Africa Soil Information Service

Diagnostic Trials
A field Guide

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This is an AFSIS publication prepared by the team of objective 4 that deals with spatial explicit and evidence based soil management recommendations. The project is implemented by International Centre for Tropical Agriculture (CIAT) under the Tropical Soil Biology and Fertility (TSBF) research area. The main partners in the project are ICRAF (World Agroforestry Centre), Earth Institute of Columbia University and ISRIC World Soil Information centre. The project is funded by the Bill and Melinda Gates Foundation (BMGF) and the Alliance for a Green Revolution in Africa (AGRA). The activities of Objective Four are implemented in 5 target countries, viz. Mali, Nigeria, Malawi, Tanzania and Kenya through its National partners, IER in Mali, IAR in Nigeria, Bunda College, DARS and DAES in Malawi, SARI in Tanzania and KARI in Kenya.

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Preface

The 'diagnostic trials' field guide provides a standard tool that is part of a structured approach for the diagnosis of soil health related constraints to crop production. It is intended for use by the national and international agricultural research systems, development partners and extensions services to ensure standard procedures in data collection that will feed to an Africa-wide database of diagnostic trials, allowing an increase in data density over time and an improvement of the reliability in the assessment of soil constraints and inferences. We hope that the concept of diagnostic trials will be adopted by a large number of institutions across Sub Saharan Africa and that they use this instruction manual for the implementation and execution of diagnostic trials and in this way that they contribute to an African database, that will enhance exchange of information and that will also allow for comparative analyses of the results.

This field guide describes the protocol as it is used in the Africa Soil information Service (AFSIS) project for diagnostic trials and provides guidelines for any other initiatives to conduct diagnostic trials in SSA. For AFSIS the trials are designed as a multi-locational setup of diagnostic trials. That does not mean that the protocol cannot be used for single or individual trials. Similarly, the treatments can also be adjusted; referring to the number of treatments included in the design as well the nature of the treatments. However, if the aim is to contribute to the African database any change in the design and treatments needs to be well documented. Thus, the diagnostic trials are intended as a flexible tool. For that purpose the field guide mentions also the considerations that lead to the choices made regarding the design of the diagnostic trials, besides just describing the protocol itself. And it is indicated where adjustments to the design and protocol can be made.

AFSIS is a collaborative effort of a number of institutions. For the further development of the diagnostic trials as a comprehensive tool for the diagnoses of soil health constraints and the distribution of the protocol we work closely together with the International Plant Nutrition Institute (IPNI). We appended 'Be your own maize doctor' to aid recognition of plant nutrient deficiencies in the field. We intend to develop a protocol for different test crops, based on the current protocol for maize. For Sorghum this implies adjustment of the plant spacing and amounts of fertilizer applied and some adjustment in the measuring protocol. This will be issued as a separate addendum to this document. Furthermore, standard prototype forms for recording field information will be made available as downloadable files.

For the development of this protocol we have consulted a number of agronomist and soil scientist who have extensive experience in conducting agronomic trials. We would like to acknowledge the input of Bernard Vanlauwe, Keith D. Shepherd and Peter Okoth.

1. Diagnostic trials: a starter

1.1. Why conduct diagnostic trials?

In order to increase the productivity of fields of smallholder farms and therefore improve food security in Sub-Saharan Africa (SSA) there is a need to identify the soil factors that constrain crop growth.

In most countries of SSA, one fertilizer recommendation applies to the whole country or a wide region. However, farmers operate under varying conditions within the agricultural landscapes of Africa and we often observe a very strong variability in yield, locally or within districts. This variability is explained largely by soil fertility gradients, either management induced or because of varying inherent soil fertility. Recognizing the wide variability in soils and management, it becomes necessary to fine tune current recommendations in order to target investments such that chance of success is improved while the risks are reduced. In order to increase the use efficiency of applied nutrients and the cost effectiveness of resource input, there is a need to target interventions whether related to soil amendments to improve the condition of the soil or to fertilizer application to address nutrient requirements. Knowing the limiting soil factors would inform about the right inputs needed.

Another cause of variation in yield is the management itself, referring to land preparation, use of improved seed, weeding operation and pest and disease management and other, and depending on the resources the farmer has available. This means that solutions need to be specific to the context of the individual farmer, to some extent. The management effects and the soil fertility status are interlocking and both need to be considered in order to explain possible yield gap that the farmer is experiencing on his fields. The farmer needs to know about the yields that can be attained on his or her fields in order to assess the yield gap and take informed decisions about investment in yield improving measures.

The diagnostic trials inform on the soil health constraints and nutrient limiting factors while it also provides information on the yields attainable under improved management. AS such it provides vital information for improving targeting of interventions for sustainable soil fertility management which is crucial to effectively address production constraints in these highly variable agricultural landscapes SSA.

The diagnostic trial guide is part of a set of tools developed by the Africa Soil Information Service[1] (AFSIS) that together allow for a comprehensive assessment of constraints to increasing productivity.

1.2. What are diagnostic trials?

The diagnostic trials are designed as nutrient omission trials to identify which of the macro-nutrients N, P and K are limiting crop growth. In nutrient omission trials, one of the nutrients is

[1] The Africa Soil Information service (AFSIS), a collaborative project led by TSBF-CIAT will attempt to diagnose the limiting soil nutrients through conducting diagnostic and integrated soil fertility management (ISFM) trials at selected sentinel sites in 5 countries in SSA, namely Mali, Malawi, Kenya, Tanzania and Nigeria. It will provide consistent, large-area mechanisms for testing the efficacy of fertilizer and soil fertility ameliorants use. The development of the tool and implementation of the diagnostic trials is part of on-going activities aimed at achievement of AFSIS objective 4 outputs and outcomes.

omitted while the others are applied at rates considered as non-limiting in all treatments. It is therefore not possible to determine the optimum nutrient applications rates[2] directly from the results from the trials.

Further treatments are added to diagnose possible other constraints related to soil chemical characteristics (e.g. acidity), availability of secondary and micro-nutrients or soil organic matter content. These treatments consist of full NPK application + soil amendments like liming or manure application. The NPK application serves to correct for possible nutrient limitations such that the 'true' effect of applying the soil amendments is observed.

Diagnostic trials are established at multiple locations to determine spatial variation, which serves two purposes: (i) the various locations where the trials are established can be considered as true replications and allow for determining the statistical variation within the sentinel site. It informs about the incidence of soil constraints within the given area, or the extent to which certain conditions are representative for the area.

More important, however, is that (ii) the replication of the trials at multiple locations allows for establishing the link between the crop response and soil characteristics, which provides the basis for predicting and mapping of crop response for the larger area where the same agro-eocological conditions apply. This is the reason why the protocol contains an operating procedure for the sampling of soils and handling of the samples. AFSIS has standard procedures for soil analyses as well, which can be found on the Web site.

1.3 Objectives of diagnostic trials

The aim of the diagnostic trials as conducted in AFSIS is to evaluate the response of crops to nutrients and ameliorants and to determine the soil factors that determine that response allowing for the diagnosis of soil health related problems. This will provide a basis for evaluating the agronomic and economic efficiency of investments in soil fertility, based on which recommendations for improved soil management will be rooted. They have the following specific objectives:

- Understand the reasons for and scope of non-responsive soils to (a) decide whether it is required to rehabilitate non-responsive soils, and (b) develop management practices required to restore productivity.
- To predict crop response to nutrient application in terms of crop growth, yield and nutrient uptake, from soil and foliar spectral and other AFSIS covariates (e.g. land degradation indices) under variable soil fertility conditions.
- To develop site-specific recommendation of ISFM practices to improve or restore soil productivity

[2] A few additional trials for nutrient response determination can be conducted alongside diagnostic trials. AFSIS has developed a set of treatments that are being used for this purpose within sentinel sites. Furthermore, data from the diagnostic trials can be used to calibrate diagnostic tools like QUEFTS (Janssen, B.H., Guiking, F.C.T., Van der Eijk, D., Smaling, E.M.A., Wolf, J. and Van Reuler, H., 1990. 'A system for quantitative evaluation of the fertility of tropical soils QUEFTS.' *Geoderma* 46: 299-318) and DRIS (Diagnosis and Recommendation Integrated System) and use these tools to predict crop response to varying fertilizer application (if N, P and K are the only limiting factors). It requires plant tissue samples to be analyzed, which is part of the protocol presented here. It is the indirect way to predict optimum fertilizer application rates.

- Establish and maintain a database of diagnostic trials' data that will allow analyses and synthesis of results from diagnostic trials across sites; that will increase data density over time allowing for improving reliability of assessments and inferences.

The data generated from diagnostic trials has wider application including (i) development rapid spectral diagnostic tests for screening soils that are responsive and non-responsive to fertilizers and for screening of nutrient limitations and chemical imbalances and physical constraints; (ii) mapping of land degradation status (including soil health) in terms of productivity decline (cost associated with yield depreciation for non- fertilized conditions and cost associated with restoring productivity) (iii) validation and improvement of crop models which are often developed on basis of national or regional scale data; and (iv) Calibration of diagnostic tools (QUEFTS and DRIS) and adapt them to predict crop response to fertilizer application under variable soil fertility conditions in sub-Saharan Africa for major food crops

1.4. Can the protocol be adjusted?

The following sections describe the standard procedures and protocols for site selection, establishment and management of the diagnostic trials as adopted by AFSIS. Choices for the specific treatments, number of trials site per sentinel site etc. were made based on consideration of relevance and efficiency, and depends on whether prior information on soil conditions is available. We have chosen to include treatments for the assessment of specific macro-nutrient limitations, based on the assumption that this may vary considerably within and between the sentinel sites. Likewise, a treatment with micro-nutrients has been included, as deficiencies of micro-nutrients are common in many soils in Africa. However, to determination of the actual micro-nutrients that are deficient in specific sites will require additional trials and adjusted treatments.

The protocol described in this document is the most practical in situations where much of the information on soil constraints is not available. One could opt for a fewer treatments, with the possible implication that the omitted treatments still need to be included in subsequent trials to obtain the specific information. The minimum set of treatments could be a 'control' and 'NPK' for example, which does not allow the diagnosis of specific nutrient deficiencies but would provide valuable information of soils that are responsive or non-responsive to fertilizers. One could subsequently conduct subsequent trials on the non-responsive soils to determine the specific character of these non-responsive soils. If this would relate to a smaller sub-set of the earlier sampled locations, gains in efficiency would be made.

In summary, yes the protocol can be adjusted. It is possible to split the trials according to treatments and phase the trials where more specific treatments are left for subsequent trials on more specific locations. It is also possible to adjust the treatments itself depending on what is expected as limiting nutrients or limiting conditions. For example, so far we did not find clear evidence of widespread K limitations, at least as far maize is concerned as a test crop, and one could consider replacing K with S in the relevant treatments if one expects S limitations to more of a problem, rather than applying S as part of a blend with other secondary or micronutrients. It also depends on the test crop being used and the nutrient requirements of that particular test crop.

Similar considerations apply to the sampling strategy. The sampling framework described in the section below is based on the framework adopted in AFSIS for the land degradation surveillance, which assumes that no prior information on soil and land characteristics is available. There are 3 scales that are considered in the trials as source of variation: field, cluster and sentinel site levels. If

prior information is available one could consider restricting the diagnostic trials to a limited number of contrasting sites. Or one could focus on the scale level that contributes most to the variation. If the clusters are the largest source of variation, the number of clusters could be increased at the expense on the number of observation per cluster. Likewise, one could consider increase the number of 'sentinel sites' at the expense of the size and number of observation per sentinel site. However, such considerations do not apply where diagnostic trial are pegged to the AFSIS sentinel sites (or where the AFSIS protocols are followed) because in those cases the same sampling procedures need to be followed in order to co-locate the diagnostic trials with the sampling points for the Land Degradation Surveillance Framework.

