	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 1 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

1. Introduction & Purpose

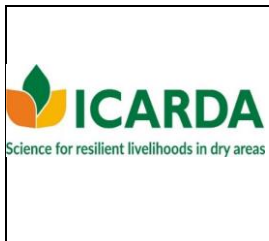
Seed regeneration is the process that leads to the generation of a new seed-lot for a given accession with the intention to increase its stored seeds in the collection (also called “multiplication”) or to increase the viability of its seeds equal to or above an agreed minimum level, which is referred to as the regeneration threshold. The latter case is often termed as “seed rejuvenation”.

Characterization is the description of plant germplasm through recording the expression of highly heritable characters (not affected by the environment) ranging from morphological, physiological or agronomical features to seed proteins and oil or molecular markers (FAO, 2013). This activity provides information on traits that allows discrimination among accessions and facilitates the verification of identity. It also includes the taxonomic identification and verification when needed.

The Genebanks of ICARDA conserve “in-trust” a total of 76,730 accessions of small-grained cereals belonging to 68 different taxa and originating from 110 countries (last update December 2021). The term “small-grained cereals” includes cultivated bread wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* Desf.), primitive tetraploid (e.g. *T. dicoccon* Schrank) and hexaploid wheat (e.g. *T. spelta* L.), barley (*Hordeum vulgare* L.) and their wild relatives (*Aegilops*, *Triticum* and *Hordeum*). It also includes accessions of triticale (x *Triticosecale* spp.), rye (*Secale* spp.) and oat (*Avena* spp). With the exception of rye and some crop wild relatives (e.g. *Aegilops speltoides*, *Aegilops caudata*, *Hordeum bulbosum*) these cereals are self-pollinated, annual and cool-season grasses. The cultivated forms are grown for human food consumption, animal feed and forage. All of these species are found as both, winter and facultative types (growing primarily during the winter months, requiring vernalization to flower, and with ability to withstand prolonged periods of below freezing temperatures) and spring types (growing primarily during the spring and summer months, normally not requiring vernalization to flower). Details on reproductive system (self vs. cross pollination, self-incompatibility) and on growth cycle (annual, biennial, perennial) for the most representative small-grained cereal species maintained by ICARDA’s Genebanks are given in Annex 1.


An essential element of seed regeneration is the maintenance of genetic integrity of the original sample. The two concerns are maintaining the occurrence of different alleles and maintaining the frequency of these alleles. Therefore, knowledge on reproductive system, growth cycle and growth habit of an accession are key elements for regeneration process. Cereal accessions at ICARDA are regenerated when seed quantity falls below 1,500 seeds in medium-term storage or when seed viability drops below 85% of initial viability of the stored seeds (75% in case of wild relative species). Regeneration is also undertaken for the newly introduced, collected or received accessions to allow to conduct characterization and multiply seeds to replenish active and base collection and to send samples for safety duplication.

Planting of cereals accessions for regeneration at ICARDA is done in the months of November and December to exploit favorable winter season precipitations and to meet the vernalization requirements for the winter type accessions. Characterization can be carried out at any stage of the

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 2 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

conservation process, as long as there are sufficient numbers of seeds to sample. However, characterization of accessions at the field is done as soon as the accession will be acquired by ICARDA's Genebank and is often combined with the regeneration process. The GRS aims to regenerate and characterize the accessions under conditions that meet recognized international standards based on current technologies and scientific knowledge.

The purpose of this SOP is to give detailed instructions and to ensure consistency on the regeneration and characterization activities of cultivated and wild cereal germplasm maintained at ICARDA's Genebank in compliance with national and international treaties and conventions.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 3 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

2. *Scope*


This SOP applies to the regeneration and characterization of cultivated and wild cereal germplasm maintained at ICARDA's Genebank and describes the following activities:

- Review of data and information available in database (e.g. seed stocks, seed viability, seed health status, characterization data, etc.)
- Selection of accessions and generate lists for accessions to be regenerated and/or characterized
- Seed sample preparation
- Field selection (choice of environment and planting season) and preparation
- Planting layout, density and distance
- Sowing (e.g. sowing rate, method, etc.) and labelling
- Crop management (fertilization, irrigation, weed control, pest and disease management, isolation, roguing off-types, etc.)
- Characterization at the field
 - Choice of most suitable descriptors
 - Choice of appropriate time and growth stage for recording plant traits
 - Taxonomic identification
 - Photos
 - *Herbarium* specimens
- Harvesting
- Spike characterization (spike specific traits)
- Seed characterization (seed specific traits)
- Threshing and seed cleaning
- Fumigation
- Data validation and uploading on ICARDA's Genebank Documentation System

Accessions may be landraces, obsolete improved varieties, advanced improved varieties, breeding materials or genetic stocks, primitive forms, wild species and may be maintained at ICARDA's genebank as populations or as pure-breeding lines.

This SOP does not describe the following activities:

- Seed processing and storage, described separately in "Conservation of Genetic Resources at ICARDA" (SOP-ICARDA-CON-001_ver1.0).
- Acquisition of accessions, described separately in "Acquisition of genetic resources at ICARDA" (SOP-ICARDA-ACQ-001_ver1.0)


	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 4 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

Seed characterization for cereal genetic resources, even though is undertaken during the seed processing into storage is described in this SOP as it is considered as an integral activity of the whole characterization procedure.

3. Terms, definitions, abbreviations and acronyms

The following terms, definitions, abbreviations and acronyms are pertinent to this SOP.

Accession	A distinct uniquely identifiable sample of botanic seeds representing a cultivar, a breeding line or a population of a particular plant species, which is maintained in storage for conservation and use.
Characterization	The description of plant germplasm through recording the expression of highly heritable characters (not affected by the environment) ranging from morphological, physiological or agronomical features to seed proteins and oil or molecular markers.
Regeneration	The process that leads to the generation of a new seed-lot for a given accession with the intention to increase the stored seeds in the genebank and/or to increase the viability of the seeds equal to or above an agreed minimum level.
Plant genetic resources	The overall genetic diversity of the cultivated and wild plant species, which have actual or potential value and can contribute to the improvement of crops.
CGIAR	Consultative Group on International Agricultural Research
FAO	Food and Agriculture Organization of the United Nations
FIGS	Focused Identification of Germplasm Strategy, a trait targeted sub-setting approach within genebank's material
GCP	Generation Challenge Programme
GRS	Genetic Resources Section at ICARDA
ICARDA	International Center for Agricultural Research in the Dry Areas
ID_Number	Identification number provided by DONOR
IG	ICARDA's germplasm number. It is a unique identifying number that applies to each accession conserved in ICARDA's genebank.
IPR	Intellectual Property Rights
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
LTS	Long term storage for base collection and safety duplicates collections
MOS	A sample of seeds from the original seed lot or the one that have undergone the lowest number of regenerations since the material was acquired by the genebank, as recommended for storage as a base collection.
MTS	Medium term storage for active collection
Ne	Effective population size (N_e) is the size of an ideal population (i.e., one that meets all the Hardy-Weinberg assumptions) that would lose heterozygosity at a rate equal to that of the observed natural population.
NARS	National Agricultural Research Systems
RH	Relative Humidity
SMTA	Standard Material Transfer Agreement

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 5 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

SOP	Standard Operating Procedure
SD	Safety duplicate collection
TTT	Trueness to Type

4. Materials and Equipment

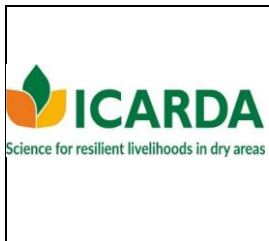
The following materials, equipment and reagents are needed to carry out this SOP.

