ICARDA
Science for resilient livelihoods in dry areas

REGENERATION AND CHARACTERIZATION OF SELF-POLLINATED GERMPLASM AT ICARDA

A.L. Tsivelikas and GRS team



Genetic material collected/acquired…

- ✓ Seldom is sufficient in quantity to secure long-term *ex situ* conservation, and to implement the different activities of the genebank.
- ✓ Health status and low seed viability can be an additional constraint for entering the new accessions into the collection.

Genetic material stored…

- ✓ Decreasing in quantity over time, due to seed removal for distribution and for germination tests.
- ✓ Seed viability is decreasing during storage (identified through monitoring).

For these reasons…

- Regeneration is a key operation for any genenbank.
  - ✓ It is a process that leads to an increase of the stored seeds and/or to an increase of the viability rates of the seeds equal to or above an agreed minimum level.
- Regeneration is the most critical operation in genebank management.
  - ✓ It involves risks to the genetic integrity of germplasm accessions due to selection pressures, out-crossing, mechanical mixtures and other factors.

Consequently…

Genebank operators will have to strike a delicate balance between avoiding regeneration as much as possible versus the potential loss of viability or depleted stock.

Self-pollinated Germplasm at ICARDA

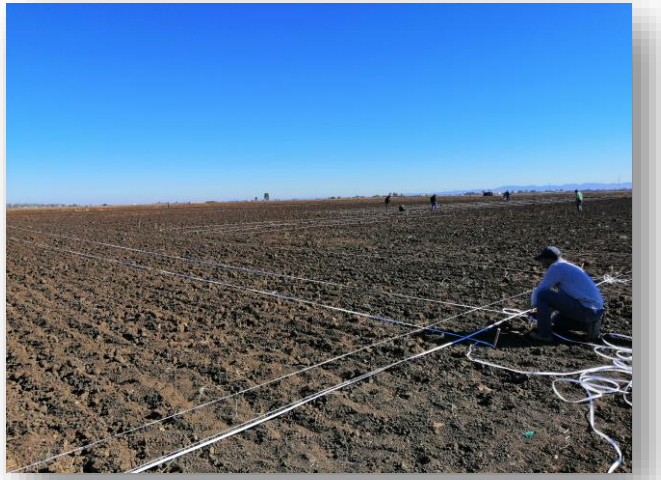
Small grained cereals (bread wheat, durum wheat, dinkel, emmer, barley, and most of their wild and primitive forms)

Self pollinated food legumes (chickpea, lentil, pea and most of their wild relatives)

Technical Aspects

Planting season

- Sow at the optimum time in order maturity and harvesting coincide with favourable weather conditions.
- Select date of planting according to recommended agronomic practices or local farmer's best practices
- Mediterranean type environment; regeneration during the rainy season (rainfed conditions).
- Keep in mind limitations due to diseases and pests (e.g. Hessian fly for cereals)



