Soybean Breeding SOPs

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SOP for Site Selection & Field Layout

1. Introduction

Selecting the right site for planting is a crucial step in the successful laying out and implementation of a trial. The quality of data collected and the results of a trial's analyses can be compromised by selecting the wrong site. Therefore, various factors must be considered in site selection before field layout.

2. Purpose

Understanding factors such as soil, topography, climatic requirements of the crop, biotic factors, and the prevalence of pests and diseases, cost of acquisition or lease in preparing the land, frequency of typhoon and other calamities, labor supply and cost, security, and political stability to determine the suitability of the site for trial establishment. The accessibility of the plot for frequent visits needs to be considered when selecting sites for the establishment of variety trials.

3. Scope

This SOP outlines a systematic procedure and technique for identifying the appropriate site for establishing variety trials. The factors to consider in site selection and trial layout, as well as the steps to transition from current practices to the new plan, need to be discussed in detail.

The site for varietal trials and nurseries shall be large enough to accommodate all the trials in one location, rather than planting trials at different sites. This will save on operational costs and create convenience and simplicity for the trials management.

4. Definition of terms

GPS Coordinates: Global Positioning System (GPS) coordinates are unique identifiers for precise geographic locations on Earth, usually expressed as alphanumeric strings. Coordinates, in this context, are points of intersection in a grid system. GPS coordinates are usually expressed as a combination of latitude and longitude.

5. Roles and Responsibilities

All staff responsible for implementing the breeding activities in the soybean improvement program at IITA must use this SOP for Site Selection and Field Layout. The program leaders should approve any alterations to the procedures unless they are exceptionally necessary. The individuals responsible for each section of the Site Selection and Field Layout SOP in the breeding data cycle are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a principal investigator (PI), or a cluster lead.
Regional Coordinator (RC): Responsible for managing and overseeing all trials and data production conducted in a region.
Trial Coordinator (TC): Responsible for proper site selection, trial design, and layout.
National Collaborator/Breeder (NC/B): Responsible for coordinating the selection of the target agro-ecological locations and trial sites to implement trials that will be sent from IITA.
Trial Manager (TM): The TM will be responsible for identifying and measuring the appropriate trial plots in consultation with the TC, NC/B, and RC.
Biometrician (BM): Instructs and guides CL, RC, and TC to use the best possible experimental design and field layout.

6. Procedure

Activity 1: Site Selection (Rain-fed Trials)

1. The CL and RC, in consultation with the TC, decide on the size of land required for the season, based on the expected field trials and activities.
2. For IITA stations, the TC, in consultation with the CL and RC, will submit the land request to the Breeding Operation unit at the right time.
3. The TC and TM, with the guidance of CL and RC, will follow up on the allocated land and confirm that the allocated land meets the requirements for soybean as listed below:
   a. The dimensions of the field can fit into the expected designs of the trials.
   b. Presence of low within-plot variability (uniform soil, where possible; avoid degraded, highly sandy or gravelly soils and high land gradient)
   c. Select land that is fully exposed to sunlight, away from any buildings or trees.
   d. Select land without any obstacles, such as pits, stumps, and rubble of demolished structures
   e. Select land that was not under soybean production in the previous season
   f. Select land with a reliable supply of irrigation water for trials
4. TC and TM measure and document the dimensions of all the allocated plots to assign the different trials to the different plots depending on the dimensions.
5. Take GPS coordinates for the sites
6. Take the soil samples for analysis

Activity 2: Sketching the Field Layout

1. Read and understand the trial design and layout
2. Identify the soil or nutrient gradient; no land is flat
3. Layout the experiment so that each replicate is on a uniform soil area, keeping replicates as compact and as close together as possible.
4. Sketch the trial design on a piece of paper, considering the field dimensions
5. Include the space for border rows and paths on the sketch.

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Technical problems with regards to understanding your layout, you can contact Olabode Kehinde K.Olabode@cgiar.org , Adeyinka Adewumi A.Adewumi@cgiar.org and Armand Yambisa A.Yambisa@cgiar.org

Experimental design and data analysis:

8. References

SOP for Land Preparation and Planting

1. Introduction

Proper land preparation is important for ensuring the soybean field is ready for proper layout and planting of soybean variety trials. A well-prepared field reduces weed infestation, recycles plant nutrients, and provides a soft soil mass for uniform stand establishment and a suitable soil surface for direct seeding.

Land preparation covers a wide range of practices, from zero-tillage or minimum tillage, which minimizes soil disturbances, to totally 'puddled' soil, which destroys soil structure.

2. Purpose

The purpose of this document is to outline the roles, responsibilities, and procedures for good land preparation practices for planting soybean breeding trials for all the phenotyping trials. This SOP aims to guide research supervisors and technicians in properly preparing the land for soybean trials.

3. Scope

This document outlines the land preparation procedures required to establish soybean breeding trials. It covers land preparation steps for both mechanical and manual planting.

4. Definition of terms

RTK (Real-Time Kinematic) - provide centimeter-level accuracy for planting, reducing seed waste by 8-12% and improving consistency

5. Roles and Responsibilities

All staff responsible for implementing the breeding activities in the Soybean Improvement Program at IITA must use the Land Preparation and Planting SOP. The procedures should not be altered unless approved by the program leaders, except in some exceptional cases. The individuals responsible for each section of the Land Preparation and Planting SOP in the breeding data cycle are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a principal investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for managing and overseeing all trials and data collection in a region.

Trial Coordinator (TC): Responsible for proper site selection, trial design, and layout.

National Collaborator/Breeder (NC/B): Responsible for coordinating the selection of the target agro-ecologies locations to implement trials that will be sent from IITA.

Trial Manager (TM): The TM will be responsible for identifying and measuring the appropriate trial plots in consultation with TC, NC/B, and RC.

Biometrician (BM): Instructs and guides CL, RC, and TC to use the best possible experimental design and field layout.

6. Procedure

Activity 1: Land Preparation

Step 1: Land clearing to remove weeds, crop residues and debris, and non-biodegradable materials from the field.

Step 2: For off-season land preparation, irrigate the field to prepare the land for harrowing.

Step 3: Ensure proper first and second harrowing, followed by marking

Activity 2: Mechanical Planting

Step 1. Arrange the seeds in seed packets for planting and place them in a wooden box in the proper order for mechanical planting, in accordance with the field layout.

Step 2: Double-check the order of the seed packets for planting before taking them to the field and again in the field before planting to avoid any arrangement errors.

Step 3. The RTK system generates straight lines within the boundary to dictate where the tractor will drive, reducing overlap

Step 4. Once the auto-start button is pressed, the tractor will steer itself on the entered heading.

Step 5. The person on each side of the planter will put the seeds in the planter as quickly as possible, – but only after hearing the cones fire.

- All the seeds from the envelope should be emptied into the cylinder immediately after the cones fire.
- The seed envelopes are always pulled from the front of each sleeve. Before beginning a pass, check the map and the envelopes to ensure they match.

Step 6. Check the soybean depth and row length after the first few plots. Adjust settings as necessary.

Step 7. The driver should raise the planter at the end of each pass and turn around to ensure the tractor is near the starting point of the next pass.

- The tractor needs to be facing the correct heading and 2 – 3 meters from the next pass to auto-steer correctly.

Step 8. The driver will then lower the planter and select “start” on the GPS monitor, then push “auto-steer”; the tractor will find the next pass and begin steering itself.

Step 9. Ideally, a runner is available to bring seed boxes once a box is empty.

- The runner should know in advance how many passes are in each box and keep track of planting progress, so he/she is ready when the next box is needed.

Step 10. The runner (or the driver and planter, if necessary) should check the accuracy of the seed supply from the cone and periodically check seed depth to ensure consistency throughout the field.

1. Hold down the blue and power buttons simultaneously to turn the unit off.
2. Turn off and disconnect headsets, and store them in the tractor.
3. Turn off the seed sensor.
4. Disconnect all the necessary wires between the tractor and the planter.
5. Disconnect the planter and remove the bucket.

Activity 3: Manual Planting

Step 1: Mark the rows for each plot by creating shallow trenches 4 m long, spaced 50 cm apart. If you use a different plot size, please note it in the field book for accurate yield calculation.

Step 2: Incorporate a minimum of 100 kg NPK ha^{-1} and 150 kg ha^{-1} TSP fertilizer, which is equivalent to 20 g of NPK and 30 g of TSP for a single row of 4m in length. Again, this provides 5 g of NPK and 7.5 g of TSP per 1m of row. You may choose to apply urea, but if so, do not apply more than 50-100 kg N/ha after the first or second weeding, depending on the need. If we decide to apply 50 kg ha^{-1} urea, we need to apply 10 g of urea to a single row 4m long, which is equivalent to 20 g at the rate of 100 kg ha-1 for a single row 4m long.

Step 3: Seed packets are numbered consecutively by plot number. Distribute the seed from one packet evenly among the rows. Planting will be done by drilling seeds in rows. The seeds should be sown 2-4cm deep. Shallow sowing is best for heavy, wet soils that are susceptible to crusting. Seed may be sown deeper on sandy soil, which does not crust.

Step 4: Plant according to the design and the trial's plot order.

Step 5: After planting, print trial names on canvas, and use A4 laminated or plastic peg labels for each trial for easy identification. The labels should bear the following information: trial name, total plot count, and number of replications.

Step 6: Collect all empty seed packets and keep them safe for reference, if any need arises

Step 9: Record planting date(s)

Step 10: Sketch a field map for reference

Step 11: After the seedlings have emerged, at 4- or 5-leaf stage, thin the plants to one seedling every 5cm (20 seedlings/m).

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Issues relating to land preparation, contact Olabode Kehinde K.Olabode@cgiar.org , Adeyinka Adewumi A.Adewumi@cgiar.org and Armand Yambisa A.Yambisa@cgiar.org and RFU Supervisor Mrs. Theresa Olusola T.Olusola@cgiar.org .

8. References

SOP for Trial Designing

1. Introduction

Proper field trial design is a crucial step in establishing efficient, cost-effective variety trials. Design properties and specific designs relevant to varietal trials are considered to ensure good control of experimental error and effective mean separation. Designs can range from unreplicated trials at a single location to multi-environment trials involving several locations and seasons. In most cases, interest focuses on genotypes as the sole treatment factor, with designs generally comparative in nature and arranged to ideally minimize variance between genotypes or to maximize selection gains.

