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On-farm Evaluation of Leaf Colour Chart and Chlorophyll Meter for Need-based Nitrogen Management in *Kharif* Maize (*Zea mays* L.)

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ABSTRACT

Background: Soil is important medium of all living beings. Due to the high doses of fertilizers application soil heath is getting deteriorated. Soil fertility variation restricts efficient fertilizer N management when broad based blanket recommendations are used in maize (*Zea mays* L.). Site-specific fertilizer nitrogen management (SSNM) could be the best management option to avoid excessive and untimely nitrogen (N) applications in maize.

Methods: A field experiment was conducted at agronomy Farm, Lovely Professional University, Phagwara during *kharif* season of 2020 and 2021 to study the nitrogen management using leaf colour chart (LCC) and chlorophyll meter in maize (*Zea mays* L.). Colour (of the first top maize leaf with fully exposed collar) as measured by comparison with different shades of green colour on a leaf colour chart (LCC) and also by SPAD meter. Need based fertilizer application at LCC5 and SPAD 50 significantly improved growth and yield attributes *viz*., plant height, dry matter accumulation plant^{-1}, number of leaves plant^{-1}, stem diameter, number of internodes plant^{-1}, leaf area index, cob length, cob girth, number of cobs plant^{-1}, number of grains cob^{-1}, grain weight plant^{-1} and 1000-grain weight along with higher grain and stover yields, grain protein content over fixed time application of 1200 kg N ha^{-1}.

Result: Evaluation of the established threshold leaf greenness revealed that fertilizer N management using LCC 5 and SPAD 50 starting from six-leaf stage to before silking stage resulted in improved agronomic efficiency in maize. There was no response to fertilizer N application at silking stage. The study concluded that in maize, fertilizer N can be more efficiently managed by applying fertilizer N dose based on leaf colour as measured by LCC than blanket recommendation.

Key words: Agronomic efficiency, LCC, Need based nitrogen, SPAD, Threshold value.

INTRODUCTION

Fertilizer nitrogen (N) has become the key input in food production. Cereals including rice, wheat and maize account for more than half of the total fertilizer N consumption in the world. As per estimates 50-70% more cereal grain will be required by 2050 to feed over 9 billion world population. This will further increase demand for fertilizer N at greater magnitude unless the fertilizer N recovery efficiency in cereals is improved. Among the cereals, maize (*Zea mays* L.) ranks third in total world production after wheat and rice and it is a staple food in many countries, particularly in the tropics and sub-tropics. Among the primary nutrients, nitrogen is very important as it is intimately involved in the process of photosynthesis and thus directly related to total dry matter production. The generally followed practice of excessive fertilizer N applications to avoid the risk of N deficiency further reduces this efficiency. Excessive N application causes nutrient imbalances and produces plants that are disease and pest-susceptible. Low recovery of N is not only responsible for higher cost of crop production, but also for environmental pollution (Kumar *et al.,* (2019). Blanket recommendations based on fixed-time application of fertilizer N doses at specified growth stages do not consider the dynamic soil N supply and crop N requirements and lead to untimely application of fertilizer N. Therefore, demand driven need based fertilizer N management in maize can help to improve N recovery efficiency and to reduce N losses. Improving fertilizer nitrogen (N) use efficiency in maize is vital not only to improve and sustain high crop yields but to reduce post field application N losses to the environment. Fertilizer N is inexpensive hence farmers have tendency to apply N in large amounts to minimize yield losses with the fear of degradation of soils due to continuous cropping Singh *et al.,* (2020) argued that recommendations based on soil tests remain ignorant about the dynamics of N release from crop residues, organic manures and irrigation water and are not very successful in maize. Efficiency of

