis as the emphasis is on genotype (G) and genotype by environment (GE) so as to arrive meaningful conclusion about the genotypic performance across varying locations. Environment centring is done by subtracting the environment mean (μ_j) from the Y_{ij} values and transforming the two-way table. Therefore, the GGE biplot analysis is based on the following model.

$$\bar{Y}_{ij} - \bar{Y}_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

where, $\bar{Y}_{ij}$ is the average phenotypic value of genotype i in the j^{th} environment; $\bar{Y}_j$ is the mean of the j^{th} environment (μ_j); $\lambda_1 \xi_{i1} \eta_{j1}$ and $\lambda_2 \xi_{i2} \eta_{j2}$ are the first and second PCs, of which λ_1 and λ_2 are the singular values, ξ_{i1} and ξ_{i2} are the genotypic PC scores, and η_{j1} and η_{j2} are the environment PC scores for the PC1 and PC2 respectively.

Biplot analysis generates a visual representation of G and GE effects that can be used in genotype evaluation, which provides information on genotype performance, stability and adaptability. This helps us to identify a group of genotypes that are widely adapted and specifically adapted, environments that are similar (mega-environment) or unique (Yan and Kang 2003). In the present study, GGE analysis of the phenotypic data generated three types of biplot visualizations, (a) which-won-where (b) mean versus stability and (c) genotype rankings. Which-won-where plots helped to identify specific and widely adapted genotypes and mega-environments, while mean versus stability plots helped to identify best performing stable genotypes and genotype rankings helped to identify the ideal genotype and rankings of the genotypes with respect to the ideal genotype over the average environment coordinate.

The which-won-where graph provides a set of vertex genotypes that are connected using a convex hull. It is the polygon that formed by connecting the outermost genotypes so that all the remaining genotypes fell within the hull. The vertex genotypes are the genotypes that formed the convex hull. The perpendicular lines originating from the centre and cutting all sides of the polygon divide the convex hull into distinct sectors. For each sector, vertex genotype is the best performing genotype for the environments that fell within that sector. Since environments are distributed based on their eigenvalues, any set of environments that are clustered in the biplot can be considered as a part of a mega-environment.

The mean vs stability plot also contains the biplot distribution of genotypes and environments. In addition, an average environment coordinate (AEC) passes through the biplot origin with an arrow at the positive end of the axis. Another perpendicular line that cuts the AEC through the biplot origin with two arrows on both the ends is called the stability axis. The lines connecting genotype points and the AEC determines the order of ranking of the genotype performance. The genotype which is extreme to the positive end of AEC is the best performing genotype while that is extreme at the negative end is the poorest among all the genotypes. The closeness of the genotypes to the AEC determines the stability. If the line connecting genotype point and the AEC is the longest then the genotype is considered highly variable (least stable) and if the length of the connecting line is the shortest then the genotype is least variable (highly stable).

Genotypes can also be compared with respect to the ideal genotype and ideal environment. A genotype that is high performing with high stability is considered to be an ideal genotype. An ideal environment is the most discriminating environment, but a representative of all the environments in the data. Therefore, both the ideal genotype and ideal environment are hypothetical points marked on the AEC. The ideal genotype is represented by a set of concentric circles drawn on the AEC, with the centre of the innermost circle representing the ideal genotype. The ideal genotype is, therefore, a projection on AEC which is equivalent to the longest genotype vector, that has above average phenotypic performance and with no variability across environments, which means that the distance between ideal genotype and AEC is zero. Hence,

any genotype that falls nearest to the ideal genotype is the most desirable genotype for selection. Further, the rest of the genotypes can be compared for their idealness for selection based on their relative distance from the ideal genotype. Similarly, the most desirable test environment can be identified from the proximity of environment points to the ideal environment. The ideal environment is depicted as a small circle on the AEC with an arrow pointing towards it (Yan and Rajcan 2002).

3.5.5 Simultaneous selection based on N use parameters

Based on the average estimates of N use parameters, the genotypes having above average estimates were set compared graphically using Venn diagrams. The analysis was carried out using the software VennPainter 1.2.0 (Lin et al. 2016).

3.5.6 Assessing mQTL diversity in rice population

The mQTLs identified were tested on the germplasm assembly using the peak SSR markers linked. The data was compared with the NUE of the genotypes to see the contribution of each mQTL in determining the NUE parameters.

3.5.6.1 Single marker analysis

Marker-trait association was carried out using single factor analysis of variance. The genotypes were classified into groups based on the marker allelic pattern, and the phenotypic variance between and within the groups was compared using ANOVA. The probability of 0.01 was fixed as the threshold for claiming the valid association. For the significant mQTL that showed conspicuous between group variation for agronomic traits under N levels and for N use parameters, the percentage of variation explained and the additive contribution of different marker alleles were computed. Analysis was carried out individually for each marker that showed sufficient polymorphism and normal distribution as per Hardy-Weinberg expectations. Analysis was performed using Microsoft Excel.

$$\text{Percent variation explained (PVE), } R^2 = \frac{\text{Between group SS}}{\text{Total SS}} \times 100$$

$$\text{Additive effect} = \frac{\text{Group 1 mean} + \text{Group 2 mean}}{2}$$

3.5.6.2 Estimation of population genetic parameters

Population genetics of the mQTLs in the germplasm assembly was estimated based on the allele distribution pattern. All the analyses were carried out using GenAlEx 6.5 addin for Microsoft Excel (Peakall and Smouse 2006, 2012).

A. Allele frequency

Based on Hardy-Weinberg expectations, the frequency of alleles generated at each mQTL locus was computed based on the peak marker pattern. The frequencies (p_x) are calculated by the formula,

$$p_x = \frac{2N_{xx} + N_{xy}}{2N}$$

where N_{xx} and N_{xy} are the number of homozygotes and the number of heterozygotes respectively for allele x and N is the total number of genotypes surveyed.

B. Heterozygosity and fixation index

Observed heterozygosity (H_o) and expected heterozygosity (H_e) of the alleles per each mQTL locus was computed from the allelic frequencies. Assuming perfect random mating, expected heterozygosity is the proportion of heterozygotes that may occur in the population.

$$H_o = \frac{N_{hetero}}{N}$$

$$H_e = 1 - \sum p_i^2$$

where, N_{hetero} is the number of observed heterozygotes, and p_i is the allele frequency of the i^{th} allele.

From the heterozygosity information, the fixation index (F) also known as the coefficient of inbreeding was calculated.

$$F = \frac{H_e - H_o}{H_e}$$

The parameter F takes the values between +1 and -1. F tends to zero under perfect random mating and move towards 1 when inbreeding occurs. The negative values of F occur when excessive heterozygosity is noted, where the

heterozygotes have a preferential selection (negative selection) over homozygotes.

3.5.6.3 mQTL diversity in the population

The mQTL based diversity of the population was determined using principal coordinate analysis (PCoA). For this, the marker allele based distance matrix was constructed using simple matching coefficients (SMC) as the distance measure (d_{ij}).

$$d_{ij} = 1 - \frac{1}{N} \sum_{k=1}^N \left(\frac{m_k}{\pi} \right)$$

where d_{ij} is the distance between genotypes i and j , N is the number of marker loci $k=1$ to N , m_k is the number of matching alleles for the marker k and π is the ploidy level of the test population.

The distance matrix computed from marker data often has no Euclidean properties, hence are not useful for factorial analyses. To correct this problem, a power transformation is done on the distance measures, so that the matrix gains Euclidean properties. In general, power transformation used $p=0.5$, which means the square root of the distance measures is computed.

PCoA is a method of factor analysis that uses distance matrices. The analysis basically reduces the multidimensional distance matrix into few components or principal co-ordinates such that estimated distances between two genotypes based on the principal coordinates remain as close as to the original distance between them. These coordinates are extracted one after the other in such a way that they remain uncorrelated and are orthogonal to each other. The first coordinate defines the maximum closeness followed by second and so on. Usually, very few coordinates (maximum 4) are required to explain the entire distance structure among the genotypes. The principal coordinates are characterised by their eigenvalues and eigenvectors that defines their variance and direction. The importance of each coordinate is calculated by the percentage of variation explained it, which is the ratio between its eigenvalue and the sum of eigenvalues, and expressed in percentage.

The closeness of each genotype to the principal coordinates are depicted by projecting the genotype on to the coordinate. In a two-dimensional graphical display between first and second coordinates, the genotypes which are close to both the axes will align closer to the origin of the graph. This we will be able to assess the diversity pattern of the genotypes based on their relative placements. Further, the clarity of the projection depends on the closeness to the axis, and therefore the square of the cosine of the angle between the projection and the genotype is taken for comparison. When the angle is narrow, $\cos^2$ values tend to one, and when the angle is wide, the $\cos^2$ value tends to zero.

Results

For identification of N efficient genotypes and to validate a set of meta-QTLs for N efficiency the present study was conducted by evaluating a set of 65 genotypes under three N regimens. The results are present hereunder.

4.1 Agronomic performance of genotypes

4.1.1 Overall performance

Analysis of variance (Table 5) revealed that for all agronomic traits studied, the genotypic component of variation was highly significant. Similarly, both the environmental and genotype x N level component were also showed significant variation.

Average performance of genotypes for nine agronomic characters and three N use related parameters such as total plant N, chlorophyll content and flag leaf N is given in Table 6.

4.1.1.1 Days to 50 % flowering

Days to 50% flowering of the genotypes ranged from 76 to 135 days in IR 64 and BPT 5204 respectively with an average mean of 100 days.

4.1.1.2 Plant height

Plant height ranged from 62.0 cm (ADT 39) to 141.6 cm (Nagina22) among the test genotypes. Plant height recorded a mean of 89.4 cm.

4.1.1.3 Tiller number

Average on of tillers among the genotypes was around 9 with a range of 5 (Taipei 309) to 13 (ADT 38) tillers per plant.

4.1.1.4 Panicle length

Panicle length also showed similar variation with length ranging from 18 cm in Vandana to 30.4 cm in Kasturi with an average of 23.0 cm.

Table 5. Combined analysis of variance for the agronomic traits and N content under three N levels

Trait	Mean squares			
	Genotype	N level	G×E	Error
DFF	1054.8**	62.5**	28.1**	0.78
GYPP	101.5**	581.8**	24.1**	0.66
NT	37.2**	562.6**	10.8**	0.59
PL	40.4**	14.5**	4.8**	0.34
PHT	2996.1**	1160.6**	89.2**	1.03
UGB	1768.1**	6997.4**	292.6**	7.46
GPP	8011.8**	8075.6**	2021.1**	11.94
SF	848.8**	7135.0**	227.7**	6.56
HI	701.9**	49.7**	61.4**	1.80
TPIN	0.2**	0.3**	0.1**	0.002

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

4.1.1.5 Upper ground biomass

Shoot biomass had high variation between the genotypes ranging from 18.82g in Rasi and Panvel to 68.7g in IR 66.

4.1.1.6 Grains per panicle

Average number of grains per panicle recorded for the genotypes was 127.0, with a range of 72 to 208 in Himdhan and Acharmati, respectively.

4.1.1.7 Spikelet fertility

Highest percentage of fertile grains was recorded among the genotypes was 91.8% in Himdhan and lowest was in IR 24 (57.5%). There was an average spikelet fertility of 78.5% in test genotypes.

4.1.1.8 Grain yield per plant

Grain yield per plant ranged from 3.6 g to 19.33 g in IR 24 and Mahamaya respectively with the average grain yield of 10.64 g.

Table 6. Mean performance of the genotypes for agronomic and plant N parameters across three levels

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	TPLN	SPAD	FLN
Acharmati	103.33	13.27	6.78	23.27	105.87	42.67	208.00	76.08	30.58	0.28	35.70	5.00
ADT 38	109.33	13.00	12.89	22.87	70.47	54.73	102.00	61.61	23.26	0.44	41.27	4.17
ADT 39	114.33	5.87	9.67	20.73	62.00	23.13	113.67	69.28	26.42	0.18	36.97	4.54
ADT 42	108.33	14.07	9.67	24.68	79.07	55.40	138.00	78.59	25.31	0.48	41.73	6.63
ASD 16	90.00	15.00	7.33	24.80	79.47	43.67	158.33	90.83	34.17	0.33	49.00	5.57
ASD 18	94.00	14.20	9.00	24.07	71.53	43.53	117.67	89.28	33.12	0.37	42.47	6.37
ASD 19	112.00	11.27	10.00	20.80	108.93	49.87	139.00	87.88	23.22	0.42	39.87	4.93
Basmati 370	103.33	7.60	7.33	24.13	118.60	36.13	108.67	66.34	20.57	0.26	32.50	4.73
BPT 5204	135.22	8.69	7.67	23.33	94.99	20.44	168.44	72.04	42.01	0.15	39.78	4.94
Chandrasahini	92.33	13.00	6.67	25.53	80.00	36.07	169.00	79.86	36.00	0.28	39.47	4.53
CO37	110.00	13.47	11.11	22.33	89.53	49.93	147.67	79.77	26.82	0.32	35.63	6.45
CO51	92.00	11.33	9.00	21.60	78.87	35.80	138.67	90.90	32.16	0.42	38.60	5.11
CR Dhan 310	104.56	9.43	6.67	25.22	117.21	21.59	170.67	87.96	43.32	0.18	46.80	3.24
CR Dhan 311	106.67	8.18	6.56	24.89	125.81	19.46	168.11	76.00	41.70	0.15	43.06	3.21
CSR27	93.00	18.13	12.67	23.40	89.67	64.47	157.33	88.41	28.16	0.70	39.93	7.83
Himdhan	80.33	9.33	9.44	19.73	100.53	44.20	72.33	91.78	21.13	0.33	34.20	3.59
Improved Sabarmati	103.00	13.53	11.00	21.67	80.07	63.40	99.67	62.23	21.11	0.60	41.00	5.20
IR 24	76.33	3.60	6.00	21.00	100.93	27.47	125.33	57.73	13.11	0.23	44.37	4.13
IR 50	84.00	9.67	9.67	21.20	70.00	31.67	104.00	82.97	30.53	0.33	53.93	5.68
IR 64	94.00	9.73	9.22	21.60	67.53	43.60	89.00	87.96	22.19	0.46	37.30	4.51
IR 66	106.00	14.60	11.89	23.93	75.73	68.67	177.33	63.73	20.56	0.73	43.80	4.74
IR 70	115.00	12.13	12.78	22.93	65.13	49.00	124.00	69.77	24.63	0.40	42.37	4.35

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPLN, total plant N in g/plant

Table 6. Contd.

Genotype	DFF	GYP	NT	PL	PHT	UGB	GPP	SF	HI	TPLN	SPAD	FLN
IR 72	88.67	13.40	10.00	22.80	67.07	34.80	138.33	77.42	37.94	0.31	39.97	5.00
Jaiphoola	121.67	7.53	10.89	20.10	99.60	65.60	147.33	83.74	12.01	0.54	35.80	5.15
Jaldi Dhan 13	81.67	9.33	7.33	20.13	62.13	25.47	91.00	70.83	37.10	0.21	44.50	4.60
Kamlesh	88.00	14.20	7.00	24.13	110.80	44.00	144.00	89.59	32.72	0.49	37.90	4.86
Kasturi	92.00	9.07	6.67	30.40	100.73	50.53	86.00	75.66	17.94	0.39	37.43	5.64
Kavya	106.33	9.60	9.22	21.00	73.67	43.27	113.67	88.38	22.22	0.27	37.70	5.75
Kranthi	82.67	10.73	8.22	23.20	79.60	33.93	112.67	89.93	32.14	0.37	38.27	2.68
Mahalaxmi	116.67	10.67	11.67	21.80	76.93	66.53	162.33	69.39	16.73	0.53	36.23	3.32
Mahamaya	95.00	19.33	8.89	20.87	83.20	61.27	127.33	83.40	31.68	0.54	41.23	5.48
MTU 1010	98.89	8.34	6.78	23.00	97.04	19.81	131.44	87.58	41.77	0.17	45.46	2.35
MTU 5249	104.00	10.00	8.56	18.60	62.53	42.27	150.33	80.17	23.58	0.29	32.70	2.98
Nagina 22	97.56	9.09	6.22	23.33	141.63	20.96	112.44	91.56	42.98	0.16	36.21	2.58
Nidhi	106.78	10.89	6.11	26.22	105.49	24.36	187.44	86.57	44.32	0.24	47.38	2.23
Pant Dhan 19	104.00	16.67	8.78	20.73	82.33	49.87	159.00	83.63	33.20	0.36	37.97	5.07
Pant Sugandh Dhan 15	102.00	12.87	7.89	26.33	95.53	53.60	122.67	67.59	24.51	0.55	34.90	4.33
Pant Sugandh Dhan 17	92.00	13.87	8.56	26.00	90.13	50.93	125.00	76.40	27.43	0.45	36.20	4.78
Parvel	111.56	7.74	5.56	24.22	93.38	18.81	157.11	89.24	40.88	0.13	44.67	4.68
PB 1121	95.00	6.67	10.44	23.93	83.80	45.73	80.33	59.08	14.53	0.57	37.87	4.24
PB 1509	88.67	5.93	8.22	23.67	71.67	26.60	93.33	64.76	21.80	0.21	39.73	5.06
PNR 162	95.00	10.47	8.67	23.73	77.07	33.47	92.67	81.73	31.42	0.28	40.80	5.88
Pokkali	105.00	9.40	6.11	23.53	135.47	50.20	109.33	77.47	19.47	0.57	40.13	2.27
Pusa 1176	108.00	8.53	8.11	23.07	92.33	40.47	133.67	86.86	20.29	0.32	40.23	5.07
Pusa 1592	93.00	7.47	6.44	22.33	75.67	32.27	83.33	71.91	23.08	0.29	37.00	4.92

DFF, days to 50% flowering; GYP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPLN, total plant N in g/plant

Table 6. Contd.

