GUIDELINE FOR SOIL BIOLOGY DATA COLLECTION IN ETHIOPIA: NATIONAL STANDARD

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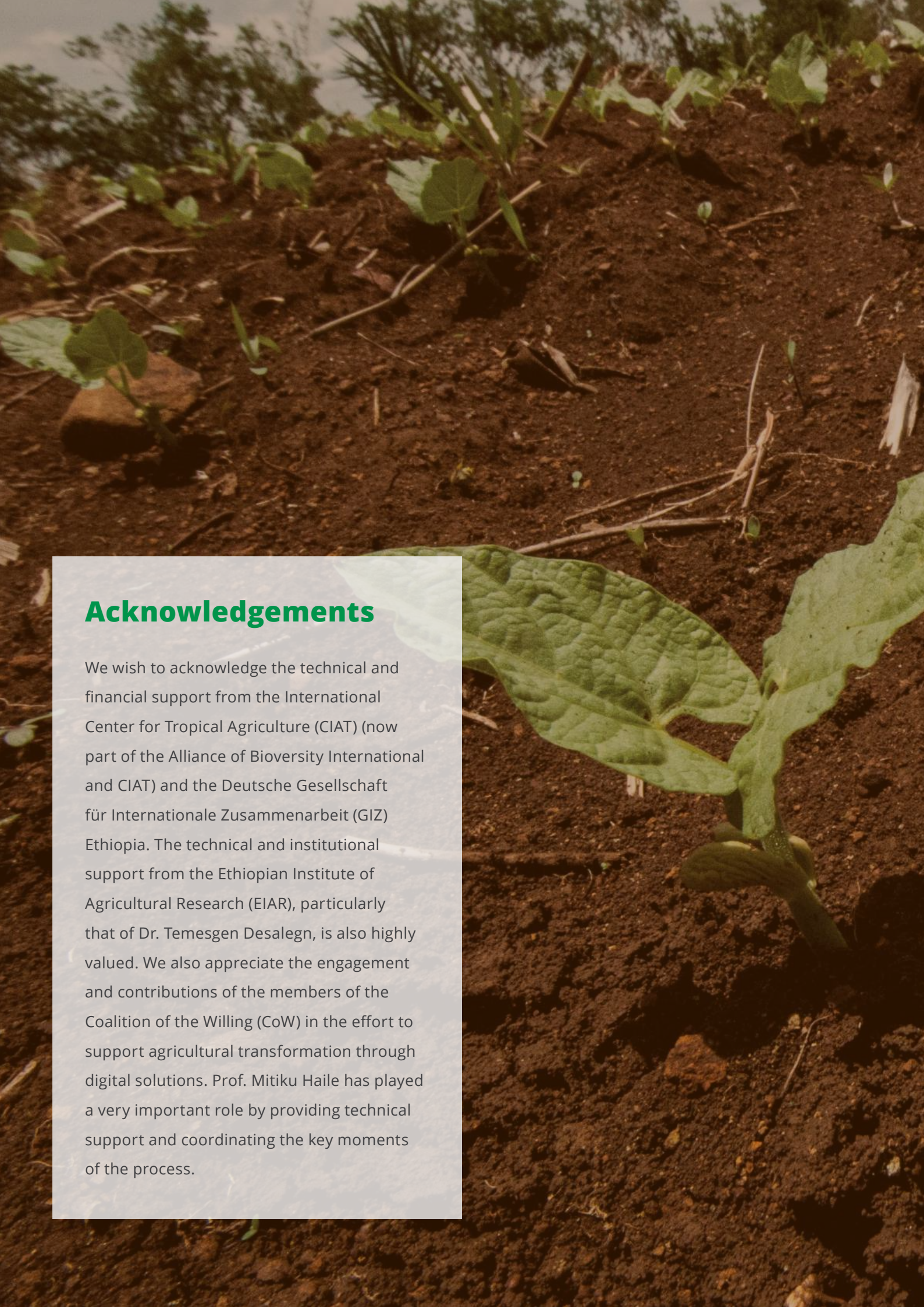

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Photo: CIAT/G. Smith

Photo: Neil Palmer

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Preface

Recently, recognition has been growing of the power of data and information for better decision-making and service provision in agriculture. To ensure good data quality, an agreed standard to collect, store, and share data along the agricultural value chain is required.

With this background, the purpose of this guideline is to provide guidance on standardizing soil biology data collection and thereby enhance temporal and spatial data interoperability.

Standard field research design, data collection, and data reporting are required for well-informed meta-analyses and syntheses of agricultural research data as well as for making these data more accessible for calibration and evaluation of process-based models. Hence, this guideline is a contribution toward enabling meta-analysis of different data collected over years and/or space to accumulate evidence and generate new knowledge or insights to facilitate informed decision-making in the agricultural sector in general and in the crop development subsector.

This guideline is compiled and intended for use by researchers, academicians, students, and other interested professionals in Ethiopia and beyond. The guideline is developed based on accepted standards and procedures in the field. Nevertheless, it is not exhaustive in its coverage of the soil biology data types and crops grown in the country. Hence, additions and updates depending on the development of research facilities, the ever-changing focus of agricultural research and production systems, and advances in technology are warranted.

Rationale

Aggregates of data are sources of technology, innovation, information, and knowledge. In addition, the generation of such data through a series of research activities and their documentation in a well-organized and usable way is the most important aspect of research and development. With the advent of agricultural research in Ethiopia, a wealth of soil biology datasets has started to be collected and will expand in extent with time.

Integration of these data enables new scientific discoveries, facilitates informed decision-making, and can transform the agricultural sector. Nevertheless, integration of data has been difficult because of the lack of uniformity in approaches and standards in data collection and measurement. Most data collected so far are held by individual researchers and only a few are published in journals and proceedings.

Many projects in the past several decades have generated data that are not accessible for data synthesis and model testing. Limited accessibility and non-interoperability of these datasets and poor infrastructure development have limited wider use of the data.

Ensuring the sustainability of agricultural systems has become increasingly complex and requires a coordinated, multifaceted approach in developing new knowledge and understanding. The collection of soil biology data using predetermined standards facilitates interoperability and integration and allows extended use of the data (Eagle et al., 2017; Kladivko et al., 2014). Hence, this guideline aims to set a standard in the collection of minimum datasets in research and development in soil biology.

The purpose and scope of this guideline are, therefore, limited to setting a standard for the collection of data and a minimum dataset on soil biology-related data for Ethiopia. It is assumed that detailed manuals for data collection and templates will be developed following the guideline.

Photo: Georgina Smith

Photo: Abera Mnalku

Introduction

The soil biota profile contains an enormous species diversity (more than 15,000 different species per gram of soil) that plays a major role in nutrient recycling and ecosystem functioning and servicing. The scope of this work, however, is limited to bacteria, fungi, and earthworms as they encompass the dominant soil-related services such as soil structure improvement (e.g., earthworms), nutrient supply regulation (e.g., diazotrophs, phosphate solubilizers, and mycorrhizae), litter transformation (e.g., micro-arthropods), and biocontrol (e.g., *Trichoderma, Beauveria*, etc.). For many years, a lot of scientific efforts have been made to manipulate soil biota to fully realize the benefits for development and environmental protection globally and locally. The hitherto data collection, measurement, and reporting approaches regarding soil biology are, however, inconsistent, without using state-of-the-art methods, and are key challenges in Ethiopia. These challenges often constrain the deployment of data sharing and metadata analysis that would ultimately help organize national information for development and policy making as a subsector.

This document contains the most important parameters and information recorded in laboratories, greenhouses, and fields across the different soil biota groups such as rhizobia, mycorrhizae, plant growth-promoting microbes (PGPMs), and earthworms from assessment (landscape) to designed (plot/farm) level research and monitoring scales. Moreover, the document attempts to include the minimum parameters to be considered during the estimation of bio-indicators of soil health and fertility at the farm or landscape level. As to the datasets, the following considerations are taken into account:

- GIS, soil and plant tissue testing, and some agronomy-related datasets are scarcely touched and details can be obtained from data standardization documents for Cross-

cutting; Soil, Water, and Plant Testing; and Agronomy themes, respectively, prepared in parallel sections.

- It is assumed that detailed dataset collection manuals and templates will be prepared as a follow-up to the guideline.
- This guideline contains datasets that can be measured in our capacities/facilities currently. Revisions can be made when more facilities or capacities are accessible.

The objective of this work is therefore to develop a standard for data collection protocols/guidelines for minimum datasets.

Rhizobia

Biological nitrogen fixation (BNF) is a process by which molecular nitrogen in the air is converted into ammonia (NH_3) or related nitrogenous compounds biologically. BNF is a viable option that can enhance crop yield sustainably, among several different types of biofertilizers that are known to affect plant growth and development. The most commonly mentioned microbes used as biofertilizers include nitrogen fixers, phosphate solubilizers, growth promotors, and decomposers. Rhizobia is a collective name for symbiotic bacteria capable of invading and forming roots or stem nodules on leguminous plants to convert atmospheric nitrogen (N_2) into ammonia (NH_3) in plant roots.