2. Purpose

The primary reason for grouping plots into uniform blocks is to minimize plot-to-plot variation and enhance the experiment's precision. Failure to adequately block a field experiment can result in unacceptably large error variance and/or biased estimates of genotype effects (See Mead, 1997, for an example). Effective control of error variance usually requires relatively small blocks. Trials with many entries, set out in a complete block experiment, where there is considerable variability among plots within a block, will likely result in very poor, possibly unusable, information on genotypes. To control field variation, especially with a large number of entries, it is essential to utilize incomplete block designs.

3. Scope

This document outlines the trial design procedure required for conducting soybean variety trials. It covers the design steps for the different testing stages (breeding nursery, preliminary variety trial, advanced multi-location variety trial, and on-farm trial). Identified/selected lines are added to Enterprise Breeding System (EBS) list, where designing the trials is performed.

4. Definition of terms

The Enterprise Breeding System (EBS) is an open-source cloud-based breeding informatics software developed for crop breeding programs serving resource-poor farmers in Africa, Asia, and Latin America. It provides applications for core breeding and data management activities, enabling a high-quality experience for decision-making processes. It is a relational database that can manage different types of breeding data and plan, create, and manage breeding trials.

Designation: an official name, description, or title.

Pedigree: the recorded ancestry or lineage of a plant or family.

Design: Whenever an agricultural experiment is conducted using a certain (statistical) procedure, it is called design, or experimental designs are various types of plot arrangements used to test a set of treatments to draw a valid conclusion about a particular problem.

Breeding Nursery: A place where plants are grown until they are large enough to be planted in their final positions.

5. Roles and Responsibilities

All staff responsible for implementing the breeding activities in the soybean improvement program at IITA must use the Trial Designing SOP. The program leaders must approve any alterations to the procedures, unless they are exceptionally necessary. The individuals responsible for each section of the Trial Designing SOP in the breeding data cycle are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a Principal Investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for establishing the list of entries based on different pipelines that need to be used for trial design. This list of entries is based on the summary results of the data analysis to be received from TC, TM, and BM.

Trial Coordinator (TC): Responsible for proper trial design and layout.

Trial Manager (TM): TM will be responsible for identifying and measuring the appropriate trial plots in consultation with the TC, NC/B, and RC.

Biometrician (BM): Facilitates the data analysis process and guides CL, RC, and TC using new statistical methods. Ensure the collected data can be interpreted to support decision-making.

6. Procedure

There are three basic principles of experimental design:

Replication: Repetition of the treatment under investigation to provide an estimate of experimental error.

Randomization: The allocation of the treatment to the different experimental units by a random process is known as randomization

Local control: The principle of making use of greater homogeneity in groups of experimental units for reducing experimental error.

Activity 1: Trial designing steps for Breeding Nursery

1. Select/identify the lines that need to be evaluated in the breeding nursery
2. Give name/designation for the lines
3. Maintain the pedigree of the line
4. Establish the list of lines in EBS
5. Design the breeding nursery in EBS

Trial protocols for breeding nursery

1. The design should be an augmented/entry list design.
2. Available check varieties will be included in the trial at both sides of the border rows and planted at the same time as the entries.
3. The number of entries may go up to 1200 or more in one set, and about three sets of breeding nurseries might be established every year.
4. The number of rows can be one or two, depending on the quantity of available seeds and the type of mechanical planter to be used.
5. The trial will not have replication.
6. The trial will be grown in a single location.
7. The trial will be conducted only for one season.

Activity 2: Trial designing steps for Preliminary Variety Trials (PVTs)

1. Establish the list of lines that need to be evaluated in PVT by advancing the entries from the previous season's breeding nursery.
2. All the entries need to be given IITA coding names.
3. Maintain the pedigree of the lines.
4. Establish the list of lines in EBS.
5. Design the PVT in EBS.

Trial protocols for PVTs

1. The design should be an alpha lattice design.
2. Available check varieties will be included in the trial.
3. The number of entries needs to be in the range of 100-150 in one set, and five to ten sets of PVTs might be established every year.
4. The trial should have two or three replications, depending on the availability of seeds and experimental plots.
5. A sparse testing strategy will be considered and implemented in at least five locations. The material will be distributed to locations based on the quantity of seeds and the size of the field at each location.
6. The trial will be conducted in at least three locations.
7. It will be conducted only for one season.

Activity 3: Trial designing steps for Advanced Variety Trials (AVTs)

1. Establish the list of genotypes that need to be evaluated in AVTs by advancing the entries from the previous season's PVTs.
2. Establish the list of genotypes in EBS.

3. Design the AVT in EBS.

Trial protocols for AVTs

1. The design should be an alpha lattice design with row-column arrangements.
2. Available check varieties will be included in the trial.
3. The number of entries needs to be 40-100 in one set, and about four to six sets are established from each maturity group every year.
4. The trial should have three replications.
5. The trial will be grown in at least six locations.
6. It will be conducted for two seasons.

Activity 4: Trial designing steps for On-farm Variety Trials (OVTs)

1. Analyze the data and summarize the results of the multi-location trials for decision-making.
2. Identify the best two to five candidate entries that are superior to the checks.
3. Prepare at least half a kg of seeds for the candidates along with the local checks.

Trial protocols for the on-farm variety Trial

1. The trial will be grown in un-replicated plots.
2. Two to three volunteer farmers need to be selected in each target agro-ecology for the trial.
3. A total of 15-20 on-farm trials need to be conducted for variety registration.
4. Available local/standard varieties need to be included as checks.
5. The number of entries can be between 2-6.
6. Liaise with the national program for the on-farm trials and submission of a variety of registration applications in the country of registration.
7. Prepare and dispatch seeds to the respective on-farm testing sites.
8. Coordinate with the national program for the planting of the on-farm trials, and evaluation of the candidates by the variety release technical committee for registration of the variety.
9. It will be conducted only for one season.

Trial Protocols for the Tricot/1000-farms On-farm Variety Trials (TOVTs)

1. **Prepare the project**. Confirm the availability of sufficient quantities of good-quality seeds of the candidate varieties and checks that will be tested.
2. **Design project**. Using the free online software ClimMob, the project implementer creates a short project profile, adds the candidate varieties and the checks to be tested, registers the field agents' names, defines which plant characteristics should be evaluated, and prepares the participant's registration.
3. **Recruit participants**. The implementer and field agents identify dedicated farmers and farmer groups interested in improving their farming practices by participating in on-farm testing of new crop varieties through the 1000 farms program.
4. **Prepare test packages**. Depending on the number of participants, an appropriate number of trial packages must be prepared. The field agents then distribute the trial packages to the

participants during an initial workshop. During this workshop, participants learn about the tricot process and can ask questions.

5. **Establish on-farm trial**. Each participant now cultivates their three technological options on three small plots on their land, otherwise treating these trial plots exactly as they would their main plots. Every participant is responsible for their plots. The participants won't know the names of their three technological options (to avoid bias), but will only know them as 'A', 'B', and 'C'.
6. **Participants record observations**. During the growing period, participants observe the trial plots and write down their observations by hand on the observation cards. What to observe and when to observe was defined in step 2 using ClimMob software.
7. **Field agents collect data**. Once the growing season is over and all questions on the observation cards have been answered, the field agents collect all data. This can be done by visiting participants and collecting their observation cards, or by making telephone calls.
8. **Data is compiled and analyzed**. The field agents upload all data to ClimMob, using the free Android smartphone app ODK Collect. Once all data is uploaded, ClimMob will analyze it and automatically prepare result sheets summarizing the overall results of the entire tricot project, as well as individual result sheets for each participant.
9. **Communicate results**. The field agents communicate the results of the tricot project to the participants. During the trial, the participants won't be aware of their technology options. Instead, each participant's three technology options will be designated as 'A', 'B' and 'C', to avoid biased evaluations. During the result feedback, the participants learn the names of the three crop varieties they evaluated. Participants can use this information independently to improve their cultivation practices.

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Technical problems with digital tools (Zebra printers, Fieldbook App, EBS), printing field labels and uploading data to EBS please contact: Simon Imoro S.Imoro@cgiar.org and Shah Trushar tm.shah@cgiar.org. For Seed Inventory Information contact: Armand Yambisa A.Yambisa@cgiar.org, Adeyinka Adewumi A.Adewumi@cgiar.org and Olabode Kehinde K.Olabode@cgiar.org

Experimental design and data analysis: Ntukidem, Solomon, S.Ntukidem@cgiar.org

8. References

Kent M. Eskridge University of Nebraska Lincoln, NE. Field Trial Designs in Plant Breeding

SOP for Field Tags and Labelling

1. Introduction

One of the crucial steps in establishing a variety trial is proper labeling and tagging, which provide details on the variety trials and each entry in the experiment, facilitating accurate data collection, tracking, and analysis throughout the field trials. Tagging and labeling are crucial for maintaining the genetic integrity of soybean genotypes used in variety trials, ensuring effective management and plot purity.

2. Purpose

The purpose of this document is to outline the roles, responsibilities, and procedures to be followed in creating field tags and labels.

3. Scope

This document outlines the procedure for soybean breeding tagging and labeling, including its functions and the importance of labeling.

4. Definition of terms

5. Roles and Responsibilities

All staff responsible for implementing the breeding activities in the soybean improvement program at IITA must use Field Tags and Labelling SOP. No alteration should be made to the procedures unless approved exceptionally by the program leaders. The individuals responsible for each section of the Field Tags and Labelling SOP in the breeding data cycle are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a Principal Investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for managing and overseeing all trials and data collection in a region.

Trial Coordinator (TC): Responsible for the preparation of tags, labels, and printing field labels.

Trial Manager (TM): The TM will be responsible for tagging and labelling each trial field and trial plots.

Seed Manager (SM) - refers to individuals or clusters who manage the seeds stored in the seed warehouse facility and ensure that the seed inventory system is up to date.

6. Procedure

The following are the step-by-step procedures for carrying out the activities for field tagging and labeling:

1. Labeling of parents in crosses:
    a. Label each parent in the crossing block for proper identification
    b. Write plot number and variety name with permanent ink marker on plastic pegs
    c. Peg down each label at the start (first row) of each plot
2. Tagging of crosses:

a. The flowers that are fertilized (crossed) with the desired parent will be tagged.

b. The tag should be placed on the main stem, beneath the crossed flower, and not on the peduncles of the leaves.

c. The tags are made up of lightweight carbon and are written with a pencil.

