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Effect of Leaf Color Chart (LCC)-based Nitrogen Application in Wheat on Available Nutrients and Enzyme Activity in Soil

Gulshan[1], Rajpaul Yadav[1], Dev Raj[1], Usha Vashisth[1] and Kiran Kumari[2*]

[1]Department of Soil Science, CCS Haryana Agricultural University, Hisar-125001, Haryana, India
[2]Krishi Vigyan Kendra, CCS Haryana Agricultural University, Karnal-132001, Haryana, India
*Corresponding author's E-mail: kirankhokar123@gmail.com

Abstract

Nitrogen management in wheat is becoming important to counteract the adverse effect of excess application of N on environment as well as on economics of the farmers. We examined the effect of different N recommendation approaches on available N fraction in the soil, urease activity at different growth stages of wheat and nutrient use efficiencies in South-West Haryana. Results showed that the available N content varied significantly during the different crop growth stages of wheat. The highest N content was reported in treatment where N was applied on the basis of STCR approach. However, the available N content in leaf color chart (LCC) based treatment was equivalent to RDN treatment at the harvest of the crop, showing 30% N fertilizer saving without sacrificing the grain yield. The trend of urease enzyme activity was followed the N availability. The activity of urease was found significantly maximum in the treatment where N was applied on the basis of STCR equation in all growth stages except at 20 and 40 days after sowing (DAS). Urease activity in LCC based treatment noticed almost equivalent as observed under RDN application after harvest of the wheat. The significantly highest amount of available P and K was recorded in the treatment where LCC guided one split of N was applied.

Keywords: Leaf color chart, Soil test crop response, Urease activity, N management

Introduction

Wheat (*Triticum aestivum* L.) is an essential staple crop, contributing around 20% of protein and calories consumed worldwide. In India, wheat witnessed acreage of 32.61 million hectares during the 2024-25 and the wheat production reached 115.43 million tones with average national productivity of 3521 kg ha^{-1} (Anonymous, 2025). There is always an increasing demand of wheat for the bludgeoning population. This has been incentivizing the farmers to earn more through meeting this demand by producing more and more. But this leads to fostering a myth among the farmers that yield always increases with increasing fertilizer application which has led to over-fertilization without significantly increasing the yield as expected by the farmer. This practice has resulted in a high price index of chemical fertilizers with their limited production, fertilizer cost, soil health, sustainability, and pollution. To cope up with that, agricultural universities have been promoting the blanket fertilizer recommendation in which a single fertilizer rate is used for large but heterogeneous areas. But the irony is that this also leads to unbalanced application of fertilizer nutrients relative to the needs of a crop, and low use efficiency of fertilizer (Rurinda *et al.,* 2020). The deterioration of sustainability of the ecosystem attributed to improper nitrogen (N) fertilization, which is one of the major concerns for agricultural production systems. Inadequate nitrogenous fertilizer application leads to lower use efficiency while excess application harms the environment.