Genotype	DFF	GYP	NT	PL	PHT	UGB	GPP	SF	HI	TPLN	SPAD	FLN
Pusa 1612	92.33	11.73	8.22	26.07	90.80	49.07	138.00	61.50	23.42	0.35	36.87	4.23
Pusa 44	104.33	11.06	9.94	22.84	72.58	42.69	135.83	86.43	25.87	0.29	39.88	3.37
Pusa 677	95.00	17.20	9.56	19.13	63.13	47.90	93.67	80.73	36.26	0.42	41.27	7.63
Pusa Basmati 1	102.33	5.13	8.22	25.40	80.07	35.80	129.67	67.11	14.04	0.29	42.13	4.80
Pusa Sugandh 5	103.00	11.73	9.56	25.60	93.67	64.80	134.33	62.96	18.41	0.47	38.20	4.43
Ranbir Basmati	87.33	8.20	8.33	25.53	123.73	36.87	97.33	83.47	21.29	0.30	36.30	4.14
Rasi	93.89	7.76	6.78	22.67	93.22	18.82	99.78	89.66	41.10	0.14	43.04	3.36
Sharbati	102.33	10.07	11.33	21.53	96.47	52.47	83.33	79.24	19.41	0.41	34.20	5.66
Sitwa Dhan	104.33	12.47	9.44	24.00	72.00	46.47	144.33	77.94	26.70	0.38	39.87	3.04
Sonasal	91.33	7.00	9.11	22.47	107.53	49.87	106.33	77.67	14.16	0.55	36.57	5.90
Super Basmati	94.00	5.20	8.22	23.47	85.33	24.87	105.33	82.70	21.91	0.22	37.07	5.35
Swarna Sub1	93.00	8.13	8.89	22.73	81.47	30.53	93.67	68.20	27.82	0.23	34.60	2.36
Taipei 309	101.56	8.36	4.89	22.89	103.76	19.80	154.78	78.40	41.91	0.14	47.14	3.60
Taraori Basmati	104.33	10.67	12.89	23.33	107.47	57.60	99.33	88.33	19.32	0.48	34.37	3.73
Thai Rice	112.00	14.93	11.89	24.80	104.87	66.60	133.67	67.23	23.78	0.34	35.33	5.41
TKM6	95.00	12.33	11.67	23.73	109.20	63.67	137.00	89.00	19.42	0.47	38.10	2.55
TKM9	95.00	16.40	12.22	23.33	81.60	41.77	96.67	81.13	39.23	0.26	38.73	6.83
Vandana	84.00	7.00	6.00	18.00	96.00	33.67	120.67	86.76	21.11	0.22	39.20	5.17
WGL 23985	104.67	10.67	7.89	20.53	71.93	36.00	180.00	62.01	29.96	0.35	41.20	5.19
White Ponni	121.67	4.97	11.56	20.40	86.53	47.40	131.00	86.00	10.28	0.26	33.47	4.35
Mean	99.73	10.64	8.86	23.01	89.40	41.94	127.27	78.47	27.16	0.35	39.50	4.61
SE 0.05 (Tukey's)	1.80	1.91	1.46	0.75	3.44	6.63	15.44	6.12	2.63	0.08	2.30	1.41

DFF, days to 50% flowering; GYP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPLN, total plant N in g/plant

4.1.1.9 Total plant N

Among the three N related parameters, Total Plant N ranged between 0.13 g/Plant to 0.73 g/ plant in Panvel and IR 66, respectively.

4.1.1.10 SPAD chlorophyll Index

Chlorophyll content in leaves as measured by SPAD Index showed an average index value of 39.5, with Basmati 370 recording the lowest value of 32.5 and IR 50 recording maximum value of 53.9.

4.1.1.11 Flag leaf N

N content in the flag leaves ranged between 2.2 mg/g in Nidhi and 7.8 mg/g in CSR 27. The average value for this trait among the genotypes was 4.6 mg/g.

4.1.2 Association between N regimens

Pearson correlation between different N regimens for all the observed traits were computed to see the genotypic performance similarity across the test environments (Table 7). Correlation coefficients showed significant agreement in performance of genotypes across the three N regimens, N0, N50 and N100. The correlation value ranged between 0.9 for Plant height and DFF. Similarly, high positive correlation was found for panicle length and harvest index. Grain yield per plant showed moderate association between N0 and remaining two N levels. However, the correlation between N50 and N100 was high (0.71) for these traits. Number of tillers had similar level of association between then regimes, which was about 0.5. A lower magnitude of correlation with N0 and other N regimens was observed for upper ground biomass.

As in the case of grain yield per plant, the biomass also showed good correlation of 0.71 between N50 and N100 treatments. A moderate level of correlation was found between N0 and N50 levels for spikelet fertility, which was further reduced between N0 and N100 levels. Spikelet fertility had a good agreement between N50 and N100 treatments. On the contrary, SPAD chlorophyll reading showed good agreement between N0 level and remaining two N levels with values 0.66 and 0.51 respectively for N50 and N100. However, SPAD readings had a lower degree of association between N50 and N100. There was significant association observed between N0 and N50 as well as N50 and N100 level for

Flag Leaf N. Further, total plant N showed almost similar agreement between all the N levels.

4.1.3 Genotypic performance under different N regimens

Based on field performance of genotypes under three N regimens the average data presented in Tables 8, Table 9 and Table 10.

Table 7. Correlation coefficients for the Agronomic and N uptake traits between different N regimes

Parameters	N ₀ -N ₅₀	N ₀ -N ₁₀₀	N ₅₀ -N ₁₀₀
DFF	0.90**	0.97**	0.91**
PHT	0.90**	0.90**	0.97**
NT	0.50**	0.49**	0.50**
PL	0.77**	0.63**	0.84**
UGB	0.57**	0.64**	0.71**
GPP	0.54**	0.33**	0.63**
SF	0.57**	0.31*	0.75**
GYPP	0.46**	0.44**	0.71**
HI	0.76**	0.71**	0.88**
SPAD	0.66**	0.51**	0.39**
FLN	-0.04	0.29*	-0.02
NHI	0.56**	0.39**	0.56**
AgNUE	-	-	0.68**
NAE	0.22	0.11	0.25*
pNUE	0.58**	0.57**	0.69**
GYEI	-	-	0.89**
NRE	-	-	-0.38**
CSI	-	0.63**	-
TPIN	0.51**	0.44**	0.49**

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant; NHI, nitrogen harvest index; AgNUE, agronomic N use efficiency; NAE, N assimilation efficiency; pNUE, physiological NUE, GYEI, grain yield efficiency index; NRE, N recovery efficiency; CSI, chlorophyll sufficiency index; FLN, flag leaf nitrogen; SPAD, soil plant analysis development chlorophyll index

4.1.3.1 Days to 50% flowering

Under N₀ condition, the days to 50% flowering was maximum in BPT 5204 (134 Days) and lowest days for this trait was recorded in IR 24 (80 Days). Under N₅₀

condition, this varied between 73 Days to 136 Days in IR 24 and BPT 5204 respectively. The lowest days for 50% flowering recorded in Jaldi Dhan13 and highest in BPT 5204 under N100 condition.

4.1.3.2 Plant height

Under N0 condition the maximum plant height recorded for Pokkali (134.6 cm) and minimum was recorded for Pusa 677 with 59.4 cm. Average height of the plants under N0 condition was 87.1 cm. Under N50 condition, Nagina 22 had the tallest plants and shortest plants was observed in Jaldhi Dhan 13 with an average height of 61.6 cm. The same genotypes produced the extreme phenotypes for plant height under N100 condition also.

4.1.3.3 Tiller number

TKM 6 produced on an average of 13.1 tiller under N0 condition with Taipei 309 recorded as low as 3.3 tiller per plant. This trait had a range of 5.17 to 14.9 under N50 level recorded in the lines super Basmati and Taraori Basmati respectively. Highest number of tiller of 18.7 was recorded under N100 condition in ADT 38. The minimum number of tillers produced under this level was 5.4 in Taipei 309.

4.1.3.4 Panicle length

The cultivar Kasturi had the longest panicles in all the N regimes tested. Under No, the average panicle length for this genotype was 31.3 cm, while 29.7 cm and 30.2 cm long panicles were recorded under N50 and N100 levels. The shortest panicle under No condition was observed in Vandana with an average length of 16.9 cm while MTU 5249 produced the shortest panicle of 19.0 cm long under N50 level. Vandana also had shortest panicle under N100 condition (17.5 cm).

4.1.3.5 Upper ground biomass per plant

Under N0 condition, the maximum shoot biomass was recorded for Pusa Sugandh 5 (74.5 g) and lowest in Taipei 309 (15.6g) with the average of 35.9 g per plant. Maximum upper ground biomass per plant was recorded in Thai rice (83.3 g) and lowest was in Super Basmati which was 19.3 g under N50 level. The average biomass in this level was 42.3 g per plant. Under N100 condition, the highest biomass recorded was 85.8 g and the lowest as 19.4 g in IR66 and Rasi respectively, with an average value of 47.8 g.

Table 8. Mean genotypic performance for agronomic and plant N parameters under N unfertilised conditions (NO level)

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	SPAD	FLN	TPIN
Acharmati	104.0	9.4	4.4	23.2	101.6	39.0	217.9	69.3	24.3	34.2	3.6	0.3
ADT 38	110.0	9.1	11.3	23.4	70.2	46.3	103.0	60.1	19.6	38.2	2.1	0.4
ADT 39	115.0	6.6	9.9	21.0	63.6	18.8	103.0	74.4	34.9	35.2	3.0	0.2
ADT 42	105.1	15.7	9.6	26.3	84.3	58.0	135.0	75.5	27.1	38.3	8.0	0.7
ASD 16	84.2	9.7	5.4	23.1	76.8	29.8	133.1	82.1	32.5	49.7	7.2	0.4
ASD 18	95.0	16.9	8.3	22.5	69.8	38.4	119.9	95.5	44.1	42.0	8.3	0.4
ASD 19	112.0	11.0	8.0	20.8	114.9	38.3	117.1	89.5	28.6	36.3	4.1	0.5
Basmati 370	104.0	5.0	4.1	23.6	114.4	28.2	90.1	54.6	17.8	29.4	2.8	0.2
BPT 5204	134.0	5.9	5.4	20.2	89.5	15.6	168.0	67.2	37.7	41.5	5.2	0.1
Chandrasahini	92.0	12.2	4.7	25.6	79.2	36.7	172.9	81.9	33.2	36.1	2.8	0.3
CO37	110.0	10.6	10.3	23.1	92.9	46.9	140.0	85.4	22.6	33.3	7.4	0.3
CO51	92.0	10.6	11.1	20.9	80.2	28.6	90.3	90.9	37.0	40.0	6.8	0.3
CR Dhan 310	104.7	6.7	5.3	22.8	97.5	16.4	135.2	79.7	40.6	48.6	3.6	0.1
CR Dhan 311	106.7	6.0	5.3	22.2	107.7	15.6	150.1	78.5	38.4	37.8	3.3	0.1
CSR27	92.0	12.3	10.1	23.0	82.1	46.3	146.0	82.9	26.6	39.5	3.5	0.5
Himdhan	83.9	7.2	6.1	19.0	90.3	29.8	90.8	87.9	24.2	30.6	2.7	0.3
Improved Sabarmati	103.0	13.8	9.7	22.7	78.8	63.5	96.0	47.2	21.7	38.3	4.6	0.6
IR 24	79.9	3.2	5.6	20.3	93.3	26.5	145.8	58.0	12.0	43.9	3.4	0.2
IR 50	84.0	8.8	8.6	21.7	71.8	29.9	113.9	92.5	29.4	49.6	5.7	0.4
IR 64	95.0	10.1	10.1	21.7	69.9	44.8	89.5	83.2	22.5	38.8	7.2	0.5
IR 66	107.9	7.9	9.1	24.2	79.7	46.3	158.1	53.9	17.1	37.7	3.9	0.9
IR 70	115.0	8.6	11.0	23.0	66.4	44.6	105.1	43.5	19.4	39.6	3.2	0.4
IR 72	84.1	8.9	5.2	22.8	65.2	26.5	123.1	70.8	33.5	33.8	3.6	0.3

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

Table 8. Contd.

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	SPAD	FLN	TPIN
Jaiphoola	120.0	10.7	10.3	20.7	100.6	55.7	229.4	88.8	19.2	33.0	2.6	0.4
Jaldi Dhan 13	80.0	9.4	7.6	20.9	59.6	22.9	90.0	90.3	40.8	45.2	4.6	0.2
Kamlesh	91.9	12.0	4.1	23.6	105.2	35.1	127.1	82.8	34.3	34.1	2.2	0.6
Kasturi	92.0	9.0	5.0	31.3	101.2	49.1	84.0	65.6	18.3	36.3	2.3	0.4
Kavya	105.0	7.8	8.0	20.8	71.6	36.4	120.9	88.3	21.4	34.5	2.8	0.2
Kranthi	84.0	10.6	5.1	22.8	72.2	31.1	104.0	81.8	33.9	35.2	2.0	0.3
Mahalaxmi	115.0	15.1	11.9	23.1	81.5	59.0	257.3	59.4	25.5	37.6	2.0	0.4
Mahamaya	95.0	16.4	7.7	19.3	80.2	64.4	139.9	93.8	25.5	40.0	6.5	0.6
MTU 1010	98.7	6.1	5.0	20.2	90.7	15.6	106.1	73.5	39.3	40.7	2.2	0.1
MTU 5249	114.7	8.4	7.9	18.2	60.4	38.2	164.9	84.5	22.0	32.3	3.6	0.4
Nagina 22	97.7	6.4	5.3	21.2	125.0	16.1	99.1	82.2	39.4	35.9	1.5	0.1
Nidhi	107.0	7.2	5.0	25.1	96.0	17.1	181.0	85.7	42.1	45.8	2.2	0.1
Pant Dhan 19	105.0	8.1	6.1	20.2	75.2	25.2	168.9	74.7	32.3	34.9	3.2	0.2
Pant Sugandh Dhan 15	102.0	12.8	8.8	27.7	94.4	64.8	117.0	51.6	19.7	34.9	3.5	0.8
Pant Sugandh Dhan 17	92.0	15.3	6.1	26.0	91.4	54.4	112.1	67.1	28.2	35.1	2.2	0.6
Parvel	111.7	5.9	4.0	23.7	82.9	15.7	158.9	77.8	37.6	40.5	7.7	0.1
PB 1121	95.0	4.6	11.8	24.5	78.6	44.3	62.1	36.0	10.4	41.1	4.5	0.7
PB 1509	90.0	3.2	8.9	23.2	69.4	22.6	87.0	36.3	14.3	37.4	2.3	0.2
PNR 162	95.0	7.2	9.2	24.9	76.6	22.9	90.0	77.8	31.6	38.3	5.9	0.2
Pokkali	102.1	10.0	4.0	23.8	134.6	39.7	111.0	73.8	25.1	38.4	2.0	0.3
Pusa 1176	102.0	5.6	8.2	25.9	77.8	34.5	130.0	57.8	16.1	43.0	5.3	0.3
Pusa 1592	108.0	4.3	7.0	23.0	92.2	24.6	83.3	78.4	17.3	37.0	2.4	0.2

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

Table 8. Contd.

