

# Progress report of the AEGIS model crops: *Avena*

## July 2008

### 1. Introduction

In 2007 during the oats (*Avena* sp.) global conservation strategy meeting at St Petersburg, Russia, a sub-group of the AEGIS *Avena* group was fully agree that the major tasks will be the development and agreement of criteria to identify the MAA and the development of a quality management system including technical standards.

In an informal meeting, at Clermont-Ferrand, France in September 2007, a discussion on elementary selection criteria of MAA has been done between present members of the ECPGR *Avena* working group. Additional question relevant of the AEGIS implementation was tackled as the assignment of accession number in order to avoid confusion, the reliability of the EURISCO database and EADB and the coordination tasks of updating data between the both.

At Foça, Turkey, in April 2008, the database manager of the *Avena* working group, C. Germeier, made a point on the current situation between the EURISCO database and EADB. Comparison among the both showed differences for entries number and level of taxonomy characterization. A first draft of criteria for AEGIS selection was discussed between the working group members. Several points were pointed as:

- the ultimate goal of assigning the MAAs is to identify most of genetic variability on a limited set of material
- AEGIS should be a collaborative task involving all genebank managers
- A most important component is a reliable database
- Available database information is not complete
- Needs in additional steps to verify, confirm, harmonize and update the EADB

This new document give the results of the *Avena* AEGIS group discussion during the meeting of the AEGIS model crops curators and database managers on 1<sup>st</sup>-3<sup>rd</sup> July 2008 in Radzików, Poland. The *Avena* AEGIS group was represented by Zofia Bulinska-Radomska (IHAR), Ayfer Tan (AARI), Igor Lokustov (VIR) and Audrey Didier (INRA).

### 2. Establishing selection criteria for the identification of the Most Appropriate Accessions (MAAs)

The Most Appropriate Accession is an accession of an original seed lot or seed sample that is genetically as close as possible to the original population. It shall be true to name, held in the country of origin or introduced material of importance for breeding and research and used in Europe, accompanied by passport data, and characterized morphologically or with markers.

The European *Avena* Database contains about 34,000 entries from more than 20 genebanks. The main problem is to identify the Most Appropriate Accession and duplicates accessions. A first identification, at the **national level**, within collection in framework national genebanks has to be done following by another one at the **international level**, between collections in framework EADB. This identification of MAA and duplicates is based on a comprehensiveness of passport data and on the FAO/IPGRI multi-crop passport descriptors which have to be as complete as possible.

- **Recommended primary selection criteria**
  - i. In the **public domain** (i.e. Annex I material that is in the multilateral system of the ITPGRFA and non-Annex I material designated to AEGIS by governments or any other holder)
  - ii. **Genetically unique**, to the best available knowledge (i.e. genetically distinct accessions; assessment based on available data and/or on the recorded history of the accession)
  - iii. Agronomically (incl. research material) and/or historically/ culturally important
  - iv. Plant Genetic Resources, including:
    - 1. Wild species (most of collected accession are unique)
    - 2. Landraces (local) (most of collected accession are unique)
    - 3. Obsolete improved varieties (all collected before 1950s are unique)
    - 4. Advanced improved varieties (to divide to unique accession and duplicates)
    - 5. Breeding/research materials (most of collected accession are unique)

The selection of accession following this criterion has to be done in two steps, the first one at national level within the collection to select accessions that have been **collected or bred in the country** where they are **being conserved**. And the second one at the international level between collections, to select unique accessions that have been **collected or bred in European countries** or accessions that have been **collected or bred in non-European countries**.

- v. European origin or introduced germplasm that is of actual or potential (breeding/research) importance to Europe

- **Comments on draft priority selection criteria**

The dominating criterion excluding accessions from being considered as MAA in any case will be lack of information.

