Indian J. Genet. Plant Breed., (2023); 83(2): 176-184
https://doi.org/10.31742/ISGPB.83.2.3
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Nitrogen dose dependent changes in leaf greenness, crop phenology, grain nitrogen content and yield in rice (*Oryza sativa* L.) sub-species

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Abstract

In the present study, 30 diverse genotypes of rice sub-species were evaluated for variations in phenology, grain protein content, grain morphology and yield under field conditions with different nitrogen (N) regimes i.e., N deficient (N=0) and N sufficient (N=120 kg ha^{-1}). N deficiency decreased the leaf greenness, panicle yield, grain protein content, altered the grain morphology and grain-related parameters. Significant variations in grain morphology-related parameters such as grain length and grain width among rice genotypes were observed for different N treatments. Changes in grain morphology related parameters were correlated with yield. The study identified Sahbhagi Dhan, BAM-759, BVD-109, Pusa Sugandh-5, and Kalinga-1 that maintained higher vegetative greenness, while Sahbhagi Dhan, Vandana, Nerica-L-44, Kalinga-1 and APO that showed higher panicle yield under N0 condition. Rice genotypes APO, Nerica-L-42 and Kalinga-1 performed well under N0 with a lesser impact on crop phenology and grain morphology. Grain protein content was found higher in BAM-759, Anjali, Thurur Bhog, IR-64, Rasi, and Kalinga-1under both the treatments. Flag leaf Soil Plant Analysis Development (SPAD) and Normalized Difference Vegetation Index (NDVI) measurements were significantly correlated with grain yield, and grain protein content. The trait specific donors suitable for low N conditions identified in the study will pave the way forward to the research in understanding underlying mechanisms and in crop improvement programs.

Keywords: Rice sub-species, N deficient, N sufficient, soil plant analysis development, normalized difference vegetation index, grain morphology, panicle yield

Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population (El Baroudy et al. 2020). Rice production and yield depend on the availability of nutrients and water, which are indispensable for its growth. The natural source of nitrogen (N) is inadequate for rice growth and yield, and it requires a large amount of chemical N fertilizer. Rice yield highly depends on the exogenous N fertilizer supply in almost all agricultural lands (Kraiser et al. 2011). The soil environment is very dynamic and available soil N to support crop growth in all agricultural land is variable. Since N is the constituent of chlorophyll molecules, key pigments involved in photo assimilatory processes, leaf growth and total biomass accumulation are determined by N availability during the crop growing period. Most N uptake occurs at the vegetative growth stage. Its deficiency during this stage results in early flowering, lower biomass accumulation, early crop maturity, lower N uptake, low yield per plant and reduced crop quality. Maintaining photosynthetic activity and higher N uptake during the

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How to cite this article: Padhan B.K., Sathee L., Kumar S., Chinnusamy V., Krishnan S.G. and Kumar A. 2023. Nitrogen dose dependent changes in leaf greenness, crop phenology, grain nitrogen content and yield in rice (*Oryza sativa* L.) sub-species. Indian J. Genet. Plant Breed., **83**(2): 176-184.

Source of support: Nil

Conflict of interest: None.

Received: Sept. 2022 **Revised:** March 2023 **Accepted:** April 2023

vegetative phase in rice is an essential determinant of outcome and grain quality (Gu et al. 2017). Leaf chlorophyll content, a critical factor in determining the photosynthetic rate, is correlated with the photosynthetically active N pool (Gu et al. 2017; Wen et al. 2019). Therefore, accurate assessment of leaf chlorophyll content is of great significance in characterizing crop nutritional status and evaluating leaf photosynthetic activity. SPAD (Soil Plant Analysis Development) readings reflect the leaf growth status and can be used indirectly to measure leaf chlorophyll content and photosynthetic activity. SPAD readings have previously been used to characterize chlorophyll content responses to N fertilization to manage crop nutrition during the growing season (Ding et al. 2015; Lemaire et al. 2008; Yang et al. 2017a). Chlorophyll content plays an important role crop physiology and therefore, increasing N fertilization is required to enhance photosynthesis by controlling the distribution of leaf chlorophyll content, resulting in improved grain yield (Gu et al. 2017).

Previous reports suggest that rice yield is closely related to sowing date (Ding et al. 2020; Hu et al. 2017) and nitrogen application rates (Li et al. 2008; Zhao et al. 2017). Rice requires N during the vegetative stage to promote growth and tillering and determine the potential number of panicles. Nitrogen contributes to spikelet production during the early panicle formation stage and sink size during the late panicle formation stage. N also plays a role in grain filling, improving the photosynthetic capacity and promoting carbohydrate accumulation in culms and leaf sheaths (Mae 1997). The post-anthesis stage in rice is a critical period for panicle growth, dry matter accumulation and N uptake which determines yield as well as quality.

The crop yield in depends on the number, shape, and size of grains and the grains per spike (Garcia et al. 2016). Grain size is characterized by grain length, width, circularity, and area (Zuo and Li 2014). As rice yield is primarily determined by grain weight, increasing the 1000 grain weight will improve rice production (Bai et al. 2011). Improving rice yield and grain quality is the principal objective of rice breeding worldwide (Qiu et al. 2015). Grain quality primarily includes grain appearance, milling, cooking and nutritional qualities. Grain appearance quality is determined by grain size, shape, chalkiness, and translucency (Bao 2014). Standard counting methods are used to record grain number (Liu et al. 2016), electronic counting devices are also used for rice grain counting (McLaughlin et al. 2011), and image based analysis (Peng et al. 2010) is an advanced and reliable non-invasive method. Grain counting based on image analysis is promising. It facilitates large scale image processing and data generation (McClung et al. 2020). The digital imaging system can be used effectively to evaluate rice grain appearance (Bao 2014) traits, including grain length, width, and circularity (Imai et al. 2013). Findings from various studies suggested that rice cultivars' nitrogen use efficiency (NUE) vary in response to N fertilizer dose, which brings the opportunity and possibility to screen diverse rice genotypes for NUE component traits. Therefore, the present study was focused on the variations observed in crop growth, phenology, grain morphological parameters, grain nitrogen, grain protein and yield due to changes in N application rates in field grown rice genotypes. Non-invasive image-based analysis of grain morphology and associated traits using Digital image analyzing software was also carried out to determine the utility of the research work.