1. Selection of sites and fields for implementation of diagnostic trials

2.1. Sentinel sites

AFSIS has randomly selected 60 sentinel sites of 10 km x 10 km each, which are surveyed in detail and based on which soil functional properties and land degradation status are mapped for the SSA region. The sites for implementation of the diagnostic trials in AFSIS are selected from these sentinel and alternate sites, and trials are co-located with the sampling points for the land degradation surveillance so that the soil, vegetation and land use data is available for the evaluation of crop response to the various treatments. That is, it allows for evaluation of difference in response between the field trial locations. The LDSF measures ecological and soil variables at both the plot and subplot level, including woody cover, visible erosion, root depth restriction, infiltration capacity, area cultivated and soil properties. The LDSF data allows for a more in depth analyses of the variation in crop response compared to the soil properties alone that are also recorded for the diagnostic trials.

The AFSIS project has selected sentinel sites in each of the five AfSIS node countries (Mali, Malawi, Kenya, Tanzania and Nigeria) for implementation of the diagnostic trials. Information on the sites is available from the 'Diagnostic Trial Site Description', available at www.africasoils.net. We very much recommend that, if possible, existing sentinel sites are selected for implementation of the diagnostic trials – as ecological and soils data is available for these sites. See for information on the location of the sentinel sites www.africasoils.net.

Depending on the purpose additional sentinel sites may need to be established, for example if the purpose is to establish a national framework for land degradation surveillance, diagnoses of soil health constraints and soil management recommendations. In such case, sites need to be selected and the standard procedures for implementing the LDSF need to be applied. AfSIS can provide support in the selection of sites and definition of the sampling locations within the newly selected sentinel site. Information on the standard procedures for the LDSF is available at www.africasoils.net.

2.2. Suitability of sites for establishing diagnostic trials

Randomly selected sentinel sites do not automatically qualify for purpose of implementing diagnostic trials. Adoption of a site will depend on

(i) Relevance of a site for agricultural production;

(ii) Opportunities for synergies with existing initiatives (and availability of relevant data and information), and

(iii) Representativeness of national and regional agro-ecological zones.

A reconnaissance mission will confirm suitability of the site. Such a suitability assessment should focus on assessment of agricultural potential and land use intensity, biophysical problems, farm types and socio-economic constraints. At the same time reconnaissance mission is used to establish contact with the local authorities and farmers to familiarize them with the project. It is important to gain the local support for conducting trials. An assessment of the available human and physical capacity in local (or regional) stations of the national agricultural research organization for the establishment and management of the trials is also done. Further, the

candidate locations of the actual diagnostic trial fields can be identified and the assurance of engagement of the land owners obtained. During the reconnaissance, random soil samples will be taken for preliminary analysis of pH as this will be useful in determining of the need for a liming treatment. Also, it needs to be explored whether manure can be sourced locally.

Plate 1. *Scientists stop to collect a random soil sample during a reconnaissance mission in Kiberashi, Tanzania*

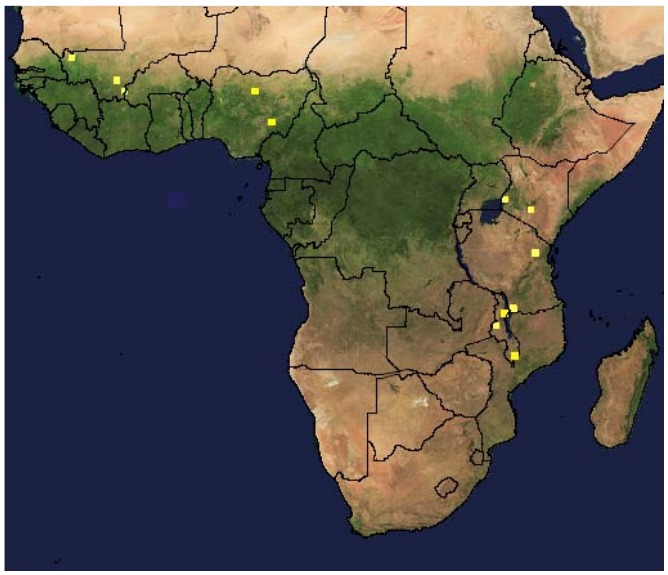


Figure 1. *Google map of the African continent with the location of the sentinel sites where diagnostic trials have been implemented*

2.3. Locations of diagnostic trials fields within a sentinel site

A sentinel site is constituted of sixteen 2.5 km by 2.5 km tiles (Figure 2). Within each tile a cluster of 10 sampling plots (white circles) are already randomly placed for the LDSF sampling, i.e., 16 clusters in total. For the diagnostic trials, 2 experimental fields are located within each of 16 clusters of a sentinel site so that responses can be related to sentinel site covariate data. Thus, a total of 32 experimental fields are expected per sentinel site. The selection of the 32 fields will follow a three-stage process to ensure close correspondence between sites for soil sampling (as part of the LDSF) and the diagnostic trials.

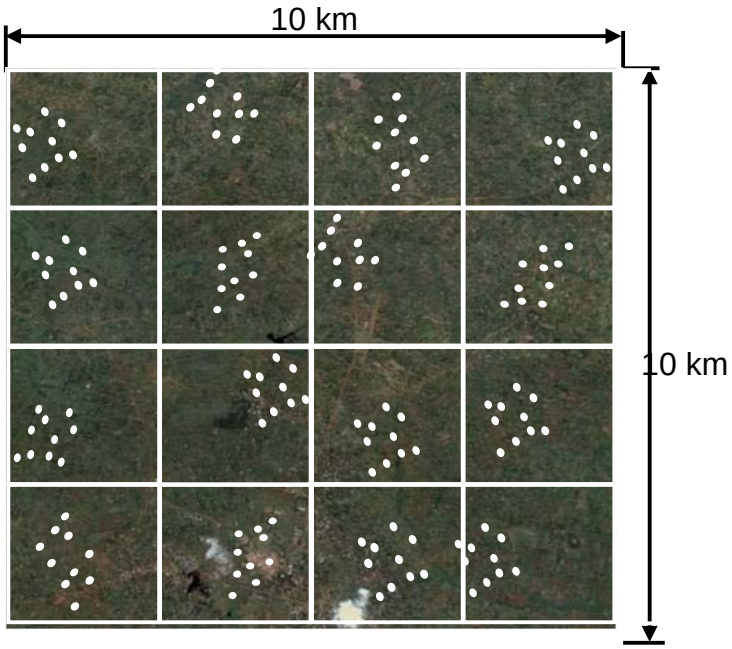


Figure 2. *Example of a sentinel site with 16 tiles (areas bounded by white line), and a cluster (10 white dots or random sampling plots) within a tile. Two fields are selected from each of the 10 random sampling plots.*

Stage 1

Within each cluster, a GPS will be used to navigate to each of the 10 sampling points and select 2 for experimental fields based on: distance from each other (the further the better); soil types (contrasting ones are preferred); position within the landscape (to represent common land forms e.g., upland and bottomland); farmer cooperation; accessibility for ease of monitoring and data collection and crop safety (protection from wild animals). The 10 sampling plots are already georeferenced.

Plate 2*. Trying to find a spot for an overview of the area*

Plate 3. Navigating to locate pre-selected fields with a GPS

Stage 2

Location of experimental fields will be limited to fields that are or have been used for arable cropping. Although priority will given to fields currently in crop production, fields that are currently under fallow (or that may have been recently abandoned) as well as fields likely to be use for cropping in the foreseeable future can be included in the sampling, provided that no major efforts are required to clear the land. If all of the 10 sampling plots within a cluster fall in land units that are not suitable (i.e., land not used for arable cropping or not currently under fallow), alternative (replacement) fields will be selected within the cluster. The selection of the alternative fields will be based on random sampling of all the potential fields, i.e., visually observe and randomly select fields by criss-crossing the cluster. Climb a hill or mast to see location of fields and better still, use local persons as these have knowledge on where arable fields are located and can serve as valuable guides (they can help to navigate to the potential and identified sites). For all the alternative fields identified, selection and re-selection will continue as in Stage 1.

Stage 3

If land within a cluster is not used for arable cropping (i.e. the above procedure will not yield enough experimental fields), closest alternative fields will be randomly selected within the 2.5 x 2.5 km tile in which the cluster falls. There may be cases where no alternative fields are available even within the 2.5 x 2.5 km tile, and in such case closest fields outside the tile can be used (i.e., have at least 3 fields in one or more of the neighbouring clusters to complement clusters without a suitable field). The total number of diagnostic trial fields for a sentinel site could be less than 32 in cases, for instance if a significant portion of the sentinel site is under forest, and it has not been possible to compensate for all clusters by additional fields.

Each field selected for diagnostic trials should be homogenous with respect to soils and management in order to ensure that responses to fertilizers are not confounded by external

factors. The complete diagnostic trial should therefore fit within an existing farmer's field. As such, the researcher can choose an alternative location off the position indicated by the GPS unit, within the farmers' field or in a neighbouring one. Reasons for such placements include ash patches due to burning of residues on-site, presence of termite mounds, localized gravel, non-similar crops (e.g., legume and a cereal) grown on a plot during the immediate past season (i.e., crops with different soil nutrient demands), different fertilization (chemical fertilizers and manure) regimes over the past season, presence of trees and tree stumps deemed to influence crop performance among other reasons.

Field selection is an important activity and should not be left to field technician. There are important decisions to be made on site that require the presence of the researcher.

The actual location of each field will be geo-referenced.

Plate 4. *Negotiating for a farmer field during field selection in Pampaida, Nigeria*

3. Experimental design

3.1. Treatments

The diagnostic trials are designed to assess the nutrients limiting crop productivity and possible effects of adding soils ameliorants such as lime to address pH constraints and manure to improve soil organic matter. They also assess the need for secondary and micro-nutrients. A modified nutrient omission design is used for the trials as follows:

Each experiment consists of six mandatory treatments and two optional treatments (Table 1). The mandatory treatments consist of:

- A 'control' with no inputs added,
- A full NPK treatment assumed to lift possible limitations in any of the macro-nutrients,
- Three omission treatments one for each macro-nutrients N, P and K, to be able to determine N, P and K limitations in the soils, and
- A NPK plus secondary and micronutrients treatment to determine whether secondary or micronutrients are limiting yields.

Two optional treatments help to diagnose other non-nutrient constraints to crop productivity. They consist of the full NPK plus either lime or manure. A treatment with lime is included if pre-analysis of soil reveals potential problems with soil acidity. As a general rule, lime will not be applied when the pH is above 5.5.

The treatment with manure is intended to determine the effects of improving soil organic matter content and should be included as treatment when low soil organic carbon concentrations are expected. The effects of manure on soil conditions that affect crop response are multiple. It is therefore not possible to directly infer conclusions on limiting soil conditions from the results of this treatment alone. This treatment plays an important role in the diagnosis procedure and it is therefore highly recommended to include the treatment. In areas where sufficient amounts of animal manure and compost are not readily available, special preparations need to be taken (see earlier comments on the reconnaissance survey).

***Table 1**. Treatments implemented in AfSIS diagnostic trials*

Treatment number	Treatment
T. 1	Control
T. 2	NPK
T. 3	PK
T. 4	NP
T. 5	NK
T. 6	NPK+CaMgSMicronutrients
T. 7	*NPK+manure (optional)*
T. 8	*NPK +lime (optional)*

Treatment responses are assessed with respect to the 'control' and the full NPK treatments. Thus, in each experimental field, treatments will be partially replicated (only the 'control' and NPK treatments will be in duplicate). Application rates for each nutrient will be constant, and will target non-limiting rates for a high yielding crop.

Nutrient application rates are provided in Table 2. The rates are defined in a way that they are not limiting for a high yielding crop in most situations.

The secondary and micro-nutrients are applied as a cocktail in ratios as indicated in Table 2. Mavuno fertilizer, which is a blend manufactured in Kenya, is used here as an example and provides nutrients in these ratios. If a blend of fertilizers, that also contains micronutrients, is commercially available its use should be considered for practical reasons. However, the amounts of the individual nutrients that are supplied in this way may not be ideal. In the paragraph below we discuss alternative solutions for the application of secondary and micronutrients. The application rates provided in the table below are a guideline. A change in the application rates can be considered as long as the rates are not limiting for the test crop and as long as the same rates are applied in all treatments and consistently over all the diagnostic trials. The diagnostic trials will then still provide valid results.

