PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Final report

A EUROPEAN REFERENCE COLLECTION OF ROSE VARIETIES

Plant Research International B.V., Wageningen
October 2006

Institution responsible of the project: Plant Research International
P.O.Box 16
6700 AA Wageningen
The Netherlands

Other institutes involved: NIAB
Huntingdon Road
Cambridge CB3 0LE
United Kingdom

Bundessortenamt
Osterfelddamm 80
30627 Hannover,
Germany

Co-ordinator and responsible scientist: Dr. Ben Vosman (PRI)

Major other contributors: Joost Barendrecht (Dutch Plant Variety Board), Danny Esselink (PRI), Huw Jones (NIAB), Elizabeth Scott (NIAB), Burkhard Spellerberg (BSA), Swenja Tams (BSA).

Funding for this project was provided by the Community Plant Variety Office, Ministry of Agriculture, Nature and Food quality of the Netherlands, DEFRA (UK) and the Bundessortenamt, Germany.

Plant Research International B.V.

Address : Droevendaalsesteeg 1, Wageningen, The Netherlands
: P.O. Box 16, 6700 AA Wageningen, The Netherlands
Tel. : +31 317 47 70 00
Fax : +31 317 41 80 94
E-mail : info.pri@wur.nl
Internet : www.pri.wur.nl

Table of contents

Executive Summary

An integrated pilot database was constructed containing administrative, morphological and molecular data as well as pictures of each variety.
All data of one variety can be shown in one screen, and the items included in the database have been evaluated by the experts of BSA, NIAB and the Dutch Plant Variety Board.
The selected morphological characteristics are all useful for selecting reference varieties, although the degree of usefullness varies according to the type of rose - for example in greenhouse cut flower roses, most varieties currently fall into the same flower and plant growth type.
The pictures taken from each variety are also considered important, although the composite photo was considered less informative for the cut flower greenhouse roses, where in comparison to the garden roses there is a more limited variation in the characteristics photographed as far as the non floral parts are concerned.
Microsatellite markers have been used to construct a database containing the molecular profiles of approx. 380 varieties. The markers proved to be very informative about the varieties. As far as we are able to tell, seedling varieties can be distinguished from each other on the basis of DNA profiles, and mutant varieties and mutant groups showed identical patterns. However, there were 2 cases in the garden roses were two varieties were suspected to be mutants of each other based on the DNA analysis, but this has not confirmed by TQ information. Further investigations are needed to shed some light on these cases. In greenhouse roses similar situations were encountered but there the mutant nature could be confirmed afterwards.
From this it is clear that the database can support and even improve the quality of DUS testing and the quality of protection.
A problem was encountered when we tried to merge data gathered in two different laboratories into one database. Differences in allele calling were observed. These problems were most likely caused by a lower quality of the DNA samples obtained from the garden roses. In spite of these difficulties we have demonstrated that two laboratories can produce substantially equivalent data and that the molecular data produced is useful as a tool for managing reference collections, prescreening and quality assurance.

Introduction

Rose is the largest ornamental crop and the most important one in many countries. Over 25,000 varieties of modern roses have been described (Cairns, 2000). The first hybrid tea rose was introduced in 1867 and since then more than 10,000 hybrid teas have entered the market. It is difficult to know how many of these varieties are still in existence, but the Plantscope database, although not yet complete, lists nearly 3,000 varieties available just in the Dutch trade, and the Combined Rose List 2002 (Peter Schneider), which aims to list all varieties actually in commerce world-wide, contains some 13,000 varieties. Such large numbers of varieties cause problems in the DUS testing context. A major issue for all countries carrying out DUS tests is the requirement to compare new varieties with an increasing number of existing reference varieties. Article 7 of the 1991 UPOV Convention says that a variety shall be considered Distinct “if it is clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of the filing of the application”. Common knowledge is broadly defined elsewhere (e.g. the recently revised General Introduction to the Test Guidelines, TG/1/3, UPOV 2002) to include all known varieties, i.e. any variety entered into or subject to an application for PBR, varieties grown commercially, varieties held in publicly accessible reference collections, or of which there is a published description. Clearly, strict adherence to this concept is logistically and financially very difficult in a species such as rose, which is cultivated around the world, although the number of varieties to be considered can be limited by climatic factors, variety type and the continued availability of material. Nevertheless, this still means that many hundreds of existing varieties should be taken into account and the number is increasing all the time, which constantly adds to the costs of testing. In order to reduce these increasing costs and to improve the exchange of information about varieties, a way of managing information about the large number of reference varieties, and of selecting most similar varieties for inclusion in the growing trials is necessary. If at the same time access to shared information about existing varieties could be improved, this would improve the efficiency of the PBR testing system. At the moment rose DUS testing on behalf of the CPVO is carried out by UK (outdoor garden types), Germany (outdoor types and pot roses) and the Netherlands (greenhouse, cut flower types). Applicants complete a technical questionnaire, which is submitted to the examination office together with a photograph of the candidate variety. Candidate varieties must be evaluated against all the relevant possibly similar existing varieties in common knowledge, and also against other contemporary candidates undergoing CPVO and national trials. Thus, a fast and systematic approach for the selection of varieties essential for direct comparison in the growing test is clearly needed to ensure robust results. This selection is currently undertaken in various ways, for example by comparison with an existing, dedicated reference collection, comparison with the collections in public rosariums, literature searches, searches in databases of descriptions and photographs, use of the expertise of the examiners, and the use of outside experts ("walking reference collections"). Having made the selection of similar varieties for a growing trial, these then need to be sourced. The examination office for greenhouse roses does not currently hold a living reference collection – mainly because of the high costs associated with maintaining such a collection, and disease problems. Therefore, the examination office needs to request reference varieties from the breeders. It is important that the examination office can quickly verify the identity of the material submitted. For this aspect of quality assurance, molecular markers are ideally suited, as they are highly discriminating and can be assayed rapidly and relatively cheaply.

Several molecular marker systems have been applied to roses. These include RFLP (Rajapakse et al. 1992, Ballar et al. 1995), fingerprint analysis with mini- and microsatellite probes (Ben-Meir and Vainstein 1994, Vainstein and Ben-Meir 1994), RAPDs (Torres et al. 1993, Cubero et al. 1996, Martin et al. 2001) and AFLP (Huylenbroeck et al 2005; Vosman et al 2004; Yan et al 2005). All these marker systems have some drawbacks for variety identification and related activities. In some, there is a lack of high levels of polymorphism, whilst other methods are difficult to reproduce, laborious and/or produce complex patterns inconvenient for database building (Vosman 1998). In contrast, DNA microsatellites (simple sequence repeats, SSRs) are highly polymorphic and have the advantage of providing a co-dominant marker system based on the PCR technology. When analysed as sequenced-tagged microsatellite site (STMS) markers, they provide simple banding patterns that are easy to record and are especially suitable for automated and objective analysis. In addition, the resultant data can be readily stored in a database. New varieties or new markers can be easily added to an existing database. The application of the STMS approach was recently successfully demonstrated in roses (Esselink et al. 2003; Nybom et al 2004; Esselink et al 2004; Rusanov et al 2005; Smulders et al 2005).

This project was aimed to produce a pilot database of rose varieties that would be available to the CPVO testing stations. The database contains not only the molecular profiles of varieties, but also photographs and information on the most important morphological characteristics.

Because the database covers different types of rose tested for PBR and the same set of molecular markers will be used for all varieties, an additional benefit will be the ability to easily cross-check applications for cut-flower glasshouse varieties against outdoor garden varieties, in those cases when varieties are mutations. Currently there is no quick way to do this, so the first part of the distinctness assessment rests on the declaration of the breeder as to the use of the variety - a situation that cannot be checked by the testing station until the first growing trial. After this, further comparative tests may be necessary. Furthermore, it enables easier exchange of information between testing stations about contemporary varieties in test, both for CPVO and national rights. At the moment this is based on morphological data and where necessary photographs, but both are prone to environmental influence and hence the information requires careful interpretation by the examiners.

This database will improve the quality assurance role outlined above and expand the data available on the range of varieties taken into consideration in each country, thus reducing the reliance on the individual expertise of "walking reference collections" and effectively improving the management of the reference collections. In addition, as the database is constructed on the basis of molecular profiles produced on the material submitted for granting PBR, the breeders will have a very effective tool (identification label) for tracing potential infringements - the database can be used to assist in the quicker technical verification of varieties after grant of rights. All of this will lead to better possibilities for enforcing PBR and technical verification.

Objectives addressed

The objective of the project was to create a unified pilot database of the most important rose varieties within the EU member states, which can be used in place of permanent living reference collections and as a way of verifying the identity of reference varieties supplied for DUS testing.

The project consisted of two phases

1. Construction of an integrated pilot database containing:
    - Key morphological descriptors (based on the CPVO protocol for roses)
    - One or more photographs of the variety in a standardized format
    - Molecular profile (based on DNA microsatellites)

2. Evaluation of the database for:
    - Characterization and cataloguing of the reference collection
    - Pre-screening and selection of appropriate reference varieties
    - Exchange of data on current candidate varieties between testing stations
    - To strongly reduce or replace permanent living reference collections at testing
    - Quality assurance within examination offices (verification of identity/authenticity)

Construction of the database

For the construction of an integrated pilot database several choices needed to be made. These concerned morphological characters to be included, markers and pictures to be used and the database structure and format. All these issues are discussed below.

1. Selection of morphological descriptors to be included in the database

During the first two project meetings we selected morphological descriptors from CPVO/TQ-EN-011 to be included in the database. Selection was determined by the robustness of the descriptor, as well as its usefulness for selecting varieties for comparison. It is important that these characters are part of the Technical Guideline (TG) and the Technical Questionnaire (TQ), which will ensure that all descriptors used are scored by the DUS stations and that breeders use them for describing their candidate variety.

The following set of descriptors was selected:

4.1 Origin
5.2 Flower: Type
5.3 Flower: diameter
5.4 Flower colour group
5.5. Plant Growth Type
7.2.1. Special conditions: Group

It was concluded that the characters 5.4 and 5.5. should/will be included in the new TG.

2. Selection of molecular markers

During the first year of the project PRI and NIAB genotyped a selected set of 23 garden rose varieties using 24 STMS markers. From these markers a subset was selected for database building. Criteria used for selection included 1) level of polymorphism, 2) robustness, 3) ease of scoring and as far as possible 4) distribution over the genome. Details on the selected markers can be found in table1. The markers RhP50, RhP518 and RhAB73 proved to be useful for garden roses but do not fit the selection criteria for glasshouse roses. Instead the markers RhM405, RhAB15 and RhO507 are included in the core set for genotyping glasshouse roses. Protocols used for marker analysis can be found in appendix 1.

