



RESEARCH ARTICLE

Influence of nitrogen at different soil depths on crop health indices and yield of rice

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Abstract

Nitrogen plays the most crucial role in the health status and yield of rice. Magnitudes of two crop health indicators, i.e., normalised difference vegetation index (NDVI) and soil plant analysis development (SPAD) values, depend on the availability of nitrogen in the soil. In coarse-textured soils, where the leaching loss of nitrogen is a major concern, the availability of nitrogen in various depths plays a prominent role in crop health indicators as well as grain yield. Rainfall amounting to 398.8 and 189.3 mm, respectively, during 0–30 and 31–50 days after transplanting (DAT) caused the presence of a notable amount of nitrogen at 40 cm depth. The influence of nitrogen levels on both NDVI and SPAD values was assessed in all observation dates (30, 50, 70 and 90 DAT) through regression analysis. The best possible relationship in terms of the coefficient of determination (R^2) value was recorded on 30 DAT. Interestingly, on that day, nitrogen at 40 cm depth showed a higher R^2 value (0.81) in relation to NDVI over the nitrogen at 15 cm depth ($R^2 = 0.78$). The relationship between soil nitrogen and SPAD was also strongest on 30 DAT and the R^2 values were 0.78 and 0.77 against nitrogen availability, respectively, at 15 and 40 cm depths. Grain yield increased continuously with an increase in nitrogen level till the N_{200} treatment. After attaining the plateau (5.27 Mg ha⁻¹) at N_{200} , the same decreased marginally at N_{240} . Available nitrogen at both depths strongly influenced the grain yield, with R^2 values of 0.88 and 0.86 for nitrogen availability at 15 and 40 cm depths, respectively.

Keywords: grain yield; normalised difference vegetation index; soil nitrogen; soil plant analysis development

Introduction

Rice (*Oryza sativa* L.) is the most commercially important staple crop, accounting for more than 25 % of global food production (1). This crop acts as a primary source of energy in terms of calories to 50 % of the global population (2, 3). Worldwide, rice is grown on more than 164 million hectare, of which around 90 % is in Asia alone (4, 5). Among individual countries, India has the largest cultivated area under rice (47.83 million hectare). India ranked second (135.76 million tonnes) next to China (145.95 million tonnes) in terms of rice production (6). At present, India is self-sufficient in rice production to feed its population. However, the steadily increasing trend of the Indian population, coupled with the deterioration of soil health and the shrinking of cultivable area, is a serious concern so far food security is a concern. To achieve the rice production target marked for 2035 and 2050, researchers have to search for the optimisation of nitrogen dose towards maximising yield. As Indian agriculture is largely monsoon-dependent and rainfed rice accounts for about 89 % of the total rice production, priority should be given to the rainfed rice ecosystem (7). Nitrogen (N), a component of protein structure, plays a key role in the rate of photosynthesis, which in turn affects grain yield (8, 9). There is a strong positive correlation between the net photosynthetic rate of rice leaves and total leaf nitrogen content (10). Crop nitrogen status is a function of nitrogen use processes and an important indicator of subsequent yield potential (11). Nitrogen

is responsible for the production of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), a CO₂-fixing enzyme that enhances the rate of photosynthesis (8). Therefore, there is a relationship between the leaf blade nitrogen concentration (LBNC) and chlorophyll content (12). Total leaf nitrogen content is related to the water status of mesophyll tissue and per percentage of chlorophyll in the leaf. The status of these two can easily be identified through the reflectance of electromagnetic radiation at near infrared (NIR) and absorption of the same at the visible range. Values of normalised difference vegetative index (NDVI) and soil plant analysis development (SPAD) are based on the principal of reflectance and absorption of electromagnetic radiation in the NIR and visible range. Measurement of NDVI and SPAD will give an idea about the photosynthetic activity in the leaf (13). These parameters serve as indicators of crop health and can aid in early yield forecasting. Nitrogen is a primary element in the synthesis of chlorophyll, which plays a crucial role in converting light energy into chemical energy (14, 15). Coarse-textured soils with high saturation hydraulic conductivity resulted in significant nitrogen leaching from surface to sub-surface soil. Thus, such soils become poor in fertility status and highly responsive to rice yield in relation to doses of nitrogenous fertiliser. A field study was carried out on a coarse-textured soil to assess the temporal status of nitrogen-induced NDVI and SPAD. The study also aimed to identify the crop stage at which soil available nitrogen exerted the highest influence on NDVI and

SPAD. In addition, the coefficient of determination (R^2) between soil nitrogen and yield was worked out.