Rhizobial biofertilizers are the most highly exploited across the globe, and their use and importance are expanding in Ethiopia. Legume-rhizobia symbiosis plays an important role in sustainable agriculture. This technology can deliver enormous benefits through the judicious use of fertilizer, for example, phosphorus, and the exploitation of genetic diversity and symbiotic effectiveness of the hosts (leguminous plants) and their corresponding endosymbionts (rhizobia). To tap the potential benefits from rhizobia-legume symbiosis, it is essential to follow sequential steps, which go from nodule collection, isolation of rhizobia, and authentication to field evaluation of pure strains for N-fixing effectiveness (Appendix Figure 1).

Nodule collection and soil sampling

Nodules can be collected from the targeted legumes through (1) bio-prospecting wild relatives or landraces, (2) plant infection technique in growth pouches, or (3) growing plants in pots that contain soils with native rhizobia (Howieson and Dilworth, 2016; Mnalku et al., 2019). The simplified initial steps include the following:

- Mapping of potential collection sites and crops.
- Collecting and preserving nodules.
- Measuring soil total N, organic matter (%), pH, and available P (ppm) (refer to the Soil, Water, and Plant Testing Data Standardization Guideline, 2020).


Photo: Abere Mnalku

Isolation and authentication of rhizobia

Rhizobia are isolated from root nodules following the updated procedures of Howieson and Dilworth (2016). Desiccated nodules must be rehydrated before isolation. The authenticity of a pure culture of rhizobia must be proven by inoculation to a compatible legume. Isolates that do not form nodules are not considered rhizobia and are therefore discarded in the next steps. Under the authentication process, the legumes are assessed for only nodules.

Characterization of rhizobial specimens

Isolates should pass through routine checks for diagnostic features on various culture media

(Howieson and Dilworth, 2016; Mnalku et al., 2019). These checks involve the following:

- Cultural characteristics: colony diameter (mm) and colony texture (nominal)
- Growth characteristics (growth rate, acid base production)
- Physiological characteristics (salt, acid, pH, etc.) with +/- for presence and absence of colony growth
- Substrate use (carbon, nitrogen, etc.)
- Agrochemical tolerance of rhizobia: growth inhibition effect (%I) is computed as:

$$(\%I) = \frac{\text{OD of Control - OD of Treated}}{\text{OD of Control}} \text{ X } 100$$

or

$$\frac{\text{Radial Growth of Control - Radial Growth of Treated}}{\text{Radial Growth of Control}} \text{ X } 100$$

Counting rhizobia

Rhizobia are counted essentially to assess rhizobial populations in soil and how they vary, to follow the growth of cultures in the laboratory, or to assess the number and viability of rhizobia in commercial inoculants for quality control (Howieson and Dilworth, 2016). The population size of rhizobia in the soil guides the need for inoculation of the soil. The number of rhizobia in the soil is dynamic and varies within and between seasons, so any enumeration must be placed in context. The process involves the following:

- Serial dilution (up to 10^{-6})
- Plate counts of rhizobia in sterile diluent
- Indirect counts by plant infection to estimate most probable number of rhizobia
- Estimate of cell number by optical density (540 nm)
- Direct counts under a microscope

Performance evaluation of rhizobial specimens

The authenticated isolates are screened for their effectiveness in fixing nitrogen in relation to standard strains (reference strains), first in the greenhouse (in pot-sterile inert material and non-sterile soil) and then under field experiments in plots (on-farm). The experiments should include + and - nitrogen controls (Howieson and Dilworth, 2016).

Photo: Georgina Smith

Photo: Georgina Smith

Greenhouse experiments

The nitrogen controls are supplied with 0.5 g L^{-1} KNO_3 during the growth period of the plants and grown for at least 35 days, after which the plants will be harvested and evaluated for nodulation (nodule number and nodule dry weight), shoot dry weight, and other parameters, as follows.

PARAMETERS	UNITS	REFERENCES	REMARKS
Vigor rating	Unitless	Friedericks et al. (1990)	Vigor rating: 0 = dead; 1 = seedling growth only; 2 = 2 to 3 leaflets with yellowing; 3 = 2 to 3 green leaflets; 4 = 3 to 4 leaflets and 1 to 2 primary leaves; 5 = > 4 leaflets and primary leaves with no yellowing.
Nodule count (active and non-active)	no. plant $^{-1}$		Pink are active whereas white, green, and brown are non-active.
Nodule dry weight	mg plant $^{-1}$		Dry for at least 2 hours at 65 °C or air-dry for 1-2 days.
Nodule volume	cm^3		Measured by water displacement method.
Nodule position			Nominal (main root, lateral roots, root hairs).
Nodulation rating	%	Nif Tal (1979)	$R = \frac{(a*10)+(b*5)+(c*1)+(d*0)}{Total\ number\ of\ plants\ uprooted}$ where a = taproot, b = close to taproot, c = scattered nodules, and d = without nodulation.
Shoot total N per plant	%	Modified Kjeldhal (1954)	
Seed N	mg g $^{-1}$		

PARAMETERS	UNITS	REFERENCES	REMARKS
N derived from air	%	Howieson and Dilworth (2016)	N difference method.
Crude protein	%		Common for forage legumes.
Root dry weight	g plant^{-1}		
Shoot dry weight	g plant^{-1}		
Root-to-shoot ratio	Unitless		Dividing root dry weight by shoot dry weight.
Symbiotic effectiveness	%	Beck et al. (1993)	Proportion of inoculated dry mass to N-fertilized treatment.
Plant height	cm		
Dry biomass yield	kg ha^{-1}		Dried at 80 °C in oven for 2 days
Seed yield	kg ha^{-1}		Moisture is adjusted to 14%

Isolates are classified into highly effective, effective, and ineffective (Equation 1 or 2 below) and those with superior growth to or the same growth as the standard strain or the nitrogen controls are selected for the field work. The top-performing isolates are selected for further nitrogen-fixing potential.

$$\text{Strain Effectiveness (\%)} = \frac{\text{DWt of inoculated - DWt of uninoculated control}}{\text{DWt of Nitrogen control - DWt of uninoculated control}} * 100$$

or

$$\text{Strain Effectiveness (\%)} = \frac{\text{DWt of inoculated - DWt of uninoculated control}}{\text{DWt of standard strain - DWt of uninoculated control}} * 100$$

Where DWt is oven- dry weight biomass

Photo: Abere Mnalku

Photo: Abere Mnalku

Field (on-station or on-farm) evaluations

A few selected isolates, often the two top-performing ones, will be evaluated in the field in the presence of reference strains and nitrogen (+ and -) control treatments following an appropriate field layout (Appendix Figure 2). The treatments are the isolates (with 10^6 to 10^9 cells, the maximum number of rhizobia cells that can be added to achieve optimal yield); the nitrogen control (100 - 120 kg N ha^{-1}), measuring the yield of the legume when nitrogen is not limiting; the uninoculated control, measuring the potential of soil nitrogen and native rhizobia; and a standard strain. Nodulation, shoot biomass, biological nitrogen fixation, and grain yield are among the data that are recorded. When possible, it is best to measure the amount of nitrogen fixed using the natural abundance method. However, other methods such as nitrogen difference are also possible (Howieson and Dilworth, 2016).

Pre-planting information

The following information is important:

- Geographic coordinates (in decimal degrees)
- Topographic information (slope, aspect, steepness, etc.)
- Farm-gate price of labor and inputs
- Land-use/cropping history
- Climate history
- Most probable number (CFU per g of soil) (Woomer, 1994; Howieson and Dilworth, 2016)
- Important soil physicochemical characteristics such as organic matter, pH, Total N, Exchangable acidity, available and total P, textural class, micronutrients, EC, etc. (appropriate methodology can be referred from to the Soil, Water, and Plant Testing Data Standardization Guideline, 2020).

Post-planting data required

The following data are required on post-planting.

PARAMETERS	UNITS
Nodule count	nodule number plant $^{-1}$
Nodule dry weight	mg plant $^{-1}$
Nodule volume	cm^3
Nodule position	Nominal (main root; lateral roots; root hairs)
Nodulation rating	%
Total N in plant	%
Nitrogen derived from the air	%
Root dry weight	g plant $^{-1}$
Shoot dry weight	g plant $^{-1}$
Root-to-shoot ratio	Unitless
Seedling vigor	Refer to Section 4.2.3
Plant height	cm
Biomass yield	kg ha^{-1}
Grain yield	kg ha^{-1}, at seed moisture adjusted to 14%
Farm-gate price of outputs and inputs (inoculant, labor, fertilizer, straw, seed, etc.)	USD
Weather information in the growing season	mm, min, and max ^{o}T, daily or monthly total rainfall (mm)
P-use efficiency	% (can be adapted from Agronomy Data Standardization Guideline, 2020)
N-use efficiency	% (can be adapted from Agronomy Data Standardization Guideline, 2020)

