Creation of a Common Maize Data Base for DUS studies through a partnership between Spain, Germany, France and the Community Plant Variety Office

Project 2003 for the Community Plant Variety Office (CPVO)
Research and Development Section

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Summary

Hundreds of new plant varieties are released each year by breeding companies. Among the conditions which have to be fulfilled to get a plant breeders right, a variety must be clearly distinct, uniform and stable (DUS). To assess these parameters, the new variety is compared to reference collections representing the variability of the cultivars already registered in national and/or European catalogs. With the development of the European Plant Variety Right System covering 25 countries, it becomes more and more relevant to develop procedures which can improve the capacity to compare each new variety to an optimized set of varieties of common knowledge and so to safeguard the quality of the protection.

In case of maize inbred lines, CPVO currently requests technical examination to France, Germany and Spain. Each of these examination centers has established a reference collection of inbred lines with 3 000 entries in France, 600 in Germany and 2 000 in Spain.

Due to the different agro-climatic conditions and the variety adaptation, these collections are not identical but a lot of entries are in common. Each country manages its own reference collection databases to run its fields trials, which generates a lot of redundancies in the DUS tests. To minimize these redundancies, reduce the functional costs and make the exchanges of seeds between countries easier, it seemed pertinent to set a frame for the examination centers to establish a partnership and share data.

The present project aimed at establishing a common maize database for DUS studies through a partnership between Spain, Germany, France and the Community Plant Variety Office. This database contains harmonised morphological and biochemical descriptions according to the CPVO technical protocol from 4471 maize lines, among which are 741 from Germany, 506 from Spain and 2395 from France (data in 2005). It will be updated regularly and will be available for electronic consultation for each partner and CPOV.

On a medium term, each partner could thus be in charge of maintaining physically at his premises only the seeds of varieties corresponding to its climatic conditions and not conserved in the other examination centres.

The created common database constitutes a model which could be extended to other species and other examination centres.

Work done

Since its start, the program unfolded through the collaborative work of all partners. Productive interactions were established between experts from all the knowledge areas involved (field, computer, biochemistry, regulation…). E-mail was used to forward documents and files, as well as to exchange on general ideas or details. Meetings on site, internet chats and conference calls were used as milestones to validate the progress done, and allowed frequent discussions between experts. During the second year, 4 meetings were organized:

- Jan 31 to Feb 01, 2005: third international meeting in Madrid, Spain [Appendix 1]
- April 29, 2005: check point on the programme via a conference call [Appendix 2]
- Oct. 13, 2005 : chat on the internet on isozyme issues [Appendix 3]
- Oct. 20, 2005: fourth and final international meeting in Angers, France [Appendix 4]

Detailed reports on all these events were written and circulated among partners. They are presented in appendix at the end of this document. In the following, we expose only a summary of the work performed during the program with reference to these reports (mentioned in brackets).

1) Harmonization of the agronomical data [1, 2, 4]

To meet the objectives of the programme, the crop experts had to, first, select a common set of example varieties, second, harmonize the testing protocols and, third, identify the lines common to several countries in order to establish the list of distinct lines to be put in the common database.

The **selection of the example varieties** that would be used by all three countries was considered as the most important task of the crop experts. It was a prerequisite to the comparison and the harmonization of the testing protocols.
Two ring tests were needed to come to an agreement regarding the number and the nature of the example varieties needed to share the same references for most characteristics and expression levels. In the future, the work will be continued to identify example varieties allowing even more communality on even more characteristics and the list of example varieties will have to be updated regularly.

Regarding the **harmonization of the testing protocols**, the agreement was easy to achieve. Experts compared their methods and came to an agreement to use the same protocol, evaluating the same criteria at the same growth stages.
Crop experts also evaluated the impact of the agro-climatic conditions on the notations by testing common inbred lines grown in Hassloch (Germany), La Minière (France) and Seville (Spain). They transformed measurements into notes and adapted their scales to obtain the same notes on the same lines. The scales are now different between countries but the notes are the same and allow all three countries to use reliably each other's data. This system of "corrected scales for comparable notes" was very useful to homogenize the evaluation of some quantitative characteristics.
This achievement demonstrates that an agreement between a small set of partners, able to use the same set of example varieties, can be reached. (It would probably not be technically doable with a larger set of countries.)
During the second year of the project, crop experts also studied new characteristics, to propose them, if useful, for their inclusion in the next UPOV and CPVO guideline. Among them, the anthocyanin coloration of the stem and the intensity of green colour of the leaves were considered as potentially useful.

The **identification of common lines** and the listing of the distinct lines present in the three countries have been performed.

6285 inbred lines were initially registered in Table T01 of the database
with 1142 inbred lines from Germany,
1931 inbred lines from Spain,
3212 inbred lines from France.

After the identification of the common lines, **4471 distinct inbred lines** were identified
with 741 inbred lines from Germany,
506 inbred lines from Spain,
2395 inbred lines from France.

