Field Chlorophyll Measurement as an Index for Nitrogen Status in Corn

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ABSTRACT

Improving N status in plant depends not only on the management of potential N supply, but also on the accurate assessment of plant N content. Therefore, this study was proposed to test the effectiveness of chlorophyll meter, as a rapid and accurate method in the field, to predict plant N status and grain yield potentials in corn. To meet these objectives, a split-plot experiment, including 3 different corn cultivars and 5 different N application rates (0, 25, 50, 75, &100 kg N/fed) was carried out under the field conditions. At the 5-6 leaf growth stage, random leaf samples of all treatments were collected to assess the leaf N status. Also, the dynamic of chlorophyll was measured using a chlorophyll meter. At the end of the growth season, yield potential and grain nitrogen content were recorded. The results have shown that remarkable variations were detected between chlorophyll meter values of the corn cultivars and the applied N rates. Increasing nitrogen fertilizer up to 100 kg N/ Fed. significantly increased corn grain yield, leaf and grain nitrogen concentration and chlorophyll meter readings. A close relation, as described by polynomial equation between chlorophyll meter readings and grain yield (R^2=0.927 to 0.994), leaf nitrogen concentration (R^2=0.924 to 0.988) and grain nitrogen concentration (R^2=0.885 to 0.997) were observed. It was concluded that chlorophyll meter is being recommended, as a rapid and promising technique, for predicting leaf N status and grain yield potentials in corn.

INTRODUCTION

Corn is an important crop because of its widespread production and utilization. It is well known that nutrient deficiency in most cultivated crops during the growth season causes imbalances, leading to reduce yield. Among the essential macronutrients, nitrogen is described as the most important element for crop growth (Shaahan *et al.*, 1999). Nitrogen plays a pivotal role in several physiological processes in the plant. It is a fundamental for the establishment of the plant's photosynthetic capacity, prolongs the effective leaf area duration, delaying senescence, and it is important for ear and kernel initiation, contributing to define corn sink capacity (Earl *et al*, 1997). To maximize grain yield, farmers often apply a higher amount of N fertilizer than the minimum required for maximum crop growth (Lemaire *et al.*, 1997). When N application is not synchronized with crop demand, N losses from the soil-plant system are large, leading to low fertilizer efficiency (Arregui *et al* 2006). The amount of N applied to corn must be carefully managed to ensure that N will be available throughout the growing season. However, the application of N at rates exceeding plant utilization, represent an unnecessary input cost to maize producer and may harm aquatic and terrestrial environments (Vidal *et al.*, 1999).

Adjusting the N input to an economically and ecologically compatible level would require reliable information on the N status of maize. Information on the N status can be obtained either from the crop side or from the soil side of the system. Crop-related indicators can be classified mainly in to three groups, namely those where the N status is monitored by (i) nitrate concentration, (ii) optical methods, or (iii) total N concentration (Herrmann and Taube, 2004). Standard methods for N determination involve tissue collection which is a destructive and time-consuming procedure. Leaf N concentration of normal plants varies from as low as 2 – 3% up to 4 – 5% depending primarily on plant species (Parvizi *et al.*, 2004).

Because of the direct relationship between N and chlorophyll contents, the portable chlorophyll meter has become a popular non-destructive means for measuring leaf N status in some crops (Costa *et al.*, 2001). The obtained chlorophyll meter values are proportional to the chlorophyll content of leaves (Kapotis *et al.*, 2003). Recent research indicates a link between chlorophyll content, leaf N status and crop yield (Cartelat *et al.*, 2005). Chlorophyll meter readings enable users to quickly and easily measure leaf greenness, which is determined by leaf chlorophyll content. However factors other than N can influence growth, chlorophyll and N relationships and thus the interpretation of chlorophyll meter reading (Arregui *et al.*, 2006).

The objective of this study is being directed to examine the possible application of chlorophyll meter in the field for predicting the leaf and grain N content and yield potentials in corn.

MATERIALS AND METHODS

Two field experiments were carried out at the experimental research station of faculty of Agriculture, Saba Basha, Alexandria University, during May-September, 2009. The physical and chemical properties of the experimental soil were analyzed (Black, 1965) and presented in Table 1.
The area of the each plot was 10.50 m^2 (3.5 m length x 3 m width), with 5 ridges 60 cm apart and 25 cm between hills. Fifteen treatments, including 3 different corn cultivars (3-way crosses 30/64. 30/84, 30/60) and N application rates in urea form (0, 25, 75 and 100 kg N/fed) were arranged in split – plot experiment with 4 replications. The Nitrogen fertilizer was portioned in two equal doses. The first dose was addressed after three weeks from sowing and the rest was added after two weeks from the first

dose.

