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Science for resilient livelihoods in dry areas

Modernization of ICARDA
Breeding Programs
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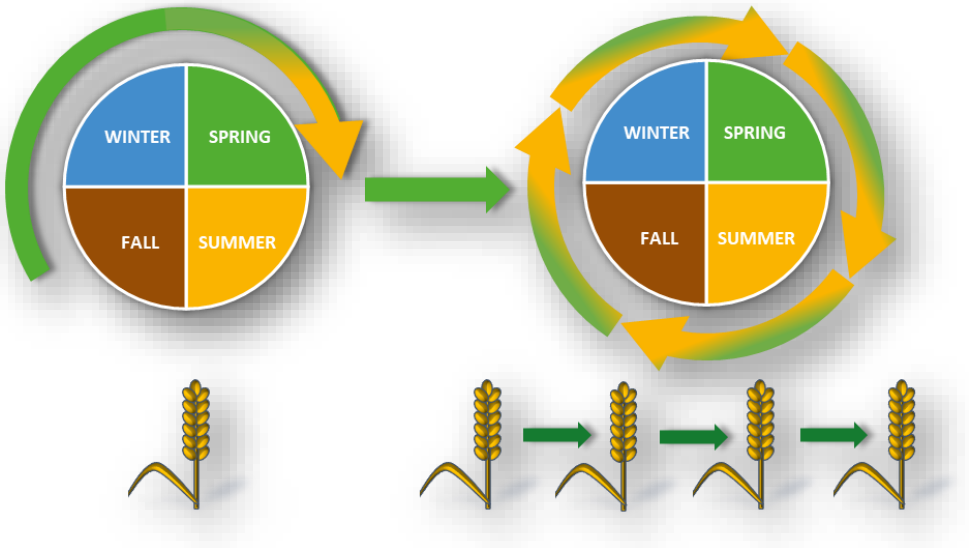
& SOCIAL DEVELOPMENT

STANDARD OPERATING PROCEDURE:

ICARDA Cereals and Legumes Speed Breeding Platform

Version: 07 June 2022



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FAO INTERNATIONAL PLANT TREATY - *Third Call for Proposals Addressing the challenges of climate change for sustainable food security in Turkey, Iran and Morocco, through the creation and dissemination of an international database to promote the use of wheat genetic resources and*

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Contents

Abbreviations

ISBP: ICARDA-Rabat cereals and legumes speed breeding platform
RS: Requesting Scientist
PL: ICARDA Rabat Platform Leader
FM: Facility manager
SB: Speed Breeding
BMS: Breeding Management System

Purpose

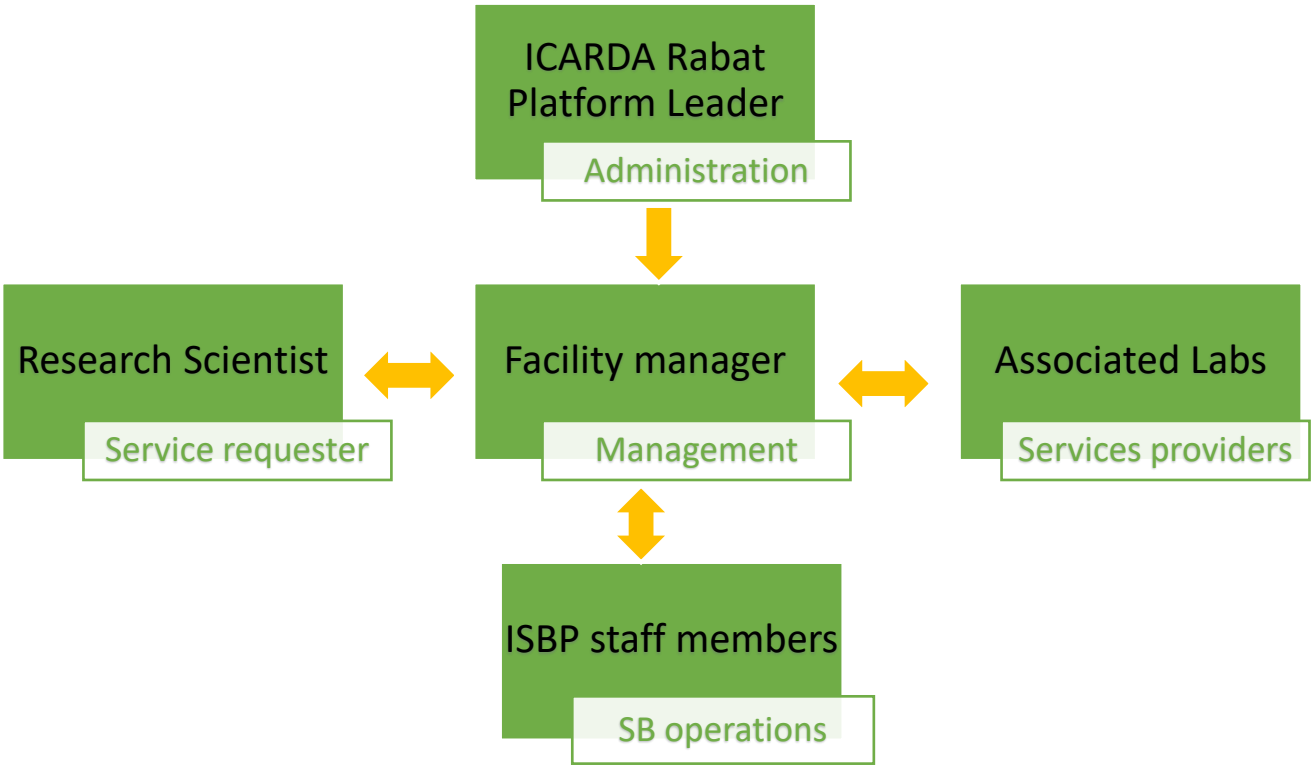
This document is intended to define the standard operating procedure for the ICARDA-Rabat cereals and legumes speed breeding platform (ISBP), inform the Requesting Scientist (RS) about the available services and the procedure to follow and serve as a guide for growth chambers protocols.

Scope

This SOP presents all details concerning the ISBP management. A general idea of the facility description is provided, together with different options available depending on the assisting disciplines and crops. The SOP details the general steps of the speed breeding process, the responsibility and coordination between the facility staff, RS and the breeding programs. This document also presents the ISBP strategy for data management, storage, and maintenance.

Oversight and management

The ICARDA speed breeding facilities are administered by the ICARDA Rabat Platform Leader (PL) and managed by the facility manager (FM).



The operations of the ISBP are organized by the FM. He / She is responsible to coordinate the projects with the RSs and the associated labs, to track the process, to program the environmental conditions to meet experiment needs, to enforce the rules and regulations of the ISBP and to carry the quality control of the outcomes and schedule regular service maintenance.
The ISBP staff members carry out and oversee the activities under the supervision of the FM.