Materials and Methods

The field study was carried out during August to October, 2024 at the Post Graduate Research Farm (18.81N latitude, 84.18E longitude, 61 m above mean sea level) of M.S. Swaminathan School of Agricultural Science, Centurion University of Technology and Management. At the family level, the soil has the nomenclature Rhodic Paleustalfs. The soil at the study site was sandy loam, with only 15.3 % clay and the saturated hydraulic conductivity was 16.7 mm d⁻¹. The pH of the soil was 6.2, coupled with low alkaline KMnO₄ oxidizable nitrogen (241 kg ha⁻¹) and Walkley–Black oxidizable organic carbon (0.42 %). The value of 0.5 M NaHCO₃ extractable-P was 12.8 kg ha⁻¹ and 1 N NH₄OAc extractable-K 126.8 kg ha⁻¹ represents the soil chemical environment. Hence, the soil is coarse-textured with a low fertility level. The climate of the study site comes under the sub-humid zone with an aridity index value of 0.64. During the entire growing period crop received a total amount of 667.7 mm rainfall, which was distributed as 398.8, 189.3, 65.4 and 14.2 mm rainfall occurred, respectively, during 0 to 30 days after transplanting (DAT), 31 to 50 DAT, 51 to 70 DAT and 71 to 90 DAT (harvest) time frame. An additional 200 mm of water was applied as life-saving irrigation, in four applications of 50 mm each. The experiment was laid out in a randomised complete block design (RCBD), with seven N levels and each treatment replicated three times. The N levels were: N₀ – where no nitrogenous fertilizer was applied i.e., control, N₄₀ – 40 kg N ha⁻¹, N₈₀ – 80 kg N ha⁻¹, N₁₂₀ – 120 kg N ha⁻¹, N₁₆₀ – 160 kg N ha⁻¹, N₂₀₀ – 200 kg N ha⁻¹ and N₂₄₀ – 240 kg N ha⁻¹. Nitrogenous fertiliser was applied as 50 % basal, 25 % on 30 DAT and the remaining 25 % on 50 DAT. Two split doses were applied after completion of observations on tillering and panicle initiation stage, respectively. Rice variety Shatabdi was taken as a test crop. Observations on available soil nitrogen at 15 and 40 cm soil depths, NDVI and SPAD (chlorophyll meter) were taken on 30, 50, 70 and 90 DAT. Grain yield was recorded after threshing of the harvested crop.

Optical sensor readings were taken with a handheld GreenSeeker TM (N Tech Industries Inc., Ukiah, CA, USA). The sensor measured canopy reflectance at red region ($\lambda = 656$ nm) and NIR region ($\lambda = 774$ nm) and NDVI was determined as:

$$NDVI = \frac{\lambda_{NIR} - \lambda_{RED}}{\lambda_{NIR} + \lambda_{RED}} \quad (\text{Eqn. 1})$$

Where NIR and RED represent the fraction of emitted NIR and red radiation reflected from the sensed area, respectively. The sensor was passed over the crop at a height of about 70 cm. Sensor height above ground increased as the crop growth progressed. The

chlorophyll meter used in the present study was the hand-held Minolta SPAD-502. The instrument measures the transmittance of the radiation from two LEDs through a 2 × 3 mm area of the leaf. The wavelength of one of these LEDs has a peak in the red visible region ($\lambda = 660$ nm), which chlorophyll absorbs. The peak from the other is in the near-infrared ($\lambda = 940$ nm), which chlorophyll transmits. The instrument uses the ratio of the transmittance from these two LEDs to calculate a numerical SPAD value that is proportional to the concentration of chlorophyll in the leaf (16). The SPAD values were recorded by inserting the middle portion of the index leaf in the slit of the SPAD meter. From each plot, readings from ten randomly selected plants were averaged. Values of SPAD were recorded at the same time along with the NDVI.

Results

Available soil nitrogen

At 15 cm soil depth, on 30 DAT, the amount of available soil nitrogen was lowest (229 kg ha⁻¹) under the control (N₀) condition. An increase in nitrogen application rates from 0 to 240 kg N ha⁻¹ continuously increased the availability of soil nitrogen. The highest value (339 kg N ha⁻¹) was recorded under the N₂₄₀ treatment. Between two consecutive treatments, the increase in availability of nitrogen was maximum (27 kg N ha⁻¹) from N₁₂₀ to N₁₆₀. On subsequent observation dates (50, 70 and 90 DAT) increase in nitrogen dose showed, in general, similar trends with available soil nitrogen as that of 30 DAT. In a temporal scale, with the advancement of time from 30–50 DAT availability of soil N decreased by 4–10 %. The per cent decrease was more (9–10 %) when N was applied at ≥ 160 kg N ha⁻¹. Application of 25 % N at 50 DAT boost the availability of soil N on 70 DAT by 1–4 %. Thereafter, grain filling caused around a 2–6 % decrease in soil N availability. On all observation dates, irrespective of treatments, the availability of soil N at 40 cm depth followed a similar trend to that observed in 15 cm depth. However, values were 4–13 % lower than those at 15 cm depth. The difference in available soil N between 15 and 40 cm depths was lower (4–5 %) under N₀ to N₄₀, moderate (7–9 %) under N₁₆₀ to N₂₄₀ N levels and high (12–13 %) under N₈₀ to N₁₂₀ N levels. Interestingly, the temporal variation of N availability between two consecutive observation dates was greater (9–12 %) at 40 cm depth than at 15 cm depth, where the variation was 4–10 % (Table 1).

