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# Multiple nutrient imbalances in ear leaves of on-farm unfertilized maize in eastern and southern Africa

H. Høgh-Jensen<sup>1, 2\*</sup>, D. Kamalongo<sup>3</sup>, F. A. Myaka<sup>4</sup> and J. J. Adu-Gyanfi<sup>5</sup>

<sup>1</sup> Department of Policy Analysis, National Environmental Research Institute, Aarhus University, Frederiksborgvej 399, DK-4000 Roskilde, Denmark.

<sup>2</sup> Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Højbakkegaard Allé 9, DK-2630 Taastrup, Denmark.

<sup>3</sup> Chitedze Agricultural Research Station, P.O.Box 158 Lilongwe, Malawi.

<sup>4</sup> Ilonga Agricultural Research Institute, P.O.Box 33, Kilosa, Morogoro, Tanzania.

<sup>5</sup> International Atomic Energy Agency (IAEA), Wagramer Strasse 5, A-1400, Vienna, Austria.

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**Maize is a major food crop in eastern and southern Africa and is often cultivated with insufficient supplies of nutrients. The current studies are aimed at diagnosing any potential nutrient limitation in maize under farmers' conditions using the nutrient content in ear leaf tissue at tasseling stage. The majority of the maize crops were considered below the critical nitrogen (N) content in the ear leaves with mean N content of 1.84%. Phosphorus (P), B and Cu content appeared critically low at three of the four sites. Unique fingerprinting by multivariate statistics was possible for each site when using the proportion of nutrients in the leaf tissue. The content of six macro elements (N, P, K, S, Mg, Ca) at the tasseling stage were found to be able to predict 83% of the variation in the grain yield at maturity whereas the inclusion of micronutrients only improved this prediction with an additional 3%. N alone predicted 50% of the variation in grain yield and the N supply of the maize crops in eastern and southern Africa must thus be the first priority before yields can be improved.**

**Key words:** Maize ear leaves, foliar tissue diagnosis, nutrient deficiencies, element interactions.

## INTRODUCTION

Maize (*Zea mays* L.) is of great importance in the household diet of African smallholders where humans consume 95% of maize production (McCann, 2005; Miracle, 1965) and it plays also an important role in household cash income (Mergeai et al., 2001). However, in large parts of the sub-continent, smallholder agricultural production has remained consistently low and food security is poor (Kumwenda, 1998; Sanchez, 2002).

It is well documented that soil fertility in African cultivated soils is declining but most attention has been focused on carbon (C), nitrogen (N), phosphorus (P) and to some extent also potassium (K) (Stoorvogel et al., 1993). Recently also sulphur (S) deficiencies has been recognized (Weil and Mughogho, 2000). As early as 1964, Bolle-Jones reported S deficiencies in maize on sandy ferrallitic soils in Malawi whereas at this time S de-

iciencies were not acknowledged in Tanzania (Bolle-Jones, 1964). Recently, Weil and Mughogho (2000) reported widespread S deficiencies in maize from farmers' fields in Malawi. Weil and Mughogho (2000) found only one report of actual crop responses to S (Shenkalwa, 1986; Weil and Mughogho, 2000) in a review but out of a very few studies. A very recent study (Nziguheba et al., 2008) used the DRIS approach but under controlled station conditions.

Despite a frequently stated lack of soil fertility (Sanchez, 2002) there is a severe lack of reliable knowledge regarding diagnosis practices and approaches under these conditions. Approaches to diagnosis of foliar nutrient status include the critical value approach (CVA), which assumes that one nutrient can be assessed individually from foliar analytical data (Bates, 1971; Reuter and Robinson, 1997). This approach was advanced to include information of interactive nutrients in the Diagnosis and Recommendation Integrated Systems (DRIS) (Walworth and Sumner, 1987) and later the Compositional Nutrient

\*Corresponding author: E-mail: [hhj@dmu.dk](mailto:hhj@dmu.dk)

Diagnosis (CND) (Parent and Dafir, 1992). The term “interactive” covers here process, which may not all be well understood physiologically but nevertheless have been established empirically, like the K-Ca antagonism (Marschner, 1986), the K-Na substitution (Høgh-Jensen, 2003), and the P-Mg competition (Marschner, 1986; Walworth and Sumner, 1987).