Materials:

- 8 x 11 cm, thickness of 0.10 mm paper craft foils (locally purchased)
- Ribbons for bar code labeling (Zebra 8000T Tuff 7.0 mil Tag; [Zebra ribbons](#))
- Cardboard boxes (locally purchased)
- Gloves (locally purchased)
- Facemasks (locally purchased)
- Safety glasses (locally purchased)
- Chemical protective clothing (locally purchased)
- Wooden sticks (locally purchased)
- Ropes (locally purchased)
- Metal sticks (locally purchased)
- Cotton bags (locally purchased)
- Doubled layer paper sacks (18 x 35 cm) (locally purchased)
- Plastic sacks of 25 and 50 Kg capacity (locally purchased)
- Chemicals (fertilizers, herbicides, pesticides, fungicides, etc.; locally purchased)

Equipment:

- Electronic balances (RADWAG model BWLC-2E0-001, max 2000 g, d=0.01 g; [RADWAG electronic balances](#))
- Seed counter (Wintersteiger DATA Count S 25; [Seed counter data count s 25](#))
- Seed counter (Pfeuffer, Contador 230 V/50 Hz, s.n. 1410 009; <http://www.pfeuffer.com/contador.html>)
- Barcode printer (ZEBRA ZM400, s.n. 10011703 –R; [Zebra Barcode printer](#))
- Liquid seed treater (Wintersteiger Hege11, s.n. H011.4014 1125; [Wintersteiger Hege 11](#))
- Disc harrow 24 discs (locally purchased “Comptoir Agricole Skhirat”)
- 1.8 m rotary hoe (locally purchased “Comptoir Agricole Skhirat”)
- Tractor-mounted plot seeder (Wintersteiger Plotseed S, s.n. 227-4014-0119; [Wintersteiger Plotseed S](#))
- Plot tractor (Wintersteiger Kubota, s.n. L5240 D 60316; [Wintersteiger Kubota plot tractor](#))
- Sprayer (12 m hydraulic booms; locally purchased “Comptoir Agricole Skhirat”)

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 6 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

- Tractor-mounted watertank (5000 lt; locally purchased “Comptoir Agricole Skhirat”)
- Hand rotovators (BCS 750 diesel Lombardini 11 hp 85 L; locally purchased “Comptoir Agricole Skhirat”)
- Laboratory thresher (Wintersteiger LD 350, s.n. 0140-4014-327; [Wintersteiger LD 350](#))

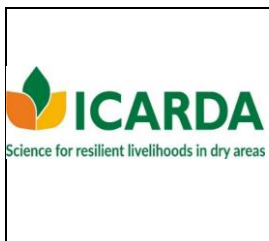
All essential genebank equipment is monitored and verified by trained staff, calibrated by a certified third party and included in the genebank’s maintenance schedule. All field equipment is maintained by the station manager. A hard copy of any equipment manual is stored in a drawer close by where the equipment is operated, while a soft copy is stored on desktops of authorized staff and operators.

5. Occupational Health and Safety

All activities performed in this SOP comply with the requirements and recommendations in ICARDA’s Health and Safety Manual.

For the activities of seed dressing with chemicals during seed preparation and for chemical application at the field, genebank dress code requires from staff the use of gloves in natural latex, facemasks and safety glasses, as well as dressing with chemical protective clothes.

Any untoward incident is reported to the supervisor and/or to the farm manager for proper action and investigation.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 7 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	


6. *Procedure*

6.1 The **Regeneration and Characterization** procedure is initiated when cereal curator review data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of the accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration and characterization activities:

- Review of data and information available in database (e.g. seed stocks, seed viability, seed health status, characterization data, etc.)
- Selection of accessions and generate lists for accessions to be regenerated and/or characterized
- Seed sample preparation
- Field selection (choice of environment and planting season) and preparation
- Planting layout, density and distance
- Sowing (e.g. sowing rate, method, etc.) and labelling
- Crop management (fertilization, irrigation, weed control, pest and disease management, isolation, pollination, roguing off-types, etc.)
- Characterization at the field
- Harvesting
- Spike characterization (spike specific traits)
- Seed characterization (seed specific traits)
- Threshing and cleaning
- Fumigation
- Data validation and uploading on ICARDA's Genebank Documentation System

Accessions are flagged automatically in the database through the monitoring system by triggering an alert notification that a particular metric is below a threshold value. Inventory of the alert notifications and review of all pertinent information from the database prompts the inclusion of accessions into the Genebank's cereals' regeneration and characterization schedule when:

1. Seed quantity in the medium-term storage falls below 1,500 seeds. This threshold value is in full compliance with the international standards that have been set on the size of collection (FAO/IPGRI, 2014), and corresponds to the concept of maintaining five times the minimum amount of seeds that approximates the effective population size (N_e) for self-pollinated crops (i.e. 5×300 ; three sets to be used for multiplication purposes, one set to be used for germination test and one set to confirm trueness to type).
2. Seed viability drops below 85% of the initial viability of the stored seeds for cultivated germplasm or below 75% of the initial viability of the stored seeds for crop wild relatives'

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 8 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

germplasm. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014).

3. Accessions are newly introduced through collection or acquisition and require regeneration to meet the above standards in terms of seed amount and viability.
4. Accessions are flagged for unknown or incomplete passport and/or characterization data to enable documentation of phenotypic traits, phytosanitary status, purity and cleanliness.

Cereal curator along with the Head of Genetic Resources Section can also identify the need to engage in an extemporaneous regeneration and/or characterization activity due to a specific request, new partnerships or projects, an emerging pathogen or the emergence of new technologies.


Selection of Accessions and List Generation

6.2 Based on the alert notifications and using all the pertinent information from the database separate lists with the accessions to be regenerated and/or characterized for each crop/species are generated by the database manager and the cereal curator according to the following grouping:

- Barley accessions (ICB list)
- Bread wheat accessions (ICBW list)
- Durum wheat accessions (ICDW list)
- Primitive wheat accessions (ICPW list)
- Wild *Hordeum* accessions (ICWB list)
- Wild *Triticum* accessions (ICWT list)
- *Aegilops* accessions (ICAG list)
- Other cereals accessions (IC list)

Each of these lists includes the following information:

- Accession number (IG)
- Full taxon name (Genus, Species, Subspecies)
- Country of origin
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (e.g. strictly winter types, wild types and pollination type requiring special seed pre-treatments are flagged)

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 9 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

Within each of the lists, accessions are further divided according to the purpose of the regeneration process into different trials, based on the following:

- i. Trials including the accessions to be regenerated due to low seed quality (e.g. low germination, phytosanitary status, etc.)
- ii. Trials including the accessions to be regenerated due to inadequate seed amount
- iii. Trials including the accessions to be regenerated due to small seed sample originally collected or donated and need immediate regeneration following receipt of the material
- iv. Trials including the accessions to be regenerated for serving specific purposes (e.g. selected FIGS or GCP subsets, confirm trueness to type, compare possible duplicates in the collection, diversity analysis, evaluation against pest and diseases and abiotic adversities, etc.)

Each of the trials receives a unique identifier, which consists of 3 letters (CEM code for Morocco, CEL code for Lebanon), followed by the cropping year (2 digits) and the number of the experiment (2 digits), the latter in consecutively order, e.g. CEM2101, CEM2102, CEM2103 etc., when it refers to the cropping season of 2021.

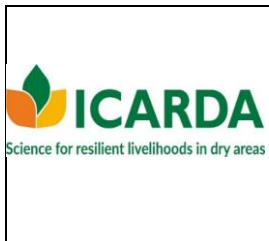
Each accession in the trials receives a plot number, which consists of the trial code followed by a serial number, e.g. CEM2101001, CEM2101002, ..., CEM2101...n for the accessions included in the trial CEM2101.

Based on the capacity and the available resources, regeneration of accessions with inadequate quality (low viability) takes priority over accessions with inadequate seed numbers. Accessions in the base collection take priority over regeneration of accessions in active collection. Furthermore, in cases where the original collection or donation is a small sample, regeneration of these accessions receives immediate priority after receipt of the material in order to obtain an adequate quantity of seeds for long-term storage.