Materials and methods

Crop growth and yield estimation

Thirty diverse rice genotypes selected from a set of 300 diverse lines screened under hydroponics differing in Nitrogen utilization efficiency (NutE) (Jagadhesan et al. 2020; Padhan et al. 2023) were evaluated in field in two consecutive years during *kharif* 2019 and 2020 at the ICAR-Indian Agricultural Research Institute, New Delhi. These genotypes include different sub-groups (Garris et al. 2005) of rice namely, Indica, Basmati and few *Oryza glaberrima** indica crosses (Nerica-L-26, Nerica-L-42, Nerica –L-44) (Supplementary Table S1). One of the genotypes Thurur Bhog could not be grouped into these categories as described in an earlier report (Neeraja et al. 2021). From the nursery, 25 days old, seedlings were transplanted in the field with two different N regimes. Three replications were maintained at nursery stage as well as during the transplanting stage. The N: P: K ratio was @ 0:60:60 kg per hectare for N0 treatment and @ 120:60:60 kg per hectare for N120 treatment. N was applied in 3 doses; 50% basal (transplanting stage), 25% at the active tillering stage and 25% at the booting stage. Yield per plant and 1000 grain weight was estimated at maturity. Days to 50% flowering was recorded for each rice genotype and under the two N treatments. Panicle yield per plant was estimated at maturity. The number of panicles per plant, number of grains per panicle and 1000 grain weight were recorded manually under both the N treatments.

SPAD and NDVI readings

Soil Plant Analysis Development (SPAD) meter readings were recorded (2019 and 2020) at the anthesis stage (within 1-7 days of anthesis) when the plant population attained 50% flowering. Flag leaf was selected for recording SPAD value reading to maintain uniformity across genotypes and readings. SPAD values were recorded from the middle part of three biological replicates. To study the leaf greenness NDVI (Normalized Difference Vegetation Index) readings were recorded at anthesis stage (50% flowering). Three biological replicates were maintained for both treatments. Data was recorded from both the cropping years.

Estimation of grain protein content and grain imaging

Grain N content (%) was estimated using the Kjeldahl method as proposed by Jones (1987). Grain protein content was calculated by multiplying total N content with the factor of 6.25 (Salo-vaananen et al. 1996; Mariotti et al. 2008). The value obtained represents crude protein content. Smart Grain version 1.1 (2012/8/1) imaging software was utilized to study the grain length and width. Rice grains were cleaned, spread uniformly on the grass on the scanner in a circular manner and scanned at 600 dpi.

Statistical analysis

Two-way analysis of variance (ANOVA) of three biological replicates was carried out in GraphPad Prism version 8 (La Jolla, California, USA) with variety, N level as treatment effects to compute adjusted P values and level of significance. Mean separation was done using Sidak's multiple comparisons test following one-way ANOVA. Graphs and heat maps were prepared using GraphPad Prism version 8 (La Jolla, California, USA).

Results

Effect of nitrogen treatments on days to flowering and leaf greenness

By recording the date of flowering (50% flowering) in all 30 diverse rice genotypes and the difference in days of flowering between two different N treatments, it was confirmed that N deficiency (N0) accelerated crop phenology. Early onset of flowering was attained in N0 in comparison to N sufficient (N120) condition (Supplementary Table S2). Early flowering was noticed in most genotypes with N0 treatment, and days to attain flowering from transplanting were thus reduced. The difference in days of flowering between treatments was from 1 to 16 days across all the genotypes. Drastic reduction in the number of days to flowering (DAT) was seen in Pusa Basmati-1under N deficiency, flowering 16 days earlier than usual. But, the days to maturity from flowering were almost similar (30–35 days). Early flowering reduced vegetative growth, early crop maturity, low panicle yield per plant, and reduced grain N accumulation and hence, grain quality. Genotypes such as KUSHAL, N-L-26, TAM-CAU-9A, Lalat, and BAM-4234 had lesser impact on crop phenology under N deficiency condition.

Flag leaf SPAD value at anthesis stage (within seven days after anthesis) showed positive interactions with N application level (Figs.1 a and b) in both the cropping years. Flag leaves, as well as the lower leaves of N120 treated genotypes, showed more greenness. Nitrogen deficient condition resulted in yellowing of the leaves. V shaped chlorotic tips were found in lower leaves. Stem also turned pale yellow. In the cropping year 2019, the highest SPAD value was recorded for rice genotype BVD-109 (71.1) with N

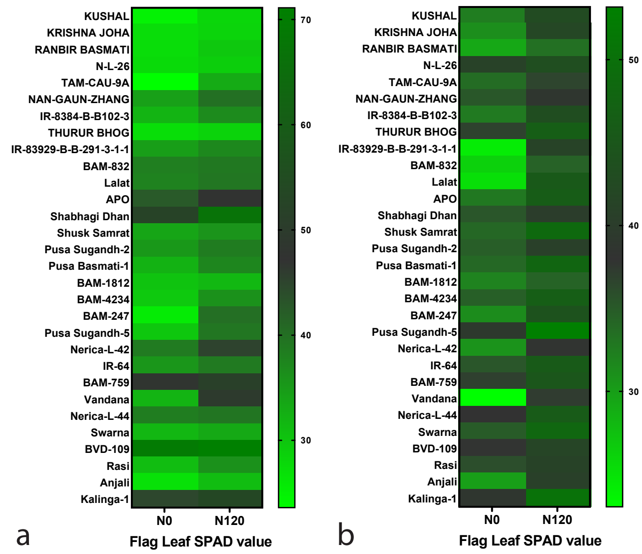

Fig. 1. Effect of nitrogen application rates (N deficient-N0, N sufficient-N120) on flag leaf SPAD value at booting during 2019 (a) and 2020 (b) cropping seasons in field grown diverse rice genotypes. Values presented are Mean ± SE with 3 replications