3. The tags should bear the following information:

    a. Date of pollination
    b. Names of female and male parents, consecutively in the cross.
    c. Name of the person who made the crosses

4. Labeling of trials

    4.1. Labels for trials are generated from the trial design in the EBS platform.

    4.2. The steps in creating planting labels in EBS include:

        a. Create and commit your experiment occurrence in Core Breeding (Experiment creation/Experiment manager).
        b. In Core System, create the necessary printout template for your plot labels.
        c. Save the created template for future use. The templates can include barcodes, images, text, tables, etc.
        d. In Core Breeding (Experiment manager), select the experiment(s) for which you want to print labels and tap on the printouts icon to select the trial occurrence(s), template, and appropriate file format for your label printer.
        e. Depending on your printer requirements, download the template and send it to your printer for further editing and printing.
        f. Review the label and ensure each label or envelope is assigned to the right variety.

5. Creating labels using Microsoft Word
        a. Go to mailings
        b. Pick a preferred format for printing by selecting “start mail merge’
        c. Select the recipient by importing the Excel dataset to work with
        d. Insert fields needed for your labels
        e. Update label
        f. Preview result

g. Finish and merge
h. Print on selected formats (envelope or sticky labels)
i. Ensure each label or envelope is assigned to the right variety.

6. Labelling of plots

Option A:

a. Print the plot information on the blue tag paper
b. Tie the blue paper tag on the first plant in the plot in a visible manner
c. Information should include plot number, designation or pedigree, entry number, trial name, and barcode labels.

Option B:

a. Design tags to the desired taste using Microsoft Word (follow the same step as how to create labels above)
b. Print exported A4 pages on Flex banner and cut each tag to size
c. Perforate holes at the top-left side of each tag
d. Attached rope to tags
e. Fix the tag to each plot by tying the tag rope to the first plant in the plot in a visible manner
f. Information on the tags should include plot number, designation or pedigree, entry number, trial name, location, and QR-Code.

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Issues relating to seed inventory management and soybean trials label and tag printing, you can contact: Olabode Kehinde K.Olabode@cgiar.org, Adeyinka Adewumi A.Adewumi@cgiar.org, and Armand Yambisa A.Yambisa@cgiar.org

For Technical problems with digital tools (Zebra printers, EBS), creating and printing field labels in EBS, please contact: Simon Imoro S.Imoro@cgiar.org , Shah Trushar tm.shah@cgiar.org and Armand Yambisa A.Yambisa@cgiar.org

8. References

Excellence in Breeding – EBS User guides - User guides - Digital Solutions

SOP for Trial Management

1. Introduction

Effective trial management requires the consistent application of sound crop management practices across all experimental plots. This uniformity is essential for generating high-quality data, reducing experimental errors, and ensuring optimal crop performance. Successful management also depends on the active involvement of all the technical personnel, including field workers, technicians, supervisors, and scientists, who work together to implement the recommended practices throughout the trial period. Key components of a well-managed trial include the use of high-quality seed, proper and timely fertilizer application, effective control of weeds, pests, and diseases, adequate irrigation, crop drying, and well-designed drainage and erosion control measures.

2. Purpose

This document outlines the roles, responsibilities, and procedures involved in managing soybean breeding trials.

3. Scope

This document contains the descriptive procedure required for effective trial management.

5. Roles and Responsibilities

All staff involved in implementing soybean breeding activities in the soybean breeding program at IITA must adhere to the Trial Management SOP. No alteration should be made to the procedures unless approved exceptionally by the program leaders. The individuals responsible for each section of the Breeding Trial Management SOP in the breeding data cycle are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a principal investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for the coordination and supervision of the proper implementation of trial management practices as recommended for the crop.

Trial Coordinator (TC): Responsible for the proper application of the trial management practices.

Trial Manager (TM): The TM will be responsible for applying the trial management practices as guided by the TC and RC.

6. Procedure

Trial Management

Activity 1. Planting time

a. In areas of short growing periods, e.g., in Northern Nigeria and Southern Zambia, planting needs to be done as early as possible, when there is adequate moisture in the soil, in mid-June and mid- to late-November, respectively.
b. In areas with a long growing period, planting can extend up to mid-July and late-December, e.g., in southwestern Nigeria and northern Zambia, respectively.

Activity 2. Spacing

The spacing between rows and plants for soybeans will be 50 cm and 5 cm, respectively. This will result in a plant population of 400,000 plants ha^{-1}, equivalent to 80 plants in a single row of 4m in length.

Activity 3: Fertilization

a. Dress 10 kg of seeds with 100 gm of NoduMax (1g of Nodumax with 100g of seeds), add a drop of water, and mix thoroughly before planting. (Please refer to SOP02 for the other aspects of fertilization.)

Activity 4: Thinning

a. After the seedlings have emerged, at the 4- or 5-leaf stage, thin the plants to one seedling every 5cm (20 seedlings/m).

Activity 5: Weeds Control

Perennial and most annual weeds can slow down growth in the early stages of soybean development. A properly timed weed control program can minimize their effects. Weed control in soybeans can be achieved by hand, chemical herbicides, or a combination of both.

Step 1: Manual weed control

a. Carry out the first weeding two weeks after planting and the second at five to six weeks after planting, depending on the types of weeds and the level of infestation.
b. Avoid weeding immediately after rainfall, as this would lead to transplanting the weeds.
c. Avoid poor hoe weeding or delays in weeding, which could cause significant reductions in soybean yield.

Step 2: Chemical weed control

a. Herbicides must be used properly for safe and effective control of weeds in soybean fields
b. Understand the predominant weed species and the availability of herbicides effective against it
c. For good weed control, twice proper harrowing contributes to good weed control

d. For applying pre-emergence herbicides, after marking the field, the field needs to be left for two to three weeks, before sowing, allowing the weeds to grow, and the pre-emergence herbicide is applied within 24 hours of planting.
e. Apply selective herbicides at post-emergence for weed control.
f. If the herbicide is applied at planting, one-time hand weeding may be required at 5–6 weeks after planting, but if it is sedges, 2–3 weeks after planting is appropriate.
g. Always refer to the user's guide for appropriate use and dosage.

Activity 6: Insect and pest control

Several insect species occur in soybean fields, but few are of significant economic importance. In the vegetative stage, the crop is very tolerant of caterpillars but susceptible to attacks by silverleaf whiteflies. From the flowering stage onwards, the soybean becomes attractive to pod-sucking bugs, which can significantly reduce seed quality.

a. Scout the soybean fields frequently for insect and pest infestation. Apply insecticides such as *Termex* (for pod bugs) and *Courage* (for whiteflies) to control infestations once thresholds are reached.
b. Observe the dominant pests and insects so as to pick the best controlling agent.
c. Always refer to the user's guide for appropriate use and dosage.

Activity 7: Disease Control

a. Continuous rouging (uprooting and destroying) symptomatic plants to reduce the incidence of insect-transmitted viruses.
b. Eradicate the weeds and volunteer plants in the vicinity of the soybean farms.
c. Treat seeds with systemic insecticides and fungicides and apply one or two foliar sprays of insecticides to reduce the insect vector activity during the pre-flowering stage (most vulnerable to virus infections).

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Issues relating to trial management, please contact: Armand Yambisa A.Yambisa@cgiar.org

Adeyinka Adewumi A.Adewumi@cgiar.org and Olabode Kehinde K.Olabode@cgiar.org

Research Farm Unit supervisor:

8. References

SOP for Harvesting and Seed Handling

1. Introduction

Various selection methods in soybean breeding are used to identify genotypes and progenies that possess the most useful combinations of desired traits. These most often include pedigree selection, which involves visually selecting the best-looking families in each generation, followed by within-family selection of one or more plants to advance to the next generation. The IITA soybean breeding program adopted the single-seed descent selection method, which involves advancing one seed or pod from each plant to the next generation to develop nearly homozygous lines that retain most of the original genetic variation in a population (Orf et al. 2004). The choice of the breeding method depends on the breeding objective and other important factors, such as the extent of genetic variability, the availability of agricultural machines and greenhouses, and the size and skill of the breeding team. Breeding objectives depend on local agroecological conditions, available acreage, production intensity, market demand, and production economics.

2. Purpose

The purpose of this document is to outline the roles, responsibilities, and procedures to be followed in selecting and harvesting soybeans at different breeding stages.

3. Scope

This document contains the harvesting procedure in the Soybean Breeding Program. It covers the steps in selecting and harvesting progeny rows, single plants, and preliminary and advanced variety trial plots.

4. Definition of terms

Progeny Rows: are plots or rows in a field where the offspring (progeny) of selected parent plants are grown to evaluate their traits.

Preliminary Variety Trials (PVTs): early-stage field trials conducted to evaluate and compare the performance of new breeding lines (varieties) under field conditions.

Advanced Variety Trials (AVTs): later-stage trials where selected lines from the PVTs are evaluated more rigorously in multiple environments and years.

5. Roles and Responsibilities

All staff responsible for implementing the breeding activities in the soybean improvement program at IITA must use the Harvesting and Seed Handling SOP. The program leaders must approve any alterations to the procedures, unless they are exceptionally necessary. The roles and responsibilities of individuals involved in the Harvesting and Seed Handling SOP in the breeding data cycle are listed below:

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a principal investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for ensuring proper harvesting of materials, the RC continuously revises harvesting approaches to improve the efficiency and speed of the process.

Trial Coordinator (TC): Responsible for proper harvesting and labeling of the seeds.

National Collaborator/Breeder (NC/B): Responsible for coordinating the harvesting of variety trials conducted in collaboration with the IITA soybean breeding program.

Trial Manager (TM): The TM will be responsible for harvesting progeny trials and seeds, ensuring proper labelling of entries.

Crossing Technician (CT): will follow up on plant maturity and ensure harvesting is done at the appropriate stage for the F1, F2, F3, and F4 progeny populations.

6. Procedure

Activity 1: Progeny row harvesting

1. Spray paints or tie colored ribbons to identify selected plants or rows.
2. Harvest only the marked plants by cutting from the base. Note that two plants close together may be mistaken for the same plant, and ensure that non-selected plants are not harvested.
3. Ensure each row is harvested separately and bundled to avoid contamination. Inspect offtypes and ensure similar plants are harvested and bundled together. Desirable and distinct-looking plants within the progeny row can be harvested separately and given different names.
4. Bundle each plot separately and attach tags with cross names and plot numbers before threshing them or taking them to the combined drying and threshing place.
5. Thresh a bundle from a progeny row together, pour it into a grain bag or clean cloth bag, and tie it up with its information tag. Ensure the label is added. Do not leave the label in the field.
6. Go through the field to ensure no selected (color-sprayed or tagged) plant rows been missed.