Seed Sample Preparation

6.3 Seed containers/foils from MTS or LTS are withdrawn and allowed to adjust to ambient temperature.

As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original-sample, i.e. LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS. In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration (see below at procedure 6.4 relevant information for sample size), then seeds from LTS are used for the regeneration of the accession and replenishment of MTS stock.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 10 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below ICARDA's threshold value (see procedure 6.1, note numb. 2), then half of the seeds stock in LTS (i.e. 750 seeds; for more information, see SOP for "Conservation of Genetic Resources at ICARDA") is withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.

Note: ICARDA's Genebank is currently under the process of reconstituting its Collection in the two new relocation sites, i.e. Morocco and Lebanon. For that purpose, ICARDA has withdrawn from the Svalbard Global Seed Vault (SGSV) the accessions previously deposited there and their provenance traced back at ICARDA's regeneration activities in Syria. Under this particular and exceptional situation, the seed stock withdrawn from the (SGSV) serves the purpose of the most-original-sample, by the time that full reconstitution of the Collection will be in place and routine processes will resume consistently.

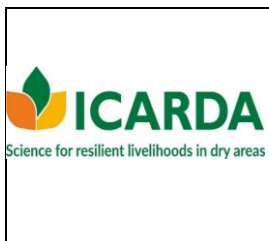
6.4 Seed samples are withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. This minimum sample size should be adequate enough to represent the genetic diversity of the accession and to capture 95% of the rare alleles. This minimal size to minimize loss of alleles and avoid the effects of genetic drift of the accession should be equal or larger than the effective size of population (N_e). According to Johnson et al. (2002, 2004) 100 plants is a minimum number of individuals which is necessary for the preservation of taxon gene pool on the regeneration of perennial allogamous species (e.g. grasses), while Crossa (1998) has shown that a sample size of 130-200 individuals will give a high probability of retaining rare alleles at low frequencies in most of the loci.

In practice for ICARDA's cereal regeneration activities, a minimum of 200 individuals for each accession is required to be established at the field for landraces and wild relative species (for pure lines and breeding germplasm, fewer plants are needed to conserve genetic integrity). This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed. For this purpose, the total number of seeds that will be used for the regeneration of an accession taken into consideration the viability of seeds is estimated based on the following equation*:

$$\text{No of seeds required} = \frac{\text{Desired plant population}}{\text{Germination (\%)} * \text{Expected field establishment (\%)}}$$

* Germination and field establishment are expressed in decimals. Plant establishment is generally 5% less than the germination percentage in poor conditions and 1% less in good conditions (Rao et al., 2006).

6.5 Database is updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 11 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

6.6 Seeds are placed in paper craft foils and identified with a barcode label, including information of the following:

- Trial code and plot number
- Accession number (IG)
- Full taxon name (Genus, Species, Subspecies)
- Country of origin
- Number of seeds

6.7 Based on the inventory of the lists, accessions that may require specific pre-treatments in order to improve seed germination, field establishment and further seed setting are marked and kept separately. The following cases can be distinguished:

6.7.1 The vast majority of the accessions (including almost all the accessions of the cultivated and most of the accessions of the wild cereal germplasm) do not require specific pre-treatments. In this case:

6.7.1.1 Seeds are treated with appropriate fungicides (Celest top (Diféconazole 25g/l + Fludioxonil 25g/l + Thiamethoxam 262.5g/l) at a rate of 200 cc/hl of seeds or Vitavax (Carboxine 200g/l + Thiram 200g/l) at a rate of 250 cc/hl of seeds), for ensuring robust plant establishment at the field and control of non-quarantine seed borne diseases (Figure 1a).

6.7.1.2 Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number (see above at procedure 6.2), placed in plastic trays and transferred to the short-term storage area to be kept until the planting date (Figure 1b).

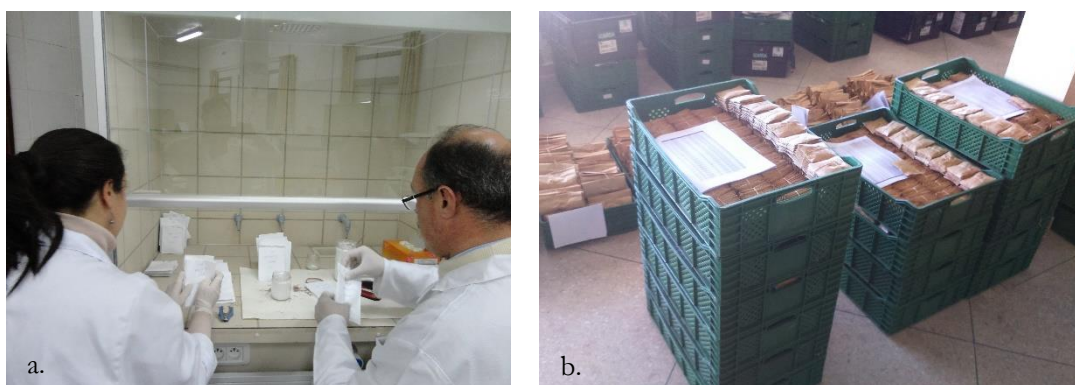



Figure 1. Cereal seeds preparation for planting. a: Seed treatment with appropriate chemicals to control seed borne diseases and ensure robust establishment at the field, b: Seeds prepared and organized into order maintained at the short-term storage area in Marchouch-Morocco prior to planting.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 12 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

6.7.2 The rest of the accessions requiring specific arrangements are further falling into one of the following categories:

6.7.2.1 Accessions with a strong vernalization requirement (i.e. strictly winter types), that may require artificial vernalization in case field conditions do not provide enough cool temperatures.

In this case pre-treatment is conducted 8-10 weeks before optimum transplanting period to the field.

- ✓ Seeds are placed on moistened blotter paper into petri-dishes and allow germination to begin at ambient temperatures
- ✓ Petri-dishes are transferred into growth chambers and adjust temperature at 1-4°C with a light source (8/16 h day/night)
- ✓ Treatment keeps up to 8 weeks
- ✓ After completion of vernalization treatment, seedlings are transplanted at the field (further details on transplanting are given at the section 6.16.1.4)


6.7.2.2 Wild accessions with irregular germination (e.g. accessions with husked seeds), that may require prior removal of the seeds from husks. In this case after removing the husks, de-husked seeds are treated by appropriate chemicals (see section 6.7.1.1) and return back to the trays with the rest of the accessions and in consecutively numbered order according to their plot number.

6.7.2.3 Accessions with limited seeds and/or very low viability (e.g. accessions from collecting missions), that may require germination in controlled conditions. In this case seeds are germinating under controlled conditions and seedlings are transplanted into pots to grow in the greenhouse or to be further transplanted at the field (for further information see section 6.15.1.4).

Field Selection (choice of environment and planting season) and Preparation

6.8 Due attention is paid to the environment in which cultivated and wild cereal germplasm is grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Field is selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and pests. For ICARDA's cereal regeneration program, cereals fields are selected after following one year of fallow or one year of planting with legume crops. Soils should have also a proper drainage and a weed-free environment is demanded at the time of sowing. Additional criteria, such as possibility of irrigation are considered essential for field selection. Under the current ICARDA's capacity all the cereal regeneration plots in both, Lebanon and Morocco are established in irrigated fields.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 13 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

Appropriate site for multiplication is selected also based on the type of the material to be regenerated, according to the following categories:

6.8.1 Wild cereal germplasm: Regeneration of all the wild cereal accessions is held in Lebanon, since this area is part of the center of origin and diversity for most of the cereal crops, where all cereal wild species occur naturally. Therefore, any potential risk of escape as invasive species is eliminated.

In this case open-pollinated accessions, such as *Aegilops speltoides*, *Ae. candata* and *Ae. longissima* (for further information see also Table Annex 1) are regenerated under isolation cages or in plastic houses in order to maintain their genetic integrity. Only one accession of each species is planted in a separate isolation cage. Some of the species having self-incompatibility like *Hordeum bulbosum* will require in addition to isolation, the use of pollinators for better seed setting. Self-pollinated accessions of wild cereal germplasm are regenerated without isolation arrangements.