Normalised difference vegetation index (NDVI) and Soil plant analysis development (SPAD)

Proximal optical sensors are a form of remote sensing in which the sensors are positioned close to the crop. These sensors do not directly measure N content in crop tissue but provide measurements or indices of optical properties of crops

Table 1. Temporal status of available soil nitrogen with grain and straw yield

Nitrogen levels	Available soil nitrogen at 15 cm depth (kg ha ⁻¹)			Available soil nitrogen at 40 cm depth (kg ha ⁻¹)				
	Days after transplanting							
	30	50	70	90	30	50	70	90
N ₀	228.8	219.5	225.2	221.8	218.2	205.3	214.2	211.8
N ₄₀	253.3	242.9	248.0	233.6	239.5	213.6	232.0	224.0
N ₈₀	273.6	259.8	264.8	259.7	247.9	227.3	244.0	247.7
N ₁₂₀	292.1	277.9	281.9	274.1	265.4	249.2	262.8	258.0
N ₁₆₀	319.0	292.1	304.8	286.5	297.8	265.4	271.7	272.7
N ₂₀₀	337.6	306.1	316.1	304.0	315.3	275.9	287.1	284.9
N ₂₄₀	349.3	319.7	326.2	310.1	332.7	288.8	297.3	297.3
SEm±	5.34	4.23	4.31	4.33	3.62	4.15	3.98	3.86
LSD ($p = 0.05$)	16.20	12.84	13.06	13.13	10.98	12.59	12.02	11.72

(i.e., canopy reflectance) that are sensitive to crop N status (17, 18). At the active tillering stage (30 DAT), an increase in N level from 0–200 kg N ha⁻¹, increased NDVI values from 0.46–0.7. Application of an additional 40 Kg N ha⁻¹ beyond the N₂₀₀ marginally reduced the NDVI value to 0.67 under the N₂₄₀ N level (Table 2). Thereafter, till harvest highest NDVI values (0.74 on 50 DAT, 0.69 on 70 DAT and 0.36 at harvest) were attained under N₁₆₀ N level. During those observation dates, minimum NDVI values were recorded as 0.55, 0.54 and 0.29. In a temporal scale, NDVI values increased markedly (4.2–21.4 %) from 30–50 DAT. After attaining the highest value on 50 DAT, NDVI values decreased marginally (1.6–10.6 %) on 70 DAT. Thereafter, at 90 DAT, a massive decrease (86–106 %) in NDVI values was recorded in all nitrogen levels. At the harvest stage, as the maximum leaf area turned yellowish, there was a massive decrease in NDVI values. It was observed that, across nitrogen levels and time, SPAD values followed trends similar to those of NDVI.

Grain yield

In the present study, the highest amount of 5.27 Mg ha⁻¹ grain yield was recorded under N₂₀₀ N level (Table 2). This was statistically at par with two nearby nitrogen levels, viz., N₁₆₀ and N₂₄₀. However, the same was significantly higher over N₀, N₄₀, N₈₀ and N₁₂₀ levels. In contrast, the lowest amount (2.28 Mg ha⁻¹) of grain yield was recorded under controlled conditions (N₀). The lowest value was statistically at par only when the crop was exposed to 40 kg N ha⁻¹ (N₄₀). A relative comparison of grain yield between two adjacent treatment levels with a difference of 40 kg N ha⁻¹ showed that, highest (37.15 %) variation occurred between N₄₀ and N₈₀ N levels. It was lowest (3.13 %) when comparison was made between N₁₆₀ and N₂₀₀ N levels. Yield data showed a continuous increasing trend from 0–200 kg N ha⁻¹. However, Fig. 1 shows that the magnitude of the yield increase from higher N levels decreased progressively. The gradient of the increasing trend was minimum at the N₂₀₀ N

level, where the yield maximisation plateau was reached. Thereafter further increase in N level gradually decreases the grain yield. In support of the physical trend of grain yield against N levels, the relationship is expressed in terms of a quadratic equation with a very strong coefficient of determination (R² = 0.98).