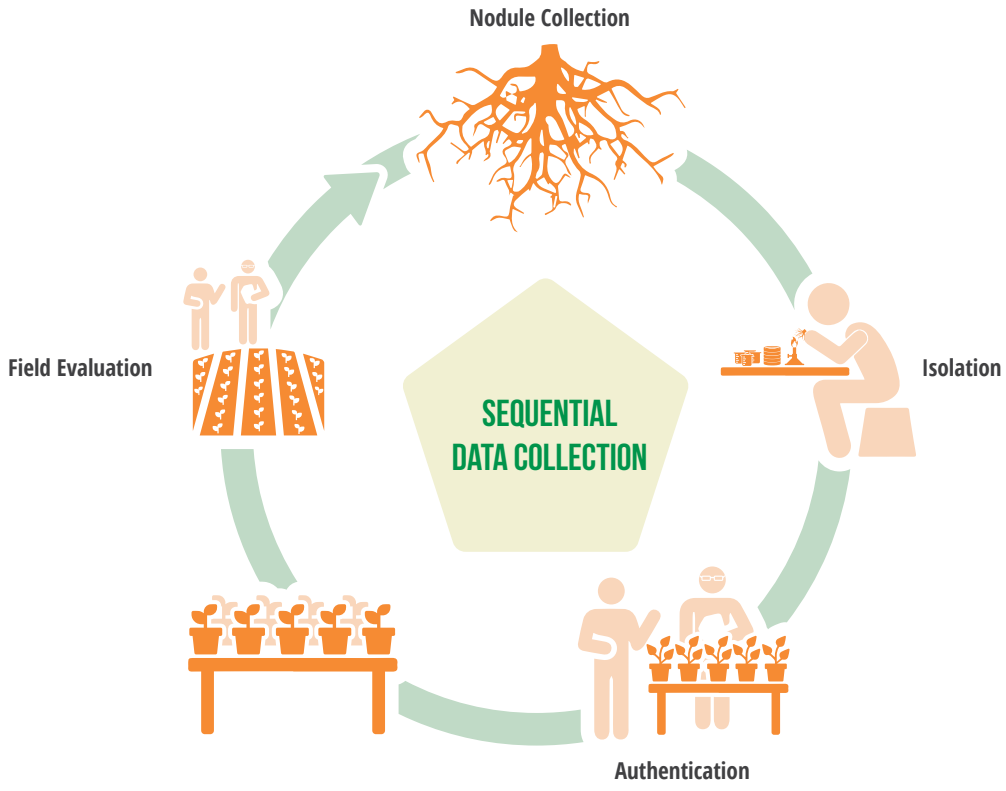


Illustration 1: Sequential data collection activities of plant growth promoting rhizobacteria.

Mycorrhizae

Arbuscular mycorrhizal fungi (AMF) are one of the mycorrhizal groups that colonize roots of more than 80% of higher plants, particularly in the tropics (Smith and Read, 2008). In relation to soil, mycorrhizal symbiosis enhances the formation and stability of soil aggregates via a complex glycoprotein (glomalin) (Wright and Upadhyaya, 1998) and uptake of N, P, and water (Jakobsen, 1999). Though growing AMF on growth medium was a great challenge and difficulty, its inoculum has been produced for use in agroforestry, horticulture, landscape restoration, and site remediation for almost two decades.
The manipulation of these organisms thus starts from acquisition from their natural habitat as indicated below.

Collection of mycorrhizae

The following steps are involved (Schenck and Perez, 1990; INVAM, 2019):

- Soil will be collected from the rhizosphere of the plant
- Spores will be extracted from the soil
- Trap culture will be used to obtain monospecific culture
- Quantification will be done via a dissecting microscope
- Identification is based on spore morphology

Characterization of mycorrhizal specimens

The following steps are involved (Schenck and Perez, 1991; INVAM, 2019):

- Spore count (no. 100 g^{-1} dry soil)
- Spore size (mm)
- Spore ornamentation/color (qualitative)
- Spore wall structure
- Hyphal attachment of spores (presence or absence of stalk)
- Root colonization of mycorrhizae (mycorrhization) (%) is measured following Wang and Jiang (2015) method: Percent colonization = (Total number of infected roots intersecting gridlines/total number of roots intersecting gridlines) × 100

Performance evaluation of mycorrhizal specimens

Estimation of AMF colonization (%) is carried out by cleaning with 10% KOH and clearing with 2% HCl and then staining with trypan blue. The gridline intersection method will be used (INVAM, 2019):

- Plant tissue total P (%)
- Seedling establishment (%)
- P-use efficiency (refer to Section 1.5.2)
- N-use efficiency (refer to Section 1.5.2)
- Plant height (cm)

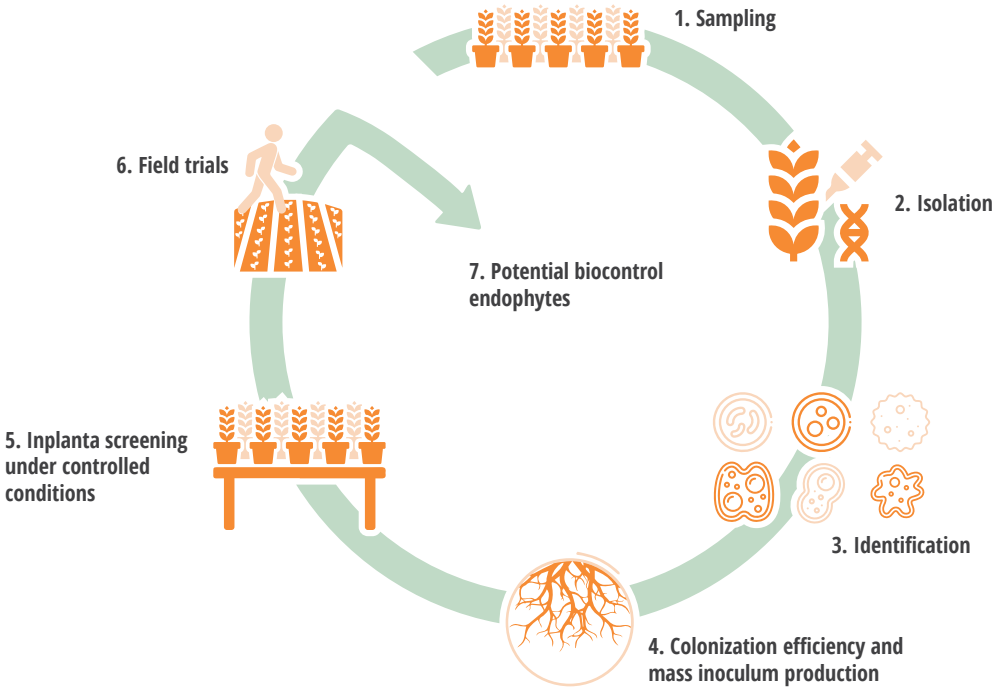


Illustration 2: Mycorrhizal development (Adapted from Cambridge university press, 2019)

Plant growth-promoting microbes

Plant growth-promoting microorganisms (PGPMs) are microorganisms that colonize the surface and inner tissues of roots and promote plant growth and health (Drogue et al., 2012; Sharma et al., 2013). Since almost 90% of these microorganisms are bacteria, they are often called plant growth rhizobacteria (PGPR). PGPR that enter and colonize interior plant tissues are known as endophytes. More than 30 bacterial genera have been recorded so far as PGPR, the most dominant ones being Pseudomonas, Bacillus, Azotobacter, and Rhizobium (Antoun and Prévost, 2005).

PGPR have many biochemical properties to stimulate plant growth (Glick, 2012). The most important direct or indirect plant growth enhancement mechanisms are nutrient acquisition (asymbiotic N-fixation, phosphate solubilization, and siderophore production), modulating phytohormones (direct mechanism), and the ability to act as a biocontrol against phytopathogens through various forms of antagonism such as competition and the production of antibiotics, lytic enzymes, and hydrogen cyanide. Nowadays, these microorganisms are selected and commercialized as bio-stimulants and bio-pesticides with different trademarks for the production of many horticultural and forest products and expected to have a market share of more than USD 5.83 billion by 2023 (Timmusk et al., 2017).

Collection of PGPMs

Their collection involves the following:

- Collection of soil sample
- Isolation of microbes in the laboratory
- Identification of microbes
- Evaluation in vitro, in pots, and in field conditions

Characterization of PGPM specimens

The following shows the items involved in characterizing PGPM specimens.