Facility description

The ICARDA speed breeding platform in Rabat covers around 500 m^2. The facility is divided into four separate buildings. Two of them are glasshouses of size 175 m^2 and 185 m^2 respectively and each one is divided into five independently controlled growth chambers, with two control boards. The control system manages the rooms' conditions, organizes the irrigation system (ebb & flow) with timing and collects environmental data. The growing conditions depend on the crop and type of selection strategy applied. Protocols for each crop and strategy can be found in Annex 1. Generally, the temperature is maintained within a range of 22 – 26 °C, humidity between 50 – 70%, light system at 22 hours light followed by 2 h dark.
Each chamber of the ten growth chambers is equipped with 6 benches, except for "Chamber 10" that has 8 tables. Each bench can accommodate 9 RL98 trays (882 SC10R cones; Stuewe and Sons Inc.) or 15 D20 trays (300 D40L cones; Stuewe and Sons Inc.). The photoperiod is set via LED lamps with a red/blue ratio of 8/1 (Apollo 8 LED Grow Light), connected to sensors. The tables use electronically controlled solenoid valves that open and close to allow water filling and draining. The draining valves are activated together with water pumps (Ref. ARO diaphragm pump) that contribute to a rapid and efficient draining. Temperature in the growth chambers is controlled by an air conditioner network and four hygrostats regulate the humidity.
The two other remaining buildings are designated as working space and storage of seeds, equipment and chemicals (Figure 1).

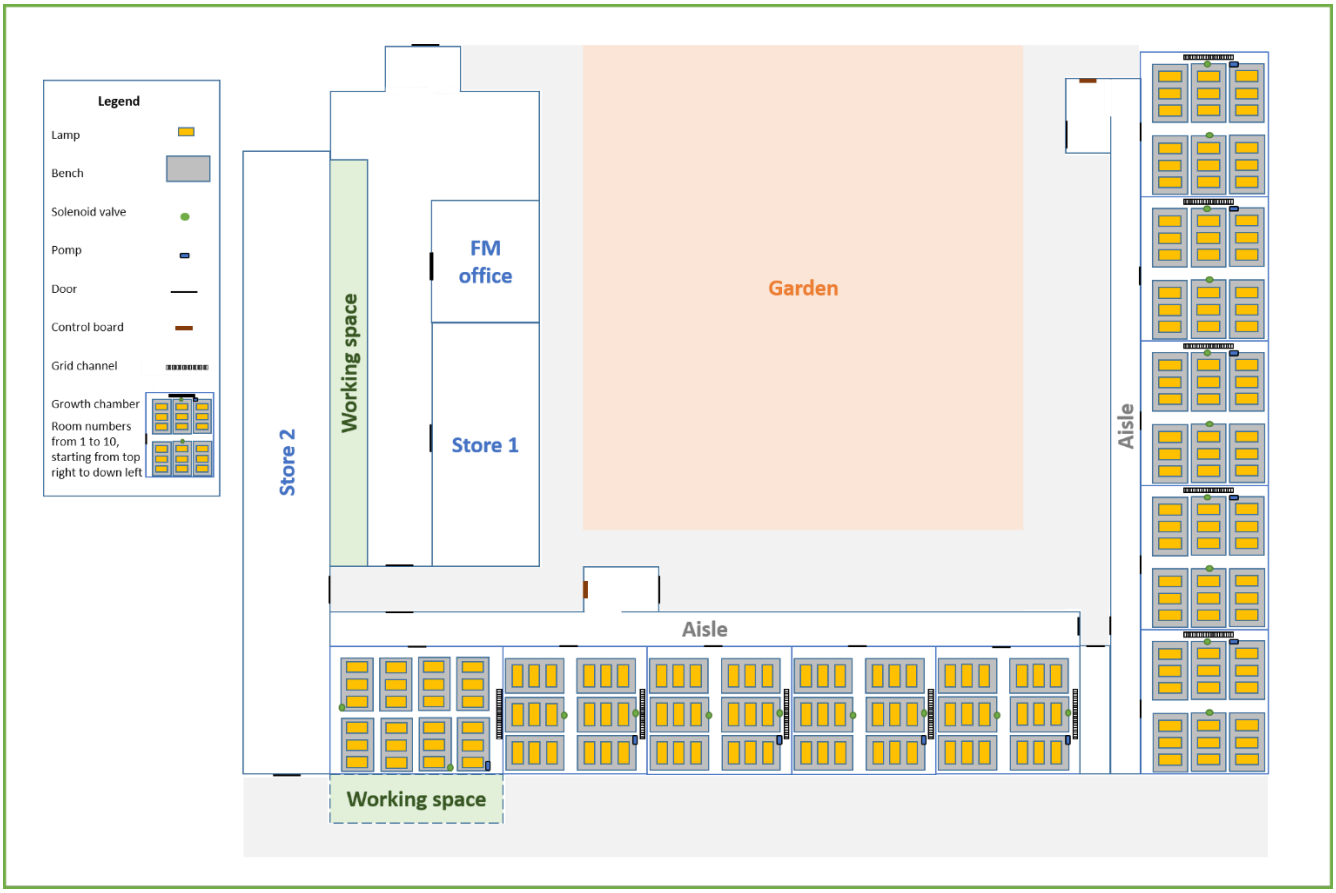


Figure 1. ICARDA Speed Breeding Platform map in 2021

Breeding programs and associated disciplines

The speed breeding facility aims at providing the breeding programs with an integrated platform to reduce the crop generation interval while offering the possibility to increase selection accuracy by teaming-up with other ICARDA disciplines. For it, the facility works in collaboration with other laboratories: phytopathology and entomology (IPM), physiology, biotechnology and nutritional and end-use quality. Constant communication between the FM and the associated laboratories is established according to the projects' requirements, to ensure accurate phenotyping.

Disciplines assisting speed breeding

The facility provides and coordinates the following services, all protocol details are reported in the annexes.

- Cereal and legume Speed Breeding (Annex 1.A)
- Disease screening-assisted Speed Breeding. (Annex 1.C)

- Physiology-assisted Speed Breeding. (Annex 1.B)
- End-Use quality-assisted Speed Breeding (Annex 1.D)
- Genomic-assisted Speed Breeding (Annex Y)
- Non-Speed Breeding Experiments (Hydroponics, Root, etc...)