The application rate for lime (1000 kg ha^{-1}) is expected to increase the pH by about one point for sandy soils and about 1.5 units on a clay soil. Apart from increasing the pH the application of agricultural lime will add Ca and Mg. And the lime treatment could be considered to replace the Ca and Mg in the multi-nutrient treatment.

Table 2. Application rates for nutrients and amendments in maize trials

Nutrient	**Source**	**Application rate (kg ha^{-1})*[1]**
N	Urea	100
P	TSP	30*[2]
K	Potash	60
Ca, Mg, S, Micronutrients	Mavuno	10 (Ca); 5 (Mg); 5 (S); 3 (Zn; B)
Lime	Agricultural Lime	1000*[3]
Manure	Animal manure or Compost	10,000

*[1] The application rate is of the nutrient element and not the fertilizer. For example, for P the application rate is that of element P (not P_2O_5 or TSP fertilizer). The rates suggested above are for a target maize yield of about 6 t ha. For arid regions where lower maize yields or sorghum is grown, rates must be reduced by 30-50%.

*[2] On strongly P fixing soils this amount should be doubled

*3 Originally this application rates was set to 500 kg ha^{-1} in order not to effect availability of micronutrients (through immobilisation), though at the same time a low application rate would affect the availability of phosphorus and 1000 kg ha^{-1} is considered the more appropriate amount

The application rate of the manure is 10 tons per ha. The manure should be well composted, dry and sand/soil content kept to minimum. The manure can be locally available or be transported from elsewhere and may be from a different source. Most important is that the manure is analyzed such that dry matter weight and composition is known.

The table below present a solution for providing secondary and micronutrients as an alternative for the multi-nutrient treatment. The chemicals can be sourced separately and blended. The target application rates are higher than indicated in the table 2, but this is desired. Using the application rates as indicated will apply sulphur at a rate of 24 kg ha^{-1}.

As a rule of thumb, all fertilizers and amendments used should be analyzed to check their composition for the elements that serve as nutrients and for contaminants. And of course the solution for the multi-nutrient treatment should be consistently applied across the diagnostic trials and well documented such that no assumptions have to be made about the application rates upon analyses of the data

Table 3. Recommended secondary and micro-nutrients application rates

Nutrient	Source	Application rate (kg ha^{-1})	S equivalent application rate (kg ha^{-1})
Zn	Zinc Sulphate	5	2.7
B	Borax	5	0.0
Ca	CaSO4	10	8.0
Mg	MgSO4	10	13.3

3.2. Field layout

Ten plots are required to accommodate the 8 treatments and two replications (one for 'control' and one for NPK). Alternative arrangements for field layout are possible depending on the size of the agricultural field. For sites where the selected experimental field is wide enough to cover all 10 plots, 5 m x 50 m land area will be marked out in each field, and divided into 10 plots of 5 m x 5 m (see Figure 3a). This will be adjusted for sites where optional treatments are not included. The field could also be 10 m x 25 m in which case plots will be in two blocks of 5 plots each (Figure 3b). For each of the experimental fields, treatments will be allocated to the 10 plots following the order as in Table 3 below.

Figure 3a. Example of plot layout in an experimental field

1	2	3	4	5
6	7	8	9	10

***Figure 3b**. Alternative layout of the experimental field and an example sketch showing key landmarks*

For the two layouts (or other layout adopted) a fixed sequence of the treatment and plot numbers is maintained to avoid any possible confusion at later stage. The variation in soil conditions between plots should be kept at a minimum. In sloping terrain, the orientation of the field and planting rows should be perpendicular to the direction of the slope to minimize erosion within the field. In the case of 2 blocks layout, the first block should be located upslope from the second row, with a small trench separating the 2 blocks. For each field, a sketch indicating the layout of the experimental plots in relation to key features or landmarks will be drawn. Plot numbers should be clearly indicated.

Pegging of the plots is best done with tape or a rope in which every 5 meters are marked with knots. Right angles are required for all plots, and can be obtained using the rule of Pythagoras. With the measuring tape or the rope, in which 4m, 3m and 5m are marked successively, and positioning and tensioning it in one of the corners of the plots, as depicted in the figure 4 below, will give a right angle. If one side of the plot is demarcated the other side can be demarcated at 90 degrees using the procedure above, and if the first plot is demarcated the others can be easily demarcated using the measuring tape or rope.

***Figure 4**. Layout of a plot and making sure you have right angles*

The allocation of treatments to these plots should follow the order given in Table 3. This is important in ensuring data from the treatments is not mixed-up especially where no labels define plots on the ground.

***Table 3**. Randomized treatments and the plot numbers assigned to each. There are 10 plots because control and NPK are in duplicate*

Plot	Treatment / replicate	Treatment code	Treatment description
1	T. 2 R1	111a	*NPK*
2	T. 8	111L	*NPK+Lime*
3	T. 1 R1	000a	*Control*
4	T. 7	111M	*NPK+Manure*
5	T. 4	110	*NP*
6	T. 6	111X	NPK+CaMgSMicronutrients
7	T. 3	011	*PK*
8	T. 5	101	*NK*
9	T. 2 R2	111b	*NPK*
10	T. 1 R2	000b	*Control*

3.3. Fertilizers and amendments

Straight fertilizers will be used to supply N, P and K in the experimental plots. For these trials, the source of N is Urea, of P is TSP, and of K is Muriate of Potash. The source for the secondary and micro-nutrients is any available multi-nutrient fertilizer; as long as it contains S, Zn and B (the Ca and Mg can be applied through agricultural lime). In Kenya, for example, 'Mavuno' is available as a multi-nutrient fertilizer. It contains N, P and K, and Ca and Mg, besides S and micronutrients and this complicates application of the right amounts for the treatment. Be careful to select the right Mavuno fertilizer. There are different types of Mavuno fertilizer that contain different amounts of N, P and K in addition to the secondary and micro-nutrients. The application rate of urea, TSP and MOP in the 'NPK + Multi-nutrients' treatment needs to be adjusted accordingly.

Alternatively, the nutrient components can be sourced separately and mixed to achieve the necessary application rates for S, Zn and B specifically (Ca and Mg can be discarded as this is applied by the agricultural lime – as a separate treatment - and the N, P and K are added through Urea, TSP and MOP). In all cases a sample of the fertilizer should be sent for analyses to confirm the composition and trace any possible impurities and additional elements. For example, TSP may contain considerable amounts of S as sulphuric acid is used in the production process.

Lime will be 'agricultural lime', which is a soil additive made from pulverized limestone or chalk. It may be of different quality depending on the active components and how finely the stone has been ground. Calcium carbonate will be the primary active components but may include calcium oxide, magnesium oxide and magnesium carbonate. Apart from raising the pH, which will have an influence on the availability of plant nutrients, this treatment can also be considered as a treatment for testing for possible limitations of the secondary nutrients Ca and Mg (to be excluded then from the Multi-Nutrient treatment). A sample of the lime used will therefore also need to be submitted for analyses.

Animal manure used should be 'composted. If the manure is collected from different sources in the area, it must be thoroughly mixed to get a homogenous quality and possibly the manure needs to be further composted. In this case preparations have to be made months in advance. This means that the manure has to be sourced and arrangements have to be made well in advance (several months) of the planting season. This could very well be done during the reconnaissance survey and implies that the reconnaissance survey should be done preferably at least three months before actual start of planting. If animal manure is not available the alternative is to use farm yard manure (FYM) or compost (from plant material only). Considerable amounts of manure (800 kg) are needed for a sentinel site (32 trial fields). The manure heap will be covered (with a canvas or plastic sheets) or packed in large bags and stored to avoid wetting in the event of rain occurrence before application. At planting time, the manure heap will be thoroughly mixed, manure for each treatment weighed in bags, broadcast and incorporated into the soil during land preparation. However, if land preparation is to take place more than three weeks from the intended planting date, manure and lime should not be applied until, at earliest, three weeks before planting.

Find out whether the required fertilizers are available in your region or country and make arrangements for acquiring these ahead of the season. TSP is not commonly available in some countries for example.

Table 4 specifies the amounts of fertilizer and manure and other inputs required in case the full 32 trials are established in order to fulfill the requirements for the application rates in the standard protocol. The amount is obtained by calculating the total area (nr of plots times the effective size of the plot per trial field where the particular element is applied, times the nr of fields per sentinel site) and then correcting for the composition and elemental content of the fertilizer. The effective plot size is determined by the number of rows per plot and the number of station per row and the spacing between and within rows. In table 4 an effective plots size of 26.25 m^2 is used (7 rows of .75m times 20 stations per row times at .25m spacing). The elemental composition is determined by converting the composition indicated on the bags into the N, P or K value. For example the composition found on bag for P indicates the weight percentage of P_2O_5 and in case of K the percentage of K_2O in the fertilizer. The element mass fraction of P in P_2O_5 is 43.6% and of K in K_2O this is 83% (in case of N this is 100%). The formula looks as follows:

$$Yi = (Ai*B*C)/10000 * (100/Ni)*(Q/M)*X_i \quad [1]$$

Where:
- Yi = Amount fertilizer required for nutrient *i* for the diagnostic trials
- A_i = nr of plots in the experimental field requiring input *i*
- B = nr of fields per sentinel site
- C = Effective plot size
- N_i = Percentage of the oxide or element *i* in the fertilizer as indicated on the bag (P_2O_5 for P fertilizers, K_2O for K fertilizer, N for urea)
- Q= Molecular mass of the oxide (P2O5) or element (N)
- M_i= Mass of the element *i* in the oxide (e.g. 2x atomic mass of P in P_2O_5)
- X_i = required amount of element per ha.

Table 4. Amount of inputs required for 32 trial sites per sentinel site

Element (application rate) (X) kg ha^{-1}	Source	Nr. of plots (A)	Nr of fields (B)	NPK value (Z)	Amount fertilizer required (Y kg)	Nr of Bags (50kg)
N (100 kg)	Urea	7	32	46-0-0	128	3
P (30 kg)	TSP	7	32	0-45-0	90	2
K (60 kg)	MOP	7	32	0-0-60	71	2
Multi-nutrient	Mavuno	1	32	10-26-10-10-4-4 (N-P-K-Ca-Mg-S)	22	1
Lime (500 kg)	Aglime	1	32		80	2
Manure (10,000 kg)	Manure	1	32		800	16

The above table gives typical values for the N-P-K in common fertilizers (Urea, TSP and Muriate of Potash respectively). However, the N-P-K value may vary for the different fertilizer brands (e.g. for TSP this may vary from 0-44-0 to 0-52-0) and the values need to be adjusted accordingly in the above calculation. This will not likely influence the number of 50 kg bags required however.

Nutrients and amendments will be applied at uniform application rates as shown in Table 2.

3.4. Test crop

Cereal crops that are suitable for diagnostic trials should have relatively high demand in nutrients and be responsive to fertilizer application (e.g. maize and sorghum). The main cereal crop grown in the study sites are used as the test crop. Within a sentinel site, it will be best that the same crop is used in all clusters to make possible direct cross-comparison of factors limiting productivity.

Improved varieties of the test crop that are already adapted or that are being used by local farmers will be used. For each site and season, the variety used should always be recorded because inherent attributes of the germplasm can help explain the observed responses to fertilizer. In these respect, cultivar characteristics of the germplasm (maturity group/days, potential yield, grain weight, height, number of leaves, harvest index, photoperiodicity, drought and disease as well as lodging resistance, etc.) should be provided. The yield potential is a particular importance as it is being used as reference value for yields obtained.

In this document we focus on maize as the test crop. Amendment to the protocol for sorghum and rice as test crop will be provided as separate appendices to this document.

4. Trials establishment and management

4.1. Preparing the land

The trials are researcher-designed and managed to ensure uniformity and optimal management of all sites. This means in practice a trained field technician will be in charge of the management of the trials. Uniformity in management should be ensured and the following standard agronomic practices should be followed.