PARAMETERS	REMARKS/REFERENCES
Cultural characteristics	Colony diameter (mm), colony texture
Physiological characteristics	Growth rate, acid base production
Functional characteristics	Plant growth promoting
Phosphate solubilization on solid medium	Edi-Premono et al. (1996)
P solubilization on liquid medium	On different inorganic P sources
Production of phytohormones (such as IAA)	Bric et al. (1991)
Siderophore production	Schwyn and Neilands (1987)
Growth inhibition	% inhibition effect over control (Landa et al., 1997)
Enzyme assay	Chitinase and protease production (Ryden et al., 1973)

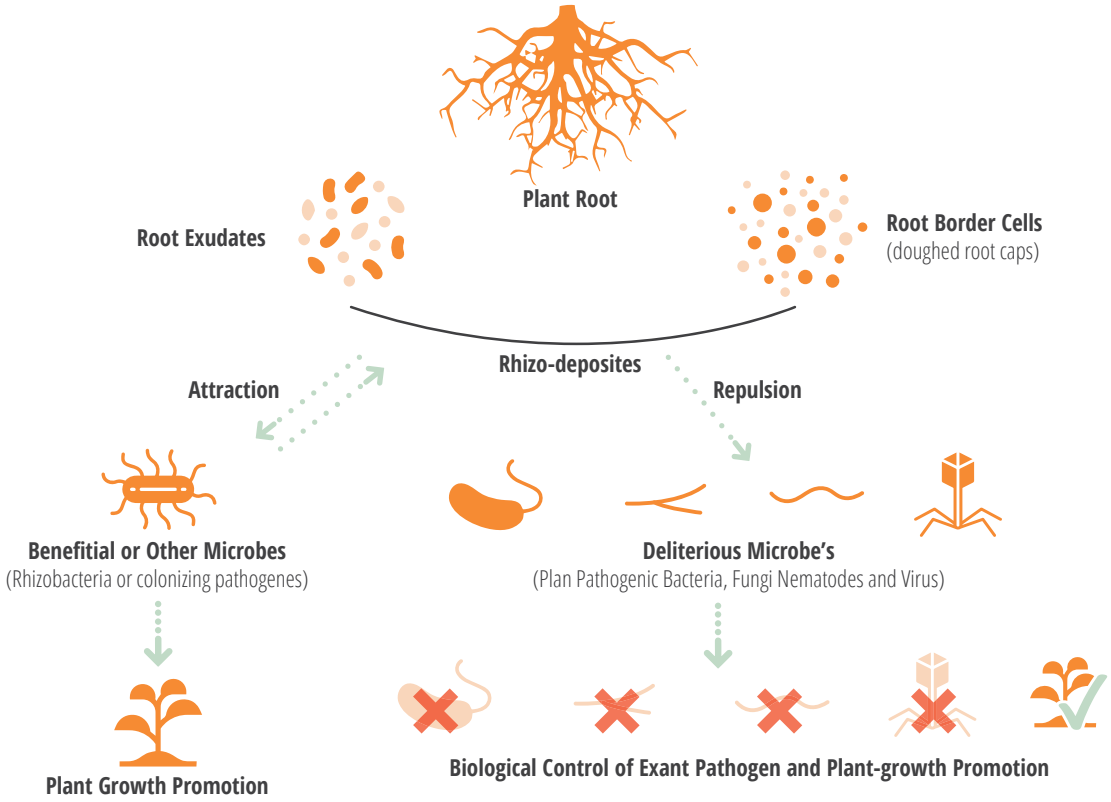


Illustration 3: Interaction of PGPMs in the rhizosphere (Smith, 2029)

Performance evaluation of PGPM specimens in greenhouse/field

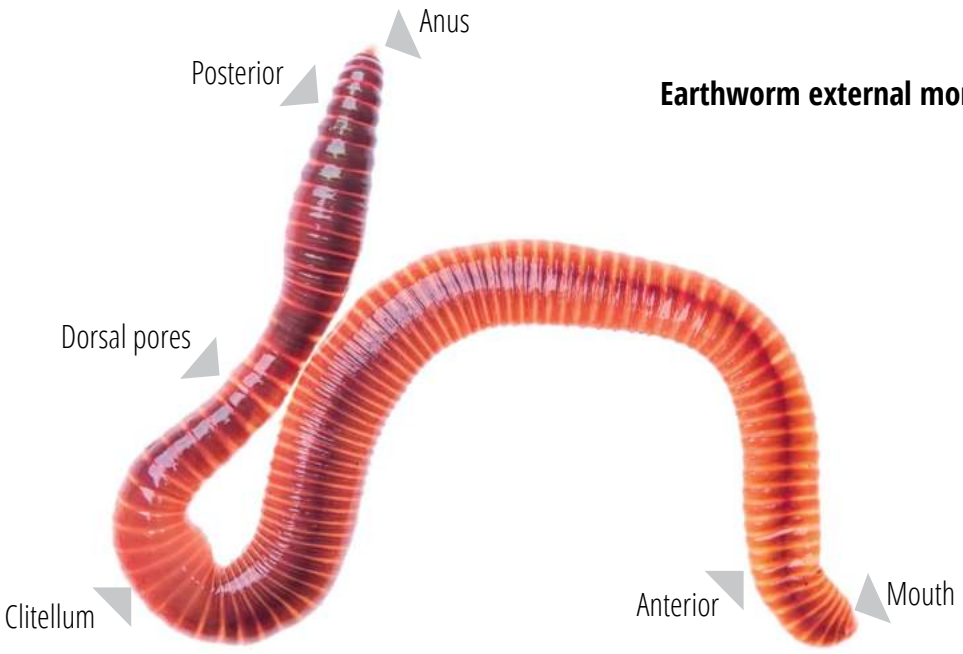
The following shows the parameters to be evaluated.

PARAMETERS	REMARKS/REFERENCES
Plant tissue N	%
Biomass yield	kg ha^{-1}
Grain/tuber/fruit yield	kg ha^{-1}
Plant tissue total P	ppm
Plant height	cm
Disease incidence and severity scaling	%
P-use efficiency	Refer to Section 1.5.2
N-use efficiency	Refer to Section 1.5.2

Earthworms and vermicompost

Environmental degradation is a major threat confronting the world, and the rampant use of chemical fertilizers contributes largely to the deterioration of the environment through excess use of fossil fuels, generation of carbon dioxide (CO_2), and contamination of water resources. Now, a growing realization exists that the adoption of ecological and sustainable farming practices can reverse the declining trend in global productivity and protect the environment (Wani et al., 1995). Earthworms are important biological organisms that help nature to maintain nutrient flows from one system to another and minimize environmental degradation. For a range of agricultural residues, all dry wastes

can be converted into vermicompost. In short, earthworms, through a type of biological alchemy, are capable of transforming garbage into gold (Crescent, 2003).



Vermicomposting is a simple biotechnological process of composting in which certain species of earthworms are used to enhance the process of waste conversion and produce a better end-product.

Vermicomposting differs from composting in several ways. Sustained vermiculture practices and the use of vermicompost improve the moisture-holding capacity of soil, which decreases water for irrigation. Vermicompost also improves the physical, biological, and chemical properties of soil, soil porosity, and softness of soil. Ample opportunities also exist for a decrease in uses of energy and greenhouse gas emissions in vermicompost production locally on farms by the farmers themselves (Hussani, 2012; Singh, 1993). The cost of producing vermicompost is insignificant compared with that of chemical fertilizers. The rejuvenation of degraded soils by protecting topsoil and the sustainability of productive soils are major concerns internationally.

Collection of earthworms

Collecting earthworms involves the following steps (Brown, 2018):

- Mapping potential collection area
- Soil pit sampling
- Total abundance (# m^{-2}): count of adult earthworms per square meter
- Biomass (g m^{-2}): live weight of adult worms per square meter
- Ratio of adults to juveniles (with no clitellum)
- Preserve, record, and identify the adults

Characterization

It is essential to characterize the earthworms, the substrate (feedstock and bedding materials), the vermicompost, and the vermiwash following standard methods. This process follows.

Characterization of earthworm specimens

MORPHOLOGICAL PARAMETERS	UNITS/REMARKS/REFERENCES
Body length	mm (total length from head to tail)
Pigmentation	Qualitative
Total number of segments	no.
Number of setae	no.
Clitellum width	mm
Position of female pore	n^{th} segment from head
Position of male pore	n^{th} segment from head
Growth/Multiplication Parameters	
Initial total matured worms	no.
Final total matured worms	no.
Initial total biomass	g
Final total biomass	g
Rate of increase in worm number	%
Rate of increase in worm weight (biomass gain)	%
Individual initial body weight	g
Individual final body weight	g
Individual weight gain	%
Individual initial length	cm
Individual final length	cm
Length increment	%
Growth rate determination	$R = \frac{\text{End EW biomass (mg) - Initial EW biomass (mg)}}{\text{Time period (days)}}$, where EW = earth worm (Suthar, 2005)

MORPHOLOGICAL PARAMETERS	UNITS/REMARKS/REFERENCES
Cocoon count	Number of cocoons laid week $^{-1}$
Count of cocoon production	Cocoon production worm^{-1} day^{-1} (Ismail, 1997)
Mortality rate of worms	%
Biomass conversion rate	Lalander et al. (2015)
Proximate Analysis (For Processed Earthworms)	
Crude protein	%
Ash	% (Srilakshmi, 2014)
Dry matter	%
Earthworm Evaluation Based On Vermicomposting	
Vermicomposting period	Number of days No of days, From day 1 to harvest
Vermicompost yield	kg kg, weight of air-dry vermicompost produced
Vermicompost quality	See section 4.2.3
Vermicomposting rate	$VR = \frac{\text{Final compost dry weight (kg)}}{\text{Initial substrate dry weight (kg)}} * 100$
Earhworms Potential As Chicken Feed	
Average daily weight gain	$g,W = \frac{\text{Wieght gained at time t (g)}}{\text{Day chicken}}$
Feed consumption	g d^{-1}
Feed conversion ratio	$FCR = \frac{\text{Average daily feed intake (g)}}{\text{Average daily weight gain (g)}}$
Average egg weight	g g
Total egg production	# no.
Egg quality	(albumen, shell and yolk weight, and shell thickness)

Characterization of feedstocks and bedding materials

The following parameters are involved in characterizing feedstocks and bedding materials.