CND builds like DRIS on a high-yielding subpopulation, something that is not always feasible under field conditions. Magallanes-Quintanar et al. (2006) have earlier used a multivariate diagnosis approach on maize in Mexico but knowledge is lacking regarding the critical levels in the organ diagnosed as well on the approaches, for example, single versus multiple nutrients versus nutrient ratios under realistic and local field conditions.

The objective of this study was to know which nutrients was determining the grain yields under low-to-no input conditions on farmers' fields in eastern Africa. The tasseling growth stage was chosen because nutrient uptake from the soil is largely finished at this time while leaves still were physiological active. The low-to-no input approach was chosen in order to avoid potential biases that high-input approach, like DRIS, may have.

## MATERIALS AND METHODS

### Experimental areas and participating farmers

The following four study areas were selected: In Tanzania, the Babati district of the Manyara Region (04°14 S, 35°35 E) and the Gairo Division of the Kilosa District of the Morogoro Region (06°13 S, 36°53 E) were selected in Tanzania. In Malawi, Nyambi (14°39 S, 35°35 E) and Ntonda (15°53 S, 34°57 E) Extension Planning

Areas were selected. The Nyambi EPA is located within the Kawinga Rural Development Projects (RDPs) and the Liwonde Agricultural Development Division (ADD). The Ntonda EPA is located within the Blantyre Shire highlands RDP and the Blantyre ADD. Totally 87 farmers participated, equally distributed between the four trial sites. The approach can be described as four clusters of trials, each encompassing 3 - 4 adjoining villages.

The soils of Babati and Ntonda are classified as ferrasols, Gairo as ferralic cambisols, and Nyambi as cambisols according to FAO/UNESCO (1990). The locations in Tanzania have a bimodal rainfall pattern with onset in November or December. For the Malawi locations, the rainfall pattern is uni-modal with onset in November or December. These four areas were similar in terms of very low fertilizer use.

### Plant material

A recommended maize variety for each area was used. In Gairo, a long duration and open pollinated maize variety “*Staha*” was used while in Babati an open pollinated variety “*Kilima*” was used. In Malawi, a hybrid maize variety “*SC 627*” was used at all sites. “*SC 627*” is recommended for its wide adaptability, intermediate maturity and tolerance to major maize foliar diseases like Grey Leaf Spot. Confounding between variety and country were non-significant ( $p > 0.05$ ) when using site as blocks.

### Crop management

Farmers managed the experimental plots but the extension agents

or technicians influenced the planting patterns. Research technicians collected all data. Each farmer planted a pure stand of maize in non-replicated plots of 10 × 10 m. Maize rows were spaced 90 cm apart. Within the rows, the recommended spacing was 60 cm between the planting stations.

### Sampling and analysis

Soils from the experimental areas were sampled just before the onset of the rains of the planting season. Twenty samples were taken with soil augers (diameter of 2 cm) diagonally across all plots to a depth of 0.15 m, bulked and analysed for total C and total N, using an elemental analyzer (ThermoQuest S.p.A., Milano, Italy). The inorganic N content was determined by shaking the soil for one hour with 2 M KCl before filtering the solution. The  $\text{NO}_3^-$  (Henriksen and Selmer-Olsen, 1970) and  $\text{NH}_4^+$  concentrations (Krom, 1980) were determined spectrophotometrically (FIAstar 5020, Tecator, Helsingborg, Sweden).

