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APPLICATION OF A CORRECTIVE DOSE OF NITROGEN AT JOINTING GROWTH STAGE OF WHEAT USING A PORTABLE CHLOROPHYLL METER

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Abstract

Collecting information about nitrogen (N) uptake in-season using tools like chlorophyll meters is promising to optimize N fertilizer management in cereals. Two field experiments were conducted during two successive winter seasons (2017/2018 and 2018/2019) on wheat at the Experimental Farm of Faculty of Agriculture, Cairo University, Giza Governorate, Egypt to quantify the relationship between N uptake at jointing growth stage with chlorophyll meter measurements and to formulate a strategy to optimize N fertilizer use efficiency. An increasing rate of N fertilizer was applied in the experiment conducted in the first season to create variability in chlorophyll meter readings determined at jointing growth stage of wheat. The data revealed that an exponential model based on the chlorophyll meter could explain 74% of the variation in N uptake. Accordingly a strategy to refine N application dose applied at jointing growth stage of wheat was suggested as guided by the meter in the second season. The suggested strategy are applying 0, 80, 120 or 160 kg N ha^{-1} corresponding to sufficiency index of chlorophyll meter values of > 0.95, 0.95-0.85, 0.85-0.75 and < 0.75. When appropriate prescriptive N fertilizer was applied (100 kg N ha^{-1} in two splits) followed by corrective dose as guided by the meter, the achieved N recovery efficiency was 67.4% compared with 53.5% in the general recommendation. This study revealed that N fertilizer could be managed more efficiently using tools like chlorophyll meters compared with the current general recommendation.

***Keywords*:** Nitrogen fertilizer, chlorophyll meter, sufficiency index, wheat

Introduction

Wheat (*Triticum aestivum* L.) is the most important grain crop in Egypt. Wheat accounts for nearly 10% of the total agricultural production value and about 20% of all agricultural imports (FAO, 2015). Use of nitrogen (N) fertilizer in Egypt has increased significantly in recent years following the general recommendation. Furthermore, farmers are often apply N fertilizer at rates higher than the general recommendation to ensure high yield levels of wheat. In 2014-2015, wheat crop consumed 22.8% of the total N fertilizer used in Egypt (Heffer, 2017). Recovery efficiency of N fertilizer has been found to be 35.6% to 51.1% in Egypt when N fertilizer was applied following the general recommendation (Ali *et al.*, 2017). It implies that high quantity of N in reactive form is lost from the soil-plant system.

Besides high cost of production, applying N fertilizer higher than the optimum may adversely affect the environment (Bijay-Singh *et al.*, 2003; Fageria and Baligar, 2005). It is well documented that N is the most difficult plant nutrient to manage because of the large number loss pathways. The loss pathways from the soil include denitrification, volatilization, surface runoff and leaching (Raun and Johnson, 1999). In fact, when broad-based general recommendations are adopted, large spatial and temporal variability restricts the efficient use of N fertilizer (Adhikari *et al.*, 1999 and Dobermann *et al.*, 2003). Site-specific N management can effectively replace the general recommendations for achieving high efficiency of N use. Application of N fertilizer which corresponds to the spatial and temporal variability should lead not only to increased N-use efficiency but also to a reduced possibility of environmental pollution (Khosla and Alley, 1999).