To get rid of the cons associated with blanket recommendation a new approach named soil test is developed for sustaining soil health. Through soil tests, soils of different regions are classified into different categories on the basis of available nutrient status and recommendations are given corresponding to each category after calibration of soil test value with crop response. The efficiency of applied fertilizer nutrients and the nutrient already present in the soil is very much location-specific and calibrations are needed for every set of the crop-soil climatic complex under the optimal agronomic practices. For this reason, soil testing has become the foundation for fertilizing our soils in a balanced proportion (Tripathi and Srivastava, 2019). All these concerns have raised the interest in precision nutrient management tools. Leaf color chart (LCC) is one of them and it is an easy-to-use and cheap diagnostic tool for monitoring the relative greenness of leaves of crop plants indicating the actual plant N status, which is much helpful to adjust N fertilizer requirement of the concerned crop (Saha *et al*., 2022). The application of nutrients on the basis of recommendations obtained from the decision support system like "Nutrient expert" and LCC proved superior over all other management practices (Bhuiya *et al*., 2020). So there is a need to understand that it's not the higher doses of fertilizer beyond a certain value but the synchronization of nutrient supply with crop demand that increases the productivity with the simultaneous increase in efficiency of fertilizer. In comparison to blanket application of N, real-time N management by LCC has been successful and efficient technique of nitrogen application in maize hybrids for increasing dry matter production and nitrogen uptake (Fayaz *et al*., 2021). Increased leaf N concentration from N management using LCC may improve plant photosynthetic efficiency. The agronomic efficiency was greater when less N was used, but this was achieved with the LCC without sacrificing the yield. The N management with LCC offers potential for improving fertilizer N use efficiency in aerobic rice and could effectively handle spatial and temporal variability while supplying nitrogen to rice (Yogendra *et al*., 2017). The need-based N management through LCC and Green-Seeker (GS) as compared to the RDN is best for efficient cultivation of wheat crop for similar grain yield and quality along with saving of N fertilizers per hectare which may also save soil environment (Ram *et al*., 2022). With an average N fertilizer savings of 20%, precision N management utilizing LCC, chlorophyll meter (SPAD), and GS sustained wheat grain yield equivalent to the soil-test based N fertilizer recommendation (Gosal *et al*., 2021; Singh *et al.,* 2020). LCC score can serve as a credible indicator of the soil's native N supply status, according to a positive and significant coefficient of correlation between LCC score and leaf N and grain production. Likewise, adoption of soil test crop response (STCR) approach fulfils the need of balanced fertilization and undermines the consequences like low yield and low nutrient use efficiency (NUE) that arise due to under and above fertilization. An appropriate dose of NPK is very essential for chlorophyll synthesis, promotion of healthy root growth, translocation of photosynthates, and enzymes activation for biochemical reaction within plant tissue, wherein the plant gets good opportunity for nutrient uptake which results in higher plant growth and development as well as yield (Kakraliya *et al*., 2022).

Long-term balanced application of fertilizers and manures either alone or through integrated approach based on the STCR concept have the ability to improve the soil's physical structure by increasing the quantity of water-stable aggregates (WSA) and the mean weighted diameter (MWD) of the aggregates as a result of increased root proliferation and biomass, which may have raised the soil organic content (OC) (Kumar *et al*., 2021). The long-term STCR study clearly showed that STCR-based integrated fertilizer use for targeting yield could produce crops with the targeted yield while conserving fertilizer without reducing soil fertility (Sharma *et al*., 2016). The current study emphasizes on study of effect of leaf color chart-based N application on available N, P, K and urease enzyme activity in soil under wheat crop.

Materials and Method

Site and location

This experiment was initiated at research farm, Department of Soil Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The experimental site is located at 29°16′ N latitude and 75°7′ E longitude in northwest part of India in the state of Haryana. The climate of the area is semi-arid with a mean annual precipitation of 443 mm and mean annual temperature of 24.8°C. During winter months, relative humidity remains significantly high. It varies from 5 to 100 percent during the year. The period from October onward until next June remains almost dry except, a few light showers received due to westerly depressions/western disturbances. The summers are generally quite hot and winters are fairly cool. During rainy and winter seasons, the average value of evaporation remains around 6.8 and 4.0 mm day $^{-1}$, respectively. The highest amount of dewfall is observed in December and January. The main characteristics of the climate in the district are its dryness, extremes of temperature and scanty rainfall.