Influence of available soil nitrogen on the status of NDVI

The normalised difference vegetation index reflects crop canopy properties related to absorption of electromagnetic radiation in the NIR and visible range. In fact, the status of leaf chlorophyll, stomatal conductance and mesophyll leaf tissue wetness determined the value of NDVI of any rice canopy. As nitrogen is an important component of leaf chlorophyll, an attempt has been made under this study to assess the dependency of NDVI value on that of soil N available at 15 and 40 cm soil depths. Data presented in Fig. 2 showed that soil N at 15 cm depth notably influences (R² = 0.78) the status of NDVI at the active tillering stage (30 DAT) of the crop. Interestingly, it has been found that, available soil N at 40 cm depth, which was leached down from the surface (15 cm) layer, showed a slightly stronger (R² = 0.81) relationship to NDVI values (Fig. 3). Coarse-textured soils having a high saturation hydraulic conductivity value (16.7 mm d⁻¹) coupled with 398.4 mm rainfall caused a good amount of nitrogen leaching from 15–40 cm layers. This was the probable reason for the slightly stronger influence of nitrogen at 40 cm depth on the status of NDVI over available nitrogen at 15 cm depth.

Influence of available soil nitrogen on SPAD

The SPAD meter works on a principle of electromagnetic radiation that works in the visible red range with a peak at λ = 660 nm, which chlorophyll absorbs. The other peak is in the near-infrared range (λ = 940 nm), which chlorophyll transmits. The instrument uses the ratio of the transmittance to calculate a numerical SPAD

Table 2. Changes in NDVI and SPAD with the advancement of growing period and grain yield under varying nitrogen levels

Nitrogen levels	NDVI				SPAD				Grain yield Mg ha ⁻¹
	Days after transplanting								
	30	50	70	90	30	50	70	90	
N ₀	0.46	0.55	0.54	0.29	28.46	32.82	27.09	17.83	2.28
N ₄₀	0.51	0.61	0.60	0.30	30.59	35.91	32.05	19.91	2.88
N ₈₀	0.55	0.67	0.65	0.33	33.75	37.49	33.85	20.16	3.95
N ₁₂₀	0.63	0.73	0.66	0.33	36.14	39.77	35.28	22.42	4.53
N ₁₆₀	0.68	0.74	0.69	0.36	41.29	41.21	36.43	22.84	5.11
N ₂₀₀	0.70	0.73	0.69	0.34	42.38	41.75	36.51	22.47	5.27
N ₂₄₀	0.67	0.72	0.68	0.33	40.66	39.82	35.66	21.32	4.87
S Em±	0.02	0.03	0.03	0.01	1.3	1.4	1.2	0.9	0.15
LSD (p = 0.05)	0.07	0.09	0.09	0.03	4.1	4.3	3.8	2.9	0.48

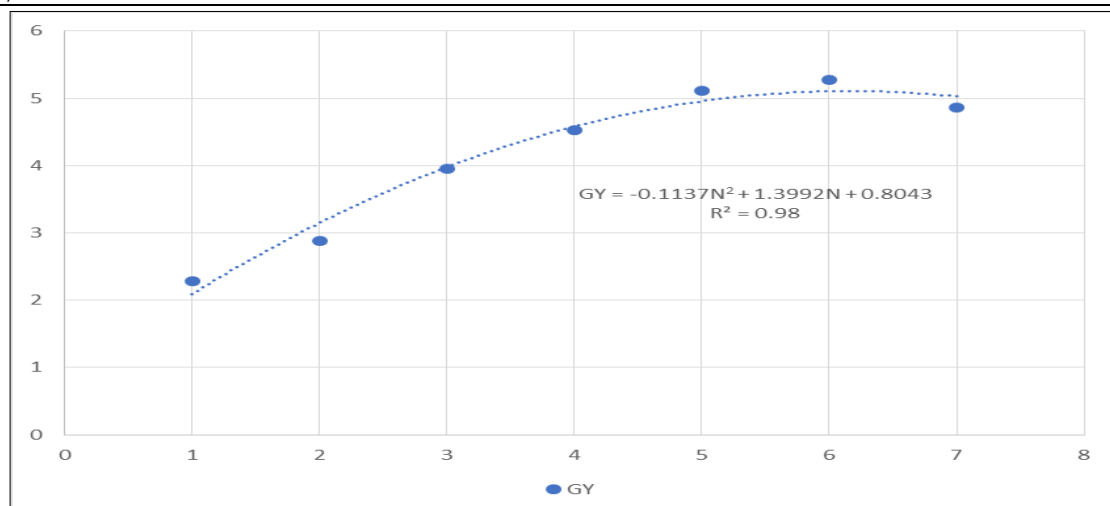


Fig. 1. Grain yield (Mg ha⁻¹) under varying nitrogen levels.

