ESTIMATION OF NITROGEN AND CHLOROPHYLL CONTENT IN WHEAT CROP USING HAND HELD SENSORS

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ABSTRACT

A Field experiment was conducted to estimate crop nitrogen (N) and chlorophyll content in wheat crop by using chlorophyll content meter (Apogee's CCM-200) and N-Tester® (Make YARA International). The experiment was conducted on wheat variety viz. PBW 550 with 6 nitrogen levels i.e. 0, 30, 60, 90, 120 & 150 kg N/ha. It was found that at all stages like tillering, booting and ear emergence, when the nitrogen rates was increased from 0 to 150 kg ha^{-1}, the correlation with both of the instruments i.e. CCM and NT for determination of plant nitrogen were better correlated having Pearson's coefficient 0.939, 0.863, 0.797 and 0.926, 0.827, 0.722 respectively. Also, at all the crop growth stages like tillering, booting and ear emergence, Lab N content in plant leaves was having strong linear relationship with CCI having coefficient of determination 0.88, 0.75 and 0.77 respectively. Coefficient of determination values for N tester and lab N-content also showed good linear relationship having values 0.85, 0.78 and 0.70 at tillering, booting and ear emergence stages respectively. The chlorophyll content measured in laboratory was having strong linear relationship with CCI at tillering, booting and ear emergence stage with coefficient of determination 0.84, 0.78 and 0.77, respectively. Coefficient of determination values between N tester and chlorophyll content showed linear relationship having values 0.82, 0.75 and 0.80 for tillering, booting and ear emergence stages respectively. There was a good correlation between plant N and chlorophyll content shown through Pearson's correlation coefficient values which were 0.890, 0.846 and 0.786 at tillering, booting and ear emergence stages respectively. This was also confirmed as there was a strong linear relationship between chlorophyll content and Lab N with coefficients of determination 0.798, 0.75 and 0.72 at tillering, booting and ear emergence stages respectively. It was concluded that both of the instruments i.e. NT and CCM are useful for the determination of N and chlorophyll in plant leaves especially at tillering stage.

keywords: Wheat, Nitrogen content, Chlorophyll content, Chlorophyll meter (CCM), Chlorophyll content index (CCI), N tester**.**

INTRODUCTION

Wheat (*Triticum aestivum*) production is about one third of India's total food grain production. India is the second largest producer of wheat next to China in global wheat production. At present total area under wheat in India is 43.4 M ha including 2.8 M ha in Punjab (Anonymous, 2012). Use of fertilizers, adoption of modem agricultural technologies and irrigation practices are some of the major factors for increased production. Among the various nutrients, nitrogen is important to increase grain yield and improve grain quality of wheat. Nitrogen (N), an essential element of the biochemical processes that drives crop production (Sinclair and Horie, 1989). Grain yield is affected due to a reduction in available N, when soil N mineralization is not enough to fulfill the crop demand. Under these circumstances, N fertilization plays an important role for achieving the highest yield in wheat in each particular environment (Cassman *et al,* 2003). N concentration and N accumulation in plant tissues are two major indicators to characterize the N status in crop plants. Plant nitrogen accumulation, as a product of plant nitrogen content and plant mass, strongly affects yield and quality formation in crop production (Zhang *et a.l,* 1996, Guo *et al.,* 2005). Since nitrogen fertilization strategy is a major consideration in field management. Hence for the proper strategy to ensure N supply at the right time and appropriate amount, it is necessary to evaluate tissue N status and recommend N dressing plan from indicative nitrogen content and nitrogen accumulation in crop plants.

Whereas the relationship between the chlorophyll content of leaves and the actual photosynthetic canopy area is well documented, comparatively little information is available about the vertical distribution of important plant parameters including chlorophyll, a key crop biophysical characteristic (Ciganda *et al.,* 2008), and nitrogen, crucial resource for plant development (Drouet and Bonhomme, 1999; Wang *et al.,* 2005).

Indeed, despite the relatively constant appearance of leaves in a canopy, the canopy itself is characterized by non-uniform vertical nitrogen distribution because the leaves are exposed to different light environments (e.g. through shading), differ in age and may develop under different nitrogen supply conditions during growth (Gastal and Lemaire, 2002).