An important element in precision N fertilizer management strategies is the use of diagnostic tools, which can assess the N need of crop plants in field. In recent decades, a number of non-destructive methods have been developed, which use leaf greenness, absorbance, and/or reflectance of light by the intact leaf to quantitatively estimate the leaf chlorophyll content. The hand-held chlorophyll meters provide a quick and non-destructive method for estimating leaf chlorophyll content in the field. And since there exists a strong correlation between leaf N concentration and chlorophyll content (Schepers *et al.*, 1992 and Schlemmer *et al.*, 2005), the chlorophyll meter can be used to assess N status of the crop, to make N fertilizer recommendations and diagnose N distribution problems (Noulas *et al.*, 2018). Generally, the chlorophyll meter has been used in managing N fertilizer following two approaches. The first approach is the sufficiency index (ratio of the meter value of the as-needed treatment and the well fertilized treatment), and when its value falls below a certain set (mostly 0.95 or 0.90) a prescribed dose of N fertilizer is applied (Varvel *et al.*, 1997; Hussain *et al.*, 2000 and Francis and Piekielek, 2019). The second approach consists of applying an N fertilizer dose whenever chlorophyll meter value is less than a critical threshold value (Peng *et al.*, 1996; Bijay-Singh *et al.*, 2002 and Ali *et al.*, 2015; Bijay-Singh and Ali, 2020).

The objectives of this study were to predict N uptake at jointing growth stage of wheat using the chlorophyll meter. Also to formulate sufficiency index strategy to use the chlorophyll meter to guide N management in order to obtain high yield levels along with increasing use efficiency of N fertilizer.

Materials and Methods

The experimental site

Field experiment was conducted on wheat (*Triticum aestivum* L.) variety Giza 171 at the Experimental Farm of Faculty of Agriculture, Cairo University, Giza Governorate, Egypt in two successive winter seasons (2017/2018 and2018/2019). Initial soil samples were collected from the experimental site and analyzed for physical and chemical characteristics as reported in Table (1) using the procedures outlined by Page *et al.* (1982).

Table 1 : Some physical and chemical properties of the topsoil (0-30 cm) of the experimental site.

Texture	pH*	EC** dS m^{-1}	Organic matter %	Available N mg kg^{-1}	Available P mg kg^{-1}	Available K mg kg^{-1}
Loamy clay	7.91	7.53	2.3	100.9	18.5	354

* pH in saturated soil paste. ** Electrical conductivity in saturated soil paste extract.

Experimental design and treatments

Prior to sowing, the soil was ploughed and levelled. In both seasons, wheat (*Triticum aestivum* L.) variety Giza 171 grains were mechanically sown in rows spaced 15 cm in mid-November and divided into 15 m^2 plots. In the first season, N fertilizer levels of 0, 40, 80, 120, 160, 200, 240, 280 and 320 kgN ha^{-1} were applied as ammonium sulfate in three equal split doses. This range was applied to establish plots with large variability in N uptake and yield of wheat. The second season was designed in order to validate the efficacy of chlorophyll meter for fine-tuning N fertilizer application. The treatments consisted on applying different scenarios of prescriptive N application at early growth stages, following by corrective dose as guided by the chlorophyll meter at jointing growth stage. The experiments were conducted in randomized complete block design with three replications. Phosphorus (as single superphosphate) was applied at sowing following the general recommendation. Potassium fertilizer was avoided because the soil contains sufficient amount of available K (354 mg kg^{-1}).

Chlorophyll meter measurement

Chlorophyll meter (atLeaf model) was used to assess chlorophyll index for the purpose of this study. Readings from ten randomly selected plants from each plot were collected and then averaged. The chlorophyll meter readings were obtained by inserting the middle portion of the fully expanded leaf in the slit of the meter. The readings were collected at jointing growth of wheat (50-60 days after sowing). This stage was selected because various studies concluded that it is the appropriate stage in wheat to make corrective N fertilizer management (Raun *et al.*, 2001; Ali *et al.*, 2019). The abnormal or diseased affected plants were avoided for measurements.

Plant sampling and analysis

Aboveground plant samples were collected at jointing growth stage from an area of 1 m^2 from each plot directly after collecting the chlorophyll meter readings. At maturity, wheat crop was harvested manually from a net area of 6 m^2 from the center of each plot. Grain and straw samples collected from each plot were dried in hot air oven at 70º C up to constant weight and ground. The samples were digested in H_2SO_4 - H_2O_2 mixture and total N was determined by micro-Kjeldahl method (Kalra, 1997).