6.8.2 Cultivated cereal germplasm: Appropriate site for regeneration of the cultivated cereal germplasm is selected according to the reproductive system of the crop. The following cases can be distinguished:

6.8.2.1 Accessions of self-pollinated cereal crops (i.e. bread and durum wheat, primitive wheat, barley and oat). These accessions can be regenerated in both sites (i.e. Lebanon and Morocco), preferably in Morocco where more land is available. No isolation arrangements needed.


6.8.2.2 Accessions of cross-pollinated cereal crops (i.e. rye). These accessions are regenerated in Lebanon under appropriate isolation cages or in plastic houses in order to maintain the genetic integrity of the accessions.

6.9 Accessions are planted during the rainy season; in Mediterranean-type environments this coincides with the winter season. Practically in both of ICARDA's sites (i.e. Lebanon and Morocco), planting season for cereal accessions starts at the middle of November and can be extended up to the middle of December. Especially in the case of Morocco, GRS staff is striving to complete planting before middle of December, in order to avoid Hessian fly infestations.

Planting during winter season rather than later at the spring is giving the advantage for the winter types to receive the low temperatures induction that are needed for vernalization and also for the grain filling to occur during the cooler period of spring and not during the first summer months.

For specific purposes though (i.e. evaluation of accessions to drought tolerance, evaluation of accessions to heat, etc.) planting can be further extended and may take place up to March depending on the scope and the purpose of each particular trial.

6.10 Early enough before planting, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing is

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 14 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

also done one or two weeks before the planting date to remove the weeds. Basal fertilizers are also applied along the second plowing. Basal fertilizers that are typically used for ICARDA's GRS cereals regeneration is the diammonium phosphate (DAP, 18-46-0) in a dose of 250 Kg/ha, 15-15-15 in a dose 250 Kg/ha or a combination between Ammonium sulphate (21%) at 200 Kg/ha and triple-superphosphate (45%) at 100 Kg/ha.

6.11 Planting rows (approx. 5 cm deep) are traced at row spacing of 30 cm.

Planting Layout, Density and Distance

6.12 Field is squared based on a fixed reference point by applying Pythagora's theorem and is divided into ranges (blocks) of 2.5 m length leaving 1.5 m alley ways between ranges. Two rows plot are delineated for planting each accession leaving four empty rows between adjacent plots, allowing a plot to plot distance of 1.5 m for adequate isolation of adjacent accessions.

6.13 For better isolation, accessions of different trials (i.e. different crops/species) can be arranged to alternate with each other; for example, one plot of *Aegilops* can be followed by one plot of wild *Triticum* to avoid mixtures at harvest.

6.14 Based on the field dimensions and the number of ranges and plots within ranges, a field layout is created (Figure 2a). Plot numbers are assigned in a serpentine pattern starting always from the left side of the field (i.e. from left to right in the first block, followed by right to left in the second block and *so on*).

Sowing and Labelling

6.15 Based on the reproduction mode (i.e. self- vs cross-pollination) of the species the following cases can be distinguished in terms of sowing/planting:

6.15.1 Accessions that are self-pollinated. For cereal crops and wild cereal germplasm this is the predominant system of pollination with many accessions to be strictly self-pollinated (cleistogamy). Almost all ICARDA's cereal germplasm belongs to this category. In this case:

6.15.1.1 Seeds are sown directly in the field using a seed rate equivalent to 150 – 200 seeds/m² (Figure 2b).

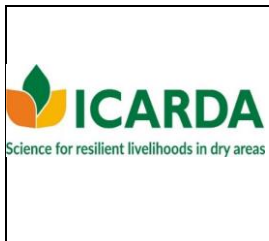
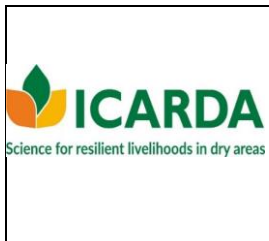
	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 15 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	



Figure 2. Field planting of cereal regeneration trials. a: Field layout prior to seed sowing, b: Seed sowing of ICARDA's cereal accessions by hand, c: Plant emergence at bread wheat regeneration fields in Marchouch, Morocco (cropping season 2017-2018).

- 6.15.1.2 Paper craft foils with seeds of the accessions are placed onto the corresponding plots based on the field layout created and the plot number given during the seed preparation.
- 6.15.1.3 Sowing is done by hand keeping in mind that seeds should be distributed evenly between and within rows.
- 6.15.1.4 Convenient space (in terms of number of plots) is kept at the end of the field for each trial for the respective accessions that may need to be transplanted when appropriate time of transplantation is applicable (i.e. middle up to end of January). These accessions are coming either due to their strong vernalization requirements receiving previously artificial vernalization (see section 6.7.2.1.), either due to their limited seed availability and/or low seed viability receiving formerly germination in controlled conditions (see section 6.7.2.3).
- 6.15.1.5 Immediately after sowing a final check is done for correspondence to the field trial layout and any planting error is noted on the field layout hard copy.
- 6.15.1.6 First and final nursery plots are marked with stakes as soon as final check is confirmed.
- 6.15.1.7 Planting is carried out under field moisture conditions. If moisture is not sufficient, planting will be followed by field irrigation.
- 6.15.2 Accessions that are cross-pollinated. A very small portion among ICARDA's cereal germplasm is considered as cross-pollinated. In particular, among the small grain cereal crops, rye (*Secale cereale*) is the only one, while among the wild cereal germplasm, *Ae. speltoides* and *Amblyopyrum muticum* (syn. *Aegilops mutica*) are outcrossing species, *Ae. caudata* and *Ae. longissima* are facultative allogamous, while *Hordeum bulbosum* is considered as a predominantly self-incompatible species. In terms of percentage this corresponds to 0.5% of the total ICARDA's cereal collection (i.e. 345 out of a total 75,934 cereal accessions). These species require special arrangements to conserve their genetic integrity. Therefore, regeneration of these species is taking place under isolation cages or in the plastic house. In this case:

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 16 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

6.15.2.1 Seeds are sown into seed trays filled with sterilized peat moss. Trays are labelled with the accession's unique identifying number (IG) and transferred to the plastic house for germination and seedling growth. During this period, trays are watered carefully to keep the substrate moist for enhanced germination and seedling establishment.

6.15.2.2 Approximately 40 days after sowing, seedlings are transplanted to their final places at the field or into pots at the plastic house or isolation cages.


- In case of field establishment, prior to the transplanting field plots are irrigated with excess of water, in order for the soil to be muddy and soft enough for facilitating transplantation. Seedlings are pegged out with their root system remaining imperturbable and simply pushed into the muddy soil at their defined plant to plant distances. Early enough and before heading time, appropriate isolation cages are used to cover the plots of these accessions to limit the air movement and to secure pollination among the plants of the same accession.
- In case of plastic house, prior the transplanting pots of 12 lt capacity (24 x 24 cm) are prepared with a soil mixture composed of sterilized soil, sterilized peat moss and perlite in a ration 1:1:1 and watered enough to keep the mixture wet. Seedlings are pegged out from the seeding trays with their root system remaining imperturbable and transplanted into the pots. Five seedlings per pot are transplanted and a minimum of ten pots per accession is used. Pots of each accession grouped together and accession by accession is allocated evenly into the plastic house. For self-incompatible species, only one accession is placed in a separate cage or plastic house.

6.15.2.3 Immediately after transplanting a final check is done for correspondence to the trial layout and any planting error is noted on the hard copy.

6.16 Once seeding/planting of a nursery is finished, each individual field plot or pot (in case of the plastic house) is labelled with a water-proof thermal plastic tag. Information printed on the tag is barcoded (using one dimensional barcode system) and includes the trial code, the plot number, the IG number, the origin of the accession, the planting date and the taxon of the accession.

6.17 A plastic board is erected at the beginning of each nursery that has a brief description of the nursery and its contents (i.e. purpose of the nursery, date of sowing, number of accessions, number of checks, experimental design).