Field layout at Marchouch (Morocco) for germplasm regeneration

Stunted wheat plants, due to infection from Hessian fly

Technical Aspects

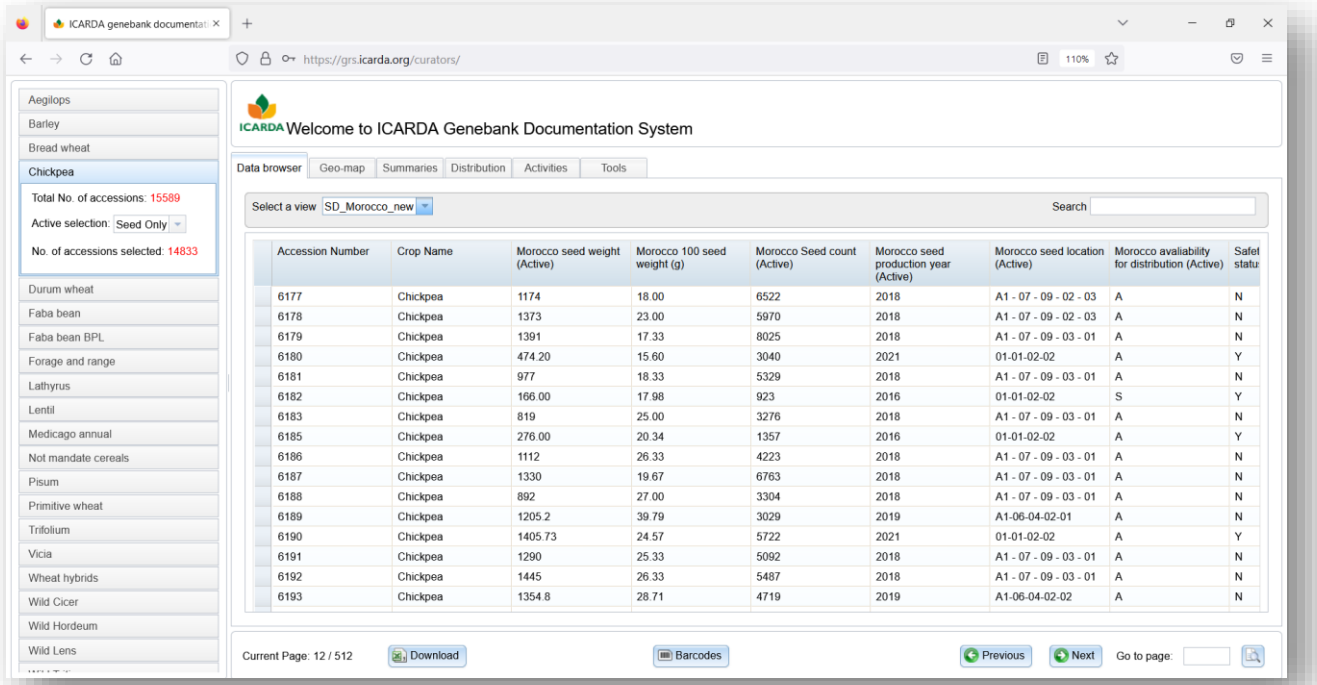
Appropriate environment

- Attention should be paid to the environment in which regeneration will be undertaken.
  - ✓ Wild populations to be regenerated in environments where occurred naturally (risk of invasive species)
  - ✓ For cultivated species, regeneration in the ecological region of its origin (if possible).
    - ❖ Need for special signals (vernalization for winter type cereals, photoperiod sensitive germplasm, etc.).
  - ✓ Alternatively seek for an environment that does not select in favour of specific genotypes.

Flagging Accessions for Regeneration

When we regenerate

- When viability rates <85% (cultivated species) and <75% (wild species)
- When the remaining seed quantity is less than what is required for five sowings of a representative population of the accession (bottom threshold value: 1200 seeds)
- Newly introduced or collected material with low seed quantity or low germination.

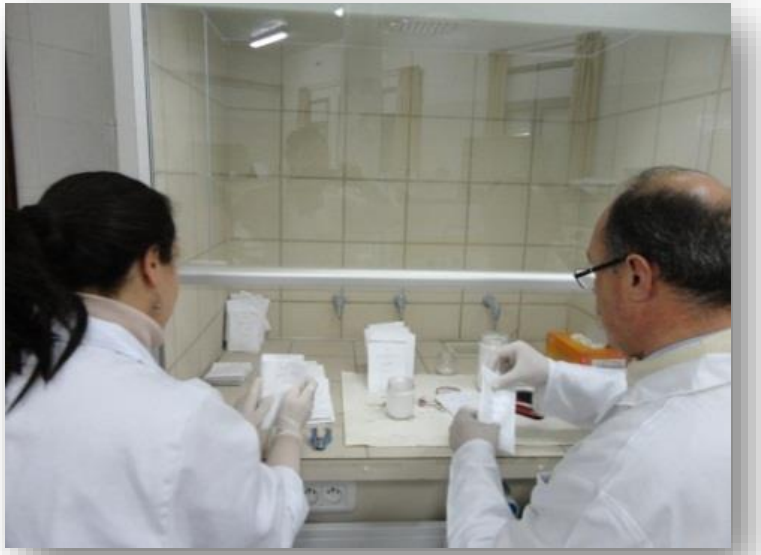


Preparation of Seed Lots

- If accessions are newly acquired (e.g. collecting mission)… dry, threshed and clean seeds needed.
- For accessions withdrawn from storage (Active collection)…
  - ✓ Identify the candidate accessions that require regeneration (monitoring through database).
  - ✓ Draw seed samples, keeping in mind the minimum sample size required for regeneration and the current level of germination.
  - ✓ Ensure absolute accuracy in identifying accessions while drawing the seeds from the genebank, packaging, and labelling seed samples.

Preparation of Seed Lots (Seed Pre-Treatments)

- Specific pre-treatment may be necessary to improve seed germination, establishment and seed setting…
  - ✓ Apply seed dressing (fungicides and insecticides) to reduce disease and insect damage.
  - ✓ For accessions with limited seeds (e.g. wild species), pre-germinate in controlled conditions and transplant the seedlings into pots with sterilized soil (grow them in a greenhouse).
  - ✓ Accessions with strong vernalization requirements may treated by artificial vernalization if field conditions not provide cool enough temperatures



Preparation of Seed Lots

- Minimum number of seeds for regeneration estimated by…
  - ✓ Standard sample size used for regeneration
  - ✓ Sample viability

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

Desired plant population = 150
Percentage germination = 85%
Expected filed establishment = 80%

$$Number\ of\ seeds\ for\ planting = \frac{150}{0.85*0.80} = 220\ seeds$$

Preparation of Seed Lots

- Desired plant population…
  - ✓ Size of the seed sample to be used in the regeneration activity has to reflect the genetic composition of the accession.

For this purpose…

Effective population size (Ne) is a key parameter that will have a bearing on the degree of genetic drift that is associated with the regeneration of the accession.

- Ne can be estimated for individual accessions based on the pollination biology
  - ✓ Perennial allogamous species Ne = 100 plants as minimum number to preserve taxon genepool (Johnoson et al., 2002; 2004)

Preparation of Seed Lots

In practice…

A sample size of 130-200 individuals will give a high probability of retaining rare alleles at low frequencies in most of the loci (Crossa, 2002).

At ICARDA…

Cereals: 200-250 seeds/m^2 (6-10 g seeds/m^2)
Food legumes (chickpea, lentil): 50-100 seeds per 2m row

Preparation of Field Plots

- Soil
  - ✓ Soil preparation based on common practices (plow and disc-cultivate soil prior to sowing, fertilizers, soil insecticides, etc.).
  - ✓ Good drainage of the field (critical factor especially for lentil).
  - ✓ Keep in mind crop rotation (e.g. cereals, legumes, fallow)
  - ✓ For specific accessions need of artificially prepared soil mixtures (wild relatives grown in pots).