Until now, the traditional method of measuring crop N status has depended on plant sampling from the field and chemical use in the laboratory (Roth and Fox 1989). Conventional methods of evaluating crop N status are time-consuming and complicated. A chlorophyll concentration index (CCI) meter (CCM-200) and nitrogen tester (N tester YARA International) are portable instruments that can be used to evaluate crop N status rapidly and in a non-destructive way. Such meters have been used to estimate foliar Chl and N content in various species, such as rice (Huang *et al,* 2008), wheat (Le Bail *et al,* 2005), beans (Abdelhamid *et al,* 2003), and fruit trees (Schaper and Chacko, 1991). The objective of the present investigation was to measure N-status and Chlorophyll content of wheat crop at its different growth stages by using chlorophyll content meter and Nitrogen tester.

MATERIAL AND METHODS

experimental planning

Field experiment to study the spectral characteristics and crop growth parameters of wheat under variable nitrogen application rates were carried out at the Research Farm of the Department of Farm Machinery & Power Engineering, Punjab Agricultural University (PAU), Ludhiana. Experimental field was having sandy loam soil in texture and was normal in pH and EC. The soil of the selected site was low in organic carbon, nitrogen and phosphorus but was medium in available K. The experiment was conducted by sowing university recommended wheat variety viz. PBW 550 with 6 nitrogen levels i.e. 0, 30, 60, 90, 120 & 150 kg N/ha. The crop was sown on 21 November 2012. The experiment was carried out in randomized completely block design (RCBD) with three replications (Table 1). All other agronomic practices were followed as per package of practices recommended by PAU, Ludhiana for wheat crop. Application time and doses of fertilizer urea (46% N) are mentioned in Table 2.

Table 1. Layout of the field experiment

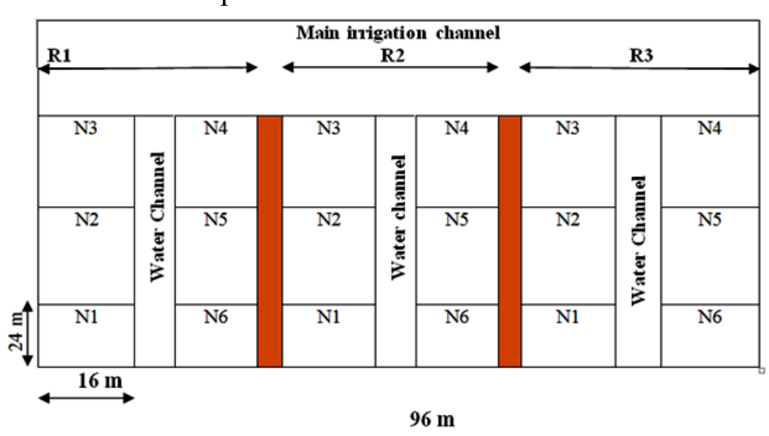


Table 2. Fertilizer urea application time and doses

Treatments	Dose (Kg N/ha)			Total (kgN/ha)
	Basal (50%)	at Ist irrigation (25%) (25DAS)	at IInd irrigation (25%) (50DAS)	
N1	0	0	0	0
N2	15	7.5	7.5	30
N3	30	15	15	60
N4	45	22.5	22.5	90
N5	60	30	30	120
N6	75	37.5	37.5	150

instruments used

Chlorophyll content meter (CCM-200) and Nitrogen Tester (Make YARA International) were used to predict chlorophyll content and N content in plant leaves. Chlorophyll meter is one of the most important used in plant N and Chlorophyll determination study. A leaf section is enclosed in a small chamber and it is exposed to two light sources (1) 640nm and (2) infrared light 940nm positioned just above the leaves. Light filtered through the leaf is captured sequentially by sensors below the leaves. The difference in the transmission of the filtered wavelengths is the chlorophyll content indicator per unit leaf area.