- **Recommended secondary selection criteria** (used when deciding which accession to accept among “quasi duplicate” or similar accessions)
  - i. Maintained in “country of origin”
  - ii. A known origin (collected and/or bred)
  - iii. Comprehensiveness of passport information
  - iv. Number of regeneration/multiplication cycles (the lowest one)
  - v. Health status (i.e. is the germplasm disease free?)
  - vi. Existence of morphological/molecular characterization data
  - vii. Existence of agronomical evaluation data
  - viii. Validated accession name (particularly relevant for perennial clonal crops where the same name can be attributed to different accessions; history of individual accessions is important; special attention to be paid to synonyms and homonyms, transparent selection is needed)
  - ix. Initial contributor/collector

- **General observations and comments on the process of developing the criteria and lessons learnt for other crops** (Germeier *et al.*, 2006)

Identification of duplicate groups and MAA will be a huge effort by itself. The management of the collections follows different methodology and fulfils different

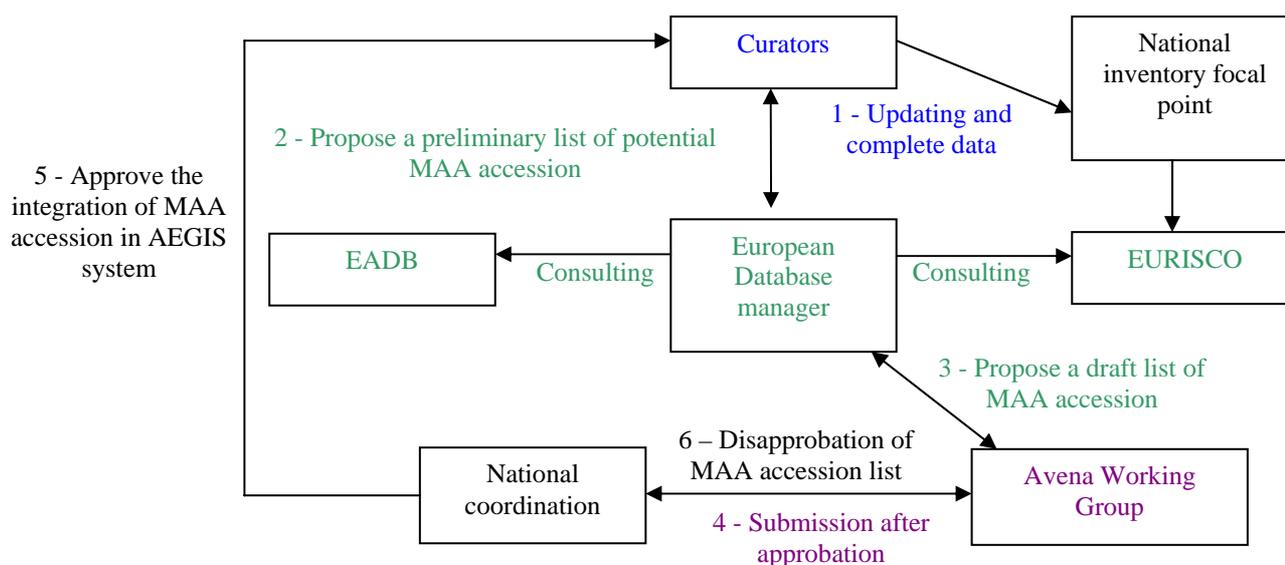
quality standards depending of the collection holder. Several methodologies could help to determinate duplicates as observation in field, protein markers or looking for genetic distances using morphological traits or molecular markers. Regrouping of unknown and mistaken accessions might be possible.

To be able to make comparison between potential duplicate accessions and choose the MAA at national and international level, some criteria have to be specified precisely as for example passport, characterization and evaluation data.

### 3. Establishing the list of MAAs

➤ **The procedure followed, including the respective roles of associated institutions, the countries (i.e. National Coordinators), the Central Crop Database manager and the Working Group**

The first step (cf. scheme 1) to establish the list of MAAs has to come from curators (1) by updating and complete the data available in European database such as Avena database, directly with the database manager or Eurisco by the intermediate of the national inventory focal point. To obtain update and complete data by curators is the first step but the hardest one because it is time-consuming and all depending of the willingness of curators. The second sensible point could be the transfer of data to the national inventory focal point (non availability, too much data ...).