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fertilizer N generally declines with increased fertilizer doses and seldom exceeds 40% (Umesh *et al.,* (2020). Lack of proper splitting of N applications and many a times over or under application of N than the crop need is one important reason for low N-use efficiency. As N requirement of maize plant is not same throughout the growth period, it is necessary to adjust fertilizer N application with the timings of plant N requirement to enhance N-use efficiency in maize. The real time N management approach can help increase N use efficiency by matching time of fertilizer application with plant need. Leaf colour chart (LCC) is a reliable tool for real time N management (Singh *et al.,* 2020). It can be used for rapid and reliable monitoring of relative greenness of the leaf as an indicator of leaf N status. The guidelines evolved using LCC helps to adopt crop demand-driven N applications and result in high crop productivity and economic returns and reduce N losses to the environment. The chlorophyll meter has proven to be another appropriate analytical tool to quickly identify N deficiencies at the beginning of the growth of the crop. It is a simple, portable, handheld device that clamps on a leaf and measures light transmittance at 650 and 940 nm. If we want to achieve and sustain high yields to meet the future need of India, it is vital to develop farmer-friendly technologies that can improve N-use efficiency and reduce N losses to a considerable extent. Adaptation of the leaf colour chart and chlorophyll meter to maize is important if we want to use these simple tools for N top-dressing for enhancing N-use efficiency and thereby yield. This requires the standardization of the critical leaf for LCC and chlorophyll meter as well as determination of the threshold LCC and chlorophyll meter values, which have not been attempted so far. Hence, the objective of this study was to standardize the critical leaf for LCC and chlorophyll meter values as well as to determine the threshold LCC and chlorophyll meter values with a view to adjust the timing of N top-dressing for maximum N-use efficiency and yield of maize.

MATERIALS AND METHODS

The experiment was conducted in farm of School of Agriculture, Lovely Professional University and Phagwara Punjab during two consecutive years, 2019-20 and 2020-21. The region receives an average 630 mm yr^{-1} rainfall about 80% of which occurs from June to September. Mean maximum and minimum temperatures are respectively, 43 and 18°C during the year. Soil pH, electrical conductivity, organic carbon, phosphorus and potassium content in surface (0-15 cm) soil layer of the experimental sites ranged from 7.8-8.1, 0.14 to 0.25 ds m^{-1}, 4.9 to 5.6 g kg^{-1}, 13 to 28 kg ha^{-1} and 112 to 146 kg ha^{-1}, respectively. During 2019, 2020, the need-based N management options using LCC were evaluated in comparison with fixed time fertilizer N application. Experiment was laid out in randomized block design and with three replications. Treatments schedule is given in a tabular statement below. Planting material used during experimentation was Parbhat. It is medium in height, medium thick stem, resistant to lodging and matures around 95days, adopting a seed rate of 25 kgha^{-1} and spacing of 60 cm × 20 cm. A basal dose of 60 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1} using SSP and MOP was applied in all the treatments. N was supplied based on LCC and SPAD values. Crop was grown under fully irrigated conditions. Two hand weeding were done 30 and 45 days after sowing to reduce the crop-weed competition. No serious incidence of any insect-pest or disease was observed in the crop. The cobs were harvested after attaining physiological maturity.

Leaf chlorophyll content was analysed as per method suggested by Gao *et al.,* (2021).

Agronomic efficiency (kg grain kg^{-1} N applied) =

$$\frac{\text{Grain yeild in N fertilized plot - Grain yeild in no N plot}}{\text{Quantity of applied in N fertilized plot}}$$

LCC measurements

A 'six panel' LCC was used to match leaf colour in five plants in each plot starting from 21 days after sowing (DAS). The LCC developed by IRRI was used in the present experiment and was standardized along with the chlorophyll meter to assess the relative accuracy of LCC in measuring the greenness of maize leaves and leaf N concentration. The LCC is made of high-impact plastic and consists of six colour shades from yellowish green (no. 1) to dark green (no. 6) and the holder is gray in colour. The leaf colours of the youngest fully expanded leaf (YFEL) blade (leaf 1), leaf below leaf 1 (leaf 2) and leaf below leaf 2 (leaf 3) were measured with the standard LCC under the same environmental conditions. The colour of a single leaf was measured by placing the middle part of the middle lobe of the leaf in front of the colour strip for comparison. Because the leaf colour reading is affected by the sun's angle and sunlight intensity, LCC measurements were made by shading the leaf being measured with the body of the person who recorded the observation. The LCC measurements were taken by the same person every time at the same time of the day. Readings of five leaves at random for each plot were taken and average LCC score was computed. If the colour of a particular leaf was between two colour shades, the mean of the two values was taken as the reading. All observations were recorded on 30, 60 and 90 days after sowing (DAS).