The NT is a chlorophyll meter based on Minolta SPAD- 502. Nitrogen tester operates by measuring the chlorophyll content of leafs as this is related to the N status of the plant. 30 random measurements from across the field taken using ‘W’ pattern, give an average value is used to indicate how much N the crop require.

agronomic data collection

The data for various parameters like total chlorophyll in terms of chlorophyll a & b and Nitrogen content were measured in laboratory at different stages like tillering (55 DAS), booting (85 DAS) and ear emergence(115 DAS) of crop and at the same time chlorophyll meter and N-tester data was also recorded. Chlorophyll meter (Make Apogee’s CCM-200) was used to measure chlorophyll concentration index (CCI). Nitrogen in plant leaves was also measured by using N-tester (Make YARA International). Photosynthetic pigments like chlorophyll a & b were estimated according to the method of Hiscox and Israelstam (1979) using dimethyl sulphoxide (DMSO) chemical in laboratory conditions. Nitrogen present in the plant leaves were calculated by using titration method in laboratory. Yield contributing parameters like plant height, ear length, number of grains per panicle were recorded for each plot before harvest of wheat crop along with grain yield and straw weight during harvesting.

statistical analysis

Various data sets were statistically analyzed by general linear model (GLM) procedure by using SAS software 9.3. All possible pairs of treatment means were compared at 5 % level of significance. Pearson's correlation coefficient was also determined by using SAS software.

RESULTS AND DISCUSSION

Laboratory data for chlorophyll content and N content at different growth stages of wheat crop and at different N-levels along with N-tester and CCI values are mentioned in Table 3. At tillering stage nitrogen content was increased with the increasing dose of Nitrogen. With increase in N level, chlorophyll content was continued to increase upto 150 Kg N ha^{-1}. The differences were however non significant at 120 and 150 Kg N ha^{-1} level and 30, 60 and 90 Kg N ha^{-1} levels. The nitrogen content in wheat plants at this stage also followed the pattern almost similar to that for chlorophyll content. Nitrogen content at 120 Kg N ha^{-1} was significantly higher than 0, 30 and 60 Kg N ha^{-1}. With further increase in N rate to 150 Kg N ha^{-1}, N content increased significantly over all the treatments. Higher N uptake at rate 150 Kg N ha^{-1} was expected due to higher biomass production and 220.3 per cent higher N content over no -N level. Higher N uptake by the crop may be attributed to its more N absorption by the root zone and higher biomass yield as nutrient uptake is function of biomass and N concentration.

At booting and ear emergence stages chlorophyll content and nitrogen content followed almost similar trend as in tillering. With the increase in nitrogen dose, chlorophyll content and nitrogen content significantly increased upto 120 Kg N ha^{-1} thereafter it showed non significant difference with 150 Kg N ha^{-1}.

Whereas, chlorophyll content at ear emergence was significantly higher in the treatment where 150 Kg N ha^{-1} was applied as compared to all other treatments. Very close link between chlorophyll and nitrogen content was observed as investigated by many investigators (Evans, 1983; Filed and Moony, 1986; Amaliotis *et al*., 2004). It is understandable, because nitrogen is a structural element of chlorophyll and protein molecules, and thereby affects formation of chloroplasts and accumulation of chlorophyll in them (Tucker, 2004; Daughtry *et al.* 2000).

correlations between different hand-held sensors values with nitrogen and chlorophyll content in wheat crop

Pearson correlation coefficients between nitrogen content, chlorophyll content, chlorophyll concentration index measured by chlorophyll meter and nitrogen tester values at tillering, booting and ear emergence stages of wheat crop are presented in Table 4. The degree of association of nitrogen content in leaves has always been useful for the prediction of yield of the crop, which is the multiplicative end product of many factors affect jointly or singly and the environment in which the crop has been grown.

Good correlations were found for both meters for determination of chlorophyll content and N content at all the stages. Although, CCI meter actually resulted in slightly higher correlations than the NT at all growth stages for the measurement of chlorophyll and nitrogen content. When the N-tester (NT) was compared to CCI over a wide range of nitrogen treatments, both meters responded in a similar manner. As the nitrogen rates (0, 30, 60, 90, 120 and 150 kg ha^{-1}) increased, leaf greenness increased and the correlation with both the CCI meter and NT for determination of plant nitrogen were better correlated having Pearson's coefficient(PC) 0.939 and 0.926 respectively at tillering stage. For measurement of chlorophyll content in the plant leaves the correlations were also better with CCI having Pearson's coefficient 0.917 and with NT having PC 0.890. For chlorophyll content measurement, both of the instruments chlorophyll meter and N-tester shown good correlations having Pearson's coefficient value ranging 0.847 to 0.917 with CCI and 0.698 to 0.930 for NT at all growth stages of crop like tillering, booting and ear emergence. It may be due to the fact that both of the instruments are used for chlorophyll measurement in plant leaves.