Land preparation will be done by ploughing to a depth of 15-20 cm using commonly used methods for each site: ox-drawn plough, hand-held hoe, etc. The method used should be recorded. If some farmers are not used to the method adopted it is important to demonstrate it to them to ensure uniformity across different fields. Especially when the hoe is used one should make sure the tilling is done to the required depth (20 cm). Except where land preparation is done after the first rains, land preparation should be done well in advance to ensure all fields are ready in time for planting. Farmers can be busy with their own parcels once the rains set, resulting in late planting of experimental fields.

Activities like and preparation, planting and harvesting of 32 diagnostic fields require a lot of work and the technician will need a team of well trained field assistants to be able to finish the work in time. Participation of the farmers and local communities should be encouraged.

4.2. Soil sampling

Soil samples will be taken from each plot separately, once the plots are marked out, and before ameliorant applications or planting. From each plot, a composite sample of three topsoil samples (0 - 20 cm) will be taken using a soil auger. The three samples will be taken along one of the diagonals of the plot by taking one sample at the centre of the 5m x 5 m plot and a sample 1.75 m away from the central in both direction on the diagonal (Figure 5). The samples should be placed into a basin, thoroughly mixed and about 500 g taken as the sample. One subsoil sample (20 - 50 cm) will also be taken from the centre hole of each plot. Samples will be put in polythene sample bags labelled with the standard sentinel site and cluster codes, a trial number, plot number, depth and date. The soil samples will be air-dried by spreading a sample out as a thin layer into shallow trays or plastic or paper sheets. The drying should commence immediately the samples arrive at the station. Drying can be done in large room, a custom-made solar dryer, or a forced-air oven at 40° C. Break up clods as far as possible to aid drying.

***Figure 5**. Location of the soil sampling points within a plot and a field technician on the job. A bucket/basin is an important field tool for mixing soils to get a representative sample.*

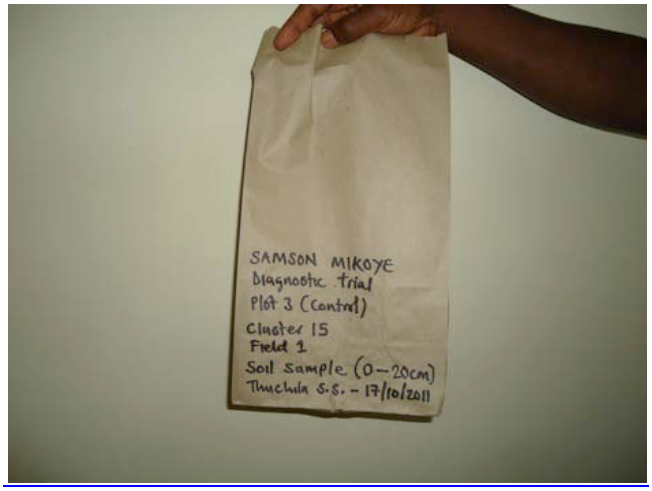


***Plate 5.** Example of a clearly labeled soil sample*

4.3. Fertilizer application

TSP, Urea, Potash and multi-nutrient fertilizers will be spot applied in the planting holes at planting, as the preferred method to maximize the nutrient recovery. The applied fertilizer should be covered with some soil before placing the seeds to avoid direct contact of seed with fertilizer. Another option is to use a stick to make a fertilizer application hole on the side of the planting hole or from the side making a hole that reaches just under the planting hole. Besides Urea application at planting, top dressing will be by spot-application (incorporated just next to the planting station) in two split applications at three and six weeks after emergence.

Fertilizers will be pre-weighed, using a suitable balance (1 g accuracy), for each plot before going to the field. Table 5 shows the amount of fertilizer required for the 5 m x 5 m plot. These amounts are based on the assumption of 7 rows and 20 planting stations (maize) per row in the plot.

***Table 5**. The fertilizers and the respective quantities to be applied per plot (5 m x 5 m) in maize treatments.*

Plot	Treatment	Treatment code	Fertilizer at planting						Top dressing[e]
			Urea (g)	Potash (g)	TSP (g)	[a]Multi-nutrient fertilizer (g)	Manure (kg)	Lime (g)	Urea (g)
1	T. 2 R1	111a	*151*	*316*	*381*	-	-	-	*210 x 2*
2	T. 8	111L	*151*	*316*	*381*	-	-	*2500*	*210 x 2*
3	T. 1 R1	000a	-	-	-	-	-	-	-
4	T. 7	111M	*151*	*316*	*381*	-	*25*[d]	-	*210 x 2*
5	T. 4	110	*151*	-	*381*	-	-	-	*210 x 2*
6	T. 6[c]	111X	*√*	*201*[b]	*√*	*693*	-	-	*210 x 2*
7	T. 3	011	-	*316*	*381*	-	-	-	
8	T. 5	101	*151*	*316*	-	-	-	-	*210 x 2*
9	T. 2 R2	111b	*151*	*316*	*381*	-	-	-	*210 x 2*
10	T. 1 R2	000b	-	-	-	-	-	-	-

[a]Application rate will vary depending on fertilizer type used
[b]Depends on the amount of potash contained in the 'multi-nutrient fertilizer, the 201g of potash is based on Mavuno being used as multi-nutrient fertilizer.
[C]Treatment 6 (plot 6) is a full NPK treatment but the N and P supplied through Mavuno is adequate to supply the needed N and P. So these nutrients are not applied.
[d]This is in dry-weight basis. Manure filled in an ordinary 50 kg packaging sack is expected to provide the 25 kg (dry weight) needed for a plot.
[e]Should be applied two times (x2) at 3 and at 6 weeks after emergence

***Plate 6**. Weigh fertilizers required for each plot before going to the field for planting*

Table 6. Amounts of fertilizers applied per station in maize plots

Fertilizer type	Fertilizer amount (g)
Urea (at planting)	1.08
Urea (1st top dressing)	1.50
Urea (2nd top dressing)	1.50
Potash (MOP)	2.26
Potash (for plot 6 if "Mavuno" is in use)	1.43
TSP	2.87
Multi-nutrient fertilizer*	4.95

1. The amounts are based on the expected plant population on per hectare basis i.e., 53333 stations for maize.
2. Lime and manure will be broadcast over the 5 x 5 m plot and incorporated before planting.
* Application rate for Mavuno fertilizer

The exact amounts of fertilizers to be applied per station are shown in Table 6. Based on a plant spacing of 0.75 m between rows and 0.25 m between planting stations, 7 rows are expected in a plot and 20 planting stations per row, giving a total of 140 planting stations per plot.

In case different application rates are used as those indicated in table 2, or alternative application rates are used in case of the multi-nutrient application as specified in table 3 the application rates per planting station can be easily calculated using equation [2]. The atomic number of Zn is 30, Ca is 20 and Mg is 12, and S is 16. For your reference, the molecular mass of the $ZnSO_4$ is 78, $CaSO_4$ is 68 and $MgSO_4$ is 60.

$$Yi = ((100/Ni)*(Q/M)*X_i)*10^3/C \quad [2$$

Where: Yi = Amount fertilizer required for nutrient *i* per planting station in grams
C = Plant density (number of plants/stations per ha – 10000 divided by plant spacing [inter row distance times intra row distance])
N_i = Percentage of the compound (oxide, salt or other of nutrient *i* - e.g. P_2O_5 for P fertilizers, K_2O for K fertilizer, N for urea) in the fertilizer or chemical product
Q= Molecular mass of the oxide (P2O5) or salt (e.g. CaSO4)
M_i= Mass of the element *i* in the oxide or salt (e.g. 2x atomic mass of P in P_2O_5)
X_i = Required amount of element/nutrient per ha.

To achieve the amounts per station and to ensure uniform distribution to all stations within a plot, fertilizers will be applied using dollop cups or bottle tops. The dollop cups and the bottle tops will first have to be calibrated (using a candle or wax) to measure the right amount of fertilizer to apply per station. This is best done by taking 10 scoops with the bottle top, weigh the total and then adjust the volume of the bottle top by dripping candle wax in it. For example, for the urea, the weight of 10 scoops should be 10.80 g for the basal application. If the balance is not very accurate it is best to increase the number of scoops for the calibration. The surface of the fertilizer in the bottle top should be flat and some routine should be obtained in measuring in a uniform way. The error margin should be within the 3%. It means that if you

would apply the fertilizer to one plot in the trials you should be maximum 3 to 4 scoops short or in excess. The balance used in calibration should be accurate to at least 0.01g. The cups or bottle tops should be marked to know which cup or bottle top is used for which fertilizer and which application. Most practical would be to use different colours of bottle tops.

Plate 7*. Dollop cups used for fertilizer application. See that some of the dollop cups are calibrated for actual fertilizer per station by use of candle wax*

Accuracy in fertilizer application will be increased by having people specialize by applying the same fertilizer throughout. For instance, one person can be applying Urea only while another applies TSP only.

When reporting on the diagnostic trials, the application rates of the fertilizers per planting station should be stated explicitly.

4.4. Planting and gapping

Planting will be done at the onset of the rainy season in each site, at the same time farmers plant their fields. Planting on ridges, in a flat seedbed, basin or other depends on what is customary in the area. However, the management of the diagnostic trials should reflect good, optimal management practices. For example, in areas with risk of rainfall shortage or dry spells, measures to increase infiltration and reduce surface runoff would be useful. If 'tied ridges' is a practice used by at least some farmers it should be applied in the diagnostic trials. The same applies for planting basins or other measures, though these might be adjusted to suite the planting densities prescribed for the diagnostic trials. The way the land is prepared and structures used should be documented. Maize will be planted at a spacing of 75 cm (inter-row) and 25 cm (intra-row). Therefore, there will be 20 planting stations per row and seven rows per 5 x 5m plot. To achieve the desired intra-row spacing, planting ropes will be marked at 25 cm intervals (using ink or knots) and planting holes (or dibbling) made adjacent to the marks. For efficiency, it is nice to have two or three planting ropes. Where planting holes are made with hoes, it should be ensured that holes are made on the same side of rope to avoid crooked rows. Planting depth should be 5-7 cm. Two seeds will be planted per station and thinned to one 10 days after emergence.

Gapping (replacement planting) will be done 5 days after emergence (or about 10 days after planting). Timely gapping will ensure that maturation of the plants within a treatment is not so staggered.

4.5. Weeding

Weeds compete for water, nutrients and light with crops and timely weeding should be undertaken to avoid this. Weeding will be done using a hoe as necessary to keep the plots clean. It is necessary to ensure that weeds do not continue growth after a weeding operation by shaking off soil from their roots.

5. Measurements

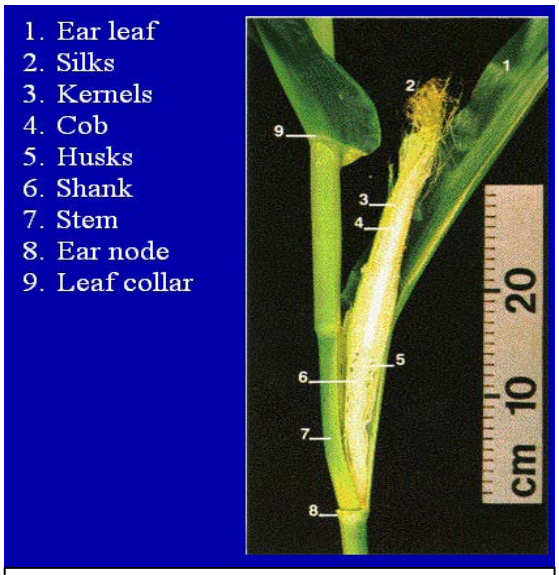
5.1. Emergence date

From the second day after the first coleoptile emerges from the soil, record the total number of stations with emerged plants within the total plot area (5m x 5m). Stop on the date when ½ of all stations have plants emerged. Record this date as the 50% emergence date. For example, with a total of 140 stations in a plot, 50% emergence date will be that date when at least 67 hills have an emerged coleoptile.