The recommended dose of phosphorus (36 Kg P_2O_5/fed) as triple phosphate, was incorporated to the soil in a single dose during land preparation. Also, potassium (90 Kg K_2O/fed) as potassium sulphate was applied to the soil in two doses. The first dose was on June. 27, 2009 and the second dose was on Junly. 11, 2009.The kernels of the three corn cultivars were seeded on May 25, 2009 and the other practices for corn growing were realized as recommended . The corn varieties were applied to the main plots and the nitrogen levels were assigned to the sub plots. Plant samples were taken on Jul. 21, 2009 to determine the leaf N content. Total nitrogen content in the dried samples was determined using the microkjeldahl technique (Bermner and Mulvaney, 1982)

Leaf chlorophyll meter readings were taken for corn plants at the five to six-leaf growth stages with a CCM-200 chlorophyll meter. Grain yield was calculated per feddan and adjusted to a standard moisture of 13 g kg^{-1}. Also, the nitrogen content of grains was determined. For prediction purposes, relationships between variables were analyzed by fitting simple linear or quadratic regressions.

RESULTS AND DISCUSSION

The grain yields of the three corn varieties were increased with increasing N application level up to 100 kg N/Fed. (Table 2). The highest grain yield was recorded for the 3-way cross 30/64 (4.40 ton/Fed.) when N rate was increased to 100 kg N/fed. However, the means of grain yields, as affected by corn variety indicated that the 3-way cross 30/84 corn was more superior in grain yield performance (3.41 ton/fed), when compared with the two other varieties (Table 3).

Table (3) showed also, that increasing N rates reflected significant increases on the grain yield. Using 100 kg N / fed rate resulted in higher grain yield over the other nitrogen rates. Varvel *et al.* (1997) demonstrated that application of N fertilizer significantly increased corn grain yield. El-Bana (2001) found that corn grain yield and its attributes were increased significantly due to increasing nitrogen fertilizer rate.

The relationship between the grain yield and the N application rate for the three corn varieties were expressed by polynomial equations. The R^2 values proved that polynomial equation was fit to describe this relation (Fig. 1).

The data in Table (3) indicated the presence of considerable variation in the values of Chlorophyll concentration index (CCI) between the three corn varieties at any given N application rate. Increasing nitrogen application rates resulted in the increase of CCI values of the respective corn varieties. The leaves of the plants from the control were found to contain the least chlorophyll content as expressed by the lower CCI values (Table 2 and Fig. 2). Similar results were observed for corn (Majid *et al*, 2008) and rice (Swain and Sandip, 2010). Also, the data presented in Table (2) showed, that increasing N rate exhibited significant increases in the chlorophyll concentration index. Majid *et al.* (2008) found that the average of SPAD values was increased with increasing of N fertilizer application, irrespective of the growth stage.

The relationship between chlorophyll concentration index and nitrogen application rates has been expressed by polynomial equations for the three corn varieties. The quadratic model was exerted best fitness to describe the relationship between chlorophyll concentration index and N application rate for the three corn hybrids. The calculated R^2 values ranged from 0.958 to o.999 (Fig.2). The statistical analysis revealed that CCI values were significantly affected by nitrogen application and corn hybrids. Waskom *et al.* (1996) and Sunderman *et al.* (1997) reported a significant difference among corn hybrids for CCI readings under similar fertilizer management conditions.

The relationship between N content of leaves and CCI readings was positively correlated, as revealed from the polynomial expressions (Fig. 3). It is devious that N concentration in leaves greatly influences both the development of plant canopies and their photosynthesis.

During field observations, the visual symptoms of nitrogen deficiency always appeared first as yellow discoloration and withering of the older parts of plant, whilst the younger parts remain green longer. As a rule, however, the younger parts are also paler than usual because the remobilized nitrogen obtained from intrinsic sources is far from adequate for normal growth or optimal chlorophyll synthesis (Bergmaun, 1992). Schepers *et al.* (1992) found that at spilling stage in corn, readings from a CCI meter was correlated well with leaves N concentration for a given hybrid and location, but that calibration of the meter was not practical due to unique greenness characteristics of hybrids. Peng *et al.* (1992) showed that the linear relationship between leaves N concentration and CCI readings was differed depending on plant developmental stage, position of the measurement on the leaf and genotype. However, the lower range of CCI meter readings are a reliable indication of nitrogen deficiency, but the higher range of CCI readings do not distinguish between adequate and excessive nitrogen levels (Wood *et al.*,1992).