Table 1: Characteristics of the selected set of microsatellite markers for genotyping garden and glasshouse roses. Selected for indicates whether the marker is selected for use in garden roses (gr), glasshouse roses (ht) or both (gr/ht).

STMS	linkage group	No. of alleles in 23 varieties	No. of allele phenotypes	Selected for	Scoring quality
RhO517	1	5	14	gr/ht	1
RhEO506	2	12	19	gr/ht	1
RhD221	4	8	12	gr/ht	1
RhE2b	6	7	12	gr/ht	1
RhB303	unknown	6	14	gr/ht	1
RhP519	unknown	7	15	gr/ht	1
RhAB40	4	11	18	gr/ht	1
RhD201	unknown	7	10	gr/ht	1
RhAB22	6	12	15	gr/ht	1
RhP50	3	11	13	Gr	1
RhP518	5	7	15	Gr	1
RhAB73	7	9	18	Gr	1
RhM405	unknown	5	13	Ht	1
RhAB15	2	10	5	Ht	1
RhO507	4	14	18	Ht	1

Figure 1: Dendrogram obtained for the 23 varieties using the 12 selected markers.

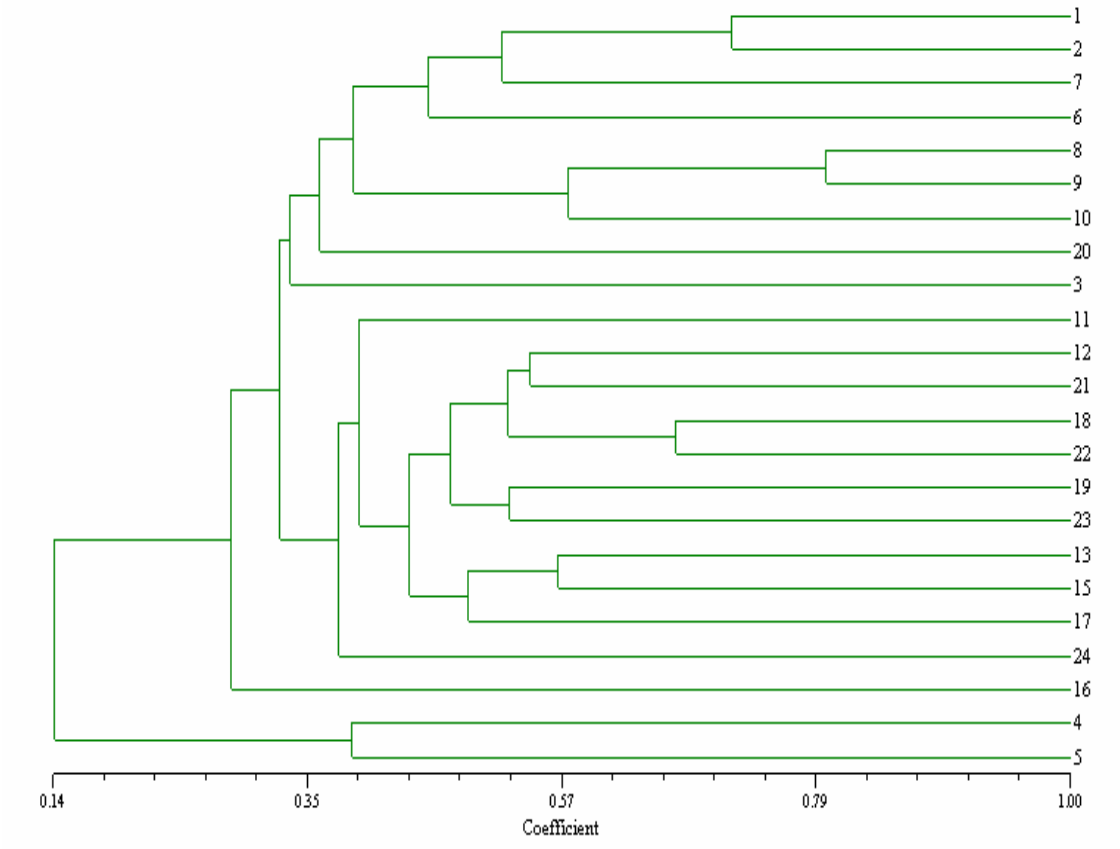


Figure 1 shows the dendrogram obtained for the 23 varieties using the 12 markers selected for garden roses. Relationships visualized by the dendrogram can as far as information is available be explained. For instance the varieties Nr. 8, 9 and 10

are very similar, morphologically. Based on the DNA analysis they cluster in one branch, indicating their relatedness, although here Nr 8 and 9 look more similar to each other than to Nr.10. The 3 varieties are coming from the same cross. In the dendrogram some varieties are placed clearly separate from the others like the two varieties 4 and 5 which are fruiting varieties for floristic purposes. Variety 1, 2 and 7 belong to a separate group in regard to the DNA results. The field results show the same relation. Variety 2 is a result from the crossing of variety 1 and variety 7. Varieties 2, 1 and 7 are distinct but very similar.

3. Standardisation between laboratories

Protocols routinely used at PRI for rose genotyping (Appendix1) were transferred to NIAB. When data obtained at NIAB and PRI for the test set of 23 varieties were compared it became evident that there were several discrepancies between the data produced at each laboratory. The discrepancies were:

1) Differences in signal intensity resulted in scoring of a peak in one lab as a marker and not scoring the same peak in the other lab. To be scored as a marker a peak needs to have a certain minimum intensity (i.e., reach a pre set threshold level). In principle 4 different alleles can be detected in one variety. A peak was considered an allele when the peak area of the smallest peak was at least 15% of the area of the highest peak. Differences in amplification efficiency resulted in differences in differences in allele calling. This problem turned out to be unsolvable within the set budget.
2) Discrepancies also arose from missing values, e.g. some samples gave amplification product within one lab and not in the other. This problem is also related to the quality of the DNA obtained. The missing data occurred at both labs, but the missing data points were not always the same in one lab and the other. The non-coincident missing data cause noise when comparing the two data sets.
3) Discrepancies originated form mis-scoring of alleles. This type of errors is easily corrected if profiles are also analysed by a second person and/or when all samples are done in duplicate.

Clearly, DNA extracts from garden roses appeared to more difficult than from glasshouse roses. Most likely the DNA extracted from the garden roses contains substances likely to interfere with the PCR stage of the analytical protocol. When the quality and quantity of extracted DNA was assessed on agarose gels a coloured co-extractant was seen on the gel. The co-extracted material was likely to include anthocyanin or phenolic substances extracted from the leaves. Two approaches were used to overcome these interferences: a secondary clean-up with a proprietary silica glass kit (Geneclean III, Q-BIOgene) at NIAB and a proprietary PCR buffer system (DNaesy plant mini kit, Qiagen) used at PRI. To circumvent the poor quality of the DNA also a different brand of Taq- polymerase was used at PRI for analysis of the CPVO applicants of 2005 of garden roses. This modified protocol is also described in Appendix 1.

Based on all these observations it was decided to run all garden rose samples that the BSA evaluated on behalf of the CPVO in the two labs (NIAB and PRI) using the 12 markers. The dataset resulting from this analysis can be found as additional data on the CD. The total dataset contain consist of marker profiles of 116 varieties. Data of marker O517 was excluded for this analysis due to the large number of missing data in the NIAB set.

From this dataset several observations can be made:

1) Missing values were observed for 68 variety/marker combinations. This is equivalent to 2.7% (4.1% in the dataset obtained at NIAB and 1.2% in the PRI dataset).
2) Some markers (like RhP518) and some varieties (e.g. no 19 from 2004 at NIAB) produced more missing values than others. The latter is most likely due to poor DNA quality.
3) In total there are 334 differences in allele calling for all variety/marker combinations. This is equivalent to 25% of the data. Interestingly, in a large number of cases this was due to a smaller number of alleles scored at NIAB, which might be caused by a combination of poor DNA amplification and the preset threshold levels for alleles (major alleles were detected, minor alleles not).
4) Within PRI a small number of varieties (10) were repeated twice. Within that set 3 differences were observed in a total of nearly 100 alleles called, which is equivalent to about 3 %. Differences in these cases were caused by missing values or different allele profiles. Similar data were obtained by NIAB.
5) When the data from Appendix 5 were used to produce a dendrogram, it was shown that in almost all cases the varieties run in the different labs cluster together in the same way. This suggests that differences between varieties were (much) bigger than differences caused by technical problems and that the data produced in the two

laboratories are largely consistent with each other. When dendrograms were produced for each laboratory separately, these were almost identical, indicating that within laboratories scoring was consistent.

From all of this it was concluded that at this moment it is not possible to produce a unified molecular database for the roses using data collected in two different laboratories. The major reasons for this were already discussed above. Therefore we choose to produce the molecular database on the data obtained at PRI only, as this lab is most experienced in using the rose markers chosen for the project.

4. Selection of photo format

Already during the first meeting of the partners it was agreed that two pictures would be made:

a. Flower from the top, on grey background, an example is shown in Figure 2.
b. A composite photo containing open flower from top and bottom, a bud, and a leaf. An example is shown in Figure 3.

Ruler should be included, in centimetres. Photos are to be labelled with CPVO number and national number. All photos are made in jpg format. The protocol and equipment used at the different stations can be found in appendix 2.

For greenhouse and garden roses approx. 100 varieties were photographed for the database.

