Notes about the selection process of the European garlic accessions for the European Collection

First of all, we should state that garlic is a clonal crop, and therefore, some features of the accession selection are specific for such types of germplasm, and they cannot simply be transferred to outbreeding populations (landraces) of seed-propagated material, seed-based varieties of other germplasm of that type. However, they could be an example for other clonal crops such as fruit trees, potato etc.

In IPK, the selection procedure has already a longer history. Several steps were undertaken and different selection processes passed, which is, for clarity, presented in the attached graph. The most interesting material came into focus in course of the first GenRes Project funded by the European Community from 1996 until 2000 in the framework of the Council Regulation No 1467/94. In course of this project characterization and virus elimination of garlic started in IPK.
Already at that time it became clear that we would not be able to treat all the accessions of our large collection (approximately 500 accessions) with the same priority, and therefore we followed the core collection concept on the basis of the knowledge we had at that time. We decided to take the country representation as the main parameter and took the accessions proportional to the numbers of representatives per country, i.e. the more accessions were present from a respective donor or country the more accessions were included into the preliminary core collection. The second parameter was the morphological diversity. Parallel
to these measures, there was a molecular analysis running in the Taxonomy Research Group based on isoenzyme and RAPD markers. When both processes were completed, we compared the results and found that there was a good correlation between the groups selected on the base of morphological characters and those circumscribed by the molecular marker grouping.

On the base of these preliminary selection activities, we attained a set of well-characterized 113 accessions. Since, we took part in the development of the IPGRI Allium minimum descriptors the description of these accessions was done according to the 16 most important minimum descriptors of that list. At the same time, 95 of these accessions were successfully cleaned of viruses by meristem culture, and the subsequent stepwise process of introduction of this material into cryopreservation started. We also started image documentation of this material.

In this GenRes project, we had already good collaboration with some other collections, namely the Spanish, Czech and Polish collections. Most intensive exchange of germplasm was implemented with the Spanish collection. Thus, we formulated a preliminary European Core Collection consisting of 25 Spanish ("Southern") and 25 German ("Northern") accessions. The 25 “Northern” accessions were the result of a further selection process from the previously mentioned 113 accessions. The Spanish genebank followed analogous procedures.

After finalization of the first GenRes project, mainly cryopreservation was continued and the Garlic Core Collection database was established in IPK containing passport, characterization data, the molecular grouping and images.

The main further progress was facilitated through the preparation and implementation of the EURALLIVEG project (2006-2011). In preparation for the project, all the IPK accessions were listed and categorized with respect to their origin, as mentioned below, mainly to define the duplicates and to separate accessions suitable for the project from those that should not be included.

In IPK the accessions were successively selected as follows:
Accepted material from the approximately 500 accessions maintained by IPK was:
a. only accessions, which were collected until 1993 or were collected in Germany to meet the conditions set by the CBD process in order not to complicate the project,
b. material, which has been part of the first project collection covering 113 accessions.

From the selected material using criteria a and b above the following material was excluded:
1. Clearly declared safety duplicates from other gene banks, in order not to create overlap with the other genebanks, which followed their own selection strategy (122 accessions).
2. Accessions with a clear indication (passport data) that they originated from another gene bank (without official 'Safety Duplicate' mark - 35 accessions)
3. Accessions from USA, mainly as donations for research purposes (32 accessions).
5. **Accessions from outside Eurasia**, mainly brought from other institutions during visits (9 accessions)

6. Real **internal duplicates** (7 accessions).

**Note:** It is not possible to summarise these exclusion figures, because in some cases there were two reasons for exclusion (e.g. all safety-duplicated material came to IPK after 1993, thus the original collection holder will have be asked for accepting such accessions as European Accessions).

Thus, a number of 196 accessions resulted from this multiple exclusion process.

In the EURALLIVEG start-up meeting it was agreed that the other participating collection holders followed a similar selection approach.

Material which successfully passed the above described selection process was subsequently used for the defined cryopreservation treatment. In course of the project activities, some further accessions (in the case of IPK about 12 %) were excluded which only weakly responded to the cryopreservation treatment. Altogether **200 accessions** from the participating countries formed then the **EURALLIVEG collection** completely stored in cryopreservation, including the mutual safety duplications.

The AEGIS Coordinator attended the first meeting of EURALLIVEG and presented the **selection criteria for MAA** (“Most Appropriate Accessions” used to be selected from sets of duplicate accessions, sometimes wrongly used as a synonym for “European Accessions”). During the AEGIS Model Crops Meeting on July 1-4, 2008, in Radzikow, Poland, the proposed MAA criteria were commented and specified for *Allium* as follows:

**ALLIUM: Primary Criteria (later called Selection Requirements)**

1. *In the public domain* (i.e. Annex I material that is in the Multi-lateral System and non-Annex I material designated to AEGIS by governments or any other holder)
   - *Allium* is not an Annex-1 species; nevertheless EURALLIVEG will use SMTA for material movement between the partners.

2. *Genetically unique* (i.e. genetically distinct accessions; assessment based on available data and/or on the recorded history of the accession) and agronomically (including research material) and/or historically/culturally important
   - For clonal crops, such as garlic, uniqueness is a key criterion. There is also agreement with the relevance of agronomic and/or historic/cultural importance.

3. *Plant Genetic Resources, including medicinal and ornamental species, and crop wild relatives* (i.e. excluding forest genetic resources; non-plant agro-biodiversity species, etc.)
   - For all AEGIS model crops including garlic, there is agreement with the relevance of this criterion.