5.2. Plant height, basal diameter and number of leaves

Plant height and basal diameter of 8 random maize plants per plot from the net plot area will be taken twice at vegetative growth stage, once at flowering stage, once at grain filling stage and at maturity. The net plot consists of 3 middle rows (leaving out 2 rows from each end) and 3m for each row (leaving out 1m at the end of each row), giving an area of 2.25m x 3m=6.75m^2. It consists of row 3, 4 and 5 and plant numbers 5 until 16 for each row. The five measurements are taken at the following points of time:

- 2 weeks after emergence (V2 stage when 2 leaves have visible collar [to understand collar, see Figure)
- 4 weeks after emergence (V6 when 6 leaves have visible collar)
- 8 weeks after emergence (Anthesis or male flowering and when pollen shed begins)
- 10 weeks after emergence (when kernels are filled with clear fluid and the embryo can be seen or when silks emerge from husk of the ear)
- 13 weeks after emergence (at dough stage when kernels are filled with a white paste, and the embryo is about half as wide as the kernel. The top part of the kernels are filled with solid starch)



Source: accessed from uoguelph.ca on 25th Nov 2010

Three plants selected from each of the 3rd and 5th rows and two plants from the 4th row (only within the netplot area) will constitute the 8 plants for measurement. For each row, every fourth plant will be sampled (measured). There is no need to measure the same plants every time the measurements are taken (i.e., no need to label the sampled plant in order to measure the same at a future date). The layout of the plot with 7 rows and 20 planting stations per row, a netplot with 3 rows and 12 plants in the row, from which the eight plants are selected, is sketched below. The effective area of the net plot in this arrangement is 6.75 m^2.

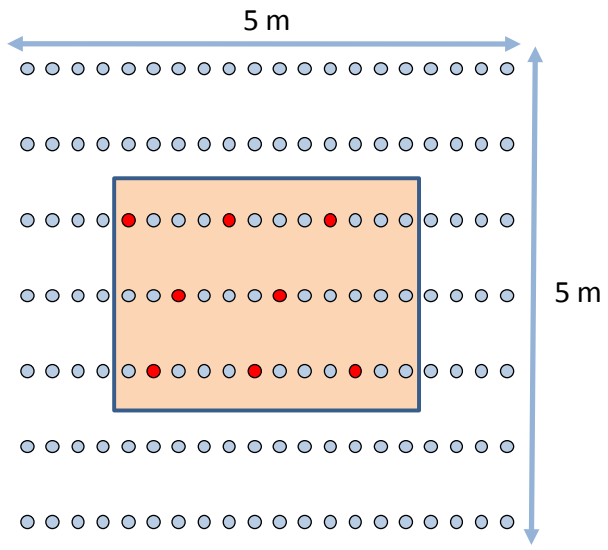


Figure 6. *A 5 m by 5 m plot showing the net plot (inner shaded area) and the expected plants (all dots). The red dots in the net plot area are an example of the 8 plants to be measured during a given visit.*

The measurement for basal diameter will be taken at about 1 cm from the soil surface using callipers. In the case callipers are not available, measuring tapes will be used to measure the basal circumference. If callipers are used, two measurements per plant have to be taken (turn 90 degrees for the second measurement) for the 2 and 4 weeks after emergence because cereal stems are oval during early growth stages. Plant height will be taken from the soil surface to the highest tip. The highest tip can be a leaf tip or the male flower. For young maize plants, an easy way to get the highest tip is to wrap the palm of the hand round the plant stem at the bottom and move the hand upwards. At later stages, when one cannot reach the tip by hand, a measuring tape is to be mounted on a long stick and used to read the highest plant tip (i.e., the tip of the male flower).

For each of the growth stages for which height and basal diameter are measured, the number of leaves on the 8 selected plants will be counted. All leaves should be counted including those that are senesced as long as this can be identified (e.g., through a visible node).

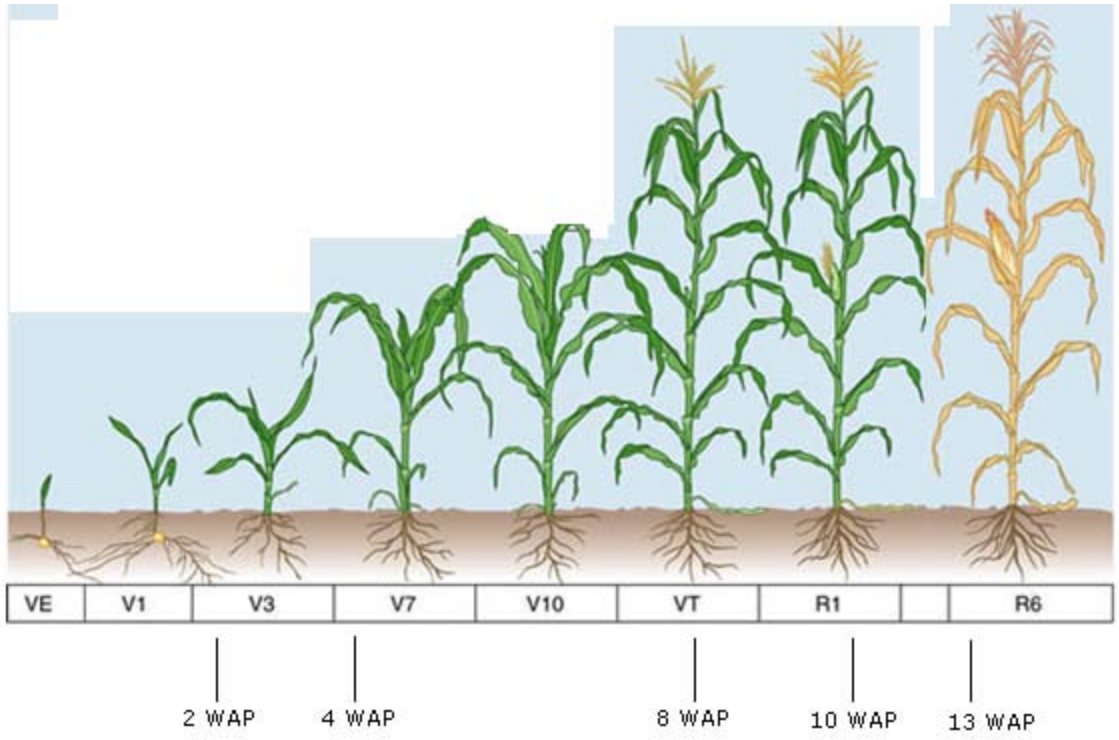


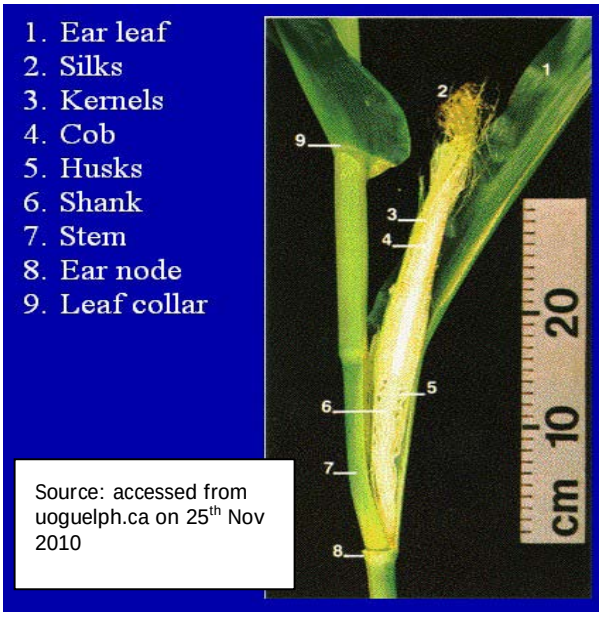
***Figure 7**. Maize growth stages indicating the measurement periods (Adapted from university of Illinois)*

5.3 Flowering

The date on which at least one flower has opened on 75% of the plants in the NPK and NPK + micronutrient plots will be recorded. Flowering is defined here as the initiation of the male inflorescences (tassel). When flowering starts, and on every subsequent 3rd day, record the number of plants that have flowered and stop on the date when ¾ of all plants have flowered. Record this as the 75% flowering date.

5.4 Plant tissue sample for nutrient analysis

Ten ear leaves will be sampled in the period between tasseling and silking (male and female flowering, respectively) immediately when the position of the ear is identified. An 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 behind the leaf base that circles the stem. A total of 10 plants next to the net plot will be sampled as follow:
Rows 2 and 6: plant 9, 10;
Rows 3, 4, 5: plant 4 and 17



The leaf samples will be placed into clearly labelled paper sample (e.g., brown) bags and carefully sealed. It is best to keep the samples cool by transporting to lab in a cool box. Where not possible to access cooler boxes, and due to bulkiness of the samples, leaf samples should be transported immediately and the paper tops opened to aid air circulation. The samples will be washed with distilled water to remove contaminants and then oven-dried at 60 ºC for 48 hrs. The samples should be sorted out in the laboratory and arranged to ensure that leaf samples from all the 10 plots of a specific field are dried at the same time (i.e., they should be put in the oven at the same time). The dried samples will be placed flat in paper bags and shipped to ICRAF-Nairobi for milling and analysis. Instructions on how to prepare and send samples are contained in the section of "handling soil and plant samples".

Do not sample soon after spraying say for termite control and also avoid disease/pest affected ear leaves.

5.5 Visual observation of deficiency symptoms

Visual nutrient deficiency symptoms will be recorded for each of the treatments at the 2, 4, 8 and 12 weeks after emergence. This information will complement foliar (leaf) analysis. A guide for visual nutrient deficiency indicators is provided as an appendix.

5.6 Harvest grain and crop residue yield

Grain and stover (above ground non-grain biomass) yields in each plot will be determined from a net plot of 3 rows x 3 m (leaving out 2 rows from each end and 1 m from each of the sides, see Figure 6). Harvesting will be done after the crop has reached physiological maturity. First, the number of plants in the net plot are counted and recorded on the harvest form. Make sure the net plot is marked properly such that plants outside the net plot are not counted. The cobs are then harvested in such a way that the husk still remain on the plant. The cobs are counted and the weight of the total number of cobs is determined. All the plants in the net-plot will be cut at the soil surface and total stover fresh weights determined in the field. This can best be done by tying the stovers together with a rope. Remember to weigh the rope and correct it from the measured weight to obtain the actual weight of the stover.

Five (5) representative stovers are selected randomly, cut into 5 cm strips, well mixed and a subsample of about 500 g taken. The weight of this fresh sub-sample is recorded. The sub-sample is bagged and taken to the lab for drying. Five (5) cobs are also randomly selected and fresh weight determined. This is best done by ordering the cobs from small to large and then selecting 5 cobs - every so many cobs (total nr of cobs divided by 5) - such that we have a representative sample of the whole range of sizes. The grains and cores of the 5 selected cobs will be separated and their fresh weights determined separately (this part can be done in the field or once samples arrive at the station). The three sub-sample plant parts (grain, cores and stover subsample) will be oven dried (60 ºC for 48 hours) and reweighed to determine moisture content. The dry weights of these sub-samples are recorded. After drying, 100 maize grains from a well-mixed sample will be counted and their weight measured for determination of the average weight per grain.

Plate 8. Chopping crop residues to obtain a subsample.

For more information on maize growth stages and characteristics, visit http://www.knowledgebank.irri.org/default.htm

5.7 Rainfall and temperature

Daily rainfall will be measured using at least three rain gauges installed within the sentinel site. If considerable variation in the amount of rainfall received is expected within the sentinel site the number of rain gauges should preferably be increased to six. One should place the rain gauges at locations where largest differences are expected. That is one should look at the orographic characteristic and consider possible dominant wind directions to determine possible rainfall shadows and areas where rainfall may be concentrated. Additional information on rainfall can be obtained from satellite data, but this will not provide the detailed information on variability of the rainfall within the area. Satellite data may be used as alternative to measurements with rain gauges especially where little internal variation is expected. The rain gauges should be acquired and installed well before the season starts. At the latest, the rain gauges should be installed at planting, placing them in an area clear of obstructions such as of trees, tall grasses and buildings. It should also be ensured that the persons to read the rain gauges are well trained to ensure good data reading and recording.