application (N120), and the lowest value was recorded for TAM-CAU-9A 23.6) with N0 treatment. The range of SPAD value under N0 was from 23.6 to 69.36; under N120, it was from 28.33 to 71.1. During 2020, the highest SPAD value recorded was 53.16 in Pusa Sugandh-5, N120 treatment and the lowest SPAD value of 24.36 in IR-83929-B-B-291-3-1-1, N0 treatment and the range from 24.36 to 41.1 under N0 and under N120 it was from 33.5 to 53.16. In both seasons, all the N120 treatments showed higher SPAD value compared to N0 treatments. During 2019, the highest difference in SPAD value between N treatments was observed in Vandana (N0- 32.26 and N120- 49.56), and during 2020, it was in the case of BAM-832 (N0-38.4 and N120-39.23). Sahbhagi Dhan, BAM-759, BVD-109, Pusa Sugandh-5, and Kalinga-1were able to maintain higher flag leaf SPAD values in N sufficient and N deficient conditions. N application improved the reading of vegetative greenness and NDVI (Normalized Difference Vegetation Index). Significant difference in NDVI was observed between N0 and N120 treatments, the difference was more evident during 2020 cropping season. Across the genotypes, significant variations were noticed during both the cropping seasons (Supplementary Fig. 1). During 2019, genotypes TAM-CAU-9A, BAM-1812, BAM-247, Vandana and during 2020, genotypes Kushal, Krishna Joha, Ranbir Basmati, N-L-26, TAM-CAU-9A, NAN-GAUN-ZHANG, IR-83929-B-B-291-3-1-1, BAM-832, Lalat, APO, Shusk Samrat, Pusa Sugandh-2, BAM-1812, BAM-4234, BAM-247, BAM-759, and the Nerica-L-44 showed more significant differences in NDVI values between N0 and N120 treatments. Higher NDVI value was reflected morphologically in the form of dark green coloration in leaves and growth. The NDVI value ranges from 0.43 to 0.66 under N0 and from 0.44 to 0.73

under N120 during the cropping season 2019. During 2019 the value ranges from 0.44 to 0.56 under N0 and from 0.49 to 0.71 under N120. A significant difference in NDVI values was observed between the cropping seasons.

Effect of N treatments on panicle yield and its components

During the 2019 *kharif* season, variations in yield attributing traits were observed among rice genotypes under N levels (Fig. 2, Supplementary Table S1). N supply increased the number of tillers per plant, the number of panicles per plant, the number of grains per panicle and panicle yield. 1000 grain weight was positively associated with total panicle yield per plant. Highest 1000 grain weight (N120-24.48 g) was recorded in rice genotype Anjali. Grain yield was lowest in BAM-247 in N0 treatment. During 2019 cropping, panicle yield per plant was significantly increased with N application and overall higher panicle yield compared to N deficient conditions. Among all 30 genotypes, the highest yield was obtained in Ranbir Basmati while, Krishna Joha showed the lowest yield in N0. During cropping season 2020, also similar results of highest yield per plant in APO and lowest in Krishna Joha, in N0 treatment. Some genotypes such as Ranbir Basmati, APO, Kalinga-1 performed well under both N0 and N120 conditions showing their high yielding ability.

Variations in grain nitrogen and protein content

Significant genotypic variations were found in grain N content, total grain protein accumulation among rice genotypes in both the cropping seasons. During 2019, Pusa Sugandh-5 showed higher grain N content (N120-2.51%). Lowest value was observed in Shabhagi Dhan (N0-1.09%). Significant increase in grain N content was found in most of the genotypes with N120 treatment. During 2020 (Fig. 3b) similar trend in grain N content was found with some exceptions with respect to treatments (except for Thurur Bhog; N0-2.63% and N120-2.53%, Pusa Basmati-1; N0-2.29% and N120-2.02%, IR-64; N0-2.71% and N120-2.40%; BAM-759; N0-2.33% and N120-2.20%, Rasi; N0-2.4% and N120-2.41%, Kalinga-1; N0- 2.33%, N120-2.31%). Highest and lowest grain N content with N120 treatment was found in rice genotypes Swarna (3.33%) and Pusa Basmati-1(2.02%), respectively. In N0 treatment it was highest for Swarna (3.08%) and lowest for Lalat (1.15%). Nitrogen content per individual grain showed different results from total grain N (%) content. Value ranges from 208. 32 µg to 717.85 µg in Lalat (N0) and Pusa Sugandh-5 (N120) respectively. Grain N content and protein content were correlated with total panicle yield. During 2019, rice genotypes that showed higher grain protein accumulation were Kalinga-1, APO, Ranbir Basmati. During 2020, the rice genotypes APO, Pusa Sugandh-5, Rasi showed higher total grain protein accumulation (Fig. 4).