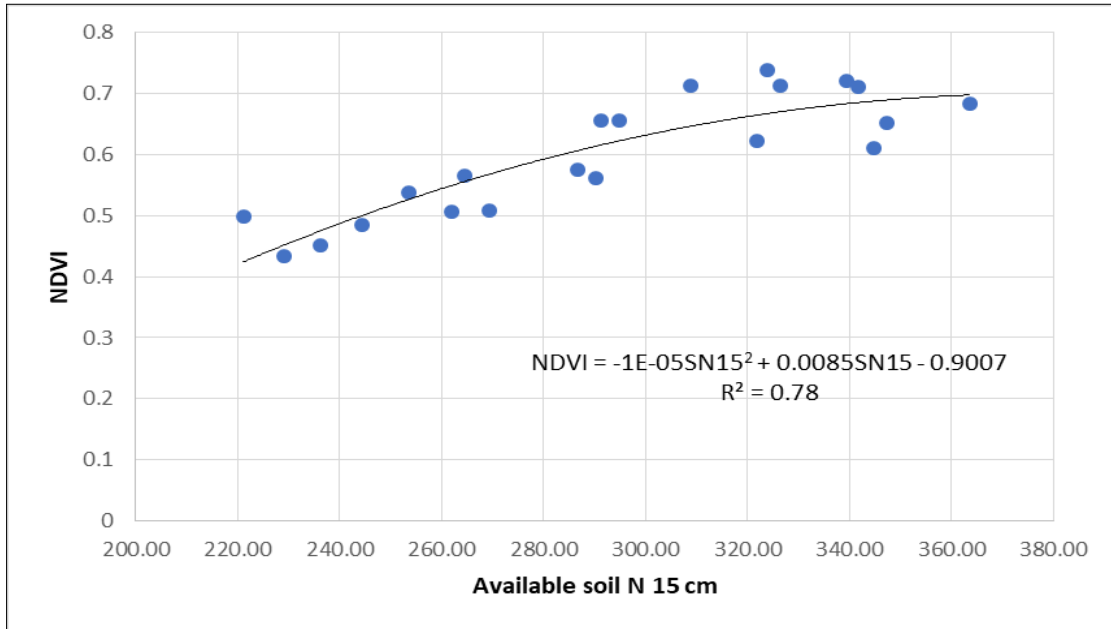


Fig. 2. Influence of available soil nitrogen at 15 cm depth on NDVI at 30 DAT under varying nitrogen levels.

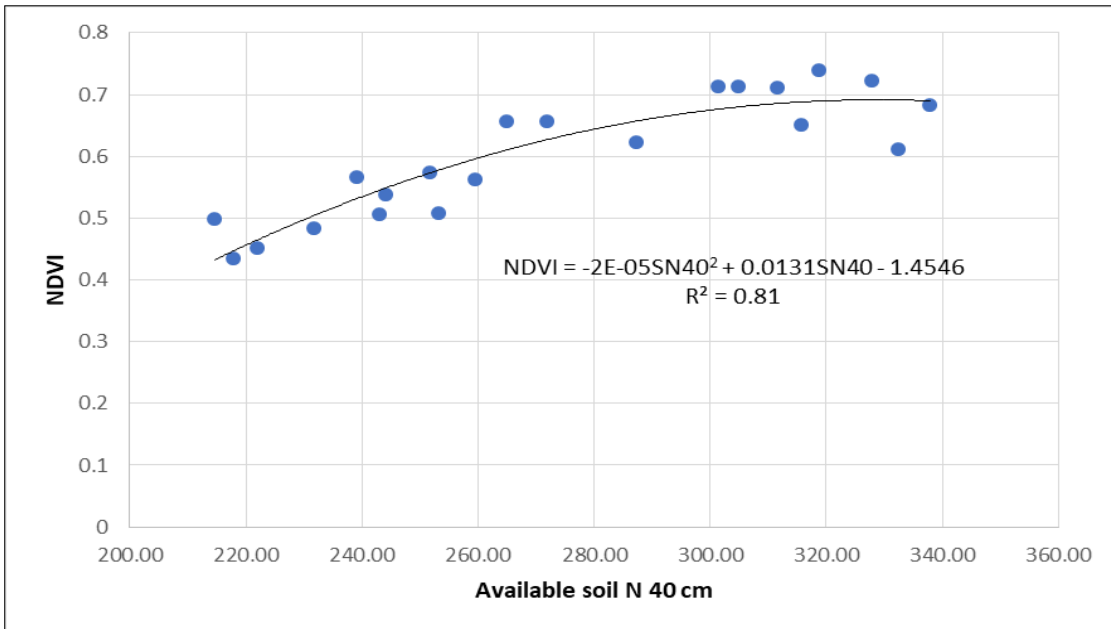


Fig. 3. Influence of available soil nitrogen at 40 cm depth on NDVI at 30 DAT under varying nitrogen levels.