Activity 2: Single plant harvest

1. Identify the field that needs to be harvested and locate the proper group of tags.
2. Attach an envelope with the plot number and genotype information printed or written with the marker for the selected plant.
3. Once an envelope is assigned to a plant, the mature plant can be uprooted or cut with hedge trimmers. Harvested single plants in each row are kept in the seed envelope, and the opening cover is properly sealed with gum and stapled.
4. Make sure all the envelopes are picked up and packed into boxes.
5. Organize the envelopes in order and enter the details of each plant harvested.

Activity 3: Advanced and preliminary trial plot harvest

1. Tie enough twine to the tags two days before harvest.
2. Make sure you have the field book or the tablet for electronic data capture.
3. Make sure you have enough harvest bags for each plot.
4. Check and confirm where the first plot of the trial starts and how the plots are arranged in the field.
5. Confirm the plot label with the trial design before starting the harvest.
6. Check the proper maturity of plots to be harvested.
7. Check for any off-types (based on differences in pod color, pubescence hair color, plant height, and maturity) missed during the previous inspection of off-types.
8. The harvesting will be done in the two middle rows.
9. Count plants at harvest to obtain data on the number of plants at harvest.
10. Put information like the number of plants harvested, harvesting date, plot number, entry number on the label, and attach the labels to the harvested plot for proper identification.
11. For threshing directly on the plot, spread the harvested plants on its plot for sun-drying before threshing.
12. In the case of threshing away the plot, arrange harvested plants of each plot into different sacks to avoid mixing of plants during transportation.
13. Manually thresh plants of each plot by beating harvested and dried plants in enclosed sack bags with a wooden stick. After threshing, pour the seeds into a bowl, remove the chaff, and then transfer the seeds into a clean cloth bag.
14. Duplicate the information tag, put a copy in a sack inside the seeds, and tie up the sack with the twined main tag.
15. Make sure all plots are harvested and labeled correctly.
16. Dry seeds in a drying cart to the proper moisture level before cleaning and storing.

Activity 4: Seed Handling

1. Once the seed is harvested, it should be taken to the seed store.
2. Then it needs to be cleaned by removing unwanted materials.

3. Make sure to arrange the harvested materials according to the trial and plot order.
4. Place the seeds in the envelopes for progeny rows and in the paper or cloth bags for seeds harvested from the trials for storage.
5. The seeds in the paper bags will be arranged in the wooden box, and then, on the shelves based on plot orders and trial name, and record the shelf and cabinet number
6. Arrange envelopes with seeds of the progeny populations, and prepare them for the next planting.

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Issues relating to selection, you can contact crop lead: Dr. Chigeza Godfree G.Chigeza@cgiar.org and regional head Abebe Abush Tesfaye At.Abebe@cgiar.org.

For technical support in harvesting, contact; Olabode Kehinde K.Olabode@cgiar.org Adeyinka Adewumi A.Adewumi@cgiar.org and Armand Yambisa A.Yambisa@cgiar.org

8. References

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US Department of Agriculture, USDA Plant Science Building, 3127 Ligon Street, Raleigh, NC, 27607 - USA

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IITA Transforming African Agriculture CGIAR	**Crop: Soybean** **Function:** Data collection	**SOP #**	IITA-SB-SOP07
		Revision #	IITA-SB-SOP07-02
		Implementation Date	15/06/2024
Page #	23 – 26	**Last Reviewed/Update Date**	29/12/2025
SOP Owner	Soybean Breeding Unit	**Approval Date**	

SOP for Data Collection

1. Introduction

Data collection in soybean variety trials involves gathering critical information on various aspects of varietal performance, including growth and development, and yield. This data is essential for identifying the best soybean varieties with good productivity and ensuring sustainability. Key areas of data collection include various growth parameters and agronomic traits, agroecological and weather data, pest and disease incidence, and genetic information. Analyzing this data helps derive valuable insights that guide decision-making, increasing yield and enhancing adaptability to diverse agro-ecological conditions.

2. Purpose

This document outlines the roles, responsibilities, and procedures of the soybean breeding program team responsible for collecting valid, high-quality data to guide genetic improvement of soybean. To make an information system more meaningful and applicable, the data must be standardized in terms of terminology and measurement.

The data preparation procedures need careful consideration, as the future universal use of existing systems for data management will depend upon them. Each descriptor must have a clear definition to facilitate meaningful information exchange among cooperating scientists. Before recording the data, the code dictionary should be prepared to give detailed information for interpreting the coded data. Automatic data validation during the input phase helps ensure data validity within permissible limits for each data item, as well as its type (alphabetical or numerical).

3. Scope

This document outlines the descriptive procedure for collecting data in soybean breeding activities.

4. Definition of terms

5. *Roles and Responsibilities*

All staff involved in implementing breeding activities in the soybean improvement program at IITA must use the Breeding Data Collection SOP. The program leaders must approve any alteration to the procedures unless they are exceptionally necessary. The individuals responsible for each section of the Data Collection SOP in the breeding data cycle are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a principal investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for coordinating and supervising the TC, TM, and NC/B for the collection of good-quality data and designing strategies that ensure data collection is standardized within the IITA soybean breeding program and across the different partners.

National Collaborator/Breeder (NC/B): Responsible for coordinating the data collection of trials sent from the IITA soybean breeding program in the target agro-ecologies and locations.

Trial Coordinator (TC): Responsible for proper data collection.

All technicians in the soybean breeding program are responsible for collecting data from the various trials.

Trial Manager (TM): The TM will be responsible for collecting quality data for the location or station assigned to them.

6. *Procedures*

Activity 1: Data Collection

Pre-harvest Data:

1. Planting Date (DATE_PLANT): record the date when the trial was planted (seeding date).
2. Days to 50% flowering (DAYS_TO_FLOWERING): collect days to flowering data when at least 50% of the plants in a plot have at least one flower. During the time of flowering, visit the trial at least three times a week to record flowering dates.
3. Flower color (FLWCOL_EST_SCALE): record the flower color as purple, white, or mixed during flowering.
4. Nodulation scoring: collected by carefully digging up about 5-6 plants from both ends of the rows in each plot about 60-75 days after sowing. Use a 1-5 scale (as described by Yates et al. 2016, URL: https://www.aciar.gov.au/sites/default/files/legacy/aciar_mn_173_web-updated_31_may_2016.pdf). Total nodule number, total nodule weight, and effective nodule weight can also be measured as required.
5. Days to maturity (DAYS_TO_MATURITY): measure days to maturity from sowing date. During maturation, visit the trial three times a week to determine days to maturity (when 95% of the pods have changed from yellow to tan, grey, or brown).

6. Plant height (PLANT_HEIGHT): Collect plant height at harvest by measuring the height (cm) of five plants of the main stem (not petioles and leaves) at the time of maturity, and take the average height of the five plants.
7. Pod clearance (POD_CLEARANCE_CM): Collect the height of the lowest pod at harvest by measuring the height of the lowest pod to the ground level
8. Lodging score (LODG_EST_1TO5): lodging data can be collected by scoring plants lying on the ground, using a scale of 1-5; where 1=all plants erect, 2=25% of plants lodged, 3=50% of plants lodged, 4=75% of the plants lodged, and5=all plants lodged.
9. Shattering score (SHATTERING): Shattering data can be recorded in two phases. The first one at harvest and the second data shall be collected two weeks after harvesting by retaining about 10 sample plants in the field. The data need to be collected in a 1-5 scale, where 1=no pods shattered, 2=25% of the pods shattered, 3=50% of the pods shattered, 4=75% of the pods shattered, and 5=all plants shattered.
10. Pubescence colour (PUBCOL_EST_1TO4):

a. For pubescence (small hair-like fibres on the pods) colour, look closely at the pods on several plants.
b. Do not mistake pod colour with pubescence colour.
c. The pubescence will be tawny (light brown), grey, or mixed (an equal 50/50 mix of tawny and grey pubescence).

11. Pod colour (PODCOL_EST_1TO4): the pods near the top of the plant are the first to lose colour, so examine the pods in the bottom 2/3 of the plant.

a. Grey – "G"
b. Tawny – "T"
c. Mixed (½ gray, ½ tawny) – "M"
d. If there are plants with colors that are different from the rest of the plot, excluding mixed plots, rogue these contaminated or off-type plants.

12. Number of plants harvested (PLNTHVST_COUNT_PLANT): collect data on the number of plants harvested by counting the number of plants in the net plot (4m in the centre of the plot contributing to the yield sample)

Post-Harvest Data:

a. 100-seed weight (SEEDWEIGHT_100): measure 100-seed weight by weighing 100 seeds sampled from the harvested plot.
b. Plot yield (AYLD_CONT): collect plot yield data by weighing in grams of seeds from the 4m plot from the middle two harvestable rows of the plot. Seeds should be uniformly dried before weighing. Use a balance with a precision of at least ±0.5g.
c. Hilum color (HILUM_COLOR): needs to be collected by hilum color observation (white, yellow, dark black, black, brown)

d. Moisture content (SDMOIST_COMP_PCT): A moisture meter should be used to check the moisture content of seeds at the time of collecting yield data. The moisture content data will be used to adjust the yield per ha to a standard moisture content of 13%.

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Technical problems with data collection, please contact: Olabode Kehinde K.Olabode@cgiar.org, Adeyinka Adewumi a.Adewumi@cgiar.org and Armand Yambisa A.Yambisa@cgiar.org

Experimental design and data analysis: Ibnou Dieng, I.Dieng@cgiar.org

8. References

Excellence in Breeding – EBS User guides - User guides - Digital Solutions

SOP for Crossing Block

1. Introduction

Soybeans exhibit a high degree of flower and pod abortion, with the greatest percentage occurring within the first seven days after flowering. Generally, the first and last few flowers to bloom are those that abort most readily (26). The cause is not definitely known. If abortion could be inhibited, yields should increase.

Flower abortion may be due, in part, to a failure in pollination. Soybeans are highly self-fertilizing, with natural crossing usually less than 1%. If successful pollination could be assured, abortion might be decreased.

2. Purpose

This document outlines the roles, responsibilities, and procedures for making and harvesting successful crosses.

3. Scope

This document contains the crossing procedure in the soybean breeding program. It covers steps to making crosses, items needed for a successful crossing, checking success in crosses, and harvesting crosses.

4. Definition of terms

5. Roles and Responsibilities

All staff involved in soybean crossing at IITA must use the soybean crossing SOP. The program leaders should approve any alteration to the procedures only if necessary. Below is the list of individuals responsible for each section of the data management SOP in the breeding data cycle.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a principal investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for coordinating and supervising the soybean crossing as per the plan and ensuring the planned timeline for rapid generation advance is achieved. Give proper support to the national partners in establishing their own crossing program.