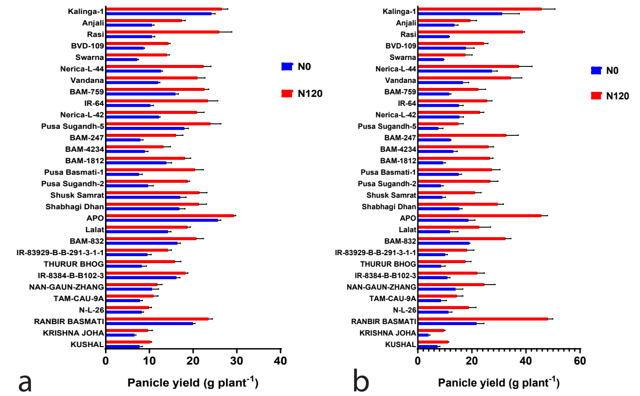

Fig. 2. Effect of nitrogen application rates (N deficient-N0, N sufficient-N120) on panicle yield per plant during 2019 (a) and 2020 (b) cropping seasons in field grown diverse rice genotypes. Values presented are Mean ± SE with 3 replications

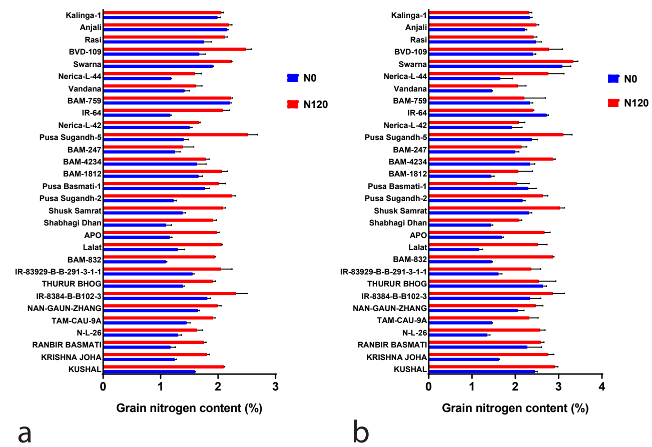

Fig. 3. Effect of nitrogen application rates (N deficient-N0, N sufficient-N120) on variations in grain nitrogen content 2019 (a) 2020 (b) cropping seasons in field grown diverse rice genotypes. Values presented are Mean ± SE with 3 replications

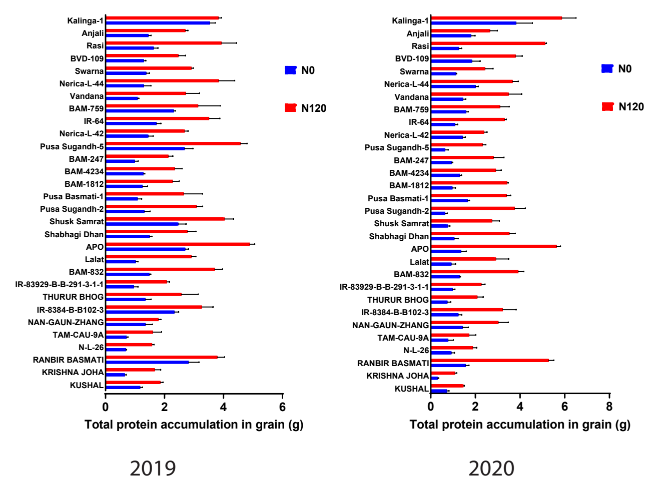

Fig. 4. Effect of nitrogen application rates (N deficient-N0, N sufficient-N120) on (a) 1000 Grain Weight and (b) grain protein content during 2020 cropping season in field grown diverse rice genotypes. Values presented are Mean ± SE with 3 replications

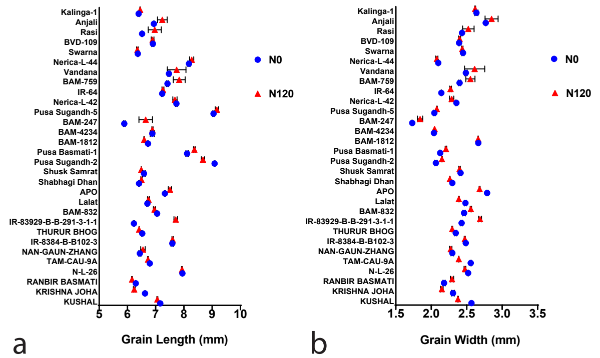

Fig. 5. Effect of nitrogen application rates (N deficient-N0, N sufficient-N120) on variations in (a) Grain Length (L) and (b) Grain (w) in diverse rice genotypes. Values presented are Mean ± SE with 3 replication

Grain morphology-related parameters

Differences in grain morphology were correlated with panicle yield per plant in both treatments (Fig. 5 a, b). Genotypes with sufficient N maintained grain morphology parameters specific to a particular genotype. In contrast, N deficient condition resulted in alteration in grain morphology, and the results were reflected in the panicle yield per plant. However, in some genotypes (APO, Nerica-L-42, and Kalinga-1), a lesser impact on grain morphology-related parameters was observed with N deficiency. A significant difference in grain length (N0- 6.22 mm and N120- 7.710 mm) and grain width (N0-2.42 mm and N120-2.68mm) was observed in IR-83929-B-B-291-3-1-1 between two N treatments. Grain length (L) and width (W) significantly increased under N120 in this genotype.

Correlation among different morpho-physiological parameters and yield

Flag leaf SPAD (Soil Plant Analysis Development) value and NDVI (Normalized Difference Vegetation Index) was significantly correlated (Fig. 6 a, b, Supplementary Fig. 1) with grain yield, and grain protein content. Grain length was significantly correlated with 1000 grain weight. SPAD value and NDVI was significantly correlated with grain protein content. Flag leaf greenness was significantly correlated with total grain N and protein content (during both *kharif 2019* and *kharif* 2020).

Discussion

Nitrogen is an essential macronutrient for rice growth and yield; hence the exogenous application of nitrogenous fertilizers is crucial to support higher growth, performance and productivity as its availability in the soil is insufficient. Doses of N application significantly influence the physiological performance of rice (Zhang et al. 2020), which is ultimately reflected in improved yield attributes. The present study displayed varying degrees in vegetative greenness, NDVI, days to flowering, grain protein content, grain morphological parameters, and panicle yield. Raun et al. (2002) demonstrated in cereal that crop reflectance measurements using optical sensors such as NDVI can be used to set more efficient and profitable fertilization levels.

