

Original Research Article

Improving Maize Yield and Nitrogen Use Efficiency Using Spectral Properties of Leaves for Need-Based Nitrogen Management

Abstract

There is a spiral increase in maize consumption in Ghana due to high population growth and changing consumer preferences. However, maize yield in the country is declining partly due to poor soil fertility and fertilizer management, resulting in low nitrogen use efficiency. A field experiment was therefore conducted at the Soil and Irrigation Research Centre, University of Ghana, Kpong during the 2016 cropping season to evaluate the effect of nitrogen management options on growth, yield and nitrogen use efficiency of maize. The experiment was laid out in a split plot design with three replicates. Variety and nitrogen (N) management were the main and sub plot factors, respectively. The varieties were; 1. Aburohemaa (V1) 2. Abontem (V2) 3. Obaatanpa (V3). 4. Omankwa (V4), while the nitrogen management levels included; 1. no N application (control, N0), 2. Conventional practice, Conv (150 kg N ha⁻¹) 3. Leaf Colour Chart, LCC (75 kg N ha⁻¹) and 4. Soil Plant Analysis Development meter, SPAD (75 kg N ha⁻¹). These results revealed that LCC and SPAD meter in maize nitrogen-based management reduced nitrogen fertilizer input by 50% without significantly affecting the growth, grain yield and nitrogen use efficiency of maize. Maize varieties significantly differed in terms of growth rate, yielding potential, N uptake and N use efficiency. The study recommended LCC to maize farmers since it is relatively cheap, easy to use and saved 50% N fertilizer input.

Keywords: Leaf colour chart, maize variety, soil plant analysis development, nitrogen uptake and nitrogen use efficiency.

Introduction

Maize (*Zea mays* L.) is one of the major cereal crops consumed all over Africa and other parts of the world. About 208 million people in Sub-Saharan African rely on maize as a source of food security and economic wellbeing [1]. Maize has several economic importance due to its wide range of uses. Maize grains can be used as food for humans as well as feed for livestock especially poultry due to its high carotenoid content. In Ghana, maize is the largest producing crop after cocoa and it accounts for half of the total cereal crops cultivated, serving as a principal food source for over 90% of the population. The yearly land area for maize production in the country is about 1,023,500 ha, with a proportional annual maize yield of 1,764,500 metric tonnes [2].

Due to the doubts associated with crop demands and fertilizer losses, which makes it difficult to figure out a single level of nitrogen that is required to achieve maximum yields. Soil testing has

been popular in many parts of the world and used as a means of blanket fertilizer recommendation to help improve grain yield and nutrient use efficiency [3]. However, these blanket recommendation rates do not always represent the spatial variability of the crop. Moreover, the recommended application rate of N fertilizer in two splits at seedling stage and at 4- 6 weeks after sowing of maize may lead to heavy nitrogen loss [2]. About 50% of N fertilizer application is used by the plants while the remaining is lost through leaching [4]. The rate of nutrient depletion in Ghana ranges from about 40 to 60 kg ha⁻¹ yearly of nitrogen, phosphorus and potassium [5] and fertilizer use is approximately 7.2 kg ha⁻¹ [6]. Therefore, there is a need for alternative lower but more efficient and cost-effective fertilizer recommendation for smallholder farmers. Time and method of nitrogen application plays a significant function in its good usage [7].

Soil Plant Analysis Development (SPAD) meter has been used to reduce N input as well as increase grain yield of maize by determining the exact time the plants need N application. SPAD meter reduces losses of nitrogen through volatilization, leaching and surface run off. However, the meter is very expensive and smallholder farmers cannot afford to purchase it. Leaf Colour Chart (LCC) has also been reported to increase maize grain yield and reduce N input by using different shades of green colours to determine when the plants need N application. LCC is relatively cheap when compared to the SPAD meter. Therefore, a reasonable, scientific, and effective leaf spectral properties technique is very important for increasing grain yield and nitrogen use efficiency of maize. The study was conducted to assess farmers nitrogen management practice and leaf spectral properties techniques on growth, grain yield and N use efficiency of four maize varieties.