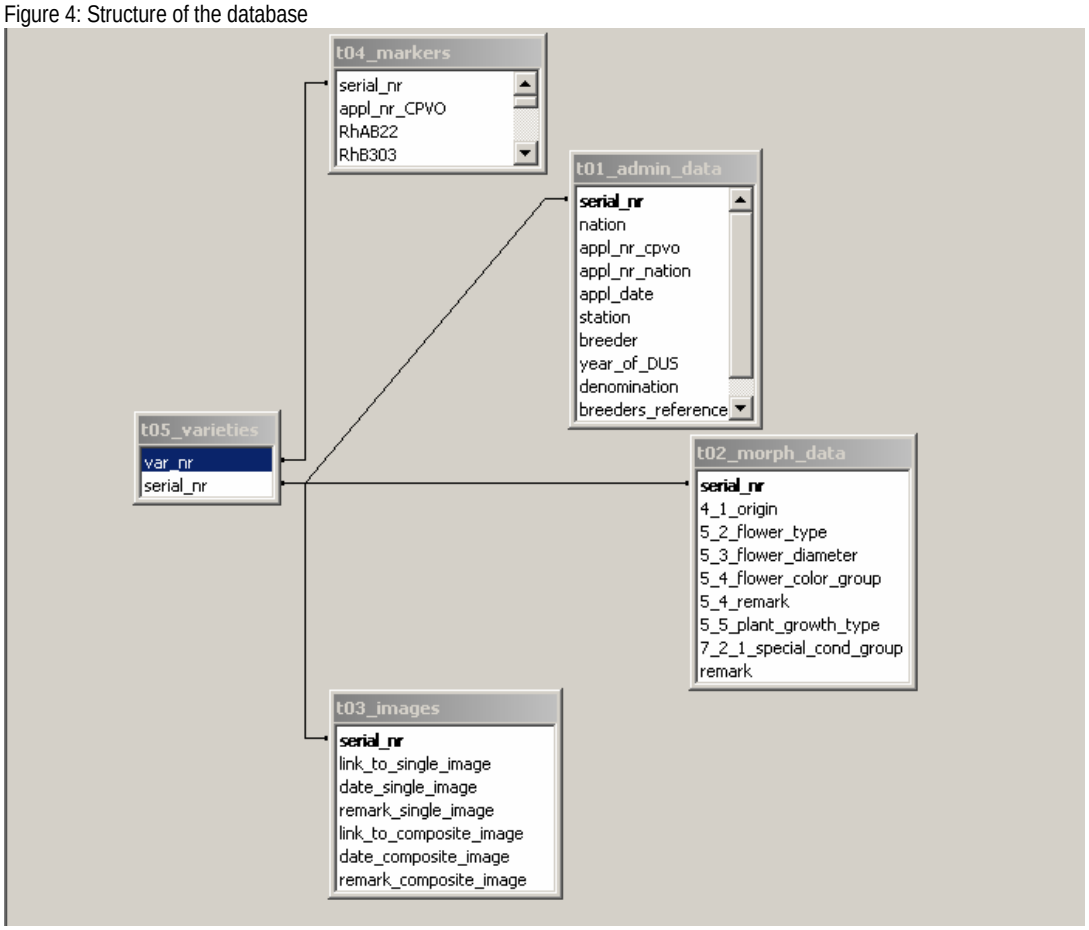
Figure 2: Flower from the top

Figure 3: composite photo

5. Selection of database format

An investigation was made of the different database systems currently in use at the testing stations. For details see the first meeting report (appendix M1). From the discussions it was concluded that the prototype database would be an Access database containing administrative data, morphological and molecular data, and a link to the photograph, as a (scanned) JPEG image. A list (Appendix 3) was made of all items to be included. Based on this list a data dictionary (description of field to be included and definition of the fields) was created. The database consists of 4 files: 1. morphological data; 2. administrative data; 3. marker data; 4. images. All files have an excel format. Links between the Excel files are made through a 'leading number' which is unique for each variety analysed. The 'leading number' (1-999, BSA- varieties, 1000-1999 PRI-varieties, 2000 – NIAB-varieties) was centrally assigned to the variety by the BSA, making it possible to integrate duplicate datasets for varieties. Pictures are stored in a separate file and are included in a data interrogation. The 4 excel files are linked together in the Access database. The final structure is shown in figure 4. All data of one variety are shown in one screen. After setting this up the database was populated with data.

Figure 4: Structure of the database



6. Filling the database

In this project we were set to populate the database with at least 200 varieties under evaluation at the testing stations on behalf of the CPVO. In the final database (Additional data: CPVO rose database) 400 varieties are included, of which 314 varieties were under evaluation on behalf of the CPVO, all varieties included are listed in Appendix 4. Of these 400, morphological data is available for all, at least one photograph for 215 varieties (193 single pictures and 184 composite pictures) and molecular profiles for 364 varieties.

Evaluation of the database

1. Evaluation of the morphological data

The different characteristics included were evaluated by the representatives of BSA, NIAB and Dutch Plant Variety Board. Their findings are summarized below.

4.1 Origin

The origin “seedling or mutant” is essential as it is used for the first selection in the reference collection and cannot be missed. This data is taken from the TQ but is challenged by the examiner during the growing trial and if necessary further comparisons are organised. Now if necessary the molecular data can also be used at an earlier stage to check in particular 4.1 and also varieties in the different 7.2.1 groups against each other.

5.2 Flower: Type

Flower type is essential for selection of reference varieties, although there is more variation for this characteristic within the garden rose group than in the cut flower group. Currently more than 90% of the latter are double flowered. There was good agreement between data provided by the applicant and the observations made by the examiner on flower type as this characteristic is probably the least influenced by environment and observer interpretation. There was one disagreement between semi-double and double but in future this situation should not occur as the new UPOV Guideline gives much firmer guidance as to where the defined border lies between the two states (within the UPOV system).

5.3 Flower: diameter

Flower diameter is a useful characteristic for the selection of reference varieties although TQ information has to be used with care by the examiner.
Flower diameter showed differences between TQ data provided by the applicant and the observations made by the examiner in 30% of cases, but they were not gross and could be expected in a quantitative characteristic where the examiner is applying a standardised system. Again, there is more variation in this characteristic within the garden rose group than in the greenhouse group, where most varieties fall in the range 3 to 6.

5.4 Flower colour group

Flower colour group is away of grouping varieties according to a defined colour classification, using known example varieties to represent the different groups on the UPOV TQ. It is essential information which is used for the first selection within the similar varieties, but it has to be used with care by the examiner and sometimes tolerances need to be applied, i.e. a variety on the borderline must be compared with more than one colour group. The groups representing the 'blend colours’ are sometimes confusing as those groups can not be described in a more or less precise way due to their blend nature, and sometimes applicants encounter difficulties in choosing the right group. The colour groups given by the applicant need a careful check by the examiners before they can be stored in the data base.
In Greenhouse roses the table illustrates how often the name of the colour group given in the TQ is changed by the examiners. From the 204 TQ forms on which a flower color group was indicated, the scoring was changed by the examiner in 77 cases.

5.5. Plant Growth Type

Plant growth type is useful for selection of reference varieties and the organisation of the trial, but more so for garden roses than for cut flower roses as currently all the latter fall into one type. Differences between data provided by the applicant on the TQ and the observations made by the examiner were found similar to 5.3. There was some uncertainty as to whether varieties were dwarf or bed roses in a small number of cases.

7.2.1. Special conditions: Group

Essential information needed to categorize the varieties in the database into garden, glasshouse and pot roses.

2. Evaluation of the photos

For the project two types of photos were made:

1. Flower seen from the top
2. A composite photo containing an open flower seen from the top and bottom, a bud, and a leaf.

The usefulness of the different photos is related to the type of variety. For greenhouse roses, leaves are less interesting compared to garden roses, as there is little variation in these characteristics, which [currently] seem to be focussed on a rather narrow morphological type. In garden roses the range of morphological variation is very great in all characteristics, and there are many different breeding aims resulting in very different types of varieties.
In this situation the composite photograph provides a great deal of extra information for the garden roses, which can be used for the screening and comparison of varieties. In fact although the UK and D have taken composite photographs according to the protocol for the project, after evaluation the UK has decided to add a stem section and dissected petals to images taken for national purposes, on a trial basis. For the greenhouse roses a picture of the lateral view of the flower is still recommended.

3. Evaluation of the molecular data

For this part we used the data obtained at PRI, for reasons discussed before.

3.1 Glasshouse roses

Molecular data were collected for 275 samples of glasshouse roses at PRI, which consisted of CPVO and National applications in the Netherlands during the years 2004 and 2005. The glasshouse roses were characterised using 11 selected markers (table 1). Marker RhO507 was deleted from the originally selected set as it showed differential amplification for some alleles in some varieties. Information for all other markers can be found in the database. From the database it can be seen that the information regarding the 275 samples is complete, e.g. no missing values. A similarity matrix was produced from the 1/0 scoring matrix using the Jaccard coefficient, with the program NTsys. All alleles were treated as independent characters. From this similarity matrix a dendrogram was draw using UPGMA, which is also incorporated in the NTsys program. The dendrogram is shown in figure 5.

Figure 5: Dendrogram showing the glasshouse roses. Varieties are indicated by their leading number (see Apendix 4)