PARAMETERS	UNITS
pH	Unitless
EC	dS m^{-1}
Total N	%
Total P	g kg^{-1}
Total K	%
Organic carbon	%
Ash	%
C/N	ratio (dividing %C by %N)
C/P	ratio (dividing %C by %P)
Water holding capacity	Ahn et al., 2005

Characterization of vermicompost

The following parameters are involved in characterizing matured vermicompost.

CHEMICAL CHARACTERISTICS	
PARAMETERS	**UNITS**
Total organic carbon	%
Total nitrogen	% (Bremner and Mulvaney, 1982)
Ammonium/nitrate (NH_4:NO_3)	Ratio
C/N	Ratio (%C divided by %N)
Total phosphorus (P_2O_5)	g kg^{-1} (John, 1970)
Total potassium (K_2O)	K_2O (%)
Total calcium (Ca)	%
Total magnesium (Mg)	%
pH	1:10 w/v (Bhat et al., 2017) vermicompost in g: distilled water in ml
EC	dS m^{-1} (1:10 w/v)
Moisture content	% (gravimetric water content): (weight of water/weight of dry vermicompost) × 100

CHEMICAL CHARACTERISTICS	
PARAMETERS	**UNITS**
PLANT GROWTH PROMOTION CHARACTERISTICS	
Vermicompost Efficacy Test in Pot/Nursery	
Shoot length	cm
Root length	cm
Germination index (GI)	$GI(\%) = x \frac{\text{Seed germination} * \text{Root length of treatment}}{\text{Seed germination \%} * \text{Root length of control}} 100$ (Bhat et al., 2017)
Seedling vigor index	*Germination percentage×(root length+shoot length)*
Shoot dry weight	g
Chlorophyll content	µg cm^{-2} , Darvishzadeh et al. (2008)
VERMICOMPOST EFFICACY TEST IN ON-STATION/ON-FARM	
Historical and Biophysical Characteristics of the Site	
Geographic coordinate	In decimal degrees
Topographic information	%
Farm-gate price of labor and inputs	USD ha^{-1}
Land-use pattern	nominal
Cropping history	nominal
Climate history	nominal
Post-planting Data Required	
Seedling vigor	*Germination percentage×(root length+shoot length)*
Plant height	centimeter (cm)
Shoot dry weight	g
Grain yield	kg ha^{-1}
Harvest index	%: (Grain yield/biomass yield) × 100
Straw biomass	kg ha^{-1}
N-use efficiency	Refer to Section 1.5.2
P-use efficiency	Refer to Section 1.5.2

Vermiwash/vermicompost tea

Vermiwash is the brownish-red liquid that comes from the body of earthworms and vermicompost filtration. The following parameters will be required to describe it in minimum detail.

PARAMETERS	UNITS/REFERENCE
pH	1:10 w/v
Electric conductivity (EC)	dS m^{-1} (1:10 w/v)
Organic carbon	%
Available N	ppm
Dissolved oxygen	mg L^{-1} (APHA, 2005)
Available P	%
Available K	%

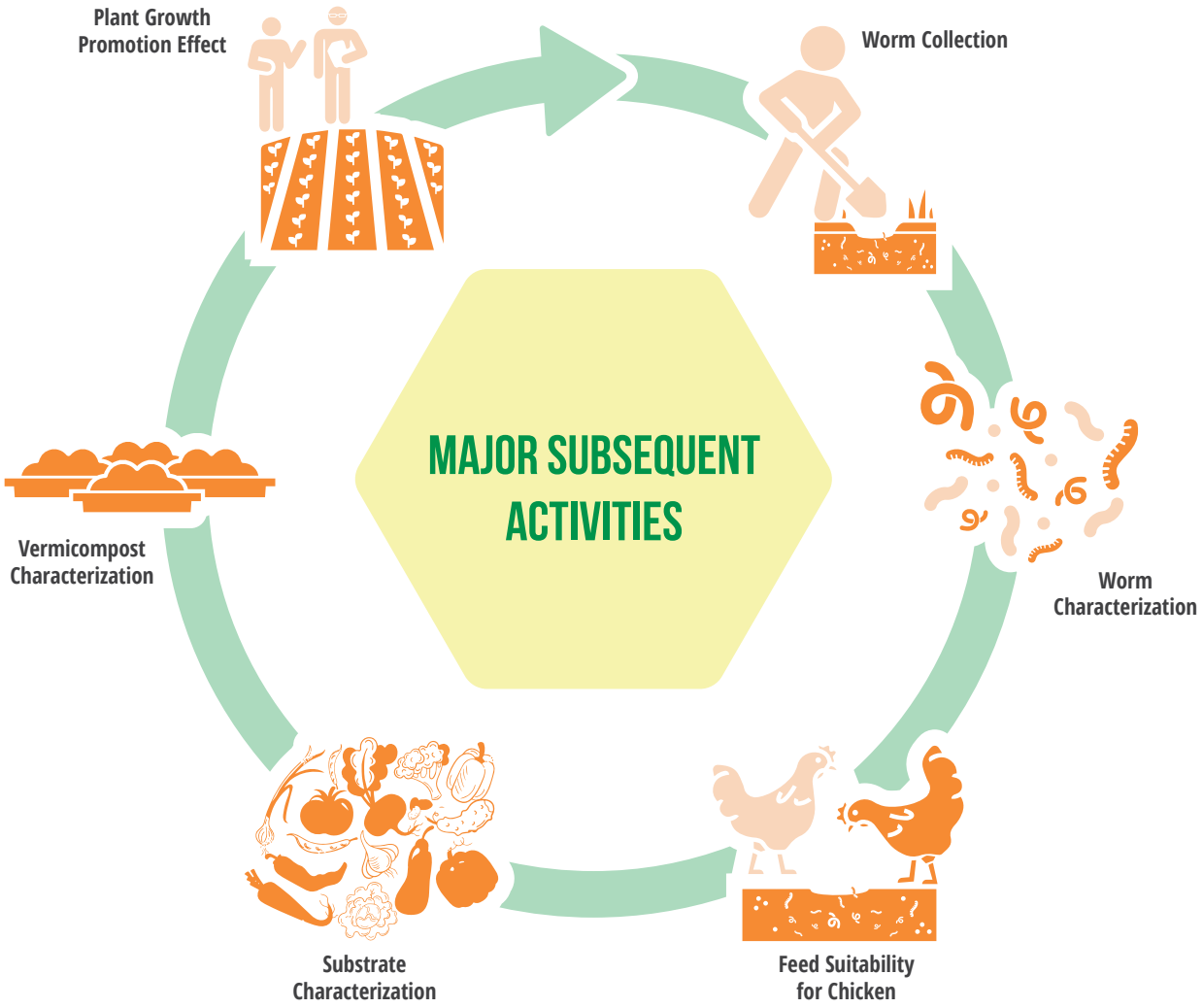


Illustration 2: Major subsequent activities of earthworm and vermicompost research.

Bio-indicators of soil quality and sustainable use

Like physical and chemical indicators, biological indicators have a relationship to soil functions and can evaluate these functions to assess soil quality. These indicators respond rapidly to soil management and land-use changes and can be candidates for soil quality indicators. Limitations exist, however, in directly measuring soil organisms as indicators of soil quality. Because of this, biological dynamic properties [respiration, POM (particulate organic matter), PMN (potentially mineralizable nitrogen), and microbial biomass] are often regarded as the minimum dataset to describe the microbial part of soil organisms while the rest measure soil quality and fertility.

Physical appearance of matured vermicompost (Photo: Abere Mnalku)

- *Soil respiration* measures the potential N or C mineralization role of soil biota (Ryan and Law, 2005).
- *Particulate organic matter (POM)* comprises all soil organic matter (SOM) particles less than 2 mm and greater than 0.053 mm in size. POM is biologically and chemically active, is part of the labile (easily decomposable) pool of SOM, and is estimated according to Diovisalvi et al. (2014).
- *Potentially mineralizable nitrogen (PMC)* (mg $N\ kg^{-1}\ d^{-1}$) is the fraction of nitrogen easily decomposable by soil microorganisms and is considered an indirect measure of nitrogen availability during the growing season (Piconne et al., 2002).
- *Soil microbial biomass (SMB)* C (µg $C\ g^{-1}$ dry soil) is measured by the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978).

where B is the mean volume of HCl consumed by blanks (mL), S is the mean volume of HCl consumed by samples (mL), 4 is incubation time (h), 100 is a conversion factor (100 g DM), 2.2 is a conversion factor (1 mL 0.1 M HCl corresponds to 2.2 mg CO_2), SW is initial soil weight (g), and DM is soil dry matter (%). A respiratory quotient of one is assumed.

- *Soil microbial biomass nitrogen* (SMBN): the fumigation-extraction procedure according to Solaiman (2007) is the determination way and often reported in mg $N\ kg^{-1}$ dry soil.
- *Soil organic matter (%):* see Nelson and Sommers (1982).
- *Soil aggregate stability index* (SASI): ,

where A and B are the weights of aggregates passed through a 0.25-mm sieve after 5 and 60 min, respectively (Pagliai et al., 1997).