Speed breeding process

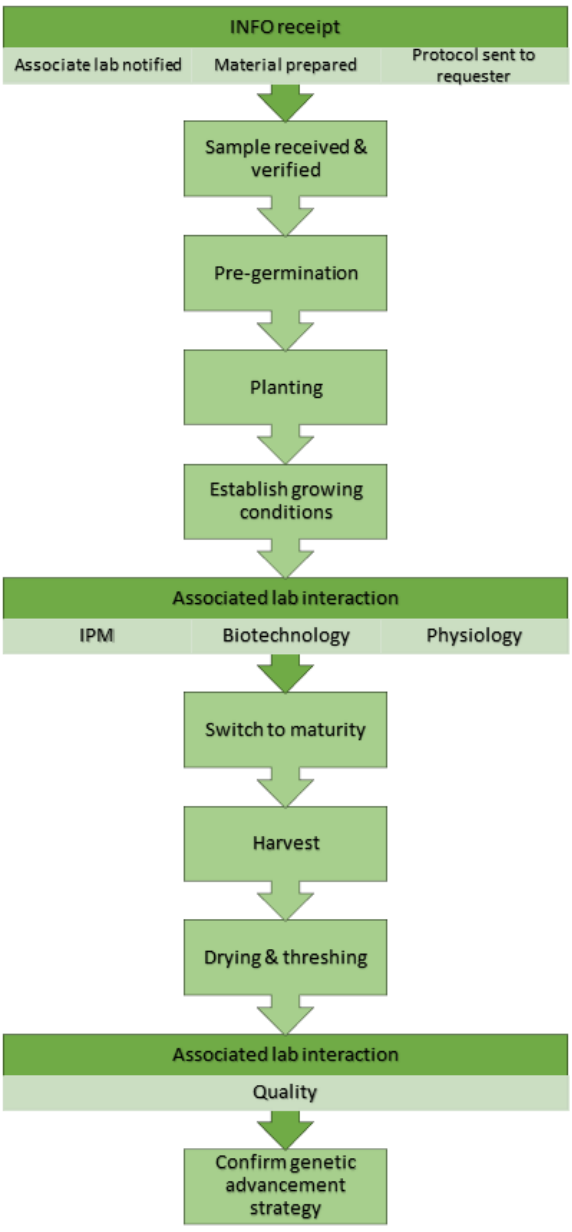


Figure 2. The general speed breeding process

1. Receiving information from the requester

Before material reception, the RS must provide full details concerning the experiment. A request form (Annex 2) must be filled, considering the following:

- Description of the material
- Advancing strategy
- Traits to be collected
- Share germplasm list via BMS or OneDrive.
- Adopt OneDrive as a tool for exchanging files.

Based on the information received, the ISBP staff will start the procedures.

a. Associated labs

Depending on the objective of the experiment, the concerned laboratories are notified. Based on the requester instructions and lab protocol (Annex 1), the ISBP and the laboratory managers create a specific working plan for the project.

b. Material and space

All the required material to perform the established protocol will be provided by the ISBP. This material includes clean petri dishes, filter papers, trays with cones, Peatmoss soil, paper bags, fertilizers, insecticides. If any additional material (not described in the protocols) is required, it must be provided by the RS. The space will be arranged aiming at optimizing resource efficiency (electricity, staff...), facilitate pest and disease control and according to the specific needs of the projects. Special requests from RS's will be considered by the ISBP and implemented if it would not incur in additional cost or significant increase of workload.

c. Project Protocol confirmation

The ISBP manager communicates to the RS the full technical details of the project, to be confirmed by RS before the start. This agreed protocol will constitute the Project Protocol.

2. Receive and check Samples

ISBP staff inspect the samples prior to placing them in ISBP Store 1 to identify and address potential pests and other problems. Then, the samples are kept at -20°C for 24h to eliminate potential insect pests in the seeds. The list of the inspected material is imported to BMS and Fieldbook Android application for data management.

3. Pre-germination

The seeds are placed on labelled petri dishes or trays at the ISBP fridges. The details concerning this step are described in Annex 1.A. The label shows: Crop, trial code, entry code, start date of incubation, and the barcode. If it is an advancement trial, the label is similar to the previous generation's harvest bag.

The results of the pre-germination process are reported to the RS as a list with number of germinated seeds and %.

4. Growing conditions

The establishment of the growing conditions will depend on the crop and type of selection strategy applied. Protocols for each crop and strategy can be found in Annex 1.

5. Planting and transfer to growth room

Once the scientist confirms the planting list, the staff start planting on cones following the agreed protocol.

6. Trait collection

The FM will notify one week in advance the concerned labs when the plants are reaching the key growing stage set in the Project Protocol for trait recording or leaf sampling. Before maturity, the relevant disciplines are IPM, physiology and biotechnology. The ISBP assisting lab protocols are described in Annexes 1.B, 1.C and 1.D. The ISBP staff will record several routinely assessed traits (Table 1). Any additional trait will be agreed during the Project Protocol set-up process (point 1).

Table1. Annotations recorded by the ISBP staff during the crop cycle.

Crop	Traits
Barley	Before harvesting: Average heading date
	After harvesting: Spike row type, Seed type, Spike color, Grain color and Number of harvested seeds per cone.
Wheat	Before harvesting: Average heading date
	After harvesting: Awn type, Grain color and Number of harvested seeds per cone
Pulses	Before harvesting: Average first flowering date and Average maturity date
	After harvesting: Grain color and Pod/plant

For traceability and data safety, data are recorded via Fieldbook Android Application, to be imported to BMS via BrApi.

7. Switch to maturity mode

After flowering the plants will enter the accelerated maturity process as per the protocols (Annex 1). Thus, the irrigation is stopped to force plant maturity. The RS will be notified of the entries with missing spikes or pods. At this stage, the FM will inform the quality lab to prepare for the reception of samples if established in the Project Protocol.

8. Harvest

The ISBP staff harvest the plants following the requester selection (if any). The harvested spikes/pods are put in labeled bags showing: Crop, trial name, harvesting date, entry code and the barcode.

9. Drying and threshing

The harvested plants are placed in ovens for 2 days at 45°C, then threshed. If the Project Protocol includes it, the seeds are sent to the quality lab for end-use quality analysis. Otherwise, the seeds are kept in Store 1 for short term storage. The FM communicates the seed number of each entry to the RS, together with all recorded data.

10. Generation advancement strategy

Based on the data collected during the experiment, the RS confirms the next step of the genetic advancement strategy. In case a selection is done, the requester provides the required information by adding the information to the request form and send it to the RS to generate a new Project Protocol.

Speed Breeding facility maintenance

All required tools for the speed breeding are facilitated by the ISBP (see section: Facility description). The ISBP staff is responsible for their cleaning and maintenance.
The planting and the oversee of the plants are the ISBP responsibility. Researchers or non-ISBP staff can also manage their experiments but must first attend a training and follow the facility's guidelines.
The FM will prepare a list on the door of each unit indicating the researchers using the unit, contact names and numbers, plus any notes on special conditions, procedures or access.
In the fridges, ovens or growth chambers, all petri dishes, bags, or any other container must have labels with sufficient information to clearly indicate project and RS.
The irrigation circuit including filters, solenoid valves and pumps are regularly cleaned and maintained following Annex 3.

Storage policy

The ISBP staff receive the material from the RS. They verify the samples and bags status. The seeds must be in good condition, clean from obvious pests or diseases and properly packed and labelled. Then the staff place the material in Store 1, awaiting provided planting date if any. Seeds will be provided no less than 10 days nor more than 1 month before planting.
At the end of each cycle, the grains are harvested, and per RS instructions new sets are prepared for the following cycle. After each cycle, the remaining seeds will be stored until the next harvest when they are returned to the RS or destroyed, according to provided instructions. For a temporary storage, the facility may (if possible) keep as reference a set of grains from the last cycle in labelled bags on dry storage at 15% moisture and less than 25° C for 6 months upon request.