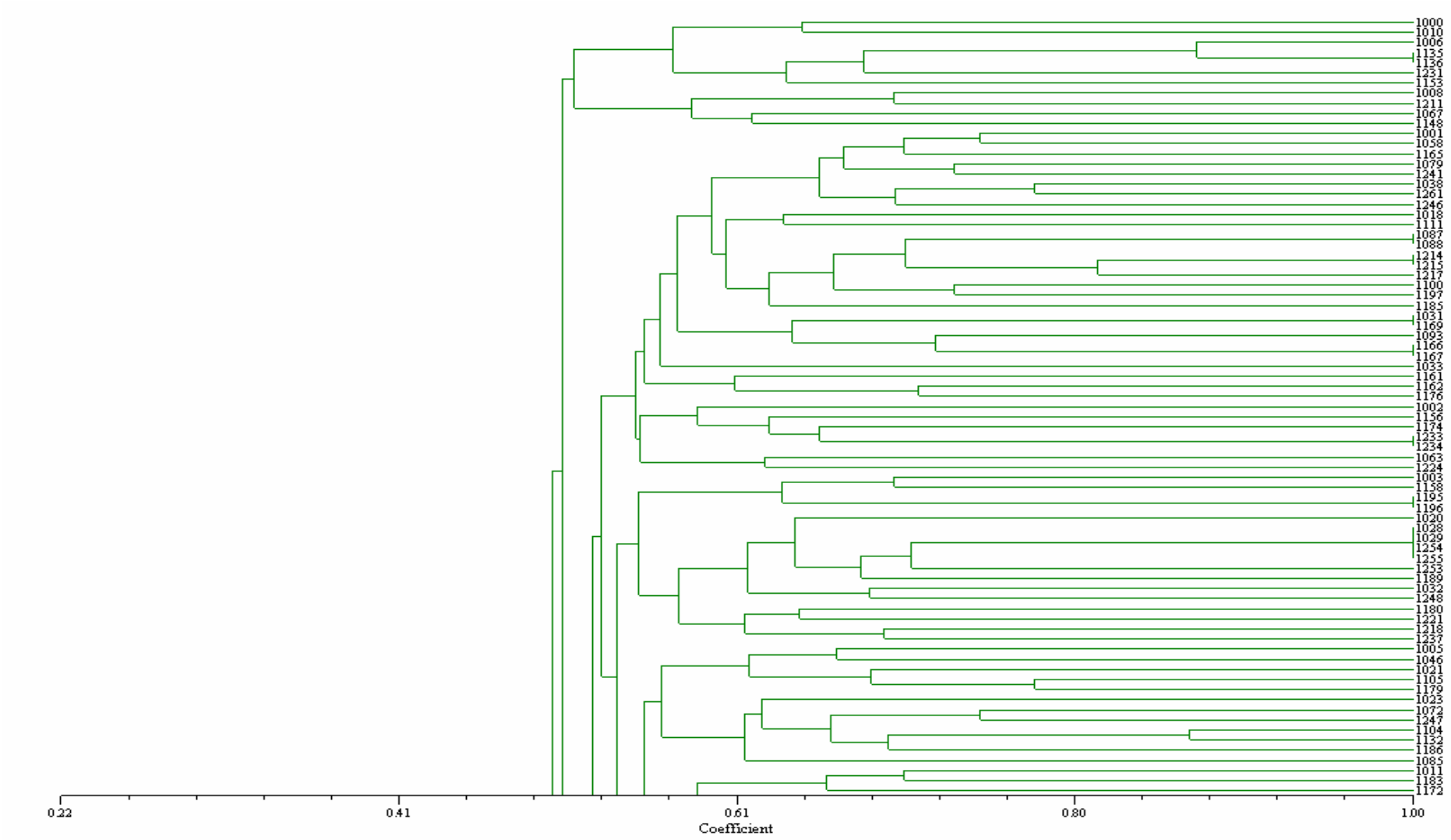


Figure 5: continued

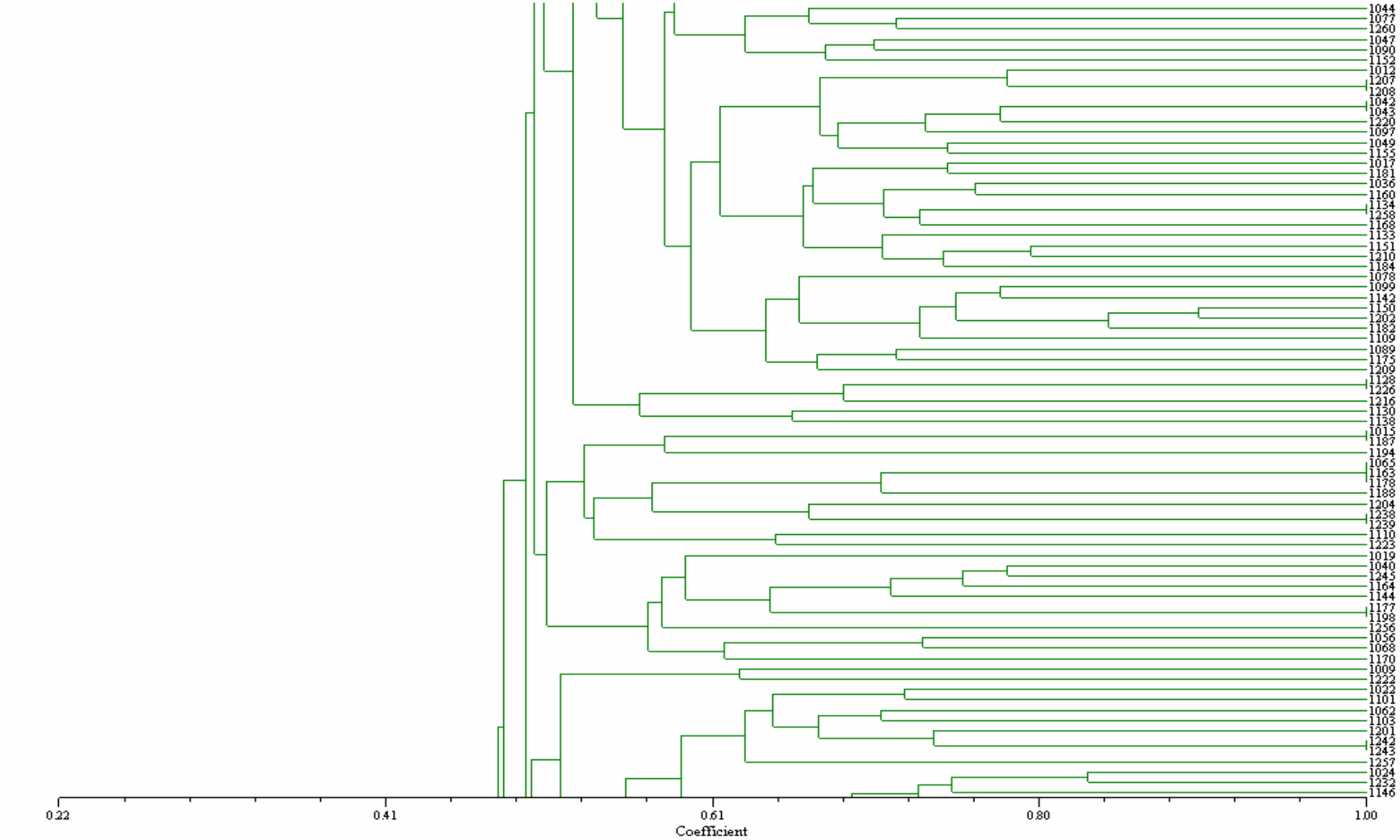
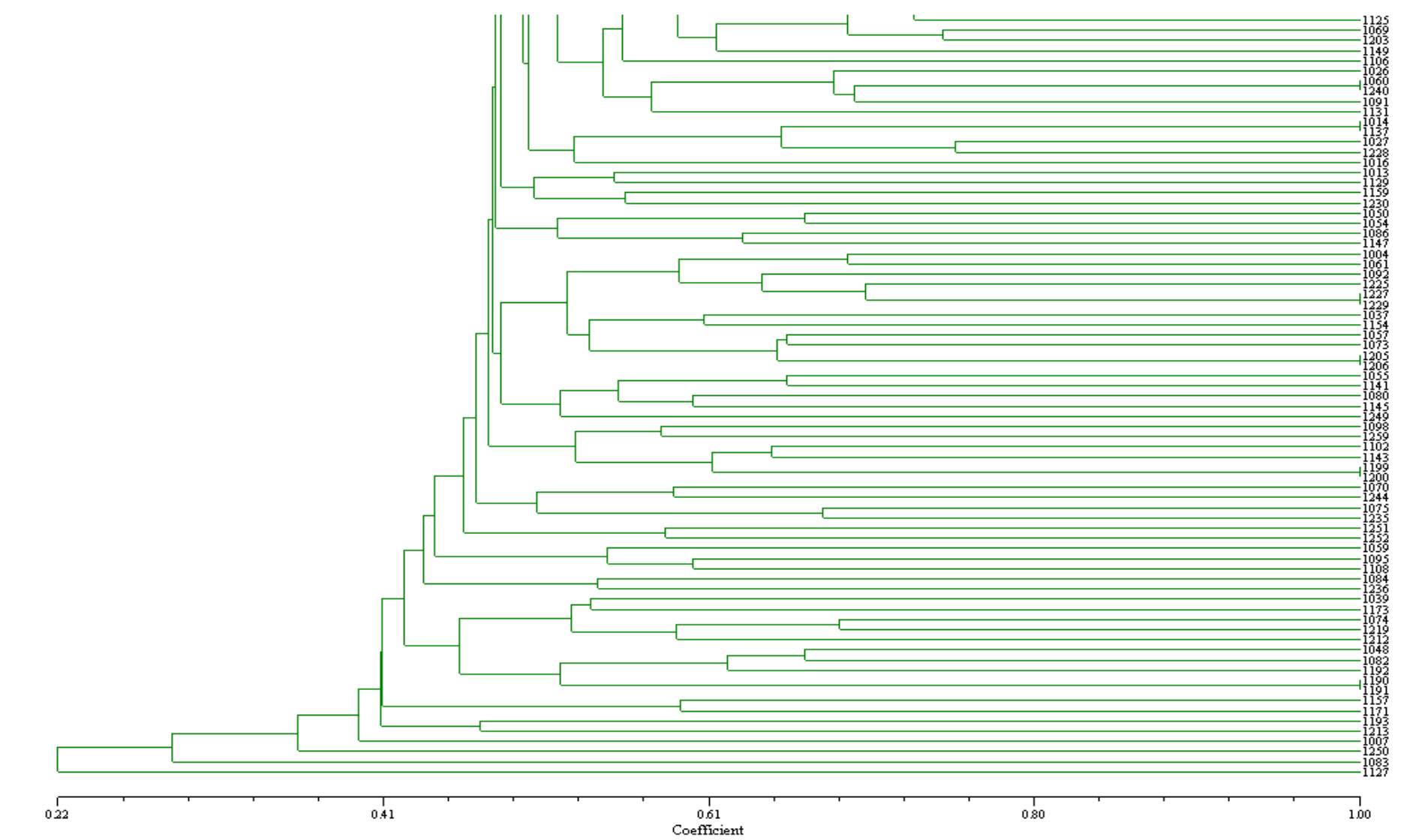


Figure 5: continued



3.2 Outdoor roses

Molecular data were collected for 145 samples, including 104 CPVO applications analysed in Germany and 2 CPVO applications analysed in the UK. The varieties were characterized at PRI using the 12 markers indicated in table 1. Again, the molecular data of the CPVO applicants can be found in the database (appendix 4). From the database it can be seen that the information regarding garden roses is largely complete. Only, In 14 cases there were missing data. This may have been caused by the DNA quality, although 'null' alleles might be present as well. A dendrogram was drawn in the same way as described for the glasshouse roses and is shown in figure 6.