Chlorophyll meter readings

A chlorophyll meter SPAD-502, soil and plant analysis development (SPAD) Section, Minolta Camera Co., Ltd, Japan) uses a silicon photodiode to detect the transmittance of light. The meter was calibrated using a reading checker supplied by the manufacturer, which standardizes the readings between different SPAD-502 meters. Four chlorophyll meter readings were taken around the midpoint of the middle lobe of each leaf, two from each side of the midrib of the middle lobe 30 mm apart. Readings of the same five leaves used for LCC measurements were used for SPAD values also and the mean of 20 readings was taken as the mean value of each plot. All observations were recorded on 30, 60 and 90 DAS.

RESULTS AND DISCUSSION

Establishment of threshold LCC and SPAD values

Threshold LCC value is defined as the LCC reading corresponding to a leaf greenness below which the crop suffers from N deficiency resulting in yield loss. Variations in LCC readings during vegetative and reproductive stages indicate the need of establishing LCC thresholds for both vegetative and reproductive growth stages. Ren, Y *et al.,* (2021) observed different threshold SPAD values for different growth stages in maize. Data for vegetative growth stages and early reproductive stage were pooled separately. Threshold value, below which the crop would suffer from N deficiency resulting in yield loss, was observed to be LCC shade 5 and SPAD 50 during vegetative growth stages to before silking stage.

Temporal changes and N effects on LCC and SPAD readings

LCC scores of the maize genotype Parbhat for the year 2020 and 2021 as a function of time and level of applied fertilizer N are shown in Table 1. During both years genotype exhibited similar trends with increasing fertilizer N levels. The LCC scores of the maize leaves were in complete accordance with fertilizer N level. In general, LCC readings increased during the early growth stage from 6 to 10-leaf stage, irrespective of the levels of N application. Indigenous sources and applied basal N dose continued to supply N for chlorophyll synthesis and thus green colour intensity of the leaves increased during early phase of the crop growth even in the no N plot. After 10th leaf stage of the crop, steep decline in LCC readings was observed in no-N control whereas LCC readings in other treatments were only slightly influenced till 13 leaf stage followed by a decline in treatments receiving 83 and 92. kg N ha^{-1}. Rapid vegetative growth during the 10-16 leaf stages and increasing demand of N for chlorophyll synthesis resulted in a decline in LCC readings of the maize leaves in low N treatments. But as the crop entered reproductive phase the LCC readings increased in all the treatments irrespective of the level of N application. This increase is attributed to the change in index leaf from topmost fully exposed leaf to ear leaf at this stage. A plot of SPAD meter readings versus LCC score across growth stages shows a close linear relation. Since both gadgets consider leaf greenness as an indicator of leaf N concentration LCC can be used as an inexpensive substitute of SPAD meter to guide need-based N applications in maize. The LCC scores and leaf N concentration at different growth stages were significantly correlated during both the years. Thus, LCC could reliably predict N concentration in maize leaves and help guide need-based fertilizer N applications by establishing threshold LCC values for different genotypes.

Effect on growth and yield

Results from experiments conducted to evaluate different need-based N management strategies during 2020 to 2021 are listed in Table 3-4. Need based N at LCC<5.0 from 6th leaf to before silking stage being at par with need-based N at SPAD<50 from 6th leaf to before silking stage significantly improved number of leaves plant^{-1}and leaf area index (LAI) compared to that in fixed time N application. This could be attributed to better synchronization of N supply with crop N demand leading to higher N uptake due to real time application of 125 kg N ha^{-1} based upon need. It is assumed that better nutrition, as indicated by higher leaf N content, improved photosynthetic rate when 122 kg N ha^{-1} was applied at LCC 5 over fixed time application of 125 kg N ha^{-1}. The results obtained during the both years indicated that using LCC threshold 5.5 and SPAD 55 lead to application of 2-3 kg N ha^{-1} more than using LCC threshold 5 and SPAD 50 during vegetative growth stages without any improvement in crop growth parameters. The LCC threshold 5 and SPAD

Table 1: Representing to treatments and detail application.