The label must include store position (shelf code), crop, trial name, cone number, harvest date, date of storage, date of discard, notes and barcode.
The staff needs to check every 6 months the stored seeds, in order to discard any stored seeds that have reached expiration date, RS will be notified once expiration date has arrived and will be able to choose to retrieve the seeds or the ISBP staff will discard them.
Store 1 and especially Store 2 will be used for soil, fertilizer, pesticides, clean trays with cones and other equipment and material.

Data management

OneDrive is set as the source for file exchange between RS and the FM.
Each germplasm list provided by the requester for speed breeding should be loaded into BMS, either by the RS or the FM. Thus, the provided information must be clear and complete for data management and germplasm tracking. The BMS will help to organize the following:

- Create labels and barcodes unique for each entry.
- Generate a layout for the experiment if needed.
- Collect phenotypic data and share it with the RS.
- Create germplasm lists for advancing segregating materials.

The Fieldbook Android application is used by the ISBP staff and the associated labs, to collect data or record any information related to the experiment. Using BrApi, the germplasm lists with the requested annotations are imported into the application from BMS. Then, similarly the data are exported from the application to BMS.
In addition, the ISBP is using other Android applications. *Coordinate* application is used to organize samples into grids using either DNA plats or seed trays. For the seed storage, *Verify* application will enable data viewing and verification by scanning entry's barcodes.
The speed breeding platform uses MEL to record project organization (capacity development activities) and advancements, sharing with donors and all interested people.

Training and visitors

All researchers aiming to use the facilities must complete a training. This will guide the access to the facility, inform of best practices for avoiding unwanted pests and diseases, crops speed breeding protocols, instructions related to storage of materials and equipment, clean-up procedures during and after experiments, how to report problems, and protocols related to an emergency.

The visits or training to the SB facilities will be organized based on the training requests as follows:

Short visit (10'-1h): targeting non-ICARDA people, NARS interested to have their own SB facility, etc. The FM will introduce the ISBP, give general information about equipment and protocols and a guided visit.

Short training (3 months or 1 SB cycle): This training is mainly dedicated to MSc students willing to use the facilities for their research project, NARS during short stay and other short stay visitors. The trainee will assist for 3 months on a full SB cycle, according to his choice and the ongoing projects. The FM informs the trainee when to start in order to pursue all cycle steps from planting to harvesting.

Long training (1 year): After attending the short training, the trainee (PhD student or NARS scientist under a long stay at ICARDA), can lead one SB project (F2 to F6) for one year. He/She will be responsible for the project and the results and will be supervised and assisted by the ISBP staff.

Cleaning, maintenance and recycling

The facility users should keep close all doors and windows in the growth chambers and stores keep internal conditions setup and prevent pests and diseases.

The growth chambers, preparation and storage spaces are regularly cleaned by ISBP staff and short and long-term trainees. The ISBP users should promptly clean up at the end of every day in order to reduce transmission of pests and diseases. All cleaning tools are available in Store 2. Failing to do this will result in repeating the training.

Hoses need to be used carefully; it is important to keep the hose's end from exposure to pathogens from the floor.

All used equipment needs to be returned to the store clean.

Staff will provide a final cleaning of the floors and benches at the end of each project, using disinfectants and pesticides.

The ISBP staff should respect the maintenance calendar in annex 3 to routinely clean and maintain the irrigation system, air conditioner, lamps, fans, sewers, sensors and glasshouse roof.

All used soil is stored in a large container for its sterilization and reuse. Water issued from irrigation is also collected to be further reused for garden irrigating.

Each chamber is equipped with a trash can, regularly emptied by staff.

Pest management

Successful experiments require preventing the outbreak of pests and diseases. Thus, both staff and researchers must take precautions to prevent invasion.

Each project should start by using clean cones, sterile tools and disease and pest-free material. It is also recommended that users make sure that all used tools are clean and disinfected before and after each use.

Users are asked to help keeping areas clean. All plant residues need to be promptly disposed.

Weekly monitoring

Users must report any pest or disease invasion. The staff will weekly carry visual inspections of all chambers to react accordingly.

To reduce the transmission of pests and diseases, once a plague is identified the chamber will be closed until treatment is applied as per IPM regulations.

Chamber Rotations

To control more effectively eventual pest and diseases, crop rotations will be favored in the chambers to break pest lifecycles. Each chamber needs to be cleaned and disinfected using the appropriate products (bleach, low residue and low duration pesticide) every 3 months or at the end of one SB cycle.

Pest Control

Attention to potential pest problems is critical since chemical controls work best when pest populations are low. Chemicals will be selected based on target pest, following IPM advice. Pesticides can only be applied by ISBP staff. The staff must wear an adequate suit, mask, gloves and glasses during treatments. Areas under treatment must be indicated with product information and closed until it is safe to access.

Record keeping

All pesticide applications must be recorded and posted on the bulletin board by the control space in buildings.

Any performed maintenance needs to be recorded.

The FM is responsible of keeping the records

The FM keeps records of facility equipment and sources to enable sourcing of parts for repairs. Whenever possible it is recommended to have at least 1 or 2 spare parts for common equipment for emergency repairs.

Alarms

The alarm messages are sent to the FM and staff. The staff will respond to the alarm as soon as possible and will send an email to the FM, and if it had implications for the experiment also to the RS, when the problem is identified. Whether the problem is solved or not by staff, the FM must physically check and record the observations.

Cost analysis

The ISBP will work as all-included service provider to the different RS. The pricing will be per entry and generation advanced and depend on the protocol used, excluding the costs of the associated disciplines whose costing will be agreed between the discipline leaded and the RS.

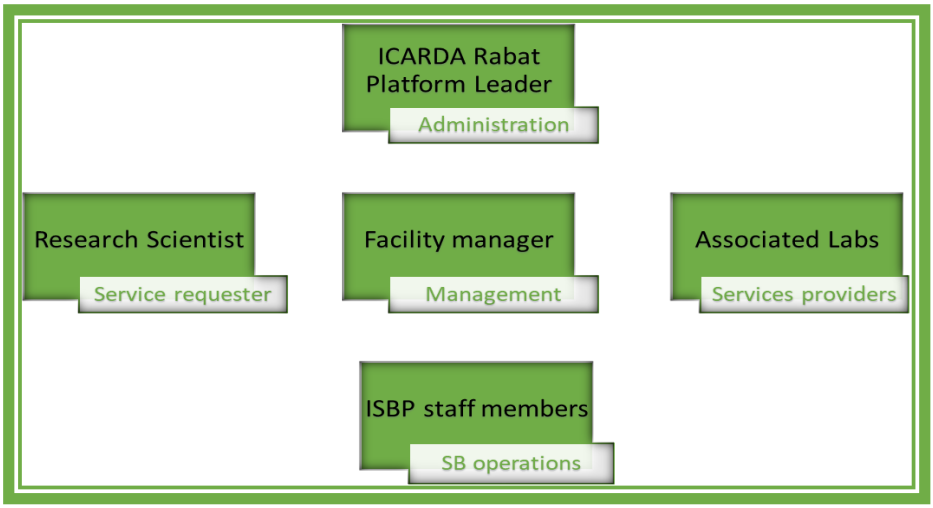
The cost of non-speed breeding experiments will be computed as the cost of two generation advancement.