Preparation of Field Plots

- Field Layout
  - ✓ Keep enough space between adjacent plots (avoiding possible mixtures)
  - ✓ Use single replicate trials and a local check variety at standard intervals (e.g. every 20 plot). Local check varieties allow relative characterization measurements with adjacent plots.
  - ✓ Ensure a stable water source for irrigation

Crop Management

Provide appropriate conditions for abundant seed setting and high quality seeds

- ✓ Eliminate weed competition (combination of manual and mechanical weed removal, use of selective herbicides)
  - ❖ Keep in mind that… diverse genebank material may react very differently to herbicides than cultivated materials.
- ✓ Preventive chemical applications to control diseases and pests.

Roguing the off-types

- ✓ Only discarding plants that are known to be contaminants or volunteers. Bear in mind that landraces are populations (genetic variability within the accession)
- ✓ Monitor for off-type plants several times throughout the growing season.

For accessions that are prone to shattering (wild relatives)

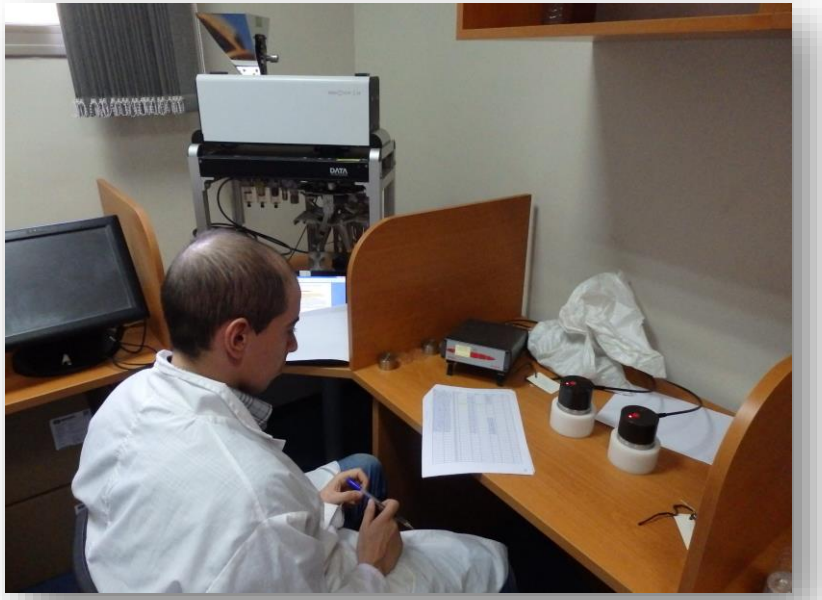
- ✓ Bag spikes (cereals) or whole plants (wild legumes) during ripening using perforated plastic or glassine bags.

Harvest and Post-Harvest Management

- Prepare cloth or plastic harvest bags. Use barcode labels to identify plots and accessions.
- Harvest at physiological maturity
- Uneven maturity in wild relatives may require repeated harvesting of individual accessions bonce individuals are at the optimum maturity stage.
- Dry plant material using ambient, non-heated air to a uniform moisture content (~12%).
- Thresh the seed using a stationary thresher
- Clean grain/seeds of straw, diseased seeds, broken seeds, weed seeds, soil, and debris using air-blown seed cleaners and appropriate sieves
- Fumigate seed to prevent insect damage prior to cold storage (usually using phosphine)

Harvest and Post-Harvest Management

- Dry the grains/seeds by placing in low humidity environment (at 15°C and 15% RH) for 6-8 weeks.
  - Cereal grains should reach an equilibrium moisture content of 5-8%
  - Legume seeds should reach an equilibrium moisture content of 3-7%
- Record seeds weights and determine 1000 kernel weight (cereals) and 100 seeds weight (legumes).



Seed Packing and Storage

Base collection (long-term storage): - 18.0 to -20.0 °C

- ✓ Cereals: 1,500 seeds per accession (two laminated aluminum foils each of 750 seeds)
- ✓ Food legumes: 1,200 seeds per accession (two laminated aluminum foils each of 600 seeds)