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	SPAD	FLN	TPIN
Pusa 1612	92.0	7.0	4.1	22.0	75.0	30.5	66.1	69.6	22.9	38.5	2.8	0.3
Pusa 44	90.1	4.3	5.1	25.4	87.0	19.7	118.1	51.8	21.9	38.4	3.5	0.2
Pusa 677	104.3	11.0	9.9	22.8	72.5	42.5	135.8	86.3	25.9	39.8	3.4	0.3
Pusa Basmati 1	95.0	14.6	6.1	17.5	59.4	36.6	94.0	76.6	39.9	33.8	6.2	0.4
Pusa Sugandh 5	104.0	8.6	9.9	26.1	99.5	74.5	114.1	57.0	11.6	34.1	4.7	0.5
Ranbir Basmati	89.9	4.1	4.5	25.0	121.8	25.7	91.0	80.1	15.8	30.2	2.0	0.2
Rasi	94.0	6.4	5.0	20.2	92.4	16.9	90.0	78.2	38.0	43.5	4.1	0.1
Sharbati	102.0	10.8	9.1	21.8	98.8	46.8	74.0	71.7	23.0	33.8	5.9	0.3
Sitwa Dhan	105.0	7.7	5.5	22.5	64.3	31.0	121.1	66.3	24.7	38.9	2.7	0.3
Sonasal	90.0	5.4	6.7	22.6	110.9	29.5	111.9	64.5	18.3	34.2	7.0	0.2
Super Basmati	92.1	6.3	7.0	25.1	89.3	25.9	95.0	78.9	24.5	38.9	5.4	0.2
Swarna Sub1	92.0	8.8	8.0	23.5	76.0	41.0	96.9	74.9	21.3	31.0	1.9	0.3
Taipei 309	101.7	6.0	3.3	20.2	101.5	15.6	89.4	74.6	38.9	47.3	1.6	0.1
Taraori Basmati	104.0	6.5	7.9	23.4	101.4	28.9	89.0	79.9	22.3	32.7	3.2	0.3
Thai Rice	112.0	15.9	11.0	24.8	100.6	49.4	124.0	62.9	32.3	33.9	2.0	0.3
TKM6	95.0	11.6	13.1	22.9	104.8	66.6	80.4	87.4	17.4	36.6	3.3	0.6
TKM9	98.9	14.6	10.0	23.0	78.8	37.7	66.2	68.4	38.8	33.1	5.5	0.2
Vandana	84.0	4.6	3.7	16.9	96.6	20.2	94.1	83.0	22.9	37.3	4.3	0.1
WGL 23985	104.0	12.1	6.0	21.5	73.4	45.7	162.1	55.0	26.6	40.5	3.4	0.5
White Ponni	122.0	8.9	10.0	22.1	98.1	46.9	146.9	73.7	18.9	30.2	2.7	0.3
Mean	9.0	7.4	22.7	87.1	35.9	120.4	73.1	37.7				
CD 0.05 (Tukey's)	3.52	1.95	2.08	2.13	2.96	6.19	11.64	4.48				

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

4.1.3.6 Grains per panicle

Under N0 condition, Mahalaxmi produced on average 257 grains per panicle which was highest among all the genotypes. The lowest number of grains was shown by PB1121 under unfertilized condition. Grains per panicle was the highest for Chandrahasini under N50 condition, while the lowest number of grains was recorded in Himdhan with average number of 51 grains per panicle. Under N100 condition, 220 grains per panicle was observed in Nidhi and lowest number of grains in this level was recorded in Jaiphoola.

4.1.3.7 Spikelet fertility

The maximum spikelet fertility recorded was 98.5%, 95.5% and 96.9% respectively under conditions N0, N50, N100 respectively. Most of the genotypes had good level of spikelet fertility in all the three N regimens. However, lowest spikelet fertility was recorded in PB 1509 under N0 condition, while IR24 and Jaldi Dhan 13 had high level of sterility under N50 and N100 conditions, respectively.

4.1.3.8 Grain yield per plant

Under N0 condition, maximum grain yield per plant recorded was from ASD 18 (16.9 g) and the lowest was from IR24 (3.2 g). The average yield under no N application was 9.0 g grain yield per plant for all the genotypes tested. Under N50 level, the maximum grain yield of 21.1g was observed in Pant Sugandh Dhan 15 and the minimum was recorded in Sonasal (3.7 g). The average grain yield at this level was 10.49 g. Maximum grain yield under N100 level was observed in Mahamaya (25.1g), while White Ponni yielded the poorest (1.6 g) with an average of 12.5 g per plant.

4.1.3.9 Harvest index

Harvest index under N unfertilized level showed a range from 10.4% in Pusa Basmati 1121 to 44.1 % in ASD 18. Maximum harvest index of 45.1% was computed for Nidhi under N50 level and 45.9% under N100 level, which was the highest value for this trait at that level among all the genotypes. White Ponni recorded lowest HI of 7.8% under N50 as well as in N100 conditions (4.1%).

Table 9. Mean genotypic performance for agronomic and plant N parameters under 50% N conditions (N50 level)

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	SPAD	FLN	TPIN
Acharmati	102.0	12.0	6.0	23.8	107.2	38.3	229.9	78.2	33.4	31.4	8.5	0.1
ADT 38	108.0	11.8	8.6	22.4	70.2	53.0	99.0	52.8	44.0	22.3	7.3	0.5
ADT 39	114.0	7.4	10.3	20.8	63.6	24.4	121.0	57.3	34.2	30.2	7.2	0.2
ADT 42	110.0	16.0	9.0	24.1	73.9	64.6	127.1	74.1	43.7	24.7	6.6	0.5
ASD 16	94.9	18.1	6.7	26.3	81.4	54.4	165.0	93.8	48.8	33.3	6.0	0.3
ASD 18	92.1	10.5	7.1	24.6	73.0	41.5	126.9	84.4	43.8	25.3	6.6	0.2
ASD 19	112.0	10.0	10.9	20.8	102.9	49.4	146.0	85.0	48.2	20.3	8.0	0.4
Basmati 370	104.0	6.8	8.3	24.4	116.0	38.0	101.1	62.5	34.2	18.0	8.3	0.3
BPT 5204	135.7	9.7	7.3	23.7	98.5	22.4	156.8	68.2	39.3	43.3	6.1	0.2
Chandrahasini	92.0	11.8	6.3	25.6	82.0	33.9	233.6	85.3	42.4	34.9	6.8	0.2
CO37	110.0	13.2	10.0	22.8	87.4	46.7	149.0	72.0	38.9	28.3	8.5	0.3
CO51	92.0	11.2	5.8	21.6	78.6	39.7	169.8	91.2	36.4	28.2	6.1	0.6
CR Dhan 310	104.0	9.7	7.6	26.0	124.6	22.0	158.4	90.0	51.3	43.9	2.5	0.2
CR Dhan 311	106.7	8.9	7.3	25.6	130.9	21.1	172.6	69.1	47.4	42.1	3.6	0.2
CSR27	92.0	19.8	13.0	23.6	92.8	79.5	169.9	89.3	42.4	24.9	15.7	0.9
Himdhan	77.1	5.9	8.1	20.7	98.2	32.9	51.2	94.0	36.2	17.9	4.7	0.2
Improved Sabarmati	103.0	8.9	10.0	21.2	79.0	45.4	98.0	54.3	41.0	19.6	4.5	0.4
IR 24	73.1	4.2	6.3	22.3	100.8	27.8	100.2	45.3	45.1	15.1	3.4	0.2
IR 50	84.0	9.0	7.8	20.7	68.8	28.9	111.0	76.8	60.3	31.2	7.4	0.3
IR 64	93.5	7.8	8.4	21.2	64.5	36.9	100.0	84.1	38.1	21.2	5.2	0.3
IR 66	105.0	15.0	11.7	23.4	68.5	73.9	192.9	60.7	40.4	20.3	5.2	0.6
IR 70	115.0	13.0	12.3	23.2	63.8	45.5	124.0	78.7	52.4	28.5	6.4	0.3
IR 72	90.0	15.4	11.9	23.6	67.0	39.1	140.0	79.2	41.6	39.3	8.3	0.3

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

Table 9. Contd.

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	SPAD	FLN	TPIN
Jaiphoola	121.0	4.9	10.0	19.6	97.0	60.3	140.1	85.6	35.1	8.1	9.4	0.6
Jaldi Dhan 13	89.8	9.0	5.4	19.6	61.6	28.9	86.0	52.9	44.1	31.1	6.7	0.2
Kamlesh	84.1	14.2	7.9	24.6	116.4	41.3	144.0	91.6	40.5	34.4	9.8	0.3
Kasturi	92.0	8.4	7.0	29.7	99.2	48.1	81.0	76.5	38.2	17.5	10.1	0.3
Kavya	106.0	10.6	10.6	20.6	73.0	50.7	95.1	86.3	38.0	20.9	11.2	0.3
Kranthi	80.1	10.8	8.0	24.1	85.7	30.5	120.0	93.2	42.0	35.4	2.7	0.4
Mahalaxmi	115.0	9.6	11.0	21.1	73.6	63.7	110.4	68.2	32.0	15.1	4.5	0.4
Mahamaya	95.0	16.5	8.0	21.6	83.0	47.9	117.1	73.8	40.9	34.4	6.5	0.4
MTU 1010	99.0	8.3	7.0	23.9	97.6	20.3	143.6	95.0	48.8	41.1	4.3	0.2
MTU 5249	82.6	9.6	8.6	19.0	65.0	39.7	140.1	71.7	29.2	24.2	4.5	0.2
Nagina 22	98.0	10.1	6.3	23.0	140.5	22.8	98.8	95.5	40.7	44.1	2.6	0.2
Nidhi	106.7	12.4	6.3	26.0	103.1	27.4	160.9	89.5	47.4	45.1	2.3	0.3
Pant Dhan 19	104.0	21.1	9.6	21.0	89.7	62.0	146.1	85.5	39.1	34.1	8.2	0.4
Pant Sugandh Dhan 15	102.0	13.6	6.7	26.2	97.6	49.3	131.0	65.7	32.2	27.6	4.3	0.4
Pant Sugandh Dhan 17	92.0	10.7	8.6	26.2	89.8	37.5	123.0	77.2	38.7	28.4	4.6	0.3
Parvel	111.0	8.0	5.3	23.1	99.1	19.5	180.5	95.3	44.6	41.2	2.7	0.1
PB 1121	95.0	5.6	8.4	23.1	82.2	46.6	75.0	59.9	34.6	12.1	4.0	0.7
PB 1509	84.1	8.4	8.0	25.5	74.8	33.1	97.0	70.9	36.7	25.2	9.9	0.3
PNR 162	95.0	13.5	6.5	23.6	74.6	38.1	101.0	81.2	45.7	35.5	8.8	0.4
Pokkali	105.0	9.6	7.6	24.2	135.2	62.5	115.0	79.0	41.3	15.3	2.4	0.8
Pusa 1176	108.0	8.4	5.5	22.6	91.4	42.6	149.9	89.9	41.3	19.7	6.7	0.3
Pusa 1592	92.0	6.4	6.0	22.2	70.5	29.1	81.0	65.6	36.3	22.1	8.6	0.3

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

Table 9. Contd.

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	SPAD	FLN	TPIN
Pusa 1612	92.0	13.8	8.9	26.0	91.8	63.3	149.9	52.2	32.6	21.7	6.3	0.5
Pusa 44	104.3	10.9	9.7	22.8	72.2	42.1	135.7	86.5	39.9	25.8	3.4	0.3
Pusa 677	95.0	15.4	6.8	19.0	62.8	51.7	66.2	78.8	39.1	29.8	7.3	0.3
Pusa Basmati 1	102.0	6.8	7.4	26.2	81.6	44.8	121.1	66.1	41.5	15.1	4.5	0.3
Pusa Sugandh 5	102.0	9.6	8.7	26.0	90.6	48.6	141.0	59.6	37.4	19.9	3.5	0.4
Ranbir Basmati	84.1	8.4	8.6	24.9	128.2	36.2	100.0	89.8	40.5	23.2	9.2	0.3
Rasi	93.7	8.4	7.3	22.1	91.8	20.1	119.5	94.3	40.4	41.9	4.7	0.2
Sharbati	102.0	10.8	12.9	21.2	91.6	62.2	75.1	79.9	31.1	17.3	5.7	0.6
Sitwa Dhan	104.0	10.1	8.1	24.4	74.6	35.4	126.1	81.6	42.2	28.4	4.2	0.3
Sonasal	92.0	3.7	7.1	21.5	99.5	39.4	95.1	81.9	31.5	9.3	4.7	0.3
Super Basmati	95.0	5.4	5.2	23.6	81.4	19.3	101.0	84.1	33.5	27.9	5.0	0.2
Swarna Sub1	92.0	8.4	9.6	22.2	80.2	25.1	92.0	52.6	33.6	33.4	3.4	0.2
Taipei 309	101.0	9.5	5.9	24.5	104.9	22.4	191.4	64.1	46.8	42.5	5.2	0.2
Taraori Basmati	105.0	11.6	14.9	23.0	113.0	70.9	90.1	94.2	31.0	16.3	5.2	0.5
Thai Rice	112.0	14.2	13.2	25.4	105.2	83.3	140.0	60.9	35.3	17.1	8.3	0.4
TKM6	95.0	11.8	7.9	24.2	105.4	58.3	154.9	90.2	33.9	20.3	3.4	0.5
TKM9	95.0	16.0	12.0	24.5	83.6	41.6	98.0	84.6	40.6	38.5	4.4	0.2
Vandana	84.0	9.9	5.7	19.7	94.6	41.1	166.7	80.4	43.2	24.2	7.6	0.3
WGL 23985	105.0	7.3	8.0	20.2	70.6	23.9	194.9	59.0	40.5	30.5	8.6	0.2
White Ponni	120.0	4.4	13.5	20.2	80.5	56.4	120.1	91.5	33.9	7.8	8.0	0.3
Mean	99.1	10.5	8.5	23.1	89.2	42.3	128.6	77.0	40.0	26.9	6.2	0.3
CD 0.05 (Tukey's)	2.98	2.43	2.36	2.03	3.04	8.56	9.32	13.92	3.46	4.6	0.93	0.077

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

4.1.3.10 SPAD chlorophyll index

SPAD Index ranged between 29.4 in Basmati 370 and 49.7 in ASD 16 under N0 condition. Under N50 level, this trait had a range of 29.2 (MTU 5249) to 60.3 in IR50. There was an increase in SAPD values for all the genotypes under N100 level, with range beginning from 32.0 in Nagina 22 to 53.4 in IR 66.

4.1.3.11 Flag leaf N

The N in flag leaf was highest in ASD 18 with average of 8.3 mg/g under N0 condition. Lowest Flag leaf N was recorded in Nagina 22 (1.5 mg/g) under N0 level. CSR 27 possessed the maximum Flag leaf N of 15.7 mg/g among all the genotypes under N50 level while Nidhi had the lowest N content of 2.3 mg/g. Flag leaf N under N100 level, ranged between 0.59 mg/g in MTU 1010 and 10.5 mg/g in TKM9.

4.1.3.12 Total plant N

Total plant N content of genotypes varied between 0.80 to 0.88 g/plant under N unfertilized condition with BPT 5204 and IR66 showing the extreme values respectively. Minimum amount of N in the plant was recorded in Panvel (0.13g/plant) and the maximum of 0.89 g/plant in CSR 27 when the plants were provided N50 level. Further, the range of total plant N fell between 0.14 (Sonasal) and 1.1 g/plant (Swarna Sub 1).

4.1.4 N Use parameters

Six N uptake and assimilation related parameters were examined with respect to the performance of genotypes under three N regimens in present study. The data is presented in Table 11, Table 12 and Table 13.

4.1.4.1 N harvest index

N harvest index under N unfertilized condition ranged between 5.9% to 80.9%. The maximum index was recorded in TKM 9 and minimum was in PB1509. Under N50 level, it ranged between 6.4% in Pusa Basmati 1121 to 78.9% in TKM9. The lowest N harvest index was computed for White Ponni (12.7%) while Nidhi showed the highest index of 68.5% when the genotypes were exposed to N100 level.