- *Soil bulk density:* see (Al-Shammary et al., 2018).
- *Soil organic carbon (SOC)*

where SOCi = soil organic carbon of a given soil depth, mg C ha^{-1}; BD (bulk density) = soil mass per sample volume, kg soil m^{-3} (equivalent to kg m^{-3}); di = horizon, depth, or thickness of soil layer, m; and CFi = % volume of coarse fragments/100, dimensionless. Coarse fragments can be determined as percentage weight of soil greater than 2 mm.

- *Microbial abundance:* The Gram-positive bacteria, Gram-negative bacteria, fungi, and actinomycetes could be enumerated using the dilution plate count technique; see (Acea and Carballas, 1996; Tateishi et al., 1989; Mabuhay et al., 2004).
- *Litter decomposition:* A common method for estimating decomposition rates is to use litter bags, detail is found on Moore and Basiliko (2006).

References

Al- shammary, A.A.; Kouzani, A.Z; Kaynak, A.; Khoo, S.Y.; Norton, M; Gates, W. (2018). Soil bulk density Estimation methods: A review. *Pedosphere* 28 (4):581–596.

Acea, M.J.; Carballas, T. (1996). Changes in physiological groups of microorganisms in soil following wildfire. *FEMS Microbiology Ecology* 20: 33–39.

Ahn, H.; Richard, T. L.; Glanville, T. D.; Harmon, J. D.; and Reynolds, D. L. (2005). Estimation of optimum moisture levels for biodegradation of compost bulking materials. Agricultural and Biosystems Engineering Conference Proceedings and Presentations. 112. https://lib.dr.iastate.edu/abe_eng_conf/112.

Anderson, J.P.E.; Domsch, K.H. (1978). A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biology and Biochemistry* 10:215–221.

Antoun, H.; Prévost, D. (2005). Ecology of Plant Growth Promoting Rhizobacteria. In: PGPR: Biocontrol and Biofertilization. Springer, Netherlands, p. 1–38.

APHA (American Public Health Association). (2005). Standard Methods for the Examination of Water and Wastewater. 21st ed. Eaton, A.D.: Clesceri, L.S.; Rice, E.W.; Greenberg, A.E. (eds.). APAH-AWWA-WEF, Washington, D.C.

Aynalem, B.; Assefa, F. (2017). Effect of glyphosate and mancozeb on the rhizobia isolated from nodules of *Vicia faba* L. and on their N2-fixation, North Showa, Amhara Regional State, Ethiopia. *Advances in Biology*, Vol. 2017, Article ID 5864598, 7 pages. DOI: 10.1155/2017/5864598

Beck, D.P.; Materon, L.A.; Afandi, F. (1993). Practical Rhizobium-legume technology manual. Technical Manual No. 19. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

Bhat, S.A.; Singh, J.; Vig, A.P. (2017). Instrumental characterization of organic wastes for evaluation of vermicompost maturity. *Journal of Analytical Science and Technology* 8:2.

Bremner, J.M.; Mulvaney, R.G. (1982). Nitrogen-total. In: Page, A.L.; Miler, R.H.; Keeney, D.R. (eds.). Methods of soil analysis. Part 2. American Society of Agronomy, Madison, Wisconsin, USA. p. 595–624.

Bric, J.M.; Bostoc, R.M.; Silverstone, S.E. (1991). Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. *Applied and Environmental Microbiology* 57:534–538.

Brown, K.D. (2017). Earthworm Recorder's Handbook. Earthworm Society of Britain. 36 p. www.earthwormsoc.org.uk.

Crescent, T. (2003). Vermicomposting. Development Alternatives (DA) Sustainable Livelihoods. http://www.dainet.org/livelihoods/default.htm.

Darvishzadeh, R., Skidmore, A.; Schlerf, M.; Atzberger, C.; Corsi, F.; Cho, M. (2008). Estimation of leaf area index and chlorophyll for a Mediterranean grassland using hyperspectral data. International Archives of Photogrammetry, Remote Sensing and Spatial Information Science 37: Part B7.

Diovisalvi, N.V.; Studdert, G.A.; Calvo, N.I.R.; Dominguez, G.F.; Belardo, A. (2014). Estimating soil particulate organic carbon through total soil organic carbon. *Ciencia del Suelo* (Argentina) 32(1):85–94.

Drogue, B.; Dore, H.; Borland, S.; Wisniewski-Dyé, F.; Prigent-Combaret, C. (2012). Which specificity in cooperation between phytostimulating rhizobacteria and plants? *Research in Microbiology* 163:500–510.

Drouin, P.; Sellami, M.; Prevost, D.; Fortin, J.; Antoun, H. (2010). Tolerance to agricultural pesticides of strains belonging to four genera of Rhizobiaceae. *Journal of Environmental Science and Health* Part B 45:780–788.

Eagle, A.J.; Christianson, L.E.; Cook, R.L.; Harmel, R.D.; Miguez, F.E.; Qian, S.S.; Ruiz Diaz, D.A. 2017. Meta-analysis constrained by data: recommendations to improve relevance of nutrient management research. *Agronomy Journal* 109(6):2441-2449.

Edi-Premono, M.A.; Moawad, X.; Vleck, P.L.G. (1996). Effect of phosphate solubilizing Pseudomonas putida on growth of maize and its survival in the rhizosphere. *Indonesian Journal of Crop Science* 11:13–23.

Friedericks, J.B.; Hagedorn, C.; Vanscoyoc, S.W. (1990). Isolation of *Rhizobium leguminosarum* (biovar trifolii) strains from Ethiopian soils and symbiotic effectiveness on African annual clover species. *Applied and Environmental Microbiology* 56(4):1087–1092.

Glick, B.R. (2012). Growth-promoting bacteria: Mechanisms and applications. *Scientifica*:1–15.

Howieson, J.G.; Dilworth, M.J. (Eds.). (2016). Working with rhizobia. Australian Centre for International Agricultural Research, Canberra.

Hussaini, A. (2012). Vermiculture biotechnology: An effective tool for economic and environmental sustainability.

INVAM (2018) International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi. West Virginia University.

Ismail, S.A. (1997). Vermicolgy: Biology of earthworms. Chennai: Orient Longman Limited.

Jakobsen, I. (1999). Transport of phosphorus and carbon in arbuscular mycorrhizas: Structure, function, molecular biology. 2nd. Edn. Springer, Heidelberg. p. 535–542.

John, M.K. (1970). Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. *Soil Science* 109:214–220.

Kladivko, E.J.; Helmers, M.J.; Abendroth, L.J.; Herzmann, D.; Lal, R.; Castellano, M.J.; ... Villamil, M.B. 2014. Standardized research protocols enable transdisciplinary research of climate variation impacts in corn production systems. *Journal of Soil and Water Conservation* 69(6):532-542. DOI: 10.2489/jswc.69.6.532

Lalander, C.H.; Allan, J.K.; Vinneras, B. (2015). Vermicomposting as manure management strategy for urban small-holder farms: Kampala case study. *Waste Management* 39:96–103.

Landa, B.B.; Hervas, A.; Bethiol, W.; Jimenez-Diaz, R.M. (1997). Antagonistic activity of bacteria from the chickpea rhizosphere against *Fusarium oxysporum* f. sp. ciceris. *Phytoparasitica* 25:305–318.

Mabuhay, J.A.; Nakagoshi, N.; Isagi, Y. (2004). Influence of erosion on soil microbial biomass, abundance and community diversity. *Land Degradation and Development* 15: 183–195.

Mnalku, A.; Negash, D.; Daniel, M.; Yifru, A.; Getahun, M. (2019). Rhizobial Inoculant Development and Management Manual. Ethiopian Institute of Agricultural Research. Addis Ababa, Ethiopia.

Moore T.R.; Basiliko N. (2006). Decomposition in boreal peatlands. In: Wieder R.K. & Vitt D.H. (eds.), Ecologial studies, Springer-Verlag, Berlin, p. 125–143.

Nelson DW.; Sommer, L.E. (1982) Total carbon, organic carbon, and organic matter. Methods of soil analysis, Part 2. Chemical and microbiological properties, 2nd edition. ASA-SSSA, Madison, 595–579.

Nif Tal (1979). Legume Inoculation Trials Procedures, Nif Tal Project, University of Hawaii, Honolulu, Hawaii, USA.

Pagliai, M.; Torri, D.; Patruno, A. (1997). Stabilita`e distribuzione dimensionale degli aggregati. In: Pagliai, M. (Ed.), Metodi dianalisifisica del suolo. Franco Angeli, Roma, Parte V, p. 1–9.

Piconne, L.I.; Cabrera, M.L.; Franzluebers, A.J. (2002). A rapid method to estimate potentially mineralizable nitrogen in soil. *Soil Science Society of America Journal* 66:1843–1847.

Ryan, M.G.; Law, B.E. (2005). Interpreting, measuring, and modeling soil respiration. *Biogeochemistry* 73:3–27.