Active collection (medium-term storage): 2.0 to 4.0 °C and 25 to 30% RH

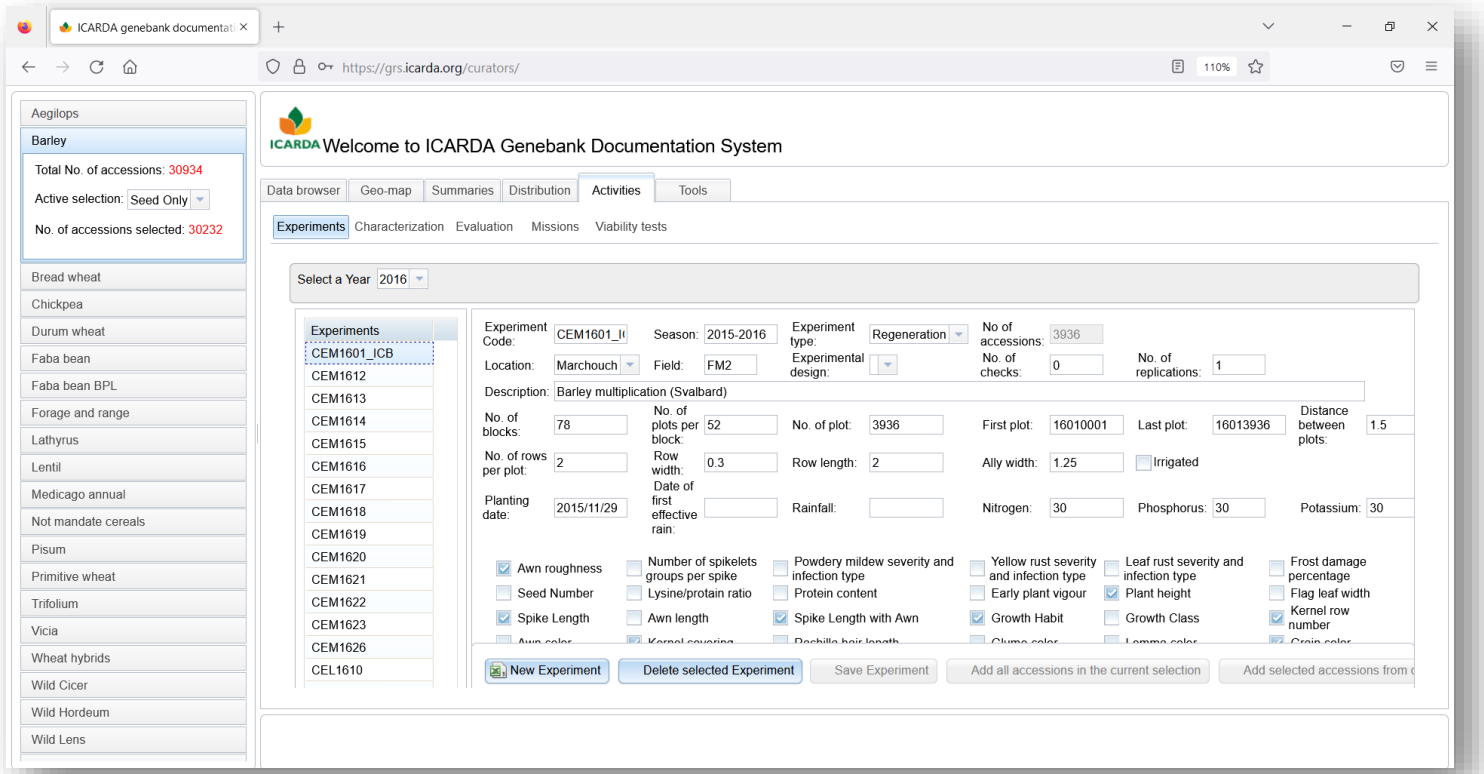
- ✓ Cereals: plastic containers with a bottom threshold of 1,500 grains
- ✓ Food legumes: plastic containers with a bottom threshold of 1,200 seeds

Safe duplication (black box)

- ✓ Cereals: one laminated aluminum foil of 750 seeds per accession
- ✓ Food legumes: one laminated aluminum foil of 600 seeds per accession

Documentation

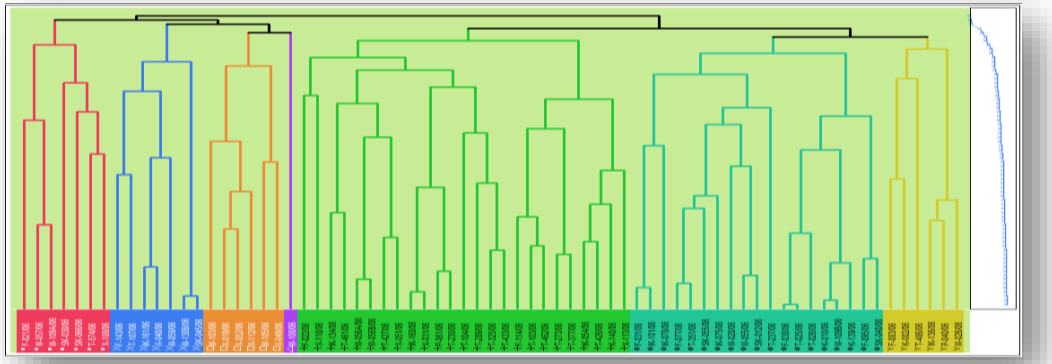
- All information gathered by regeneration activities documented
  - ✓ Regeneration site
  - ✓ Seed treatments
  - ✓ Plot reference
  - ✓ Field treatments
  - ✓ Sowing date
  - ✓ Number of plants established
  - ✓ Harvest date
  - ✓ Quantity of seeds harvested (base, active collection, safe duplication)



Germplasm Characterization

Characterization is the description of plant germplasm. It determines the expression of highly heritable characters ranging from morphological, physiological or agronomical features to seed quality traits or molecular markers and high throughput genotyping.

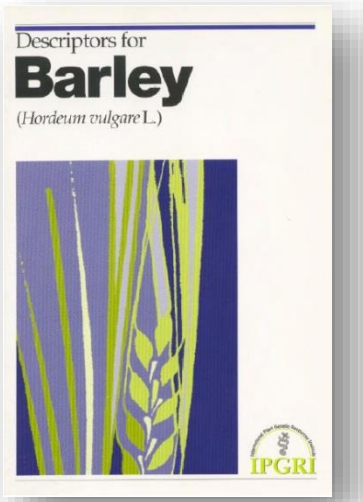
- Added value to the accessions maintained in the genebank
- Rationale management of accessions
  - ✓ Easy and quick discrimination among accessions
  - ✓ Allow grouping of the accessions (tool to create core and mini-core collections)
  - ✓ Check on the trueness-to-type
  - ✓ Identification of duplicates for appropriate management
  - ✓ Identification of accessions (wild species)