Table 4: Pearson correlation coefficients for determination of nitrogen and chlorophyll content with chlorophyll concentration index and nitrogen tester readings different growth stages of wheat crop

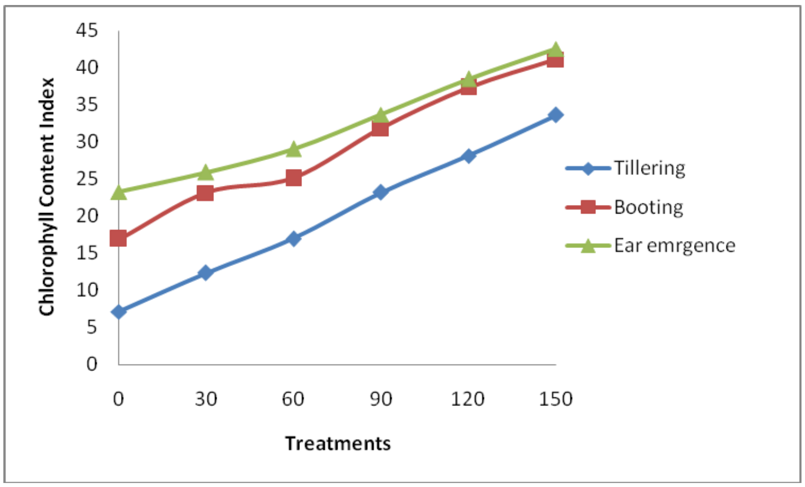
Variables	Attributes	Lab N	CC	CCI	NT
	Tillering		**0.89**	**0.939**	**0.926**
		-	<.0001	<.0001	<.0001
	Booting		0.846	0.863	0.827
		-	<.0001	<.0001	0.0053
	Ear emergence		0.786	0.797	0.722
Lab N		-	0.0002	0.0019	0.0009
	Tillering			**0.917**	0.89
			-	<.0001	<.0001
	Booting			0.861	0.698
			-	<.0001	0.0013
	Ear emergence			0.847	**0.93**
CC			-	<.0001	<.0001
	Tillering				**0.971**
				-	<.0001
	Booting				0.733
				-	0.0005
	Ear emergence				0.954
CCI				-	<.0001

Table 5 shows the range of CCI and NT values for different treatments. For tillering stage, the value of CCI and NT range from 6.3 to 35.9 and 456-634 for N0 to N150 treatments. For the booting stage, the values of CCI and NT range from 13.4 to 42.8 and 531-646 for N0 to N150 treatments. The incresase in value of both instruments with change in stage may be due to the more uptake of nitrogen at booting stage. Fig 1 shows the ralationship between different N levels with CCI and NT at different growth stages.

Table 5: Range of CCI and NT values for different treatments

Treatments	CCI	NT
Tillering Stage		
N0	6.3-8.0	456-490
N30	11.9-12.8	505-525
N60	14.5-19.4	546-583
N90	21.9-24.5	564-597
N120	26.8-29.5	608-615
N150	31.4-35.9	624-634
Booting Stage		
N0	13.4-20.4	531-542
N30	19.6-26.7	555-565
N60	22.1-28.1	580-591
N90	30.5-33.2	592-605
N120	35.8-38.9	619-635
N150	39.5-42.8	631-646
Ear Emergence Stage		
N0	21.8-24.7	543-555
N30	22.8-29	557-569