Calculations and statistical analysis

Regression models were fitted using Excel software (a component in Microsoft Office 2016). The analysis of variance (ANOVA) was used to identify the effects of N treatments on the generated data. Duncan's multiple range test (DMRT) at probability level < 0.05 was used to test the differences between means as described by Gomez and Gomez (1984). The recovery efficiency of N (RE_N) as described by Cassman *et al.* (1998) was computed as:

$$\mathrm{REN}(\%) = \frac{\text{Total N uptake in fertilized plot} - \text{Total N uptake in zero N plot}}{\text{Quantity of applied N fertilizer}}$$

Results and Discussion

Effect of N fertilizer application rate on grain yield of wheat

Grain yields of wheat obtained from the first season experiment were plotted against the increasing rate of N fertilizer (Fig.1). As shown in the curve, the relation exhibited a second-degree response function. Derivation analysis of the function revealed that maximum grain yield of 8881 kg ha^{-1} can be obtained by N fertilizer application rate of 215.8 kg N ha^{-1}. The N fertilizer rate required to obtain economic grain yield (8437 kg ha^{-1}, 95% of maximum yield) was computed to be around 155 kg N ha^{-1}. The widely adopted general recommendation of N fertilizer for wheat in the region is 180-240 kg N ha^{-1}. Furthermore, farmers are generally apply N fertilizer levels even higher than the general recommendation, which implies that unnecessary amounts of N fertilizer are being applied. In addition to susceptibility of the excess N fertilizer to be lost from the soil-plant system, it can also lead to deterioration of soil health (Bijay-Singh, 2018). These findings suggest that there is a need to develop in-season site-specific management strategies that have the potential to adjust N fertilizer application rate according to the crop actual need.

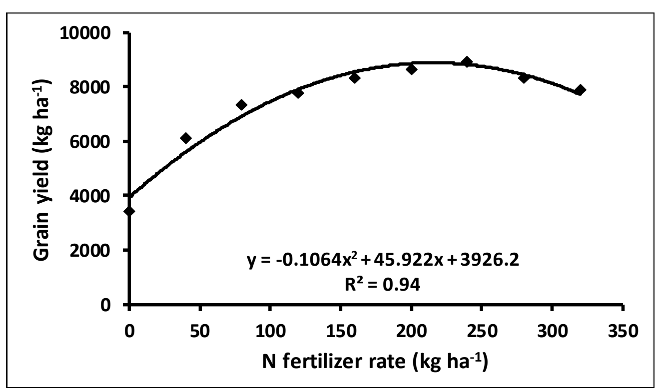

Fig. 1 : Response of wheat to increasing rate of N fertilizer fitted to quadratic function.

Prediction of N uptake at jointing growth stage using chlorophyll meter

Rapid acquiring information about N uptake where plant can response to N inputs prior to harvest is crucial for developing successful precision N fertilizer management strategy. The increasing rate of N fertilizer applied in the first season experiment created variability in N accumulation at jointing growth stage of wheat. These variability's were reflected in increments in readings of chlorophyll meter. Different regression models were tested to explain this relation, and the highest R^2 value was found for the exponential model (Fig. 2). The R^2 value for the relationship between N uptake at jointing growth stage and chlorophyll meter readings was 0.74. It means that 74% of the variation in N uptake at jointing growth stage of wheat can be explained by the chlorophyll meter. The empirical exponential model that can be used to predict N uptake in this stage is:

N uptake (kg ha^{-1}) = 0.1079 e 0.1156 x chlorophyll index

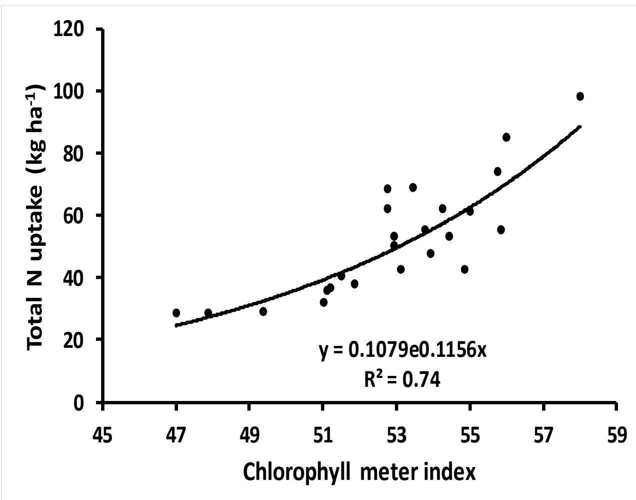

Fig. 2 : Relationship between chlorophyll meter values and total N uptake at jointing growth stage of wheat fitted to exponential function

Monitoring of N uptake during the season is essential for developing strategies to optimize N fertilization and reduce the environmental risks associated with applying high amounts of N fertilizer. Inadequate prediction of N uptake may result in over- or under-applications of N fertilizer than the actual requirements (Yao *et al.*, 2012). Several studies have shown that N status and grain yield of many crops can be estimated in-season from spectral measurements of leaves (Varvel, 1997; Raun *et al.*, 2001; Ali *et al.*, 2014). In fact, hand-held portable sensors like chlorophyll meter have opened up a new approach to make in-season accurate decisions rapidly.

Sufficiency index approach for managing N fertilizer using chlorophyll meter

Leaf greenness may vary among varietal groups, seasons or regions. Therefore, one fixed threshold value of chlorophyll meter may not work efficiently. Sufficiency index (defined as ratio of chlorophyll reading of tested plot and that of a reference N-rich plot) approach allows practicing precision N management using dynamic values rather than a fixed threshold value. This approach has the potential of being self-calibrating according to the variation in soil properties and seasons.

Nitrogen nutrition index and sufficiency index of chlorophyll meter were calculated by dividing the N uptake and chlorophyll meter readings in the tested plot by reference values. Boxplot diagram was used to identify the reference values of N nutrition index and sufficiency index as the upper interquartile values (Fig. 3). The generated reference values of N nutrition index and sufficiency index were 63 and 54.5 kg N ha^{-1}, respectively. The relationship between both indexes was tested and a highly significant linear relation was obtained with R^2 value of 0.68 (Fig. 4). This relation suggest that sufficiency index calculated from the chlorophyll meter can reflect the variation in N nutrition index.