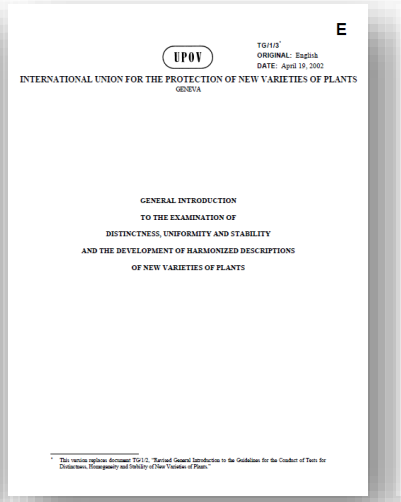


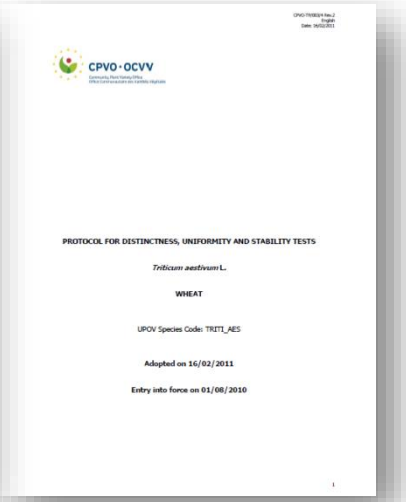
"Basic" Characterization at Genebank

Descriptor Lists

- Elaboration of a minimum set of phenotypic, physiological and seed qualitative traits.
  - ✓ Bioversity International (IPGRI) Crop Descriptor List.
  - ✓ International Union for the Protection of New Varieties of Plants (UPOV) Test Guidelines.
  - ✓ Community Plant Variety Office (CPVO) Technical Protocols







Characterization of Cereals

A. Vegetative traits

- Growth class (seasonality)
  1. Winter
  2. Facultative (intermediate)
  3. Spring

Characterization of Cereals

A. Vegetative traits (continue…)

- Growth habit
  - 3. Prostrate
  - 5. Semi-erect
  - 7. Erect
- Early vigour
  - 1. Very weak
  - 3. Intermediate
  - 5. Vigorous
- Stem pigmentation
  - 1. Green
  - 2. Purple (at base)
  - 3. Purple (> half)

Characterization of Cereals

A. Vegetative traits (continue…)

- Plant height (excluding awns, measured on March and during maturity)
  a. Recorded in cm (quantitative value)
  b. Recorded in a scale, 1: very short, 3: short, 5: intermediate, 7: tall, 9: very tall (categorical variable)

- Auricle pigmentation
  1. Green
  2. Pale purple
  3. Purple
  4. Dark purple

Characterization of Cereals

B. Reproductive traits

- Photoperiodical sensitivity
  1. Very low or no sensitivity
  2. Low sensitivity
  3. Intermediate sensitivity
  4. High sensitivity

- Days to heading

  Counted in number of days from showing up to when 50% of the plants in the plot have started heading

Characterization of Cereals

B. Reproductive traits (continue…)

- Row number/lateral florets

1. Two rowed, large sterile lateral florets
2. Two rowed, small sterile lateral florets
3. Irregular, varietal lateral florets development
4. Six rows, awnless or awnletted lateral florets
5. Six rows, long awns on lateral florets
6. Other (specify)

Characterization of Cereals

B. Reproductive traits (continue…)

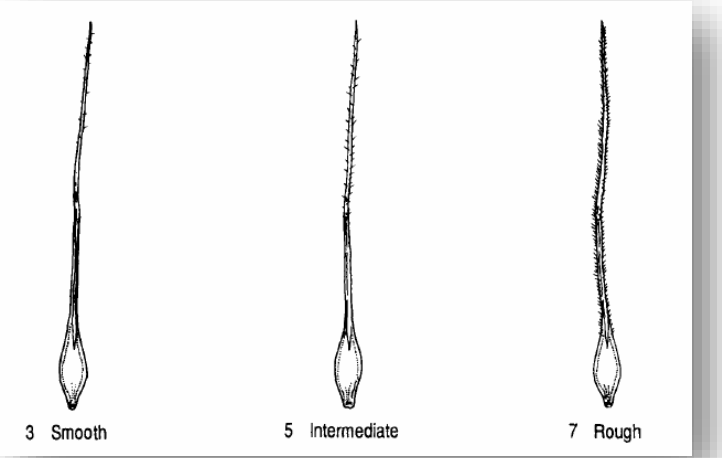
- Spike density
  1. Very lax
  2. Lax
  3. Intermediate
  4. Dense
  5. Very dense

Characterization of Cereals

B. Reproductive traits (continue…)

- Lemma awn/hood
  1. Awnless
  2. Awnletted
  3. Awned
  4. Sessile hoods
  5. Elevated hoods

- Lemma awn barbs
  1. Smooth (few barbs at tip)
  2. Intermediate (small barbs upper part)
  3. Rough



Characterization of Cereals

B. Reproductive traits (continue…)

- Glume and glume awn (outer glume)