Daily maximum and minimum temperatures will be measured in strategic locations using at least three min/max thermometers that will be installed with the rain gauges.

In some areas, weather stations exist with long-term climatic information. Such information is important and wherever possible should be acquired as well.

6. What to do in case of stress, drought and other anomalies

6.1. Rating pest and disease problems

Plant diseases and pests impact on quantity and quality of produce. The severity of a disease or pest attack is assessed as a scale representing no or low attack to high or "very severe". It provides important information for evaluating and interpreting the results from the trials. This assessment will be done for each plot at flowering and at physiological maturity, and at any time as deemed necessary, e.g., when an attack is noted. The observations will be done for the net plot (or in case of damaged plots for the harvested area, generally the whole plot [See section 6.3])

For each condition, a rating scale is provided as follows:

1. For bird damage, count and record separately the number of cobs slightly, moderately or severely damaged by bird eating in the net plot. This assessment will be undertaken at physiological maturity.

Damage rating	Description
Slightly	small opening at the tip of ear and less than 10 kernels damaged
Moderately	large opening of the ear, about 1/3 of cob exposed
Severely	greater than 1/3 of cob exposed and most kernels destroyed

2. For termite damage, count the number of plants in the net plot that are lodged as a result of termite attack.

3. For attacks of stem/stalk borer, a 1-5 scale should be used to assess damage on each plant in net plot according to the below rating:

Rating	Description
1	No visible larva feeding damage
2	Small amount of shot-hole-type lesions on a few leaves
3	Shot-hole injury common on several leaves
4	Several leaves with shot-hole and elongated lesions
5	Several leaves with elongated lesions, severe stem damage or death of plant

4. For diseases, such as maize streak virus, record for each plant affected within the net plot the severity of the disease. Severity rating should be based on the percentage leaf area that is affected by the disease (i.e., proportion of area with lesions according to table below). Note that the area is given as a proportion of the total leaf area of the plant and not as a proportion of the particular leaf affected.

Rating	Proportion of area with lesions
1	0% i.e., No visible signs of disease
2	1 - 10% of total plant leaf area affected
3	11 - 25% of total plant leaf area affected
4	26 - 50% of total plant leaf area affected
5	>50% of total plant leaf area affected

5. At harvest, cob rot and termite feeding on cob will be visually assessed by grouping all the affected cobs into five sets according to the proportion of kernels affected (Table below). The number of cobs in each of the five groups will be counted and recorded.

Rating	Cob rot classes
1	0-5%- no or very few kernel affected
2	6-15% kernels affected
3	16-40% kernels affected
4	41-70% kernels affected
5	>75% kernels affected

6.2. What to do if plants are in different growth stages

If the plants have germinated poorly and significant replacement planting and gap filling has taken place the crop will be in different growth stages. If replacement planting is almost complete, the date of the replacement can be considered as the planting date and the timing of all subsequent measurements based on it. Alternatively, measurements or sampling is timed based on the growth stage and when majority of the plants show the described plant characteristics of that growth stage, rather than using a fixed time schedule. Also, the particular time (days after planting) to reach the different grow stages will also depend on the cultivar and should therefore not be adhered to too strictly. The growth stages have been described earlier (in above sections).

Ear/flag leaf sampling under non-uniform plant conditions should proceed with caution. Only plants that have just produced an ear should be sampled for ear or flag leaf.

6.3 Harvesting damaged plots

Crops damaged by animals, disease or pest attack, severer lodging because of windfall etc lodging can result in under reporting of the observed yields or misrepresentation of plant nutrient concentrations. In many cases it is still possible to gather some relevant data

In case that many plants have been damaged within one or more plots of a specific field, the procedure needs to be adjusted. The same procedure then needs to be followed for all of the plots in the experimental field, in order to ensure comparability of the data and results from the various plots. In this case 12 healthy and best performing plants (or more if they can be found on each of the plots) are harvested. These should preferably be harvested from the net plot, but if needed a larger area can be considered up to the whole plot area if required. Record what procedure (the area as specified by the number of rows and planting stations per row) is followed. Harvesting should be done according to the procedure explained in previous sections,

and data recorded in the same forms used for harvesting under normal conditions. A comment has to be made however that only 12 plants are used.

The harvest results of the 12 plants will be extrapolated to account for the expected plant populations. Note that this procedure only applies to fields where sporadic damage to crops has been meted. Whereas the yields obtained following this method of harvesting can be useful to assess treatment effects, comparisons with other fields can only proceed with caution due to overestimation of yields.

We often see compound effects in damaged plots. For example, we see a treatment effect confounded by the drought effects on poor soils. It will cause poorly developed plants in the control and nutrient omission treatments that are also more susceptible to lodging and subsequent termite attack for example. This means that for the better, less or none damaged plots in the field the normal procedure needs to be followed beside the adjusted procedure as described above. The data generated in this way will still allow to compare treatment effects between the various field locations.

Plant counts (those still standing and those that have lodged or lost) remain important. In the case that many plants have lodged (and partly lost), or drought has badly affected the area such that the above procedures are no longer suitable, all plants (standing and lodged) in the entire 5 m x 5 m plot will be counted, harvested and used for determination of biomass and grain yield. In this case, remove all the ears and record their weight. Then cut all the stover at the surface and weigh together with the lodged ones. If the ears are damaged make sure to record for each plot the number of damaged ears according to size category (small, medium or large) and damage category as described above. Some cobs may be soiled (plastered) by termites; these should not be weighed, but should be counted and recorded.

For maize, cobs should be arranged in order of increasing size and divided into 3 groups of equal number of ‘small’, ‘medium’ and ‘large’ cob sizes. Select one representative cob from the ‘small’, 3 from the ‘medium’ and one from the ‘large’ cob size groups. Weigh these 5 cobs and then shell the grain and take grain and core weights. These should be dried and dry weights taken. In the same way, select a sample of 5 stover and cut into 10 cm pieces and record the weight. This sample will be dried and dry-weights taken. Note: use ‘Maize Harvest Data Recording Form (drought modified)’ for harvesting drought-affected fields.

Take only clean stalks and cobs as sub-samples to ensure accurate nutrient analysis results

7. Recording field management history

For each experimental field, details of management history (previous 5 seasons) are collected using simple standard survey forms. The information is collected during site selection, preferably immediately after deciding to use a particular field. Care should be taken to get information that relates to the actual section of the field to be used for diagnostic trials and not for other sections of the field which may be different. The recording of management history is intended for explaining the possible differences in responses to fertilizers observed in the different diagnostic trial fields and that may not be explained by obvious differences in soil type or other, or to explain varying functional soil properties on the same type of soil. The information to be acquired relates to:

- Period of cultivation for that field (i.e., the number of years since field was converted from natural vegetation to cultivation. It would also be nice to just record the year in which conversion took place)
- Crops grown during the previous 5 seasons (record the crops grown for each season).
- Type and amount of fertilizer used in the past 5 seasons (the fertilizer used should be specified including, the actual seasons in which these were applied and to which specific crops). Fertilizer may be applied to one crop and not the other in an intercrop system for example and it is nice to capture this.
- Organic nutrient resources used (type of manure [cattle, goat, chicken, and cow dung], compost, crop residue, biomass transfer etc.). It may be the case that the manure is not actually supplied but that the field has been used to corral cattle at night, for example. The use of organic resources can explain differences in soil fertility status on the same type of soil and within the farm, and will have an effect on fertilizer responses.
- Crop residue management (buried/incorporated, burned on site, removed/taken away from the field)
- Fallow periods (record the actual period the land was under fallow in order to determine how many seasons ago this took place and determine the duration of the fallowing. The type of fallow should also be specified, e.g. improved fallow (specific species), natural fallow)
- Estimated distance from the homestead expressed in kilometres or meters
- Cropping system (intercropping, rotation systems – list the crops, cereal, tubers and legumes that are part of the crop rotation-, continuous monocropping, agroforestry system etc., that has been used by the famer should be stated).

In addition, the topographic position and slope and direction of the slope of the site should be noted together with the drainage conditions. This is of relevance to the hydrographic characteristics of the site (e.g. whether there is any possible lateral supply of water or supply from groundwater). A field may be located in a flood plain, or bottom land with even risk of inundation, or in a valley bottom where soil moisture conditions can be good for longer periods of time even under conditions of relative drought/dry spells and where deposition can occur during flooding events. Gently sloping field can experience erosion. Otherwise we assume that relevant site characteristics are recorded during the LDSF.

8. Data collection and management

8.1. Forms for recording the data in the field

All the data from the diagnostic trials are recorded using pre-designed field forms. The following forms have been prepared to record the data that is needed for each sentinel site and for each season. These forms can be obtained as separate documents and can be obtained from the www.africasoils.net website. These forms serve as an example and can be used as bases for designing your won customized forms. It is advised that a separate logbook is maintained in which the events and activities are recorded, which will assist in the progress reporting and which will be used as reference for the observations made.

1. General information on the sentinel site. One form per sentinel site in which the location and general information on the agro-ecological conditions is described
2. General information on the cultivar used in the diagnostic trials. One form for each cultivar to record yield potential and other attributes.
3. Experimental field administrative data. One form to record location of the experimental field, name of the farmer, the cluster, and the village. This form provides also further information on soil type and general topographic information.
4. Experimental field management history survey forms (A & B) in which the crops and management in the previous 5 seasons is described.
5. Management of the diagnostic trials. One form that records the dates of management operation (planting, fertilizer application, weeding, etc) and a form for recording the crop growth stage (the emergence and flowering dates).
6. Crop growth data. Form to record plant height, basal diameter and leaf counts. One form for each diagnostic trial/field and for each date of recording
7. Crop harvest data. A form for recording harvest data for normal season and forms to use in case of drought or animal damaged on plots.
8. Notes on anomalies. General form to record pest and diseases phenomena, or nutrient deficiency symptoms or plant and ear damage at time of harvesting. One form for each field and each event that is recorded
9. Daily rainfall and maximum and minimum temperature registration. One form for each station.

We have provided general forms for recording plant pest and disease symptoms, nutrient deficiency symptoms and for recording damage to plant and cobs. We recommend these forms are customized to suit the particular circumstances if incidence rate is of certain pest and diseases quite high. Conditions will often apply to several or most of the fields, if these relate to drought, windfall or other. In such cases, where damage to the cobs is quite widespread it would be advantageous to have forms to record the damage to the cobs per plot in a systematic manner (record number of cobs per damage category and per size category).

All the above forms can be customized to the particular circumstances to ease the data collection process.

8.2. Data organization and storage

Data will be entered into Excell worksheets. We have prepared excel templates that are in line with the forms developed for data recording as described above. The file has the following worksheets: SiteInfo (for sentinel site specific information such as climate), GenTrialsInfo (general information on the diagnostic trials), FieldInfo (for data related to previous management of the field), PlantGrowth (for plant measurement data), HarvestData, FieldOperations (for all data related to dates of field activities) and WeatherInfo (for weather data recorded during experimentation). The excel file once populated with all the data should be emailed to the data manager of AFSIS for the diagnostic trials (s.kinyanjui@cgiar.org).

AFSIS maintains a database to manage all the data related to the diagnostic trials. This relates to diagnostic trials that have been conducted under the auspices of the AFSIS project, but also of the results of diagnostic trials that are conducted by partner organizations and associated projects. The data is available to third parties upon request. AFSIS can also provide the database itself (design and structure) for parties that aim to implement diagnostic trials in a number of sites and that need to invest in the data management. The database is implemented in Filemaker Pro. The figure below illustrates the basic structure of the database. Further information can be obtained from the web site.