N60	23-35.1	570-588
N90	31.5-35.9	604-629
N120	37.6-39.4	621-645
N150	41.5-43.6	634-665



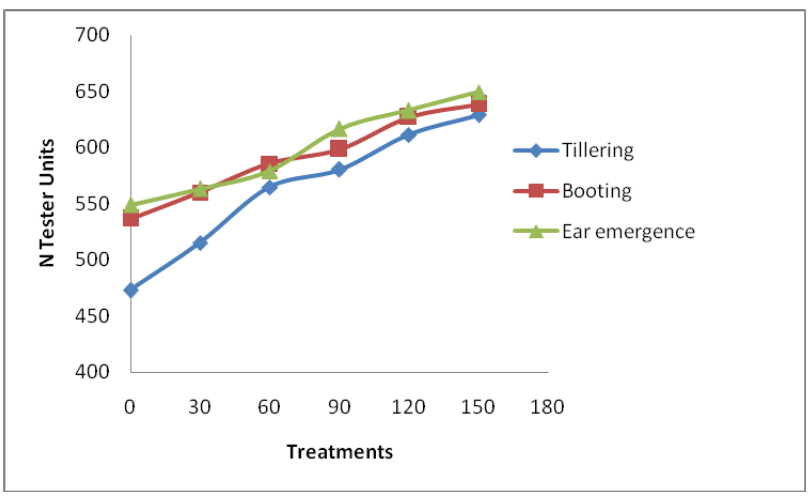
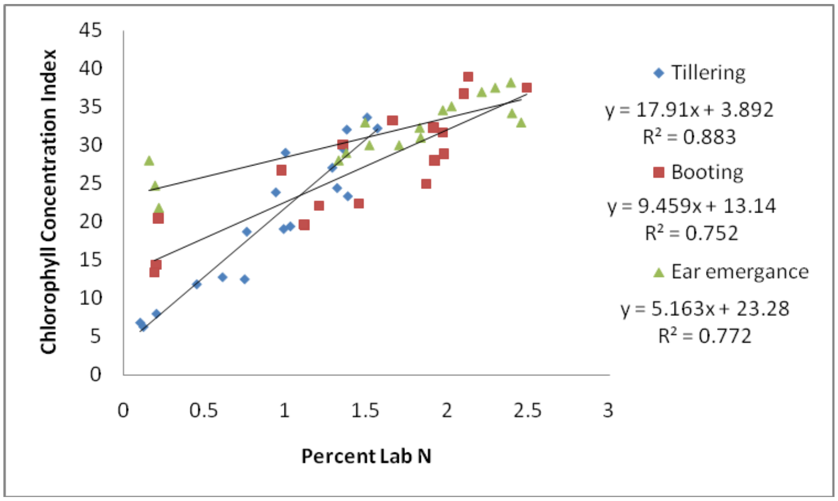


Figure.1 Relationship between chlorophyll content index and N tester Units with different N treatments in wheat leaves at different growth stages

Relationship for N Tester readings and Chlorophyll concentration index with per cent lab N and chlorophyll in wheat leaves at different growth stages are shown in Figures 2 and 3. The relationship for N Tester and chlorophyll concentration index with per cent N and chlorophyll content was linear in nature with different values of the coefficient of determination. Lab N content showed a strong linear relationship with CCI at tillering, booting and ear emergence stages with coefficient of determination 0.883, 0.752 and 0.772 respectively.