Materials and Method

Description of Study Area

The study was carried out at the Soil and Irrigation Research Centre (SIREC), University of Ghana, Kpong in the Eastern Region of Ghana. The research centre has a land size of 1,024 hectares and it is located at longitude 00° 04' E, latitude 6° 09' N with an altitude of 22 m. The site has a bi-modal seasonal rainfall pattern with a yearly precipitation of about 1200 mm. The main rainfall seasons start from April to July and minor rainfall season also begins from September to December. The centre has a maximum temperature of 33.3°C and a minimum temperature of 22.1°C. The relative humidity at the centre is based around 70% to 100% from night to morning and from 20% to 65% in afternoon throughout the year. The soil at the site belongs to the Akuse series with the following chemical properties; pH in water 1:1 (7.88), organic carbon (1.63%), total nitrogen (0.07%), C/N ratio (24.3), available potassium (4.72%), available phosphorus (2.09%), magnesium (1.26 mg kg⁻¹), calcium (22.8 mg kg⁻¹), and electrical conductivity (0.54).

Land Preparation, Experimental Design and Crop Establishment

The land was prepared by ploughing to bury all vegetation, loosen and aerate the soil, and to bring leached nutrients back to the soil surface. The land was ridged for planting. The main plots were divided into twelve sub-plots with an alley of 1 m both vertical and horizontal while the spacing between each main plot was 5 m. The experimental area measured 104 m x 52 m. A 4 x 3 factorial experiment was arranged in a split plot design and replicated three times. Maize variety and nitrogen management were the main and sub-plot factors, respectively. The maize varieties were; 1. Aburohema (V1), 2. Abontem (V2), 3. Obaatanpa (V3) and 4. Omankwa

(V4). Nitrogen management levels included; no N application (control, N0), 2. Conventional practice, Conv (150 kg N ha⁻¹) 3. Leaf Colour Chart, LCC (75 kg N ha⁻¹) and 4. Soil Plant Analysis Development meter, SPAD (75 kg N ha⁻¹). The sub-plots treatments were completely randomized in each main plot. The spacing between each sub-plot was 1m both horizontal and vertical while the spacing between each main plot was 5 m.

Maize seeds were obtained from the Crops Research Institute and healthy uniform seeds were selected for the study. Two seeds were planted per hill at a planting distance of 70 × 40 cm and thinned to one plant per hill at two weeks after planting. The study was carried out from October 2016 to January 2017. Selective herbicide (Nicotine) was applied at three weeks after emergence to prevent weeds from competing with the crops for nutrients, light, space and water. Insect pests were controlled with bifenthrin insecticide (Akate master) at a rate of 250 mL ha⁻¹ at three and five weeks after emergence due to heavy caterpillar infestation.

Nitrogen Fertilizer Management

Seventy-five (75) kg ha⁻¹ of NPK (15:15:15) was applied per treatment as basal at two weeks after emergence, and 75 kg ha⁻¹ of ammonium sulphate was applied as top dress for the conventional practice. For LCC treatment, 35 kg ha⁻¹ of ammonia sulphate was applied as a basal at two weeks after planting. Ten fully expanded leaves of different plants per hill in the same sub-plot was selected for the colour at 10 days intervals after twenty-one days of emergence. Twenty (20) kg ha⁻¹ of ammonium sulphate was applied, whenever the greenness of six or more out of the ten leaves were lighter than shade 4 of the colour chart. For SPAD meter treatment, 35 kg N ha⁻¹ of ammonia sulphate was applied as basal at two weeks after planting and three different plants per hill were selected randomly in each sub-plot for leaf chlorophyll content determination. The chlorophyll content in the upper, middle and lower portions of the leaves were determined and their average was calculated. Twenty (20) kg ha⁻¹ of ammonium sulphate was applied whenever the average chlorophyll content of the leaves fell below the critical value of 35.9. All the treatments received the same amount of phosphorus and potassium fertilizer of Seventy-five (75) kg ha⁻¹, respectively.