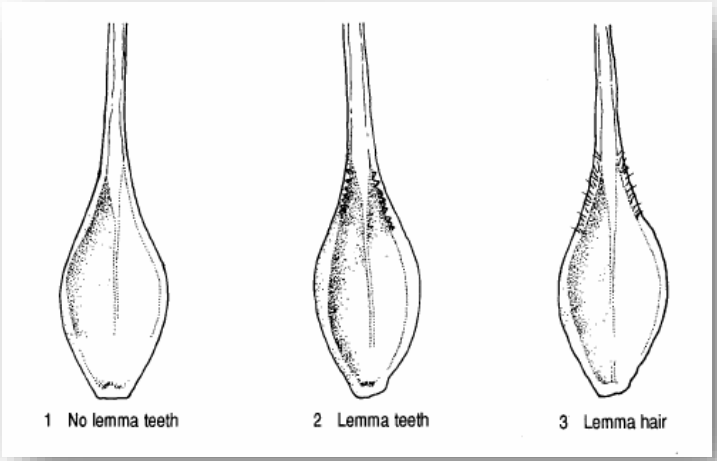
1. Length of glume and awn shorter than kernel
2. Length of glume and awn as long as kernel
3. Glume plus awn longer than kernel
4. Glume plus awn nearly twice as long as kernel
5. Lemma like
6. Other (specify)

Characterization of Cereals

B. Reproductive traits (continue…)

- Glume colour
  1. White
  2. Yellow
  3. Brown
  4. Black

- Lemma type
  1. No lemma teeth
  2. Lemma teeth (barbs or lateral nerves)
  3. Lemma hair



Characterization of Cereals

B. Reproductive traits (continue…)

- Awn colour
  1. Amber/White
  2. Yellow
  3. Brown
  4. Reddish
  5. Black
  6. Other (specify)

- Number of spikelets per spike
- Number of seeds per spikelet (wheat)

Characterization of Cereals

B. Reproductive traits (continue…)

- Kernel covering (lemma and palea adhere to the caryopsis)
  1. Naked grain
  2. Semi-covered grain
  3. Covered grain

- Lemma colour
  1. Amber (=normal)
  2. Tan/red
  3. Purple
  4. Black/grey
  5. Other (specify)

Characterization of Cereals

B. Reproductive traits (continue…)

- Grain colour
  1. White
  2. Tan/red
  3. Purple
  4. Black
  5. Other (specify)

- Aleurone colour (difficult to observe, used for market type classification)
  1. White
  2. Blue

Characterization of Cereals

B. Reproductive traits (continue…)

- 1000 kernel weight
- Grain size
  1. Very small
  3. Small
  5. Intermediate
  7. Large
  9. Very large
- Seed vitreousness (glass like appearance when seeds transversely sectioned)
  1. Non vitreous (bread wheat)
  2. Partly vitreous
  3. Vitreous (durum wheat)

Novel Tools for Characterization at the Genebanks

Spectral imaging instruments to determine seed traits

- Primary traits
  - ✓ Seed length
  - ✓ Seed width
  - ✓ Seed perimeter
  - ✓ 1,000 kernel/seed weight
  - ✓ Seed color

Total cost: ~14,000 USD

Grainscan from CSIRO
https://research.csiro.au/workspace/grainscan/
Total cost: 0.00 USD – Free software the basic edition

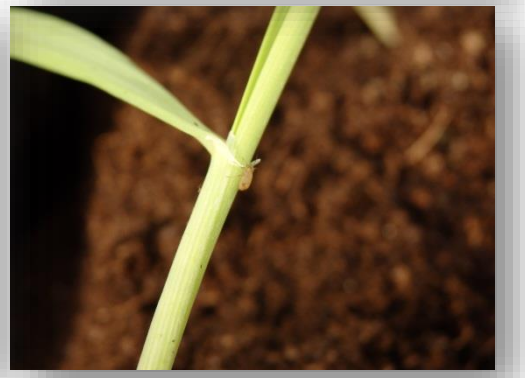
- Secondary traits
  - ✓ Seed purity
  - ✓ Seed health
  - ✓ Seed germination
  - ✓ Seed shape
  - ✓ Vitreousness
  - ✓ etc.

Videometer instrument

Total cost: ~50,000 to 70,000 USD

Agronomic Characters

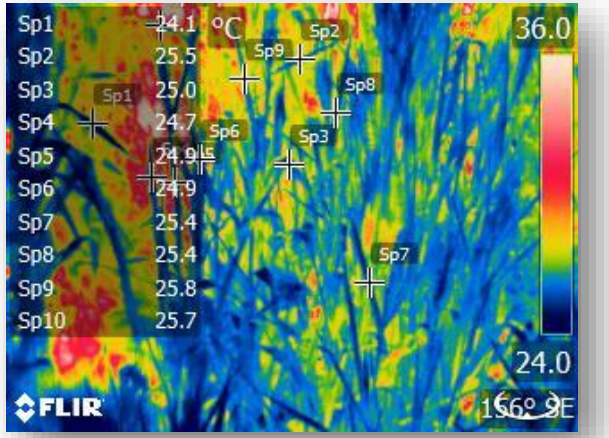
- Physiological seed maturity:
  1. very early, 2. early, 3. intermediate, 4. late, 5. very late
- Yield per plant (g).
- Tillering capacity
- Harvest index (%).
- Resistance to pest and diseases (fungi, insects, nematodes, bacteria, viruses)
- Tolerance to biotic adversities (e.g. salinity, drought, waterlogging, heat, etc.)



Plant Physiology Characters

- Leaf chlorophyll content
- Canopy temperature
- Leaf fluorescence
- Stomatal conductance
- Canopy width (or related parameters e.g. LAI)
- etc.