4. *European origin or introduced germplasm that is of actual or potential (breeding/research) importance to Europe*
   - Garlic originated in Central Asia, but is of significant importance to European agriculture, commerce and health.
**ALLIUM: Secondary Criteria**

1. **Maintained in “country of origin”**
   - Garlic originated in Central Asia. On the accession level no selection pressure is caused in vegetative maintenance. Therefore, the country of origin is not critical.

2. **A known origin (collected and/or bred; pedigree data?)**
   - Having a known origin is generally a useful characteristic because origin is an indicator of certain characteristics (e.g. daylength requirements). In some circumstances material with no passport data is maintained because of special characteristics making it of high importance.

3. **Comprehensiveness of passport information**
   - Passport data are very useful.

4. **Number of regeneration/multiplication cycles**
   - Garlic has to be grown and replanted in some year-periods, mostly annually, thus is criterion is of no relevance.

5. **Health status (i.e. is the germplasm disease free?)**
   - This criterion is imperative for vegetative material.

6. **Existence of morphological/molecular characterization data**
   - In the context of vegetative material and the objectives of the AEGIS Allium group, the use of morphological and molecular characterisation is essential.

7. **Existence of (agronomical) evaluation data**
   - This is useful but not essential.

8. **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)**
   - The validation of accession name is of limited use for garlic and this criterion turned thus out to be of little use to garlic.

In 2009, the ECPGR Steering Committee discussed the Selection Requirements in detail and decided on the following wording:

a. Material under the management and control of the member countries and their Associate Members, in the public domain and offered by the associate members for inclusion into AEGIS.

b. Genetically unique within AEGIS, to the best available knowledge (i.e. genetically distinct accessions; assessment based on available data and/or on the recorded history of the accession).

c. Plant genetic resources for food and agriculture as defined in the International Treaty as well as medicinal and ornamental species.

d. European origin or introduced germplasm that is of actual or potential importance to Europe (for breeding, research, education or for historical and cultural reasons).

The *Allium* WG has subsequently adopted these Selection Requirements, which was also in accordance with the EURALLIVEG project implementation.

Since IPK excluded all known duplicates from the management, it was avoided that some of these duplicate accessions entered the Core collection.

In course of the EURALLIVEG project (AGRIGENRES 050 of European the Council Regulation (EC) N° 870/2004) the intention failed to fingerprint all accessions of the
participating countries (~ 1600) by SNP markers. Thus, we could not use these markers for circumscribing the candidates for the European Accessions (which we termed as “EURALLIVEG Collection”). The most appropriate way was to concentrate on all those accessions which were included into the cryopreservation set according to the above-mentioned procedure. We needed to confine the morphological description to these 200 accessions. In order to exclude total duplicates, we performed an AFLP analysis covering 72.5 % of the 200 EURALLIVEG accessions. At least in these accessions, the AFLP tree showed that there was not a total congruence of any of the accessions.

Most important is that the 200 accessions of the EURALLIVEG Core collection including the already finally flagged accessions are safety duplicated, whereby the three participating institutions distributed the germplasm so that each partner received material from one of the other two, a rather random process as far as the accessions were concerned. This safety collection is completely present in cryopreservation. Additionally to that each country has its own collection part in the field.

The 61 first European garlic accessions are the German component of the EURALLIVEG Collection.

Since Joachim Keller was the EURALLIVEG project coordinator and since last year he became the Chairman of the Allium Working Group, the flagging procedure was simplified for Germany. Joachim sent the accession list to our National Coordinator and he forwarded it to the EURISCO focal point.

After this initial step of including the first garlic accessions in the European Collection, further follow-up is expected. The second partner, Czech Republic, also flagged already its 82 garlic accessions. They also stem from the EURALLIVEG project. It is expected that Poland will be do the same in due time. Furthermore, the EURALLIVEG catalogue includes a list of more candidate garlic accessions which will be considered later this year, after they will have been introduced into cryopreservation and having been safety-duplication.

Another action will be undertaken with shallots, which were also included in the EURALLIVEG project as field genebank accessions, but without cryopreservation. For these accessions a strategy needs to be agreed upon how to safety-duplicate the accessions that are maintained as field cultures. After having resolved and implemented this, these accessions can also be included in the European Collection and flagged accordingly.

What has been documented here is a process of how accessions of a particular crop maintained by a number of genebanks have been selected, treated and finally included in the European Collection. Having (even simplified) selection procedures proposed by AEGIS, this account demonstrates that the selection process is actually more complicated than the theory is, since all activities have to be done with concrete material considering the specificity of each single accession and the routine procedures applied by the collaborating genebanks. However, we do not forget the general intentions we have in mind when we think about our
great goal to preserve the valuable European germplasm for future generations in an efficient and effective manner.

**Summary:**

The main aspects of the process to flag accessions as European Accessions can be summarized as follows:

- characterization of the material as perfect as possible,
- selection of a representative core set with which further work would be manageable (criteria in case of IPK: geographical representation, highest morphological and molecular diversity),
- use of the selection requirements (i.e. availability; genetic and agronomic/historic uniqueness; PGRFA as per the political definition; the geographic origin),
- assessment of the material using the appropriate selection (MAA) criteria to choose the best accession among duplicates,
- collaboration with other collections/genebanks from the beginning, including the long-term storage in cryopreserved conditions,
- safety-duplication of the material with partners that you trust because of this collaborative experience.