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Deciphering the Stability and Association of Ear Leaves Elements with Nutrients Applied to Grain Yield of Maize

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ABSTRACT

Ear leaf as a vegetative part has proven to be useful for evaluation of nutritional indices in maize and for making predictions about yield. This study was conducted to determine the stability of ear leaves nutrient under varying fertilizer applications and their relationship with grain yield. Thirty-five (35) nutrient omission trials were established in four locations using two maize varieties; IWD (OPV) and Oba Super-9 (hybrid) making a total of eight environments in 2015 wet season across the Guinea Savannas of Nigeria. Ten ear leaves were sampled in the period between tasseling and silking immediately when the position of the ear was identified and analyzed for macro and micro elements. The results showed that environment contributed to most of the variability observed in all the elements rather than the treatments. The GGEbiplot showed that Mg, Mn, and Cu are positively associated with grain yield and are the most stable elements. The confirmatory analysis also showed the importance of these elements in predicting grain yield. The environment has demonstrated to be a major determinant of ear leaves elements in maize. Therefore, accurate envirotyping of maize producing regions in Nigeria is important for better classification of maize-growing regions.

Keywords: Ear leaf, enviryotyping, grain yield, nutrients, stability

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INTRODUCTION

Fertilizer research is usually conducted to determine the yield responses of crops to fertilizer and to make better predictions for several soil and management conditions.

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Availability of nutrients in the soil is majorly determined from the nutrient composition of the plant. The plant is also used to determine key nutrient and their levels required for growth and development of the plant were further increase will have low or no yield response, and a decrease will result in low yield. In other to make accurate predictions of this yield response using plants, knowledge of the interrelationships among different nutrient omissions and compositions of the plants with yield responses are required. Ear leaf; the leaf covering the upper cob of a maize plant, is a vegetative part of the plant and have proved to be useful for evaluation of nutritional indices in maize and for making predictions about yield. It is the most sensitive stage that usually affects kernel formation on a cob (Jones, Schreiber, & Roessler, 1996). The ear leaf can be greatly exploited to predict grain yield which is an advantage of ear leaf analysis (Soltanpour, Malakouti, & Ronaghi, 1995). However, a major disadvantage is the low practical applicability of obtained models using ear leaf. The lack of knowledge about the stability of the ear leaf elements under different nutrient management and environment sometimes make the applicability of the model low. The ear leaf stage is close to flowering and marks the end of the vegetative stage. So for nutrient diagnostic purposes, the ear leaves should be sampled at the earliest stages, just before the inflorescences set up. It has been reported that maize plants reach their highest rate of absolute growth and are significantly affected by nitrogen supply and other elements at this stage (Grzebisz, Baer, Barłóg, Szczepaniak, & Potarzycki, 2010). Several studies have shown the importance of the nutritional status of maize ear leaves to grain yield (Grzebisz, Wrońska, Diatta, & Dullin, 2008; Mallarino & Higashi, 2009). Based on the importance of the ear leaf nutrient composition in predicting yield, the present study was conducted to determine the stability of ear leaf nutrient composition under varying fertilizer applications and the relationship of these nutrients with grain yield with a view of increasing the applicability of the model in predicting grain yield.

MATERIALS AND METHODS

Thirty-five (35) nutrient omission trials (NOTs) were established in four locations, Zango Kataf (5), Karu (10), Kokona (10), and Tsafe (10) in 2015 wet season across the Guinea Savannas of Nigeria (Supplementary Table 1). The NOTs consisted of six treatments (Table 1) with plots sizes of 5 m by 6 m used and two maize varieties were used, IWD (OPV) and Oba Super-9 (hybrid), which were considered as environments. Nitrogen was applied in three split application (planting, 3 weeks, and 6 weeks after planting).