Figure 6: Dendrogram showing the outdoor roses. Varieties are indicated by their leading number (see Appendix 4)

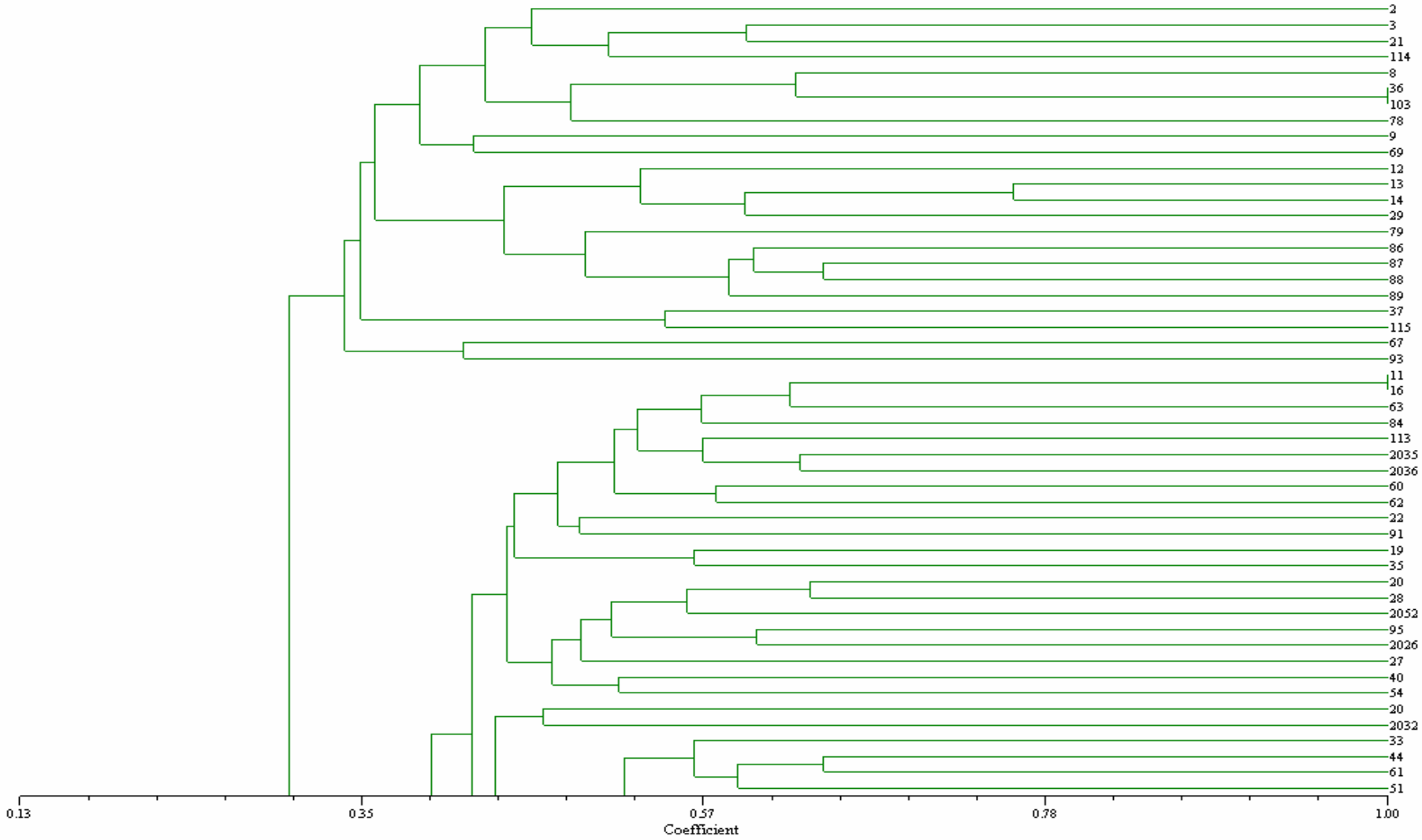
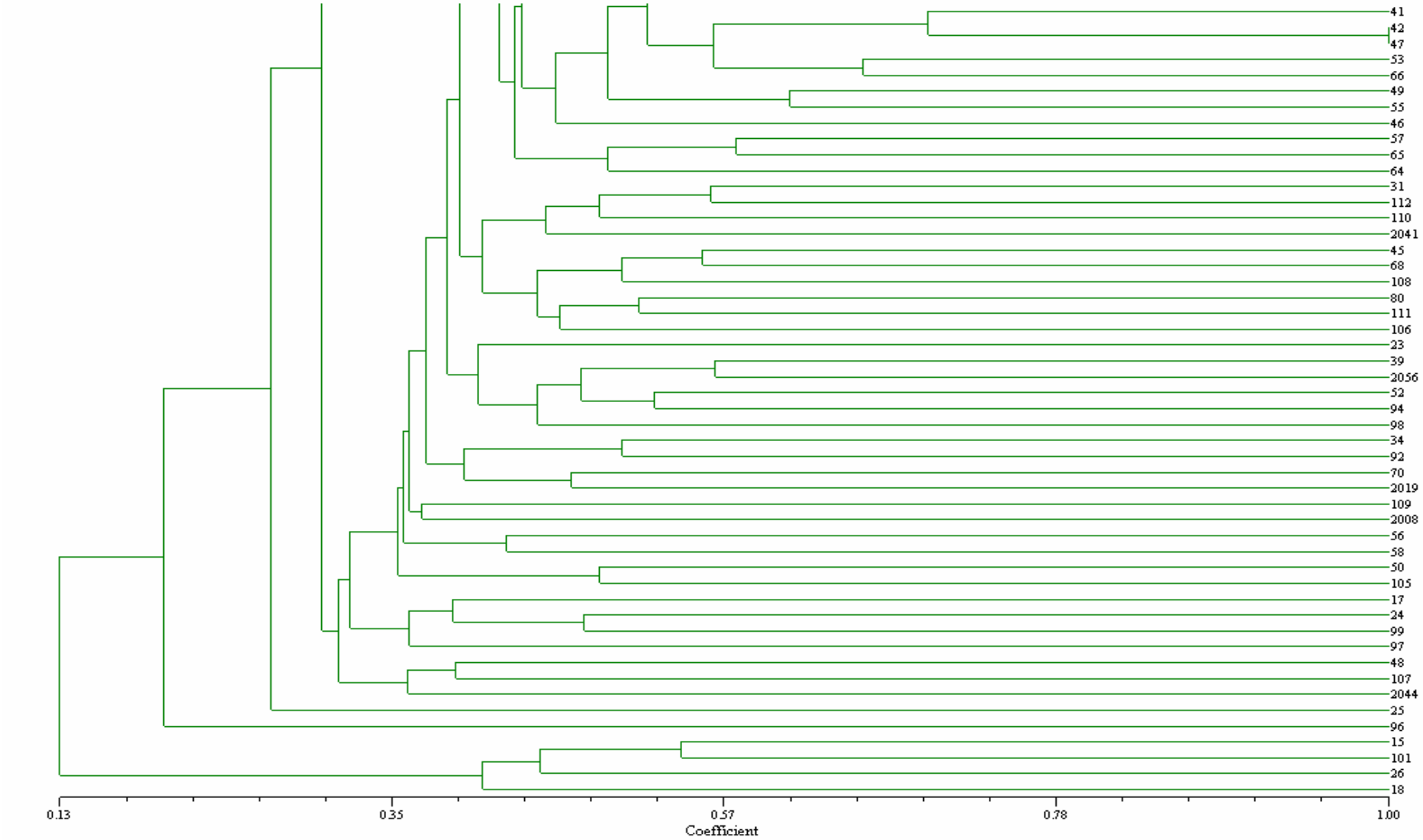


Figure 6: continued



3.3 Consistency of the molecular dataset

An assessment has been made for reproducibility of molecular genotyping in glasshouse roses: duplicate analyses were made for 35 accessions; identical profiles were obtained for 34 out of 35 varieties (1 allele difference on approx. 800 alleles) In addition, analyses were made for 37 mutants and their parents; in all cases identical profiles were obtained. Consistency of data was also noted by comparing the profiles of standard and candidate varieties with the glasshouse roses database.

When an equivalent assessment was made for reproducibility of molecular genotyping in garden roses duplicate analyses were made for 10 accessions; identical profiles were obtained for 8 out of 10 accessions. Two varieties generated profiles that differed between the duplicates; the differences between duplicates included profiles where different numbers of alleles were called for marker and profiles where data were recorded for one duplicate but not for the other.

This pattern of good reproducibility in glasshouse roses and poor reproducibility in garden roses is consistent with the supposition that the quality of DNA extracted from garden roses is less good than that for glasshouse roses. For the future a protocol needs to be worked out that includes the initial processing of the leaves at the place where they were collected.

3.4 Evaluation of the markers

Markers can be characterised using the number of alleles and the number of allelic phenotypes detected. Table 2 shows the results obtained. From this table it can be seen that, although the number of varieties analysed is considerably lower for the garden roses, the number of alleles and allelic phenotypes detected is considerably higher, indicating a much larger genetic diversity in the group of garden roses. The number allelic phenotypes detected in the latter group is almost twice as high as in the glasshouse roses.

Assuming an independent breeding history it is possible to calculate the chance that two varieties have an identical profile. This value can be approximated by multiplying the frequency of the most common allelic phenotype for each of the markers. Using the 12 markers these chances are lower than 10^{-8} and lower than 5.10^{-11} for hybrid tea and garden roses, respectively.

Table 2: Characteristics of the selected microsatellite markers in garden (gr) and glasshouse (ht) roses. Data for glasshouse roses are based on 275 varieties. The garden rose data on 145 varieties

STMS Marker	No. of alleles in ht	No. of allelelic phenotypes in ht	No. of alleles in gr	No. of allelelic phenotypes in gr
RhO517	5	28	7	26
RhEO506	7	28	16	63
RhD221	7	28	11	40
RhE2b	7	29	9	43
RhB303	7	45	8	35
RhP519	6	25	9	28
RhAB40	10	55	16	75
RhD201	5	15	12	37
RhAB22	8	25	12	58
RhP50			13	39
RhP518			10	38
RhAB73			17	62
RhM405	4	10		
RhAB15	9	25		
RhO507	11	69		
Average	7.2	31.8	11.7	45.3

Based on the 9 common markers we can analyse whether the glasshouse and garden rose varieties are based on similar germplasm. In figure 7 the dendrogram based on the 9 common markers is presented. This dendrogram shows that the two types are completely mixed. No representatives of the two groups had identical profiles.