6.18 Promptly after sowing/planting, an electronic list (xls. file) is prepared for each individual trial. This list includes the following fields with information for each accession planted at the nursery:

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 17 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

A) Trial code; B) Plot number; C) Accession number (IG); D) Crop ID; E) Genus; F) Species; G) Subtaxa; H) Sowing/Planting date; I) Seed treatment; J) Number of rows/plot; K) Number of seeds/row; L) Number of seeds/plot; M) Row by row distance; N) Plot length; O) Country of origin of the accession; P) Number of seeds; Q) Source of seeds (i.e. active or base collection, collecting mission, acquisition, etc.); R) Remarks.

An accompanying word file with more detailed description of the nursery established is also prepared (e.g. experimental design; time and amount of fertilizers application; date of first effective rainfall; total rainfall; timing and amount of irrigation, etc.).

These two electronic files prepared for each individual experiment are sent to the database manager to be uploaded into the database.

- 6.19 The regeneration field book is prepared for each individual nursery and incorporated into appropriate electronic application. The field book lists the trial code, the plot number with the corresponding accession number and/or germplasm name and all the characterization and evaluation data that are going to be recorded.

Crop Management


Water regime, as well as nutritional and health status of the plants are monitored regularly during the regeneration process. Any kind of application or field treatment is decided by the cereal curator along with the station manager. All chemicals applied are selected appropriately and are included in the annually updated list of approved chemicals issued by national authorities in each country where cereals regeneration activities are taking place.

Irrigation

- 6.20 All cereal regeneration plots are irrigated to establish a full moisture soil profile immediately after sowing in case that soil moisture is not sufficient enough and rainfall will not come within a period of 15 days after sowing.
- 6.21 Subsequently, plots are irrigated when needed based on the allocation of rainfall throughout the cropping season. Irrigation can be also combined when superficial fertilizers are applied or in case of granular soil insecticide applications for their incorporation into the soil.
- 6.22 Due attention is paid when crop has reached booting stage (i.e. immediately prior to flowering). At this stage the soil moisture conditions of the ground should be at the optimum point (i.e. reaching the water capacity of the soil). Therefore, if needed, supplementary irrigation is given at this stage up to the point of establishing a full moisture soil profile.

Weed management

- 6.23 All cultural practices applied during field preparation are intended to prepare a uniform and weed-free seed bed immediately prior to sowing. After sowing the weed management on cereals' nurseries depends on the type of the crop, especially during the initial stages of crop establishment. The following cases can be distinguished:

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 18 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

6.23.1 Cultivated varieties (landraces, obsolete and modern varieties) of wheat and barley for which selective post-emergence herbicides have been developed and are available at the market. In this case:

6.23.1.1 A selective post-emergence herbicide is applied at the initial stages of the crop and before 4th leaf stage to control the narrow leaf weeds (e.g. fenoxaprop, clodinafop-propargyl). After 4th leaf stage application of this class of herbicides is strictly prohibited, since they could have adverse effects to the genetic integrity of the accessions. For the growth stage of tillering and later stages narrow leaf weeds within the plots are eliminated by hand weeding.

6.23.1.2 Broad leaf weeds are controlled using appropriate selective herbicides that belong to the class of “plant growth regulators” (site of action: IAA like) with applications at any stage of the crop that can extend if need be up to the booting stage. Examples of these herbicides are those belonging to the family of MCPA and 2,4-D. For the latter stages of the crop, broad leaf weeds within the plots are eliminated by hand weeding.

6.24.1.3 All weeds between plots and within the alley ways are controlled through regular soil cultivation using hand rotovators.

6.23.2 Cultivated varieties (landraces, obsolete and modern varieties) of oat and rye, as well as wild accessions and primitive forms of all cereal crops for which no selective post-emergence herbicide is available in the market. In this case:


6.23.2.1 Both, narrow and broad leaf weeds are eliminated within the field plots by regular hand weeding, starting before the initiation of the tillering stage (i.e. approximately at the 3rd leaf stage). Selective herbicides of the MCPA or 2,4-D family can be applied to control the broad leaf weeds, however it is strongly recommended to avoid the use of any herbicide treatment, especially for the wild and primitive material that can cause temporary suspension of growth and possible malformations.

6.23.2.2 Weeds between plots and within the alley ways are controlled through regular soil cultivation using hand rotovators.

Fertilization

In general, for cereal crops, the recommended amounts of fertilizer are ranged between 120 - 160 units/ha for nitrogen (N), 50 - 60 units/ha for phosphorus (P₂O₅) and 70 - 80 units/ha for potassium (K₂O) depending on the soil and the cultural practices used. Basal fertilizers applied during field preparation added into the soil 40 to 45 units/ha of nitrogen and 35 to 115 units of phosphorus (see also section 6.10). The balance of the total amount needed (for nitrogen and potassium) is added as superficial fertilizer. In particular:

6.24 Nitrogen is applied twice, once at the jointing stage (75% of the quantity) and once at the booting stage just before heading (25% of the quantity). On total superficial fertilization is

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 19 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

intended to add into the soil 80 to 120 units/ha of nitrogen. Landraces, tall materials and wild relatives which are not adapted to high nitrogen levels are receiving less amounts within the recommended range of nitrogen fertilization.

- 6.25 Potassium is applied along with nitrogen applications depending on the selected fertilizer. Most common superficial fertilizers used by ICARDA for cereal regeneration is ammonium nitrate (NH_4NO_3 , 34-0-0) and potassium nitrate (KNO_3 , 13-0-46).

Pest and Disease management


Regular monitoring of pests and diseases occurrence is conducted by cereal curator and the station manager. Periodic visits at the cereals' regeneration nurseries are coordinated also with the experts from pathology, entomology and seed health laboratory, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects (for further information see related SOP on Seed Health Testing).

- 6.26 For the diseases that are of major risks and/or are consistently present almost every growing season in the region where cereals' regeneration nurseries are placed (e.g. powdery mildew on barley, leaf rust on wheat, etc.), a preventive strategy with systematic chemical applications is adopted. In this case, treatments with appropriate products start early enough and before the disease is present and are repeated every 2 weeks' time interval up to the time that the conditions will be preventive for the spreading of the disease as shown in the Table below.

	Developmental stage			
	Leaf emergence and Tillering	Stem elongation	Head emergence and Flowering	Grain development
Diseases	-	Powdery mildew, Septoria, Stripe rust, Stem rust, Fusarium		-
Insects	Leafhoppers of cereals, Aphids, Hessian fly	-	-	Aphids
Kind of Treatment	Insecticide	Fungicide	Fungicide	Insecticide
Active compounds	Carbofuran, Spinosade, Imidaclopride	Epoxiconazole, Fluxapyroxade, Pyraclostrobine, Chlorothalonil, Cyproconazole, Propiconazole, Flutriafol, Azoxystrobine		Pyrimicarbe, Cyperméthrine, Pipéronyl butoxyde

- 6.27 For diseases occurring occasionally in the cereals' fields (e.g. septoria and tan spot on wheat), chemical treatments are decided based on the severity of the disease and the prevalent weather conditions.

- 6.28 Insects and pests are effectively controlled with appropriate crop management applied during the whole growing season (e.g. completing planting in Morocco before middle of December to avoid Hessian fly infestations, weed control to avoid insects' winter hope up shelters).

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 20 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

However, in case of severe insect infestations appropriate soil or foliar insecticides (depending on insect biology) can be applied to avoid any potential risk for the regeneration of the accessions.

For the special case of bird damage, bird watchers are used during the grain filling and maturity period to generate noise by using improvised structures or devices.

6.29 Viruses are controlled mainly through the appropriate crop management (e.g. weed control for avoiding aphid-infested grasses). When virus infected plants are spotted, these are rogued and discarded immediately to prevent contamination of healthy plants.