Trial Coordinator (TC): Responsible for supervising the crossing activities that are performed as per the plan.

Crossing technicians (CTs): Responsible for planting the parental lines in a staggered manner and making crosses as per the plan.

Genotyping Coordinator: Responsible for sample collection, preparation, and shipment to the genotyping service provider to check and confirm success in crossing.

6. Procedure

Activity 1: Creating Crosses in EBS

1. Click the dropdown field under Experiment Type and select Intentional Crossing Nursery.
2. Fill in the necessary fields, especially those marked with an asterisk (*).
3. Once done, click **'Save'**.
4. Go to the Parent List tab
5. Next, add and manage a parent list
    a. Click the Add Entries [+] icon from the toolbar.
    b. In the Add Entries modal window, choose between four methods of adding entries. A simpler way to add entries is to use the Saved List method.
    c. Add entries from a saved list
    d. Click Saved List
    e. Locate the desired saved list, and then click Select.
    f. The entries should now be displayed in the Entry List table
    g. Once the entries have been added, users may proceed to update their entries, especially when identifying the parent roles (i.e., female, male, or female-and-male)
    h. Specify the required values under the **Parent Role** (required), **Parent Type**, and **Description** columns. The changes will be automatically saved.
    i. Once done, click Next in the upper-right corner of the page. Users will then identify the crosses

Activity 2: Planting the parental lines

1. Ensure enough plots are prepared in the screenhouse to plant the parental lines
2. Prepare seeds of the selected parental lines
3. Label the parents planted in each plot.
4. Plant the parental lines in a staggered manner (only two staggered plantings to align with the speed breeding plan).

Activity 3: Making crosses

1. Ensure all the crossing tools are available in the crossing kit
2. Check the possible crossing combinations to identify if female flowers are available on the date of crossing to make sufficient crosses.
3. Then, pollination will be made between the selected male and female flowers after making the female flower ready for pollination by opening the stigma
4. The minimum number of crosses an experienced person makes shall not be less than 25 per day, depending on the availability of sufficient male and female flowers.
5. During warm weather, the crossing needs to be done twice a day, between 8:00 am and 10:00 am during the daytime, and 5:00 pm and 7:00 pm during the evening; however, during cool weather, the crossing can be done any time of the day, depending on the availability of flowers.
6. During the warm weather, watering the plots is required twice a day.
7. Tie a red thread on the successful crosses after confirming successful pod development in the cross (this needs to be done 15-30 days after crossing).

a. Items needed for crossing:

i. Blank tags
ii. Pencil
iii. Pencil and pencil sharpener
iv. Garden marker
v. Forceps
vi. Head lens (Optivisor)
vii. 90% ethanol
viii. Digital tablet with barcode reader for data collection
ix. Colored threads

7. Go to your appropriate female row and be certain that you are facing the correct row before you begin to cross.
8. Set up your seat, if desired.
9. Select a healthy-looking female plant from the row and look for 1-2 buds that are not all green nor completely flowered (will flower within 1-3 days). Pinch off the other buds nearby that are young or flowering, and leave the 1 or 2 buds that will be used for crossing.

10. Pull off the five sepals using forceps. Sepals are the green leaf-like structures below the petals (refer to Figure 1). If these are not removed, the petals will be difficult to remove. Sepal removal can later serve as a marker (forming a brown scar) for identifying successful crosses.
11. Gently remove petals. Pinch the petal close to the base with forceps and slowly pull the petal out and away from the bud in the direction of the straight (vs. curved) side of the bud. After removing it, you should see the stigma and style as in Figure 2.

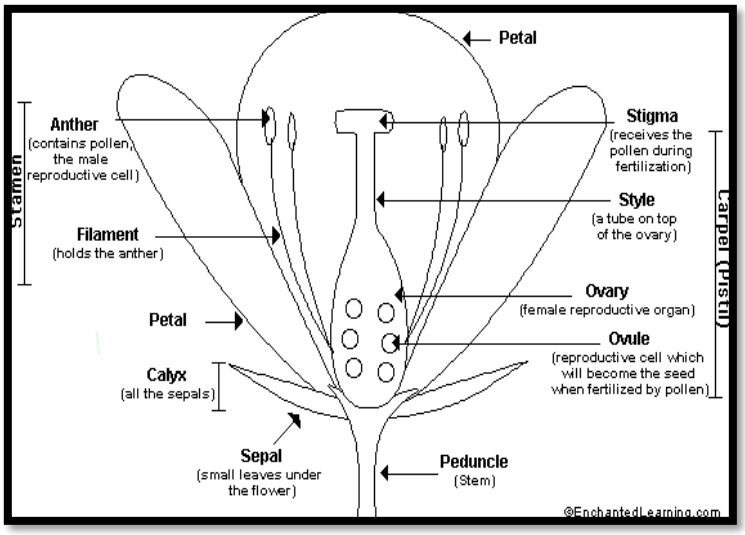


Figure 1. Flower anatomy

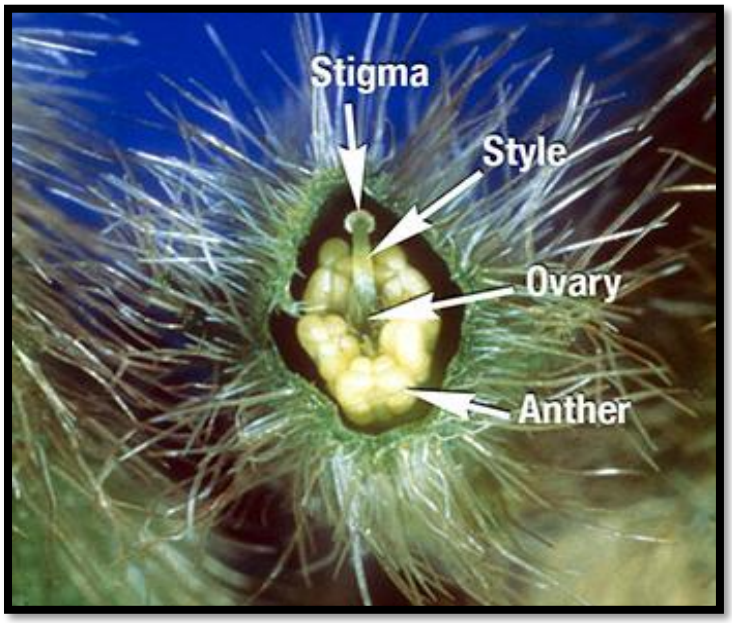


Figure 2. The stigma and style

12. Remove the anthers carefully if they interfere with your view of the stigma. This is called emasculation. However, in soybean, even if you don't remove the anthers (you don't emasculate), there is very little chance (almost zero) of self-pollination, and hence there is no need to emasculate soybean flowers. Do not touch the stigma during

emasculation. The stigma is the apical end of the style where deposited pollen enters the pistil—this is where you pollinate the female with the male flower.

13. Select a male flower from the male parent rows. Identify freshly opened male flowers with abundant pollen. Remove the sepals and open the petals for access to the anthers.

a. To check if the flower is ready to pollinate, touch an anther to your fingernail. If there is pollen residue, it is ready.

14. Gently brush or touch the male anthers against the female stigma – this is pollination.

15. The crossed flowers need to be labelled, and the labelling of the crosses needs to follow the following steps

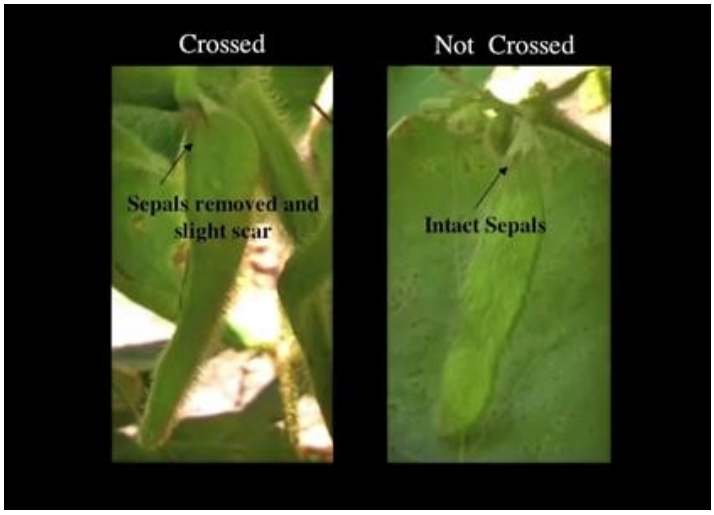
- The female parent needs to be written first, and then the male parent
- The date of the crossing needs to be written
- Write the number of crosses you made on one side of the tag
- Write the initials of the name of the person who made the crosses
- Put the tag on the stem immediately above the crossed flower, not on the leaf, as the leaf can fall at any time during the growth stages

16. Be very careful to avoid damaging your most recent crosses when you move or change plants.

Activity 4: Checking crosses

1. Look at the list of crosses
   a. Check the crosses that have been pollinated 7-10 days ago.
   b. Don't check crosses in a row if there are freshly made (<1 day old) crosses in the same row. Wait at least 1 day before checking the row to avoid disturbing fresh crosses.
   c. Make a note of which rows you need to check and the name of the cross.
   d. Check the oldest crosses first.
2. Find the tags labelled with the appropriate cross name (and crosser, if applicable). Be certain that you are looking at the correct row. This is another opportunity for quality control.
4. Check the tags for the crosses you are checking to make sure they are labelled correctly and on the correct row.
   a. If you are on the wrong row, you may end up removing successful crosses.
   b. If the crossers used an incorrect row for a specific cross name, you need to remove those tags.
5. Find the tags.

6. Clean up the area around the potential cross.



   a. Pay attention to the number of crosses that were done per tag to ensure that the correct number of buds are covered by the loop of the string. There are buds that were not pollinated (by us).

7. Repeat clean-up for each tag.
8. Check the pollination for the emerging bud.
9. Keep a tally of the number of successful crosses to compare against later.
10. Check each row to make sure you went through all of the tags.
11. Repeat for each cross until all crossings are finished and double-checked.

12. The important part of double-checking is to make sure your crosses are labelled correctly for that row, that there are successful crosses, and that they are cleaned. If you go through a row and each bud has died, then more crosses of that combination need to be made again.