Since most of the leaf N is in enzymes association with chlorophyll (Chapman and Barreto, 1997), so it follows that chlorophyll concentrations reflect crop N status (Blackmer and Schepess, 1995), which reflects the soil N fertility status.

The average CCI value of each N treatment was determined (Tables 2 and 3) and its relationship was established with the grain yield for the three varieties. The results indicated a significant and positive correlation between the average CCI values and grain yield for the three varieties (Fig. 4). Also, a clear relationship between CCI values and grain nitrogen concentration was found for the three corn varieties (Table 4). Varvel *et al.*, (1997) and Fox *et al.*, (2001) using the CCI meter to assess corn nitrogen status showed that CCI is reliable indication of nitrogen status and consequently of the relatively relative yield.

As shown in Tables 2 and 3, the lowest grain yields, for the three corn varieties were associated with the lowest grain N concentrations and then gradually increased with increasing N application rate.

There was a good relationship between N in grain and N in leaves (Fig. 5). By increasing CCI values and N concentration in leaves, the N in grain has been increased, because a large proportion of the N in grain is remobilized from leaves and stems after anthesis, rather than being taken up from the soil.

Since the CCI readings are closely related to leaf nitrogen concentration, the CCI meter can be used to monitor the N status of corn thereby to adjust the rates of N fertilization in order to increase nitrogen use efficiency (Hussain *et al.*, 2000 , Varvel *et al.*, 2007 and Majid Rostami *et al.*, 2008) . Lopez – Bellido *et al.* (2004) concluded that the chlorophyll meter could be used to predict the grain N concentration of wheat in England.

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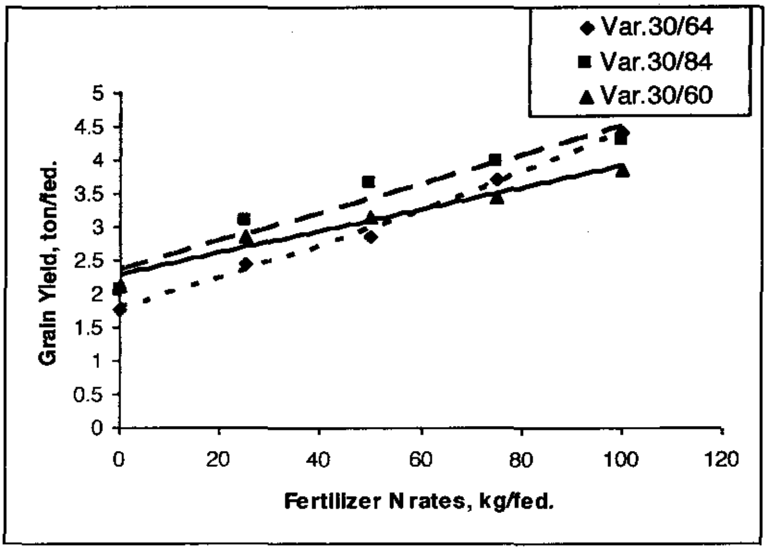

Fig (1):The relationship between the grain yield and nitrogen application rates for the three corn varieties as described by the polynomial equations :($Y_{var.\ 30/64} = 5E^{-05}x^2 + 0.0212x + 1.7806$, $R^2 = 0.995$; $Y_{Var.\ 30/84} = -0.0002x^2 + 0.0397x + 2.118$, $R^2 = 0.993$; $Y_{var.30/60} = -7E^{-05}x^2 + 0.0233x + 2.1893$, $R^2 = 0.982$).