Table 10. Mean genotypic performance for agronomic and plant N parameters under 100% N conditions (N100 level)

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	SPAD	FLN	TPIN
Acharmati	104.0	18.3	9.9	22.8	108.8	50.8	176.3	80.8	39.5	36.1	2.9	0.4
ADT 38	110.0	18.1	18.7	22.8	71.0	64.9	104.0	72.0	41.6	27.9	3.1	0.5
ADT 39	114.0	3.7	8.8	20.4	58.9	26.3	117.0	76.1	41.5	14.0	3.4	0.2
ADT 42	110.0	10.5	10.4	23.7	79.0	43.6	151.9	86.2	43.2	24.1	5.4	0.3
ASD 16	91.0	17.2	10.0	25.0	80.2	46.9	176.9	96.6	48.5	36.7	3.5	0.4
ASD 18	95.0	15.2	11.6	25.1	71.8	50.8	106.1	88.0	41.7	30.0	4.2	0.5
ASD 19	112.0	12.8	11.1	20.8	109.0	61.9	153.9	89.1	35.1	20.7	2.7	0.3
Basmati 370	102.0	11.0	9.6	24.4	125.4	42.2	134.9	81.9	34.0	26.0	3.1	0.3
BPT 5204	136.0	10.5	10.3	26.1	97.0	23.4	180.6	80.8	38.5	45.0	3.5	0.2
Chandrabhasini	93.0	15.0	9.0	25.4	78.8	37.7	100.5	72.4	39.9	39.8	4.0	0.3
CO37	110.0	16.6	13.0	21.1	88.2	56.2	154.0	81.9	34.7	29.5	3.5	0.4
CO51	92.0	12.2	10.1	22.4	77.8	39.1	155.9	90.6	39.4	31.3	2.4	0.4
CR Dhan 310	105.0	12.0	7.1	26.9	129.5	26.4	218.4	94.2	40.5	45.5	3.7	0.3
CR Dhan 311	106.7	9.7	7.1	26.9	138.9	21.7	181.6	80.4	43.9	44.6	2.7	0.2
CSR27	95.0	22.4	15.0	23.6	94.2	67.7	156.0	93.0	37.9	33.0	4.3	0.8
Himdhan	80.0	14.9	14.2	19.4	113.1	70.0	75.0	93.5	35.8	21.3	3.4	0.5
Improved Sabarmati	103.0	17.9	13.3	21.1	82.4	81.3	105.0	85.2	43.7	22.1	6.5	0.8
IR 24	76.0	3.4	6.1	20.5	108.8	28.1	130.0	69.8	44.2	12.2	5.6	0.3
IR 50	84.0	11.2	12.6	21.2	69.4	36.2	87.2	79.6	51.9	30.9	3.9	0.3
IR 64	95.0	15.1	11.2	21.8	70.0	60.1	93.0	87.4	42.5	25.1	3.8	0.7
IR 66	105.0	20.9	14.9	24.2	79.0	85.8	181.0	76.6	53.4	24.4	5.2	0.7
IR 70	115.0	14.8	15.0	22.6	65.2	57.0	142.9	87.1	35.0	26.0	3.4	0.5
IR 72	91.9	16.0	12.9	22.1	69.0	38.8	151.9	82.3	44.6	41.2	3.1	0.4

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

Table 10. Contd.

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	SPAD	FLN	TPIN
Jaiphoola	123.9	7.1	12.4	20.0	101.2	80.8	72.6	76.8	39.2	8.7	3.4	0.7
Jaldi Dhan 13	75.2	9.6	9.0	19.8	65.2	24.5	97.0	69.3	44.2	39.3	2.6	0.2
Kamlesh	88.0	16.4	9.0	24.2	110.8	55.7	160.9	94.5	39.1	29.4	2.6	0.6
Kasturi	92.0	9.8	8.0	30.2	101.8	54.4	93.0	84.9	37.8	18.0	4.6	0.4
Kavya	108.0	10.4	9.1	21.6	76.4	42.7	125.0	90.5	40.6	24.4	3.3	0.3
Kranthi	84.0	10.8	11.6	22.7	80.8	40.2	114.0	94.8	37.7	27.0	3.4	0.4
Mahalaxmi	119.9	7.3	12.1	21.3	75.6	76.9	119.3	80.5	39.1	9.5	3.5	0.8
Mahamaya	95.0	25.1	11.0	21.7	86.4	71.5	125.1	82.6	42.8	35.1	3.5	0.6
MTU 1010	99.0	10.6	8.4	24.9	102.8	23.6	144.6	94.2	46.9	44.9	0.6	0.2
MTU 5249	114.7	12.0	9.1	18.6	62.2	49.0	146.1	84.3	36.6	24.5	0.8	0.4
Nagina 22	97.0	10.8	7.1	25.8	159.4	23.9	139.5	96.9	32.0	45.4	3.7	0.2
Nidhi	106.7	13.1	7.1	27.6	117.3	28.6	220.5	84.6	48.9	45.9	2.2	0.3
Pant Dhan 19	103.0	20.8	10.7	21.0	82.0	62.5	162.0	90.7	40.0	33.2	3.8	0.5
Pant Sugandh Dhan 15	102.0	12.3	8.1	25.1	94.6	46.7	120.1	85.5	37.6	26.3	5.3	0.4
Pant Sugandh Dhan 17	92.0	15.6	11.0	25.8	89.2	60.9	139.9	84.9	34.8	25.6	7.5	0.5
Parvel	112.0	9.3	7.3	25.9	98.1	21.2	131.9	94.7	49.0	43.8	3.7	0.2
PB 1121	95.0	9.8	11.1	24.2	90.6	46.3	103.9	81.3	37.9	21.1	4.2	0.3
PB 1509	91.9	6.2	7.8	22.3	70.8	24.2	96.0	87.0	45.1	25.8	3.0	0.2
PNR 162	95.0	10.6	10.3	22.7	80.0	39.4	87.1	86.3	38.3	27.0	3.0	0.3
Pokkali	107.9	8.7	6.7	22.7	136.6	48.4	102.1	79.5	40.7	17.9	2.4	0.6
Pusa 1176	108.0	12.9	11.9	23.6	93.4	54.2	167.8	92.3	42.5	23.9	6.2	0.4
Pusa 1592	95.0	9.0	9.3	22.8	81.6	37.2	102.9	80.5	36.2	24.2	3.4	0.3

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

Table 10. Contd.

Genotype	DFF	GYPP	NT	PL	PHT	UGB	GPP	SF	HI	SPAD	FLN	TPIN
Pusa 1612	94.9	17.1	10.6	26.8	93.6	64.2	146.0	80.4	39.6	26.7	2.9	0.4
Pusa 44	104.3	11.3	10.3	22.9	73.0	43.5	136.0	86.6	40.0	26.0	3.4	0.3
Pusa 677	95.0	21.5	15.7	20.9	67.2	55.4	120.8	86.8	50.9	38.9	9.4	0.5
Pusa Basmati 1	103.0	3.1	9.1	24.1	80.8	28.1	138.0	77.4	41.9	11.0	4.6	0.3
Pusa Sugandh 5	103.0	16.9	10.1	24.7	90.9	71.4	147.9	72.2	43.2	23.7	5.1	0.5
Ranbir Basmati	88.0	12.2	11.9	26.7	121.3	48.7	101.0	80.5	38.3	25.0	1.1	0.4
Rasi	94.0	8.4	8.0	25.8	95.5	19.4	89.8	96.5	45.3	43.3	1.2	0.2
Sharbati	103.0	8.7	12.1	21.6	99.0	48.4	100.9	86.1	37.7	17.9	5.4	0.4
Sitwa Dhan	104.0	19.7	14.8	25.1	77.2	73.0	185.7	86.0	38.5	27.0	2.2	0.6
Sonasal	92.0	11.9	13.5	23.3	112.2	80.7	112.0	86.5	44.0	14.8	6.0	1.1
Super Basmati	95.0	3.9	12.5	21.7	85.2	29.4	119.9	85.1	38.9	13.1	5.7	0.3
Swarna Sub1	95.0	7.3	9.1	22.4	88.2	25.4	92.0	77.1	39.3	28.5	1.8	0.1
Taipei 309	102.0	9.5	5.4	23.9	104.9	21.5	183.5	96.5	47.3	44.3	4.1	0.2
Taraori Basmati	104.0	14.0	15.9	23.6	108.0	73.0	118.9	90.9	39.5	19.1	2.7	0.6
Thai Rice	112.0	14.6	11.5	24.3	108.8	67.1	137.0	78.0	36.8	21.8	6.0	0.3
TKM6	95.0	13.6	14.0	24.2	117.3	66.1	175.8	89.4	43.8	20.6	0.9	0.4
TKM9	91.1	18.6	14.6	22.5	82.4	46.0	125.8	90.4	42.6	40.4	10.5	0.3
Vandana	84.0	6.5	8.6	17.5	96.8	39.8	101.2	96.9	37.1	16.2	3.6	0.3
WGL 23985	105.0	12.6	9.7	19.9	71.8	38.5	183.0	72.0	42.7	32.7	3.6	0.4
White Ponni	123.0	1.6	11.1	18.9	81.1	38.9	126.1	92.9	36.3	4.1	2.3	0.2
Mean	100.2	12.4	10.8	23.2	91.7	47.8	132.3	85.1	41.0	27.6	3.8	0.4
CD 0.05 (Tukey's)	2.6	2.2	2.5	1.7	3.5	6.8	13.6	4.5	3.9	4.2	1.3	0.1

DFF, days to 50% flowering; GYPP, grain yield per plant; NT, number of tillers; PL, panicle length in cm; PHT, plant height in cm; UGB, upper ground biomass in g; GPP, grains per panicle; SF, spikelet fertility in %; HI, harvest index; TPIN, total plant N in g/plant

4.1.4.2 N assimilation efficiency

N assimilation efficiency was the lowest for IR66 (52.4) and highest for Panvel (202.3) in N0 condition while PB1121 had the lowest efficiency of 65.0 and Acharmati had the highest efficiency of 297.7 in Under N50 condition. N assimilation efficiency of genotypes varied between 73.4 in Sonasal to 202.7 in Thai rice under N sufficient condition.

4.1.4.3 Physiological N Use Efficiency

Physiological NUE ranged from 6.8 to 76.1 under N unfertilized condition with the lowest value recorded in PB1121 and highest in Panvel. This is increased to 7.8 to 93.46 respectively in PB1121 and Acharmati when the plants were exposed to N50 level. The lowest value for this estimate further increased to 8.38 in White Ponni while it was found decreased to 56.46 in TKM9 under N100 condition.

4.1.4.4 Agronomic N Use Efficiency

Agronomic NUE was minimum to a level of -32.17 in ASD 18 and highest for Pant Dhan 19 (64.77) under N50 condition. Under N100 level, efficiency shifted to a minimum of -19.4 in Mahalaxmi, while it was maximum at 32.5 for IR66.

4.1.4.5 Grain yield efficiency index

Grain yield efficiency index ranged between 0.14 to 2.85 under N50 condition with IR 24 recording lowest and Mahamaya recording highest. This estimate for the genotypes exposed to N100 condition ranged from 3.66 in Mahamaya to the lowest value of 0.10 in IR24.

4.1.4.6 N recovery efficiency

N recovery efficiency of genotypes varied between -2.06 to 2.43 with Pant Sugandh Dhan 15 and Pokkali showing highest and lowest values respectively under N50. This range was further narrowed down to -1.03 (PB1121) to 1.95 (Sonasal), Under N100 condition.

4.1.5 Interrelations of N use parameters

N use efficiency parameters showed, under N0 condition, positive and significant association for grain yield per plant and N harvest index, however,

Table 11. Average estimators of N use parameters in the test germplasm under NO level

Genotype	NHI	NAE	pNUE	Genotype	NHI	NAE	pNUE
Acharmati	41.6	144	34.9	Nidhi	61.6	118.8	50
ADT 38	42	120	23.5	Pant Dhan 19	51.1	130.4	42.2
ADT 39	64	114.5	40	Pant Sugandh Dhan 15	40.9	77.3	15.2
ADT 42	54.8	87.6	23.8	Pant Sugandh Dhan 17	44.7	93.9	26.5
ASD 16	65.5	85.1	27.7	Panvel	62.1	202.3	76.1
ASD 18	67.9	109.1	48.1	PB 1121	10	65.3	6.8
ASD 19	29.5	79.6	22.8	PB 1509	5.9	118.4	16.9
Basmati 370	28.8	120.7	21.5	PNR 162	51	129.2	40.8
BPT 5204	63.3	180.4	68.1	Pokkali	45.2	132.7	33.3
Chandrasahini	50.8	116.3	38.7	Pusa 1176	32.1	114	19.7
CO37	46.3	145.8	33	Pusa 1592	46.3	96.9	22.2
CO51	54.3	106.9	39.5	Pusa 1612	43.2	123.1	27
CR Dhan 310	59.8	170.2	69	Pusa 44	46.5	149.1	38.5
CR Dhan 311	63.5	183.2	70.3	Pusa 677	58.8	91.3	36.5
CSR27	40.1	103.3	27.5	Pusa Basmati 1	17.7	114.5	18.4
Himdhan	34.9	116	28.1	Pusa Sugandh 5	24.8	151.6	17.6
Improved Sabarmati	38.9	107.1	23.2	Ranbir Basmati	19.8	122	19.2
IR 24	21.8	119.1	14.3	Rasi	70.4	174.7	66.4
IR 50	41.1	81.1	23.8	Sharbati	45.2	146.4	33.6
IR 64	28.1	85.7	19.3	Sitwa Dhan	39.2	112.1	27.7
IR 66	15.6	52.5	9	Sonasal	29	134.4	24.6
IR 70	38.1	123.5	23.9	Super Basmati	46.9	114.4	28.1
IR 72	59.4	107.6	36.1	Swarna Sub1	35.5	126.8	27
Jaiphoola	39.5	158.5	30.4	Taipei 309	64.8	162.3	63.1
Jaldi Dhan 13	51.1	111.7	45.6	Taraori Basmati	25.9	87.4	19.5
Kamlesh	56.9	61.3	21	Thai Rice	72.5	189.3	61.1
Kasturi	38.1	113.7	20.8	TKM6	26.7	114.7	19.9
Kavya	42.4	149.8	32.1	TKM9	80.9	184.2	71.4
Kranthi	64.6	100	34	Vandana	43.1	141.1	32.3
Mahalaxmi	66	161.5	41.2	WGL 23985	52.5	101.9	27.1
Mahamaya	47.7	111	28.3	White Ponni	40.7	152.6	28.9
MTU 1010	70.8	190.2	74.7	Mean	45.4	124	34.2
MTU 5249	30.3	105.5	23.2	CD 0.05 (Tukey's)	5.88	8.31	5.04
Nagina 22	55.7	165.8	65.3				

NHI, nitrogen harvest index; NAE, N assimilation efficiency; pNUE, physiological NUE

Table 12. Average estimators of N use parameters in the test germplasm under N50 level

Genotype	AgNUE	GYEI	NRE	NHI	NAE	pNUE
Acharmati	12.88	1.20	-0.71	42.1	297.7	93.5
ADT 38	13.86	1.13	0.40	40.7	114.2	25.4
ADT 39	4.06	0.51	0.14	65.5	126.7	38.3
ADT 42	1.13	2.65	-0.79	56.5	127.9	31.6
ASD 16	42.25	1.85	-0.46	45.0	210.8	70.3
ASD 18	-32.17	1.87	-0.64	49.6	185.2	46.8
ASD 19	-4.75	1.16	-0.25	28.7	114.8	23.3
Basmati 370	8.96	0.36	0.16	37.9	142.7	25.6
BPT 5204	18.91	0.60	0.50	65.6	119.4	51.7
Chandrasahini	-1.81	1.52	-0.42	67.4	146.2	51.1
CO37	12.88	1.48	-0.21	56.8	167.2	47.3
CO51	3.08	1.25	1.68	22.8	65.7	18.5
CR Dhan 310	15.00	0.68	0.40	59.6	124.5	54.7
CR Dhan 311	14.51	0.56	0.40	64.4	128.5	54.1
CSR27	37.36	2.56	2.21	49.1	89.3	22.2
Himdhan	-6.71	0.45	-0.16	40.1	145.6	26.0
Improved Sabarmati	-24.33	1.29	-0.82	35.4	105.7	20.7
IR 24	5.04	0.14	-0.22	39.6	156.5	23.6
IR 50	1.13	0.84	-0.37	56.7	98.1	30.6
IR 64	-11.30	0.83	-1.06	35.3	118.7	25.2
IR 66	35.40	1.25	-1.35	42.5	120.4	24.4
IR 70	21.69	1.18	-0.12	62.2	134.6	38.4
IR 72	32.46	1.44	0.38	58.7	121.5	47.7
Jaiphoola	-29.23	0.55	1.26	13.0	100.1	8.1
Jaldi Dhan 13	-1.81	0.89	0.17	62.3	121.3	37.7
Kamlesh	10.92	1.80	-1.38	53.7	139.2	47.9
Kasturi	-2.79	0.80	-0.47	43.4	142.5	24.9
Kavya	13.86	0.87	0.31	48.6	166.3	34.7
Kranthi	1.13	1.20	0.44	43.8	76.3	27.0
Mahalaxmi	-27.27	1.53	0.35	47.7	146.1	22.1
Mahamaya	0.15	2.85	-0.84	57.2	116.5	40.0
MTU 1010	11.08	0.54	0.50	59.5	111.4	45.8
MTU 5249	6.02	0.85	-1.03	56.6	254.0	61.5
Nagina 22	18.59	0.68	0.33	71.0	139.9	61.7
Nidhi	25.93	0.94	0.53	67.9	110.0	49.6
Pant Dhan 19	64.77	1.81	1.23	55.3	141.1	48.1
Pant Sugandh Dhan 15	4.06	1.83	-2.06	46.4	115.3	31.8

NHI, nitrogen harvest index; AgNue, agronomic N use efficiency; NAE, N assimilation efficiency; pNUE, physiological NUE, GYEI, grain yield efficiency index; NRE, N recovery efficiency

Table 12. Contd.