Experimental design and sample collection

The experiment was laid out in randomized block design with three replications. Sowing of wheat was completed on 26th November, 2022. The 7 number of nitrogen management treatments were tested as T1- Control, T2- recommended dose of nitrogen (blanket recommendation of 150 kg N ha^{-1} in two splits as basal and at first irrigation), T3- 125% of recommended dose of nitrogen (blanket application of 187.5 kg N ha^{-1} in three splits as basal (75 kg), at first irrigation (75 kg) and t 40-42 DAS (37.5 kg), T4- soil test based nitrogen management (application of 123.50 kg N ha^{-1} in three splits as basal (23.46 kg), at first irrigation (50.02 kg) and at 40-42 DAS (50.02 kg), T5-STCR based nitrogen management (application of 215 kg N ha^{-1} in three splits as basal (23.46 kg), at first irrigation (95.77 kg) and at 40-42 DAS (95.77 kg), T6- LCC based nitrogen management -3rd split guided by LCC [104.02 kg N ha^{-1} in three splits as basal (23.46 kg), at first irrigation (46 kg) and at 40-42 DAS (34.56 kg)], T7- LCC based nitrogen management- 2nd and 3rd split guided by LCC [104.02 kg N ha^{-1} in three splits as basal (23.46 kg), at first irrigation (46 kg) and at 40-42 DAS (34.56 kg)]. Wheat variety WH-1184 was selected for the experiment. In treatment T6 and T7, LCC reading of 4.5-5.0 was considered for N fertilization before the irrigation. At these readings, application of 75 kg urea ha^{-1} was done on the basis of LCC guidelines as per treatments. Plot size of 8m × 6m was maintained for each treatment. Initially, the soil pH, EC, OC, available N, P and K in experimental soil were 8.1, 0.36 dS m^{-1}, 0.54%, 115 kg N ha^{-1}, 18 kg P ha^{-1}, 335 kg K ha^{-1}, respectively. The cultivation practices like land preparation, sowing, weeding, irrigation, and insect-pest management for wheat crop were done as per the package of the practice of Haryana Agricultural University, Hisar except fertilizer management and scheduling. Soil samples were collected before sowing, during different crop growth stages and after the harvest of wheat crop from 0-15 cm soil depth. The samples were collected from 3-4 sites of each plot of each treatment. Soil samples were then air-dried, grinded and sieved (2 mm) for analysis.

Determination of available N, P and K

Available N (kg ha^{-1}) in soil samples were determined by micro-kjeldhal method as suggested by Subbiah and Asija (1956). A known amount of soil was distilled with alkaline $KMnO_4$ solution and finally the absorbed ammonia in boric acid was titrated against the standard sulphuric acid. Under alkaline conditions, available P (kg ha^{-1}) was extracted with 0.5 M $NaHCO_3$ (pH 8.5) and was determined calorimetrically (Olsen *et al.*, 1954). The reduced phosphomolybdate complexes by $SnCl_2$ of the soil filtrate provides blue color complex whose intensity was measured with the help of spectrophotometer at 660 nm wavelength. Available K was determined neutral ammonium acetate (NH_4OAC) as the extractant (Jackson, 1973). The suspension was shaken for 5 minutes on a mechanical shaker. Ammonium ions (NH_4^+) from ammonium acetate solution replaced the potassium ions (K^+) on exchange sites of soil particles until equilibrium and replaced K^+ ions enter the solution concentration of which was measured with flame photometer.

Determination of urease activity

Urease activity was measured following the method described by Tabatabai and Bremner (1972). Five gram of soil was incubated with 9 ml Tris hydroxymethyl amino methane (THAM) buffer, 0.2 ml toluene and 1 ml of 0.2% of urea solution at 37°C for 2 hours. Then 50 ml KCl-$AgSO_4$ solution was added, and shaking was done for 30 minutes. Soil suspension was filtered. Taking 20 ml aliquot from filtrate, NH_4-N was determined by steam distillation method (Keeney and Nelson, 1982).

Statistical analysis

The data recorded during the course of investigation were subjected to statistical analysis using analysis of variance technique (ANOVA) for RBD on OPSTAT software. Standard error of mean in each case and critical difference only for significant cases was calculated at 5% levels of probability.

Results and Discussion

Available N at different growth stages

The availability of N in the soil, which is crucial for plant uptake, depends on external inputs and several other factors. In this study, as the amount of N applied through different approaches in each treatment had variable effect on N availability in the soil at different crop growth stages (Table 1). In the control plot, soil available N consistently declined over time, from an initial level of 115 kg N ha^{-1}. However, in all other treatments, increase in available N was observed up to 40 DAS due to external N application, followed by a decline. The highest available N at 20 DAS (Crown root initiation stage) was recorded in the 125% RDN treatment T3 (131 kg N ha^{-1}). The maximum soil available N across all the stages was observed in treatment T5 at 40 DAS i.e., tillering stage (158.7 kg N ha^{-1}). At 70 DAS, the soil available N in each treatment was observed to be lower than that observed at 40 DAS except in treatment T3. The highest N at this stage was observed in T5 (154 kg N ha^{-1}). This trend continued up to 100 DAS (Milking and dough stage) and post-harvest, with T5 showing the highest post-harvest N (120 kg N ha^{-1}), which was at par with the available N in T3 (118 kg N ha^{-1}).