Figure 7: Dendrogram showing the outdoor and glasshouse roses together. Varieties are indicated by their leading number (see Appendix 4)

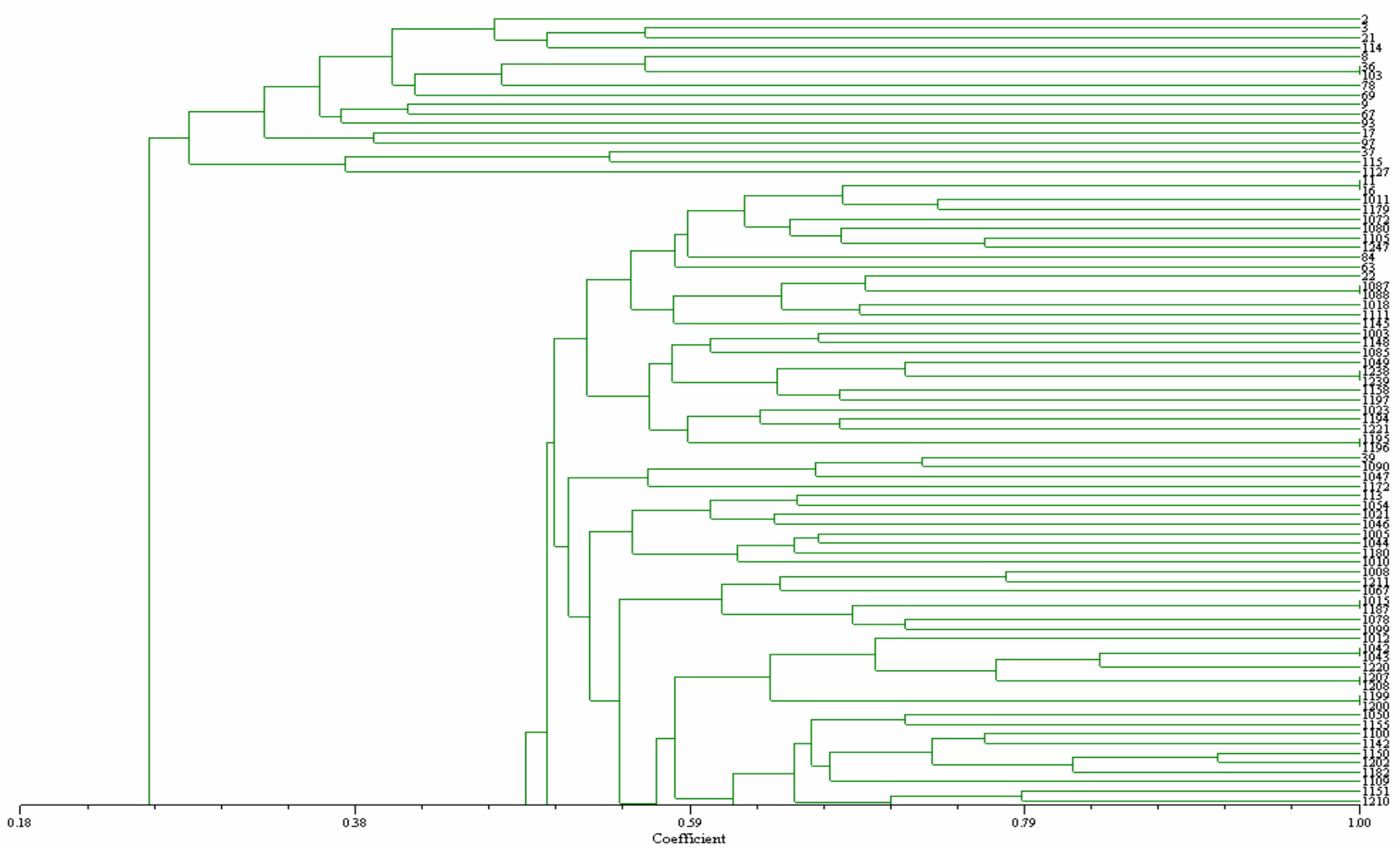


Figure 7: continued

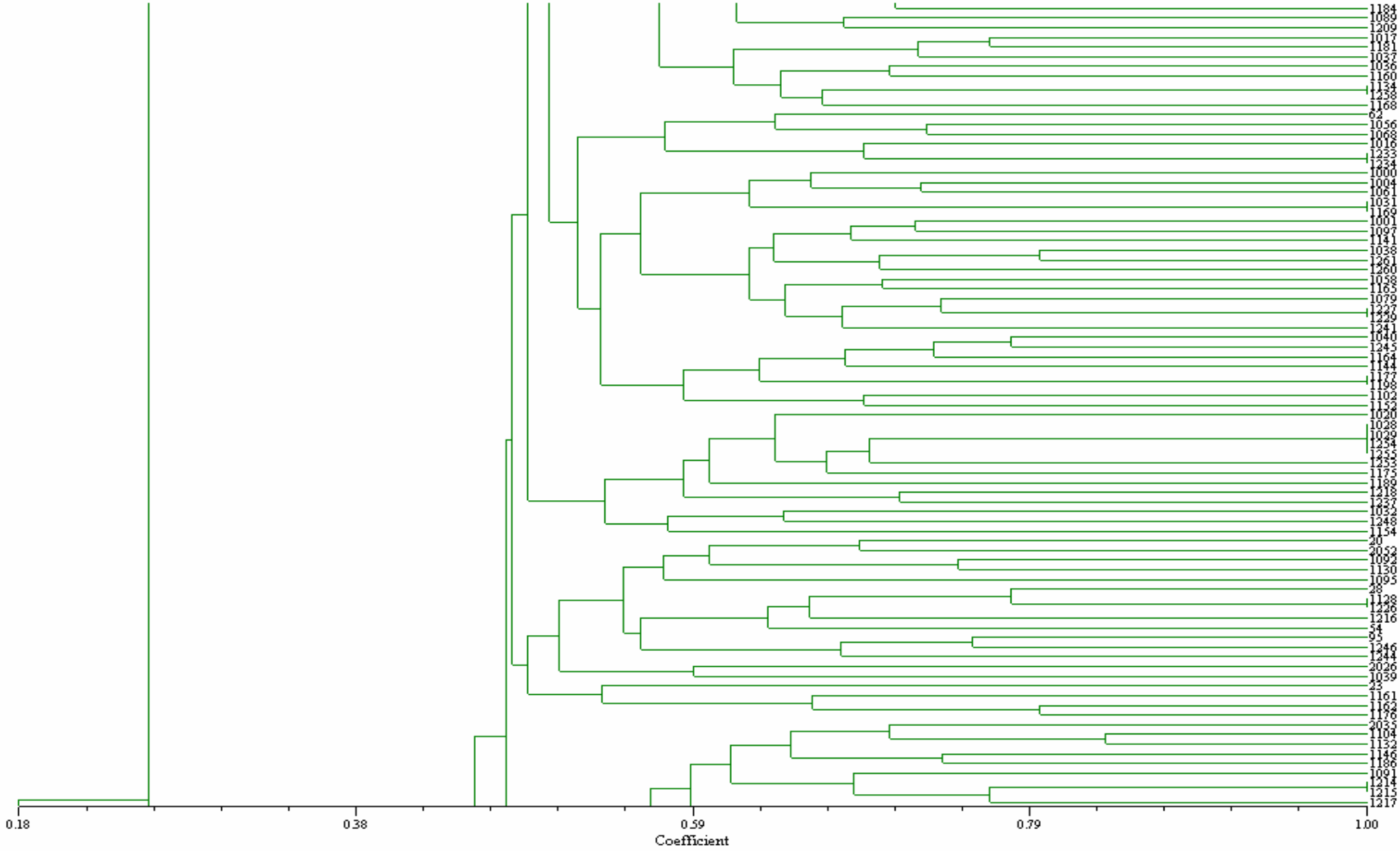


Figure 7: continued

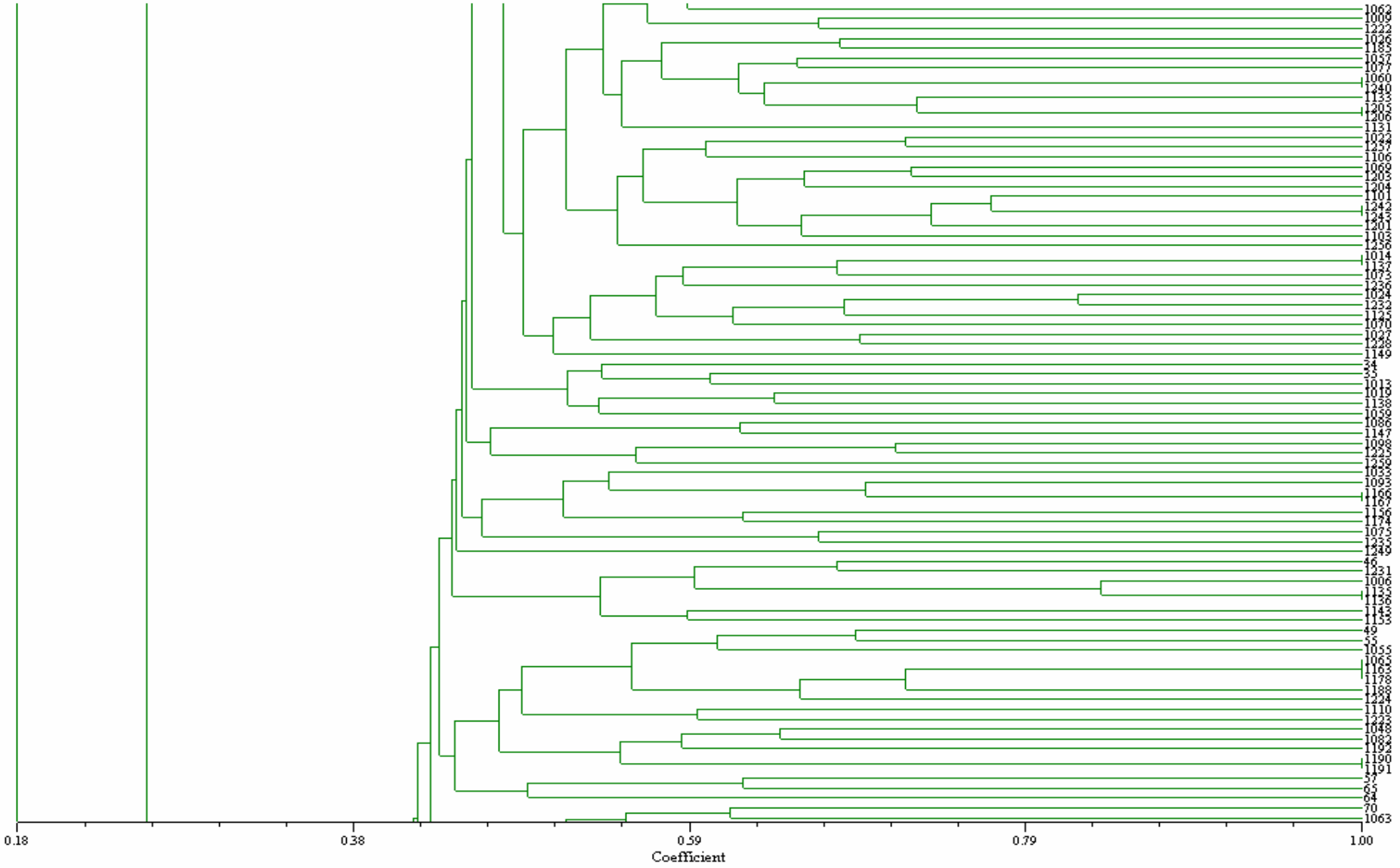
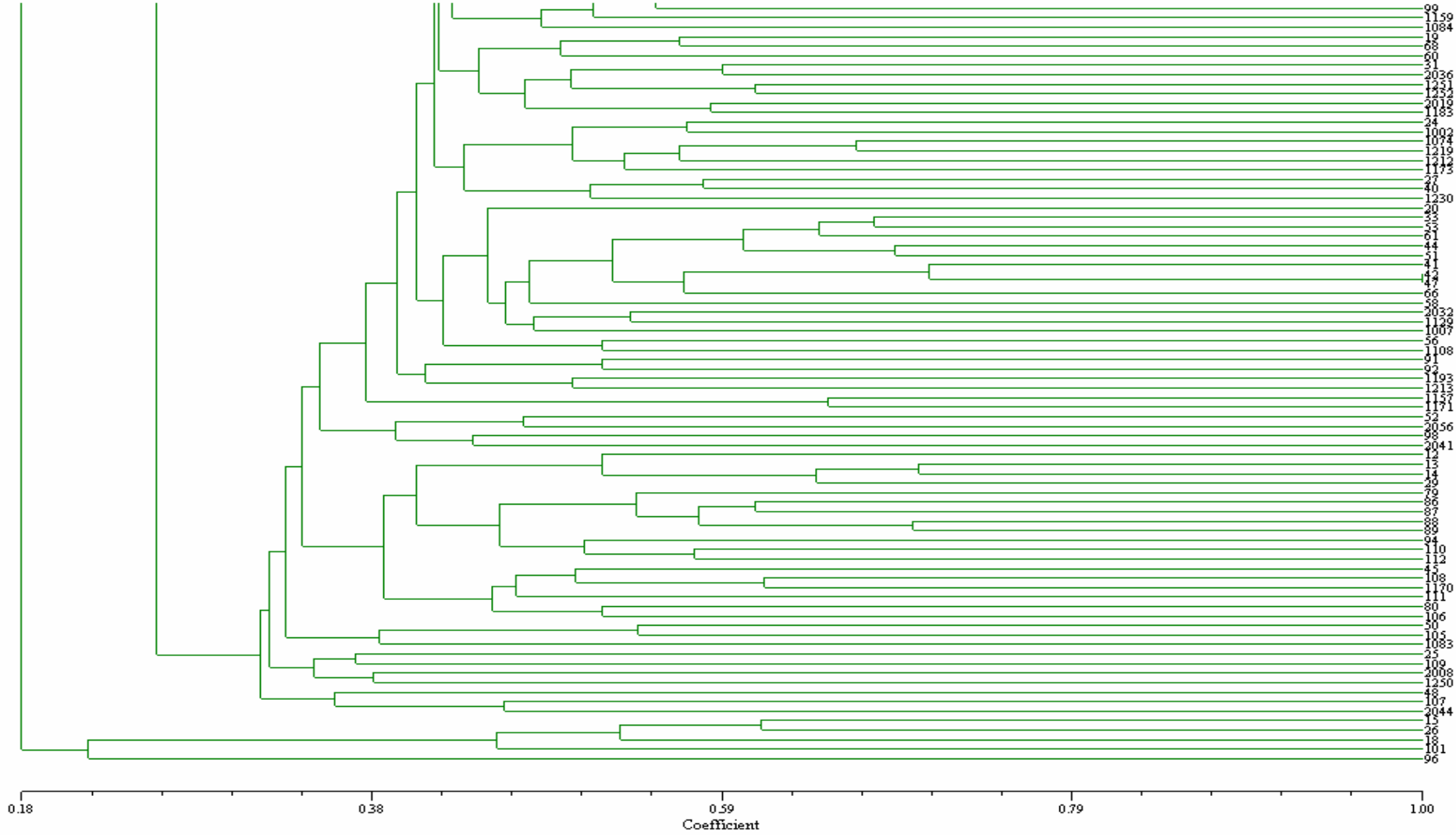


Figure7: continued



3.5 Evaluation of molecular in relation to the morphological data.

These evaluations were carried out for each DUS station independently.

We have addressed the following questions related to the use of the molecular data, as was discussed in the Cambridge meeting.

- Do all original varieties and mutants derived from them come out together
- Try to explain all similarities with values larger than 0.8-0.85
- Check whether varieties that were used for comparison with a particular variety also group with that variety

Analysis of the glasshouse roses data indicated that all original varieties and mutants derived from them came out together. Coming out together too were a few varieties that were described by the applicant in the TQ as seedlings but turned out to be mutants. During the growing trial the accuracy of the TQ information on variety origin had already been challenged, and the molecular data confirmed the inconsistency.

Finally another strange situation could be explained: one of the standard varieties (a seedling) used for calibration came out together with a mutant of another family and its parent and by coincidence that standard was rather closely related to the mutation family by being one of the parents of the seedling parent of that family.

A further look at the order of the plots in the greenhouse showed that the standard was growing next to the mutation family. Apparently material of the neighboring variety had been collected erroneously.

Unfortunately not all similarities with values larger than 0.8-85 could be explained as the TQ 's did not give any relevant information:'unknown seedling x unknown seedling' or just 'number x number'. Looking at their descriptions and photographs some of the varieties in the cluster look rather close.