N deficiency was visible as chlorotic leaves and senescence was early. As estimated by flag leaf SPAD values, leaf chlorophyll content was used as a measure of leaf senescence (Erley et al. 2007). In N0, the rice genotype showed early leaf senescence. During booting and anthesis, rice requires a sufficient amount of water and N to support physiological functions, and the yield potential of rice is defined during these stages (Fageria 2007). N scarcity during this growth stage in N0 treatment accelerated the physiology of rice as a survival mechanism. A decrease in chlorophyll content or chlorosis was observed in the flag leaf in the form of a low SPAD value. However, low N tolerant genotypes managed to sustain high leaf greenness and normal yield. N deficiency also affected grain morphology as N is the critical nutrient involved in enzyme activity and transcriptional factors responsible for grain growth and development. This was reflected as final yield reduction. Non-availability of N during grain filling resulted in defects in grain shape, size, nutrient and protein content.

Crop phenology and growth are directly associated with N availability during the crop growth stage. Imbalance in management and availability of N during the critical stages of crop growth changes the phenology and overall performance. N deficiency during crop growth or stress imposed in the form of N0 treatment resulted in acceleration of crop phenology, early crop maturity, and yield reduction. N deficient conditions activate the factors such as senescence-associated genes (SAG), proteolysis (autophagy, proteasome), N recycling activity, and phase change (phenology) that regulate the yield and crop quality in rice (Feng et al. 2010; Ueda et al. 2020). N application increased flag leaf SPAD reading (Yang et al. 2014b; Ye et al. 2022) as N availability triggered chlorophyll and other pigment biosynthesis leading to higher greenness and photoassimilatory activity. Chlorophyll content of leaf is affected by N availability as N is the integral component of chlorophyll porphyrin ring structure. SPAD reading can be used as a simple tool for evaluating the N concentration of the leaf and investigating the effect of N application, N distribution (Yang et al. 2014a) and vegetative greenness. Therefore, chlorophyll content or SPAD value could indicate N status in rice at different crop growth stages. SPAD value has been extensively used to study crop status to improve yield and N use efficiency (Khurana et al. 2007; Peng et al. 2006; Wang et al. 2008). A reduction in SPAD value was observed in N scarcity, most probably due to a disturbance in chlorophyll biosynthesis. N efficient genotypes absorb available soil N and maintain growth and photosynthetic activity. But, crop growth was not achieved up to the optimum level with minimal N availability. The genetic potential of crop growth is achieved when necessary

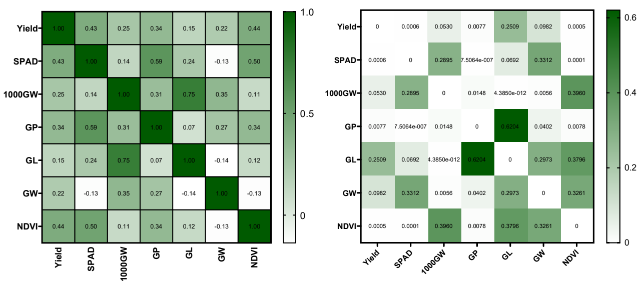

Fig. 5. Pearson correlation matrix showing relationship between yield, SPAD value at booting, 1000 grain weight, grain length, grain weight, NDVI at booting.

environmental factors are favorable. Nitrogen nutrition index (NNI) is an essential index for crop N nutrition diagnosis, and is a quantitative indicator for fertilization decision-making (Yang et al. 2014b). It was found that SPAD and NDVI values are well correlated with chlorophyll content, N status and growth in rice. Thus both NDVI and SPAD values can be considered as a surrogate for crop NNI.

Among the yield components in rice, tiller number or the number of panicles per plant, number of grains number per panicle, and 1000 grain weight determine panicle yield and can closely reflect N use efficiency. Several studies support that the tiller number, rather than other parameters displays a significant positive response to increasing N fertilizer application (Liu et al. 2021). Increasing or decreasing the expression of N metabolism-associated genes can consequently affect rice tillering activity (Liu et al. 2021). Studies have shown that N uptake of rice interacts with biomass accumulation, leaf area, and flowering activity, and the application of N fertilizer can increase the N accumulation of above-ground dry matter, thereby achieving higher panicle yield; however, excessive application of N fertilizer may have a negative impact on plant performance (Padhan et al. 2020). The reproductive growth stage is the most sensitive to biotic and abiotic stresses (Fageria 2007).

Grain quality (N and protein content) of rice is strongly linked with N fertilization. Vegetative stage N uptake in rice depends on its availability in soil and its form of availability (oxidized or reduced), but reproductive stage N uptake mainly depends on the organic N content in plant tissues which further remobilized for grain development and seed filling (Lee 2021). When sufficient resources are supplied, i.e., N and water, the growth is attained at a maximal level with higher vegetative biomass accumulation and improved N uptake (Kraiser et al. 2011). The vegetative storage N pools have an essential function in high input situations where they can enhance N uptake efficiency when pre-reproductive stage supplies are abundant. The requirement for N is higher after anthesis. Stored N is a buffer pool for maintaining leaf expansion and synthesizing photosynthetic proteins in early leaf growth (Lehmeier et al. 2013). Higher N uptake leads to high biomass accumulation, photosynthetic activity, source-sink translocation and yield. As the post-anthesis N uptake in rice is low (Ju et al. 2009; Ye et al. 2022), fertilizer application after flowering may lead to wastage. The N supplied during the vegetative stage in this experiment (N sufficient-N120) with three divided doses, 50% at transplanting, 25% tillering, and 25% at anthesis, were sufficient enough to support vegetative growth in rice. N remobilization after anthesis from vegetative organs (flag leaf, lower leaf, stem) to developing grains resulted in higher grain N accumulation, protein content and panicle yield, which is reported in rice and other cereal crops (Padhan et al. 2023). In the first crop season, BAM-832, APO and second season, TAM-CAU-9A, THURUR BHOG, APO**,** and Vandana performed better than other genotypes in N0. In the high-performing genotypes, crop growth in N0 was at par with N120, probably through a nutrient mining mechanism with a well-developed root system. N storage and remobilization to developing grain with N120 treatment increased final grain N and protein content. The grain's final N and protein content was reduced, whereas N deficiency rested in higher remobilization activity.