Ten ear leaves were sampled in the period between tasseling and silking (male and female flowering, respectively) immediately when the position of the ear was identified. Ear leaf is removed by plucking downwards (at roughly an adjacent angle of <30°) with moderate force as this allows the leaf to cut at the collar, leaving

Table 1

Treatment structure of NOT in Guinea Savanna of Nigeria

Treatment code	Treatment	Nitrogen (kg N/ha)	Phosphorus (kg P/ha)	Potassium (kg K/ha)	Secondary and micronutrients
1	Control	0	0	0	0
2	NK	140	0	50	0
3	NP	140	50	0	0
4	NPK	140	50	50	0
5	NPK (S-Ca-Mg-Zn-B)	140	50	50	24-10-10-5-5, respectively
6	PK	0	50	50	0

behind the leaf base that circles the stem. A total of ten plants in the two rows next to the net plot (5 in each row) were sampled, taking representative samples. The leaf samples were placed into clearly labelled large khaki paper sample bags and carefully sealed. The samples were washed with distilled water to remove contaminants and then oven-dried at 60^{o}C for 48 h.

Total nitrogen in the ear leaves samples was determined using the Micro-Kjeldahl digestion method (Bremner & Mulvaney, 1982). While P, K, Mg, Ca, Cu, B, and Zn were digested with hot nitric acid (HNO_3) and their concentration was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES).

Surface soil samples were collected at 0–20 cm depth from each field. Soil pH, total soil organic carbon, total nitrogen, available phosphorus, and exchangeable cations (Ca, Mg, and K) were analyzed using the methods of Gee and Or (2002), Heanes (1984), Bremner (1996), and Mehlich (1984), respectively.

Grain yield data were collected from the field. The data collected for grain yield and nutrient concentration in the ear leaves element were analyzed using JMP 10.1.2. Variance components of each of the factors were estimated to determine the contribution of each factor to grain yield. The treatments were treated as fixed effect while the replication, environment, and interaction of treatments with the environment were considered as random effects. The GGE biplot analysis was done using R GGE biplot graphical user interface package. Partial least square regression was done to determine the important variables contributing to grain yield.

RESULTS

Sand was the dominant soil textural fraction in all the study locations with a median value of >60% (Table 2). Kokona and Tsafe have a moderately acid soil pH, while Zango and Karu have strongly acidic and moderately acidic reactions, respectively. All the four locations have low organic carbon (<1%), low N (<0.1%), and low available P, respectively, according to the Esu (1991) soil fertility classification. Calcium is low in Karu, Kokona, and Zango (<2 cmol/kg) and moderate in Tsafe (2–5

Table 2
Physical and chemical properties of the soil

Locations	Sand (%)	Silt (%)	Clay (%)	pH	OC (%)
Karu	76 (71.80)	9 (4.12)	16 (12.20)	6.1 (5.7,6.2)	0.59 (0.44,1.24)
Kokona	71 (65.77)	12 (7.15)	16 (14.22)	5.9 (5.5,6.1)	0.63 (0.42,1.22)
Tsafe	61 (54.66)	25 (19.25)	15 (14.21)	6 (5.8,6.6)	0.48 (0.38,0.81)
Zango	60 (53.67)	13 (12.16)	26 (20.34)	5.1(4.9,6.1)	0.75 (0.41,1.06)

Locations	N (%)	Meh_P (mg/kg)	Ca (cmol/kg)	Mg (cmol/kg)	K (cmol/kg)
Karu	0.05 (0.03,0.07)	3.08 (2.06,4.11)	1 (0.19,2.46)	0.59 (0.41,0.83)	0.11 (0.09,0.12)
Kokona	0.04 (0.03,0.04)	2.26 (2.06,4.93)	1.31 (0.38,3)	0.6 (0.23,0.66)	0.15 (0.11,0.26)
Tsafe	0.03 (0.02,0.04)	3.49 (2.88,5.69)	2.44 (0.56,4.31)	0.85 (0.46,0.96)	0.15 (0.11,0.17)
Zango	0.04 (0.04,0.05)	2.06 (1.65,2.67)	0.56 (0.38,1.69)	0.26 (0.19,0.26)	0.17 (0.12,0.26)

Numbers in parenthesis “( )” are minimum and maximum values, respectively

cmol/kg). Soil Mg concentration was in moderate condition (0.3–1.0 cmol/kg) in all the four locations. Soil K concentration were low (<0.15 cmol/kg) in Karu and moderate (0.15–0.30 cmol/kg) in Kokona, Zango, and Tsafe, respectively.