**Scheme 1:** Procedures to establish the MAAs list

After the update, the European database manager will be able to elaborate a preliminary list of potential MAAs (2). By several exchanges with curators, European database manager will validate the draft list of Avena MAAs and submit it to the Avena working group (3). The working group will take the first decision to approve the list. Two possibilities are considered, the first one and simplest is that the Avena working group transfers the final MAA list to the national coordinators for approval (4). Exchanges are expected between the Avena WG and the national coordinators to solve potential problems. The second possibility, the most probable is that the ECPGR secretary does the intermediate between both. Finally the national coordinators approve the list (5) and give it back to the curators. Several problems can occur at this stage, first of all the approbation by the national coordinator of the MAA list,

depending of the national policy and of the signature of the MoU. Another one is the availability and the implication in the national program of the national coordinator.

The needs in meeting and/or coordination for each step of the elaboration of MAAs list have been listed in the following table.

	Step	Number	Who	Details
<b>Meeting</b>	1	1	All curators + CCDB manager	To explain the needs of AEGIS and give/explain criteria for the MAA selection
	3	1 or 2	Avena WG	Approval of the draft list
	6	1 or 2	Avena WG	Resolution of potential or temporary problem with the approval of the final MAA list
<b>Coordination</b>	1	As much as necessary	All Curators + CCDB manager	Contact by email between database manager and curators
	2	As much as necessary	Voluntary curators + CCDB	Contact by email between database manager and curators
	4	As much as necessary	Avena WG chairman or ECPGR Secretary? + national coordinator	Approval of the final list
	5	As much as necessary	National coordinator + curators	Approve the integration of MAA accession in AEGIS system
	6	As much as necessary	Avena WG chairman or ECPGR Secretary? + national coordinator	Return list for several reasons (MAA accession could not be included in the national program, non signature of the MoU,...)

**Table 1:** Needs in meeting and/or coordination to establish the MAAs list

➤ **Generated list of MAAs (for the model crop in question and based on Central Crop Database)**

The working group was not able to give a final list to the ECPGR secretary. Furthermore, we strongly believe that several meetings and correspondence between curators and CCDB manager will be necessary before the generation of the list.

➤ **Experiences with the use of the selection criteria while establishing the list (I. Loskutov)**

Database manager was absent at this meeting and AW group presents practical approaches to create the list of Most Appropriate Accessions based on VIR Oat collection.

N. I. Vavilov Institute of Plant Industry is harbored one of the largest oat collection. There are more than 12 700 accessions there. It presents world genetic diversity of *Avena* genera with wide variability of morphological, agronomical and biochemical characters for oat breeding and crop industry.

Tools for identification MAA and duplicates in this case we use VIR Oat database in Paradox 9 for Windows software amounted to 18 178 Kb and contained over 12 700 records in 43 fields.

Types of accessions included in collection are:

- Wild populations
- Landraces (local)
- Obsolete (old) improved varieties
- Advanced improved varieties
- Breeding/research materials/genetic stocks