6.30 In case of seed borne diseases, i.e. those transmitted by seeds (e.g. leaf stripe on barley, loose smut on wheat, black chaff on wheat, etc.), infected plants are rogued and discarded immediately from the plots and covered by soil. If possible, symptoms are recognized during vegetative stage and before heading time for avoiding sporulation and further dispersal of the inoculum.

Particular attention is paid also by the experts of ICARDA's seed health laboratory for specific seed borne diseases. Therefore, additional visits for field inspections at ICARDA's Genebank cereal regeneration trials are implemented by the experts of the seed health lab starting by the end of March, when first accessions are starting to head up to harvesting. These field inspections are held on a daily basis and plants are inspected for common bunt (*Tilletia foetida* and *T. caries*) and smut (*Ustilago* spp.). In case of infections, infected plants are rogued and discarded immediately from the plots and covered by soil.

Roguing off-types


6.31 Meticulous monitoring for off-type plants within each plot is conducted several times throughout the growing season. Outmost care is taken to discard only the plants that are well confirmed to be contaminants or volunteers within the plots (e.g. by searching in the database for previous collection and/or characterization data).

Harvesting

6.32 Prior to harvest a second plastic tag same with the ones used for labelling the field plots is printed for each accession, including information on trial code, plot number, IG number, origin of the accession, planting date and taxon (see also section 6.16). Appropriate plastic sacks, which provide ample ventilation, are also prepared labelled with the trial code, plot number and the number of the accession (IG number).

6.33 Harvesting time and management depends on the type of the material to be handled. Two cases can be distinguished:

6.33.1 Cultivated types (landraces and primitive forms, obsolete and modern varieties) with uniform maturity. In this case:

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 21 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

6.33.1.1 Accessions are harvested when spikes are mature and dry, i.e. when 90% of spikes in the plot are yellow and the grains are hard when pressed between finger nails.

6.33.1.2 Accessions are harvested by hand and placed into the corresponding labelled plastic sacks. The plot tag is placed into the plastic sac and the new one prepared is firmly tightened outer of the sack.

6.33.2 Wild types which are prone to shattering and uneven maturity. In this case:

6.33.2.1 Accessions are harvested when spikes are in their physiological maturity. This stage is determined visually from the loss of the green color of the uppermost internode of the peduncle, which turns into very light green or yellow. At this stage transportation of water and nutrients to the spike is cut off and accession is reaching maximum grain fill.

6.33.2.2 Accessions are harvested by hand requiring though repeated harvesting in order to reduce seed loss. As a rule, spikes of these accessions are harvested early in the morning, every second day. Mature spikes (physiological maturity) are placed into the labelled plastic sacks that are fixed to wooden pegs at the beginning of each plot.

Note: The ICARDA's Genebank had traditionally harvested whole plots during its regeneration procedure. Methodologies will be modified in 2019 to ensure that the genetic composition is maintained by harvesting one representative spike from the largest possible number of individual maternal plants of each plot.

6.34 A separate sample composed from ten distinctive spikes is also harvested from each individual accession and placed into appropriate paper sacks labelled with a self-adhesive tag that indicate the trial code, the plot number and the accession number. These samples will be used in a later stage for further spike and seed morphological characterization, molecular characterization, as well as for confirming trueness to type.

Particularly for the latter case, i.e. confirming trueness to type photos from typical spikes and threshed seeds of each individual accession are taken for use as a reference (Figure 3a, b). In addition, two individual representative spikes are threshed separately and seeds are scanned using the GrainScan software (Whan et al., 2014). Scanned photos and data are also used as a compliment source for confirming trueness to type (Figure 3c, d).

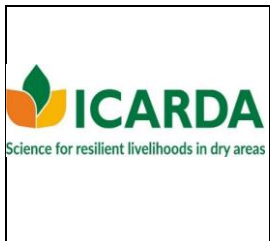
	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 22 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	



Figure 3. Means for confirming trueness to type for ICARDA's cereal genetic resources. a: Spike photos at physiological maturity in the field, b: Seed photos after threshing and cleaning, c and d: Scanning of seeds using appropriate software.

Furthermore, for the wild accessions, a voucher plant sample is maintained at the herbarium of ICARDA's Genebank in Lebanon and in cases needed reference of this sample can be also used to confirm trueness to type.

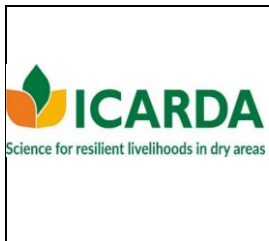
Moreover, for all the cereal accessions at ICARDA, the original seed material withdrawn from SGSV and has not been used for regeneration purposes (approximately 350 seeds in the cases of cereal genetic resources), is archived in the long-term storage, to be used in case needed for reference purposes.

6.35 After harvest, plastic sacks are transferred to the threshing area, where they are kept under natural ventilation for further drying for a period from seven up to ten days.

Threshing and cleaning

6.36 Threshing is done using laboratory threshers with appropriate concaves (5 x 15 mm) for cereal seeds (Figure 4).

6.37 Particular care is received when threshing accessions of hull-less barley and naked oats as the germ is more susceptible to mechanical removal or damage than in the case of hulled cereals or wheat. In this case drum speed is adjusted to lower levels.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 23 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

- 6.38 A preliminary cleaning of the threshed grain from chaff, straw and soil is done immediately after threshing of the accession, using appropriate sieves.
- 6.39 After initial cleaning, grains are placed into cotton bags using for labelling the same inner and outer plastic tags used previously at the original respective plastic sacks.
- 6.40 Seeds are transferred in a designated area, where a second more meticulous cleaning is conducted aiming to the removal of diseased and broken seeds or other weed seeds.




Figure 4. a: Hand harvesting of ICARDA's cereal accessions at Marchouch, Morocco, b: Threshing area at ICARDA's Genebank premises in Marchouch, Morocco.

Fumigation

- 6.41 Cotton bags with threshed and cleaned grains are transferred to the fumigation chamber. Seeds are fumigated with phosphine (in the form of tablets, 3 gr each) to prevent insect damage prior to processing to conservation. The recommended dose for this application is 4-6 gr of phosphine tablets per m³ of room space.
- 6.42 Seeds remain inside the hermitically closed chamber for 3 to 5 days (depending on the air temperature) until the process is complete and then the chamber opens and ventilated using the natural current of the air for 24 hours before any staff entrance.
- 6.43 Immediately after phosphine treatment, bio hazard labels are placed around the fumigation chamber warning about the use of poisonous gas indicating also the starting and ending day of fumigation and the subsequent ventilation of the space.

Data validation and uploading on ICARDA's Genebank Documentation System

- 6.44 Cereal curator validates all data from cereal regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 24 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

A. Seed preparation and planting data


1. Seed source
2. Trial code
3. Crop ID
4. Full taxon name
5. Number of accessions planted and number of checks (if applicable)
6. Date of planting
7. Number of replications, Number of ranges and number of plots within each ranges
8. Number of rows per plot and seeding rate per plot
9. Row length, Row width, Plot to plot distance
10. Seed treatment (e.g. use of appropriate chemicals for seed dressing prior to planting)
11. Date of first effective rainfall or first irrigation

B. Crop Management and Harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (e.g. late frost, severe drought, etc.)
5. Any other event related to the accessions planted (e.g. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. Total grain weight per plot

Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

The regeneration procedure for ICARDA's cereals accessions is considered finalized when threshed grains have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by the cereal curator so as to start the conservation procedure.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 25 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

Characterization

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

Note: As of 2018, the ICARDA's genebank plans to modify its planting methodologies by planting at the margins of each regeneration field two check varieties with extreme expression of particular traits (e.g. early and late maturity) to be used as reference for the regenerated accessions and making characterization generated data comparable.

The characterization for ICARDA's cereal accessions is starting from the field during the seed regeneration process. Beginning of tillering (i.e. 4th to 5th leaf stage) is triggering the initiation of this procedure.

6.46 Characterization of accessions at the field is done as soon as the accession will be acquired by ICARDA's Genebank and is often combined with the regeneration process.