Genotype	AgNUE	GYEI	NRE	NHI	NAE	pNUE
Pant Sugandh Dhan 17	-23.35	1.73	-1.47	47.2	131.6	37.4
Panvel	10.59	0.50	0.28	63.1	146.0	60.1
PB 1121	5.04	0.27	0.19	6.4	65.0	7.8
PB 1509	25.61	0.28	0.36	52.6	126.3	31.9
PNR 162	31.48	1.03	0.95	60.7	103.6	36.8
Pokkali	-1.81	1.01	2.43	15.4	79.6	12.2
Pusa 1176	20.71	0.38	0.54	54.5	131.4	25.9
Pusa 1592	-2.79	0.47	-0.13	56.0	100.7	22.2
Pusa 1612	47.15	0.63	1.74	59.0	124.6	27.1
Pusa 44	-0.67	1.26	-0.07	26.9	155.0	40.1
Pusa 677	4.06	2.38	-0.34	45.4	155.1	46.3
Pusa Basmati 1	6.02	0.40	-0.12	33.5	161.9	24.4
Pusa Sugandh 5	5.04	0.88	-0.40	31.4	117.9	23.4
Ranbir Basmati	21.69	0.36	0.21	50.1	143.3	33.2
Rasi	9.94	0.57	0.28	66.5	131.3	55.0
Sharbati	0.15	1.22	1.16	37.7	112.8	19.6
Sitwa Dhan	11.90	0.81	-0.11	60.7	139.1	39.4
Sonasal	-8.67	0.21	0.49	20.7	124.3	11.6
Super Basmati	-4.75	0.36	-0.19	52.8	102.3	28.6
Swarna Sub1	-1.81	0.78	-0.55	53.8	117.6	39.3
Taipei 309	17.28	0.61	0.29	65.7	145.7	62.0
Taraori Basmati	25.61	0.79	1.04	27.1	131.5	21.5
Thai Rice	-8.67	2.39	0.81	61.0	196.4	33.5
TKM6	1.13	1.44	-0.59	37.8	126.1	25.5
TKM9	7.00	2.47	0.19	78.9	170.9	65.8
Vandana	26.59	0.48	0.70	50.4	145.5	35.2
WGL 23985	-24.33	0.93	-1.27	63.8	122.6	37.3
White Ponni	-22.37	0.41	-0.17	25.8	206.2	16.1
Mean	7.3	1.1	0.1	48.5	134.7	36.2
CD 0.05 (Tukey's)				6.43	12.65	5.34

NHI, nitrogen harvest index; AgNue, agronomic N use efficiency; NAE, N assimilation efficiency; pNUE, physiological NUE, GYEI, grain yield efficiency index; NRE, N recovery efficiency

Table 13. Average estimators of N use parameters in the test germplasm under N100 level

Genotype	AgNUE	GYEI	NRE	NHI	NAE	pNUE
Acharmati	22.21	1.54	0.77	56.32	116.40	42.04
ADT 38	22.70	1.46	0.07	46.55	132.22	36.93
ADT 39	-7.17	0.21	-0.03	40.39	144.67	20.30
ADT 42	-13.04	1.47	-0.56	43.19	155.56	37.53
ASD 16	18.78	1.48	0.29	66.78	124.65	45.74
ASD 18	-4.23	2.28	0.78	44.60	94.81	28.42
ASD 19	4.58	1.25	-0.23	53.86	181.98	37.67
Basmati 370	14.86	0.49	0.06	51.54	144.75	37.62
BPT 5204	11.52	0.55	0.01	68.05	121.14	54.45
Chandrasahini	7.03	1.62	0.15	62.87	128.46	51.11
CO37	14.86	1.56	0.18	55.84	160.45	47.32
CO51	4.09	1.15	-0.59	34.39	105.67	33.06
CR Dhan 310	13.31	0.71	0.24	66.03	96.19	43.74
CR Dhan 311	9.23	0.51	0.08	68.78	110.90	49.49
CSR27	25.14	2.44	-0.29	63.66	87.22	28.81
Himadhan	19.27	0.96	0.71	48.13	136.82	29.17
Improved Sabarmati	10.46	2.19	0.86	34.39	105.33	23.24
IR 24	0.66	0.10	0.25	23.43	100.74	12.33
IR 50	6.05	0.87	0.08	54.24	111.67	34.54
IR 64	12.46	1.35	1.00	32.00	84.53	21.19
IR 66	32.49	1.47	0.25	38.55	119.79	29.20
IR 70	15.35	1.13	0.40	47.56	114.53	29.73
IR 72	17.80	1.26	0.12	61.76	104.95	43.19
Jaiphoola	-9.13	0.67	0.16	16.24	120.87	10.54
Jaldi Dhan 13	0.66	0.80	-0.11	61.89	125.14	49.11
Kamlesh	10.94	1.75	0.76	32.22	92.69	27.29
Kasturi	2.13	0.78	0.17	35.28	133.91	24.16
Kavya	6.54	0.72	-0.14	41.27	172.67	42.10
Kranthi	0.66	1.02	0.01	54.72	99.50	26.83
Mahalaxmi	-19.41	0.98	0.87	18.87	98.30	9.34
Mahamaya	21.72	3.66	0.51	62.30	115.93	40.72
MTU 1010	11.19	0.57	0.15	61.40	97.78	43.86
MTU 5249	8.99	0.89	0.49	52.54	139.33	34.12
Nagina 22	11.19	0.61	0.11	67.02	116.40	52.78
Nidhi	14.86	0.84	0.17	68.51	89.84	41.28
Pant Dhan 19	31.51	1.50	0.04	46.05	137.20	45.59
Pant Sugandh Dhan 15	-1.29	1.39	-0.08	44.23	117.77	30.92

NHI, nitrogen harvest index; AgNue, agronomic N use efficiency; NAE, N assimilation efficiency; pNUE, physiological NUE, GYEI, grain yield efficiency index; NRE, N recovery efficiency

Table 13. Contd.

Genotype	AgNUE	GYEI	NRE	NHI	NAE	pNUE
Pant Sugandh Dhan 17	0.66	2.12	0.46	45.65	130.07	33.32
Panvel	8.50	0.49	0.11	62.90	120.65	52.86
PB 1121	12.90	0.40	-1.03	60.52	152.62	32.20
PB 1509	7.52	0.18	-0.21	51.54	135.12	34.83
PNR 162	8.50	0.68	-0.15	48.03	127.09	34.31
Pokkali	-3.25	0.76	-0.38	18.74	76.46	13.68
Pusa 1176	21.72	0.49	0.20	51.04	133.63	31.90
Pusa 1592	5.07	0.56	-0.05	47.11	139.01	33.64
Pusa 1612	32.00	0.66	-0.30	58.39	165.79	44.22
Pusa 44	0.75	1.10	0.10	64.43	140.11	36.42
Pusa 677	17.31	2.80	0.48	61.97	105.46	41.04
Pusa Basmati 1	-6.19	0.15	-0.01	30.63	102.70	11.27
Pusa Sugandh 5	20.74	1.30	0.23	45.90	141.28	33.50
Ranbir Basmati	20.25	0.44	0.46	48.29	111.17	27.76
Rasi	4.99	0.48	0.03	67.13	116.50	50.48
Sharbati	-5.21	0.83	-0.45	33.96	129.89	23.26
Sitwa Dhan	30.04	1.34	0.90	42.19	118.45	31.96
Sonasal	16.33	0.57	1.95	14.88	73.44	10.86
Super Basmati	-6.19	0.22	0.14	27.58	119.29	15.67
Swarna Sub1	-3.74	0.56	-0.18	19.86	178.07	50.81
Taipei 309	8.66	0.51	0.05	66.58	125.32	55.49
Taraori Basmati	18.78	0.80	0.10	28.45	125.97	24.11
Thai Rice	-3.25	2.07	-0.23	55.75	202.74	44.24
TKM6	5.07	1.40	-0.23	44.13	178.49	36.77
TKM9	9.97	2.41	0.21	67.75	139.79	56.46
Vandana	4.58	0.26	-0.08	35.57	160.19	25.96
WGL 23985	1.15	1.36	0.50	41.25	97.63	31.97
White Ponni	-18.19	0.13	-0.20	12.65	202.61	8.38
Mean	8.4	1.1	0.1	47.2	126.2	34.1
CD 0.05 (Tukey's)				6.76	8.37	4.42

NHI, nitrogen harvest index; AgNue, agronomic N use efficiency; NAE, N assimilation efficiency; pNUE, physiological NUE, GYEI, grain yield efficiency index; NRE, N recovery efficiency

there was no significant association between grain yield, N assimilation and Physiological N use efficiency observed in the level. N harvest index showed significant and positive correlation with both N assimilation efficiency and physiological N use efficiency (Figure 2).

Similarly, N assimilation efficiency and physiological N use efficiency was found strongly and positively associated at N50 level. Grain yield per plant had positive and significant association between N harvest index and physiological N use efficiency. Agronomic N use efficiency was positively associated with grain yield efficiency index, grain yield per plant, N harvest index and physiological N use efficiency. N recovery efficiency was positive and significantly associated with agronomic N use efficiency and negatively associated with N assimilation efficiency. N harvest index also showed significant association with physiological N use efficiency while N assimilation efficiency had strong association with Physiological N use efficiency under this N50 level.

Interrelation between N related parameters under N100 indicated agronomic N use efficiency having significant positive relation with grain yield per plant, N harvest index, physiological N use efficiency and grain yield efficiency index. Grain yield efficiency index has positive association with N recovery efficiency, and grain yield per plant. Association of N recovery efficiency with grain yield per plant is positive and significant as that of N assimilation efficiency, however which was having a negative direction. Grain yield per plant found positively associated with N harvest index and physiological N use efficiency. N harvest index was positively associated to physiological N use efficiency.

4.1.5 Genotype x N level interactions

4.1.5.1. Grain yield per plant

Genotypic grouping based on Grain yield per plant is done as observed by GGE biplot analysis which showed that under N50 and N100 condition average grain yield was similar for all genotypes (Figure 3). The cultivar Mahamaya gave better yield under N0 and N50 condition followed by the genotype CSR 27. However, CSR27 may be better suited for the N100 condition than the other two levels.

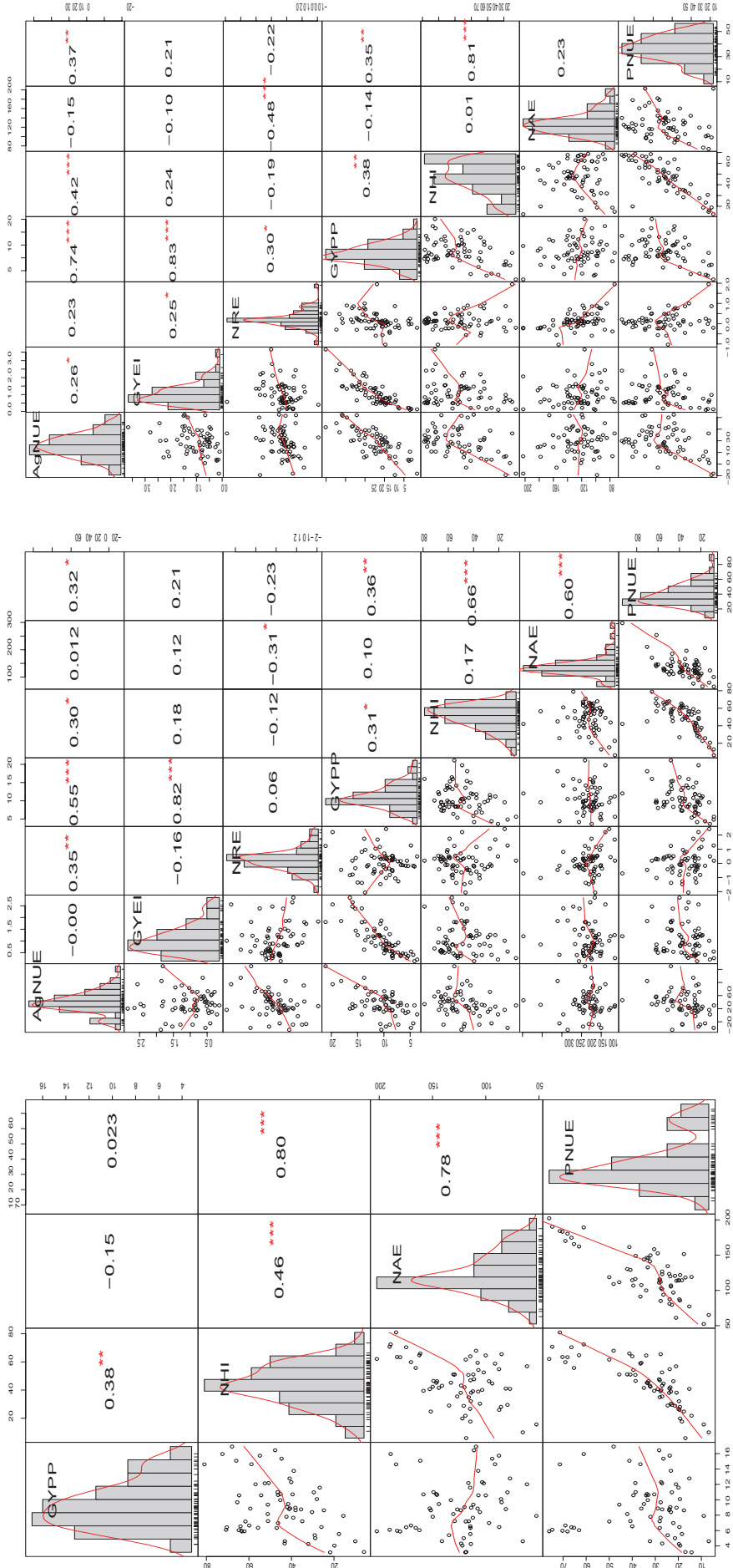


Figure 2. Interrelationships between N use parameters under three N levels

Genotypic stability under N0 condition was low and cultivars such as TKM 9, Thai rice and TKM6 were found to perform better for grain yield. Under N0 condition, however, their mean value was low as compare to the other genotypes which performed well under N0 and N50 conditions. The cultivar Pusa 677 was found to be better stable in the average environment based on its position on the average environmental co-ordinate. Besides, under average fertilized condition, this genotype has yield rank of 3 and can be considered as better suited for average N fertilization condition. Mahamaya which had the highest mean yield performance under high fertilized condition may be better suited to N100 level. Among the genotypes, IR 24 was the lowest mean performer for yield.

4.1.5.2 Upper ground biomass

The N levels, N50 and N100 showed little distinction with respect the trait upper ground biomass. While N0 level had an apparent variation for this trait. has distinctly different influence on the genotypes for the trait. Two genotypes, Ranbir Basmati and IR 66 were found to produce better biomass yield under N0 condition, followed by other genotypes such as Pusa Sugandh 5, Mahamaya, Pant Sugandh Dhan 15 and Pant Sugandh Dhan 17. Average environment axis revealed better stable genotype like Jaiphoola, Mahalaxmi, ADT 38 and Himdhan, however, the genotypes with better rank were mostly unstable.

4.1.5.3 Nitrogen harvest index

N harvest index across all the three levels did not show significant variation between three N levels. The genotype TKM9 had better NHI under N50 and N100 condition followed by genotype Rasi, Taipei309, BPT5204 and Thairice of which Rasi was more stable. As far as NHI is concerned N50 level showed intermediate performance than N0 and N100 level.