		<b>*Wild species</b>	<b>*landraces (local) or/and obsolete (old) improved varieties</b>	<b>*advanced improved varieties</b>	<b>*breeding/research materials/genetic stocks</b>
<b>Taxonomical Information</b>		Has to be corrected	Has to be corrected	Has to be corrected	Not so important
<b>Geographical Information</b>	<b>Country of origin (ORIGCTY)</b>	National or European or non-European countries	National or European or non-European countries	National or European or non-European countries	National or European or non-European countries
	<b>Donor country (DONORCODE)</b>	The same that origin country	The same that origin country	The same that origin country	The same that origin country
<b>Numeric Information</b>	<b>Collecting number (COLLNUMB)</b>	Preferably filled	Preferably for landraces		
	<b>Acquisition date (ACQDATE)</b>	Same dates (year)	Has to be unique for obsolete varieties		
	<b>Collecting date of sample (COLLDATE)</b>	Same dates (year)	Preferably for landraces		
	<b>Donor accession number (DONORNUMB)</b>	Have to be empty	Has to be empty	Has to be empty	Has to be empty
	<b>Other identifications (numbers) (OTHERNUMB)</b>	Have to be empty	Has to be empty	Has to be empty	Has to be empty
	<b>Accession name (ACCENAME)</b>			Has to be unique	Has to be unique
	<b>Accession number (ACCENUMB)</b>				Has to be unique
<b>Genetic group</b>	<b>Ancestral data (ANCEST)</b>			Preferably filled	Preferably filled

\*Selection unique accessions that have been collected or bred where they are being conserved or/and in European countries or/and in non-European countries.

**Table 2:** Information needed to establish a selection of MAA sample

For this selection we use the list of the most important fields of descriptors:

**1. Taxonomical group**

- Genus (GENUS) I-V
- Species (SPECIES) I-V
- Species authority (SPAUTHOR) I-V
- Subtaxa (SUBTAXA) I-IV
- Subtaxa authority (SUBTAUTHOR) I-IV

**2. Geographical group**

- Country of origin (ORIGCTY) I-V
- Location of collecting site (COLLSITE) I-II
- Donor (country) institute code (DONORCODE) I-V

### 3. Name and Numeric group

- Accession number (ACCENUMB) I, V
- Collecting number (COLLNUMB) I, II
- Accession name (ACCENAME) III, IV
- Acquisition date [YYYYMMDD] (ACQDATE) I-V
- Collecting date of sample [YYYYMMDD] (COLLDATE) I, II
- Donor accession number (DONORNUMB) I-V
- Other identification (numbers) associated with the accession (OTHERNUMB) I-V

### 4. Genetic group

- Ancestral data (ANCEST) III-IV and some genetic information V

### 4. Establishing the quality management system (QMS)

- **General observations on establishing a QMS for model crop** (Germeier *et al.*, 2006)

The structure of the collections has an impact on the establishment of the QMS. Methodological considerations will be different considering wild or cultivated material. Wild accessions are very hard to handle and need special attention not to lose valuable material. Collection holders of wild accessions have to deal with several problems related to safety duplicates, proper regeneration and conservation. These problems in the maintenance of wild accessions result from the following facts:

- Some of the wild *Avena* species are noxious weeds and their growing is restricted by quarantine considerations
- Several of them are difficult to regenerate thus the regeneration methodology needs to be worked out.
- Europe offers good regeneration conditions only for *A. fatua*.

A list of minimum standards has to be done concerning collecting, regeneration, storage, conservation and distribution methodology. To help in this task, some basic standards have already been given by FAO genebank standards (Anonymous 1994) and described by Engels and Visser (2003). Additional guidelines are available (e.g. Sackville-Hamilton *et al.*, 1997; Rao *et al.*, 2006) giving advice in management of maintenance and regeneration of accessions in seed collections. Nevertheless, these basic standards have to be adapted to wild species which need specific cares and the current methodology of participating genebanks have to be taken in account too.

- **Recommendations on crop specific technical standards**

Definitions of minimum technical standards for *Avena* species have been carried out during the meeting.

- **Collecting methodology** (Guarino *et al.*, 1995)
  - Fine grid collection to fill the gap,
  - Optimum maturity (seed vigour, drying tolerance, viability level),
  - Random sampling to collect as much variation/genotype to reach minimum effective population size if very narrow population, leave some plants and collect others and record the population size (single plant collection should be recorded),
  - Complete information on collection and passport data (documentation of site, date and methodology) in EADB and EURISCO database.