Ten maize ear leaves were collected diagonally across each plot at the time of maize tasseling. All samples were dried at 60°C to constant weight and stored. After grinding in a titanium-coated mill (SuperMill 1500, Newport Scientific Europe Ltd., Macclesfield, UK), sub-samples of the plant materials was digested in an open vessel system using 70 ml HD polyethylene vials (Capitol Vial, Fulton Ville, NY, USA) and a graphite-heating block (Mod Block, CPI International, Amsterdam, Holland). In brief, the plant materials were first digested for 15 min in 35%  $\text{HNO}_3$  at 95°C. After cooling additional 70%  $\text{HNO}_3$  was added together with  $\text{H}_2\text{O}_2$ . During digestion, the vials were covered with HD-PE watch glasses. Samples were cooled overnight and diluted to 50 ml with ultra pure water. After appropriate dilution, the samples were analyzed by ICP-MS (Agilent 7500c, Agilent Technologies, Manchester, UK) for the following twelve: B, Ca, Cr, Cu, K, Mg, Mn, Mo, Na, Ni, P, and S (Rodushkin and Huhtassari, 1999). Zn was excluded due to pollution of part to the samples by using non-titanium coated grinding equipment. Total N content of the plant material was analysed using an elemental analyzer (ThermoQuest S.p.A., Milano, Italy).

### Statistics

A uni-variance analysis was carried out on the data using the GLM procedure of the SAS software (SAS Institute Inc. 1993). Mean comparisons for the individual treatments were done using a Waller-Duncan *t*-test.

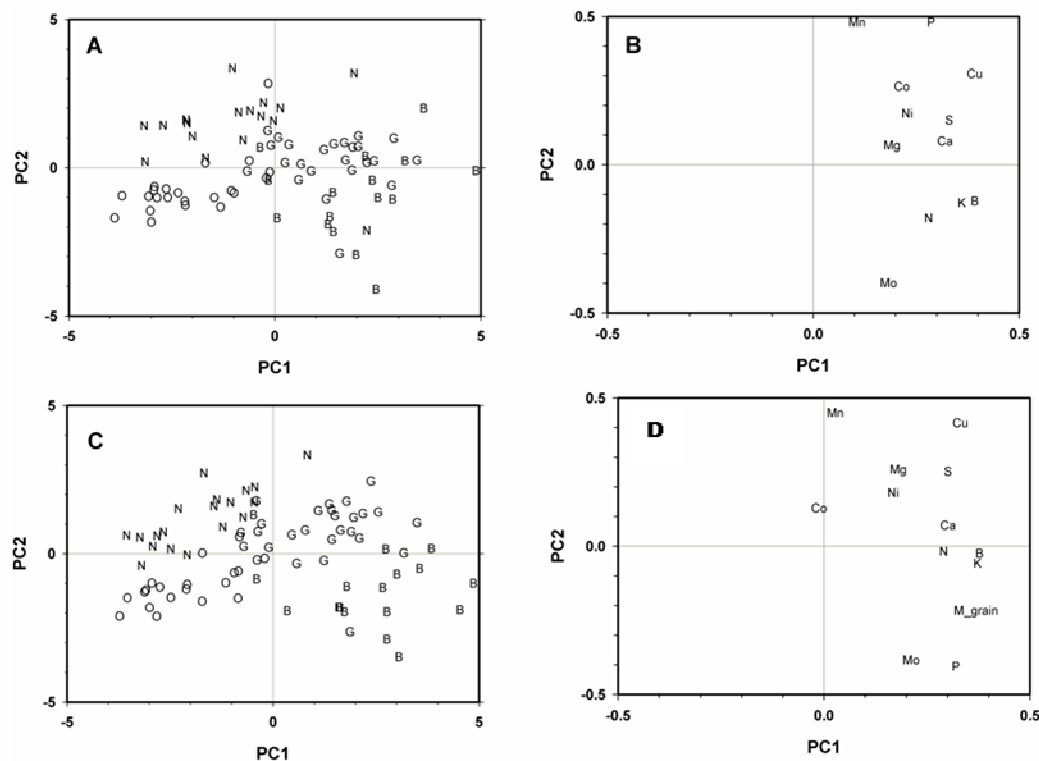
Multi-variance analysis was conducted using the Unscrambler® (CAMO Process A/S, version 9.2, Oslo, Norway) for principal component analysis (PCA) and classification on mean-centred and normalised variance data. PCA models were validated using cross-validation by the segment or the leave-one-out procedure when appropriate. Suspected outliers were identified based on both their position in the Score-plots and on their leverage and residual values (Esbensen, 2002).