ANNEXES

STANDARD OPERATING PROCEDURE:

ICARDA Cereals and Legumes Speed Breeding Platform

Version: 5 June 2022



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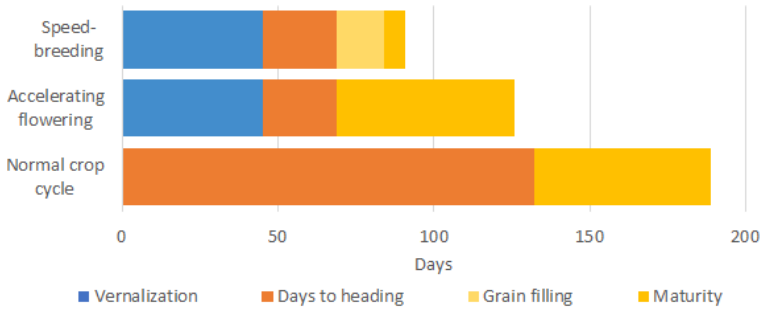
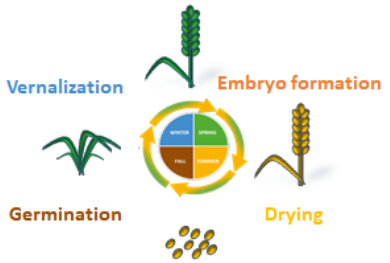
Annex 1. Protocols

Annex 1.A. Cereal & Legume speed breeding protocol

The present protocol for cereal and legume including wheat, barley, lathyrus sativus, lentil and chickpea is based on a modified ICARDA winter wheat speed breeding protocol.





Cereal and legume can be classified into spring sown (does not require vernalization) and winter sown (requires vernalization), according to their requirement for sensing cold before flowering.

1. In the case of spring cereal, dry seeds are left to germinate in the dark for 7 days at 5°C in petri dishes (or trays) on sterile, wet filter paper, to break the eventual dormancy. While winter wheat needs 45 days of vernalization at 5°C. Then one extra day under ~ 20°C to facilitate acclimation.

1. In the case of legume, seeds are first scarified, sterilized with Sodium hypochlorite solution 5% (bleach) and distilled water, and put to germinate for 2 days at ~ 20°C in Petri dishes on sterile and wet filter paper. Then, transferred to fridge set at 5°C for 7 days.

2. Two germinating seeds are planted in special cones / trays (Ref. Small: SC10R / RL98. Big: D40L / D20; Stuewe and sons, Inc.) that allow growing 529 plants/m^2 using small cones (3.8 x 21 cm) or 196 plants/m^2 using big cones (6.9 x 25.4 cm). Legumes are mainly transplanted into big cones. A thinning is done to keep one healthy plant in each cone after 1 week.

Regular Pittmoss is used as soil, with a base pre-sowing fertilizer application of NPK 14.7.17% (Ref. Engrais Bleu Universel NovaTec® Algoflash).

3. The trays are transferred to the ICARDA speed breeding platform chambers. The plants are grown in a controlled environment room depending on the type of selection strategy applied (Figure 3). Generally, the growth chambers are set to have long photoperiod 22 hours light followed by 2 h dark using LED lamps with a red/blue ratio of 8/1 (Ref. Apollo 8 LED Grow Light). The temperature is set at a range of 23-26°C and the humidity ranges 50-70%.

The plants are hand-watered daily during the first 14 days. After the development of roots, the plants are automatically irrigated using the Ebb-flow irrigation system. The irrigation is done once every 2 days, to keep the soil well-watered. Approximately 14 days after the flowering stage, water supply is stopped to accelerate grain ripening, leading up to harvest after one week. Same irrigation protocol is applied for legumes, water supply is stopped after pods formation, then plants are harvested after pods ripening.

Foliar fertilizations are done as needed. In the cereal case, applications are scheduled at the tillering stage, the flag leaf stage and weekly application of calcium.

4. When plants are fully senesced, pods are harvested. While spikes are harvested 3 weeks after flowering. Then Pods and spikes are counted and put in labeled bags, then dried in the oven for two days at 45°C to reduce the moisture content. Grains are threshed and stored at room temperature.

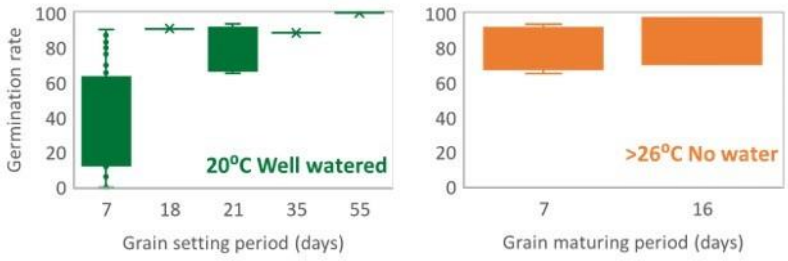


Figure 2. Relationship between duration of wheat grain setting (Left) and grain maturing (Right) stages and the grain germination rate. Germination rate was tested to be high when extending grain setting period beyond 2 weeks with enough irrigation at 20°C, and grain maturity for 1 week without watering at 26°C.

5. The recovered seeds are planted using the above-described protocol to obtain next generation seeds.

This figure represents the standard speed breeding procedure followed in ICARDA, with the different collaborating disciplines.