- **Conservation**
  - Cleaning (for wild hand cleaning),
  - Drying - Dehumidification to reduce the seed moisture to 6-8% – For small seed samples two steps slow drying (oven drying; ISTA, 2005)
  - Packing - Preferably glass jar / aluminium cans for medium and long term (paper and poly-urethane plastic bag are also used – the relative humidity of cold room had to be under 15%, if not use moisture proof containers)
  - Moisture proof containers or silica gel (if moisture of the cold room is not controlled)
  - Storage rooms
    - Long term condition for base collection -18°C/-20°C
    - Medium term condition for active collection 0°C
    - Short term for temporary storage if necessary, for the material need multiplication and regeneration
  
- **Variability testing**
  - Initial variability testing for collecting seed: minimum 2 x 100 seeds
    - ISTA, 2005/AOSA, 2005 for cultivated species (*A. sativa*, *A. byzantina*, *A. abyssinica*, *A. strigosa*)
    - Dormancy assessment for wild species
    - Successful break of dormancy with gibberellic acid solution (ISTA rules, 2005, with using the highest recommended concentration (0.1%) in the recommended buffer solution). If the seed lot is small use tetraolium test
  - Periodical viability testing
    - Each 5 years for active collection
    - Each 10 years for base collection
  
- **Regeneration and multiplication**
  - Regeneration and multiplication is in similar ecology where the material collected or controlled conditions for keeping the population as it is and not to lose any genotype
  - Not close to disease/ pest evaluation plots - for this effective population size: 100/500 seeds according to the germination rate of the accession
  - Bulk harvest to keep populations as it is (at least 50 plants)
  
- **Characterisation / evaluation**
  - Morphological characterisation: follow the IPGRI/Bioversity descriptors list and if necessary use UPOV descriptors otherwise **minimum characterization descriptors of Avena should be prepared**
  - Evaluation follows the IPGRI/Bioversity scale of descriptors list otherwise **useful evaluation descriptors of Avena should be prepared**
  - Molecular characterisation: use most suitable method to assess the molecular variation

- For cultivated species: SSR markers (RAPD/ RFLP are currently used)
  - For wild species: to determine most powerful marker methodology (RAPD/ RFLP are currently used)
- **Distribution**
    - Packaging
    - Minimum information attached to sample
      - In case of collected accession
        - Accession number
        - Documentation of site (country, geographic coordinate, type of environment)
        - Collect date
        - Viability capacity (date of the last test and percentage)
      - In case of bred accession
        - Accession number
        - Accession name
        - Pedigree
        - Viability capacity (date of the last test and percentage)

➤ **General comments and observations**

The implementation of these standards will need several steps. The first one will be collecting information on the methodology used at institutions holding *Avena* collections (Germeier *et al.*, 2006). In a second one, the results should be summarised and minimum standards should be agreed on in a workshop on maintenance methodology for *Avena* accessions in genebanks. This could be some certification procedures implemented by single genebanks or provided by AEGIS system.

The cultivation standards have to be divided in two subgroups: cultivated species and wild species due to the specific treatment required by wild species. No specific information are available for these species concerning technical standards or the effective population size which has to be collected, maintained and distributed, this work is **still to be carried out**.

For both cases, several methodological points need to be specified by the working group as:

- **Collection**  
How to evaluate the effective population size in a narrow population of wild and/or cultivated species?
- **Viability test**  
What are the dormancy mechanisms in wild and/or cultivated species?  
Which minimum level of viability is required according to each species?
- **Conservation**  
Standards concerning packaging, drying conditions, seed moisture percent and storage conditions had to be defined specially for wild species.
- **Multiplication/Regeneration**  
Is there any isolation need?  
What is the effective population size to sow to preserve the genetic integrity of the accession?

Which harvest quantity to be able to fulfil request?