## RESULTS

### Grain yields and soil characteristics

Grain yields varied ( $p < 0.05$ ) between sites with 4880, 2280, 1270, and 690 kg DM  $\text{ha}^{-1}$  at Babati, Gairo, Ntonda and Nyambi, respectively. This ranking followed the total N content of the upper 0.15 m soil layer were 1.03, 0.79, 0.77, and 0.64 g  $\text{kg}^{-1}$  dry soil for Babati, Gairo, Ntonda and Nyambi, respectively.

The C:N ratio for Babati and Gairo were approximately



**Figure 1.** Score and loadings plots from principal component (PC) analysis for all 80 samples showing maize ear leaf elements content (B, Ca, Co, Cu, K, Mg, Mn, Mo, N, Ni, P, and S) at the tasseling stage expressed in concentration of dry matter. The score plot is depicted with the identification of site of cultivation (G, Gairo; B, Babati; N, Nyambi; O, Ntonda) and without (A and B) or with (C and D) grain yield at maturity.

12.5 but the ratio was approximately 15.1 for Ntonda and Nyambi. The soil content of  $\text{NaHCO}_3$ -extractable P (Olsen et al., 1954) was on average 31.4, 5.4, 31.3, and 12.7  $\text{mg kg}^{-1}$  dry soil for Babati, Gairo, Ntonda and Nyambi, respectively. The soil pH and electric conductivity ranged from pH 5.7 to 6.4 and 1.2 to 2.4  $\text{dS m}^{-1}$ , respectively.

### Proportion and ratios of elements in maize ear leaves

A uni-variance of analysis revealed that the concentration of all elements except Co and Al declined ( $P < 0.05$ ) in maize ear leaves when going from Babati in Tanzania to Nyambi in Malawi (Table 1). Al were solely included as an indicator of soil or soil dust pollution but the concentrations were in all cases very low (Table 1). The importance of the differences in Co concentrations is minor and will not be discussed further in this context. The N content in the ear leaves explained 51% of the variation in grain yield at maturity. When calculating the ratios of N versus the other nutrients, P and K seemed particular low in Nyambi while S seemed high only in Nyambi (Table 2).

The experimental approach by having trial farmers in clusters that were identifiable as villages in administrative and physical terms could mean that soil fertilities differed

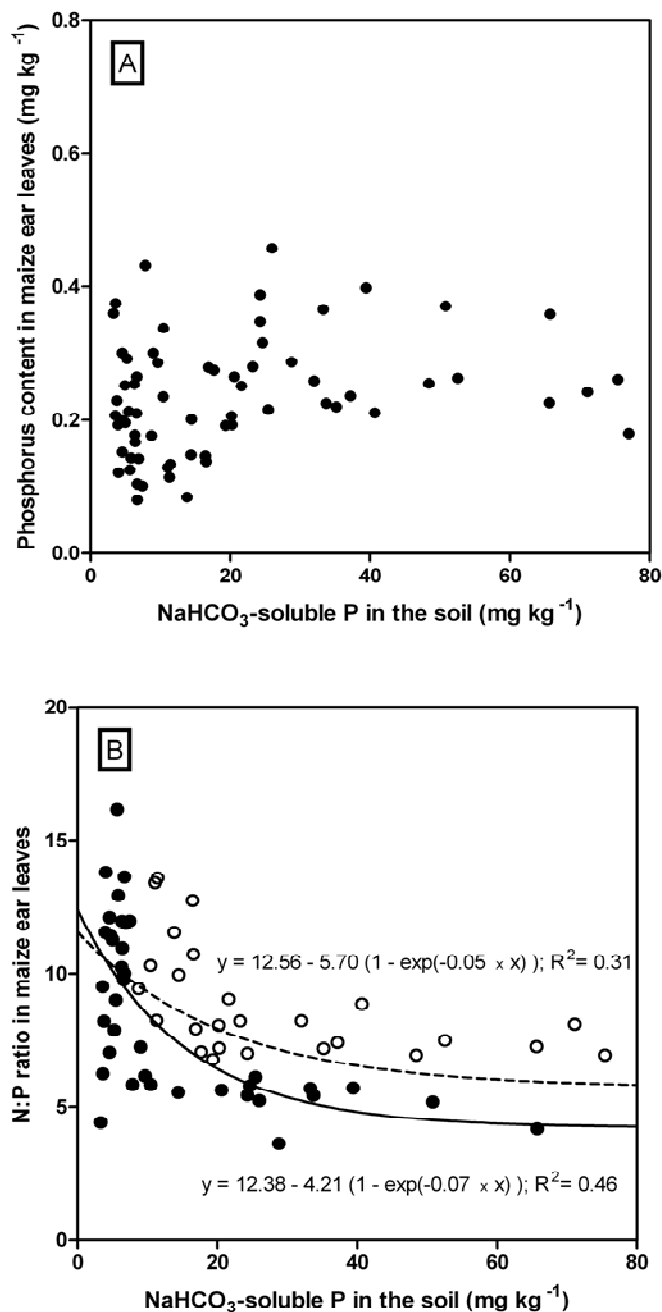
between these clusters. However, both in terms of the concentrations of the elements and in terms of the N-other nutrient ratios (data not shown) no clear distinction could be made between the villages within each site.