The variance components and percent contribution of each of the factors to macro elements in maize grain yield are presented in Table 3. The minimum percent variance contribution was from the treatment by environment interactions (TEI) for yield and all the macro elements of ear leaf. The TEI contribution ranges between 0.04% for grain yield to 11.96% for Ca. The unexplained variability for grain yield as a result of noise was 31% this was followed by the variability due to treatment effect (28%) and variability as a result of the environment (24%). The unexplained noise effect for N and P were 35% and 39%, respectively, followed the effect of environment (32% and 24%, respectively). The treatment effect also explained some percentage of the variability observed in the amount of N and P in the ear leaves. The variability in K for the ear leaves was majorly from the environment (56%) followed by the replication within environment effect (35%). The treatment effect was 0.32% which is an indication that any difference observed in K was not the result of the treatment but majorly due to the environment (combined effect with replication was about 90%). The addition or omission of K from the treatments did not affect the response of maize ear leaves to K. About 39% and 33% of the variability in Ca was accounted for by the unexplained noise and replication within environment effects. While for Mg, the replication within environment contributed 51% of the variability observed followed by the residual effect. The treatments only explained 0.7% of the variability while the environment explained just 0.2% of the variability in Mg content of the ear leaves.

The contribution of the treatment and the TEI to the variability observed in the micro nutrients were very low except for Mn where they accounted for 9% and 20% of the variability, respectively (Table 4). The noise variance was 31% of the total variability observed for Mn while the environmental variance was 27.84%. The environmental variance for Zn, Cu, and B were 24%, 40%, and 35%, respectively. The noise variance for Fe was very high (93%) followed by that of Zn (64%) and for Cu and B, the environmental variance was higher than the noise variance.

The principal axis explained about 78% of the treatment and treatment by ear leaves element interaction (Figure 1). Basically, in GGE biplot, the smaller the angle between two variables the closer the association. Copper has the highest association with grain yield while other elements in close association with grain yield in decreasing order are Mg, Mn, and N. In the polygon view (Figure 2), the vertex treatment in each sector represents the highest yielding treatment for the ear leaves element that falls within that sector. Five sectors were identified in the biplot. The response of Fe contents in the ear leaves was closely associated with treatment 2 (NK) followed by K and Zn. None of the element showed a response to the control (treatment 1) and B

Table 3
Variance components and percent contribution of factors to yield and macro elements in ear leaves of maize

Random effect	Grain yield	N	P	K	Ca	Mg
Replication (environment)	452806.52 (17.17)	0.02 (7.94)	0.00087 (18.13)	0.3169 (35.31)	0.0049 (33.01)	0.00243 (50.90)
Environment (E)	639461.22 (24.25)	0.09 (31.92)	0.00116 (24.16)	0.5022 (55.97)	0.0011 (7.10)	0.00001 (0.21)
Treatment (T)	727618.72 (27.59)	0.06 (19.55)	0.00073 (15.22)	0.0029 (0.32)	0.0013 (8.67)	0.00003 (0.70)
T × E	1169.28 (0.04)	0.01 (3.14)	0.00017 (3.57)	0.0029 (0.32)	0.0018 (11.96)	0.00014 (2.93)
Residual	816244.04 (30.95)	0.11 (37.46)	0.00187 (38.92)	0.0725 (8.07)	0.0058 (39.27)	0.00216 (45.26)
Total	2637299.79	0.28	0.00480	0.8974	0.0149	0.00476

Number in parenthesis “( )” are percentage contributions

Table 4
Variance components and percent contribution of factors to yield and micro elements in ear leaves of maize

Random effect	Mn	Fe	Zn	Cu	Br
Replication (environment)	164.15 (11.42)	976.68 (3.30)	7.89 (8.40)	10.10 (33.21)	115.04 (31.31)
Environment (E)	400.00 (27.84)	582.80 (1.97)	22.41 (23.86)	12.22 (40.18)	126.19 (34.35)
Treatment (T)	136.60 (9.51)	616.37 (2.08)	−0.18 (0.00)	−0.10 (0.00)	12.11 (3.30)
T × E	291.49 (20.28)	−579.68 (0.00)	3.47 (3.70)	0.88 (2.89)	20.64 (5.62)
Residual	444.81 (30.95)	27444.21 (92.65)	60.12 (64.04)	7.21 (23.72)	93.39 (25.42)
Total	1437.05	29620.06	93.89	30.41	367.38