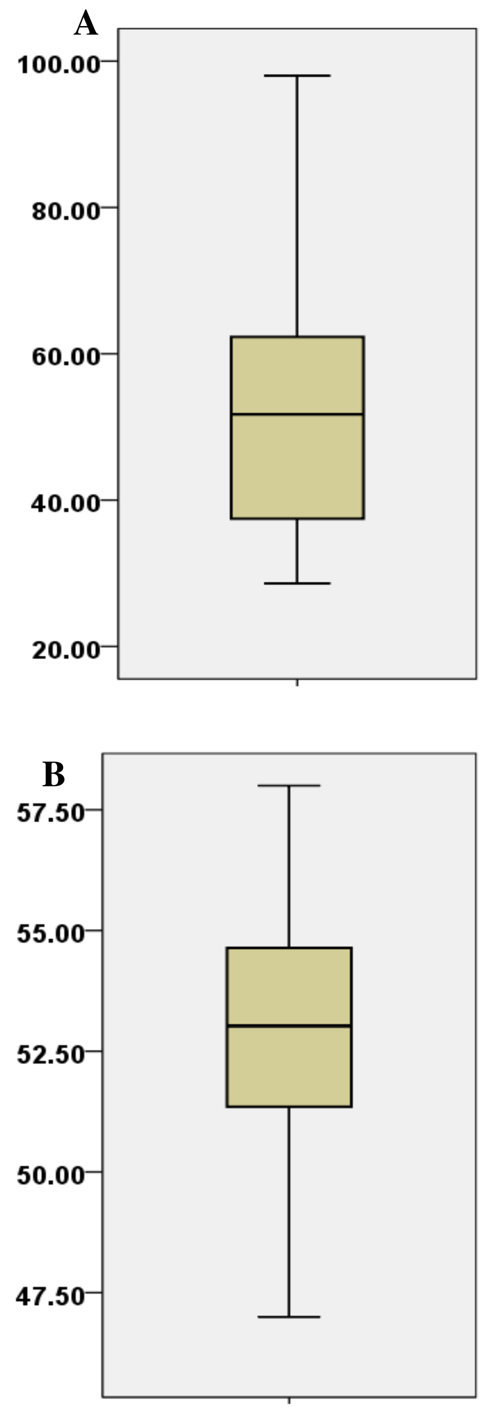

Fig. 3 : Boxplot diagram of (A), Total N uptake and (B) chlorophyll meter readings collected at jointing growth stage of wheat. The box represents the interquartile range (IQR), the solid line within the box indicates the median, and the whiskers represent 1.5 times the IQR.

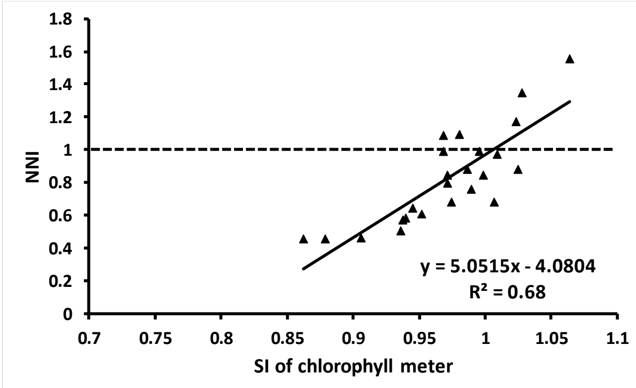

Fig. 4 : Relationship between sufficiency index (SI) of chlorophyll meter and nitrogen nutrition index (NNI) at jointing growth stage of wheat

Keeping up these findings, a strategy to refine N application dose was suggested to be applied at jointing stage of wheat as guided by the chlorophyll meter in the second season (Table 2). An N-rich strip was maintained as a reference by applying N fertilizer at a rate of 320 kg N ha^{-1}. The suggested strategy is applying 0, 80, 120 or 160 kg N ha^{-1} corresponding to sufficiency index of chlorophyll meter values of more than 0.95, 0.95-0.85, 0.85-0.75 and less than 0.75. These ranges of N application doses were suggested to challenge the current blind general recommendation that does not account for the variation in field-to-field soil properties and other management practices that effect the N fertilizer need.

Table 2 : The suggested corrective doses of N fertilizer corresponding to sufficiency index of the chlorophyll meter

Sufficiency index	Corrective dose of N (kg ha^{-1})
> 0.95	0
0.95-0.85	80
0.85-0.75	120
< 0.75	160

The chlorophyll meter readings at jointing growth stage of wheat in this study matched with Feekes 6 growth stage (around 50 days after sowing) and it is considered to be the appropriate stage to obtain information and make in-season corrective N fertilizer management decisions. For example, Raun *et al.* (2001) reported that relationships between optical sensors readings and grain yield of wheat were the highest between Feekes 4 and 6 stages. Also, Zhang *et al.* (2019) reported that leaf dry matter in wheat during Feekes stages 4 to 7 are more variable than other stages and agronomic information can be reliably obtained. Also, Bijay-Singh *et al.* (2011) and Varinderpal-Singh *et al.* (2017) reported that Feekes 6 stage of wheat in northwestern India is the appropriate stage to decide the amount of site-specific N fertilizer to be applied as topdressing.