Rice grain appearance quality, starch content, amylose content, and protein content are associated with factors like N availability and grain N content. Grain morphology is one of the most important traits affecting rice yield (Huang et al. 2017; Wang et al. 2012). A large number of measurements are needed to obtain accurate seed morphological data because there is little difference in size among seeds from one plant. Our study was performed using *Smart Grain* software (Tanabata et al. 2012), making it easier and significant results were obtained in 30 diverse rice genotypes grown with two different N regimes in the field with a minimal level of available soil N. In previous studies conducted using image based analysis, it is evident that morphological and colour variations in grains among diverse rice genotypes were observed (Kuo et al. 2016). We noticed variations in grain length, grain width, grain length to width ratio, grain perimeter length, and grain area size across the genotypes studied. These variations were due to N-mediated stimulation of mechanisms and enzymes responsible for grain growth. Grain N content is directly associated with variations in all these grain morphology-related parameters. Non-availability of N made the grains shriveled, unfilled or partially filled. Changes in grain filling were reflected in the grain morphological parameters, such as grain circularity. Variations observed in grain circularity and distance between IS (intersection of length and width) and CG (centre of gravity) were due to the grain-filling ability of rice genotypes. Properly filled grains maintained their shape, were circular in appearance, and could be distinguished from unfilled or partially filled grains. Individual and 1000 grain weights are essential components of crop yield attributes (Garcia et al. 2016; Lian et al. 2005; Slafer et al. 2014). Single grain weight, 1000 grain weight and total panicle yield per plant were increased because N supported the grain growth and development through post anthesis enzyme activities. N is also involved in tillering actions mediated through cytokinin, leading to more tillers per plant, more panicle numbers and improved panicle architecture (Cerutti and Delatorre, 2013). Though grain length, width, and grain length to width ratio are the genetic characteristics of a genotype, disturbances in environmental factors or N deficiency change the grain length and result in the grain's overall size. Grain Area Size (AS) and Grain Perimeter Length (PL) were also affected due to N deficiency. Regulation of seed size is a crucial strategy for improving crop yield. However, the molecular mechanisms by which plants determine their seed size remain elusive. DEP1 (dense and erect panicle 1) controls grain size, shape, and N use efficiency (NUE) in rice (Huang et al. 2009; Sun 2014). Grain size is increased when the DEP1 allele is over expressed, whereas small grains are produced when the *dep1* allele is over expressed. The high sink capacity of the *dep1* mutant was not met by a low photosynthetic rate, leading to the reduced grain filling, grain set, grain length, 1000 grains weight and finally, panicle yield (Zhao et al. 2019). To optimize the grain development process, C and N flow from source to sink should be optimized in a controlled manner (Chen et al. 2021). The ubiquitin-proteasome pathway also plays a crucial role in controlling seed size.

In rice, the genetic variations that offer changes in NUE have been reported. The allelic variation in N transport and assimilation genes are major determinants of NUE divergence of *indica* and *japonica* rice. NRT1.1B-indica allele, indica allele of GRF4/ngr2, indica allele of OsMYB61, OsNR etc are associated with high NUE of indica rice (Hu et al, 2015, Liu et al. 2021). Liu et al (2021) evaluated a diverse panel of rice landraces spanning all the rice subgroups and identified an allelic variant in the promoter region of OsTCP19 that regulates tillering response to nitrogen (TRN). The haplotype H (OsTCP19-H) associated with high TRN is found in aus varieties while low TRN-associated haplotype L (OsTCP19-L) is seen in japonica and most of the indica varieties. NUE is a quantitative trait controlled by multiple genes (Yang et al. 2017b). Multiple sets of genes in crop plants regulate the mechanisms such as N absorption, accumulation and remobilization associated with NUE. Therefore, NUE of cereal crops could be improved by employing fundamental genetics that involve both conventional breeding and QTL mapping in combination with marker-assisted selection (Sandhu et al. 2021). Significant variability and marker-trait associations have been reported in genome-wide association studies for N uptake and N use efficiency (Liu et al. 2016; Monostori et al. 2017). Also, several genetic regions that are associated with nutrient uptake have been detected in rice (Ming et al. 2000, Yang et al. 2017b), wheat (Su et al. 2009; Dubey et al. 2022) and other cereals.

The present study comprising of indica, basmati and Nerica (Indica**Oryza glabberima),* genotypes*,* significant variations were found in the traits considered. For example*,* N deficiency did not affect flowering time in the Indica (KUSHAL, TAM-CAU-9A, Lalat, and BAM-4234) and Indica*O glabberima genotype N-L-26. Indica genotypes, Sahbhagi Dhan, BAM-759, BVD-109, Pusa Sugandh-5, and Kalinga-1 maintained higher vegetative greenness in comparison to other sub groups. The genotypes Sahbhagi Dhan, Vandana, Kalinga-1, APO and Indica**O. glabberima* genotype N-L-44 showed higher panicle yield under N0 condition. Indica genotypes APO, Kalinga-1 and Indica**O glabberima* genotype N-L-42 showed a lesser impact on grain morphology under N0. Grain protein content was found higher in BAM-759, Anjali, Thurur Bhog, IR-64, Rasi, and Kalinga-1 under both the treatments, suggesting the superiority of indica lines over other subgroups. Evaluation of diverse rice genotypes belonging to different subgroups will provide new insights into rice NUE. For example, the *NRT1.1B-indica* and *OsTCP19*-H alleles are deployed to develop "green super rice varieties" that perform better under low N input conditions without compromising yield (Hu et al. 2023). Among the two subspecies of Asian cultivate rice, indica varieties require less N input than japonica varieties (Hu et al. 2023). The findings in the present study also support that *indica* varieties out-perform other subgroups in majority of the traits studied, however evaluation of a larger and diverse set of genotypes are required to draw robust conclusions. Worldwide, NUE for cereal production (wheat, corn, rice, barley, sorghum, millet, oat, and rye) is approximately 33% (Raun and Johnson 1991). Increased cereal NUE must accompany increased yields needed to feed a growing world population that has yet to benefit from the promise of N fixing cereal crops.