4.1.5.4 Physiological N use efficiency

Physiological N use efficiency differentiated N levels as distinctly different. Genotypes such as TKM9 and IR50 had better physiological NUE under N0 condition followed by Taipei 309, Thai rice and Rasi. Under fertilized conditions, Mahamaya, CR Dhan 311, CR Dhan 310, Chandrahasini and Co 37

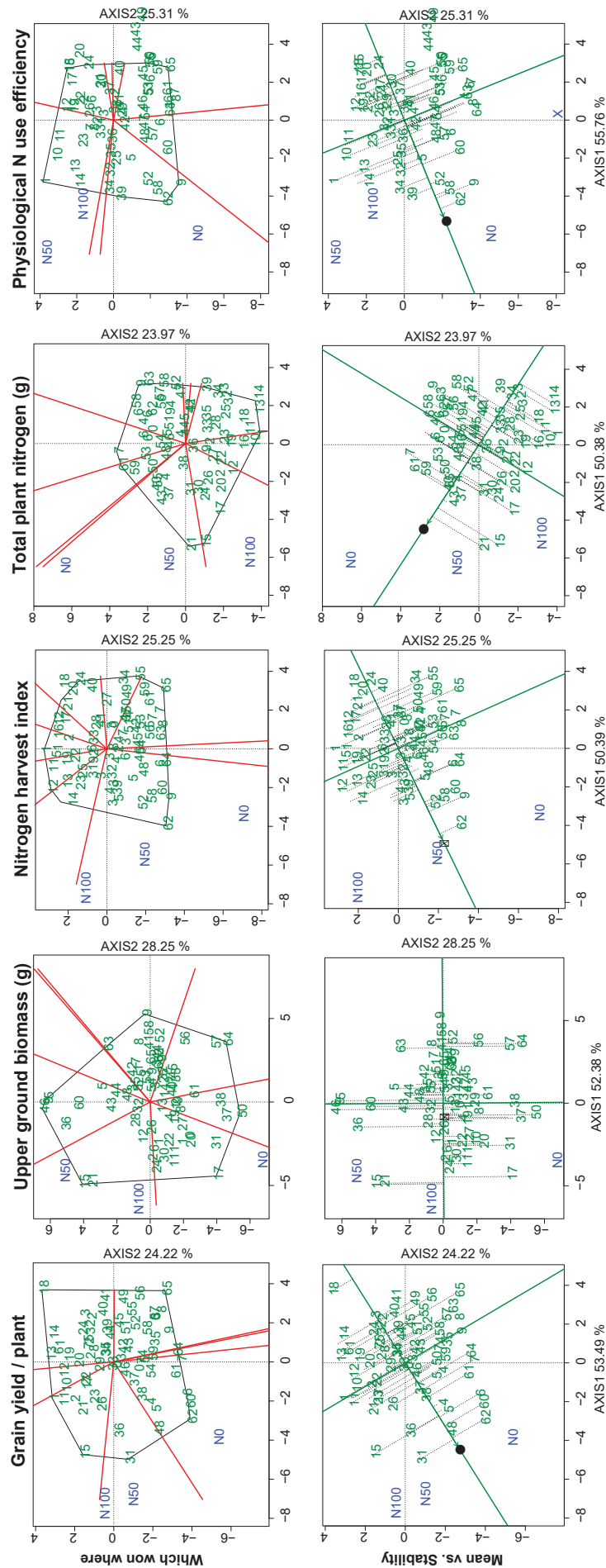


Figure 3. GGE biplots of nitrogen use related parameters across three levels of nitrogen, No - No N fertiliser applied, N50 - 50kg N/ha, and N100 - 100kg N/ha. Genotypes are numbered as given in Table 1

exhibited better pNUE. Mean performance of the genotypes with reference to this trait indicated that TKM 9 having first rank followed by Super Basmati and Rasi.

4.1.5.5 Grain N content

This trait also showed a G+GE pattern similar to the physiological NUE. The cultivars Acharmati, Mahamaya and IR66 could accumulate better grain N content under fertilized conditions, whereas genotypes such as Thai rice, TKM9 and ASD 18 showed relatively better grain N content under N0 condition. Comparing the mean performance of genotype based on grain N content, CSR 27 was ranked first with respect to the average performance followed by genotypes ADT42 and Mahamaya. Among this, ADT 42 may be better adapted to N0 condition.

4.1.5.6 Total plant N

With respect to total plant N, N100 level was distinctly placed away from the other two N Levels. Although the genotypes IR66 and CSR 27 had the better level of Plant N under both N50 and N100 conditions, their performance was relatively less adapted to N100 condition. Cultivars such as Improved Sabarmati, IR64, Jaiphoola, Kamlesh, ADT 38 and Co 51 distinctly has high plant N content under N100 condition. Plant N content was significantly low under N0 condition for all the genotypes. Ranking of the genotypes based on average environment, showed that IR 66 having ranked first based on the mean performance, followed by CSR 27. However, CSR 27 was less stable than IR 66 for this trait. Stable genotypes with better plant N were Pokkali and Pant Sugandh Dhan 15.

4.1.6 Simultaneous selection for N uptake and assimilation parameters

Considering the above average estimates for N uptake parameters such as agronomic NUE, grain yield efficiency index and N recovery efficiency in relation to grain yield per plant, it was found that four genotypes were having grouped together for having the better estimates for the all the parameters tested (Figure 4). These genotypes were Pant Dhan 19, CSR 27, IR 72 and ADT 38. Grouping with respect to N assimilation parameter such as N harvest index,

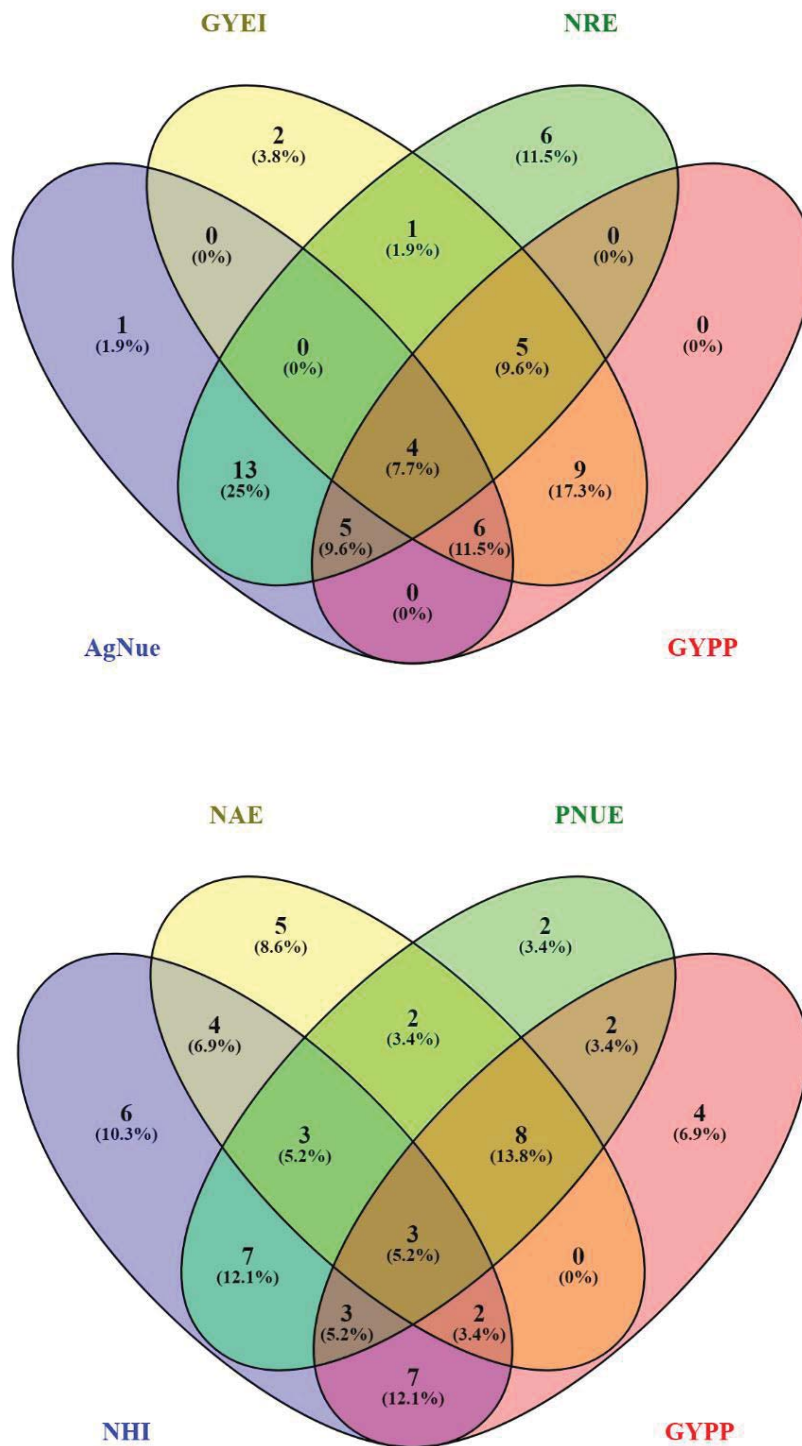


Figure 4. Selection of genotypes having above average estimates of N use parameters under N50 conditions.

physiological NUE, N assimilation efficiency and grain yield per plant showed three genotypes having more than average performance for the parameters tested, such as Pant Dhan 19, TKM 9 and ASD 18.

Under N100 condition, grouping based N uptake parameter had 12 genotypes having more than average performance for component trait such as IR66, Sitwadhan, Acharmati, Mahamaya, Pusa Sugandh 5, ASD16, Pusa 677, IR 70, IR 64, Kamlesh, Improved Sabarmati and TKM9. For N utilization traits, six genotypes such as TKM9, Chandrahasini, Pusa 1612, CO37, Thai Rice and ASD 19 were found more efficient with better than average rankings for all the parameters (Figure 5).

4.2 Meta –QTL analysis and its validation in genotypes

4.2.1 Meta-QTL analysis

Meta-analysis of QTLs revealed that there were 16 QTLs having consensus among the reported N related parameters. These QTLs are located on chromosome 1,3,4,8,9 and 11 (Figures 6-10). The peak marker and position of QTL with estimated confidence interval is given in Table 14.

Table 14. MetaQTLs (mQTLs) identified in the study

mQTL ID	Chromosome	Position (Mb)	Peak marker	Distance (Mb)	CI (95%)
mQTL1.1	1	27.6	RM576	1.72	2.06
mQTL1.2	1	34.49	RM575	2.07	0.69
mQTL1.3	1	43.42	RM578	0.91	4.57
mQTL3.1	3	35.91	RM6483	3.97	4.44
mQTL3.2	3	55.75	RM487	1.89	1.52
mQTL3.3	3	63.08	RM16	1.4	3.94
mQTL3.4	3	68.34	S03096A	1.28	2.06
mQTL3.5	3	72.77	RM514		0.03
mQTL4.1	4	1.70	RM417	2.75	1.2
mQTL4.2	4	11.91	RM273	5.02	4.53
mQTL4.3	4	36.98	RM252	1.42	2.57
mQTL4.4	4	43.35	RM451	2.39	2.22
mQTL8.1	8	16.63	RM80	3.32	5.66
mQTL9.1	9	43.81	RM108	4.74	9.61
mQTL11.1	11	8.68	RM479	5.05	2.92
mQTL11.2	11	32.04	RM202		0.57

Mb, million base pair

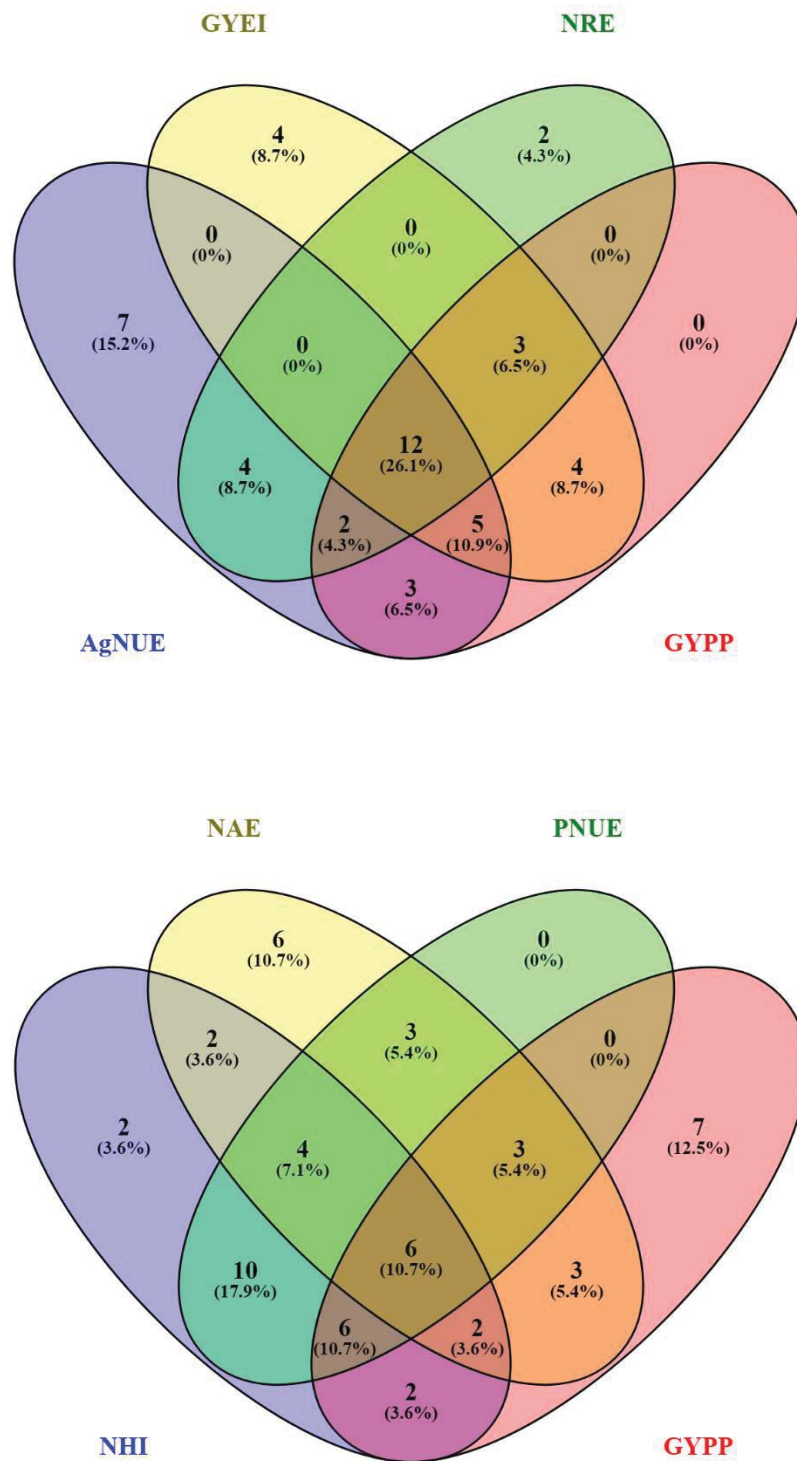


Figure 5. Selection of genotypes having above average estimates of N use parameters under N100 conditions.

4.2.2 Validation of the mQTL linked marker

Sixteen peak markers linked to mQTLs were surveyed across the random set of germplasm lines showed that five markers having distinct polymorphism across the genotypes. Based on the grain yield and N assimilation efficiency of genotypes one marker with common effect on both these traits has been identified by using single marker analysis. This marker RM 202 is located on chromosome 11 at 32.04 mb position was found to have an estimated narrow confidence interval of 0.57 mb. This marker produced two alleles 179 bp and 193 bp of which the allele 179 bp was associated positive effects towards grains per panicle under N0 condition and N assimilation efficiency under N50 condition. The phenotypic variation explained (R^2) values were 19.1% for grains per panicle and 8.53% for N assimilation efficiency. Respective additive effects were 21.6 for grain number and 18.2 for N assimilation efficiency.

4.3 Assessment of mQTL diversity in the population

MetaQTL diversity was tested using population genotypic parameter for the polymorphic marker profiled across 65 genotypes assembled into a single test population.