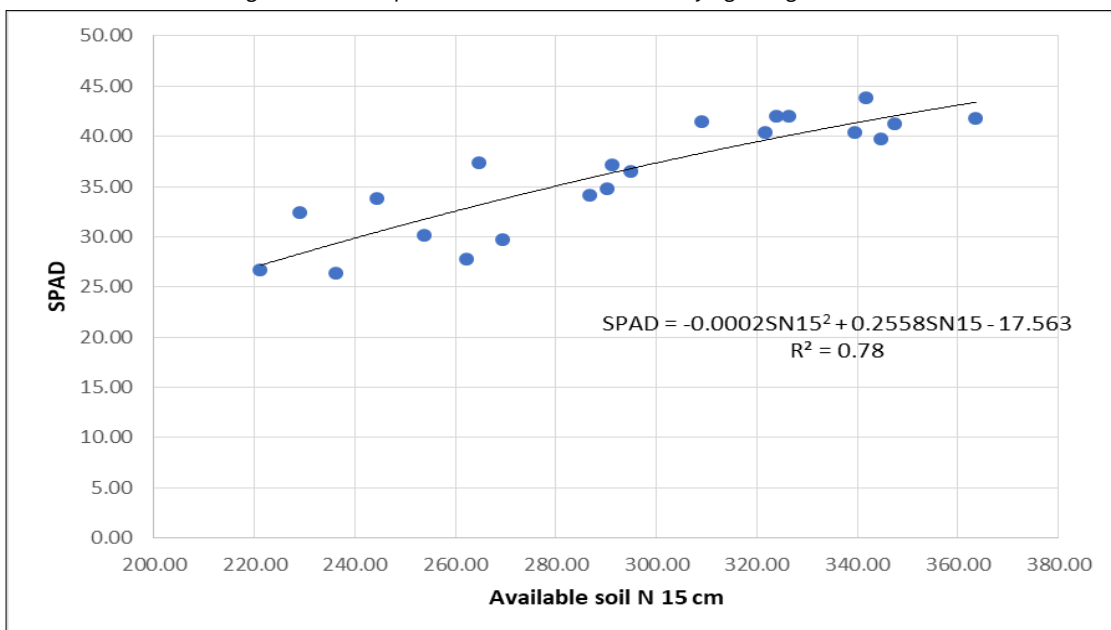


Fig. 4. Influence of available soil nitrogen at 15 cm depth on SPAD at 30 DAT under varying nitrogen levels.

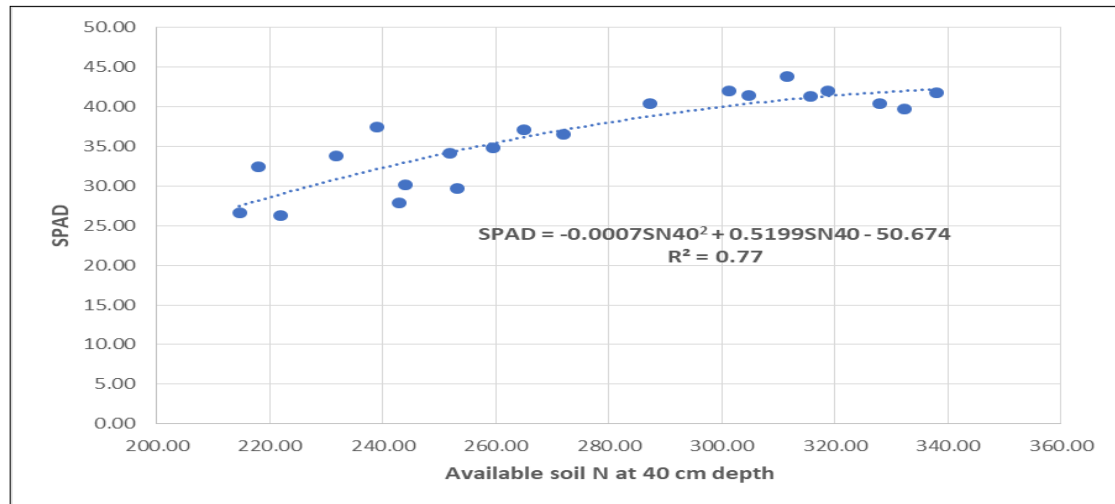


Fig. 5. Influence of available soil nitrogen at 40 cm depth on SPAD at 30 DAT under varying nitrogen levels.

value that is proportional to the concentration of chlorophyll in the leaf. Data presented in Fig. 4 revealed that, at the active tillering stage (30 DAT), the available soil N at 15 cm depth influences the SPAD value to a notable extent, with a R^2 value of 0.78. Unlike the influence of soil N at 40 cm depth on the magnitude of NDVI, the R^2 value representing the relationship of soil N at 40 cm depth to SPAD was slightly lower, i.e., 0.77 (Fig. 5).