### Multivariate and correlations

A principal component analysis (PCA) of maize ear leaves sampled from 80 plots across Malawi and Tanzania with 12 variables containing the analyzed element content were computed (Figure 1). The principal component (PC) numbers 1, 2 and 3 explained 37, 17 and 13%, respectively, of the total variance in the data but it took 6 PC's to explain 80% of the total variance. Contributing factors to this low explanation may be errors due to sampling or the analytical uncertainty related to several of the micro elements. Clustering in the data matrix of 80 plots x 12 elements were nevertheless investigated.

The PCAs present the data in loadings, which represents variables, and scores, representing the organizing entity for samples. The organizing entities for the samples were sites with clear separate clusters

(Figure 1A and 1B). The first principal component (PC1) separated Babati based on N, K and B in particular while PC2 was the main separator of Nyambi (low P, high



**Figure 2.** Critical phosphorus content boundaries (A) and critical ratio (nitrogen:phosphorus) in maize ear leaf at tasseling stage (B). Broken regression line include all data (○, ●) while full line only include the boundary data (●).

high Mn) and Babati (high P, low Mn). PC3 indicated that Nyambi also were associated with higher contents of micronutrients like Cu, Co and Ni.

A principal component analysis (PCA) of maize ear leaves' elemental content which further included the grain yield at harvest did not add more explanatory power as PC 1, 2 and 3 explained 38, 16 and 11%, respectively, of the total variance in the data and it took still 6 PC's to

explain 80% of the total variance (Figure 1C and 1D). The PC1 related Babati with higher grain yield.

A multiple regression (PLS) demonstrated that the elements content in the ear leaves at the tasseling stage could predict grain yield at harvest ( $r^2 = 0.86$ ) quite accurately. If only the macro elements (N, P, K, Ca, S, and Mg) were included in the regression, still 83% of the variance in grain yield at harvest could be predicted based on the concentrations of the elements in the ear leaves dry matter ( $r^2 = 0.83$ ).

Following the general importance of P supply for plant growth at these sites, the relation between the P content in the ear leaves and NaHCO<sub>3</sub>-soluble P in the soil was tested. Using the boundary-line approach described by Walworth et al. (1986), no critical limits of NaHCO<sub>3</sub>-soluble P kg<sup>-1</sup> soil could be identified (Figure 2A) despite some very low P concentrations, that is, frequently < 0.2 mg kg<sup>-1</sup> dry matter. Possibly this can be ascribed to the N limitation of the maize crop. Thus, a plot of the N:P ratio versus the NaHCO<sub>3</sub>-soluble P in the soil using the boundary-line approach indicate that a critical limit of approximately 10 mg NaHCO<sub>3</sub>-soluble P kg<sup>-1</sup> soil may exist (Figure 2B).