- **Characterisation and evaluation**  
What are the compulsory descriptors for characterisation and evaluation?
- **Distribution**  
Which amount of seed by sample has to be sent to partner (fixed or variable) and for which species?
- **Safety duplication**  
How to share responsibility for safety duplication (cooperation between several institutes, one depositary institute...)?
- **Standardization of database management**

## 5. Observations on the framework and tool for the assessment of operational costs for collection maintenance

To have an idea of operational costs for the collection maintenance, the working group has listed the needed requirements to implement the European collection. Five thematic have been pointed out as quality standard, coordination, contribution of national government, information management and knowledge transfer.

### a. Quality standard

All Avena collection holder taking parts to the European collection have to be agreeing to common or similar procedures and standards based on current knowledge. This concerns all the genebanks tasks: conservation, multiplication/regeneration, viability test, characterisation and evaluation including molecular marker analysis, distribution and safety duplication.

Nevertheless, current situations are very diverse between institutes. Some equipment and supplies will be required to standardize procedures. Writing guidelines will be helpful to describe lack in equipment and supplies, in building and knowledge capacity too. It is indeed conceivable that a holder institute will not have the ability to receive all accessions originating from his country and/or the specific knowledge of this species.

- ⇒ For the implementation of the system, **additional costs** are considered at the beginning to standardize procedures, equipment and building/knowledge capacity.
- ⇒ A drop of these costs are expected with time if a comprehensive and complete characterization (include molecular marker analysis) of all collection and data collection is performed. The drop is in narrow relation with the number of characterization and evaluation descriptors, the level of precision expected to these descriptors and the involvement of participant institute in the tasks sharing.

### b. Coordination

- **At European level** an exhaustive list of lead European institute in genetic resources management, conservation and scientific work with Avena species have to be done to implement the coordination and the sharing responsibility.
- **At National level** a better coordination between the national inventory focal point, the national coordinator and the genebanks curators is required

at the beginning to implement AEGIS and in a second time to update and integrate additional accessions in the system.

- To obtain similar progress and update in the AEGIS system, **regular meetings**, between curators in charge of MAA, will be necessary to coordinate activities.
- **Communications** have to be done at national and international level by all involved partners (i.e. curators, coordinator ...) to promote the work done in collaboration at European level and by this way, try to persuade national coordinator and government of legitimacy of a European collection and a **better integration of AEGIS activities in National activities**.
- Human resources for different positions and works

c. In kind contribution of national government

d. Information management/ Database management

An improvement system (EURISCO, CDB center, National) which could be web enabled.

e. Knowledge transfer

All Avena collection holder institute do not have expert and this explains some lack or mistake in taxonomy and/or characterization and evaluation descriptors. It would be helpful to have a taxonomical key and perhaps exchange of experience in the use of IPGRI/Bioversity descriptors list.

**6. Proposal on the involvement of all the relevant stakeholders of the European Region in establishing and operating the European Collection for Avena (including on services to be provided; rationalization aspects; coordination; etc.) (Germeier *et al.*, 2006)**

Gene banks curators, Avena database manager and breeders within or out the working group might be involved to elaborate the MAA list and/or participate to further characterization of AEGIS accessions.

A financial support of EU by the way of scientific European program will be necessary to obtain common and high quality descriptors and standards.

A strong commitment of the national coordinators is expected to insure a co-ordinate participation of institution at national level.

During the meeting in Roma, the Avena group had suggested additional centralized elements as:

- A **co-ordinating European institution** for each crop i.e. a co-ordination European institution for Avena genetic resources.
- A central (European) **safety duplicate store**.

**7. Proposed “general workplan”, whenever possible costed, for the model crop Avena Working Group activities**

A. Monitoring all QMS procedures applied in each genebanks for the listed management targets in the EADB

Different methodology occurs among Avena collection holders. An inventory has to be done to go to a standardization of the procedures. Each step of the genetic resource conservation is affected: collection, viability test, conservation,

multiplication and regeneration, characterisation and evaluation including molecular marker analysis, distribution and safety duplication. Especially methodology for germinability testing and seed rejuvenation would be interesting also for users of genetic resources.