Treatments	Fertilizer N application at each split					
	Basal	30 DAS	45 DAS	60 DAS	Total N applied	Saving of fertilizers over RDF
T0	-	-	-	-	-	0
T1	42	41.5	-	41.5	125	0
T2	62.5	30			92.5	32.5
T3	62.5	30	30		122.5	2.5
T4	62.5	21.5	21.5	21.5	127	-2
T5	41.5	41.5			83	42
T6	41.5	28	28		97.5	27.5
T7	41.5	28	28	28	125.5	-0.5

** T0- Absolute control (N_0), T1- Fixed time N application (42 kg N ha^{-1} as a basal dose+41.5 kg N/ha at knee high stage + 41.5 kg N/ha at pre tasselling), T2- Need based N at LCC<4.5, T3- Need based N at LCC<5.0, T4 -Need based N at LCC<5.5, T5- Need based N at SPAD<45, T6- Need based N at SPAD<50, T7- Need based N at SPAD<55.

"LCC and SPAD readings were taken from 6th leaf stage onwards and Fertilizer N application was made whenever LCC/SPAD score was below specified threshold.

50 guided fertilizer N applications of 122 kg N ha^{-1} (during vegetative growth stages) improved crop growth as compared to fixed time application of 125 kg ha^{-1}. Improvement in leaf chlorophyll content due to application of 122 kg N ha^{-1} at LCC threshold 5 and SPAD 50 over fixed time application of 125 kg N ha^{-1} supports improved photosynthetic rate leading to higher growth and biomass production. The enhanced growth with LCC based nitrogen application was also reported by Bana *et al.,* (2020). The LCC threshold 5 and SPAD 50 need-based application of 122 kg N ha^{-1} significantly increased yield attributes *viz*., number of grains cob^{-1}, grain weight plant^{-1} and 1000- grain weight and ultimately grain (3069 kg ha^{-1}) and stover yield (4045 kg ha^{-1}) of maize as compared to fixed time application of 125kg N ha^{-1} (Table 4). The results obtained with maize variety Parbhat during the years 2020-21 indicated that using LCC threshold 5.5 lead to application of 2-3 kg N ha^{-1} more than using LCC threshold 5 during vegetative growth stages without any yield benefit. The LCC threshold 5 guided fertilizer N applications of 122 kg N ha^{-1} (during vegetative growth stages) produced grain yield comparable with fixed time application of 125 kg N ha^{-1}. Blanket applications at fixed growth stages were not able to match fertilizer N supply with plant N demand and thus produced yield comparable with need-based N management strategy only if 40 kg or more fertilizer N was applied at fixed growth stages. However, in LCC<4.5 and SPAD 45 the amount of N was less and plant suffer for want of N at critical stages of crop growth (4th leaf, 8th leaf stage, tasselling and silking) where N is most required at these stages as we seen from the uptake of N. Agronomic efficiency also recorded highest (12.2% and 11%) at LCC threshold 5 and SPAD 50 need-based application. It was found that need based application of whenever (starting from 6th leaf stage to before silking stage) leaf greenness is less than LCC threshold value 5 and SPAD 50 is the appropriate need-based fertilizer N management strategy along with a basal dose of 40 kg N ha^{-1}. These results confirm the findings of Chittapur *et al.,* (2015).

Table 2: Simple correlation between LCC shades, SPAD, leaf chlorophyll content and leaf nitrogen content in maize (Averaged over two years).

Variable	r
LCC and leaf chlorophyll content	0.765**
LCC and leaf N content	0.350**
Leaf chlorophyll content and leaf N content	0.870**
SPAD and leaf chlorophyll content	0.381**
SPAD and leaf N content	0.509*
Leaf chlorophyll content and leaf N content	0.397**

- *Indicates significant at 1% level of significance.
- ** Indicates significant at 1% level of significance.

Effect on quality

The LCC threshold 5 and SPAD 50 need-based application of 122 kg N ha^{-1} significantly enhanced leaf chlorophyll content, leaf N content and grain protein content over fixed time application of 125 kg N ha^{-1} (Table 3). This could be explained based on better availability of N in the crop root zone and enhanced N uptake and consequent increase in photosynthetic and metabolic activities resulting in better

Table 3: Effect of LCC and SPAD based real time N management on leaves per plant, leaf area index, leaf chlorophyll content, leaf N content and grain protein content in maize (Pooled over two years).