Activity 4: Harvesting on EBS

1. Go to Harvest Manager on EBS
2. Under the search parameters,
   a. Select the **program**
   b. Select the **experiment**
   c. Select **occurrence**
   d. Then click on '**crosses**'
3. Indicate the harvest method as **'Bulk'**
4. Click the field under **Harvest Date** to use the calendar widget and select the date
5. Once the crosses have their harvest data updated, click 'Next' in the upper-right corner of the browser. The **'creation'** tab will open for users to commit the harvest data.

Activity 5: Harvesting crosses

1. Have your book of crosses out and ready. You should have numbers written by all your crosses, so you know how many to expect.
2. Before going to a row, make sure you have the envelope with the correct cross name for that row.
3. If the rows are still green, they are not ready to be harvested; if you find the pods shattering, it is too late to start. As the plants in the crossing block begin to brown, you should consistently check the crossing block/screenhouse to see if crosses are ready to be harvested.

a. If a pod has any green on it, give it more time to dry well.

b. If you have any doubt that a cross is ready for harvest, do not harvest it.

4. Starting with the oldest crossing dates, go through the appropriate row and find tags with the matching cross name.
5. To remove the pod(s), place your hand around both the pod(s) and tag, and gently pull them off. You need to be careful not to rip the pod(s) or lose the tag. If it's truly ready to harvest, it should snap off easily.
6. Wrap the tag around the pod(s) and place it in the corresponding envelope.
7. When you have collected all the crosses from a row, make sure the opening is closed using gum or a stapler, label the envelope with the number of pods collected, and place it in the box/cloth bag. The harvesting date needs to be written on the envelope.
8. Repeat this for each cross name.

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Issues relating to crossing block, you can contact the crop lead, Dr. Chigeza Godfree G.Chigeza@cgiar.org and the regional head, Abebe Abush Tesfaye At.Abebe@cgiar.org. Contact Mr. Sunday Ojo S.Ojo@cgiar.org , Ajayi Ademola Ebenezer Ea.Ajayi@cgiar.org and Adeyinka Adewumi A.Adewumi@cgiar.org for specific technical crossing-related information for the West Africa program, and Armand Yambisa A.Yambisa@cgiar.org and Nanku Chilima N.Chilima@cgiar.org for the Southern Africa program.

IITA Transforming African Agriculture CGIAR	**Crop: Soybean** **Function:** Parental Selection	**SOP #**	IITA-SB-SOP10
		Revision #	IITA-SB-SOP10-01
		Implementation Date	15/06/2024
Page #	34 – 36	**Last Reviewed/Update Date**	29/12/2025
SOP Owner	Soybean Breeding Unit	**Approval Date**	

SOP for Parental Selection

1. Introduction

Selecting the best parental lines with good *perse* performance and a wide genetic base is one of the most crucial steps in a breeding program. Visual evaluation of genotypes for performance across various trials, including single- and multi-site analyses, along with results from previous diallel analyses, can be used to select the best parental lines for crossing. However, the need to conduct numerous crosses and experiments with many hybrids and parental lines limits the use of diallel crosses for selecting parental lines. Thus, assessing the genetic divergence of the available germplasm before making any crosses may help breeders concentrate their efforts on the most promising combinations.

2. Purpose

This document aims to outline the roles, responsibilities, and procedures for parental selection, providing the raw materials for creating each new generation of genetic improvements. Ultimately, the probability of successfully meeting the breeding objectives depends on the choice of parents for intermating.

3. Scope

This document outlines the criteria for selecting parents for the soybean crossing program.

4. Definition of terms

5. Roles and Responsibilities

The breeders responsible for implementing the breeding activities in the soybean improvement program at IITA must use the Parental Selection SOP. The program leaders must approve any alterations to the procedures, unless they are exceptionally necessary. The individuals responsible for each section of the parental selection SOP in the breeding program are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a Principal Investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for selecting the parental lines for crossing and coordinating the TC and NC/B on the timely data availability for curation, analysis, and summary of data from multi-location trials to guide parental line selection.

National Collaborator/Breeder (NC/B): Responsible for the timely collection and submission of data from trials sent to the respective locations, for the timely analysis of data, and for summarizing the results.

Trial Coordinator (TC): Responsible for timely data collection and summarizing the results to guide parental selection.

All technicians in the soybean breeding program are responsible for timely data collection.

Data Analyst (DA): curates and analyzes data from a single or multiple locations and summarizes the results in tables that will guide the breeder to make proper parental selection.

6. Procedure

Activity 1: Parental selection

1. Parental selection is one of the most important steps in the soybean hybridization program. The breeding objectives, or product concept (PC), need to be considered when selecting parents. Two major product concepts were developed by the soybean breeding program of IITA.

PC1: Parental lines will be selected for high yield, medium to late maturity (maturity dates ranging between 100-120 days), and resistance to major foliar diseases, such as rust, red leaf blotch, brown spot, bacterial pustule, and promiscuous nodulation.

PC2: Parental lines for early to extra early maturity that can escape/tolerate moisture stress and give reasonably good yield under drought conditions with promiscuous nodulation and resistance to major diseases, such as rust, red leaf blotch, brown spot, and bacterial pustule, and can adapt better to short growing season environments to be selected.

2. A maximum of 10-20 parents are to be selected for each breeding center (WAH and SAH) and per pipeline, and the crossing shall be done in the screenhouse facilities of IITA Zambia, Lusaka, for the Southern and Eastern African regions, and Nigeria, Ibadan, for the Western and Central African regions.
3. The selection can be made based on the results of previous season multi-location or single-location yield trials data analysis and output summary.
4. Parental selection also needs to consider the breeder's critical visual field evaluation of the trials to identify parents with the desired specific merits based on actual field performance evaluation.
5. The best genotypes that showed consistently high performance and low disease scores for the above-listed diseases and high scores for nodulation would be selected as parents.

6. Genotypes that showed unique/specific merits for some of the desired or priority traits, e.g., disease resistance or high quality, need to be included as parents to ensure the contribution of the specific desirable genes to the progeny populations.
7. Parental selection also needs to consider the genetic divergence or the genetic distance of the parents that can be established based on the genotyping and phenotyping data to ensure high genetic recombination and complementarity. This can be done using genotypic cluster analysis, the breeder's field evaluation of the trials, and summary tables from the variety trials to ensure complementarity among the parental lines.

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Issues relating to parental selection, you can contact crop lead: Dr. Chigeza Godfree G.Chigeza@cgiar.org and regional head Abebe Abush Tesfaye At.Abebe@cgiar.org

8. References

Soybean Parent Selection Based on Genetic Diversity
Valéria Carpentieri-Pípolo, Antônio Eduardo Pípolo, Flavio André Martins da Silva and Marcos Rafael Petek

IITA Transforming African Agriculture CGIAR	**Crop: Soybean** **Function:** Seed Inventory Management	**SOP #**	IITA-SB-SOP11
		Revision #	IITA-SB-SOP11-02
		Implementation Date	15/06/2024
Page #	37 – 39	**Last Reviewed/Update Date**	29/12/2025
SOP Owner	Soybean breeding team	**Approval Date**	

SOP for Seed Inventory Management

1. Introduction

Seed is one of the most important agricultural inputs, playing a crucial role in enhancing crop production and productivity, thereby improving the livelihoods of farming communities. It is the repository of the genetic potential of crop species and their varieties resulting from continuous improvement and selection over time. The potential benefits of seeds in ensuring crop productivity and food security can be enormous.

In addition, production increase resulting from the adoption of improved varieties improves farmers' income when market linkages exist. Food security is heavily dependent on the seed security of the farming community.

A sustainable seed system will ensure that high-quality seeds of a wide range of varieties and crops are produced and made fully available to farmers and other stakeholders in a timely and affordable manner.

2. Purpose

The purpose of this document is to outline the roles, responsibilities, and procedures for seed inventory management, including storing, maintaining seed records, and creating and managing the seed inventory database system.

3. Scope

This document outlines the procedures for managing seed inventories, which are essential for seed lot creation and seed monitoring activities involved in maintaining soybean germplasm and harvesting seeds from variety trials in the IITA soybean breeding program and collaborating partners.

4. Definition of terms

5. Roles and Responsibilities

The personnel responsible for the use and implementation of this SOP are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a Principal Investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for managing and overseeing the proper seed lot creation and storage in the database system for all the trials conducted in a region.

Trial Coordinator (TC): Responsible for the preparation of seeds, proper storage and labelling, and seed database management. TC is also responsible for regularly checking the seed lots' status and viability ratings in the system.

Seed Manager (SM): refers to individuals or clusters who manage the seeds stored in the seed warehouse facility and ensure that the seed inventory system is up to date.

Seed Warehouse Worker (SWW): All technicians, interns, and skilled casual staff are responsible for preparing seeds for dispatch. This includes validating the seed lots to be withdrawn, preparing the seed container for use, cleaning the seeds, and ensuring they are properly labeled and stored.

6. Procedure

The following are the step-by-step procedures for carrying out the Seed Inventory Management:

1. Ensure all seeds are sorted, cleaned, and the moisture level is maintained at the utmost 13%.
2. Manage harvested seeds by packaging them in paper bags and cloth bags for short-term storage and plastic bags, Ziploc bags, and jars for long-term storage.
3. Properly label the seeds with all important information, such as

a. Trial name
b. Variety name
c. Entry number
d. Year of production
e. Stock ID/GID
f. Quantity
g. Moisture Percent

4. Arrange all packages into boxes
5. Label boxes with the box code number
6. Each shelf and partition in the shelf should be coded.
7. Update all information above into the database.

Seed rejuvenation

Soybean seeds older than two years are likely to lose more than 50% of their viability. Replant seeds within two years to maintain a fresh, high-quality seed stock.

Seed discarding
Trial seeds stored for more than three years should be discarded after sample seeds are retained for germplasm maintenance. In the unlikely happening of seed quality deterioration during storage, the seeds require rejuvenation, and the old seeds must be discarded.

Regular updating of the seed inventory database
The seed database for all the new entries needs to be updated annually.

The quantity of seeds in the database needs to be updated whenever additional multiplication is done.

Imported seed lot received

All imported germplasm samples must go through the IITA Germplasm Health Unit (GHU) and must include an appropriate Material Transfer Agreement (MTA) or Standard Material Transfer Agreement (SMTA) document to ensure that the use of each of the samples complies with the conditions set for the sample. The imported seed lot packages can only be added to inventory after their compliance with all the regulatory requirements has been validated and cleared by the GHU.