## DISCUSSION

The hypothesis tested in the current study was if multi-element analysis of maize ear leaves at tasseling growth stage can be used in diagnosis of nutrient deficiencies affecting maize grain yield at maturity under field conditions without reference values from high-external-input sub-populations.

The relevance for such approaches is that smallholder agricultural production has remained consistently low and food security poor in large parts of the sub-continent (Kumwenda, 1998; Sanchez, 2002) as well as the low use of fertilizers in Sub-Saharan Africa (Kherallah et al., 2002). The current testing took place under farmers' conditions because the average use of fertiliser is as low as 9 kg ha<sup>-1</sup> in the sub-continent. None of the participating farmers used fertilizers of any kind. Thus, it is important to understand the likelihood of the crops to respond to additional supplementation of nutrients under the yield responses normal for resource-poor farmers in eastern and southern Africa.

## Concentrations versus ratios

Nitrogen, P and S are the most clearly related to the nutrient status of the crops (Ulrich, 1952) and they are integral components of virtually all biochemical compounds that make life possible and few options appear to exist to diminish the crop requirements substantially for specific elements (Sinclair and Vadez, 2002). Thus without supplementation in organic or inorganic form at soils of poor fertility, crop yields will remain low. The soils tested in the current study varied substantially in fertility and in the

**Table 1.** Contents of elements (g kg<sup>-1</sup> DM) in maize ear leaf at tasseling stage. Data is showing mean (n=18-22) where different notations indicate statistical difference on 5% level.

	<b>N</b>	<b>P</b>	<b>S</b>	<b>K</b>	<b>Ca</b>	<b>Mg</b>	<b>Mn</b>	<b>Cu</b>	<b>Ni</b>	<b>B</b>	<b>Mo</b>	<b>Co</b>	<b>Al</b>
<b>Babati</b>	20.3a	3.50a	1.73a	26.2a	5.47a	4.17a	4.35a	1.15a	0.49a	1.20a	0.32a	0.06a	14.8a
<b>Gairo</b>	20.5a	2.54b	1.77a	24.4a	4.55b	2.40b	5.87a	1.34b	0.54ab	1.15a	0.07b	0.06a	13.6a
<b>Ntonda</b>	18.1b	2.27b	1.56b	19.2b	4.55b	2.35b	2.72a	0.68c	0.23c	na	0.03b	0.03ab	7.8a
<b>Nyambi</b>	13.6c	1.43c	1.47b	17.2b	3.72c	2.08b	10.8b	1.11b	0.37ac	0.00b	0.06b	0.10c	24.2b

na - the sample values were too low to measure.

**Table 2.** Ration of elements against the nitrogen content (nitrogen:element) in maize ear leaf at tasseling stage. Data is showing mean (n=18-22) where different notations indicate statistical difference on 5% level. The ratios of nitrogen versus micronutrients are divided by a factor 10<sup>6</sup>.

	<b>N:P</b>	<b>N:S</b>	<b>N:K</b>	<b>N:Ca</b>	<b>N:Mg</b>	<b>N:Mn</b>	<b>N:Cu</b>	<b>N:Ni</b>	<b>N:B</b>	<b>N:Mo</b>	<b>N:Co</b>	<b>N:Al</b>
<b>Babati</b>	6.01a	12.0a	0.82ab	3.97a	9.40a	5.76a	19.5a	47.5a	62a	121a	407a	1.61a
<b>Gairo</b>	8.51b	11.8a	0.87a	4.66ab	5.22c	3.79b	15.4b	47.7a	23a	676b	403a	1.77a
<b>Ntonda</b>	8.03b	12.8a	1.07c	5.21b	8.10ab	7.07a	26.9c	108b	na	762b	621b	2.51b
<b>Nyambi</b>	10.2c	8.7b	0.69b	3.04c	6.62bc	1.55c	12.8b	42.1a	1065b	281a	270c	0.85c

na - the sample values were too low to measure.

average harvested crop yield.