In greenhouse roses seedlings [i.e. varieties derived from crossing] are very seldom selected and used as comparison varieties, as it is unusual for new seedling candidates to be very similar to existing varieties. On the other hand, mutants are always compared to the original variety and other known mutants derived from it. The reason is that mutation families often are large in number and sufficiently close in colour to require direct comparison. To identify mutation families molecular markers are a very powerful tool, as indicated above.

For the garden varieties, from the NIAB part, within the candidates examined there were no closely related pairs, so it is not surprising that the varieties seem to be scattered through the dendrogram. An additional dendrogram (not shown) based on data which includes a lot of national and reference varieties, showed pairs of mutations and a much greater clustering of morphologically very similar varieties from a particular related group.

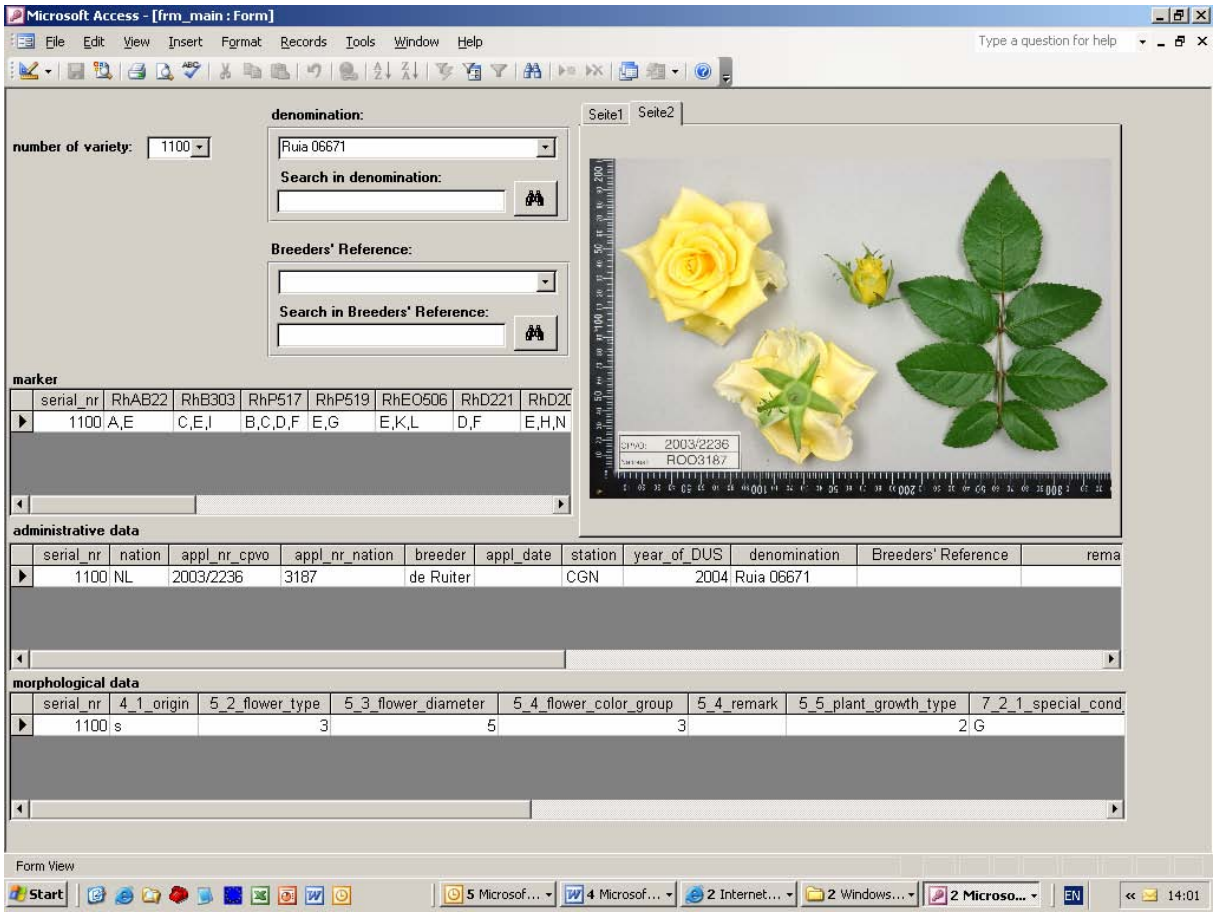
Form the BSA part a number of results have already been discussed in relation to Figure 1. The marker analysis of the varieties Noatraum, Korsilan and Heidestar show a specific grouping in the dendrogram because Heidestar is a crossing of Noatraum and Korsilan. The varieties 8 (in figure 1), 9 and 10 have their origin in crossings. They are similar but very distinct on the base of the flower colour (red, pink, white). On the basis of the marker data there is a close relationship between variety 8 and variety 9 but a rather greater distance between the mentioned varieties and variety 10. Another situation is given with the varieties 3 (figure 1) and 6 (figure 1). The varieties have there origin in different crossings from different breeders and they are very similar but in the dendrogram the level is very low. The origin of the two varieties goes back on different parents but they have the same parental variety Immensee in their background, which may be a hint on selecting on the Immensee- type within the offspring. The dendrogram allows at best a finite grouping of the two varieties 3 and 6 but not a classification as reference varieties.