*Avena* collection curators are expected to do the reporting of their current methodology, by this way, it could be the first step of the QMS process – Say what you do.

A synthesis of all methodologies will enable to highlight lacks or problems in performing the QMS procedures.

#### B. Role of the *Avena* working group

The working group will give an expertise of the synthesis and propose some revisions of standards to adapt them to *Avena* species. Experts could also give technical and management advices in several points necessary to determine the MAA list such as:

- **Taxonomic standards**

*Avena* cultivated species include many inter-specific forms or botanical varieties. Among collection holders, there are different levels of curator's knowledge in inter-specific taxonomy and so taxonomic characterization is various between genebanks. To be able to make a comparison at national and international level to determine the MAA list, the curators need to proceed at a validation or a correction of inter-specific and specific taxonomy.

- **Characterization and evaluation standards**

To allow the functioning of the system and sharing tasks, curators need to know that they will find descriptors they need of in AEGIS system. A list of obligatory and additional standards has to be defined.

- **Specific standards for wild species**

As no specific information for wild species is available about different criteria such as effective population size, technical standards, curators need guidelines to prevent the loss of genetic resource especially for this material who demand special care.

- **Methodological procedures of multiplication/Regeneration of MAAs**

It would be helpful for curators that the working group in collaboration with *Avena* expert writes guidelines for an optimal multiplication or regeneration of wild species.

In the future, curators in charge of MAA will have the responsibility to distribute accession to all partners. Like a minimum cycle of multiplication or regeneration is preferable to guaranty the genetic integrity, an estimation of the minimum amount of seed available has to be defined.

- **Distribution of MAAs accession**

To obtain a standardization of distribution procedures, three information have to be common to all collection holder: the maximal amount of seed distribute per request, which would be the lowest level of germinability acceptable and for which species and, which kind of minimal information has to be provided with the accession samples.

In parallel, the working group has to define precisely and by importance order the criteria for selection of the MAA in case of duplicate samples.

### C. Completion of passport information by curators

The first step will be to specify exactly on which passport data curators should base their selection of MAA.

In the AEGIS Final Report of Avena subgroup (Germeier et al., 2006), some descriptors out of the Multicrop Passport data have been identified, which need to be available for a search of duplicates in a strict sense:

- **Collecting information**  
Collecting number, collecting date, collecting site.
- **Breeding information**  
Breeding stock number, release year, breeder institution, pedigree.
- **Donation information**  
Donor institution, donor number, acquisition date.
- **Botanical identification**

This information needs to be completed and delivered to the CCDB for all accessions to be considered for becoming AEGIS accessions. This could imply newly entering the information from old catalogues or collecting documents into a computerised system, reformatting and transliterating it for delivering it to the European *Avena* Database.

### D. Provide a list of potential list of MAA accessions

**At national level**, curators establish the list of potential MAAs available in their genebanks collection and try to identify duplicate (see point 3. establishing the list of MAAs – step 1).

**At European level**, database manager in relation with curators search for duplicates. The procedures currently available based on a VBA application operating within MS Access on an Oracle database connected via ODBC allows passport data update and duplicate search for about 150-170 accessions per day.

### E. Selection of optimal list of MAAs

CCDB manager in cooperation with the WG will create an optimal list of MAA for the European collection with name of most appropriate holder for each MAA. After approval by the WG, the list will be submitted to the national coordinator (see point 3. establishing the list of MAAs – step 3 to 6).

### F. Definition of the coordinator Institute (Germeier et al., 2006)

The WG in collaboration with the ECPGR steering committee validates the idea of co-ordinating European institute, inventory the criteria this institute should fulfil and define precisely those tasks.

### G. Definition of the centrally stored safety duplicates (Germeier et al., 2006)

The aegis avena group supports a similar idea for centrally storing safety duplicates. It recommends that one or two specialised European facilities should be created for storing safety duplicates.

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