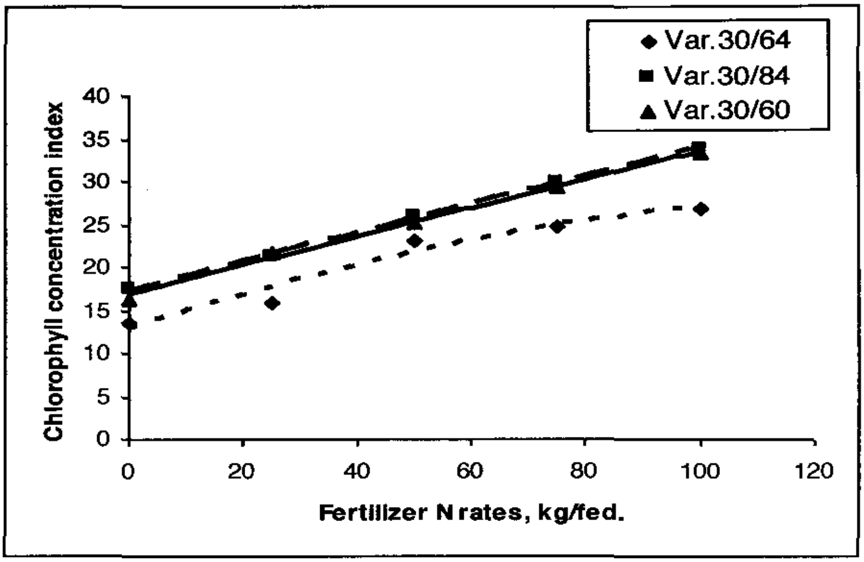

Fig (2): The relationship between chlorophyll concentration index and nitrogen rates for the three varieties as described by the polynomial equations : ($Y_{var.\ 30/64} = -0.0007x^2 + 0.2127x + 12.787$, $R^2 = 0.958$; $Y_{Var.\ 30/84} = -5E^{-05}x^2 + 0.1707x + 17.262$, $R^2 = 0.999$; $Y_{Var.\ 30/60} = -0.0002x^2 + 0.189x + 16.499$, $R^2 = 0.996$).

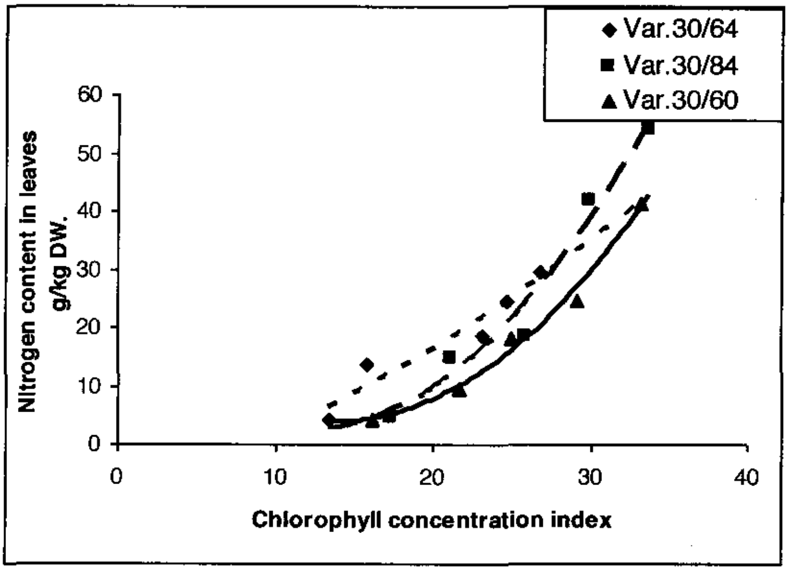

Fig (3): The relationship between nitrogen content in leaves and chlorophyll concentration index values for the three varieties as described by the polynomial equations ($Y_{var. 30/64} = 0.022x^2 + 0.7513x - 7.652$, $R^2 = 0.924$; $Y_{var. 30/84} = 0.1129x^2 - 2.735x + 19.018$, $R^2 = 0.969$; $Y_{var. 30/60} = 0.0989x^2 - 2.7447x + 22.854$, $R^2 = 0.988$).