Number in parenthesis “( )” are percentage contributions

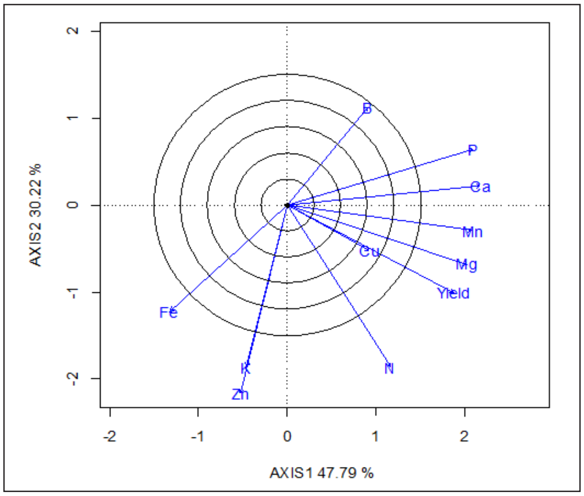

Figure 1. Relationship between the ear leaves element and grain yield

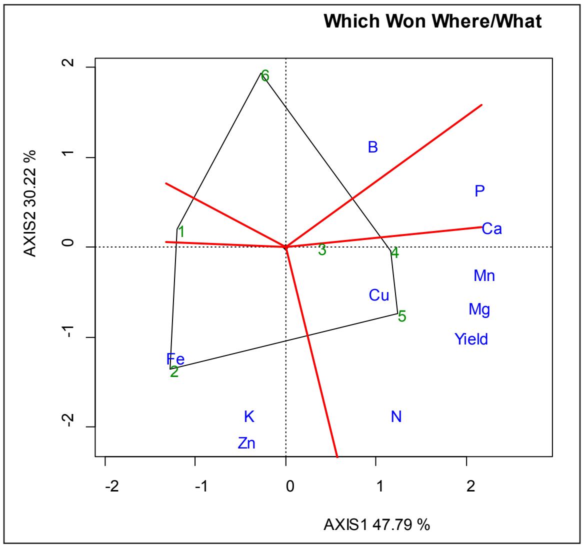

Figure 2. Which won where/what Biplot of NOTs and ear leaves of maize

slightly showed a response to PK (treatment 6). Grain yield per se was more influenced by treatment 5 (NPK + S-Ca-Mg-Zn-B) and 4 (NPK) while the response of Cu and Mg was more affected by treatment 5.

The length of the vector of elements describes its discriminating power, whereas the angle between an element and the thick horizontal axis measures its representativeness. Most of the elements had long vectors indicating that they are able to discriminate among the treatments but only Mg and Mn concentrations had smaller angles with the horizontal axis describing their representativeness. Also, grain yield had a good discriminating and representative power (Figure 3).

The most stable nutrient omission treatment was treatment 5 followed by treatment 3 while treatment 2 and 6 were highly unstable (Figure 4). Copper was the most stable among the ear leaf elements followed by grain yield.

Six factors were identified to explain the variability in grain yield and factor 1 was loaded more with Mg, Mn, Ca, P, and N (Figure 5[a]). Among all the six factors, factor 1 accounted for about 90% of the variability observed (Figure 5[b]). A variable important plot was plotted using partial least square regression to identify most important elements in predicting grain yield (Figure 6). From the plot, Mn, Mg, N, Ca, and P were the most critical elements in determining grain yield of maize.