6.47 Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages, using tablets and appropriate applications. A set of morphological and agronomical descriptors are observed following internationally agreed standards (IPGRI descriptors) (Annex 2).

6.48 Traits showing discrete discontinuous states of expression (e.g. growth habit, spike density, awn color, etc.) are assessed based on the expression of the trait in all plants of the plot and mixtures are recorded in case that different expression among plants of the same accession will be identified.


Traits showing a continuous range of expression (e.g. plant height, flag leaf length, spike length, etc.) are recorded as the mean of five representative plants (or spikes when appropriate) of each accession.

6.49 Spike specific traits (e.g. awns, palea, lemma, etc.) in addition to seed characterization are scored in the laboratory.

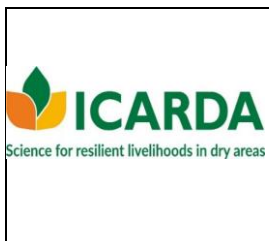
6.50 Identity is monitored by comparing the accession with previous passport or morphological data.

6.51 All characterization information is verified by the cereal curator and the genebank manager.

6.52 Characterization data is uploaded on the on-line ICARDA's Genebank Documentation System by the Documentation specialist. ICARDA's system is linked to GENESYS portal making automatic update and at the same time validated characterization data is becoming publicly available.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 26 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

The characterization procedure is considered finalized when all the data has been uploaded to ICARDA's Genebank Documentation System.

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 27 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

7. Related Flowcharts, Documents and Links

The following **flowcharts** are pertinent to this SOP:


- Flowchart for Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA (Annex 3)

Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA is in compliance with the following standards and policies:

- [FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture](#) (2014)
- International Rules for Seed Testing. [International Seed Testing Association](#), ISTA (2016)

Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA adheres to the following national and international frameworks:

- [ITPGREA](#)
- [SMTA](#)
- [International Plant Protection Convention \(IPPC\)](#)
- [WTO Agreement on the Application of Sanitary and Phytosanitary Measures \(SPS Agreement\)](#)
- [European and Mediterranean Plant Protection Organization \(EPPO\)](#)
- [UPOV](#)

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 28 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

8. *Staff Training and Competency*


Training and competency requirements to perform this SOP have been met by the following staff:

- Zakaria Kehel, Research Team Leader, Head of Genetic Resources Section. Contact: Z.Kehel@cgiar.org
- Mariana Yazbek, Senior scientist, Contact: M.Yazbek@cgiar.org
- Athanasios Tsivelikas, Scientist, Contact: A.Tsivelikas@cgiar.org
- Adil Moulakat, Research Associate, Contact: A.Moulakat@cgiar.org
- Oumaima Zaher, Research Assistant, Contact: O.Zaher@cgiar.org
- Rama Jawad, Senior Research Assistant, Contact: R.Jawad@cgiar.org
- Bashir Al-Awar, Research Associate, Contact: B.AlAwar@cgiar.org

Genebank staff undergo periodic training. Competency testing is done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9. *References*

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	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 29 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

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10. Revision History

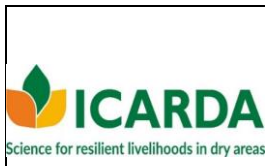
Effective Date of the SOP	Version #	Description	Reviewed By
December 20, 2017	1.0	Original SOP	<ul style="list-style-type: none"> • Dr Ahmed Amri • Dr Janny van Beem
December 20, 2019	2.0	Updated version	<ul style="list-style-type: none"> • Dr Ahmed Amri
December 20, 2021	3.0	Updated version	<ul style="list-style-type: none"> • Dr Zakaria Kehel

This SOP is reviewed and approved annually as part of activities in staff workplans and performance evaluations.

11. Citation, Copyright and Access Agreement

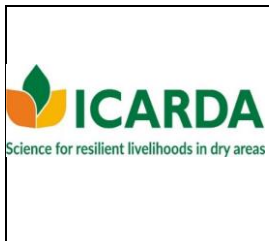
Citation: ICARDA, Genetic Resource Section. 2021. Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA (SOP ICARDA-REG & CHAR_CE001_ver3.0). Contributors: A. Tsivelikas, M. Yazbek, Z. Kehel, A. Moulakat, R. Jawad, B. Al-Awar, O. Zaher, I. El-Miziani.

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	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 30 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	




Annex 1. Major cross pollinated small-grained cereal species maintained by ICARDA's Genebank

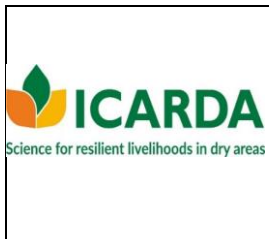
s.n.	Taxon	ICARDA Crop ID	Genome	Pollination	Growth
1.	<i>Aegilops caudate</i>	ICAG	C	Cross-pollination	Annual
2.	<i>Aegilops longissima</i>	ICAG	S	Cross-pollination	Annual
3.	<i>Aegilops speltoides</i> var. <i>ligustica</i>	ICAG	S	Cross-pollination	Annual
4.	<i>Aegilops speltoides</i> var. <i>speltoides</i>	ICAG	S	Cross-pollination	Annual
5.	<i>Avena fatua</i>	IFMI		Cross-pollination	Annual
6.	<i>Avena sterilis</i>	IFMI		Cross-pollination	Annual
7.	<i>Hordeum bulbosum</i>	ICWB		Cross-pollination	Perennial
8.	<i>Secale cereale</i>	IC		Cross-pollination	Annual


	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 31 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	


Annex 2. List of Descriptors assessed during the characterization process of ICARDA's Genebank cultivated and wild cereal germplasm



A. Data related to Regeneration/Characterization site	
1.	Regeneration site name and map/GPS reference
2.	Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
3.	Climate data of regeneration/characterization site (daily and monthly temperature range, daily and monthly precipitation, date and duration (in days) of frost temperatures, etc.)




B. Plant Descriptors	
B1. Vegetative traits	
1.	Growth class (seasonality) <ul style="list-style-type: none"> 1. Winter 2. Facultative 3. Spring
2.	Growth habit <ul style="list-style-type: none"> 1. Erect 2. Intermediate 3. Prostrate  <p>Figure 1. Variation for growth habit between wheat accessions (left: intermediate growth, middle: prostrate growth, right: erect growth).</p>
3.	Stem pigmentation (barley accessions only) <ul style="list-style-type: none"> 1. Green 2. Purple (basal only) 3. Purple (>half)  <p>Figure 2. Variation for stem pigmentation between barley accessions (left: green stem, middle: purple at base, right: purple at half or more of the stem).</p>
4.	Auricle pigmentation (barley accessions only) <ul style="list-style-type: none"> 1. Green 2. Pale purple 3. Purple 4. Dark purple  <p>Figure 3. Variation for auricle pigmentation between barley accessions (left: green auricles, right: purple auricles).</p>




	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 32 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

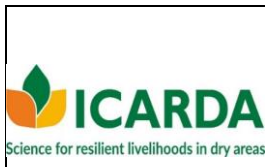
5.	Node pigmentation <ol style="list-style-type: none"> 1. Present 2. Absent 	 <p>Figure 4. Variation for node pigmentation between <i>Triticum</i> spp. accessions (up: absent, down: present of pigmentation).</p>
6.	Plant height (cm) Measured at maturity from five representative plants of each accession as the distance in cm from the ground level to the tip of the spikes excluding awns.	
7.	Flag leaf length (cm) Measured at heading time at the flag leaf of five representative plants of each accession in cm.	
8.	Flag leaf width (cm) Measured at heading time at the flag leaf of five representative plants of each accession in cm.	
9.	Flag leaf attitude <ol style="list-style-type: none"> 1. Erect 2. Medium 3. Horizontal 	
10.	Tillering capacity Measured at maturity from five representative plants of each accession as number of fertile tillers.	
11.	Plant waxiness (for <i>Triticum</i> spp. and <i>Aegilops</i> spp. accessions only) <ol style="list-style-type: none"> 1. Waxy 2. Intermediate 3. Not waxy 	
12.	Stem solidness (for <i>Triticum</i> spp. accessions only) <ol style="list-style-type: none"> 1. Solid 2. Hallow 	
13.	Lodging resistance <ol style="list-style-type: none"> 1. Excellent 2. Good 3. Fair 4. Poor 5. Very poor 	