Validation of chlorophyll meter in managing N fertilizer

The experiment conducted in the second season was used to evaluate the performance of the sufficiency index of chlorophyll meter as suggested in this study. To create growth variability in biomass and N uptake at jointing growth stage of wheat, different doses and timings of N fertilizer were applied preceding applying the corrective dose as guided by the chlorophyll meter.

The data listed in Table (3) show that grain yields obtained in Treatment #3 (applying 40 and 60 kg N ha^{-1} at 0 and 30 DAS, respectively, followed by a corrective dose as guided by the chlorophyll meter) is statistically similar to the yield obtained in the general recommendation, but with 60 kg N ha^{-1} less. Other treatments proved the efficacy of the meter in increasing or decreasing the N fertilizer levels according to the plant need at jointing growth stage. The meter-based N management successfully overcame the heterogeneity in wheat growth caused by different prescriptive N management, but with using less N fertilizer amounts.

Data pertaining to RE_N show that the meter-guided N treatments resulted in higher use efficiency as compared to the general recommendation. For example, when appropriate prescriptive N fertilizer was applied (Treatment #3) followed by a corrective dose as guided by the meter, an increase of 13.9 % RE_N compared with the general recommendation. Therefore, using the chlorophyll meter in guiding N management could effectively manage N fertilizer in order to obtain higher yield along applying less N fertilizer.

Table 3 : Wheat grain yields, total N uptake and N recovery efficiency as influenced by different N fertilizer (kg N ha^{-1}) treatments as guided by the chlorophyll meter

Treatment	N fertilizer		Sufficiency index	Corrective dose kg N ha^{-1}	Total amount of N fertilizer kg N ha^{-1}	Grain yield kg ha^{-1}	Total N uptake kg ha^{-1}	RE_N^+ %
	At sowing	30 days age						
T1 (zero-N)	**0**	**0**	-	0	0	3208 d	96.6 c	-
T2 (general recommendation)	**80**	**80**	-	80 (fixed)	240	8172 a	225.1 a	53.5 c
T3	**40**	**60**	0.91	80	180	8141 a	218.6 a	67.4 a
T4	**100**	**0**	0.82	120	220	7413 b	230.1 a	60.6 b
T5	**0**	**100**	0.87	80	180	7642 b	215.2 a	65.7 a
T6	**0**	**0**	0.73	160	160	6215 c	177.3 b	50.4 c
T7	**100**	**100**	0.96	0	200	7613 b	199.4 b	51.4 c

$^+$ RE_N = Recovery efficiency of nitrogen fertilizer

Means followed by the same letter within the same column are not significantly different at the 0.05 level of probability by Duncan's multiple range test (DMRT)

The sufficiency index approach of chlorophyll meter has be used successfully in previous studies. For instance, Hussain *et al.* (2000) monitored sufficiency index in rice and whenever chlorophyll meter reading of the field was less than the threshold corresponding to 0.90 of the reading of the N-rich strip, 30 kg N ha^{-1} less fertilizer was applied with statistically similar yield. Bijay-Singh *et al.* (2006) also followed sufficiency index approach using the chlorophyll meter for guiding N fertilizer application in rice in north-western India and found that by following the criteria of 0.90 sufficiency index, 50 kg N ha^{-1} less fertilizer was used in comparison to the general recommendation without any reduction in the grain yield.

Conclusions

The chlorophyll meter is proved as a reliable tool for predicting N uptake in wheat from measurements made at jointing growth stage. By fitting an exponential regression model, chlorophyll meter could explain 74% variability in N uptake. Thus, this hand-held meter can be used reliably for managing N fertilizer in wheat on a field specific manner. Accordingly, a strategy was suggested by applying corrective doses of 0, 80, 120 or 160 kg N ha^{-1} corresponding to sufficiency index of the chlorophyll meter values of > 0.95, 0.95-0.85, 0.85-0.75 and < 0.75, respectively. The suggested strategy efficiently used in managing N fertilizer, compared with the general recommendation, and resulted in an increased N recovery efficiency level by 13.9% with statistically similar yield.

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