Ryden, A.; Lindberg, M.; Philipson, L. (1973). Isolation and characterization of two protease-producing mutants from Staphylococcus aureus. *Journal of Bacteriology* 116:25–32.

Schenck, N.C.; Perez, Y. (1990). Manual for identification of vesicular arbuscular mycorrhizal fungi. (INVAM). University of Florida, Gainesville.

Schwyn, B.; Neilands, J.B. (1987). Universal CAS assay for the detection and determination of siderophores. *Analytical Biochemistry* 160:47–56.

Sharma, S.K.; Ramesh, A.; Johri, B.N. (2013). Isolation and characterization of plant growth-promoting *Bacillus amyloliquefaciens* strain sks_bnj_1 and its influence on rhizosphere soil properties and nutrition of soybean (*Glycine max* L. Merrill). *Journal of Virology and Microbiology* 2013:1-19.

Singh, R.D. (1993). Harnessing the Earthworms for Sustainable Agriculture. Institute of National Organic Agriculture, Pune, India. p. 1–16.

Smith, S.E.; Read, D. (2008). Mycorrhizal Symbiosis. 3rd edition. https://doi.org/10.1016/B987-0-12-370526-6.X5001-6

Solaiman, Z. (2007). Measurement of microbial biomass and activity in soil. *Advanced Techniques in Soil Microbiology* 11:201–211.

Srilakshmi, G.P. (2014). Determination of physicochemical properties and morphological observation in *Syzygium cumini* leaf. *American Journal of Phytomedicine and Clinical Therapeutics* 2(2):174–179.

Suthar, S. (2005). Potential utilization of guar gum industrial waste in vermicompost production. *Bioresource Technology* 97:2474–2477.

Tateishi, T.; Horikoshi, T.; Tsubota, H.; Takahashi, F. (1989). Application of the chloroform fumigation-incubation method to the estimation of soil microbial biomass in burned and unburned Japanese red pine forests. FEMS Microbiology Ecology 62: 163–172.

Timmusk, S.; Behers, Lawrence, Muthoni, j.; Muraya, A.; Aronsson, Anne-Charlotte. (2017) Perspectives and challenges of microbial application for crop improvement. *Frontiers in Plant Sciences* 8:49. DOI: 10.3389/fpls.201700049

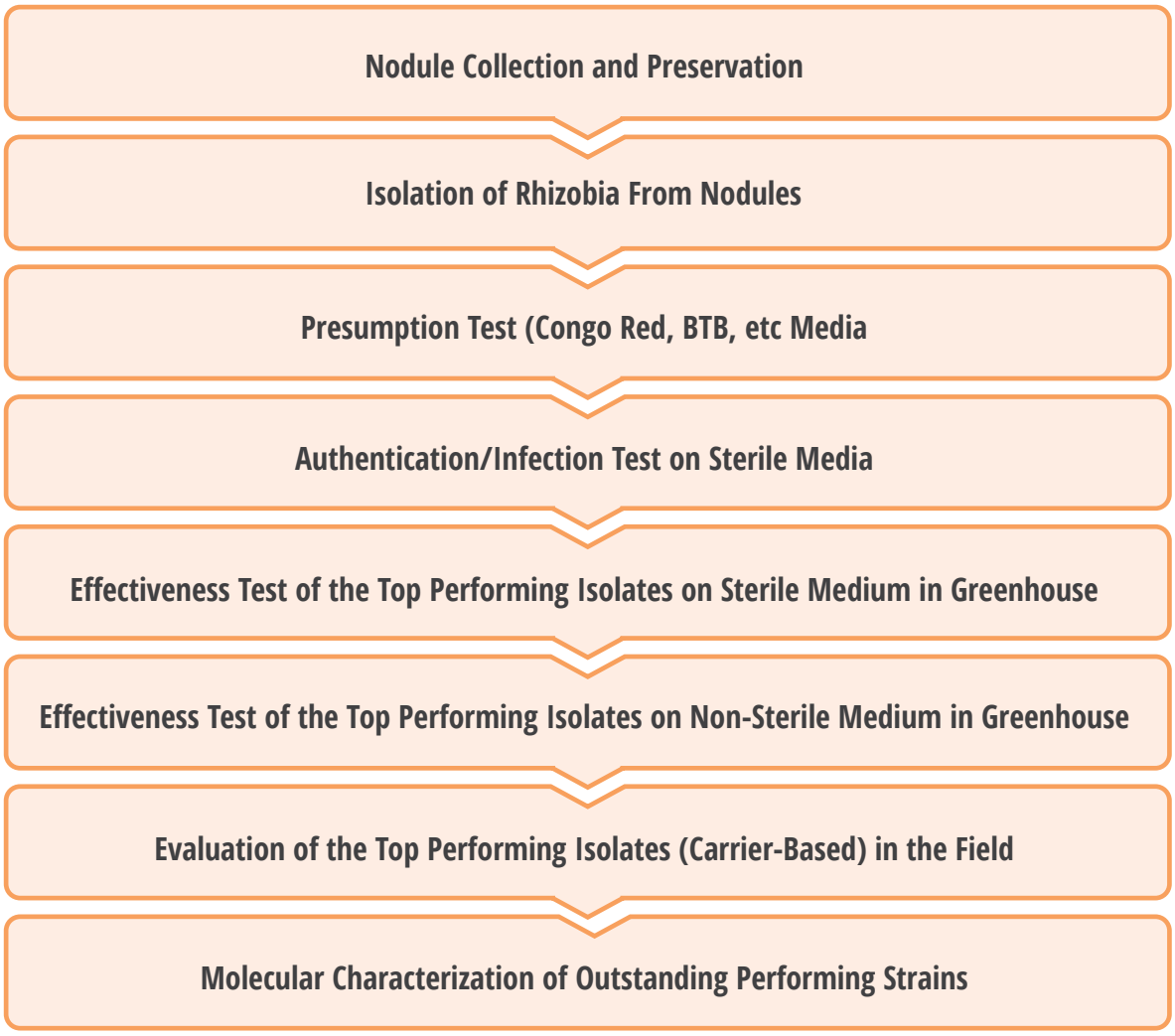
Wani, S.P.; Rupela, O.P.; Lee, K.K. (1995). Sustainable agriculture in the semi-arid tropics through biological nitrogen fixation in grain legumes. *Plant and Soil* 174:29–49.

Woomer, P.L. (1994). Most probable number counts. In: Methods of Soil Analysis, part 2. Weaver, R.W.; Angle, S.; Bottomley, P.; Bezdicek, D.; Smith, S.; Tabatabai, A.; Wollum, A. (Eds.). Soil Science Society of America, Inc., Madison, Wisconsin. p. 59–79.

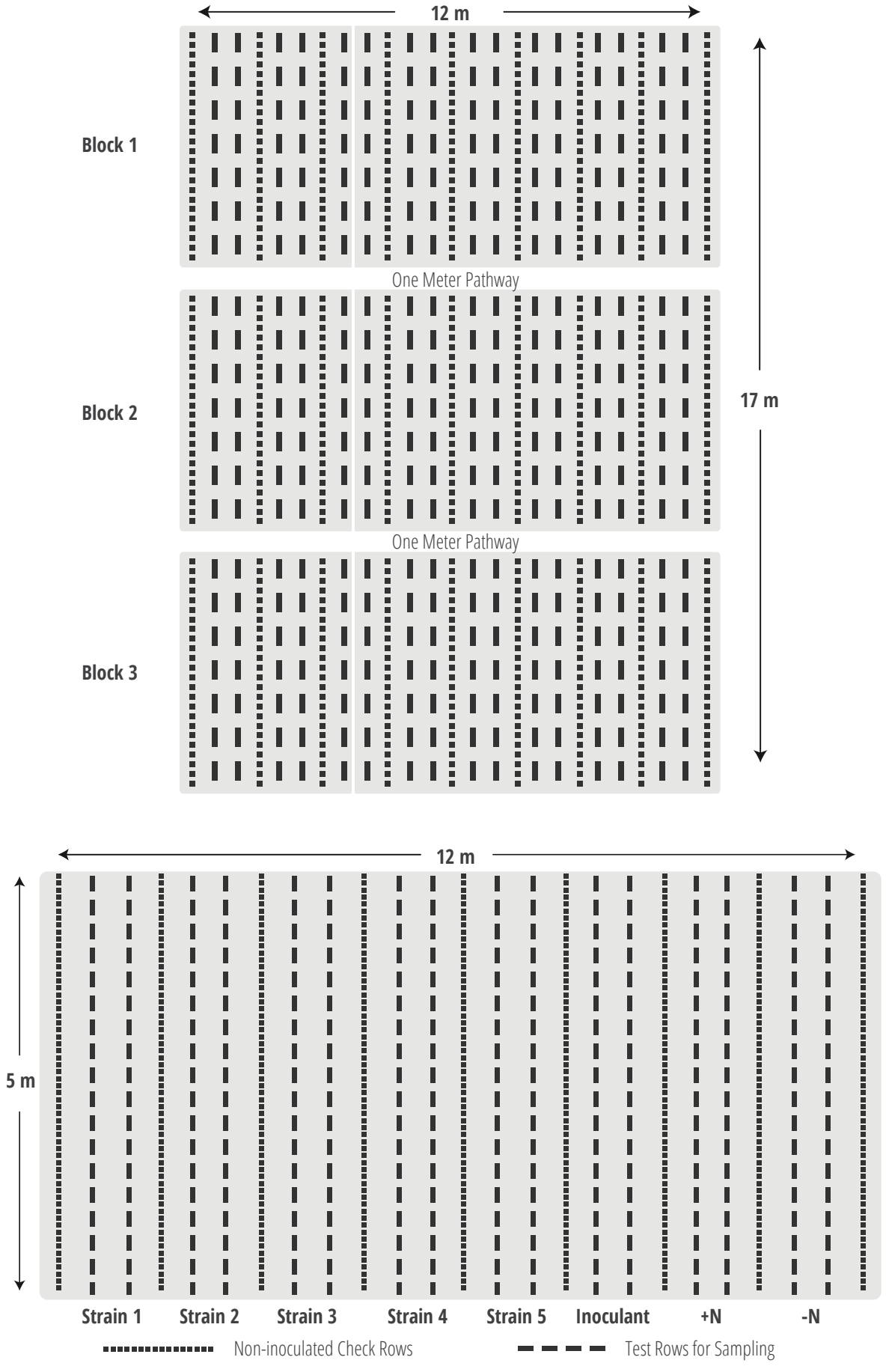
Wright, S.F.; Upadhyaya A. (1998). A survey of soils for aggerigate stability and glomalin, a glycoprotein produced by hyphae of AMF. *Plant Soil* 198: 97-107.