4.3.1 Hardy-Weinberg equilibrium

Test of Hardy-Weinberg equilibrium showed that all the markers were significantly distributed across the population with the observed heterozygosity being minimum (Figure 6)

4.3.2 Allele frequencies

All the five markers were biallelic in the nature in the population tested. The marker RM80 produced an allele with size of 129 bp having a frequency of 0.44 in population, its corresponding allele with the fragment size of 143 bp had a frequency of 0.56. Marker RM 202 produced two alleles of amplicon sizes 179 bp and 193 bp having frequency of 0.23 and 0.77 respectively. The alleles of marker RM 250 were observed to have an allele frequency of 0.35 and 0.65, and amplicon sizes of 200 and 210 bp respectively in the population. Marker RM 273 produced alleles of sizes, 225 bp with a frequency of 0.48 and 250 bp with frequency 0.52. The remaining marker, RM 451 had one of the allele with a

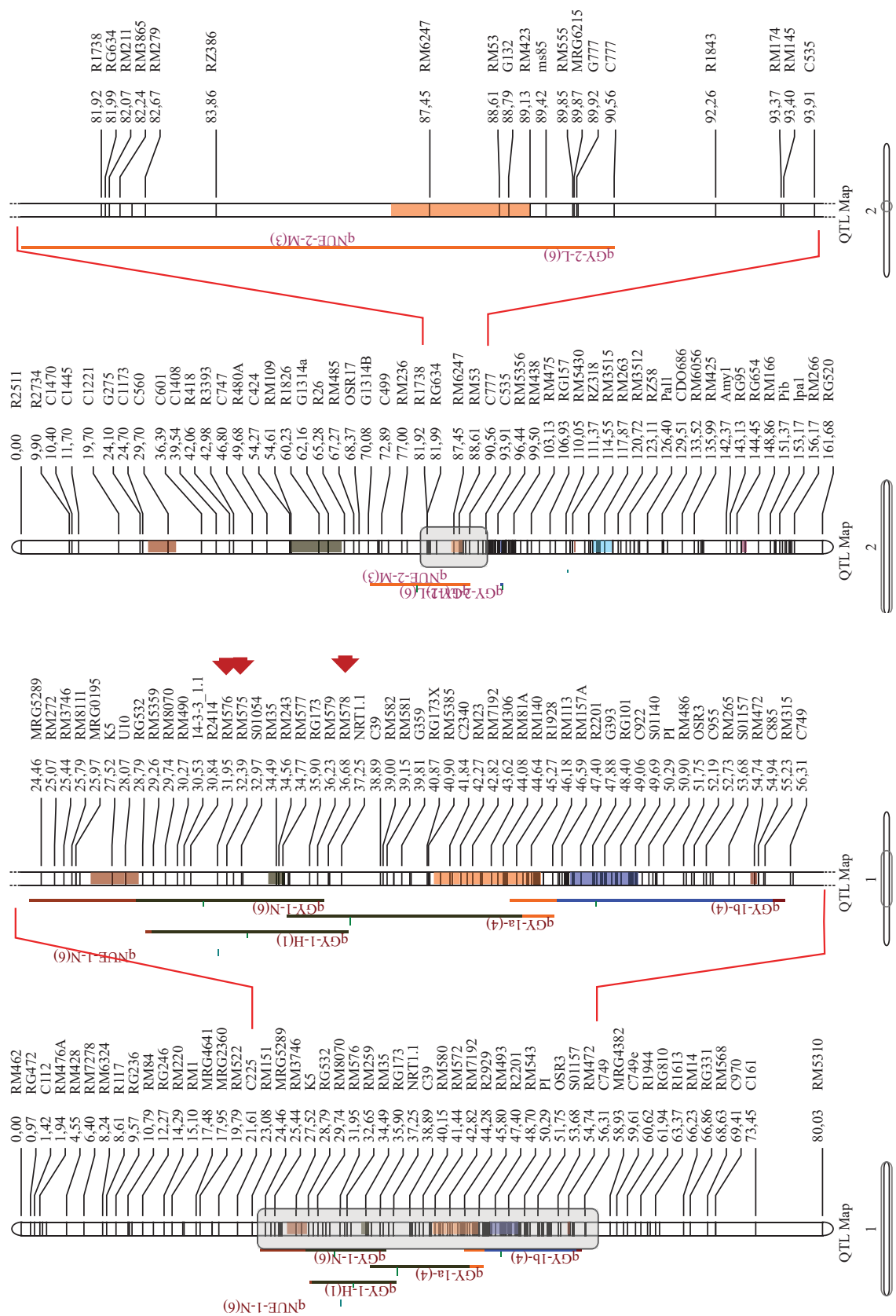


Figure 6. Consensus map showing the meta-QTLs detected on chromosomes 1 and 2 for N use efficiency and grain yield



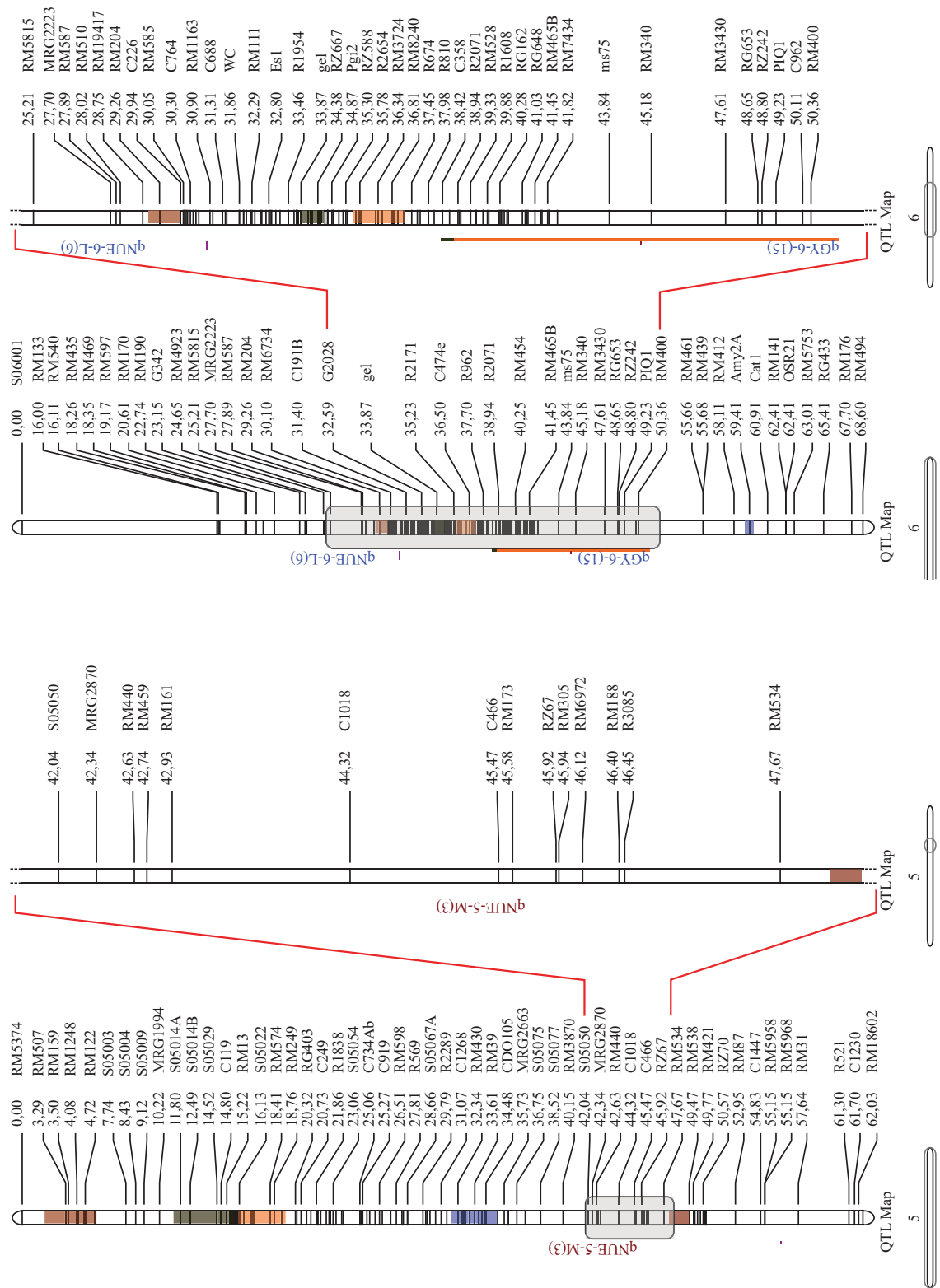
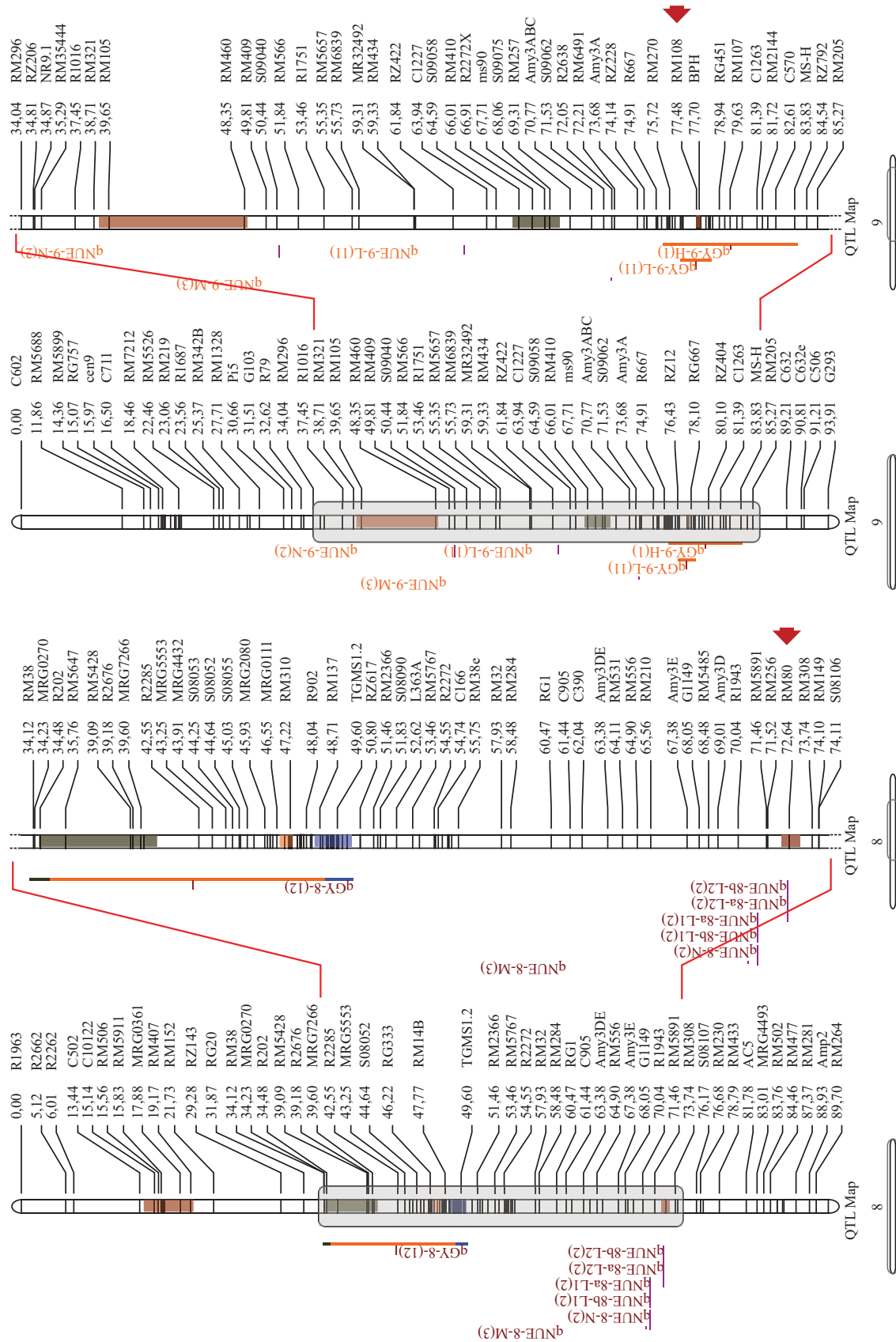


Figure 8. Consensus map showing the meta-QTLs detected on chromosomes 5 and 6 for N use efficiency and grain yield



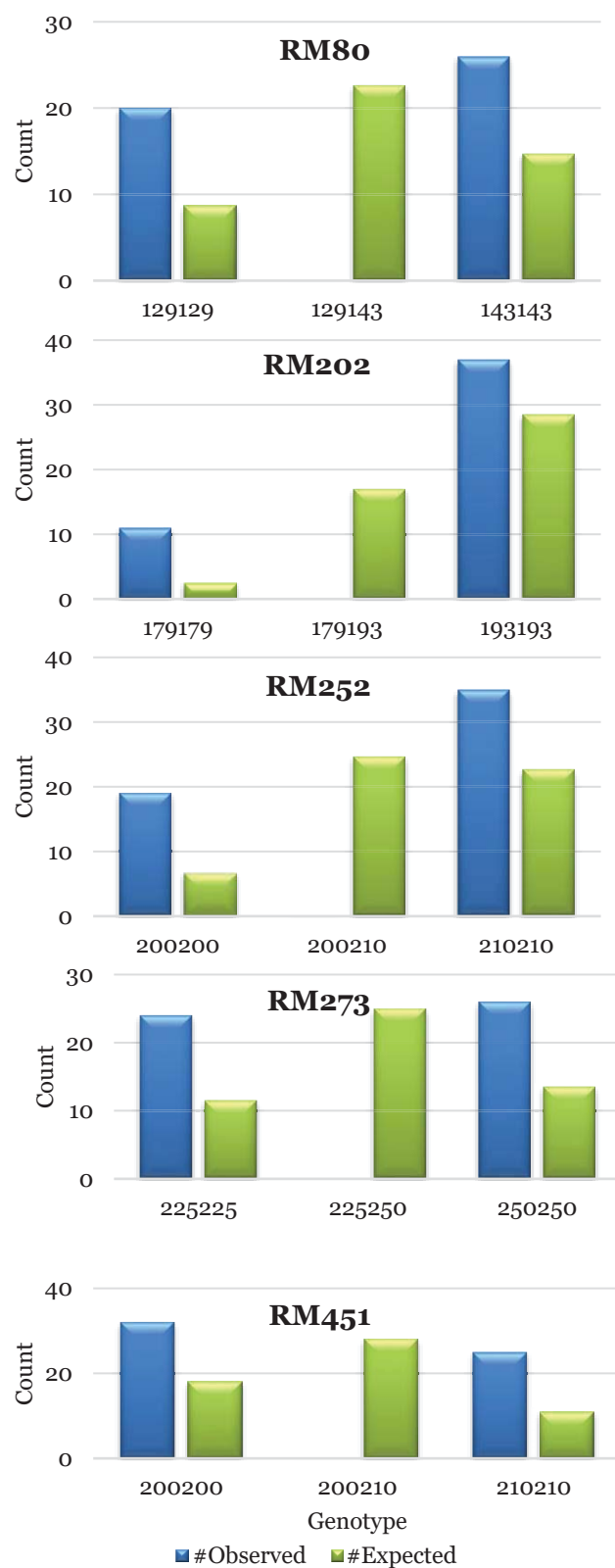


Figure 11. Expected and observed allele frequencies for the marker alleles under Hardy Weinberg equilibrium

frequency of 0.56 having an amplicon size of 200bp and its corresponding allele with frequency of 0.44 and product size of 210 bp (Table 15).

Table 15. Allele frequencies and sample size by populations for the mQTL linked markers

Locus	mQTL	Chromosome	Allele (bp)	Pop	Frequency
RM80	<i>mQTL8.1</i>	8	129	46	0.435
			143		0.565
RM202	<i>mQTL11.2</i>	11	179	48	0.229
			193		0.771
RM252	<i>mQTL4.3</i>	4	200	54	0.352
			210		0.648
RM273	<i>mQTL4.2</i>	4	225	50	0.480
			250		0.520
RM451	<i>mQTL4.4</i>	4	200	57	0.561
			210		0.439

mQTL, meta QTL; Pop, population size of the allele carriers

4.3.3 Allelic pattern within the population

The analysis of allelic pattern across population revealed that number of different alleles (N_a) per marker in population remained two. Number of effective alleles (N_e) was calculated as 1.86, with Shannon's information index (I) of 0.65. Number of private alleles were two. The average expected heterozygosity for whole population was estimated to be 0.46 (Figure 12).

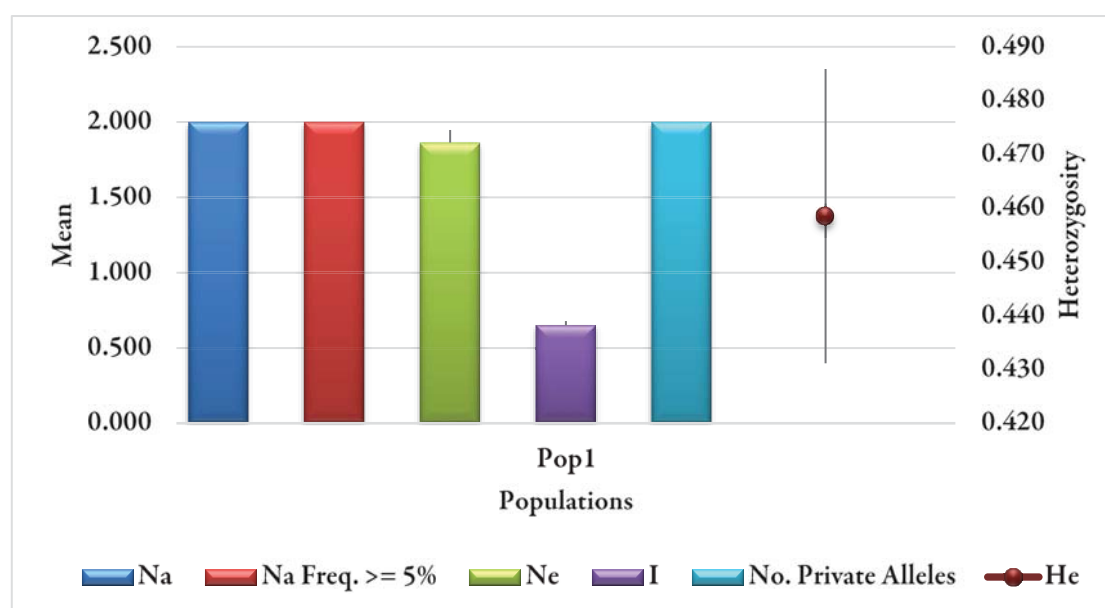


Figure 12. Allelic Patterns across populations for the mQTL linked markers

Discussion

N is an indispensable element for rice growth and its availability is continued to shrink because of the continuous cultivation. The limitation of N in agriculture is being compensated by the application of nitrogenous fertilizer which is dominantly derived from Petroleum industries. N use efficiency of the Rice genotypes hovers around 30-35% providing way for continuous loss of applied N into environment worth millions of rupees. Compensating N loss through continuous fertilization is no longer going to be judicious approach, either we have to improve the N Use Efficiency of the genotypes beyond the current limit of 35% and also, we need to reduce the import of fertilizer N. The N use efficiency of rice genotypes have been extensively studied and it is found that two major component of N use efficiency mainly N uptake efficiency and N utilization efficiency are controlled by separate genetic process. Several QTLs have been reported in different genetic background for either or both the N use efficiency parameter. Over different studies have reported different genomic regions hardly finding any conspicuous locus regulating the N use. Finding the consensus QTLs with significant effects can help in identification of underlying genes, processes (Xu 1997) and can help in rice breeding for N use efficiency through marker driven approaches (Ashikari and Matsuoka 2006).