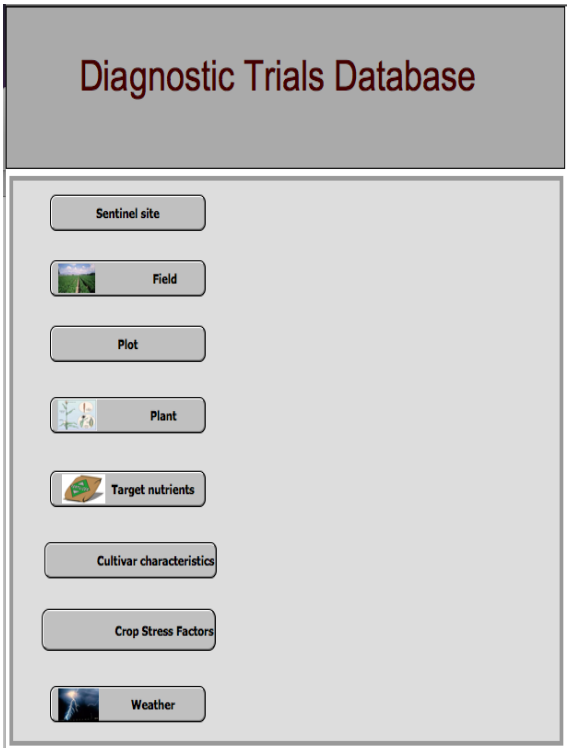


Figure 8. *Basic structure of the diagnostic trials database (version '0', naming conventions may change in subsequent versions)*

The diagnostic trials database requires that we will be able to uniquely identify the site, cluster, field and plot. It will allow to link individual observations related to the diagnostic trials, but also to link to observations related to other activities, like the LDSF. It enables to effectively query the database and retrieve data according to particular selection criteria. It requires that a system for the coding of the individual fields and plots is used.

For the site a four letter code is used. This is generally derived from the first four letters of the name of the sentinel site. For the AFSIS sentinel sites the name is assigned by AFSIS and generally is the name of the most important town or village in the area. For any new site we are able to adopt any name suggested by the organization that is implementing the diagnostic trials. AFSIS will however assign the four letter code in case the code already exists for another sentinel site.

A 'year' tag will be added to make distinction between the various years in which the diagnostic trials are repeated at the same site (though a possible different locations within the site). Cluster ID is the cluster number (1-16), again as assigned by AFSIS when the randomization is done. The cluster number is the same number as for the respective tile, since there is only one

cluster for each tile. The fields within a cluster are sequentially numbered 1, 2 or 3 during field selection. This number is preceded by the prefix ‘a’ or ‘b’ to indicate whether the diagnostic trials are conducted during the early season (or in case there is only one seasons in the year) or the late seasons in the year. The letter prefix for field number is to avoid confusion with the numbering for the sampling points in the LDSF. Because the location of the diagnostic trial is not necessarily co-located with the sampling point of the LDSF we adopt separate numbering for the fields for the diagnostic trials. Links to the corresponding sampling location for the LDSF is established through the coordinates (spatial correlation). Adding the plot number subsequently provides for unique identification of the plot and herewith also the treatment is identified.

Therefore, the code for a particular diagnostic trial and plot within that trial will consist of the first 4 characters of the sentinel site name, the year in which the diagnostic trials are established, a letter code to indicate early or late seasons, the cluster number and number assigned to a specific diagnostic trial field within the cluster (each cluster has 2 or 3 fields, rarely more than 3) and then the number of the plot. For example, plot 10 in the first field in cluster 16 in the Pampaida site would have the following ID:

“Pamp2010a16d110”

The data will be entered as separate data fields and will be generated automatically, while entering data in the database. The plot ID will subsequently be used to reference all measurements done at the plot level, whether related to the soil sampled, the plant tissue samples as well as measurements related to crop growth and yield. Measurements that are repeated within the plot (e.g. crop growth characteristics) are foreseen with a time stamp.

A schema of the structure of the database is given below for illustrative purposes. It illustrates how the various tables are linked through the identifiers. Note, updated information on the organization of the database and content of the database (recent version) is found on the AFSIS web site.

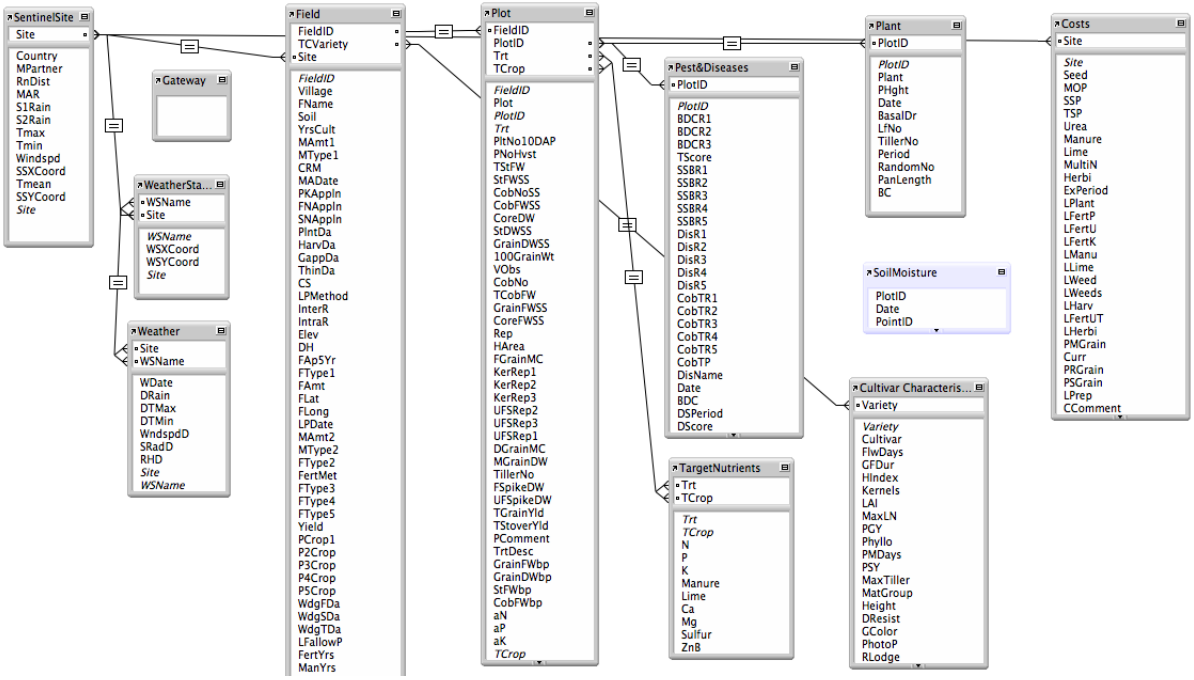


Figure 9. Relationships between the various tables in the DT database.

8.3 Data quality control

Data from each sentinel site should be accompanied by a data report explaining how data issues have been handled and reporting on any events that may have impacted on the quality of the data, or that may be relevant for the interpretation of the data.

Good data is collected by team members who have a clear understanding of the project and purpose of the diagnostic trials. This means errors are minimized through appropriate training and briefing. Thus, once identified, team members should be familiarized with the entire protocol: from objectives to data reporting. The structure of the study especially the specific numbers assigned to the clusters should be clearly understood by the team. Nevertheless, all data reporting should include the name of the farmer in which field a trial is located.

For quality assurance, the persons collecting data should understand exactly how to go about the data collection before setting out to the field. The researcher in-charge of the trials in each country should train/demonstrate the exact procedure to follow in measurements and data recording to the technical assistants and confirm this is clearly understood. The data collection team should be asked to take measurements (e.g. basal diameter) on the same plant and same plot and to compare results as a form of training and assuring that measurements are done in a consistent manner. Retrain until every member of the team achieves similar results. Ensure also that each member can reproduce similar values on repeated measurements.

Data inspection should be done at two stages: recording stage and after entry into a computer. At the recording stage, the national researcher assigned to manage diagnostic trials should inspect the field recording sheets used by the technical assistants. This should be done every time the researcher is visiting the field. It is not rude to ask the assistants to demonstrate how they collected a given dataset. Any procedures used that are not as described in these protocols should be noted and reported in the final dataset. Nothing should be ignored as a small aspect of a procedure can have a huge impact on the values recorded. Re-training should be done if data collection does not proceed as expected. One should minimize the need to interpret what is recorded on the recording sheets before entering data on the computer.

One aspect is how to deal with zero values and missing data (data is not available). What should be recorded if plants in a plot have no cobs, for example? A zero (0) should be recorded for the number of cobs in the plot and 'not applicable' (N/A) for the weight of the cobs consequently. One could use the 'NULL' value to indicate that no data is available and could be reserved for those cases when data has not been recorded to make the distinction between when it is not applicable (not relevant and no data is expected). The NULL value would apply, for example, if cobs would have fallen off the van (it must not happen) before recording in the datasheet. It also makes sense to check what has been recorded in the field forms after each field harvest, to confirm possible extreme or deviating values.

In stage 2, data that has been entered in the computer (the provided Excel file) should first be inspected and errors corrected immediately. One way of inspecting the data is by plotting the data. One could plot of the data from all the trials for each treatment and check values that are abnormally high or low. A more formal criterion would be to calculate the frequency distribution and reject all values that are more than two times the standard deviation. Possible errors should be corrected by consulting the field data sheets. If there is any doubt about the correct

value (e.g. because of interpretation of the hand writing or field forms having been smudged) this should be flagged. Any corrections made on the data not based on field data sheets should be flagged as well and documented in the data report.

Advanced data inspection will be achieved by using simple 'if' statements in a spreadsheet. It is expected that sub-sample stover weight is less than net plot stover weight, or that the dry weight is less than the fresh weight, etc. One can also generate summary statistics for specific variables (data columns) and check the minimum and maximum values against expectation. Relationships (scatter graphs) between datasets, for example biomass vs. grain, etc., will further show data points that are off the general trend. For such observations, check which of the two data have error and correct them. Always, for all inconsistent data, go back to the source (data sheets). Care should be taken to always have a back-up file. The best way to do this is to save a new copy of the file to work with each day and its file name should end with the calendar date (yyyymmdd) for ease of tracking.

It is the responsibility of national researcher to ensure completeness of the final data. That is an integrity check needs to be done to see whether there is any missing data in the data set.

The type of scale will affect precision of data. As a rule, all weighing balances used for weights reported in kilograms and grams should have at least 1 decimal. The balances/ scales should be checked for consistency in measurements. Also, care should be taken when weighing samples, ensuring that scales are tarred for paper weight, basins or other as necessary. Some of the spring balances are notoriously instable and need to be checked and corrected (zeroed) before each measurement when necessary.

A data report should accompany all the data sets sent to AFSIS-Nairobi. It should include the details of data processing and how data inconsistency has been handled, map of the sentinel sites, indicating location of diagnostic trial sites and objective 3 sampling points.

8.4 Data analysis

In this section we provide some general comments or guidelines on the analysis of the data from the diagnostic trials. Data analysis refers to different levels of interpretation in which increasingly we add meaning to what is observed. At the first level the data itself is explored and we characterize the area (sentinel site) in terms of the variables measured. In case of the diagnostic trials this refers to crop performance on the control plots, the crop response to the various treatments and attainable yield under the prevailing conditions for that particular season. The crop response should be measured as crop performance for that particular treatment compared to the control. We can describe the area in terms of the mean value for that particular variable and some measure of the variation. Subsequently we can start exploring the sources of variation (though that is entering the next level already). The set up of the diagnostic trials is such that it can be explored whether the main source of variation is from the field, cluster or sentinel site level.

Typical outputs at this stage are graphs that display the distribution of yield and crop growth variables per sentinel sites. This can be box plots, bar charts, scatter plots and other. Particular illustrative are scatter plots in which response to a treatment (e.g. NPK) is plotted against the control. In case data from several sentinel sites are displayed, or for different seasons from the same site, it is important to normalize the data, especially when it concerns response to treatments, by normalizing against the control normalizing against the attainable yield (the

maximum yield obtained in that particular trial for that particular season). The outcomes will generally inform whether some kind of action is desired.