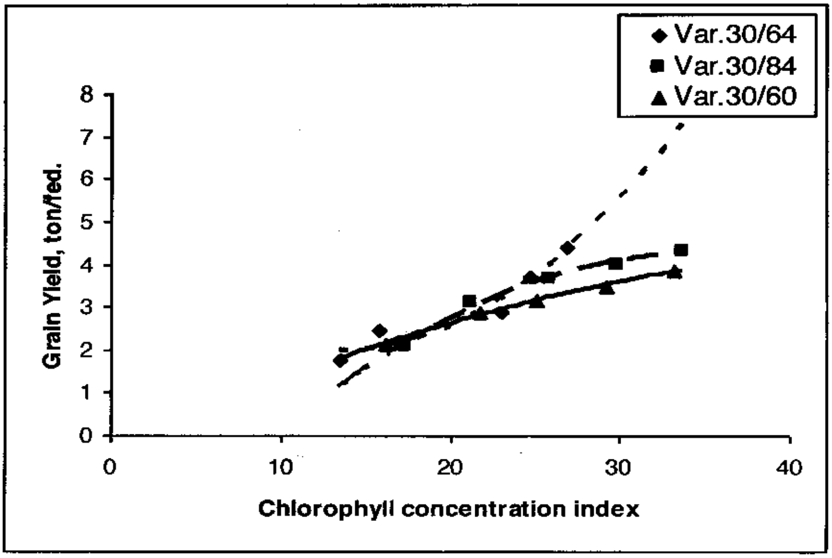

Fig (4):The relationship between grain yield and chlorophyll concentration index for the three varieties as described by the polynomial equations for ($Y_{var.\,30/64} = 0.0127x^2 - 0.3388x + 4.2351$, $R^2 = 0.927$; $Y_{var.\,30/84} = -0.0067x^2 + 0.4695x - 8.4699$, $R^2 = 0.987$; $Y_{var.\,30/60} = -0.0018x^2 + 0.1854x - 0.3966$, $R^2 = 0.994$).

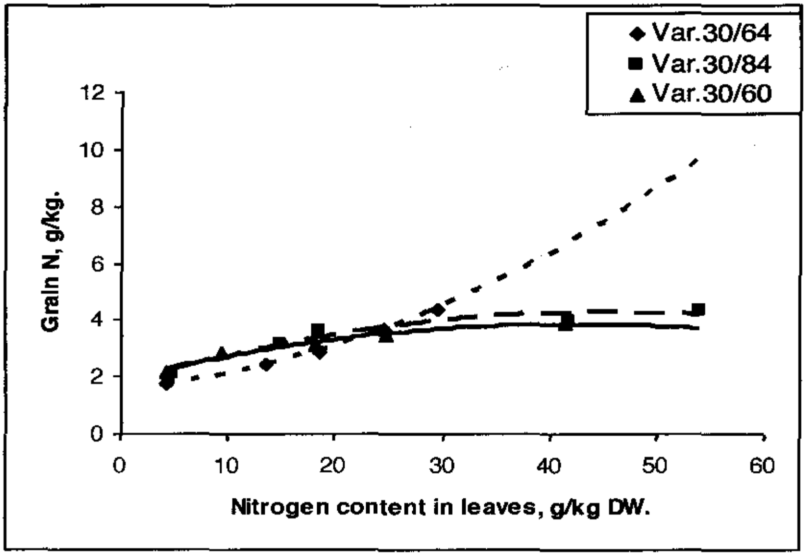

Fig (5): The relationship between grain N and nitrogen content in leaves of the three varieties as described by the polynomial equations were define by: ($Y_{var.\ 30/64} = 0.0053x^2 + 0.317x + 6.6128$, $R^2 = 0.991$; $Y_{var.\ 30/84} = -4E^{-05}x^2 + 0.2303x + 8.4239$, $R^2 = 0.885$; $Y_{var.\ 30/60} = -0.0054x^2 + 0.5884x + 6.4363$, $R^2 = 0.997$).

Table 1: The main physical and chemical characteristics of the experimental soil.

Soil properties	Values
particle size distribution	
Sand %	36.27
Silt	18.91
Clay	44.82
Soil texture	Clay
pH (Measured in 1:2.5 soil-water suspension)	8.33
EC (dS/m) (Measured in 1:1 soil-water extract)	3.80
Organic Mater (%)	0.79
Soluble cations (meq/L)	
Ca^{++}	4.8
Mg^{++}	2.8
Na^{+}	7.8
K^{+}	1.4
Soluble anions (meq/l)	
$CO^{-3} + HCO^{=}_{3}$	2.60
Cl^{-}	14.30
$SO^{=}_{4}$	0.45
Total Calcium Carbonate (%)	7.44
Available K (ppm)	198
Available N (ppm)	86.31
Available P (ppm)	5.20
Total N,%	0.13

Table (2):Grain yield, grain nitrogen content, chlorophyll concentration index and leaf-N content as affected by corn variety and nitrogen application rates.