Data collection and statistical analysis

Data were taken on the following parameters after tagging ten plants per plot; plant height, leaf area and dry matter accumulation from two weeks after planting to harvest. One thousand (1000) grains weight, grain yield, number of kernels per cob, number of kernels per row and number of rows per cob were taken after harvest. Plant height was measured from the base of the plant to the tip of the highest leaf with a tape measure. Leaf area was recorded by measuring the breadth and length of the upper, middle and bottom leaves on each tagged plant and multiply by a constant 0.75 [8]. Days to 50% tasseling was recorded by counting the number of days half of the total number of plants on the plot flowered. Five plants per hill were uprooted and dried in an oven at 70°C for 72 hours and the weight was recorded as dry matter accumulation. Number of rows and kernels per cob were recorded by manually counting the total number of rows and kernels per cob, respectively. Number of cobs per plant were recorded by manually counting the total number of cobs per plant. One thousand (1000) grain weight was taken by measuring 1000 grains from the tagged plants and weighed. Grain yield was recorded by removing all the grains from the cob harvested from the plot excluding the border plants and weighed.

Plant samples (root, straw and grains) were ground through a mm sieve after drying in an oven at a temperature of 70°C to a constant weight and used for N uptake and N use efficiency

determination. Nitrogen uptake in the samples (root, shoot and grains) were determined using micro Kjeldahl digestion method as described by [9]. Total N uptake was calculated as the sum of N uptake in the root, straw and grains. Nitrogen use efficiency (NUE) was determined based on physiological N use efficiency (PNUE) and agronomic N use efficiency (ANUE) by using the formulas below;

$$ANUE = \frac{\text{Grain yield with N application} - \text{Grain yield with no N application}}{\text{Amount of N applied}}$$

$$PNUE = \frac{\text{Grain yield with N application} - \text{Grain yield with no N application}}{\text{Total N uptake and N application} - \text{Total N uptake and no N application}}$$

The data collected on various parameters were entered in Microsoft excel spreadsheet for treatment means and subjected to Analysis of Variance using IBM SPSS software package for Windows (version 25.0). Where significant differences were observed among treatments, LSD (5%) was used to separate the mean.

Results

Effect of variety and nitrogen management on maize growth

The main effects of variety and nitrogen management and their interaction significantly ($p=0.04$) affected dry matter accumulation at only 4 weeks after planting (Table 1). V2 x N0 treatment interaction produced significantly the lowest dry matter accumulation while the rest of the treatment interactions produced similar dry matter accumulation. Plant height was significantly ($p=0.049$) influenced by only the main effects of variety and nitrogen management (Table 1). Plants from V2 recorded the tallest plants, followed by V3, V4 and V1 treatments, respectively. Plants from Conv, LCC and SPAD treatments produced significantly the tallest plants from 2 weeks after sowing to harvest while the plants from the control produced the shortest plants. Moreover, only the main effects of variety and nitrogen management significantly ($p=0.04$) influenced leaf area at 8 and 12 weeks after sowing (Table 1). Plants from V3 and V2 treatments produced the highest and lowest leaf area, respectively. Plants from Conv and N0 treatments produced significantly the highest and lowest leaf area, respectively. Days to 50% tasseling was significantly ($p=0.03$) influenced by only the main effects of variety and nitrogen management (Figure 1a-b). Varieties were ranked as follows; V3>V1>V4>V2. The control recorded significantly the highest days to 50% tasseling, while N1, N2 and N3 treatments recorded statistically similar days to 50% tasseling.

Comment [A1]: Please add the explanation of V1, V2, V3, and V4 in the text

Comment [A2]: Please add the explanation of N1, N2 and N3 in the text

Means with the same letter within a column are not significantly different from each other. V1: Abrohema; V2: Abontam; V3: Obaatanpa; V4: Omankwa; N0: no nitrogen application (control); Conv: Conventional practice; LCC: Leaf colour chart; SPAD: Soil and Plant Analysis Development meter; WAS: Weeks after sowing; LSD: least significant difference; * means significant at 5%; NS means not significant at 5%.

Comment [A4]: Please add the remarks regarding the uppercase and lowercase letters in table and explain in the text.