Treatments	Leaves plant^{-1}	Leaf area index	Leaf chlorophyll	Leaf N% (mg/g)	Grain protein (%)
T0- Absolute control (N_0)	8^{d}	1.86^{c}	1.29^{c}	1.7^{d}	5.32^{e}
T1- Fixed time N application	12.9^{ab}	2.78^{a}	3.93^{b}	3.35^{b}	8.02^{c}
T2- Need based N at LCC<4.5	10.2^{c}	1.97^{c}	1.86^{c}	2.81^{c}	7.79^{d}
T3- Need based N at LCC<5.0	13.6^{a}	2.94^{a}	4.23^{a}	3.91^{a}	10.73^{a}
T4 -Need based N at LCC<5.5	12.8^{ab}	2.48^{ab}	3.79^{b}	3.2^{b}	9.25^{b}
T5- Need based N at SPAD<45	11.9^{b}	2.64^{a}	3.73^{b}	3.7^{ab}	9.28^{b}
T6- Need based N at SPAD<50	13.2^{a}	2.86^{a}	3.99^{b}	3.86^{a}	9.98^{b}
T7- Need based N at SPAD<55	11.5^{b}	2.3^{ab}	3.83^{b}	3.29^{b}	9.26^{b}

Table 4: Effect of LCC and SPAD based real time N management on yield attributes and agronomic efficiency of maize (Pooled over two years).

Treatments	Grains cob^{-1}	1000 grain weight	Grain yield (kg ha^{-1})	Harvest index (%)	Agronomic efficiency (%)
T0- Absolute control(N_0)	189^{e}	95.6^{g}	1602^{e}	38.42^{d}	-
T1- Fixed time N application	257^{c}	120.6^{f}	2756^{c}	41.30^{b}	3.0^{e}
T2- Need based N at LCC<4.5	203^{d}	118.6^{f}	2698^{d}	40.59^{c}	3.4^{e}
T3- Need based N at LCC<5.0	283^{a}	142.3^{a}	3069^{a}	43.14^{a}	12.2^{a}
T4 -Need based N at LCC<5.5	254^{c}	124^{e}	2913^{b}	42.91^{b}	6.1^{d}
T5- Need based N at SPAD<45	257^{c}	128.3^{d}	2749^{c}	41.54^{b}	9.6^{c}
T6- Need based N at SPAD<50	275^{b}	135.6^{b}	2977^{ab}	43.40^{a}	11.0^{b}
T7- Need based N at SPAD<55	255^{c}	130.6^{c}	2807^{bc}	42.82^{b}	10.0^{c}

partitioning of photosynthates to sinks, which got reflected in quality enhancement in terms of leaf chlorophyll content, leaf N content and grain protein content. These results are supported by the findings of Kumar *et al.* (2021).

Correlation between LCC shades, SPAD value, leaf N content and leaf chlorophyll content

A positive and highly significant correlation was found between LCC shades, SPAD value, leaf N content and leaf chlorophyll content (Table 2) which indicates that the LCC and SPAD could be effectively used to decide the timings of fertilizer N application in standing crop for better synchronization of crop N demand with supply. Leaf N status is closely related to photosynthetic rate and biomass production and it is a sensitive indicator of changes in crop N demand within a growing season. The chlorophyll or soil plant analysis development (SPAD) meter and LCC can be used for rapid and reliable monitoring of relative greenness of the leaf as an indicator of leaf N status (Singh *et al.,* 2020). LCC being cheaper and user friendly thus could be used to improve fertilizer N use efficiency and improve productivity largely in developing countries where farmers cannot afford the costly SPAD meter.

CONCLUSION

The generally followed blanket recommendation of applying fixed N dose at fixed time intervals is not adequate for obtaining high agronomic efficiency of fertilizer N in maize. Matching fertilizer N supply with crop demand using threshold LCC shade 5 and SPAD 50 saved 25-50% fertilizer N. This study provides evidence for the usefulness of LCC guided need -based fertilizer N management technology in assuring high yields and improvement in fertilizer N recovery efficiency. LCC being a cost effective, simple and farmer's friendly gadget can be easily used even by small and illiterate farmers. Thus, the blanket recommendations of applying fixed N dose at fixed time intervals should be replaced with need based fertilizer N management technology using LCC and SPAD in maize. It can be concluded that higher yield and net returns in maize can be secured by need based application of 122 kg N ha^{-1} at LCC 5 and SPAD 50.

Conflict of interest: None.

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