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For Issues relating to seed inventory management, you can contact: Armand Yambisa A.Yambisa@cgiar.org, and Olabode Kehinde K.Olabode@cgiar.org

8. References

IITA Transforming African Agriculture CGIAR	**Crop: Soybean** **Function:** Seed Packaging for Planting	**SOP #**	IITA-SB-SOP12
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		Implementation Date	15/06/2024
Page #	40 – 43	**Last Reviewed/Update Date**	29/12/2025
SOP Owner	Soybean Breeding Unit	**Approval Date**	

SOP for Seed Packaging for Planting

1. Introduction

The aspect of seed processing and packaging in breeding operations cannot be underemphasized. A well-organized seed package will accelerate breeding operations and improve the time required for the process. Seed packaging before dispatch can be easily overlooked as non-essential for trial establishment. However, seed packaging procedures are fundamental in ensuring cost-effective, timely, and well-organized seed dispatch and trial establishment. This document outlines the standard operating procedures (SOPs) for implementing seed-handling processes and serves as a living document, subject to updates to accommodate current learnings and workflows within the breeding cycle.

2. Purpose

This document outlines the roles, responsibilities, and procedures for seed packaging for planting soybean breeding trials to support effective phenotyping. This SOP also intends to guide the Seed Processing Coordinators.

3. Scope

This document contains the seed packaging procedure required for soybean breeding activities. It covers the labeling of packages, proper packing, sorting, and arrangement of seed envelopes to suit the purpose for which the seed is packaged.

4. Definition of terms

5. Roles and Responsibilities

All staff responsible for seed packaging and preparation in the soybean breeding program at IITA must use the seed packaging SOP. The program leaders should approve any alteration to the procedures, unless they are exceptionally necessary. The individuals responsible for each section of the seed packaging SOP in the breeding data cycle are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a Principal Investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for managing and overseeing the proper seed packaging of all the trials conducted in a region.

Trial Coordinator (TC): The TC is responsible for ensuring seed viability before packaging and for properly preparing, packaging, and labeling seeds.

Seed Manager (SM) – In alignment with the TC and RC, the SM is responsible for verifying seed viability before preparing, packaging, and labeling seeds.

Seed Warehouse Worker (SWW) – all the technicians, interns, and skilled casual staff are responsible for preparing and packaging the seeds.

6. Procedure

Activity 1: Preparing seeds for packaging

1. Obtain an entry list
2. Check for the quality and uniformity of seeds to be packed and ensure off-types are removed, if any, based on:
    a. Seed size
    b. Hilum colour
    c. Seed coat colour
3. Weigh the seeds of the entries in the list to check if they have enough seeds for the purpose for which they are packed.
4. Submit the list with the available quantity of seeds
5. Prepare enough seed packets or seed bags, staplers, measuring cups, a seed counter, and a seed sieve for packaging
6. Lay out the bags in the new entry order for easy identification during packing
7. If an envelope printer is available, print the label directly on the envelopes
8. Make sure you put the envelopes according to the entry numbers
9. Put the envelopes at the top of the corresponding bag of seeds.
10. Make sure that the information on the envelope matches the information on the label inside the bag

Activity 2: Packing seeds

1. Count and pack seeds in the envelope

2. Depending on the trial's nature, ensure the right quantity of seeds is poured into the seed packet or seed bag.
3. Ensure you use all the seeds from one replication or bag before opening another one. Please note: Once you open seeds from another replication or bag, confirm the similarities with the original bag.
4. Make sure each envelope is stapled.
5. Once all the envelopes are packed, sort by location number, followed by plot order for each location
6. Double-check the seed arrangement to ensure you have not missed or left out any envelopes
8. Using a rubber band, tie four or five envelopes together to help pack them into plastic for shipment.
9. Pack into plastic bags and fit into boxes, ready for shipping and planting

Activity 3: Packing envelopes in planting order (Mechanical planting)

1. Have your field map ready
2. Count the number of fillers for the field and package many filler envelopes.
3. Using the field maps, place envelopes on the table and ensure they follow the field order.

a. Two (2) people are necessary to put envelopes in the field order: one person to place the envelopes in the lines and the other to read the field map and check correct envelope placement.

b. Three people are best because an additional person can obtain the box for each test, check the plot numbers, and pass the envelopes to the envelope placer in the correct order.

4. Read the field layout from left to right and bottom to top (follow the serpentine pattern when taking notes in the field). Place envelopes in the sleeves in that order, starting at the bottom of each sleeve (or pass).
6. Put the envelopes in order, starting from left to right. Always start from left to right
7. Indicate box number
8. After all the seed packages have been put in the boxes, triple-check that the envelope placement matches the planting order.

a. Two people are necessary - one person checks the map, while the other reads the plot numbers of each envelope in the planting order

b. Write a small check mark on the label each time the box is checked. After three check marks, the box is ready for planting.

9. Put a copy of the field map into Box 1 for each field.

10. The seed is now ready for planting.

7. Forms/Templates to be used for monitoring and data collection

7.1 **Contacts for support**

For Issues relating to seed packaging, you can contact: Armand Yambisa A.Yambisa@cgiar.org

Adeyinka Adewumi A.Adewumi@cgiar.org and Olabode Kehinde K.Olabode@cgiar.org

8. References

SOP for High-throughput phenotyping

1. Introduction

Soybean is a crop with the highest nutritional composition (protein and oil), making it an ideal crop in the fight against malnutrition and in meeting growing market demand. However, the attention given to nutritional quality by African breeding programs, including the IITA soybean-breeding program, has been limited, primarily because nutritional composition is given low priority in the soybean value chain. However, processors and end users are increasingly demanding nutritionally enhanced soybean varieties to meet the poultry and fishery industries' growing demand for high-protein feeds. composition in soybeans, with proper calibration based on wet-chemistry results. In addition, the use of high-throughput phenotyping with drone technologies has been commended for its rapid, high-throughput workflow for estimating soybean yield, biomass, plant height, and days to maturity.

2. Purpose

The purpose of this document is to outline the roles, responsibilities, and procedures to be followed in soybean high-throughput phenotyping for grain compositional analysis and estimation of yield and other parameters.

3. Scope

This document outlines the steps for utilizing Near-Infrared Spectroscopy (NIRS) and drone technologies in high-throughput phenotyping in the soybean breeding program.

4. Definition of terms

5. Roles and Responsibilities

All staff responsible for implementing breeding activities in the soybean improvement program at IITA must begin using the high-throughput phenotyping SOP. No alteration should be made to the procedures unless approved exceptionally by the program leaders. The individuals responsible for each section of the SOP in the breeding data cycle are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a Principal Investigator (PI), or a cluster lead.

Regional Coordinator (RC): Responsible for coordinating and supervising the TC, TM, and NC/B for the calibration of the NIRs machine for rapid phenotyping of soybean genotypes for

nutritional composition and collaborating with the nutrition and GIS Units for nutritional analyses and UAV-based soybean data collection.

National Collaborator/Breeder (NC/B): Responsible for sending samples for nutritional composition analysis once the NIRS machine is calibrated. As needed, the NC/B facilitates the collection of UAV data from their respective stations.

Trial Coordinator (TC): Responsible for sample preparation and submission for analysis as advised by the CL and RC. The TC will support and facilitate the UAV-based data collection in collaboration with the GIS unit.

All technicians in the soybean breeding program: Responsible for sample preparation for nutritional composition analyses, and supporting the UAV-based data collection in collaboration with the GIS unit

GIS and RS expert: to collect drone-based data for yield, biomass, plant height, and days to maturity-related data and run the required analyses.

Food scientist: who will collect spectral data and develop NIRS calibration models for the nutritional properties of interest.

Nutrition lab manager: The lab manager will take care of the laboratory analysis (the chemical (Reference data) analysis and NIRS spectra data collection in the laboratory) for the calibration of the NIRS machine

Data Analyst (DA): Supports and collaborates with GIS and RS experts to facilitate the analysis and interpretation of UAV-based data.

6. Procedure

Activity 1: Flying drones for phenotyping data capturing

1. Discuss the trial details (trial locations and experimental layout) for data collection.
2. Plan to fly the drone (flight path design)
3. Fly the drone with a multi-spectral RGB camera
4. Post-processing of drone data
5. Analysis of drone data with machine learning algorithms and the actual agronomic data collected from the field
6. Write reports based on the results of the analysis.

Activity 2: NIRs analysis for compositional analysis

1. Calibrate the NIRS machine at IITA nutritional lab for compositional analysis
2. Prepare samples for wet chemical analysis to generate reference data to calibrate the NIRs machine
3. Conduct the wet-chemistry analysis on a sufficient sample population to obtain a good calibration model

4. Develop the prediction models based on the wet analysis with the spectral data, and ensure the prediction level is acceptable
5. Analyze the nutritional composition of the soybean genotypes in the breeding program using the NIRS technique.
6. Testing the developed model for accuracy using an independent sample set to validate the model developed
7. Analyze the nutritional composition of the soybean genotypes in the breeding program to validate the developed NIRS models
8. The priority compositions for analysis are total protein content, amino acid profile, total oil content, fatty acid profiles, and anti-nutritional factors

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For issues relating to nutritional composition analysis and flying drones for UAV-based data requirements, contact the crop lead, Dr. Chigeza Godfree G.Chigeza@cgiar.org, and regional breeder Abebe Abush Tesfaye At.Abebe@cgiar.org, the IITA GIS unit Mr. Alabi Tunrayo T.Alabi@cgiar.org, Nutrition Unit Dr. Mercy Lungaho Mg.Lungaho@cgiar.org,

And for technical support on sample preparation and submission and facilitating flying the drone, contact Olabode Kehinde K.Olabode@cgiar.org, Armand Yambisa A.Yambisa@cgiar.org and Adeyinka Adewumi A.Adewumi@cgiar.org

8. References

Jegor MILADINOVIĆ, Joe W. BURTON, Svetlana BALEŠEVIĆ TUBIĆ, Dragana MILADINOVIĆ,Vuk DJORDJEVIĆ, Vojin DJUKIĆ

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(16) (PDF) Soybean breeding: Comparison of the efficiency of different selection methods. Available from: https://www.researchgate.net/publication/265415202_Soybean_breeding_Comparison_of_the_efficiency_of_different_selection_methods [accessed Mar 28 2022].