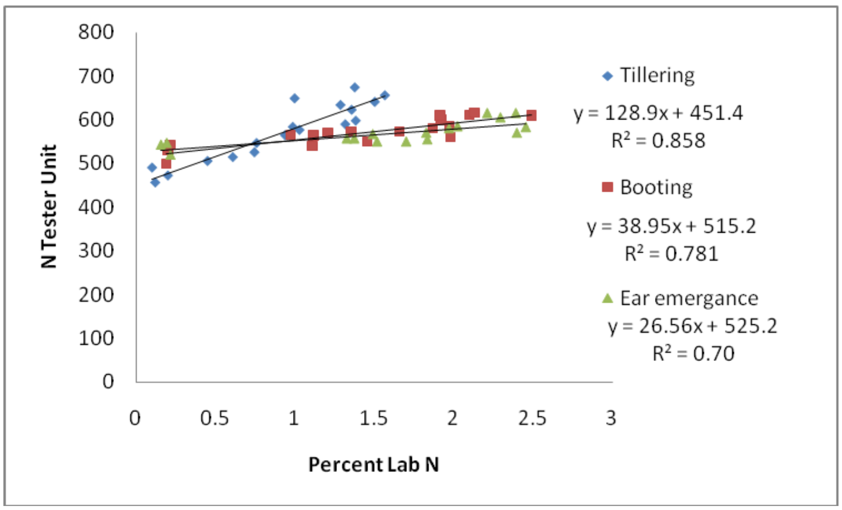
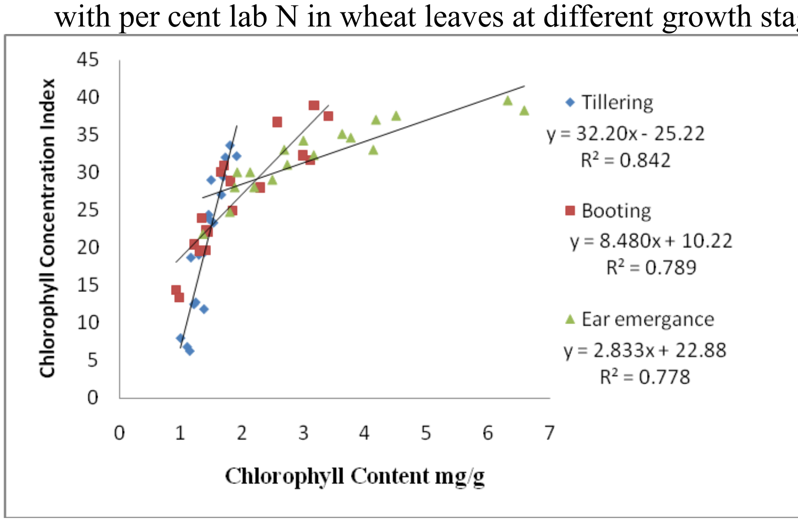


Figure 2: Relationship for N Tester readings and Chlorophyll concentration index with per cent lab N in wheat leaves at different growth stages



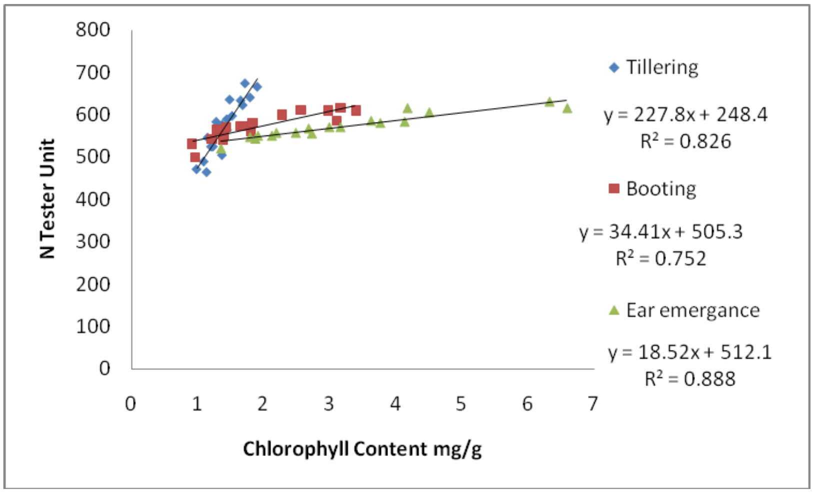


Figure 3: Relationship for chlorophyll content index and N tester values with Chlorophyll content in wheat leaves at different growth stages

Coefficient of determination values for N tester and lab N-content also showed linear relationship having values 0.85, 0.78 and 0.70 at tillering, booting and ear emergence stages respectively The chlorophyll content showed a strong linear relationship with CCI at tillering, booting and ear emergence stage with coefficient of determination 0.84, 0.78 and 0.77, respectively. Coefficient of determination values for N tester and chlorophyll content also showed linear relationship having values 0.82, 0.75 and 0.88 for tillering, booting and ear emergence stages respectively. All the above discussion leads strong support for the use of NT and CCM as N and chlorophyll content recommendation tool.

relationship between n-content and chlorophyll content

Relationship between chlorophyll content and per cent N in wheat leaves at different growth stages of wheat crop are shown in Fig. 4. There was a good linear relationship between chlorophyll content and lab N with coefficients of determination 0.79, 0.75 and 0.72 at tillering, booting and ear emergence stages respectively. Different nitrogen based indices in which chlorophyll concentration index and nitrogen tester found good relation between themselves. This correlation was determined according to the formula developed by Pearson (Petz, 1985). Very close link between chlorophyll and nitrogen content has been proved by many investigators (Evans 1983, Filed and Moony 1986 and Amaliotis *et al* 2004). A close relationship between chlorophyll and nitrogen content was because nitrogen is a structural element of chlorophyll and protein molecules which affects formation of chloroplasts and accumulation of chlorophyll in them (Tucker 2004). Cabrera (2004) also observed that nitrogen content in the leaf was in relation with colour of the leaf.