Wide variety of instruments can be employed (different price levels)



Seed Quality Traits

- Seed protein (starch, fiber, etc.) content (g/100 g DW)
- Carotenoid content (durum wheat)
- β – glucane content (barley)
- Malt suitability (barley)
- Fodder digestible fibber content (g/100 g DW) (dual purpose barley)

Easy, rapid, reliable and non-destructive methods by employing spectral image analysis

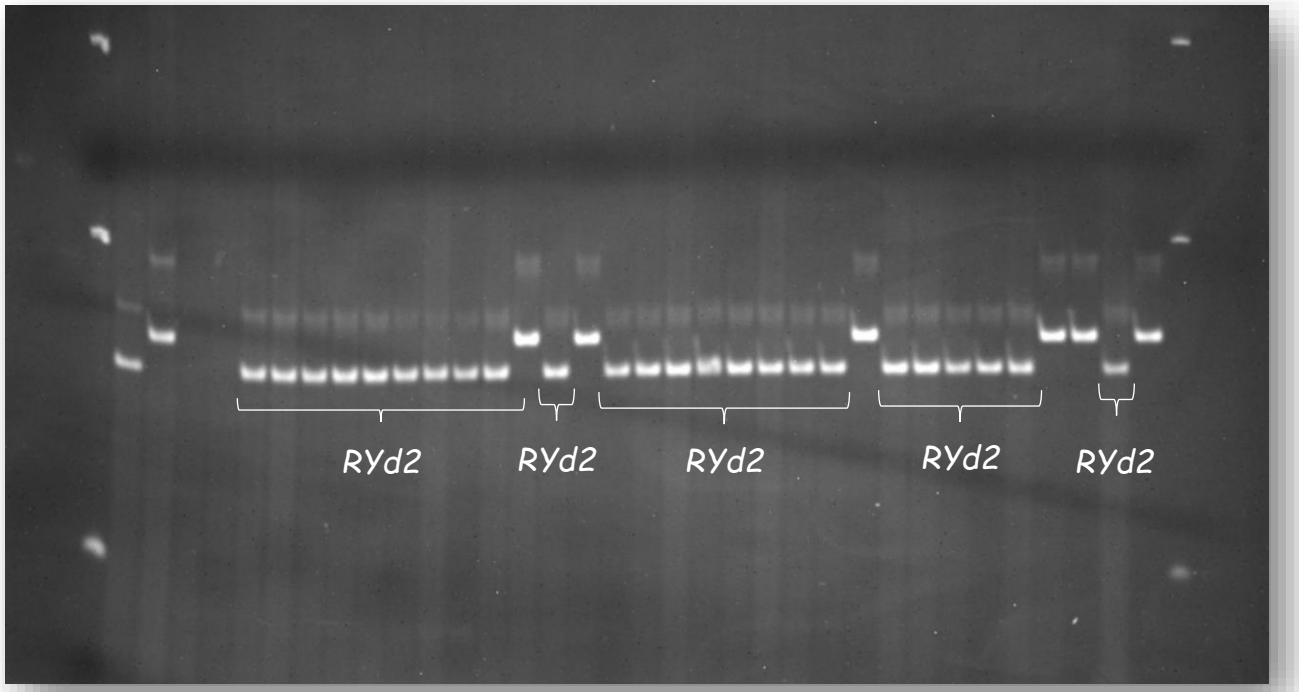
Videometer instrument

Near-infrared (NIR) instrument

Molecular Characterization

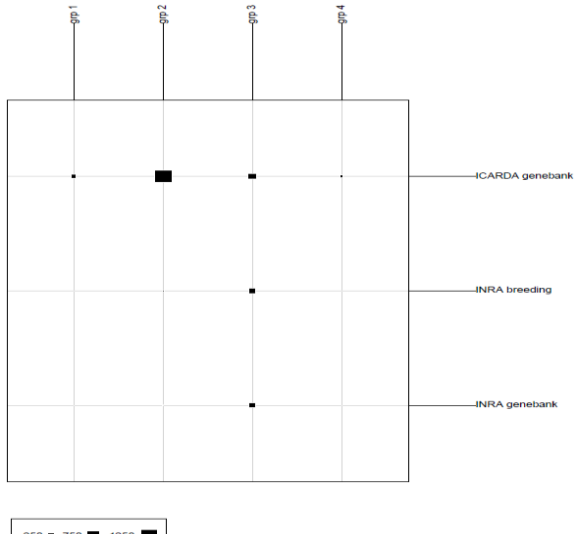
Employment of molecular markers (e.g. SSRs, SNPs, etc.)

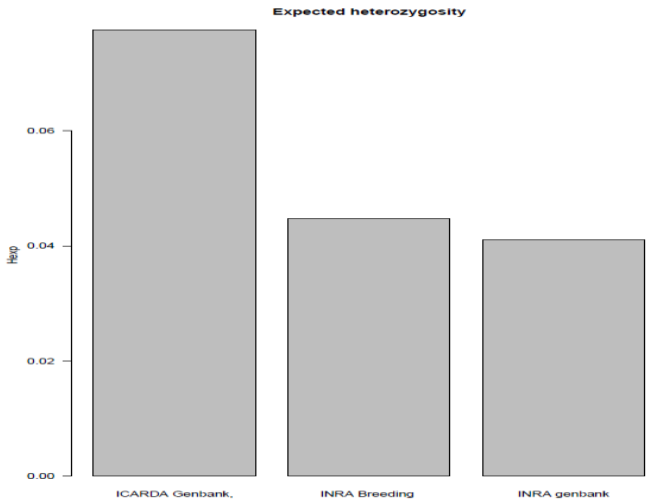
- Genetic diversity studies
- Duplicates identification
- Alleles identification linked to specific traits