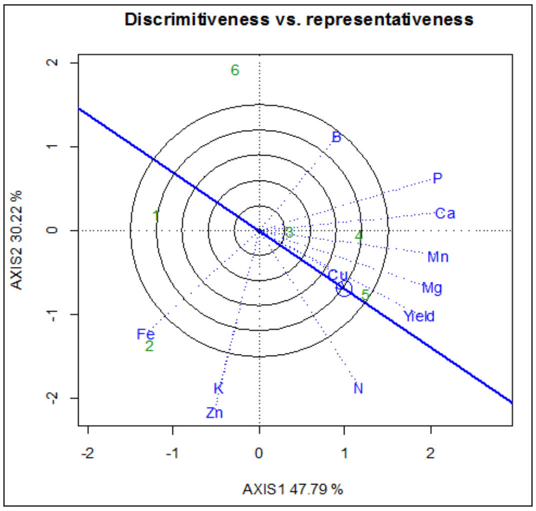

Figure 3. Discrimitiveness and representativeness of ear leaves element

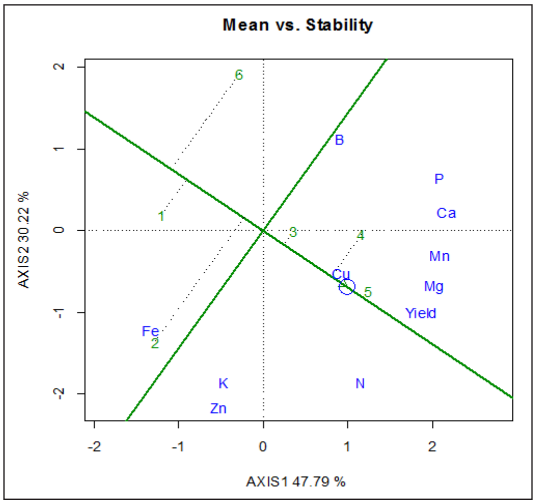

Figure 4. Stability of NOTs and ear leaves element of maize

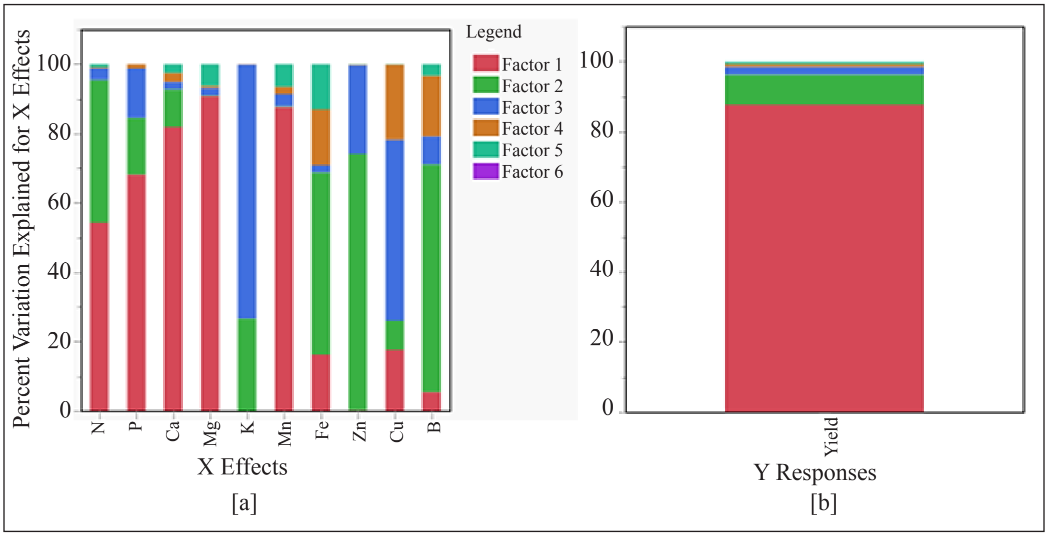

Figure 5. Variation of grain yield explained by the ear leaves element of maize

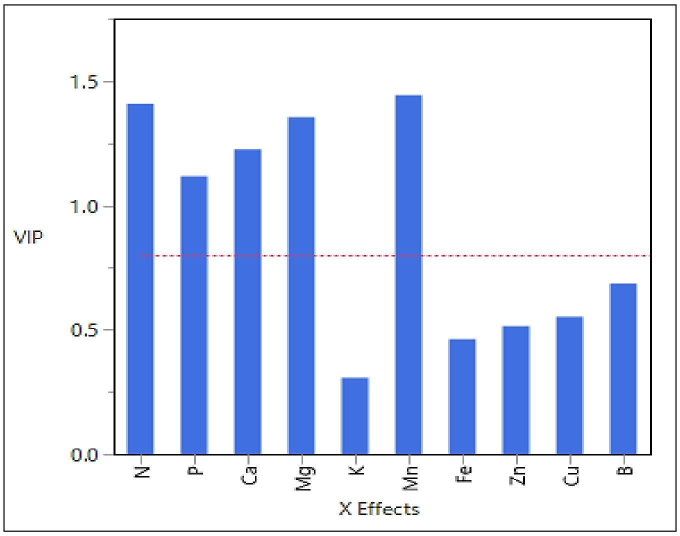

Figure 6. Variable important plot for ear leaves element in relation to yield

DISCUSSION

The high sand content in the locations is attributed to the parent material as the soils were developed largely on deeply pre-Cambrian basement complex rocks such as sandstone. The low O, C, N, and P could be related to the inherent low status as the parent materials where dominated by a low activity clays such as Kaolinite and complete removal of plant residue materials by the farmers in the study locations (Manu, Bationo, & Geiger, 1991; Shehu, Jibrin, & Samndi, 2015). Low Ca in Karu, Kokona, and Zango indicates the potential development of Ca deficiency. Moderate K in Kokona, Zango, and Tsafe have been attributed to the appreciable amount of K-bearing feldspar minerals in the sand and silt fractions in the northern Nigerian Savanna soils (Møberg & Esu, 1991) and/or residual effect of past K application through NPK fertilizers.

The variability in grain yield was majorly explained by the treatment structure because of the high signal/noise ratio. The signal here includes the combined effect of variance component in the model with the exception of residual variance which is the noise component. Also, the signal/noise ratio was high for N and P and the environment contributed a higher percentage. For K, the environment accounted for most of the variability observed not the treatment effect per se. This is an indication that there is a high level of K variability in the environment and the environment, not the treatment was responsible for observed differences of K in the ear leaves. The environment can be further inferred to be highly variable for N, P, and K and it is responsible for the differences in these elements in the ear leaves. For Ca and Mg, high signal/noise ratio was observed with a larger proportion of the environment contributing the signal effect.