Influence of available soil nitrogen on grain yield

Available soil N at 15 cm depth during harvesting time of the crop strongly influenced ($R^2=0.88$) the grain yield of rice (Fig. 6). Available soil N at 40 cm depth also showed a strong relationship and its influence on grain yield. However, the R^2 value was slightly lower (0.86) than that under the relationship between available soil N at 15 cm depth with grain yield (Fig. 7).

Discussion

The sand content of the experimental site was 58.2 % coupled with a lower bulk density (1.43 kg m^{-3}) resulted in the dominance of macro pores. This was well reflected in the value of the saturated hydraulic conductivity of 16.7 mm d^{-1} . In such soil, nutrients like nitrogen move through mass flow and a high mobility rate can easily be leached from the surface layer to the lower layers. At the time of transplanting, 50 % of the total nitrogen dose was applied to the soil. During the early vegetative phase, when the crop spends 12–14 days overcoming the

transplanting shock, the crop may uptake a smaller amount of nitrogen. During 0–30 DAT, on 5 occasions, more than 60 mm of rainfall occurred in a day (with a total value of 398.4 mm). The status of available soil nitrogen was measured on 30 DAT and it was found that nitrogen availability was relatively low. At the same time, available soil nitrogen at 40 cm depth was only 4–7 % lower than the nitrogen status at 15 cm depth. These two trends strongly support the vertical movement of a notable amount of nitrogen through leaching. Loss of nitrogen through leaching is very much dependent on soil type (19). Under lower doses of N leaching loss of N beyond 40 cm depth was less; as a result, the difference in N content between 15 and 40 cm depth was less. However, higher N doses resulted in greater leaching losses beyond 40 cm depth, increasing the difference in N content between the two depths.

Application of another 25 % of nitrogen as a first split on 30 DAT, as well as 189.3 mm rainfall during 30–50 DAT, caused only a 4–5 % reduction in nitrogen availability in the root rhizosphere at 50 DAT compared to 30 DAT. This happened when nitrogen was applied at 40–120 kg N ha^{-1} . However, at the upper level of nitrogen application (N_{160} to N_{240}) greater amount of nitrogen uptake made the difference by 9–10 %. Application of the remaining 25 % nitrogen on 50 DAT (when the crop enters its reproductive phase), coupled with only 65.4 mm rainfall, resulted in a marginal (1–4 %) increase in nitrogen availability in the soil. NDVI provides detailed feedback on plant light absorption and reflection, indicating plant health and the effectiveness of nitrogen

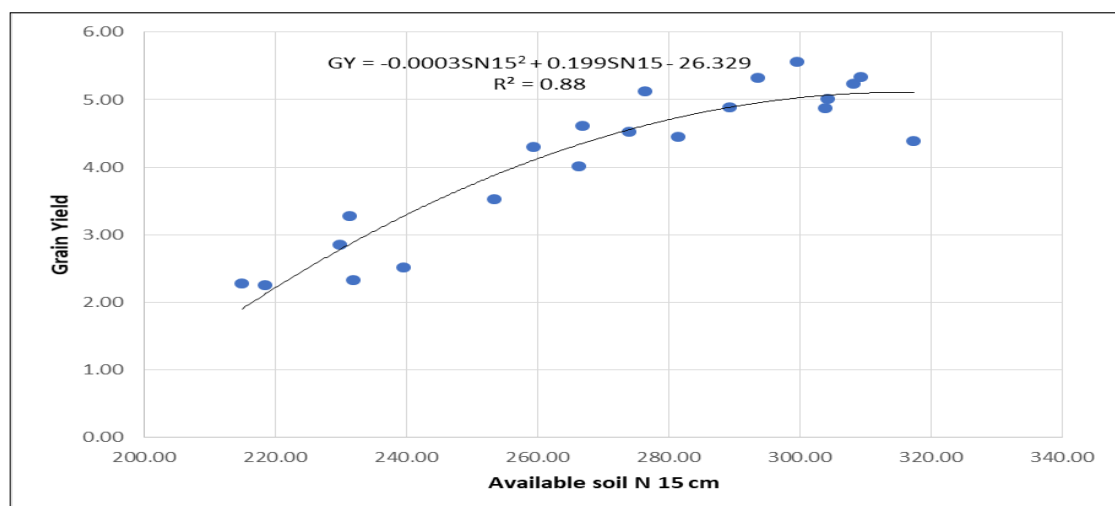


Fig. 6. Influence of available soil nitrogen at 15 cm depth on grain yield under varying nitrogen levels.