In T6 (110 kg N ha^{-1}), where only one dose was given based on LCC, and T7 (110.6 kg N ha^{-1}) with two LCC-guided doses, the soil available N was statistically similar to T2 (112.5 kg N ha^{-1}). Treatments T2 and T4 also showed comparable available N levels from 70 DAS onwards. Except for the control, all treatments observed an increase in available N from the initial 115 kg N ha^{-1} up to 40 DAS. The highest available N at 20 DAS was recorded in the 125% RDN-based treatment T3 and RDN-based treatment T2, both receiving 75 kg N ha^{-1} as basal dose through urea. In contrast T4, T5, T6, and T7 received only 23.46 kg N ha-^{1} via DAP, with no basal urea, leading to lower available N levels at 20 DAS. The highest available N at 40 DAS was observed in STCR based treatment T5, due to 84.77 kg N ha^{-1} applied during the first top dressing after the first irrigation.

At 70 DAS, available N was low across all treatments compared to 40 DAS, except in T3, despite the second top dressing after the second irrigation. This decline may be due to crop uptake during peak vegetative growth and critical physiological stages. Krishna Kumar and Haefele (2013) also noted that soil N increases up to the tillering stage and then declines up to harvest, likely due to N release from organic matter and concurrent crop uptake. However, STCR based treatment T5 maintained significantly higher available N even at 70 DAS, which may be attributed to the higher N application through urea. The higher available N in STCR-based treatment aligns with findings of Mohanty *et al*.

Table 1. Effect of different approaches of N application on available nitrogen status during different growth stages and after harvest of wheat

Treatments	Available N (kg ha^{-1})				
	20 DAS	40 DAS	70 DAS	100 DAS	Afterharvest
T1 (Control)	111.5	113.0	105.0	95.5	90.0
T2 (RDN)	122.4	133.5	123.6	117.0	112.5
T3 (125% RDN)	131.0	142.0	151.7	126.5	118.0
T4 (Soil test based)	118.1	129.5	121.5	118.0	113.0
T5 (STCR)	119.6	158.7	154.0	129.0	120.0
T6 (LCC-3rd split)	118.5	130.0	115.0	113.0	110.0
T7 (LCC-2nd and 3rd split)	117.6	130.7	116.0	113.2	110.6
CD (p=0.05)	2.13	2.38	2.2	2.161	2.846

Table 2. Effect of different approaches of N application on post-harvest available phosphorus and potassium status in soil

Treatments	Available P (kg ha^{-1})	Available K (kg ha^{-1})
T1 (Control)	15.53	332
T2 (RDN)	19.73	340.5
T3 (125% RDN)	18.57	338.0
T4(Soil test based)	19.10	342.0
T5 (STCR)	18.26	336.0
T6 (LCC-3rd split)	20.87	341.6
T7 (LCC-2nd and 3rd split)	20.37	342.3
CD (p=0.05)	2.658	1.30

(2016), who reported significantly greater NH_4^+ and NO_3^- concentrations at 0-15 and 15-30 cm soil depths under STCR. This increase may be attributed to external N inputs and enhanced organic matter decomposition driven by greater microbial activity in treatments receiving higher N doses.

Available P before sowing and after harvest of crop

The availability of P in the soil was significantly affected by N management with different recommendation approaches (Table 2). Before sowing the crop, the available P in soil was 18 kg P ha^{-1}. But after the harvest of the crop, there was an increase in available P in the soil in all treatments except control and it has been seen that the available P in the soil had varied from 15.53 kg P ha^{-1} in control to 20.87 kg P ha^{-1} in LCC based treatment T6. The available P in treatment T5 was increased by 1.44% to 18.26 kg P ha^{-1}. While the highest increase of 15.94% to 20.87 kg P ha^{-1} in available P was recorded in LCC-based T6 treatment followed by the 13.17% increase in the case of LCC-based T7 treatment.