IITA Transforming African Agriculture CGIAR	**Crop: Soybean** **Function:** Soybean genotyping	**SOP #**	IITA-SB-SOP14
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SOP Owner	Soybean Breeding Unit	**Approval Date**	

SOP for Genotyping

1. Introduction

Due to their high nutritional value (40% protein and 20% oil), soybeans have high commercial value globally. Hence, it has received increasing attention from the private and public sectors for advanced genomic studies. Several molecular genomic resources, including single-nucleotide polymorphisms (SNPs), such as Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html), SoyKB (http://soykb. org/), and Soybase (https://soybase.org/), are available in the public databases. To utilize the opportunities presented by genotyping for genetic diversity studies, marker-assisted selection, and mapping populations for traits of interest, and to implement genomic selection, the IITA soybean-breeding program plans to genotype the available working germplasm regularly.

2. Purpose

The purpose of this document is to outline the roles, responsibilities, and procedures to be followed in soybean genotyping at different breeding stages.

3. Scope

This document contains the genotyping procedure in the soybean breeding program. It covers steps for extracting DNA and submitting it for genotyping from available soybean germplasm in the breeding program.

4. Definition of terms

5. Roles and Responsibilities

All staff responsible for implementing the breeding activities in the soybean improvement program at IITA must use the genotyping SOP. No alteration should be made to the procedures unless approved exceptionally by the program leaders. The individuals responsible for each section of the SOP in the breeding data cycle are listed below.

Crop Lead (CL): Responsible for the overall management of the trial and for delegating team responsibilities. The CL could be a lead breeder, a Principal Investigator (PI), or a cluster lead.

Soybean Breeder: Responsible for all the activities related to genotyping and molecular breeding in the soybean breeding team.

Trial coordinators: Participate in preparing the seeds for planting and coding the genotypes for sampling and shipment to the genotyping service provider.

Genotyping Coordinator: Coordinate the proper leaf sample collection, proper leaf sample storage, proper DNA extraction using kits and in-house DNA extraction protocols, proper DNA integrity and quantity checks, in-house genotyping using SSR, SNP, and KASP markers, adequate shipment for leaf and DNA samples to genotyping service providers, such as EIB, Intertek, LGC, DArT, or other service providers, and analysis of the genotyping results.

All soybean technicians are responsible for planting genotypes to be genotyped and for participating in sample collection.

6. Procedure

Activity 1: Leaf sampling and preparation

Collection using leaf sampling bags

A single plant per genotype will be tagged with a genotype code name for the molecular assay and monitored. For liquid nitrogen and dry ice methods, four young leaves will be collected from the target plant and then placed in well-labelled leaf sampling bags. The leaf samples will be stored in liquid nitrogen or dry ice for 1 hour during field collection, then transferred to a -80°C freezer for 72 hours before being lyophilized (freeze-dried) at -196°C for 96 hours using a freeze-drying machine. For leaf sample collection using silica gel, leaves will be placed in 20 g of granular silica gel mixed with a colour indicator and stored in the dark at room temperature for 72 hours. For each genotype, 2 g of dry leaf will be removed from a sampling bag containing the silica gels for DNA extraction. After drying, four leaf discs or more, depending on the requirement, will be punched into well-labelled DNA extraction plates or Eppendorf tubes for genotyping.

Collection using DNA extraction plates

The leaf samples may also be collected into DNA extraction plates, as specified in the service request. The DNA extraction plates will first be prepared and arranged according to the genotype list in the field, and the list should be matched to the letters and numbers on the plates. The well-arranged DNA extraction plates will be placed on a dry ice box or bag, and four-leaf discs or more of approx. 0.5 cm^2 to 1 cm^2 will be collected from each genotype using a clean leaf puncher placed on top of a clean plastic mat and punched into each well of the strips according to the arranged list. For each genotype collection, the punchers and mats must be cleaned with 70% ethanol to avoid contamination. Care should also be given to ensure that the appropriate genotype is placed in the appropriate well of the DNA extraction plate. The extraction plates containing the leaf samples will be transferred to a -80°C freezer for 72 hours before lyophilization (freeze-drying) at -196°C for 96 hours using a freeze-drying machine. The samples will then be sent to service providers for genotyping.

Activity 2: DNA Extraction

Conventional or In-house DNA extraction

1. Collect 2g of lyophilized leaf samples into 2 ml microcentrifuge or Eppendorf tubes or 1.5 ml DNA extraction strip plates containing steel balls.
2. Grind the leaf samples in the tubes using a genogrinder and avoid grinding to prevent shredding of DNA samples.
3. Remove the steel balls after grinding.
4. Add 700 µL of extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 1% SDS, 300 mM NaCl) and mix thoroughly by vortexing using a vortex machine.
5. Keep the mixture in a water bath for 10 mins at 65°C.
6. Add 500 µL of chloroform: isoamyl alcohol (24:1) and mix thoroughly
7. Centrifuge for 10 min at 6000 RPM.
8. Collect the supernatant in a new micro-centrifuge tube or DNA extraction strip.
9. Add another 500 µL of chloroform: isoamyl alcohol (24:1) to the supernatant and mix thoroughly
10. Add 800 µL of ice-cold isopropanol and mix by inverting the tube.
11. Place the mixture in a -20°C freezer for 2 hours or in a -80°C freezer for 1 hour
12. Centrifuge the mixture for 10 mins at 4000 RPM and discard the supernatant
13. Wash the pellets with 70% alcohol twice for 5 minutes at 4000 RPM and dry on the bench to remove traces of ethanol.
14. Dissolve the pellets in 50 µL TE buffer and store them at -20°C.
15. Samples ready for analysis

Kit DNA Extraction Protocol

1. Disrupt samples (≤100 mg wet weight or ≤20 mg lyophilized tissue) using the TissueRuptor®, the TissueLyser II or a mortar and pestle.
2. 400 µl Buffer AP1 and 4 µl RNase A. Vortex and incubate for 10 min at 65°C. Invert the tube 2–3 times during incubation. Note: Do not mix Buffer AP1 and RNase A before use.
3. Add 130 µl Buffer P3. Mix and incubate for 5 min on ice.
4. Recommended: Centrifuge the lysate for 5 min at 20,000 x g (14,000 rpm).
5. Pipet the lysate into a QIAshredder spin column placed in a 2 ml collection tube. Centrifuge for 2 min at 20,000 x g
6. Transfer the flow-through into a new tube without disturbing the pellet if present. Add 1.5 volumes of Buffer AW1, and mix by pipetting.
7. Transfer 650 µl of the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge for 1 min at ≥6000 x g (≥8000 rpm). Discard the flowthrough. Repeat this step with the remaining sample.

8. Place the spin column into a new 2 ml collection tube. Add 500 µl Buffer AW2, and centrifuge for 1 min at ≥6000 x g. Discard the flow-through.
9. Add another 500 µl Buffer AW2. Centrifuge for 2 min at 20,000 x g. Note: column does not come into contact with the flow-through.
10. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.
11. Add 100 µl Buffer AE for elution. Incubate for 5 min at room temperature (15–25°C). Centrifuge for 1 min at ≥6000 x g.
12. Repeat step 11.

Activity 3: Gel electrophoresis

1. To make a gel, add 0.8-1g agarose tablets or powder into 100mL of 1XTBE buffer and add 2ul of save-view or SYBRsafe stain or ethidium bromide.
2. Microwave for 5 minutes, pour into gel tray, and let it cool for 30 minutes.
3. Fill the electrophoresis tank with 1XTBE buffer and place the casted gel into it, ensuring it is submerged in the tank
4. Use 2µL of your DNA samples and 3µL loading dye to check the integrity of DNA samples on the gel.
5. Use a 10µL pipette to dispense the 5µL DNA samples and the loading dye into the wells on the casted gel.
6. Set the voltmeter machine to at most 100 volts and allow it to run for up to 1 hour
7. Check the quality or integrity of the DNA samples under gel documentation

Activity 4: RNA extraction

1. Disrupt a maximum of 100 mg of plant material according to step 1a or 1b.

1a. Disruption with a mortar and pestle. Immediately place the tissue in liquid nitrogen. Grind thoroughly. Decant tissue powder and liquid nitrogen into RNase-free, liquid-nitrogen–cooled, 2ml micro centrifuge tube. Allow liquid nitrogen to evaporate, but do not allow the tissue to thaw.

1b. Disruption using the Tissue-Lyser II, TissueLyser LT, or TissueRuptor®

2. Add 450 µl Buffer RLT or Buffer RLC to a maximum of 100 mg tissue powder. Vortex vigorously.
3. Transfer the lysate to a QIA shredder spin column (lilac) placed in a 2 ml collection tube. Centrifuge for 2 min at full speed. Transfer the supernatant of the flow-through to a new micro centrifuge tube (not supplied) without disturbing the cell-debris pellet.
4. Add 0.5 volume of ethanol (96–100%) to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Proceed immediately to step 5.
5. Transfer the sample (usually 650 µl), with any precipitate, to an RNeasy Mini spin column (pink) in a 2 ml collection tube (supplied). Close the lid and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard the flow-through.

6. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
7. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid and centrifuge for 15 s at ≥ 8000 x g. Discard the flow-through.
8. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at ≥8000 x g. Optional: Place the RNeasy spin column in a new 2 ml collection tube (supplied). Centrifuge at full speed for 1 min to dry the membrane.
9. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid and centrifuge for 1 min at ≥ 8000 x g to elute the RNA.
10. If the expected RNA yield is >30 µg, repeat step 9 using another 30–50 µl of RNase-free water. Alternatively, use the eluate from step 9 (if high RNA concentration is required). Reuse the collection tube from step 9.

Activity 5: Genotyping and shipment of leaf and extracted DNA samples

1. Identify the appropriate SNP or SSR markers for genotyping.
2. Identify the genotyping service providing companies.
3. Communicate with the service-providing company on the details of the genotyping service
4. Prepare the extracted DNA samples for shipment
5. Ship the samples to the service-providing company.
6. Follow-up on the genotyping process
7. Receive the results of the genotyping
8. Inspect and check the integrity of the data
9. Perform statistical analysis

7. Forms/Templates to be used for monitoring and data collection

7.1 Contacts for support

For the genotyping plans of the soybean breeding program, you can contact crop lead: Dr. Chigeza Godfree G.Chigeza@cgiar.org and regional head Abush Tesfaye Abebe At.Abebe@cgiar.org. For details of sample preparation and DNA extraction: Adewumi Adeyinka A.Adewumi@cgiar.org.

For technical support: Armand Yambisa A.Yambisa@cgiar.org and Olabode Kehinde K.Olabode@cgiar.org

8. References

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Qiagen DNeasy Plant Mini Kit (2024) handbook
Qiagen RNeasy Plant Mini Kit (2016) handbook

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