Effect of variety and nitrogen management on grain yield and yield components of maize

The main effects of variety and nitrogen management as well as their interactions significantly ($p=0.001$) influenced the number of kernels row⁻¹, number of rows cob⁻¹ and number of kernels cob⁻¹ (Table 2). Plants from V3 x Conv and V2 x N0 treatments produced significantly the highest and lowest number of kernels row⁻¹ and cob⁻¹, respectively. All the treatment interactions did not differ significantly from each other with the exception of V2 x N0 which recorded significantly the lowest number of rows cob⁻¹. Plants from LCC, Conv and SPAD treatments had higher number of rows cob⁻¹ than the control. One thousand grains weight was significantly ($p=0.04$) influenced by only the main effects of variety and nitrogen management (Table 2). Plants from V3 and V2 varieties produced the highest and lowest 1000 grains weight, respectively. Nitrogen treatments produced similar 1000 grain weight except for the control which recorded significantly the lowest weight. Grain yield was significantly ($p=0.03$) affected by the main effects of variety and nitrogen management as well as their interaction (Figure 2). Plants from V3 x LCC and V2 x N0 treatments produced significantly the highest and lowest grain yield, respectively. Plants from SPAD, LCC, and Conv treatments produced similar grain yield while the control produced significantly the lowest grain yield.

Table 2: Effect of variety and nitrogen management on number of kernels per cob, number of kernels per row, number of rows per cob and 1000 grains weight of maize.

Variety (V)	Nitrogen magt. (N)	Kernels cob ⁻¹	Kernels row ⁻¹	Rows cob ⁻¹	1000 grain weight (g)
V1	N0	301f	23ef	11ab	238.1gh
	CONV	348c	26cd	12a	249.2defg
	LCC	335d	29ab	12a	246.8efgh
	SPAD	320e	27bcd	11ab	245.8fgh
	Mean	326B	26B	12A	245.0B
V2	N0	255i	22f	10b	201.3j
	CONV	294g	25de	11ab	252.0cdefg
	LCC	299g	25de	11ab	226.7i
	SPAD	353c	29ab	12a	230.3hi
	Mean	300C	25B	11A	227.6C
V3	N0	375a	24ef	12a	254.4cdefg
	CONV	354c	30a	12a	275.4ab
	LCC	287h	23ef	12a	283.1a
	SPAD	294g	24ef	12a	276.4ab
	Mean	328B	25B	12A	272.3A
	N0	294g	26cde	11ab	238.1ghi
	CONV	363b	29ab	12a	269.7abc

V4	LCC	350c	28abc	12a	257.3cdef
	SPAD	363b	28abc	12a	263.4bcde
	Mean	343A	28A	12A	257.1AB
LSD (5%)	V	5*	1*	NS	15.6*
	N	6*	2*	1*	17.1*
	V x N	10*	3*	1*	NS

Means with the same letter within a column are not significantly different from each other. V1: Abrohemaa; V2: Abontam; V3: Obaatanpa; V4: Omankwa; N0: no nitrogen application (control); Conv: Conventional practice; LCC: Leaf colour chart; SPAD: Soil and Plant Analysis Development meter; WAS: Weeks after sowing; LSD: least significant difference; * means significant at 5%; NS means not significant at 5%.

Comment [A5]: Please add the remarks regarding the uppercase and lowercase letters in table and explain in the text.

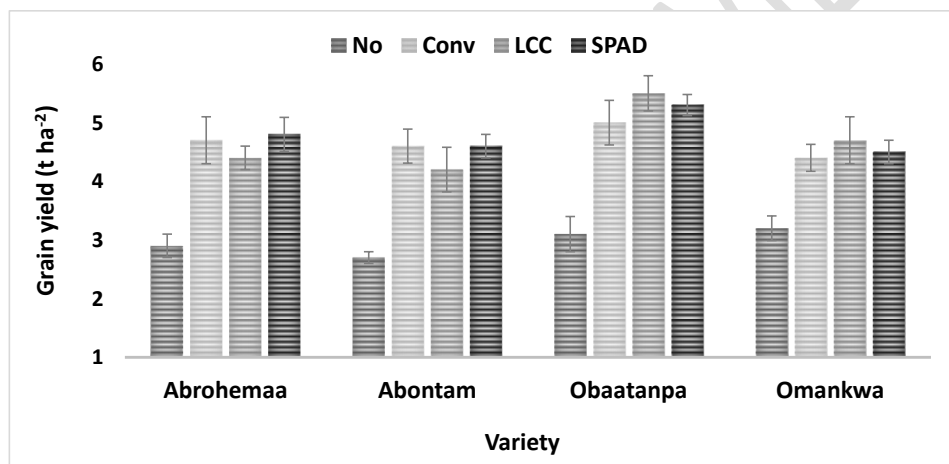


Figure 2: Effect of variety and nitrogen management on grain yield of maize

Effect of variety and nitrogen management on Plant N uptake, ANUE and PNUE (kg ha⁻¹)