The signal/noise ratio in the micro elements of maize ear leaf was also high except for Fe and Zn which were having a very low signal/noise ratio and may be as a result of high insolubility of Fe and Zn. For Cu and B, the environment contributed majorly to the variability because plants respond to Cu deficiency or limitation by increasing Cu uptake and, where possible, switching to non-Cu-requiring protein.

GGE biplot was used in this study to determine the association of the ear leaves element with grain yield of maize, identifying the elements that could represent and discriminate grain yield, determine possible treatments that could increase grain yield and finally understand the stability of the nutrient omission treatments. Some elements such as Cu, Mg, Mn, and N showed a positive association with grain yield. Among these elements, Cu and Mg were majorly associated with the application of NPK + S-Ca-Mg-Zn-B followed by NPK which also have a high association with Mn. Application of NP had shown positive association to Cu, Mg, and Mn but not as high as the two NPK treatments. Kayode and Agboola (1985) reported that in addition to NPK, Mg and Cu were necessary for high yield of maize and in some rare cases the inclusion of Fe and Zn to NPK might be required. However, in this study, the high response of Fe and Zn was majorly from the control plots and both elements together with the control treatments were highly unstable and show no association with grain yield. Cu was the most stable elements while Mg and Mn were found to be the highly representative elements of ear leaves for all the treatments and they could discriminate among the treatments. The most stable treatment across the environments and in terms of ear leaves element was NPK + S-Ca-Mg-Zn-B followed by NP.