Treatments Corn variety	Nitrogen rates Kg N/ fed.	Grain yield ton/fed	Grain N content g/kg	Chlorophyll concentration Index	leaf-N content g/kg
	0	1.752	7.90	13.45	4.11
	25	2.436	12.10	15.87	13.60
3-way	50	2.862	14.60	23.08	18.50
cross	75	3.708	16.80	24.70	24.50
30/64	100	4.404	21.00	26.84	29.60
	0	2.070	85.00	17.39	4.70
3-way	25	3.096	12.10	21.19	14.90
cross	50	3.636	14.60	25.82	18.60
30/84	75	3.984	15.70	29.87	41.90
	100	4.308	21.90	33.73	54.10
	0	2.130	87.00	16.25	4.21
3-way	25	2.856	11.80	21.69	9.40
cross	50	3.144	14.90	25.02	18.10
30/60	75	3.450	17.90	29.20	24.70
	100	3.858	21.50	33.19	41.50
Statistical Significant $LSD_{0.05}$					
Variety (V)			0.005	0.21 1.04	1.01
Nitrogen rates (R)			0.116	0.47 1.35	1.21
V X R			0.202	0.82 N.S	2.11

Table (3): Means of grain yield, grain nitrogen content, chlorophyll concentration index and leaf-N content as affected by corn variety and nitrogen application rates.

Treatments	Grain yield, ton/fed	Grain N content, g/kg	Chlorophyll concentration Index	leaf-N content, g/kg
		cultivars of Corn		
3-way cross 30/64	3.091	14.49	20.77	18.06
3-way cross 30/84	3.419	14.55	25.60	26.83
3-way cross 30/60	3.085	14.98	25.07	19.57
$LSD_{0.05}$	0.005	0.21	1.04	1.01
		Nitrogen rates, Kg N/fed		
0	1.980	8.35	15.69	4.33
25	2.796	11.98	19.55	12.63
50	3.213	14.72	24.64	18.41
75	3.712	16.82	27.92	30.36
100	4.190	21.49	31.25	41.70
$LSD_{0.05}$	0.116	0.47	1.35	1.21

Table (4): The relationship between grain nitrogen percentage and chlorophyll concentration index for the three corn varieties .

source	Varity of corn	Regression equation X = Chlorophyll concentration index Y = grain nitrogen g/kg grain	R^2
Urea	3-way cross 30/64	$Y = -0.039 + 0.073\ X$	0.897
		$Y = 2.084 - 0.156\ X + 0.006\ X^2$	0.941
	3-way cross 30/84	$Y = 0.074 + 0.056\ X$	0.888
		$Y = 1.324 - 0.061\ X - 0.003\ X^2$	0.957
	3-way cross 30/60	$Y = -0.019 + 0.062\ X$	0.948
		$Y = 1.039 - 0.038\ X + 0.002\ X^2$	0.994

الملخص العربي

قياس الكلوروفيل فى الحقل كدليل لحالة النيتروجين فى الذرة

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يعتمد تحسين حالة النيتروجين في النبات ليس فقط على ادارة التسميد بعنصر النيتروجين ولكن أيضا على دقة تقييم محتوى النيتروجين فى النبات. ولذلك تم إجراء هذه الدراسة لاختبار مقياس الكلوروفيل كوسيلة سريعة ودقيقة فى الحقل للتنبؤ بحالة النيتروجين فى النبات و محصول الحبوب فى الذرة. ولتحقيق هذه الأهداف, أجريت تجربة حقلية فى قطاعات منشقة, تشمل ثلاث أصناف ذرة مختلفة وخمس معدلات نيتروجين وهى صفر, 25, 50, 75, 100 كجم نيتروجين للفدان. وعند الورقة الخامسة و السادسة لمرحلة النمو لأصناف الذرة الثلاثة, أخذت عينات من الأوراق من جميع المعاملات وتقدير محتوى النيتروجين فيها, كما تم أيضا قياس الكلوروفيل باستخدام مقياس الكلوروفيل. وفى نهاية موسم النمو, تم تقدير المحصول ومحتوى النيتروجين فى الحبوب. ولقد أضحت النتائج أن هناك إختلافات واضحة بين قياسات الكلوروفيل للثلاث أصناف ذرة و معدلات اضافة النيتروجين. كما وجد أن زيادة عنصر النيتروجين إلى 100 كجم / فدان له تأثيرمعنوى على زيادة محصول الحبوب، تركيز النيتروجين فى الحبوب و الاوراق وقراءات الكلوروفيل. وقد وجد أن هناك علاقة وثيقة يمكن وصفها بالمعادلة متعددة الحدود بين قراءات الكلوروفيل ومحصول الحبوب (R^2 =0.927:0.994), تركيز النيتروجين فى الاوراق (R^2= 0.924 : 0.988) و تركيز النيتروجين فى الحبوب (R^2=0.885 : 0.997). ويمكن الاستنتاج أن مقياس الكلوروفيل وسيلة مفيدة وسريعة وللتنبؤ بحالة النيتروجين و محصول الحبوب فى الذرة.