The main effects of variety and nitrogen management and their interaction significantly ($P=0.001$) influenced plant N uptake and physiological N use efficiency (Table 3). Plants from V3 x Conv recorded the highest plant N uptake while plants from V2 x N0 recorded the lowest plant N uptake. Plants from V3 x SPAD produced the highest PNUE, followed by V2 x SPAD, V3 x LCC, respectively however, there were no significant differences among these treatment interactions. V4 x Conv produced significantly the lowest PNUE. ANUE was significantly ($p=0.001$) influenced by only the main effects of variety and nitrogen management (Table 3). Plants from V3 recorded the highest ANUE, followed by V2, V1 and V4, respectively. SPAD recorded the highest ANUE, followed by LCC and Conv, respectively.

Table 3: Effect of variety and nitrogen management on Plant N uptake, ANUE and PNUE (kg ha⁻¹)

Variety	Nitrogen magt.	Plant N uptake	ANUE	PNUE
---------	----------------	----------------	------	------

(V)	(N)	(kg ha ⁻¹)	(kg ha ⁻¹)	(kg ha ⁻¹)
V1	N0	61.8i	–	–
	CONV	159.0bc	11.9c	18.5ef
	LCC	122.4fg	22.1b	24.8cd
	SPAD	141.5de	27.6ab	23.8cd
	Mean	121.2B	20.5B	22.4BC
V2	N0	61.2i	–	–
	CONV	145.1d	12.6c	22.6de
	LCC	110.2h	23.5b	30.6ab
	SPAD	117.8gh	29.0ab	33.6a
	Mean	108.6C	21.7B	28.9A
V3	N0	65.2i	–	–
	CONV	191.4a	15.6c	15.1fg
	LCC	149.8cd	35.9a	28.4bc
	SPAD	129.7f	33.1a	34.1a
	Mean	134.0A	28.2A	25.9AB
V4	N0	73.1i	–	–
	CONV	167.0b	10.4c	12.8g
	LCC	133.4ef	23.5b	24.9cd
	SPAD	122.3fg	20.7	26.4bcd
	Mean	124.0B	18.2B	21.4C
LSD (5%)	V	7.9*	5.7*	4.1*
	N	11.6*	8.3*	4.8*
	V x N	15.4*	NS	7.6*

Means with the same letter within a column are not significantly different from each other. V1: Abrohema; V2: Abontam; V3: Obaatanpa; V4: Omankwa; N0: no nitrogen application (control); Conv: Conventional practice; LCC: Leaf colour chart; SPAD: Soil and Plant Analysis Development meter; ANUE: Agronomic nitrogen use efficiency; PNUE: Physiological nitrogen use efficiency; WAS: Weeks after sowing; LSD: least significant difference; * means significant at 5%; NS means not significant at 5%.

Comment [A6]: Please add the remarks regarding the uppercase and lowercase letters in table and explain in the text.

DISCUSSION

Effect of nitrogen management and variety on maize growth

The control took the highest number of days to 50% tasseling and it could be due to the absence of additional nitrogen from the fertilizer to the plants which consequently led to retardation of the plant growth and development. This finding disagrees with [10, 11] who reported that nitrogen fertilizer application delay days to tasseling. Plants from LCC and SPAD treatments produced similar dry matter accumulation and days to 50% tasseling as Conventional treatment, however the former received lower N rate than the latter. This might be due to better utilization of N, since N fertilizer was only applied when the plants need it [12]. Plants from V3 produced higher dry matter accumulation than the other varieties and it might be due to its genetic characteristics. Varieties differ in terms of growth rates as a result of their ability to form roots, compete for growth factors and convert them into physiological development [13]. This finding

was in conformity with [14, 15] that growth parameters of maize plant varies due to differences in their genetic make-up. Plants from V2 produced the tallest plants, however it recorded the lowest leaf area index and it might be due to its smaller leaf area (length and width) and leaf number. V3 recorded the lowest days 50% tasseling since the variety is an early maturing type. This explains why V3, a late maturing variety, recorded the highest days to 50% tasseling. Plants from V2 x Conv interaction produced the highest dry matter accumulation at 4 weeks after planting and it may be attributed to the genetic characteristics of the variety [13] as well as the half recommended N rate applied as basal. V2 is an early maturing variety and consequently might have grown faster than the other varieties.