As a confirmatory analysis, the partial least square regression was done and six factors were identified as important factors. Factor 1 accounted for about 90% of the variability observed and can be used alone to explain the variability in grain yield. The most important elements in factor 1 were Mn, Mg, N, Ca, and P. These components of the ear leaves can be used in predicting grain yield of maize. Mn is very important for photosynthesis, pollen germination, and pollen tube growth. It is also an activator or cofactor for more than 30 other enzymes in plants (Millaleo, Reyes-Diaz, Ivanov, Mora, & Alberdi, 2010). Mg deficiency directly limits photosynthesis and causes leaf chlorosis that is aggravated by high light due to the production of reactive oxygen species (Shaul, 2002).

Generally, from the study, the environment has demonstrated to be a major determinant of ear leaves elements in maize. There is, therefore, a need for accurate envirotyping of maize producing regions in Nigeria in other to classify the environments base on the availability of nutrients that have demonstrated a strong association with grain yield through ear leaves and are major determinants of yield. Phenomics will always depend on the accurate envirotyping and without accurate envirotyping, phenomics will be meaningless. Enviryotypic data can also be used in environmental characterization, genotype by environment interaction analysis, predicting plant phenotype under variable environments, construction of near-iso-environment, precision agriculture, and breeding (Xu, 2016). In some parts of Africa, selection of the trial sites that are best suitable for different stresses such as drought, low nitrogen, low pH, stem borer, and Striga has been done (Xu, 2016). Bänziger, Setimela, Hodson and Vivek (2006) identified eight maize mega-environments in South Africa using maximum temperature, season precipitation, and subsoil pH. For the US corn-belt target population of environments, Cooper et al. (2014) identified typical temporal modes of environmental variation for the soil–plant water balance.

CONCLUSION

As much as we are interested in increasing grain yield on maize through the nutrient application, this study demonstrated that the environment should be greatly put into consideration as the phenotype is the sum total of genotype + environment. If the environmental effect is high, the observed phenotype will be only a reflection of the environment rather than the genotype (treatment). To increase the applicability of ear leaves in predicting grain yield, the environment needs to be well understood.

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APPENDIX

Supplementary Table 1
Coordinates of sites used for the experiments

S/N	State	LGA	Longitude (N)	Latitude (E)
1	Kaduna	Zango Kataf	9.74969	8.39246
2	Kaduna	Zango Kataf	9.75623	8.37620
3	Kaduna	Zango Kataf	9.75573	8.37736
4	Kaduna	Zango Kataf	9.75487	8.37769
5	Kaduna	Zango Kataf	9.75802	8.36775
6	Nasarawa	Karu	9.17998	7.87595
7	Nasarawa	Karu	9.18196	7.87068
8	Nasarawa	Karu	9.17766	7.91596
9	Nasarawa	Karu	9.17055	7.86985
10	Nasarawa	Karu	9.12460	7.94194
11	Nasarawa	Karu	9.11256	07.9358
12	Nasarawa	Karu	9.13739	7.94287
13	Nasarawa	Karu	9.14620	7.94759
14	Nasarawa	Karu	9.03674	7.91573
15	Nasarawa	Karu	9.03813	7.91309
16	Nasarawa	Kokona	8.8401	8.00863
17	Nasarawa	Kokona	8.85228	7.99768
18	Nasarawa	Kokona	8.84577	7.99223
19	Nasarawa	Kokona	8.84382	7.99171
20	Nasarawa	Kokona	8.83967	7.98729
21	Nasarawa	Kokona	8.83965	8.00084
22	Nasarawa	Kokona	8.83650	8.00208
23	Nasarawa	Kokona	8.83571	8.00152
24	Nasarawa	Kokona	8.83821	7.98671
25	Nasarawa	Kokona	8.83911	8.01819
26	Zamfara	Tsafe	12.02667	6.88916
27	Zamfara	Tsafe	12.02775	6.88679
28	Zamfara	Tsafe	12.02656	6.88577
29	Zamfara	Tsafe	12.02842	6.89183
30	Zamfara	Tsafe	12.04519	6.88357
31	Zamfara	Tsafe	12.04295	6.88122
32	Zamfara	Tsafe	12.03496	6.87785
33	Zamfara	Tsafe	12.03779	6.87315
34	Zamfara	Tsafe	12.04519	6.88357
35	Zamfara	Tsafe	12.03632	6.87102