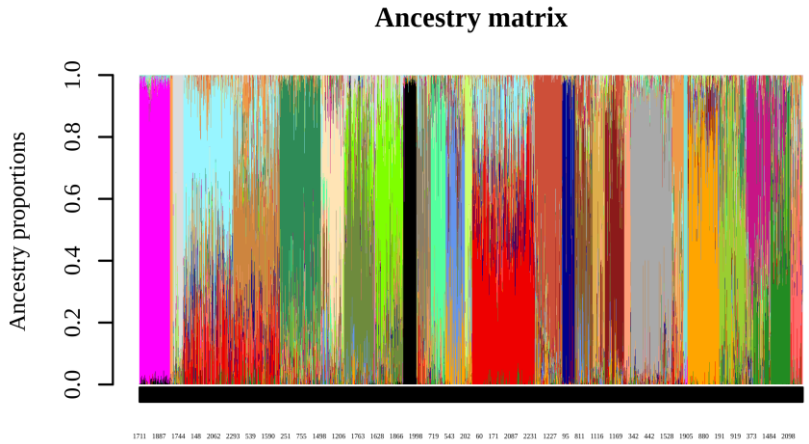


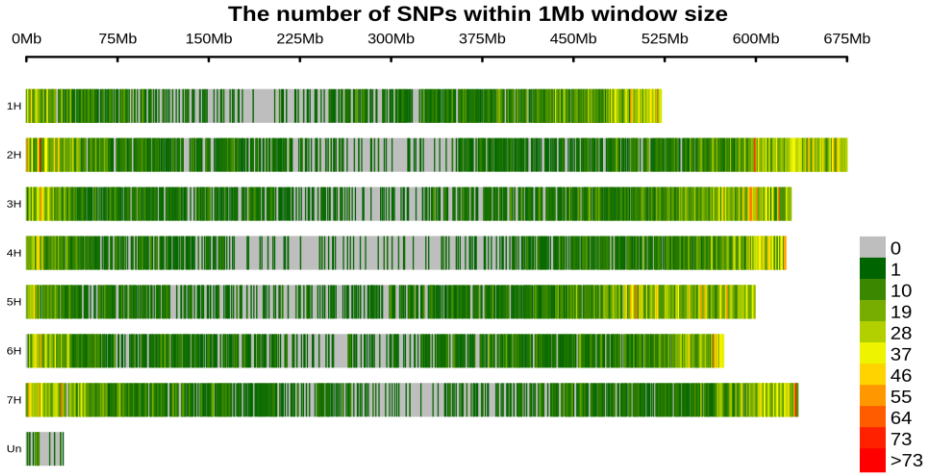
Detection of the *RYd2* allele for resistance to BYDV in barley accs.

Genomic Characterization

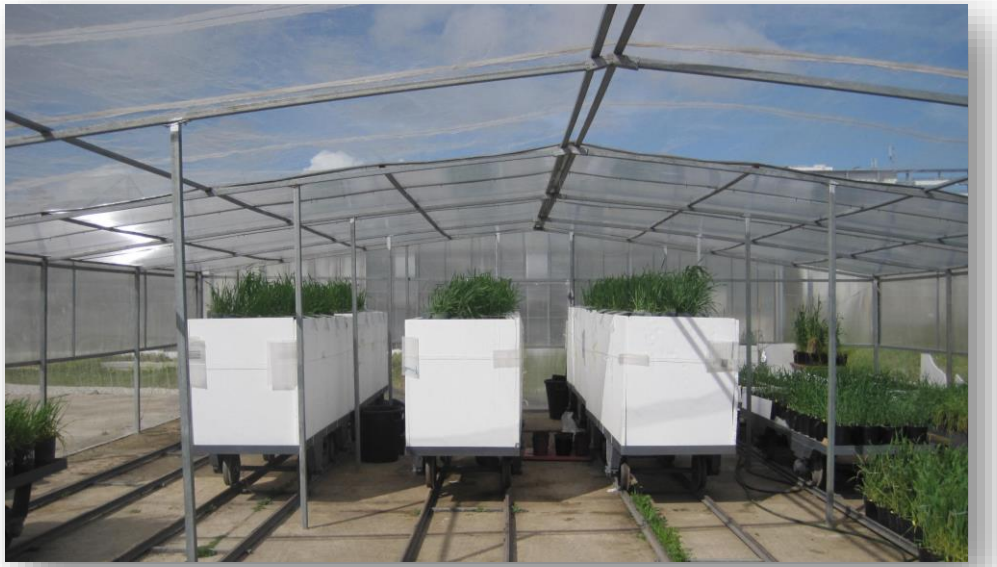








THANK YOU FOR YOUR KIND ATTENTION!



Artificial Vernalization

- ✓ Conduct the treatment 8-10 weeks before optimum field sowing date to allow to the transplanted seedlings optimal growth and development at the field.
- ✓ Identify the genotypes (strictly winter genotypes) that require the treatment
- ✓ Place seeds on moistened blotter paper into petri-dishes and allow germination to begin at ambient temperatures
- ✓ Transfer the petri-dishes into growth chambers and adjust temperature at 1-3^{o}C with a light source (8/16 h day/night)
- ✓ Keep the treatment for 4-8 weeks depending on the vernalization demands of the accs.
- ✓ Carefully transplant seedlings into the field