Appendices

Appendix Figure 1: Sequential steps in the collection and isolation of nodules, isolation and authentication of rhizobial strains, and field testing of their N-fixing effectiveness.



Appendix Figure 2: Field layout and dimensions of a trial with three replicate blocks containing five test strains and three controls (a) and a replicate block containing five test strains and three control treatments, a commercial inoculant, nitrogen fertilizer, and a non-inoculated control (b).



Appendix Table 1: Sample data recording sheet of MPN count.

DILUTION LEVEL	REPLICATES				TOTAL
	1	2	3	4	
5^{-1}	+	+	+	+	4
5^{-2}	+	+	+	+	4
5^{-3}	+	+	+	+	3
5^{-4}	-	-	+	-	1
5^{-5}	-	-	-	-	0
5^{-6}	-	-	-	-	0

Experimental results = 4-4-3-1-0-0, replications = 4, tabular MPN = 165.
Population estimate = 165 cells per gram of sample. Inoculation volume = 1 mL.

Appendix Table 2: Nodule sampling passport data.

COLLECTOR			AUTHORITY		
Side ID		**Data Collected**		**Location**	
Latitude		**Longitude**		**Altitude (m)**	**Rainfall (mm)**
Soil Colour		**pH (Kit)**		**pH (Water)**	**Photo Ref.**

Parent Material
- ☐ Granite
- ☐ Basaltic
- ☐ Schistic
- ☐ Calcareous
- ☐ Limestone
- ☐ Alluvial
- ☐ Sandstone
- ☐ Dune

Habitat
- ☐ Pasture
- ☐ Fallow
- ☐ Crop
- ☐ Wood
- ☐ Market
- ☐ Roadside

Soil Type
- ☐ Sand
- ☐ Sandy Loam
- ☐ Loamy Sand
- ☐ Loam
- ☐ Clay Loam
- ☐ Clay
- ☐ Stoney
- ☐ Gravel
- ☐ Organic

Soil Depth
- ☐ 0 - 10 cm
- ☐ 10 - 20 cm
- ☐ 20 - 40 cm
- ☐ >40 cm

Aspect
- ☐ Flat
- ☐ North
- ☐ South
- ☐ East
- ☐ West

Slope
- ☐ Level 0-3%
- ☐ Ondulating 3-8%
- ☐ Rolling Gently 8-16%
- ☐ Sloping 16-30%
- ☐ Steep >30%

Water Relations
- ☐ Free Draining
- ☐ Water Table
- ☐ Swamp

Area Sampled
- ☐ 1 m^2
- ☐ 1 - 10 m^2
- ☐ 10 - 100 m^2
- ☐ 100 - 1000 m^2
- ☐ >1000 m^2

Grazing Pressure
- ☐ Nil
- ☐ Light
- ☐ Moderate
- ☐ Heavy

ACCESSION	HOST	COMMON NAME	BOTTLE NUMBER	NOTES

Appendix Table 3: Observation recording sheet of rhizobia study (adapted from N2 Africa-Ethiopia).

TREATMENT DESCRIPTIONS				INSTALLATION			CROP ESTABLISHMENT				PHENOLOGY								
Rep	Trt	Plot No.	Gross Plot Size	Planting Date			Date of Germination Count			Germination Percentage	Date of 50% Flowering			Date of 50% Podding			Date of Full Maturity		
#	#		[m2]	DD	MM	YY	DD	MM	YY	[%]	DD	MM	YY	DD	MM	YY	DD	MM	YY

BIOMASS SAMPLING AT MAXIMUM BIOMASS

Date of Biomass Sampling			Area of Biomass Sampling Plot	No. of Plants in Sampled Area	Fresh Weight of all above Ground Biomass From Biomass Plot	Fresh Weight of Biomass Subsample	Dry Weight of Biomass Subsample	Above Ground Biomass Weight (Calculated)	Nodule Mean Score From 10 Plants	Active Nodules (Sum of Pink, Red, Brown Colour) From 10 Plants	Inactive Nodules (Sum of White, Green, Black Colour) From 10 Plants	Nodule Dry Weight From 10 Plants
DD	MM	YY	[m2]	#	[kg]	[g]	[g]	[kg Dry]	#	#	#	[g]

FINAL HARVEST

Date of Harvest			Area of Harvest Plot	No. of Plants in Harvested Area	Total Fresh Weight of all Pods in the Harvest Plot	Total Fresh Weight of all Haulms (Without Pods) in the Harvest Plot	Fresh Weight of a Subsample of Pods	Fresh Weight of a Subsample of Haulms	Dry Weight of Grains of the Subsample	Dry Weight of Empty Pods (Husks) in the Subsample	Dry Weight of Haulms in the Subsample	Dry Weight of 100 Seeds	Grain Yield (Calculated)	Haulm Yield (Calculated)	Empty Pod Yield (Calculated)	Total Stover Yield (Haulm + Empty Pods) (Calculated)
DD	MM	YY	[m²]	#	[kg]	[kg]	[g]	[g]	[g]	[g]	[g]	[g]	[kg/ha]	[kg/	[kg/h]	[kg/ha]

GENERAL INFORMATION RECORDING SHEET FOR RHIZOBIAL STUDY

Farm ID												
Season/Year												
Country	Ethiopia											
Woreda												
Kabela												
	Latitude	**Longitude**	**Altitude (m)**									
Experiment site GPS Coordinates				(e.g. Coordinate in decimal degrees 7.2458)								
Name of organization implementing the trial	**EIAR-HARC**											
Data Entry by												
Type of Experiment	**Test Legume**											
	Soil Data (Composite Soil Samples Before Planting)											
pH (H_2O)	**Total Carbon**	**Total Nitrogen**		**P (Olsen)**	**CEC**	**Exch. K**	**Exch. Ca**	**Exch. Mg**	**Exch. Na**	**Sand**	**Silt**	**Clay**
	%	%		ppm	cmol/kg	cmol/kg	cmol/kg	cmol/kg	cmol/kg	%	%	%

Appendix Table 4: General information (metadata) sheet.

FIELD HISTORY

	PREVIOUS SEASON	SEASON BEFORE LAST SEASON
Crops Grown in the N2A Plot:		
Mineral Fertilizer Used:		
Organic Input Used:		
Inoculant Used:		

Location of the Plot in the Landscape:		*1) Plains, 2) Valley bottom, 3) foot slope, 4) slope, 5) plateau*
Soil Drainage in the Plot:		*1) Good, 2) Moderate, 3) Poor*
Are there Signs of Soil Erosion in the Plot:		*1) Yes, 2) No*

Please fill in the dates at which the following events occurred on the strain by legume Varieties Field Trials.

ACTIVITY

	DD	MM	YYYY
Date of Land Preparation			
Date of Organic Manure Application			
Date of Planting			
Date of Mineral Fertilizer Application			
Date of 1st Weeding			
Date of 2nd Weeding			
Date of 3rd Weeding			
Date of Pesticide Application			
50% Flowering			
50% Maturity			
Date of (final) Harvest			

Pesticide Name	

Please record rainfall data in the growing period using the following table. The data can be sourced from the nearby weather stations either owned by research center or NMO.

	NAME	LATITUDE	LONGITUDE	ALTITUDE (M)
Nearby Weather Station GPS Coordinates				

(e.g. Coordinate in decimal degrees 7.2458)

RAIN (MM)	DD	MM	YYYY

Partners:


german
cooperation
DEUTSCHE ZUSAMMENARBEIT


giz


CABI


Alliance
Bioversity
International
CIAT
International Center for Tropical Agriculture
Since 1967 Science to cultivate change


CGIAR
RESEARCH
PROGRAM ON
Water, Land and
Ecosystems