At the second level of interpretation involves identification (or classification) based on the observed values. In case of the diagnostic trials this refers to identification of the soil fertility status in terms of inherent soil fertility and response characteristics. Inherent soil fertility is determined by crop performance of the control 'treatment'. Response categories are determined by the response to NPK application. In practice there are three categories of soil, *viz.* fertile non-responsive soils, poor responsive soils and poor non-responsive soils. It does require some kind of criteria against which the crop performance is evaluated in order to determine the status. In second instance the more specific character of the limiting conditions is determined. This may refer to the particular limiting nutrient, N, P or K in case of the responsive soils (we may want to evaluate the effect of the nutrient omission) and in case of the poor-non responsive soils whether limitations are related to the secondary and micro-nutrient concentrations, chemical imbalances or physical characteristics of the soil. One aspect of this level of interpretation will also be the identification of the spatial domains where these constraints (or combination of constraints) occur. Part of the analyses will involve comparative analyses, e.g. to develop the evaluation criteria. Outcome of these analyses will inform about the incidence of particular constraining factors and will tell what and type of action is required where

The third level of interpretation is about explaining the phenomena observed. For the diagnostic trials we would like to know what determines the crop response and what explains the variation observed. In this case we are interested especially in the soil and land degradation characteristics. The analyses will typically involve different kind of regression analysis and principal component analysis. It makes sense to develop a number of hypotheses for the explanation of the phenomena observed. The formulation of the hypotheses should be informed by the environmental context and land use history and testing of the hypothesis will require contrasting conditions (in soil erosion and soil carbon saturation for example). The outcome of these analyses will typically allow predicting crop response for larger areas based on soil and environmental characteristics. It will also tell what type of ISFM options to consider for further experimentation or demonstration to address the constraints identified.

The first step or level in the analyses requires crop data from the multi-locational trials. The second level of data analyses will require additional data in the form of rainfall, data on the crop variety used etc. and preferably over different seasons for reference purposes. The last step in the analyses will further require soil data and information on land characteristics that is catered for (partly) in the protocol for the diagnostic trials.

9. Handling soil and plant samples

9.1. Preparation of samples for shipping

Soil samples

All samples from the agronomic trials will be transported to regional laboratories. In these laboratories, samples will be processed (grinding, sieving and sub-sampling) according to AFSIS standard operating procedure for soil sample processing at regional laboratories. NIR analyses will be performed on all of the samples in the regional labs.

From each plot for all fields (trials sites), one sub-sample of at least 20 g of the top soil will be prepared for shipping to ICRAF-Nairobi for MIR measurements. Assuming that 32 trials have been established per sentinel site, this would amount to 32 x 10 = 320 samples weighing in total around 6.4 kg. Standard codes should be applied for each of the sample (e.g. 'kolo2010a01.1.04-topsoil')

From each field (trial site) a sample of 350 g should be prepared for shipment to ICRAF-Nairobi for reference analyses. This sample is obtained by taking 350 g from the processed soil sample from one plot in the trial (plot 5 if plots are laid out sequentially, from plot 3 or 7 in case of blocked layout of the plots). A sample will only be taken for the topsoil (0-20 cm) in case LDSF is implemented on the site. A sample for the subsoil will also be shipped if the LDSF has not been conducted. The reference analyses consist of: TXRF (Total X-ray fluorescence), X-ray refraction and Laser diffraction measurements. "AfSIS Standard Wet Chemistry Soil Analyses" will be conducted for cross reference. The standard wet chemistry soil analyses will be done to determine pH(water), EC, Exchangeable acidity, Mehlich 3 (Al, P, K, Ca, Mg, Na, S, Fe, Mn, Cu, B, Zn,), CEC plus %Ca, %Mg, %K, %Na, %OB, %H + Ca:Mg ratio, and P sorption index. Assuming 32 trials sites per sentinel site, the total weight for shipping will add up to 2 x 11.2 kg is 22.4 kg.

NB: in the case that a regional laboratory is not able to conduct NIR on the samples (e.g., a malfunctioning instrument in the laboratory can delay results), samples of 50 g from each of the plots and depths should be sent to ICRAF-Nairobi for NIR analysis. This has to be done in consultation with the appropriate AFSIS scientist.

Plant samples

All plant samples should be oven dried at 60 ºC for 48 hrs (or to constant weight) after preparing them appropriately (e.g., ear/flag leaves should first be rinsed with distilled water to flush off pollen and dust). The dried plant ear and flag leaf samples will be milled to 0.5 mm within the collaborating institution or regional laboratories and sent to ICRAF-Nairobi already milled. Where this is not possible, the unmilled samples should be placed flat in paper bags and shipped to ICRAF- Nairobi for milling and analysis. Care must be taken to avoid contact with soil dust or other contaminants. Grain and stover samples will also be milled (to pass 0.5 mm sieve). The milled samples should be placed into 50 g zip-lock paper bags and shipped to ICRAF-Nairobi for IR, XRF and reference analyses. Label the bags with the code for site, year, field and plot number, date and sample type. Indicate whether it concerns a plant tissue (Pl), grain (Gr) or stover (St) sample. Assuming 32 trial sites a total 320 plant tissue samples would be expected (or 960 samples for grain, stover and ear leaves). Of the samples 10%, i.e. one out of the 10 plot per experimental field, will be send for cross reference "AFSIS Standard Leaf Analysis" to determine Plant N, P, K, Ca, Mg, Fe, S, Zn, Mn, Cu, B, Na).

An overview of the all the samples to be taken and shipped to one of the laboratories is given in table 7.

Table 7. Overview of the samples to be submitted to regional lab and the Nairobi labs for analyses

	NIR analyses regional lab	(NIR &) MIR analysis Nairobi	Wet chemistry (for reference) Nairobi
Soil samples per field			
Topsoil (0-20cm)	10 x 50g	10 x 20g	1 x 350g
Subsoil (20-50cm)	10 x 50g	10 x 20g	1 x 350g
Plant samples per field			
Ear/Flag leaf		10 x 50g	1 x 50g
Grain		10 x 50g	1 x 50g
Stover		10 x 50g	1 x 50g
Fertilizer & amendments samples per site			
Urea			5 x 50g
TSP			5 x 50g
MOP			5 x 50g
Multi-Nutrient			5 x 50g
Manure			5 x 200g
Lime			5 x 50g

Amendments and fertilizer samples

At the time of manure application, five manure samples of 200 g for chemical, ash and moisture content analysis will be collected and submitted to the AfSIS labs in the node countries (Mali, Malawi, Tanzania and Kenya) for logging and milling to pass a 1-mm sieve. Sampling should be taken from different parts of a well-mixed manure heap. The samples will be labelled with (1) site name, (2) “Applied Manure”, (3) Sample number (1, 2, 3, 4 or 5) and (4) date of sampling. Fifty gram of the milled samples will be sub-sampled and submitted to ICRAF-Nairobi for reference analyses.

Five 50 g samples of bulked lime will be labelled and submitted to ICRAF-Nairobi for analysis.

For each type of fertilizer applied, five 50 g samples will be taken, clearly labelled with site, name of fertilizer and date and submitted to ICRAF-Nairobi for analysis.

9.2 Shipping procedures

Shipping procedures: The shipping procedure for samples is detailed in AfSIS operating procedure for regional laboratories and the steps are briefly explained:

1. In advance of shipment, send the details of your samples to the ICRAF Soil-Plant Spectral Diagnostic Lab at ICRAF Headquarters to: Keith Shepherd (k.shepherd@cgiar.org) copied to Elvis Weullow (e.weullow@cgiar.org). The information required is (a) a description of the material (e.g. air-dried 2 mm-sieved soil samples), (b) the number of samples, (c) the total

weight of the samples in the batch, and (d) name, institutional address and fax number of the scientist shipping the samples.

2. Obtain a phytosanitary certificate from your country's plant inspectorate authorities indicating that the samples are specifically meant for research purposes only and have no commercial value. Send the phytosanitary certificate or letter to the ICRAF laboratory.
3. Based on the above documentation, the ICRAF laboratory will obtain a Kenya import permit for the samples and will email you a scanned copy of the permit. The samples should be shipped together with a copy of the KEPHIS permit and your phytosanitary certificate or government letter.
4. The samples to be shipped should be carefully double-packed into strong polythene bags that cannot be easily ripped or damaged in transit, and packed into strong shipping cartons.
5. Ship the samples accompanied by the import permit and your phytosanitary certificate.
6. Immediately fax or email the shipping details (e.g. airway bill number) to Samuel Gaturu (s.gaturu@cgiar.org), Elvis Weullow (e.weullow@cgiar.org), and Mercy Nyambura (m.nyambura@cgiar.org), copied to Dr Keith Shepherd (k.shepherd@cgiar.org).

The ICRAF laboratory charges US$100 to cover all the expenses involved in sample clearance protocols, including KEPHIS fee, visits to the KEPHIS office, and clearance when the samples arrive.

10. Budgetary implications and practical considerations

10.1. Cost for shipment of samples and analyses

About 20 kg of soil will be shipped (in case no subsoil samples are included – otherwise add approx. 10 kg); the plant tissue, grain and stover samples will in total add another 4.5 to 5 kg and the fertilizer and manure and lime sampled together will add an additional 1.5 kg. The total weight to be shipped will be around 26.5 kg, with an estimate cost of 80$ per Kg this will amount to around US$ 2120.

The price for the AfSIS standard Wet Chemistry Soil Analysis' is around US$ 45 per sample. The price for the AfSIS Standard Leaf Analyses is around US$ 31.25 with the current exchange rate. Analysis of the 32 soil samples will amount to US$ 1440, with US$ 1000 to be added for the Standard Leaf Analyses, another US$ 1000 each for the grain and stover samples (reduced number of samples to be sent for reference analyses) and another US$ 300 for the analysis of the fertilizers, manure and lime. This will amount to a total cost of US$ 5860 per sentinel sites, based on 32 trails sites per sentinel site. The total cost will reduce proportionally if less trials sites have been established.

Cost of MIR for all the 1280 soil and plant samples from a sentinel site is about US$ 2000. Another 10% of the samples will cost about US$ 1100 for XRF analysis. Further, the 32 soil samples for X-Ray refraction analysis will cost about US$ 1500. Thus, these cost result to an additional US$ 4600.

Please note also that an additional cost at US$ 1.5 and US$ 2.5 per soil and plant sample, respectively, can result for samples sent to ICRAF before being processed.

10.2. Cost for fertilizers

In total 10 bags of fertilizers are needed for a full set of diagnostic trials. If we assume a cost of maximum 20US$ per bag the total cost will not exceed US$ 200. Then the agricultural lime and the manure still need to be purchased. The cost for manure will of course vary but generally will just cost a fraction of fertilizer. As mentioned, some fertilizer may not be readily available. If the fertilizer has to be imported the cost will be a multiple of the cost of the fertilizer itself. Also, if the fertilizer has to be transported from elsewhere the transport cost can contribute significantly to the cost.

10.3. The team, planning and labor requirements

Our experience is that the diagnostic trials (32 per site) can be conducted with a good and well trained team. If you would want to increase the number of diagnostic trials further you might easily be confronted with some logistical constraints. The windows of opportunities for planting and harvesting are often quite small. Also, you would not want too much time to elapse between the planting of the first field and the last field. This limits the number of trials that can be established by one particular team. One well trained team can establish and plant 32 trials within two weeks or 7 to eight days. The same applies for the harvesting of the trials or even a bit less if needed. It does require a well organized and prepared team. It also requires that transport is available for these days.

Please note that when requested, the AfSIS scientists can be very helpful in training teams to appropriately conduct diagnostic trials.

One field technician is required to manage and oversee all activities related to the diagnostic trials per sentinel site. The technician needs to be stationed in the field and should be facilitated with transport. The technician needs to be assisted by a team of preferably 5 or 6 persons. A team of 5 to 6 persons would be able to do the land preparation, planting, etc. Alternative arrangements exist where land preparation and other field activities like weeding is done by the farmer. Planting, fertilizer application and harvesting needs to be reserved for assistants trained for this task. In such a construction use could be made of extension workers for example, who could then also conduct the survey of management history, do the agronomic survey and other. In short, different constructions are possible that need to be grafted on the local situation and presence of staff of the NARES in the area. Based on the organization of the team, cost implications could be easily determined.

Further costs are associated with the cost for the scientist in charge and transport needed for regular visits to the field.