Effect of nitrogen management and variety on maize grain yield and yield components

Plants with nitrogen fertilizer application produced better vegetative growth than the control which might have facilitated the partition of assimilate and therefore increased grain yield and yield components. Plants with higher vegetative growth produce more photosynthate partitioning and better source - sink relationship, and consequently leads to high grain yield and yield components [15]. The absence of yield loss from SPAD and LCC treatments compared with Conventional practice might be attributed to their better N utilization, since N fertilizer was applied only when the plants demand it. This outcome is in line with [12] who reported that LCC and SPAD nitrogen management reduced fertilizer input without decreasing grain yield. Grain yield was significantly influenced by the varieties used and it might be due to their genetic characteristics. The yield potential of varieties varies as a result of many physiological processes which are mostly controlled by their genetic characteristics and the environment. This finding is supported by previous studies [13, 15, 16] who reported that grain yield differs significantly among maize varieties. For the interaction between variety and nitrogen management on grain yield, plants from V3 x Conv produced significantly the highest grain yield and it might be attributed to its higher rate of N fertilizer applied as well as its higher yielding ability due to its genetic make-up. Moreover, the plants recorded the highest kernel number per row and cob, and therefore might have contributed to its high grain yield [11].

Effect of variety and nitrogen management on shoot N uptake, agronomic use efficiency (ANUE) and physiological use efficiency (PNUE) of maize.

Plants from Conventional practice recorded the highest N uptake and it might be due to its high rate of nitrogen fertilizer applied [17]. Moreover, it may be attributed to their larger leaf area and dry matter accumulation than the other treatments which might have increased their transpiration rate and consequently increased its nitrogen uptake. [18] reported that nutrient uptake is directly proportional to plants transpiration rate. Plants from SPAD and LCC treatments significantly produced higher nitrogen use efficiency (ANUE and PNUE) than plants from the Conventional method however, the latter produced higher N uptake statistically and similar grain yield with the former treatments. This could be attributed to the fact that; Conventional practice received a higher rate of the fertilizer than SPAD and LCC treatments. SPAD and LCC had similar N uptake and it may be due to fact that both treatments received the same rate of N fertilizer as well as their better synchronization of N supply with plant N demand [19]. Nitrogen use efficiency and N uptake were significantly influenced by the varieties tested and it may be attributed to their genetic constitutions. Similar findings were reported by [13, 20]. Plants from V3 variety recorded the higher N uptake than the other varieties, and it could be due to its high dry matter accumulation and leaf area which might have increased its transpiration rate and consequently its

higher N uptake [17]. Plants from V3 produced the highest ANUE and it could be attributed to its higher grain yield. For the interaction between variety and nitrogen management on N uptake, ANUE and PNUE, plants from V3 x LCC recorded the highest N uptake and ANUE and it may be due to their high grain yield as well as the small rate of N fertilizer applied.

Conclusion

The use of Leaf Colour Chart (LCC) and Soil Plant Analysis Development (SPAD) meter in maize nitrogen management reduced nitrogen fertilizer input by 50% without significantly affecting maize growth, grain yield and nitrogen use efficiency. Maize varieties significantly differed in terms of growth rate, yielding potential, N uptake and N use efficiency. Maize farmers should use Leaf Colour Chart in N management since it is very affordable, save 50% N fertilizer input as well as produced grain yield at par with the recommended N rate (conventional practice).