Nutrient interactions may however alter crop responses to environmental alterations like supply of nutrients. Ideally, foliar diagnosis can provide information that may allow managers to make intelligent decisions and recommendations based on perceptions of imbalances, insufficiencies and excesses. However, foliar diagnoses do not make it possible to quantify the amounts needed to alleviate such conditions (Walworth and Sumner, 1987).

Based on the concentrations in the dry matter of the maize ear leaves (Table 1 - 2), P and K were found to be low but S high in Nyambi. This may indicate that S may be of importance at the weathered leached soils that Ntonda represents. Weil and Mughogho (2000) concluded that more than 5 kg S ha<sup>-1</sup> would be sufficient to for maximum response by at maize yields < 6 mg ha<sup>-1</sup>, which is just the conditions this study was conducted under. Multivariate statistical analysis indicated a negative correlation between the P content in the maize ear leaves and Mn (Figure 1A and 1B) in agreement with a hypothesis that a better supply of P will reduce the allocation to the root systems and thus reduce Mn uptake. The correlation coefficient (r<sup>2</sup>) was however only -0.32. Phosphorus and K showed a positive interaction with an r<sup>2</sup> of 0.93, which confirm the observation by Magallanes-Quintanar et al. (2006). The commonly observed K-Ca antagonism was however very weak (r<sup>2</sup> = 0.09).

The concentration of elements in the dry matter of foliar is critically dependant on the physiological age of the sampled tissue (Reuter and Robinson, 1997). Using tasseling is considered a well-defined physiological age although variations must be expected. Using the ratios of

the concentrations of N versus other elements in turn is relative to the dry matter and more constant with age whereby we eliminate some of the error associated with variation in age and sampling.

The ratio of N:S in the maize ear leaf gave in an earlier study a good prediction of maize yield in a study on farmers' fields (Weil and Mughogho, 2000). The current study does not conform to this (Table 2). A multiple regression demonstrated that in addition to N, P and S (Ulrich, 1952) also K, Ca and Mg should be included in foliar diagnostic approaches of maize crops under such environmental conditions.

The critical content of NaHCO<sub>3</sub>-soluble P in the soil for the crop means that a reliable leaf diagnosis can be done at in maize ear leaves at tasseling stage (Figure 2B) when using N:P ratios. The boundary effect must however be interpreted with some care due to the lack of sufficient data to enable a more correct statistical analysis (Shatar and McBratney, 2004).

### Tissue contents of elements at an early growth stage versus grain yield

The tissue N contents at tasseling were normal distributed around a mean of 1.83% ranging between (±sd) 0.98 and 2.66%. This is however below the critical values of 3.1 - 3.6% (Reuter and Robinson, 1997) although Forde, (1976), Hanway and Dumenil (1965) report that values could go as low as 2.2% before criticality arose. Based on values reported by Reuter and Robinson (1997) the tissue content of P must be considered critical except for the Babati site, whereas S, K, Ca, and Mg (the later except for Nyambi) is considered sufficient for maize

growth at some sites. The Cu content was however only 25% of the critical level and Mo was also very low, with the exception of Babati (Table 1). The inclusion of these last nutrients did however only contribute little (3%) to the prediction of grain yield at maturity.

## Conclusions

The N content of the ear leaf at tasselling stage of maize grown under field conditions with a low supply of nutrients explained 51% of the variation in grain yield, which comply with a critical low N content in the ear flag leaves. However, the content of the other single nutrients could not be used to diagnose deficiencies. Nevertheless, inclusion of N, P, K, Ca, S and Mg in a multi-element approach enabled a good (83%) prediction of grain yield at maturity. Micronutrients that appeared to be at critical low levels influenced only marginally the grain yield at maturity. Thus, a multiple regressions approach captures the interactions between nutrients when using the major elements of tissue at an early growth stage to identify the limiting factors for final crop yield. In conclusion, before improving the N supply of the maize crops in eastern and southern Africa there is no need to worry about other element deficiencies.

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