Among the common lines identified by the experts, 760 were common to two countries and 69 were common to the three countries.

The description of all these inbred lines have been put in the common database which now provides an almost exhaustive representation of the variability of maize in Europe and a good representation of the variability of maize in the world.

In conclusion, the harmonization of the technical protocol has been achieved and common lines descriptions are now available for all three countries. Crop experts also have a better sense of which characteristics are more reliable or more susceptible to change under different ecological conditions.

2) Harmonization of the electrophoresis data [2, 3, 4]

A ring test on a selection of maize cultivars was performed between the 3 institutes. Isozyme experts exchanged pictures of their gels to compare their conclusions and had several discussions via emails. Eventually, a chat on the internet was organised between them to come to a final agreement.

Before the chat, Germany had proposed a table presenting an interpretation of all the isoenzyme systems used to describe maize inbred lines and hybrids. It was discussed during the chat. After the chat, the interpretation of the systems for inbred lines had to go through some minor changes to comply with the French and Spanish views and an agreement was reached (see Table 1 in Appendix 4). No full agreement could be reached for the hybrids. To come to a conclusion in the delay, all partners decided to focus only on the specific needs of the database (which does not contain any hybrids) and to continue the work in this area after the end of the programme.

To comply with the actual requirements of the UPOV and CPVO guidelines, the common database contains descriptions of isoenzyme characteristics expressed only through notes. If the regulation evolves this way and if the partners could come to an agreement, the database could be adapted to include descriptions of the genotype of the lines. On the long term, a description of the genotypes would also be needed if the regulation eventually included molecular aspects.

3) Design of the common database [1, 2, 4]

a) Creation of the structure and feeding

In close collaboration, the partners created a database model, selected the data that it would contain and wrote a common data dictionary. The model was implemented in software and fed with the appropriate data (Figure 1).

The protocol used in the common database was defined following exactly the database model and the data structure used by CPVO.

The common database will be hosted individually at each partner’s premises and will use Access97 as an exchange tool between countries. Each country will keep its own database and the common database will contain only a compilation of data extracted from the three national databases. This way, the national databases and the common database will be totally disconnected. The data will regularly be put on a CD-rom that will be circulated among partners after each update.

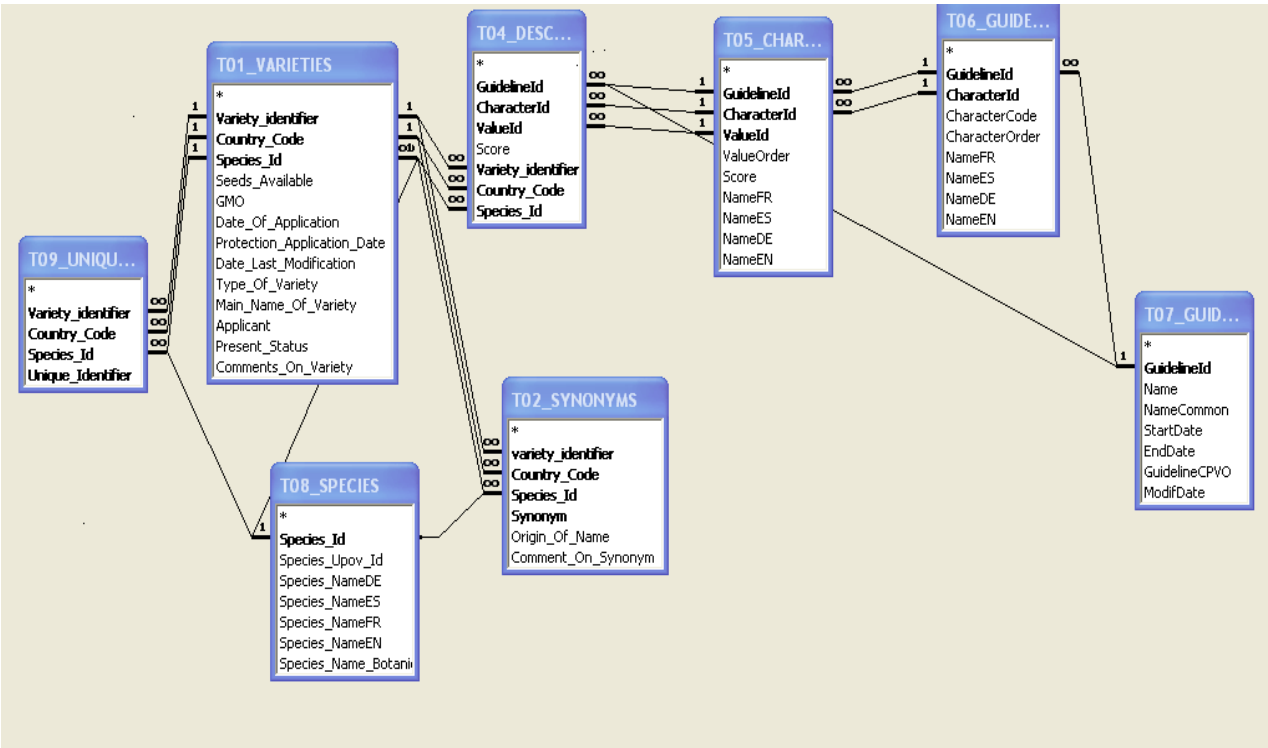

Figure 1: Structure of the SFG maize common database.