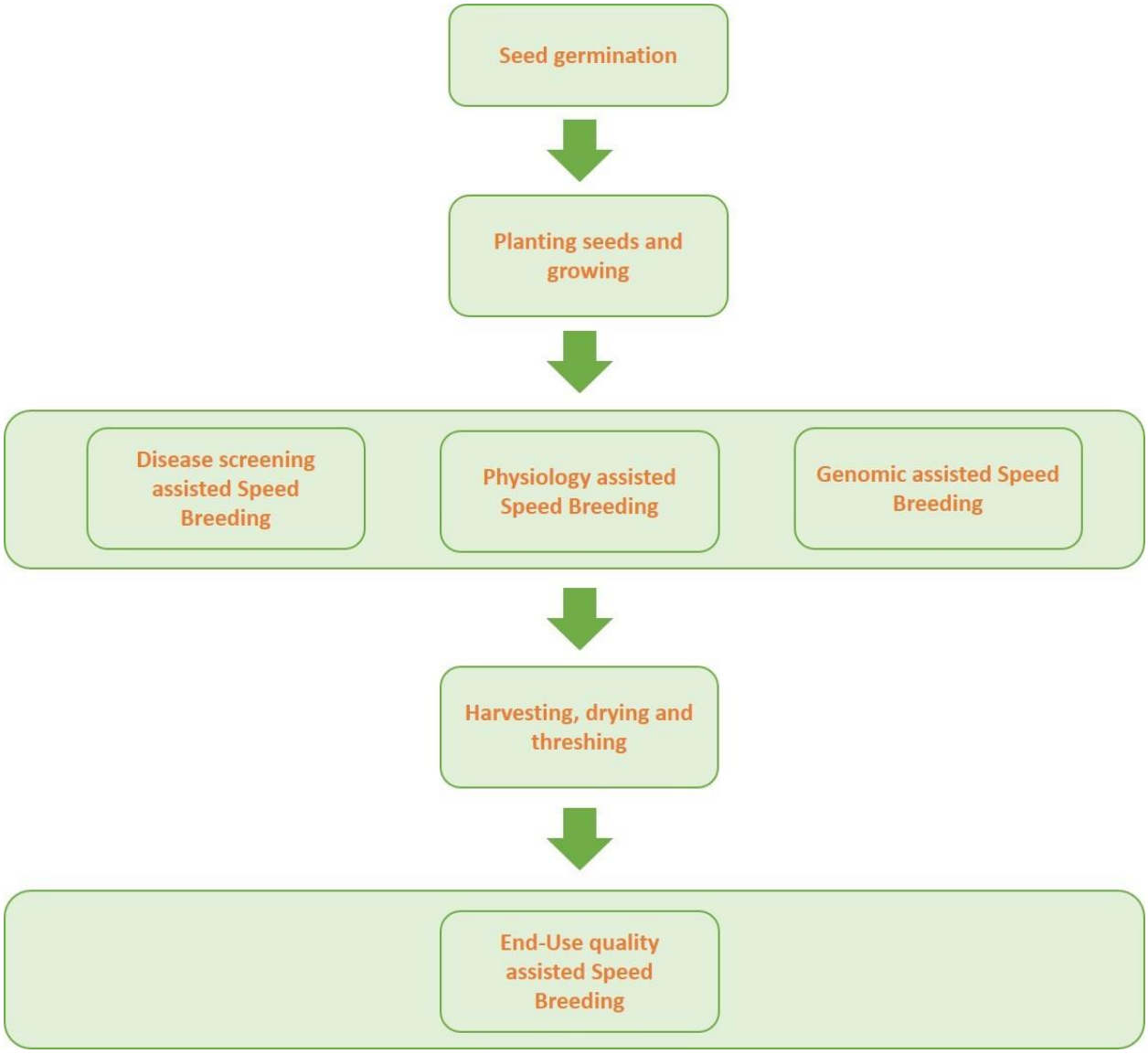


Figure 3. The standard speed breeding procedure

Annex 1.B. Physiology measurements assisted Speed Breeding

The following table presents the physiology measurements that can be assisted in the speed breeding facilities. Additional measurements can be added depending on the RS.

Measurement	Device	Method description
Chlorophyll content	Hand-held SPAD meter 	**Conditions:** Measurements can be taken at any environmental conditions, most important is that the leaf surfaces must be clean and dry. **Plant developmental stage:** It depends on the experimental objectives and the timing of treatments, for the: Peak of chlorophyll content for cereals two measurements should be taken between the start of heading and mid grain filling, for the *legumes* the measurement should be taken in the vegetative stage, during the flowering stage and mid grain filing stage. Determination of the stay green or Senescence frequent measurements should start at mid grain filing until the physiological maturity. **Number of samples**: Three averages of five leaves per cone.

Measurement	Device	Method description
Photosynthesis measurements (CO2 exchange measurement)	LI-6400 XT portable photosynthesis system 	**Conditions:** Measurements can be taken at any environmental conditions. Usually the Li-cor has its own conditioned room. For the dark measurements, it should be taken at dark adapted leaves using clips. Leaves must be dry and clean from any dust or pollen. **Plant developmental stage:** It depends on the aim of the measurements but usually it can be taken at any developmental stage for both cereals and legumes. **Number of samples:** At least two to six leaves per cone.
Chlorophyll Fluorescence	OS30p+ chlorophyll fluorometer 	**Conditions:** Data can be recorded under any environmental conditions. The leaves should be adapted to darkness using the special clips provided with the machine. **Plant developmental stage:** Measurements can be taken at any developmental stage from the mid seedling to the mid grain filling stage. **Number of samples:** At least two to six leaves per cone.

Measurement	Device	Method description
Stomatal conductance	Porometer AP4 	**Conditions:** Measurements can be taken under any environmental conditions. **Plant developmental stage:** It depends on the objectives but usually the measurements can be taken at any developmental stage **Number of samples:** Three readings at random leaves per cone.
Leaf Area	LI-3000C Portable Area meter 	**Conditions:** Values can be recorded under any environmental conditions. The most important is that the leaves must be dry and clean from any dust or pollen. **Plant developmental stage:** Measurements can be taken at any developmental stage from the vegetative stage to the mid grain filling stage. **Number of samples:** Three measurements per cone.

Annex 1.C. Disease screening assisted Speed Breeding protocol

The protocols developed by the IPM program for diseases and Hessian fly screening under the speed breeding facilities, are based on a response tests correlation with field data. All details concerning the experiment and results are reported in the annex 4.

The protocols of seedling resistance screening to foliar and soil-borne diseases and Hessian fly under the speed breeding, are presented in the following two tables.

Table 1: Protocol for seedling screening for each crop under different controlled conditions

Diseases	Pathogen	Stage for inoculation	TEM during incubation (°C)	TEM after incubation	HMD (%)	Light regime	Days for scoring after incubation	duration to multiply enough inoculum	Rating scale	Descriptions-rating scales
Barley										
Scald	*Rhynchosporium commune*	2 leaves	15	20	80	16/8 h light/dark	12 -14	30 days	0_5	0: Immune, 1: Resistant, 2: Moderately resistant, 3: Moderately susceptible, 4-5: Susceptible
Net blotch	*Pyrenophora teres*	2 leaves	20	20	80	16/8 h light/dark	7	30 days	1_9	0: Immune, 1-3: Resistance, 4-5: Moderately resistant, 6-7: Moderately susceptible, 8-9: Susceptible
Spot blotch	*Cochliobolus sativus*	2 leaves	20	20	80	16/8 h light/dark	7	30 days	1_9	0: Immune, 1-3: Resistance, 4-5: Moderately resistant, 6-7: Moderately susceptible, 8-9: Susceptible
Leaf rust	*Puccinia hordei*	1-2 leaves	20	20	80	16/8 h light/dark	10	2-3 months	0_4	0: Immune, 1: Resistance, 2: Moderately resistant, 3: Moderately susceptible, 4: Susceptible
Stem rust	*Puccinia graminis f.sp. tritici*	1-2 leaves	20	20	80	16/8 h light/dark	12	2-3 months	0_4	0: Immune, 1: Resistance, 2: Moderately resistant, 3: Moderately susceptible, 4-5: Susceptible
Powdery mildew	*Blumeria graminis f. sp. hordei*	Fully expanded first leaf	22±2	22±2/16	80	16/8 h light/dark	8-10	20 days	0_4	0: Immune 1: Resistance 2: Moderately resistant 3: Moderately susceptible 4-5: Susceptible
Yellow rust	*Puccinia striiformis f. sp. hordei*	1-2 leaves	10	18	80	16/8 h light/dark	17	2-3 months	0_9	0-1: Resistance 2-4: Moderately resistant 5-7: Moderately susceptible 8-9: Susceptible