	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 33 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

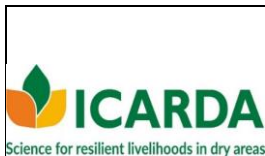
B. Plant Descriptors	
B2. Reproductive traits	
14.	<p>Days to heading</p> <p>Counted as the number of days from the sowing date up to the date when 50% of the plants of the accession have reached heading (Zadoks stage 59) (see Zadoks et al., 1974).</p>
15.	<p>Days to maturity</p> <p>Counted as the number of days from the sowing date up to the date when 50% of the plants of the accession have reached maturity (Zadoks stage 89) (see Zadoks et al., 1974).</p>
16.	<p>Grain-filling period</p> <p>Recorded as the difference in days between Days to Maturity and Days to Heading.</p>
17.	<p>Row number (for barley accessions only)</p> <ol style="list-style-type: none"> Six rowed Two rowed large or small sterile lateral florets Two rowed rudimentary sterile lateral florets Mixture <div data-bbox="852 880 1417 1061">  </div> <p>Figure 5. Variation for row number between barley accessions (left: two rowed barley, right: six rowed barley).</p>
18.	<p>Lemma awn/hood (for barley accessions only)</p> <ol style="list-style-type: none"> Sessile hoods Elevated hoods Awnless, or awnleted (2 cm), on all rows Awned (on central rows only for two-rowed forms, on all six rows for six-rowed forms) Awned on central rows only, lateral rows awnless or awnleted (for six rows only) Mixture <div data-bbox="866 1198 1396 1552">  </div> <p>Figure 6. Variation for lemma awn/hood trait between barley accessions (upper left: sessile hoods, upper right: elevated hoods, lower left: awnless, lower right: awned).</p>
19.	<p>Awn roughness (for barley accessions only)</p> <ol style="list-style-type: none"> Smooth Rough Mixture

20.	<p>Awnedness (for <i>Triticum</i> spp. and <i>Aegilops</i> spp. accessions only)</p> <ol style="list-style-type: none"> 1. Awnless or very short awns 2. Intermediate 3. Long awns 	 <p>Figure 7. Variation for awnedness trait between bread wheat accessions (left: awnless, right: long awns).</p>
21.	<p>Spike density</p> <ol style="list-style-type: none"> 1. Very lax 2. Lax 3. Intermediate 4. Dense 5. Very dense 	 <p>Figure 8. Variation for spike density between barley accessions (left: very lax, middle: lax, right: dense).</p>
22.	<p>Spike length (cm)</p> <p>Measured in cm at five mature representative spikes from each accession excluding awns.</p>	
23.	<p>Awns length (cm)</p> <p>Measured as the length of awns in cm at five mature representative spikes of each accession.</p>	
24.	<p>Number of spikelets per spike</p> <p>Counted as the number of spikelets per spike from five representative spikes of each accession.</p>	
25.	<p>Number of kernels per spikelet (for <i>Triticum</i> spp. accessions only)</p> <p>Counted as the number of kernels from two spikelets taken by the middle of the spike from five representative spikes of each accession.</p>	
26.	<p>Lemma color</p> <ol style="list-style-type: none"> 1. White/brown 2. Black/purple 3. White 4. Brown 5. Black 6. Purple 7. Mixture 	 <p>Figure 9. Variation for lemma color between barley accessions (upper left: white, upper right: black, lower left: brown, lower right: purple).</p>

27.	<p>Awn color</p> <ol style="list-style-type: none"> 1. White 2. Yellow 3. Brown 4. Reddish brown 5. Black 6. Mixture 	 <p>Figure 10. Variation for awn color between barley accessions (left: white awns, middle: yellow awns, right: black awns).</p>
28.	<p>Rachilla hairiness (for barley accessions only)</p> <ol style="list-style-type: none"> 1. Short 2. Long 3. Mixture 	
29.	<p>Glume hairiness (for <i>Triticum</i> spp and <i>Aegilops</i> spp. accessions only)</p> <ol style="list-style-type: none"> 1. Absent 2. Low 3. High 	
30.	<p>Kernel covering (for barley accessions only)</p> <ol style="list-style-type: none"> 1. Naked grains 2. Covered grains 3. Mixture 	 <p>Figure 11. Variation for kernel covering trait between barley accessions (left: naked grains, right: covered grains).</p>
31.	<p>Grain color</p> <ol style="list-style-type: none"> 1. White 2. Blue 3. Black 4. Brown 5. Purple 6. Mixture 	 <p>Figure 12. Variation for grain color between barley accessions.</p>
32.	<p>Grain vitreousness (for wheat accessions only)</p> <ol style="list-style-type: none"> 1. Non vitreous (bread wheat) 2. Partial vitreous 3. Vitreous (durum wheat) 	
33.	<p>One-thousand kernel weight (g) Measured on precision scale and expressed in g as the weight of 1,000 grains counted per accession.</p>	

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 36 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

C. Further Evaluation and Characterization traits	
1.	<p>Biological yield (kg/ha)</p> <p>Measured in g as the total biomass (straw dry matter and grains) produced by each accession in each individual plot and expressed as kg/ha.</p>
2.	<p>Grain yield (kg/ha)</p> <p>Measured in g as the total weight of dry grains produced by each accession in each individual plot and expressed as kg/ha.</p>
3.	<p>Harvest Index</p> <p>Expressed for each accession as the ration of grain yield to the respective biological yield.</p>
4.	<p>Grain size</p> <p>Expressed as the average of major ellipse and minor ellipse of 50 grains per accession, assessed through the GrainScan software (CSIRO Enquiries) (Whan et al., 2014).</p>
5.	<p>Yellow Rust reaction</p> <ol style="list-style-type: none"> 1. Resistant (R) 2. Moderate Resistant (MR) 3. Moderate Susceptible (MS) 4. Susceptible (S) 5. Very Susceptible (VS) <div data-bbox="774 1310 1401 1541" data-kind="parent">  </div> <p>Figure 13. Susceptibility of bread wheat accessions to yellow rust.</p>
6.	<p>Powdery Mildew reaction (mainly for barley accessions)</p> <ol style="list-style-type: none"> 1. Resistant (R) 2. Moderate Resistant (MR) 3. Moderate Susceptible (MS) 4. Susceptible (S) 5. Very Susceptible (VS) <div data-bbox="774 1608 1401 1832" data-kind="parent">  </div> <p>Figure 14. Left: Resistance reaction to powdery mildew, Right: Susceptible reaction to powdery mildew (photo credit to Dr Sajid Rehman).</p>

	Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA		
	SOP Number: ICARDA-REG & CHAR_CE001	Version: 1.0	Page 37 of 37
	SOP Owner: Athanasios Tsivelikas	SOP Approver: Zakaria Kehel	

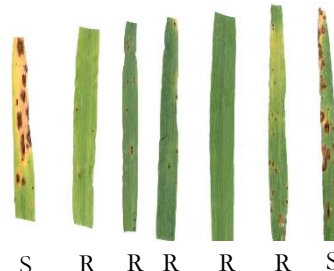
7.	Spot Blotch reaction (mainly for barley accessions)	
	1. No symptoms 3. Low symptoms 5. Moderate symptoms 7. High symptoms 9. Very high symptoms	

Figure 15. Reaction on leaves of barley accessions due to spot blotch infection (photo credit to Dr Sajid Rehman).

Note: The above-mentioned diseases are the ones most commonly occurred under the prevalent conditions at ICARDA's regeneration/characterization field sites. A number of different other biotic and/or abiotic adversities can be also assessed during the characterization/evaluation procedure of ICARDA's Genebank cultivated and wild cereal germplasm based on particular research objectives and specific demands.