A unique identifier was given to each distinct inbred line. This identifier is neither the breeder's reference, nor the countries' identifier. For each line, each country kept its own identifier and a specific unique identifier was attributed for the database (see Table T09 of Figure 1).

A document describing the database and user's instructions will be written, in collaboration with all partners, by the end of 2006.

b) Updating of the common database

At each update, partners will extract the totality of their data and a new database will entirely be re-created. The former database will be stored. To identify the changes in the common database, partners will just have to make a comparison by request between the former and the new databases.

The common database will contain the date of its "creation", meaning the date of the last updating.

The updating will be done according to the yearly procedure described in Table 1. The updating will be performed in three phases that will generate each year, three "versions" of the common database: versions A, B and C. For that matter, crop experts will communicate by phone or chat as much as needed.

In order to maintain a good level of collaboration, at least one meeting per year by phone/chat or in person will be organised by France at the beginning of November between crop experts of the three countries. A short report for each of these meetings will be written and sent to all partners including

CPVO, as well as for each change decided along the year in the structure of the base or in the data definition, to keep track of the modifications.

Table 1. Yearly DB updating procedure
(3 phases of updating per year generating 3 database "versions" (SFG DB v. A, B and C) each year)

Database version	Deadline	Update activity
	28/02/year *(31/03 for 2005)*	Definition of a unique identifier agreed between crop experts for each new line.
SFG DB v.A	05/03/year *(05/04 for 2005)*	Distribution of updated T09
	01/06/year	Sending of update of status and addition of new lines (status = NEW) to coordinator
SFG DB v.B	10/06/year	Distribution of new database by coordinator for B
	15/12/year	Sending of new descriptions to coordinator for C
SFG DB v.C	20/12/year	Distribution of new database by coordinator for C

A whole cycle has been performed in 2005 by France (SFG DB v.A) and Germany (SFG DBv.B and v.C).

The three countries will share the responsibility of the coordination of the different phases of the updating procedure. A planning was defined as described in Table 2.

Table 2. Planning for coordinating countries
(3 phases of updating per year generating 3 database "versions" (SFG DB v. A, B and C) each year)

Year	**SFG DB v.A** (05/03/year)	**SFG DB v.B** (10/06/year)	**SFG DB v.C** (20/12/year)
2004	-	-	SPAIN
2005	FRANCE	GERMANY	GERMANY
2006	SPAIN	FRANCE	FRANCE
2007	GERMANY	SPAIN	SPAIN
2008	FRANCE	GERMANY	GERMANY
etc…	**…**	**…**	**…**

Conclusion and perspectives

During the programme, all partners worked efficiently and in a very cooperative and open way to build the SFG maize common database that is now operational.

This new tool should help the testing offices to manage :

a) the improvement of the quality of the reference collections;

b) the improvement of the quality of DUS testing for national and European listing. The database indeed broadens the range of available reference varieties and allows the exchange of data between countries for the identification of the candidate lines in test after the first year of observation;

c) the improvement of the efficiency of the maintenance of the reference collections: each country will not have to physically keep the totality of the reference collection but only the lines not already present in the other countries.

The database is already being used and fulfils all the requirements of the partners of the programme. It is very useful to chasse the right inbred lines, know the right characteristics to be used for foreign inbred lines, and know the characteristics that are strongly impacted by environmental conditions. Moreover, it emphasises the European dimension of the DUS experts' activities.

The implementation of the common database and its integration in the testing systems of the partners still requires some efforts. Its usefulness will be fully measurable in two to three years. At term, the common database should allow lowering the cost of DUS testing and will strengthen the protection.

It remains now to be seen if the database could be extended to maize hybrids in the future.

The creation of this database was a very good start for the efforts of harmonisation that should be done in the following years. It was in particular a very good opportunity to start to harmonise the isoenzyme characteristics which are, for maize, the first way to group the varieties. In that perspective, experts will continue to work together to finalize a harmonised system between countries, for inbred lines but also for hybrids.

The SFG maize common database has now started its life and will be updated regularly by all three countries who will share the maintenance activities each year from now on.

In case of any evolution of the UPOV and CPVO guidelines, the database could be adapted to new characteristics (which would nevertheless trigger a significant amount of work).

The model of the database could also be used for other species and potentially other kinds of data (for example using. molecular markers). A programme supported by the CPVO on winter oil seed rape and initiated in 2005 is presently exploring these possibilities.