Authors' Contributions

Conceptualization of research (LS); Designing of the experiments (LS, VC, GK, SK, AK); Contribution of experimental materials (SK, AK, VC, GK); Execution of field/lab experiments and data collection (BKP); Analysis of data and interpretation (BKP, LS); Preparation of the manuscript (BKP, LS).

Acknowledgments

The first author acknowledges the ICAR-Indian Agricultural Research Institute for the fellowship support and NAHEP-CAAST funding during the study.

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Supplementary Table S1. Details of rice genotypes used in the study

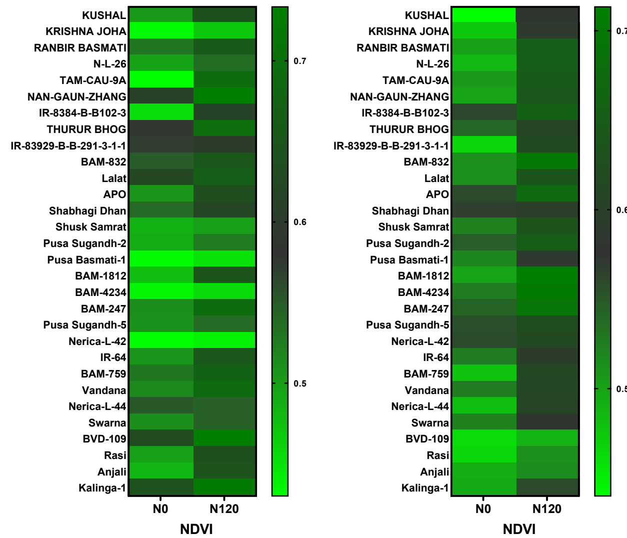
SI. No	Name of genotypes	Parentage/ IC No/EC No	Oryza sativa Subgroup (Garris et al. 2005)
1	Kushal	Pankaj / Mahsuri (IC443804)	Indica
2	Krishna Joha	Traditional aromatic landrace of Assam	Indica
3	Ranbir Basmati	Selection from Basmati 370	Basmati
4	NERICA-L-26	TOG5681/4*IR64	Indica**O glabberima*
5	TAM-CAU-9A	IRGC 8228	Indica
6	NAN-GAUN-ZHANG	-	-
7	IR83383-B-B-129-3	IR 72022-46-2-3-3-2/IR 57514-PMI 5-B-1-2	Indica
8	THURUR BHOG	Landrace	Yet to be sub grouped (Neeraja et al. 2021)
9	IR 83929-B-B-291-3-1-1	IR78878-53-2-2-2/CT6510-24-1-2	Indica
10	BAM 832	-	-
11	Lalat	OBS 677/IR 2071//VIKRAM/W 1263	Indica
12	APO	UPL RI 5/IR12979-24-1 (Brown)	Indica
13	Sahbhagi Dhan	IR 55419-4*2/WAY RAREM	Indica
14	Shusk Samrat	C1064-5/Kalkari//IR54	Indica
15	Pusa Sugandh- 2	Pusa 1238-1/Pusa 1238-81-6	Basmati
16	Pusa basmati-1	Pusa 150/Karnal Local	Basmati
17	BAM 1812	319449	Indica
18	BAM 4234	497171	Indica
19	BAM 247	123683	Indica
20	Pusa Sugandh- 5	Pusa 3A/Haryana Basmati	Basmati
21	Nerica-L-42	TOG5681/4*IR64	Indica**O glabberima*
22	IR 64	IR 5657-33-2-1/IR 2061-465-1-5-5	Indica
23	BAM-759	-	Indica
24	Vandana	CO 22/KALAKERI	Indica
25	Nerica-L-44	TOG5681/5*IR64	Indica**O glabberima*
26	Swarna	VASISTHA/MAHSURI	Indica
27	BVD109 (Birsa Vikas Dhan 109)	Kalinga IIIxIR64	Indica
28	Rasi	T(N)1 x Co.29	Indica
29	Anjali	Sneha/RR 149-1129	Indica
30	Kalinga 1	Dunghansali/ IR 8	Indica

= Not known

Supplementary Table S2. Effect of nitrogen application rates (N deficient-N0, N sufficient-N120) on days to flowering and yield parameters (number of tillers per plant, number panicles per plant and panicle length) in field grown diverse rice genotypes. Value presented are Mean ± SE with 3 replications.