Before the start of the experiment, the available P in soil was 18 kg ha^{-1}. But after harvesting the available P content in the soil under different treatments was higher than it was before sowing. The phosphorus in all treatments was significantly higher than the control. The highest amount of available P was recorded in treatment where LCC guided only one split (T6). It might be due to the release of phosphorus because of the decomposition of higher root biomass formed as a result of better physiological and metabolic processes in plants where balanced nutrition takes place on basis of LCC. The released organic compounds also have the potential to convert the unavailable form of P into the available form. Kaur and Singh (2022) also came out with similar findings and reported that fertilization with LCC resulted in maximum refinement of soil fertility along with saving of N fertilizer. The improvement with LCC was observed to be 20.9% for P compared to the control. The lowest increase of 1.44% over the initial status was noticed in STCR might be due to the residual effect of applied phosphorus after the excessive uptake due to higher crop growth. Nearly the same results of the increased Olsen-P by 22.4% in treatment where NPK fertilization done on basis of the STCR approach were reported by Mohrana *et al.* (2012). Nitrogen addition promoted the dissolution of immobile P_i (mainly Ca-bound recalcitrant P) to more available forms of P_i (including Al- and Fe-bound P fractions and Olsen P) by decreasing soil pH from 7.6 to 4.7, but did not affect P_o (Wang *et al.,* 2022).

Available K before sowing and after harvest of crop

The available K has builds up in the soil of all treatments except treatment T1 (Table 2). The available K in the soil of control plots was 332 kg K ha^{-1} and stands up to 342.3 kg K ha^{-1} in the plot of treatment T7. The available K in LCC-based treatment T7 was 342.3 kg K ha^{-1} which was significantly higher than available K in treatments T3, T5 and T2 whereas it was statistically at par with LCC based treatment T6 and soil test based treatment T4.

The available K (Table 2) in the soil after harvest was found highest in LCC-based treatment in comparison to other treatments. The similar findings of Krishna Kumar and Haefele (2013) stated that the LCC 4 (115 kg N ha^{-1}) treatment showed higher available potassium at all the growth stages of crops which might be due to the synergetic effect of N on potassium. Kaur and Singh (2022) also gave evidence of refinement of soil fertility in terms of K by using LCC and the increased amount of available K in their study was 7.2% over unfertilized plots.

Table 3. Effect of different approaches of N application on urease enzyme in soil activity at different growth stages and after the harvest of wheat

Treatments	Urease activity (µg N g soil^{-1} h^{-1})				
	20 DAS	40 DAS	70 DAS	100 DAS	After Harvest
T_1 (Control)	88.64	84.01	82.13	77.56	70.64
T2 (RDN)	117.00	130.88	112.76	101.45	88.19
T3 (125%RDN)	116.00	128.16	133.48	123.33	105.56
T4 (Soil test based)	92.08	116.81	122.08	113.80	94.48
T5 (STCR)	92.50	125.60	141.02	134.82	115.7
T6 (LCC-3rd split)	91.78	108.78	114.80	103.42	89.25
T7 (LCC-2nd and 3rd split)	90.38	106.63	113.00	102.00	87.83
C.D. (p=0.05)	2.503	2.204	2.576	2.57	2.489

Urease activity at different growth stages and after harvest

In different N application strategies under our experiment, urea was added as a source of N for plant growth and as the dose of N being supplied through urea varies with different treatments the urease activity in soil of different treatments had also shown variation. Among different treatments, the lowest urease activity was noticed in the control plot at different growth stages as given in Table 3. Among different treatments, the significantly highest urease activity was observed in STCR-based treatment T5 during the complete growth period of the wheat crop except at 20 and 40 DAS. Except for control and RDN, the urease activity in all treatments successively increases during the first 70 days thereafter a decline has been seen in the activity. In RDN-based treatment, the activity increases up to 40 DAS and then declines at later stages. The highest activity ever had been noticed during the whole growth period was 141.02 µg N g soil^{-1} h^{-1} in treatment T5 at 70 DAS. At 20 DAS the urease activity in RDN-based treatment T2 was 117 µg N g soil^{-1} h^{-1} which was significantly high than other treatments except for T3. It was increased by a magnitude of 13.88 to touch the value of 130.88 µg N g soil^{-1} h^{-1} at 40 DAS which is significantly higher than T_4, T5, T6 and T7 treatment except T3. At 70 DAS the urease activity was significantly high in STCR-based treatment T5 than in other treatments. The LCC-based treatments were at par with RDN-based treatment at 70 DAS and afterward. The enzymatic activity mostly depends on the amount of substrate present in the medium as well as on the prevalent temperature. Likewise, the activity of the urease enzyme in the soil varies with the amount of urea present in soil.