Diseases	Pathogen	Stage for inoculation	TEM during incubation (°C)	TEM after incubation	HMD (%)	Light regime	Days for scoring after incubation	duration to multiply enough inoculum	Rating scale	Descriptions-rating scales
Bread Wheat										
Septoria leaf blotch	*Zymoseptoria tritici*	2 leaves	20	20-25	80	16/8 h light/dark	20	20 days	1_5	0-1: Resistance 2: Moderately resistant 3: Moderately susceptible 4: Susceptible
Yellow rust	*Puccinia striiformis f. sp. tritici*	1-2 leaves	10	18	80	16/8 h light/dark	17	2-3 months	1_9	0-1: Resistance 2-4: Moderately resistant 5-7: Moderately susceptible 8-9: Susceptible
Leaf rust	*Puccinia recondita f. sp. tritici*	1-2 leaves	20	20	80	16/8 h light/dark	10	2-3 months	0_4	0: Immune 1: Resistance 2: Moderately resistant 3: Moderately susceptible 4: Susceptible
Stem rust	*Puccinia graminis f.sp. tritici*	1-2 leaves	20	20	80	16/8 h light/dark	12	2-3 months	0_4	0: Immune 1: Resistance 2: Moderately resistant 3: Moderately susceptible 4-5: Susceptible
Tan spot	*Pyrenophora tritici-repentis*	2 leaves	20	20-25	80	16/8 h light/dark	10	20 days	1_5	1: Resistance 2: Moderately resistant 3-4: Moderately susceptible 5: Susceptible
Durum wheat										
Septoria leaf blotch	*Zymoseptoria tritici*	2 leaves	20	20-25	80	16/8 h light/dark	20	20 days	1_5	0-1: Resistance 2: Moderately resistant 3: Moderately susceptible 4: Susceptible
Yellow rust	*Puccinia striiformis f. sp. tritici*	1-2 leaves	10	18	80	16/8 h light/dark	17	2-3 months	0_9	0-1: Resistance 2-4:Moderately resistant 5-7: Moderately susceptible 8-9: Susceptible

Diseases	Pathogen	Stage for inoculation	TEM during incubation (°C)	TEM after incubation	HMD (%)	Light regime	Days for scoring after incubation	duration to multiply enough inoculum	Rating scale	Descriptions-rating scales
Durum wheat										
Leaf rust	*Puccinia recondita f. sp. tritici*	1-2 leaves	20	20	80	16/8 h light/dark	10	2-3 months	0_4	0: Immune 1: Resistance 2: Moderately resistant 3: Moderately susceptible 4: Susceptible
Stem rust	*Puccinia graminis f.sp. tritici*	1-2 leaves	20	20	80	16/8 h light/dark	12	2-3 months	0_4	0: Immune 1: Resistance 2: Moderately resistant 3: Moderately susceptible 4-5: Susceptible
Tan spot	*Pyrenophora tritici-repentis*	2 leaves	20	20-25	80	16/8 h light/dark	10	1 month	1_5	1: Resistance 2: Moderately resistant 3-4: Moderately susceptible 5: Susceptible
Fusarium root rot	*Fusarium culmorum*	spike stage	20-25	20-25	70 -90	16/8 h light/dark	3 months	2 months		
Faba bean										
Ascochyta blight	*Aschochyta fabae*	10-15 days old seedlings	20±1	20±1	>80	12/12h light/dark	10 -15	10 days	0_5	0: No visible lesion 1: 1-2 small lesion (5mm) 2: 1 to 2 lesions with pycnidia 3: More than 2 lesions with pycnidia or 25-50% of pod or leaf area covered 4: 50-75% of pod or leaf covered stem cut 5: Stem cut dead plant
Chocolate spot	*Botrytis fabae*	10-15 days old seedlings	20±1	20±1	>80	12/12h light/dark	10	10 days	0_5	0: Plants without chocolate spot 1: Leaf with few chocolate spot 2: Number of spots increased and scattered 3: Spots combined together and became larger 4: The necrotic spots reached half of the leaf 5: Majority of the leaf was necrotic and drop

Diseases	Pathogen	Stage for inoculation	TEM during incubation (°C)	TEM after incubation	HMD (%)	Light regime	Days for scoring after incubation	duration to multiply enough inoculum	Rating scale	Descriptions-rating scales
Faba bean										
Rust	***Uromyces fabae***	10-15 days old seedlings	20±1	20±1	100	14/10h light/dark	10-15	20-25 days if enough spores are not available	1_9	1: No disease 3: Few pustules with poor sporulation 5: Many pustules with moderate sporulation 7: Pustules across the whole plant with leave withering 9: Severely infected plant up to 100 per cent leaves withering
Lentil										
Stemphylium blight	***Stemphylium botryosum***	10 to 12 days old seedlings or early flowering stage	20±2	25±2	>80	12/12h light/dark	10-15	10-15 days	1_9	1: No infection or tiny non-spreading lesions 2: Few chlorotic lesions leaf drying affecting ≤5% leaves 3: Expanding lesions on 6 to 15% of leaves turning necrotic 4: 16 to 30% leaves infected and defoliation 5: 31 to 45% leaves infected and defoliation 6: 46 to 60% leaves infected and defoliation 7: 61 to 80% leaves infected defoliation and stem lesions 8: 81 to 90% leaves infected stem lesions and plant left with terminal leaves only 9: Whole plant death
Rust	***Uromyces fabae***	10 to 12 days old seedlings	20±2	22±1	100	14/10h light/dark	10-15	20-25 days if enough spores are not available	1_9	1: No pustules visible 3: Few scattered pustules usually seen after careful search 5: Pustules common on leaves and readily observed but causing no apparent damage 7: Pustules very common and damaging but not observed on petioles and stems 9: Pustules extensive on all parts seen on leaves petioles and stems and kill leaves and other plant parts
Ascochyta blight	***Ascochyta lentis***	10 to 12 days seedlings	20±2	22±1	>80	12/12 h light/dark	10-15	12 days	1_9	1: No symptoms 3: Leaf lesion only chlorosis of affected leaves <10% leaf drop 5: Leaf lesion up to 25% leaf drop stem flecks or lesions <2mm 7: Leaf lesion up to 50% leaf drop stem flecks or lesions >2mm 9: Leaf lesion potential defoliation stem girdling and potential plant death

Diseases	Pathogen	Stage for inoculation	TEM during incubation (°C)	TEM after incubation	HMD (%)	Light regime	Days for scoring after incubation	duration to multiply enough inoculum	Rating scale	Descriptions-rating scales
Chickpea										
Ascochyta blight	*Ascochyta rabiei*	10 to 15 days after planting	20±1	20±1	>80	12h/12h light/ dark	10-15	12 days	1_9	1: No visible symptoms 2: Minute lesions prominent on the apical stem 3: Lesions up to 5 mm in size and slight drooping of apical stem 4: Lesions obvious on all plant parts and clear drooping of apical stem 5: Lesions on all plant's parts defoliation initiated breaking and drying of branches slight to moderate 6: Lesions as in 5 defoliation broken dry branches common some plants killed 7: Lesions as in 5 defoliation broken dry branches very common up to 25% of plants killed 8: Symptoms as in 7 but up to 50% of the plants killed 9: Symptoms as in 7 but up to 100% of the plants killed
Fusarium wilt	*Fusarium oxysporum f. sp. Ciceris*	30-40 days old seedlings	22±1	25±1		12h/12h light/ dark	40 -50 days after planting	–	% of Mortality	

Table 2. Protocol for seedling screening for insect pests in durum and bread wheat based on the experiment.