Reference

1. Food and Agricultural Organisation Statistical Database, FAOSTAT. Food and Agricultural Statistical Pocketbook. 2015. www.fao.org/3/a-i4691e.pdf
2. Ministry of Food and Agriculture, MoFA. Agriculture in Ghana. 2015. <https://agrihomegh.com/agriculture-in-ghana-facts-figures-2015/>
3. He, P., Li, S.T., Jin, J.Y., Wang, H.T., Li, C.J., Wang, Y.L., & Cui, R.Z. Performance of an optimized nutrient management system for double-cropped wheat-maize rotation in North-Central China. *Agronomy Journal*. 2009;101:1489-96.
4. Asghari, H.R. & Cavagnaro, T.R. Arbuscular mycorrhizas enhance plant interception of leached nutrients. *Funct. Plant Biol.* 2011;38:219-26.
5. Food and Agriculture Organization, FAO, (2005). Fertilizer use by crop in Ghana. Rome. 2005. www.fao.org/3/a0013e/a0013e00.htm
6. International Fertilizer Research Center, IFDC. Local Organisation part of food security group. 2002 <https://ifdc.org/2012/07/13/local-organization-part-of-food-security-group/>
7. Bundy, L.G. Side dressing nitrogen: Useful soils? Proc of the 2006 Winsconsin Fertilizer, Agrilime & Pest Management Conference. 2006;45:39-43
8. Watson, D.J. The dependence of net assimilation rate on leaf-area index. *Annals of Botany*, 1958;22(1):37-54
9. Nelson D.W. and Sommers L.E. Total carbon, organic carbon and organic matter. In *Chemical and Microbiological Properties. Part 2. Agronomy Series.* (A. L. Page, ed.). Madison, USA. 1982;9:570.
10. Amanullah, Khattak R.A. & Khalil S.K. Effects of plant density and N on phenology and yield of maize. *Journal of plant nutrition* 2009;32:246-60
11. Imbran, S., Arif, M., Khan, A., Khan., M.A., Shah, W., & Latif, A. Advances in Crop Science and Technology. Effect of Nitrogen Levels and Plant Populations on Yield and Yield Components of Maize. *Advances in Crop Science and Technology.* 2015;3(2):3-9 <http://doi.org/10.4172/2329-8863.1000170>.
12. Maiti, D., Das, D.K., Karak, T., & Banerjee, M. Management of nitrogen through the use of leaf color chart (LCC) and Soil Plant Analysis Development (SPAD) or chlorophyll meter in rice under irrigated ecosystem. *The Scientific World Journal.* 2004;4:838-46.

Comment [A7]: Should be italic style

Comment [A8]: Should be italic style

Comment [A9]: ???

Comment [A10]: Should be italic style

13. Dogor, M.K. The effect of fertilizer formulation on yield component and yield of hybrid maize (*Zea mays L.*) varieties in the Guinea Savana Zone of Ghana. *Thesis submitted to the Department of Crop Science, University of Ghana in partial fulfillment of the requirement for the degree of Masters of Philosophy in Crop Science.* 2013;1-17.
14. Enujeke, E.C. Effects of variety and spacing on growth characters of hybrid maize. *Asian Journal of Agriculture and Rural Development.* 2013;3(5):296.
15. Kumar, V., Singh, A.K., Jat, S.L., Parihar, C.M., Pooniya, V., Sharma, S., & Singh, B. Influence of site specific nutrient management on growth and yield of maize (*Zea mays L.*) under conservation tillage. *Indian Journal of Agronomy.* 2014;59(4):657-60.
16. Buah, S.S.J., & Mwinkaara, S. Response of sorghum to nitrogen fertilizer and plant density in the Guinea Savanna Zone. *Journal of agronomy,* 2009;8(4):124-30.
17. Swamy, M., Umesh, M.R., Ananda, N., Shanwad, U. K., Amaregouda, A., & Manjunath, N. Precision nitrogen management for rabi sweet corn, (*Zea mays saccharata L.*) through decision support tools. *Journal of Farm Science* 2016;29(1): 14-18.
18. Haefele, S.M., Jabbar, S.M.A., Siopongco, J.D.L.C., Tirol-Padre, A., Amarante, S.T., Cruz, P.S., & Cosico, W.C. Nitrogen use efficiency in selected rice (*Oryza sativa L.*) genotypes under different water regimes and nitrogen levels. *Field Crops Research.* 2008;107(2):137-46.
19. Garcia, B.A., Mollah, S., Ueberheide, B.M., Busby, S.A., Muratore, T.L., Shabanowitz, J., & Hunt, D.F. Chemical derivatization of histones for facilitated analysis by mass spectrometry. *Nature protocols.* 2007;2(4):933.
20. Reena-Dhyani, V.C., Charturvedi, S., & Gouda, H. S. Growth Yield and Nitrogen Use Efficiency in Wheat as Influenced by Leaf Colour Chart and Chlorophyll Meter Based Nitrogen Management. *International Journal of Current Microbiology and Applied Sciences* 2017;6(12): 1698-1704

Comment [A11]: Should be italic style