The urease activity at 20 DAS was highest in RDN based T2 and 125% RDN based T3 treatment. It was because urea was added as an extraneous source of N during sowing in these treatments. The urease activity further increases at 40 DAS and 70 DAS progressively in all treatments except control and T2 because of the continuous addition of urea just after 1st and 2nd irrigation. In all the crop growth stages, except 20 DAS and 40 DAS the urease activity was significantly high in STCR based T5 treatment because of the addition of total 367.9 kg urea per hectare. Till 40 DAS the highest dose of urea had been supplied in treatment T2 and T3 which was 325.5 kg urea per hectare deemed to be accountable for higher activity of urease in these treatments during the inception of crop growth. The urease activity under medium and high N rates were 42.9% and 23.6% higher compared to low N rates (Sekaran *et al*., 2019).

Afterward, 70 DAS the urease activity starts declining due to cessation of the addition of urea. Hence, this indicated that the amount of urea along with the prevalent temperature has the potential to alter the activity of the urease enzyme. The most probable reason behind the higher urease activity in treatment where a higher dose of urea was applied is vigorous growth during the 70 DAS in wheat that leads to the release of root exudation, secretions, mucigel, mucilage, etc. which is more evident during this period in the rhizosphere due to their long residence time. These

Table 4. Effect of different approaches of N application on agronomic, recovery and physiological efficiency of nitrogen

Treatments	Agronomic efficiency (kg grain kg^{-1} N applied)	Recovery efficiency (%)	Physiological efficiency (kg grain kg^{-1} N absorbed)
T1 (Control)	-	-	-
T2 (RDN)	8.20	27.23	30.11
T3 (125% RDN)	9.50	38.72	24.51
T4 (Soil test based)	11.07	37.73	29.36
T5 (STCR)	8.89	36.63	24.28
T6 (LCC-3rd split)	12.47	42.49	29.33
T7 (LCC-2nd and 3rd split)	12.53	41.81	29.97

organic compound in rhizosphere resulted in increased microbial and enzymatic activity (Ankush *et al.*, 2020). The urease enzyme activity after harvest in LCC-based treatment was statistically at par with activity in RDN-based treatment and is in conformity with other study where enzymatic activity in prilled urea plus FYM-based treatment was at par with the treatment in which 100 kg N ha^{-1} was applied on basis of customized LCC (Mohanty *et al.*, 2017). The trend followed by urease activity during the growth of wheat crop was almost similar to the trend that was followed by available N. The urease activity as well as available N both first increased during the initial days of crop growth until the urea is added in soil and then started declining at later stages. The similarity between trends reflects the importance of the urease enzyme in making N available through the hydrolysis of urea.

Nitrogen use efficiency

The highest agronomic efficiency i.e. 12.53 kg grain per kg N applied was obtained in treatment where N dose was applied on the basis of LCC (Table 4). However, low agronomic efficiency was obtained in RDN treatment. Likewise, highest recovery efficiency of 42.49% was obtained with LCC based treatment and on other hand lowest one lie in the court of RDN based treatment. While the highest physiological efficiency was observed in RDN based treatment and the lowest one was calculated in STCR based treatment. Further it has been indicated that the recovery efficiency and agronomic efficiency of LCC-based treatments were increased by 56.04% and 52.07% respectively in comparison to RDN-based treatment which results in a saving of 46 kg N ha^{-1} over RDN-based treatment without sacrificing yield and N content of soil.

Conclusion

The study revealed that STCR and LCC-based nutrient management effectively sustained soil fertility, with higher post-harvest soil N and K compared to the control. LCC-based N management optimized nutrient availability while saving 46 kg N ha^{-1} in wheat crop over the RDN treatment. Hence, LCC-guided N application can replace fixed RDN or soil-test approaches, reducing fertilizer costs and minimizing environmental impacts.

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