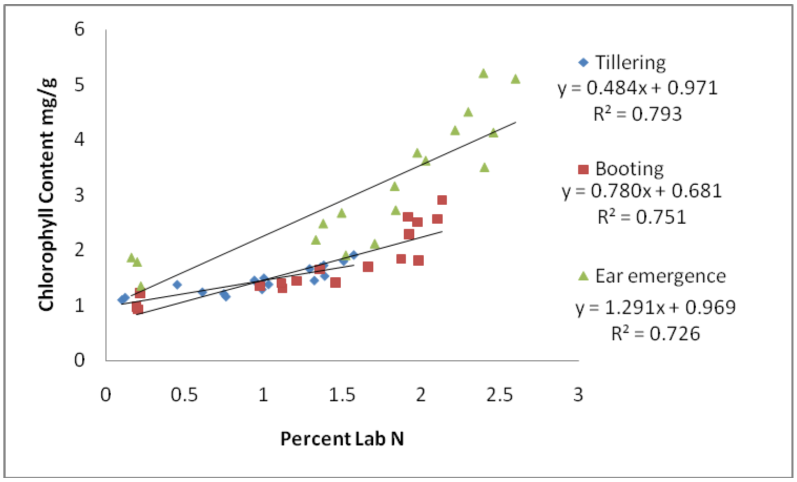


Figure 4: Relationship between chlorophyll content and per cent N in wheat leaves at different growth stages

effect of different nitrogen rates on growth and yield characters of wheat

Plant height at wheat harvest increased significantly with graded dose of N application upto 120 Kg N ha^{-1} (Table 6). Plant height in N150 treatment was significantly higher than N0, N30, N60 and N90 treatments. Plant height in N120 treatment was 24.5 per cent higher than that in control. With further increase in N application upto 150 kg ha^{-1} there was no significant increase in plant height over 120 Kg N ha^{-1}. Kibe and Singh (2003) have observed increase in plant height of wheat with N fertilization. Similarly, ear length was increased significantly upto 120 kg ha^{-1} of N applied and further increase in N application was not significantly affected the ear length of wheat. The number of grains per panicle and grain yield were also significantly increased upto 120 kg N ha^{-1} but with further increase in rate of N application upto 150 Kg N ha^{-1} number of grains per panicle and grain yield did not increased significantly over 120 Kg N ha^{-1}. The grain yield obtained with N application of 120 Kg ha^{-1} was 91.1, 38.4 and 10.2 per cent higher over the application of 30, 60 and 90 Kg N ha^{-1}, respectively. Similar trend was observed for straw weight of wheat which also significantly higher in the treatment where 150 Kg N ha^{-1} was applied and was statistically at par with straw weight at 120 Kg N ha^{-1}.

Table 6: Yield and various yield contributing parameters of wheat crop as affected by different nitrogen levels

Treatment	Plant height (cm)	Ear length (cm)	No. of grains per panicle	Yield (Kg ha^{-1})	Straw weight (Kg ha^{-1})
N1 (0)	83.0 c	7.0 d	26.7 c	2266.7 e	4333.3 c

N2 (30)	88.9 bc	8.1 dc	28.6 c	2922.3 de	5132.7 c
N3 (60)	90.2 bc	8.8 bc	30.6 c	4036.3 dc	4153.7 c
N4 (90)	93.1 b	9.4 b	35.5 bc	4719.0 bc	5606 bc
N5 (120)	103.4 a	11.1 a	41.1 a	5585.0 a	8198 a
N6 (150)	107.2 a	11.2 a	45.4 a	6216.6 a	8600.4 a
F (p)	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**

CONCLUSIONS

An experiment was conducted to estimate crop nitrogen (N) status and chlorophyll content in wheat crop by using chlorophyll content meter and N-tester by sowing wheat variety viz. PBW 550 with 6 nitrogen levels i.e. 0, 30, 60, 90, 120 & 150 kg N/ha. The following conclusions were drawn from the study

1. Tillering stage was the most important stage where the values of both of the instruments were better correlated with nitrogen and chlorophyll content in the plant leaves. At tillering stages when the nitrogen rates was increased from 0 to 150 kg ha^{-1}, the correlation with both of the instruments i.e. CCI meter and NT for determination of plant nitrogen were better correlated having Pearson's coefficient 0.939 and 0.926 respectively.