Insect	Plant material	Rearing of HF	Stage for infestation	TEM during and after infestation (°C)	HMD (%)	Light regime	Days for evaluation after infestation	Description of plants resistance /susceptible to H.F
Hessian fly	Ten seeds of each entry including the susceptible wheat variety and resistant check were sown in rows in plastic flats containing a mixture of soil and peat.	The Hessian fly population used in the screening is derived from a population collected from Chaouia-Ouardigha region and reared on the susceptible wheat variety under growth chamber.	1 leaf (5 - 6 days after germination)	22 - 24	70 - 75	14/10 h light/dark	21 days after infestation	Susceptible plants showed stunting, a dark green color, and contained live larvae. The resistant plants exhibited a normal growth, a light green color, and contained dead first-instar larvae. The escaped plants contained no dead or live larvae.

Annex 1.D. End-Use Quality-assisted Speed Breeding protocols

The quality laboratory at ICARDA Rabat receives the harvested seeds from the speed breeding facilities for quality analysis. Clean seeds from any foreign material are packed on labeled paper bags. The labels show the following: Quality Lab Plot Number, Cone Number, Trial, Location, Crop, Harvested Date (MMYY) and the barcode. The samples are analyzed using non-destructive methods, except the SDS-PAGE which needs just one grain to be crushed. First, samples are regularly scanned with Near-Infrared Reflectance Spectroscopy (NIRS) using the FOSS 2500 cup (REF: 60047234) and FOSS 2500 IH-0337S adapter (REF: 60013320) allowing the analysis of down to 10 seeds. The quality traits are predicted based on the NIR predictive models available at the ICARDA Cereal and Legume nutritional and end-use quality laboratory.

This table summarizes the quality parameters available for Speed breeding:

Test	Facilities Used	Procedure Used	Accuracy
Protein content	NIR / Foss : DS 2500	AACCI Method 39-25.01 / ISO12099	0.97
Moisture content	NIR / Foss : DS 2500	AACCI Method 44-19.01	0.95
SDS- sedimentation	NIR / Foss : DS 2500	Internal Calibration	0.46
Grain beta Glucan	NIRS / Foss : DS 2500	Internal calibration	0.88
Area	GrainScan - Canon Lide 220	Alex P Whan et al, 2014	-
Perimeter	GrainScan - Canon Lide 220	Alex P Whan et al, 2014	-
Length	GrainScan - Canon Lide 220	Alex P Whan et al, 2014	-
Width	GrainScan - Canon Lide 220	Alex P Whan et al, 2014	-
Zinc	X-SUPREME 8000	Internal Calibration - Gupta et al 2016	-
Iron	X-SUPREME 8000	Internal Calibration - Gupta et al 2016	-
Selenium	X-SUPREME 8000	Internal Calibration - Gupta et al 2016	-
Magnesium	X-SUPREME 8000	Internal Calibration - Gupta et al 2016	-
Aluminium	X-SUPREME 8000	Internal Calibration - Gupta et al 2016	-
Potassium	X-SUPREME 8000	Internal Calibration - Gupta et al 2016	-
Calcium	X-SUPREME 8000	Internal Calibration - Gupta et al 2016	-
SDS-PAGE	Electrophoresis vertical slab gel	Singh et al., 1991	-

	unit, Hoefer SE-600		

Annex 2. Request form

Principal Investigator Name	
Phone number	
Email	
Submission date	
Department600133	
Project	
Crop(s)	
Assisting disciplines involved:	☐ Cereal and legume speed Breeding (see Annex 1.A) ☐ Physiology assisted Speed Breeding (see Annex 1.B) ☐ Disease screening assisted Speed Breeding (see Annex 1.C) ☐ Genomic assisted Speed Breeding ☐ End-Use quality assisted Speed Breeding (see Annex 1.D)
Germplasm list available in BMS	☐ Yes ☐ No
Desired start date	
Expected end date (Firm or flexible)	
List employees who will be working in the facility on a regular basis. It is the manager's responsibility to ensure users have completed SB training before working in the facility	
Special Instructions	
Breeding strategy	
<u>Generation 1</u>: a. Number of pops b. Number of entries per population, If customized list must be provided c. Discipline involved d. Trait(s) to score e. Advancement strategy f. Number of seeds advanced per entry, If customize list must be provided g. What to do with remaining seeds? h. Comments	
<u>Generation 2</u>: a. Number of pops b. Number of entries per population, If customized list must be provided c. Discipline involved d. Trait(s) to score	

e. Advancement strategy f. Number of seeds advanced per entry, If customize list must be provided g. What to do with remaining seeds? h. Comments	
Generation 3: a. Number of pops b. Number of entries per population, If customized list must be provided c. Discipline involved d. Trait(s) to score e. Advancement strategy f. Number of seeds advanced per entry, If customize list must be provided g. What to do with remaining seeds? h. Comments	
Generation 4: a. Number of pops b. Number of entries per population, If customized list must be provided c. Discipline involved d. Trait(s) to score e. Advancement strategy f. Number of seeds advanced per entry, If customize list must be provided g. What to do with remaining seeds? h. Comments	
Generation 5: a. Number of pops b. Number of entries per population, If customized list must be provided c. Discipline involved d. Trait(s) to score e. Advancement strategy f. Number of seeds advanced per entry, If customize list must be provided g. What to do with remaining seeds? h. Comments	
Generation 6: a. Number of pops b. Number of entries per population, If customized list must be provided c. Discipline involved d. Trait(s) to score e. Advancement strategy f. Number of seeds advanced per entry, If customize list must be provided	

g. What to do with remaining seeds? h. Comments	

Annex 3. Maintenance Calendar

Equipment	Interval for maintenance	Activity
Irrigation system	6 Months	The irrigation circuit including filters, solenoid valves and pumps are regularly cleaned and maintained
Air conditioner	3 Months	Clean cooling pads
Lamps	As needed	Check HID bulbs and replace them as needed
Fans	As needed	Check HAF fans
Sewers	6 Months	Clean the sewers
Sensors	3 Months	Calibrate the sensors of all growth chambers
Glasshouse's roof	6 Months	Clean the roof of the glasshouse

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