There are several genes identified in Rice genome that regulate N uptake and assimilation (Baligar et al. 2001). Most of these genes are constitutively expressed, hence are the part of fundamental genetic framework of rice plant that help in growth and in survival. Therefore, the genes that contribute to the improved N use efficiency must be beyond this fundamental framework. This study attempted to explore the quantitative trait loci reported for N use parameters from 16 studies beginning from 2001 to 2016. There were 16 consensus QTLs identified from 155 N use efficiency QTLs which estimates to 10.32 %. Identification of several consensus QTLs indicated that N use in Rice

is governed by complex genetic regulation. The percent of variation explained by these QTLs were all less than 25% indicating that they had minor to moderate influence. Therefore, one of the possible way to improve N use efficiency must be to pyramid most of moderately influencing QTLs together with few of minor QTLs. In order to validate identified mQTLs we have picked up the peak marker linked to these QTLs. We hypothesizing validating these QTLs over random set of genotypes that vary for N use efficiency would be one of the logical step in validating these QTLs over wide range of background as well as identifying potential donors for the mQTLs. For this purpose, the germplasm assembly screened under three different N levels. One which possessed only the native reservoir of N and other two supplemented respectively with 50% and 100% of recommend fertilizer dose that is 120 kg/ha. The higher variation of genotypes for N level and their interaction implied that the germplasm assembly contain genotype with significantly different N efficiency. Further these genotypes performing different under three different N levels. The variation observed in genotype but N levels due to genetic makeup of individual lines which could include sensing of N deficiency and N sufficiency. The LATS and HATS system for N uptake and Transport play a major role in the uptake variation.

The genotypic variance for N response was also computed by correlating different agronomic traits between the N levels. In order to characterize the Rice genotypes for N efficiency we have employed the N use parameters that were apparently related to N uptake and assimilation. The uptake efficiency was evaluated by using parameter such as agronomic N use efficiency which measured the quantum grains produced per unit application of N. Further another parameter N recovery efficiency was used which measured the level of N accumulated per unit of applied N. In addition, the grain yield efficiency Index was also used to evaluate the genotypes for their response to added N. Comparing the average values of these parameters and evaluating the genotypes based on their above average performance, few genotypes such as Pant Dhan 19, CSR 27, IR 72 and ADT 38 were found uptake efficient under N50 condition, while none of these were found uptake efficient at N100 levels. This indicated that the genotypic response under applied N condition differed with the level of N applied. The N50 level which supplement the N, half of the recommended level would have been the optimum level because this level could possibly meet

the N requirement of growing crop, while under N100 condition, N level was more than needed which compelled the plant to uptake more N but without apparently making better yield. These were only few genotypes which responded maximum for high N level. Whereas general response was wider under N50 level for N assimilation parameter such as physiological N use efficiency which measured the amount of grains produced per unit plant N content and N harvest index which is the ratio of grain N to plant N. A distinct indicator of source to sink conversion and also the N assimilation efficiency which denoted quantum of biomass produced per unit plant N.

The genotype which produced better for all these component under N50 and N100 condition was only the cultivar TKM 9. TKM9 is reported to be N responsive variety by Krishnaprabhu and Grace (2017). TKM9 also found to be performing better under N0 condition than the fertilized condition however with lesser stability. This genotype probably could be carrying low N responding genes which could be exhibiting significant Gene $\times$ environment interaction .It was also found that TKM 9 has better N harvest Index under fertilized condition . Another Genotype was not worthy with respect to N use parameter . Nidhi was found N efficient under applied N condition.Nidhi produced better yield when more N given to the plant .

The diversity and distribution of mQTLs linked marker indicated that they were widely distributed among the germplasm . Marker RM 80 , RM273 and RM451 linked to mQTL 8.1 , mQTL4.2 and mQTL4.4 respectively with allele frequency varying from 0.44 to 0.56 counted not have contributed much to the N use variation in the germplasm tested because of the equal distribution among the germplasm.Marker RM252 which is linked to mQTL4.3 showed allelic frequency between 0.35 to 0.65 which also could be a low priority candidate marker. The remaining marker RM 202 which was linked to mQTL 11.2 showed allele frequency 0.23 to 0.77 which indicated at least 23% of genotype carrying a particular allele in this germplasm could possibly define the N use variation in the present assembly. Accordingly the validation test revealed that RM 202 having a allele of size 179 bp with 23% distribution in germplasm was found to associated to two N use parameters such as grains no. per panicle under N0 condition and N assimilation efficiency under N50 condition.

RM 202 was mapped for N use efficiency by Senthilvel et al. (2001). Based on the presence of allele of size 179 bp the genotypic profiling of germplasm have showed that 11 genotypes which among this allele include Pusa 44, Nidhi, Nagina 22, ADT 38, ADT 42, ASD 16, Acharmati, Chandrahasini, CR Dhan 310, Jaiphoola and Kamlesh which were found grouped together in the scatter plot generated from the genotypic data. Thsesse genotypes were rather high input efficient than the low N tolerant . So it is evident from the result that low N response shown by the genotype such as TKM9 could not be accommodate by meta-QTL profile .

In conclusion, we have identified a set of genotype that are uptake and assimilation efficient under low as well as high N condition. The consensus QTL have been identified based on the reported QTLs for N related parametrs under different background condition not account for all the variation observed in genotypes.It implies that there could be genes specifically available in the N effiecient genotypes identified in the study which are yet to be mapped . Further all the mQTLs are not validated in the test germplasm because of the ambigyuty in the polymosrphism observed for some of the marker , therefore identifying alternate marker in this region may provide some vital clues . So the study lead us to conclude that the more QTLs and marker remain to be identified and their profiling in Indian Rice germplasm.

Summary and conclusion

A study was conducted to identify meta-QTLs for N efficiency that are reported in various populations in rice. To validate and assess the distribution of the identified QTLs, a set of 65 Indian rice germplasm (*Oryza sativa* L.) was assembled. In order to characterize the N efficiency of these genotypes, they were field evaluated under three different N regimes. The salient findings and conclusion drawn are mentioned below.

- In order to find out the most significant genomic regions (meta-QTLs) governing the N use efficiency, 15 reports on the N efficiency related 453 QTLs between the years 2001 to 2016 were used.
- Meta-analysis identified sixteen mQTLs related to NUE and grain yield under N deficient conditions which were located on six chromosomes 1,3,4,8,9 and 11.
- It was hypothesized that validation of the mQTLs in a germplasm set would help in identifying the mQTLs that are working under different genetic backgrounds as well as will help in identifying the donors of such QTLs in the local germplasm. The objectives of the study were formulated based on this hypothesis.
- Field screening of the selected germplasm under three different N conditions such as no applied N, 50% and 100% of the recommended dose (120 kg/ha) helped to identify different adaptation behavior of the germplasm assembly.
- Study revealed significant variation in germplasm for N response as revealed by the difference in agronomic performance under different levels of N.
- Comparing the N efficiency trait such as N uptake and assimilation few genotypes such as Pant Dhan 19, CSR 27, IR 72 and ADT 38 were found uptake efficient under N50 condition.

- The N50 level was found optimum for most of the genotypes, because this level could possibly meet the N requirement of growing crop, while under N100 provided N levels more than that is required for some of the genotypes. Increased availability of N compelled plants to uptake more N but without apparently making better yield.
- The genotype which produced better for all these N use efficiency component under N50 and N100 condition was only the cultivar TKM 9. The cultivar Nidhi was found N efficient under applied N condition. Nidhi produced better yield when more N given to the Plant .
- Genotyping of the germplasm with mQTLs marker showed wide distribution of meta-QTLs among the germplasm.
- The marker RM202 which was linked to *mQTL11.2* showed allele frequency 0.23 to 0.77 which indicated 23% of genotype carrying a particular allele in this germplasm define the N use variation in the present assembly.
- RM 202 had a allele of size 179 bp with 23% distribution in germplasm was found to associate with two N use trait such as grains no. per panicle under N0 condition and N assimilation efficiency under N50 condition.
- Based on the presence of allele of size 179 bp of marker RM 202 the genotypic profiling of germplasm have showed that 11 genotypes which among this allele include Pusa 44, Nidhi, Nagina 22, ADT 38, ADT 42, ASD 16, Acharmati, Chandrahasini, CR Dhan 310, Jaiphoola and Kamlesh which were found grouped together in the scatter plot generated from the genotypic data.
- In conclusion we have identified a set of genotype that are uptake and assimilation efficient under low as well as high N condition. The consensus QTL identified did not account for all the variation observed in genotypes. It implied that there could be genes specifically available in these N effiecient genotypes which are remaining to be mapped .

नाइट्रोजन दक्षता के लिए मेटा- क्यूटीएल (एमक्यूटीएल) की पहचान और भारतीय चावल के जननद्रव्य में एमक्यूटीएल विविधता की रूपरेखा

चावल के विकास के लिए नाइट्रोजन को सबसे महत्वपूर्ण तत्व माना जाता है और यह चावल के पौधे की सभी शारीरिक और चयापचय प्रक्रिया को नियंत्रित करता है। पौधे के अस्तित्व में महत्वपूर्ण मात्रा में नाइट्रोजन की आवश्यकता है, क्योंकि यह प्रोटीन का एक प्रमुख घटक है, हालांकि चावल की नाइट्रोजन उपयोग दक्षता बहुत कम है जो लगभग 30-35% है। शेष 65-70% नाइट्रोजन पर्यावरण में बहाव, अनाइट्रीकरण तथा औटना के माध्यम से खो जाता है। चावल में नाइट्रोजन उपयोग दक्षता के सुधार के लिए कई क्यूटीएल की पहचान की गई है जो आम तौर पर इस नाइट्रोजन उपयोग विशेषता पर मामूली प्रभाव डालती हैं। इसलिए चावल में नाइट्रोजन उपयोग विशेषता को नियंत्रित करने वाले सबसे महत्वपूर्ण अनुवन्शिक क्षेत्रों (मेटा-क्यूटीएल) की पहचान करने के लिए, यह अध्ययन किया गया। अनुसंधान कार्य तीन नाइट्रोजन स्तर नाइट्रोजन 0, नाइट्रोजन 50 और नाइट्रोजन 100 के तहत सस्य विज्ञान और नाइट्रोजन उपयोग संबंधित लक्षणों के लिए 65 जीनोटाइप के मूल्यांकन के साथ शुरू हुआ। अध्ययन ने तीन अलग-अलग नाइट्रोजन स्तर के तहत नाइट्रोजन उपयोग दक्षता के लिए जीनोटाइप के बीच महत्वपूर्ण भिन्नता का खुलासा किया। पिछले अध्ययनों से एकत्रित नाइट्रोजन उपयोग संबंधित क्यूटीएल के बड़े सेट से नाइट्रोजन उपयोग विशेषता के लिए सबसे मजबूत क्यूटीएल को खोजने के लिए क्यूटीएल का मेटा-विश्लेषण भी किया गया। सोलह एमक्यूटीएल की पहचान की गई जो नाइट्रोजन उपयोग क्षमता और अनाज उपज जैसे गुणों से संबंधित थे और छह गुणसूत्र 1, 3, 4, 8, 9 और 11 पर स्थित थे। एमक्यूटीएल से जुड़े मार्कर का उपयोग फील्ड मूल्यांकन के लिए इस्तेमाल किए गए समान जर्मप्लाज्म सेट को जीनोटाइप करने के लिए किया गया। मार्कर आरएम 202 जो एमक्यूटीएल 11.2 से जुड़ा था, ने एलील फ्रीक्वेंसी 0.23

से 0.77 दिखाया जो इस जीवाश्म में एक विशेष एलील ले जाने वाले जीनोटाइप का 23% इंगित करता है तथा वर्तमान जर्मप्लाज्म संग्रह में नाइट्रोजन उपयोग भिन्नता को परिभाषित करता है। तदनुसार सत्यापन परीक्षण से पता चला कि आरएम 202 जर्मप्लाज्म में 23% वितरण के साथ आकार 179 बेस पेयर का एक एलील था, जो अनाज संख्या जैसे दो नाइट्रोजन उपयोग पैरामीटर से जुड़ा हुआ था। नाइट्रोजन 0 स्थिति के तहत प्रति पैनिकल अनाज संख्या और नाइट्रोजन 50 स्थिति के तहत नाइट्रोजन आकलन क्षमता। इन मार्करों को चावल में नाइट्रोजन उपयोग क्षमता के लिए प्रजनन के लिए आगे नियोजित किया जा सकता है। नाइट्रोजन दक्षता के संभावित वाहक की भी पहचान की गयी, जो अचारमती, एडीटी 38, एडीटी 42, एएसडी 16, चंद्रहासिनी, सीआर धान 310, जयफूला, कमलेश, नगिना 22, निधि और पुसा 44 थे। आगे इन जीनोटाइपों को नाइट्रोजन कुशल विविधताओं के प्रजनन के लिए भविष्य में दाताओं के रूप में उपयोग किया जा सकता है।

प्रमुख शब्द: नाइट्रोजन उपयोग दक्षता, मेटा- क्यूटीएल, विविधता, जननद्रव्य, चावल

Abstract

Identification of meta-QTLs (mQTL) for nitrogen efficiency and profiling of the mQTL diversity in Indian rice germplasm

Nitrogen (N) is considered one of most important element for rice growth it is fundamental for the plant physiological and metabolic processes. N is required in significant quantities for crop production. Because the N use efficiency (NUE) of rice is low which is about 30-35%, there is a considerable quantum loss of N from the crop field to the tune of 65-70 %. Improving the NUE of crops would therefore will save considerable fertilizers which is expensive and becoming scarce. For the improvement of N use efficiency in rice several QTLs have been identified which generally have minor effect. Most of these are identified in different genetic backgrounds. Therefore, in order to identify most significant genomic regions (meta-QTLs) which govern N use trait in rice, this study was undertaken. We have assembled 453 QTLs reported from 16 studied published between 2001 to 2016 for the meta-analysis. Sixteen meta-QTLs (mQTLs) related to traits such as N use efficiency and grain yield per plant under N deficit conditions have been identified distributed over six chromosomes 1, 3, 4, 8, 9 and 11. The mQTLs showed varying confidence intervals. In order to validate and study the diversity of the mQTLs in local rice germplasm, 65 genotypes were field evaluated under three N level N0, N50 and N100 for agronomic and N use related traits. The study revealed significant variation among genotypes for N use efficiency under three different N level. The mQTLs linked marker were used to genotype the same germplasm set used for field evaluation. The marker RM 202 which was linked to *mQTL11.2* showed allele frequency 0.23 to 0.77 which indicated 23% of

genotype carrying a particular allele in this germplasm. This marker was validated to be associated with two N use parameters such as grains number per panicle under N0 condition and N assimilation efficiency under N50 condition. The positive allele for this marker with 179 bp size was found distributed in genotypes such as, Acharmati, ADT 38, ADT 42, ASD 16, Chandrahasini, CR Dhan 310, Jaiphoola, Kamlesh, Nagina 22, Nidhi and Pusa 44. There are other N efficient genes identified in the study which may carry novel alleles for N efficiency traits which needed identification.

Keywords: Nitrogen use efficiency, meta-QTL, diversity, germplasm, rice

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