2. For measurement of chlorophyll content in the plant leaves at tillering stage, the correlations were also better with CCI having Pearson's coefficient 0.917 and with NT having 0.890.

3. At all the crop growth stages like tillering, booting and ear emergence, Lab N content was having strong linear relationship with CCI having coefficient of determination 0.88, 0.75 and 0.77 respectively.

4. Coefficient of determination values for N tester and lab N-content also showed good linear relationship having values 0.85, 0.78 and 0.70 at tillering, booting and ear emergence stages respectively.

5. The chlorophyll content measured in laboratory was having strong linear relationship with CCI at tillering, booting and ear emergence stage with coefficient of determination 0.84, 0.78 and 0.77, respectively.

6. Coefficient of determination values between N tester and chlorophyll content showed linear relationship having values 0.82, 0.75 and 0.8 for tillering, booting and ear emergence stages respectively.

7. It was concluded that both of the instruments i.e. NT and CCM are useful for the determination of N and chlorophyll in plant leaves especially at tillering stage.

8. There was a good correlation between plant N and chlorophyll content shown through Pearson's correlation coefficient values which were 0.890, 0.846 and 0.786 at tillering, booting and ear emergence stages respectively. This was also confirmed as there was a strong linear relationship between chlorophyll content and lab N with coefficients of determination 0.798, 0.75 and 0.72 at tillering, booting and ear emergence stages respectively.

9. The grain yield of wheat crop obtained at N application rate of 120 Kg ha^{-1} was 91.1, 38.4 and 10.2 % higher over the application of 30, 60 and 90 Kg N ha^{-1}, respectively.

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Abbreviations:

CCI: Chlorophyll Concentration Index

NT: Nitrogen Tester

Lab N: Nitrogen in leaves of wheat determined in laboratory

CC: Chlorophyll content in leaves of wheat determined in laboratory

PC: Pearson's Coefficient

RCBD: Randomized completely block design

N : Nitrogen

Table 3: Laboratory data for chlorophyll content and N content at different N-levels and growth stages of wheat crop along with N tester and CCI values

Treatment	Chlorophyll content (mg/g)			N content (%)			N Tester Values			CCI		
	Tillering	Booting	Ear Emergence	Tillering	Booting	Ear Emergence	Tillering	Booting	Ear Emergence	Tillering	Booting	Ear Emergence
N1 (0)	1.07 d	1.04 c	1.67 d	0.14 e	0.20 c	0.19 d	472.6 c	496.6 c	536.6 d	7.03 f	16.0 e	23.2 c
N2 (30)	1.27 dc	1.34 bc	2.19 cd	0.60 d	1.06 b	1.41 c	514.6 c	537.3 bc	548.6 cd	12.3 e	22.0 d	25.9 bc
N3 (60)	1.27 dc	1.50 bc	2.65 cbd	0.92 c	1.33 b	1.67 bc	568.3 b	541.6 bc	557.6 cb	19.05 d	24.8 dc	30.1 bac
N4 (90)	1.47 bc	1.78 b	3.3 bc	1.21 b	1.83 a	1.94 b	583.3 b	552.5 bac	569.3 b	23.8 c	29.0 bc	30.8 ba
N5 (120)	1.61 a	2.64 a	3.8 b	1.22 b	1.99 a	2.30 a	634.3 a	602.9 ba	591.6 a	28.5 b	32.1 ba	34.7 a
N6 (150)	1.81 a	3.17 a	5.6 a	1.48 a	2.17 a	2.48 a	656.0 a	617.0 a	609.3 a	32.6 a	36.2 a	36.3 a
F (p)	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**