Genotypes	Days to flowering		Number of tillers (per plant)		Number of grains per panicle		1000 grain weight (g)	
	1st flowering	Difference (days)	N0	N120	N0	N120	N0	N120
KUSHAL	N0	1	6.667 ± 0.882	7.667 ± 0.333	21.667 ± 1.453	24.000 ± 1.528	18.483 ± 0.905	20.133 ± 0.685
KRISHNA JOHA	N0	3	4.333 ± 0.882	7.000 ± 0.577	17.667 ± 0.667	20.333 ± 1.202	16.733 ± 0.538	18.133 ± 0.107
Ranbir Basmati	N120	2	10.333 ± 1.202	17.667 ± 0.882	22.667 ± 1.453	24.333 ± 0.333	17.627 ± 0.866	19.780 ± 0.376
N-L-26	N120	1	9.667 ± 0.882	11.667 ± 0.667	20.333 ± 0.333	21.000 ± 1.155	19.653 ± 0.658	20.267 ± 0.576
TAM-CAU-9A	N120	1	6.667 ± 1.202	8.667 ± 0.882	20.667 ± 0.882	23.667 ± 1.202	18.090 ± 0.315	18.507 ± 0.335
NAN-GAUN-ZHANG	N0	2	7.000 ± 0.577	9.333 ± 0.882	21.667 ± 0.882	22.333 ± 0.882	17.073 ± 0.870	18.817 ± 0.497
IR-8384-B-B102-3	N120	3	7.000 ± 1.000	12.000 ± 1.000	19.333 ± 0.333	23.333 ± 0.882	20.387 ± 0.721	22.767 ± 1.056
THURUR BHOG	N0	7	8.333 ± 0.882	12.667 ± 1.856	18.333 ± 0.667	20.000 ± 1.000	19.027 ± 0.859	22.273 ± 0.507
IR-83929-B-B-291-3-1-1	N120	4	4.333 ± 0.333	6.000 ± 0.577	20.667 ± 0.667	24.000 ± 0.577	19.983 ± 0.876	23.347 ± 0.638
BAM-832	N120	2	8.667 ± 0.333	10.667 ± 0.882	24.000 ± 1.000	24.333 ± 0.333	14.713 ± 0.846	19.077 ± 0.593
Lalat	N0	1	6.000 ± 1.528	7.667 ± 1.453	23.333 ± 0.882	24.333 ± 1.202	16.767 ± 0.198	18.690 ± 0.212
APO	N0	4	6.000 ± 1.000	11.000 ± 0.577	23.333 ± 0.667	22.667 ± 0.882	17.283 ± 0.643	20.440 ± 0.871
Shabhagi Dhan	N120	11	5.667 ± 0.667	8.333 ± 0.882	23.667 ± 0.882	24.667 ± 0.882	16.770 ± 0.191	17.367 ± 0.764
Shusk Samrat	N0	10	6.667 ± 0.882	9.667 ± 1.453	20.667 ± 0.667	25.000 ± 1.155	18.397 ± 1.041	20.363 ± 0.112
Pusa Sugandh-2	N0	6	6.000 ± 0.577	9.000 ± 0.577	23.000 ± 0.577	22.667 ± 0.882	16.543 ± 0.898	19.840 ± 2.458
Pusa Basmati-1	N0	16	8.000 ± 0.577	10.333 ± 0.882	20.333 ± 0.882	25.333 ± 0.667	19.000 ± 0.770	21.223 ± 0.119
BAM-1812	N120	10	6.667 ± 0.667	10.000 ± 0.577	21.333 ± 0.667	22.000 ± 1.155	17.270 ± 0.452	20.963 ± 0.902
BAM-4234	N0	1	6.333 ± 0.882	10.000 ± 0.577	20.000 ± 0.577	21.333 ± 1.202	18.710 ± 1.451	22.420 ± 2.280
BAM-247	N0	5	7.667 ± 0.333	10.667 ± 1.202	25.667 ± 0.882	26.000 ± 1.000	14.077 ± 1.172	17.670 ± 0.613
Pusa Sugandh-5	N120	13	6.000 ± 1.155	9.000 ± 0.577	20.000 ± 0.577	23.000 ± 0.577	18.127 ± 1.010	21.223 ± 1.275
Nerica-L-42	N120	3	6.667 ± 0.667	8.333 ± 0.333	21.333 ± 0.667	23.667 ± 0.333	17.627 ± 0.725	17.993 ± 0.515
IR-64	N120	10	6.000 ± 0.577	8.667 ± 0.667	21.000 ± 0.577	23.667 ± 0.333	15.483 ± 0.454	17.987 ± 0.565
BAM-759	N0	9	6.667 ± 0.333	10.667 ± 0.882	19.667 ± 1.202	23.333 ± 0.882	18.573 ± 1.307	23.407 ± 1.392
Vandana	N0	3	7.333 ± 0.882	11.667 ± 1.453	24.667 ± 0.882	24.667 ± 0.667	23.480 ± 1.259	24.547 ± 0.641
Nerica-L-44	N0	3	10.000 ± 0.577	13.333 ± 1.202	21.333 ± 0.882	24.333 ± 0.667	20.797 ± 0.746	21.847 ± 0.159
Swarna	N0	2	8.333 ± 0.333	12.667 ± 1.764	20.333 ± 0.333	20.667 ± 0.333	16.183 ± 0.592	19.140 ± 0.618
BVD-109	N0	2	7.000 ± 0.577	9.667 ± 0.667	22.667 ± 0.882	27.000 ± 1.155	17.423 ± 1.003	19.327 ± 1.312

Rasi	N0	2	9.000 ± 0.577	16.000 ± 0.577	22.667 ± 0.882	24.333 ± 0.667	21.607 ± 0.696	22.413 ± 0.558
Anjali	N0	3	11.000 ± 1.000	13.000 ± 1.528	21.66 ± 0.3337	22.000 ± 1.000	14.920 ± 0.737	19.823 ± 0.115
Kalinga-1	N0	2	15.000 ± 2.517	17.667 ± 0.667	23.667 ± 0.667	26.333 ± 0.882	17.260 ± 0.471	20.247 ± 0.672
Source of Variation			P value	P value summary	P value	P value summary	P value	P value summary
Variety (V)			<0.0001	****	<0.0001	****	<0.0001	****
Nitrogen (N)			0	***	0	*	0	*
N*V			0.1	ns	0	*	0.4	ns



Supplementary Fig. 1. Effect of nitrogen application rates (N deficient-N0, N sufficient-N120) on NDVI at booting during 2019 (a) and 2020 (b) cropping seasons in field grown diverse rice genotypes. Values presented are Mean ± SE with 3 replications