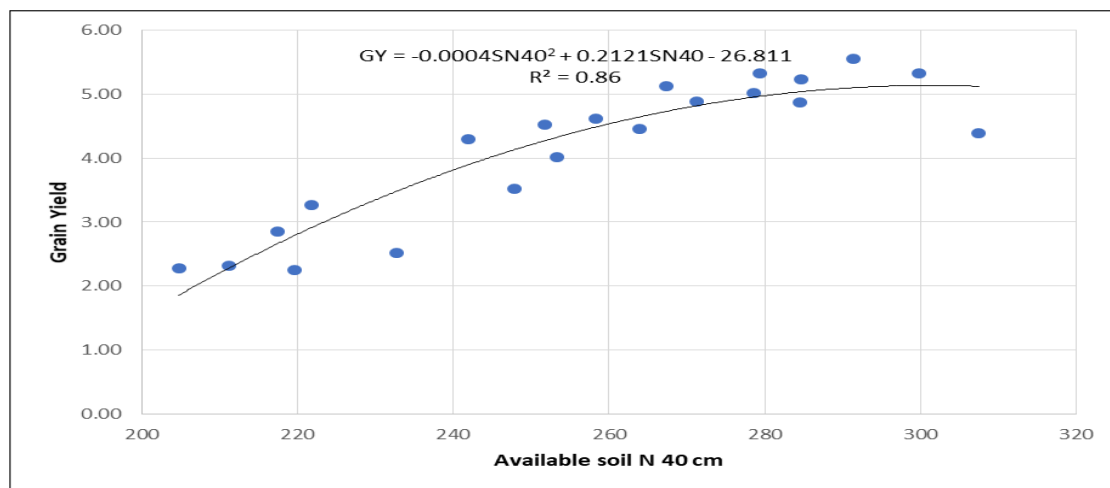


Fig. 7. Influence of available soil nitrogen at 40 cm depth on grain yield under varying nitrogen levels.

status in soil (20). As NDVI status depends on the greenness of the leaf blade, in general and chlorophyll content, in particular, besides N is a component of chlorophyll. Therefore, the magnitude of NDVI gives a clear idea about the status of soil N (21). Due to transplanting shock, as well as a higher amount of nitrogen leaching, this may be the reason for lower NDVI values on 30 DAT over 50 and 70 DAT. Peak vegetative stage, moderate rainfall and appropriate supply of nitrogen may boost the nitrogen uptake rate by the crop.

This was the reason for the highest NDVI values on 50 DAT. SPAD can rapidly measure the leaf chlorophyll content more accurately. As N is one of the staple elements of chlorophyll, the plant N content, which is very much dependent on available soil N, could influence the SPAD values (22). As both NDVI and SPAD follow the same principle, the trend of SPAD against nitrogen level or time behaves in the same fashion as that of NDVI. In this study influence of soil N at 40 cm depth influenced the NDVI value more strongly ($R^2 = 0.81$) than at 15 cm depth ($R^2 = 0.78$). It is well known that, NDVI value is related to chlorophyll content in leaves and the chlorophyll status is monitored by nitrogen. The higher R^2 value between NDVI and soil N at 40 cm depth indicates greater leaching losses of soil N, likely caused by 398.4 mm rainfall during the first 30 DAT. During the reproductive stage, the entire nutrient sink is towards the grain. Moreover, nitrogen is a key component of proteins, which are essential for the formation and development of the grain. Consequently, increasing nitrogen application rates results in higher rice grain yields. As the increase in quantity reduces the efficiency level, the rate of increase in grain yield decreases with each increment of 40 kg N ha⁻¹ (23).

Conclusion

In the early vegetative stage (0–30 DAT), approximately 400 mm of rainfall caused the maximum movement of nitrogen from the 15–40 cm soil layer. Leaching continues to a notable extent until the panicle initiation stage of the crop. Split application of 25 % nitrogen at panicle initiation stage marginally enhanced the available soil nitrogen in both layers. On 30 DAT, the status of NDVI, SPAD and grain yield were influenced to the maximum possible extent (highest R^2 values) by the soil available nitrogen. Interestingly, at 30 DAT, available soil nitrogen at 40 cm depth had a greater effect on NDVI than soil nitrogen content at 15 cm depth. However, for SPAD, the influence of soil nitrogen was nearly

similar at both depths. Like earlier researchers, the values of both NDVI and SPAD attained the highest level at the panicle initiation stage. The same at active tillering and post-flowering stages were marginally lower. However, at the time of harvest, the magnitude of NDVI and SPAD had reduced by 50 % over the values recorded at the panicle initiation stage. Grain yield increased steadily with nitrogen application rates from 0–200 kg N ha⁻¹. After attaining the yield plateau against 200 kg N ha⁻¹ the same decreased when 240 kg N ha⁻¹ was applied to the crop. Available soil nitrogen in both 15 and 40 cm layers strongly influenced the grain yield of rice.

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Authors' contributions

SS conceptualised the study, contributed to methodology development, validation and data curation and supervised the research. ST assisted in conceptualisation, validation and overall supervision of the study. CB contributed to methodology, analysis and data curation and prepared the original draft of the manuscript. MS and JG participated in the review and editing of the manuscript and provided critical feedback during revision. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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