From the dendrogram presented in figure 6 we can draw the following conclusions. In one case varieties, which are bred for special purposes are grouped according to the DNA-results. This refers to the varieties 15, 18, 26 and 18 which are cut berry roses for floristic purposes. The result of the marker analysis of varieties 36 and 103, which show identical profiles, is not in line with the information in the TQ. The same situation is given with the varieties 42 and 47. They are also described as seedling varieties, whereas the marker data suggest that they are mutants of each other. Further inquiries with the breeders might give an answer to these questions. The DNA analysis of the varieties 11 and 16, is in line with the TQ information that they were mutants. From Figure 6 it can be seen that all other varieties, even the ones that are known to originate from one cross, have similarities that are lower than 0.8. This strongly suggests that the 2 pairs mentioned with conflicting results between DNA analysis and TQ are indeed mutants, although morphological differences are clear.

Discussion and Conclusions

An integrated pilot database was constructed containing administrative, morphological and molecular data as well as pictures of each variety.
The database consists of 4 excel files containing these different data types, which are linked together in an Access database. All data of one variety can be shown in one screen. For an example see Figure 8. All items included in the database have been evaluated by the experts of BSA, NIAB and the board for plant varieties in the Netherlands.

Figure 8: Screen showing all information available for a particular variety.



Morphological data

Usefulness of the morphological data in the Database

It is essential to have some basic morphological categorisation to assist in sorting and screening of candidate varieties, particularly when the database grows in size. The selected morphological characteristics are useful for this purpose; although tolerances are of course needed as there can be influences of the environment on TQ characteristics 5.3 and 5.4 and even sometimes on 5.2 and 5.5. As usual, experts will have bear this in mind when using the data coming from different test stations, and check across an appropriate range of groups. It is obvious that the influence is the same on the candidate and the reference varieties at one testing station. A "sufficient" reference collection in the field, supported by a data base with pictures and other relevant information can reduce the impact of environmental influences. These are the kind of differences which have also shown up in comparison of data from different trial stations in the UPOV projects on evaluation of variety descriptions. They show the value of the morphological data but also the need for it to be used by expert examiners knowing the need to compare a range of groups.

Which data to include in the database

It is suggested that the standard characteristics chosen should be included for all types of variety because, even though currently a particular group may seem to consist largely of one type, one cannot pre-judge the future and also the full information is needed to compare data from varieties of different types.
Comparison of data supplied by the applicants via the TQ with data obtained from the trial stations shows the importance of the examiner's expertise in assessing the varieties in a standardised way. While this could be expected,for inclusion in the database it seems to be preferable that for 5.2, 5.3, 5.4, and 5.5 only the data from the examination offices are entered into the database, to reduce the possible deviations to a minimum.

Pictures

Usefulness of the pictures

The pictures taken from each variety are very important, although the composite photo was considered less informative for the greenhouse roses, because in comparison to the garden roses there is very little variation in the extra characteristics photographed. By contrast for the garden varieties the composite adds very useful information.
A point to consider in the use of photographs is the effect of the screen on which they are displayed; to improve the database consideration could be given to the use of a colour standard in all images as a point of reference.
Another consideration is the labour that is involved in taking the pictures.
The time investment in the chain from collecting the leaves to the storage of the pictures should not be underestimated, but can hopefully be balanced against efficiency gains elsewhere.

Use of the database

Currently pre-screening involves a number of different approaches as described in the introduction. A database containing a small number of varieties would not contribute much at the beginning but would rather be used for detailed information about pairs of similar varieties tested in different years, to check the necessity for further tests, and also for information about varieties tested elsewhere. As the coverage increases, the usefulness for pre-screening would also increase.
In a future large database, issues which would need addressing would be image management and ranking for similarity, as the number of varieties in each group would become too large to assess easily by eye.

Molecular data

Microsatellite markers have been used to construct a database containing the molecular profiles of approx. 380 varieties. The markers proved to be very informative about the varieties. Large numbers of allelic phenotypes (on average 32 for glasshouse varieties and 45 for garden roses per marker) have been detected (table 2). As far as we are able to tell, all seedling varieties can be distinguished from each other on the basis of DNA profiles, whereas mutant varieties and mutant groups showed identical patterns.
Molecular data can inform experts about genetically similar materials which might be truly mutants. One such example case was identified, also during the trial these varieties appeared very similar in their morphological characteristics. From this it is clear that marker data can add too and improve the quality of the DUS work.
Based on the frequency with which the different allelic phenotypes occur it is possible to calculate the chances that two varieties have identical profiles, assuming an independent breeding history. Using the 12 markers these chances are 10^{-8} and 5.10^{-11} for glasshouse and garden roses respectively, which is extremely low. Effectively, this means that when two identical profiles are detected the chance that the two samples are identical or belong to the same mutant group is almost a 100%. When we combined the data of greenhouse and garden roses it was observed that all of them (excluding the mutants within a given set of garden or greenhouse roses) showed different profiles.

A problem was encountered when we tried to merge data gathered in two different laboratories into one database. Several differences in allele calling were observed. These problems were most likely caused by a lower quality of the DNA samples obtained from the garden roses. For this reason we have decided to use only the data obtained at PRI for the database. Clearly, more effort is needed to harmonize the molecular marker analysis between different laboratories, including the development of good protocols for taking and handling leaf samples, extracting DNA, applying clean-up methods as required, PCR of fragments, standardising allele calling and co-ordinating allele nomenclatures for databasing. In spite of these difficulties we have demonstrated that two laboratories can produce substantially equivalent data and that the molecular data produced is useful as a tool for managing reference collections.

In the project proposal we identified 5 possible uses of the integrated database

- Characterization and cataloguing of the reference collection
- Pre-screening and selection of appropriate reference varieties
- Exchange of data on current candidate varieties between testing stations
- Strong reduction or replacement of permanent living reference collections at testing stations
- Quality assurance within examination offices (verification of identity/authenticity)

The database produced will be helpful for all aspects. However, not every part of the database (morphological data, molecular data and picture) is equally useful for each application.
For characterization and cataloguing of the reference collection all types of data are valuable. In addition to this one might consider to store a DNA sample as well, for use for future verification of replacement samples for the trials and to provide the applicant an opportunity to show a firm link with the DUS tested material in the case of suspected infringement. This issue was also discussed during the Cambridge meeting (see appendix M3).
For pre-screening and selection of appropriate reference varieties also all data types are useful. Especially the morphological data and the photograph are valuable for this, and marker data can be used to identify or confirm mutants and thus possible varieties for comparison. Thus, the database allows to categorize the reference collection in the sense of storing information on morphology, photograph and molecular data This information can then be used for screening and selection of necessary similar varieties to grow in the test.

In addition, the database will facilitate the exchange of data between the different testing stations. However, a lot more research will be necessary to standardize sample handling, DNA extraction and scoring for the molecular data. On the morphological side, ring tests will be useful to ensure continued consistency of scoring.

Although the BSA still sees the necessity to have a living reference collection for garden roses, in principle the database can in future be used to reduce the living reference collections. In that situation, reference varieties will need to be obtained from the breeders each time they are needed; with the aid of molecular markers these replacement samples can easily be verified before being used in the trial.

A system for the possible practical implementation of the database into the DUS testing procedure.

The database contains administrative data, TQ information and a photograph, so in principle all information needed to select varieties for comparison in the traditional way is there. In addition, the database contains molecular data. Having all this information is very valuable. Prescreening can be carried out using TQ data and the photo. The molecular data can be used to trace mutants and for identification, e.g. to check the identity of material that was ordered from breeders and should be used for comparison (Quality control).
During the last project meeting in Cambridge (see also appendix M3) a number of practical points related to possible practical implementation of the database were discussed. CPVO could enter the administrative data, TQ information and the photograph into the database before the request for analysis is send to one of the testing stations. The advantage of this is that all testing stations have information on what is under study in the other station as well. However, legal issues related to (and restrictions on the use of) the pictures of candidates varieties need to be addressed first. Also some concerns were raised regarding the scanning in of the photograph, as colors might change. Recently it was decided with the ornamental experts that the CPVO will ask for a printed photo submitted by the breeder to send to the station that will carry out the DUS test of the candidate variety. For reasons discussed above, the TQ data and the photograph provided by the breeder that are included in the database at the start of the testing should be replaced in the end by a photograph and data collected by the testing station. Molecular data can best be produced by companies that are specialized in these analysis. However, effective protocols for sampling and analyzing especially the garden roses need to be developed first. Costs related to these analysis depend on the company. It is expected that these will be 150€- 200€ per sample for 9 markers, when more than 30 samples are supplied in one batch.

The database produced is only a pilot database. It was felt necessary that at least all EU protected varieties and all varieties that are still in trade should be included in the database. CPVO should maintain the database, testing stations should be able to read/write. PVRO and board for BVR in the Netherlands should also have access to central database. Also breeders might, subject to resolution of any confidentiality issues. As the molecular data are produced on the plant material used for

DUS testing, these data are an identification label that can be used by the breeders to trace infringements, giving them effective tools to enforce their rights.
Another issue related to the database is maintaining a DNA sample of the material analyzed for DUS. For the moment CGN stores leaf samples. It is questioned whether this is the best solution. Probably storing clean, freeze dried DNA samples is better. DNA samples could be used to screen reference varieties, used in the case of technical verification and for the enforcement of rights. It seems that database information of DNA profiles is not sufficient. Analysis need to be run again. Storing DNA has the advantage that it can be used to obtain DNA profiles in future with newly developed tools. Issues to be addressed include, who should maintain these samples, who is liable when something happens to the samples and should samples be stored at 2 places to reduce the risk of loosing the material. Cost related to storing DNA include deprecation of equipment and running cost of freezer. At the moment it is unclear what these cost will be.

A model that could be applied to DUS testing of other species

The framework developed with the rose database can in principle be introduced in any vegetatively propagated crop, provided that molecular markers are available.

Literature

Ballard R, Rajapakse S, Abbott A, Byrne D (1995) DNA markers in rose and their use for cultivar identification and genome mapping. Acta Hort 424:265-268

Ben-Meir H, Vainstein A (1994) Assessment of genetic relatedness in roses by DNA fingerprint analysis. Sci Hort 58:115-121

Cairns, T (2000) Modern Roses XI, The World Encyclopedia of Roses. Academic Press

Cubero JI, Millan T, Osuna F, Torres AM, Cobos S (1996) Varietal identification in Rosa by using isozyme and RAPD markers. Acta Hort 424: 261-264

Esselink, D., H. Nybom & B. Vosman (2004) Assignment of allelic configuration in polyploids using the MAC-PR (Microsatellite DNA Allele Counting - Peak Ratios) Method. Theor. Appl. Genet. 109:402-408.

Esselink, G.D., M.J.M. Smulders & B. Vosman (2003) Identification of cut rose (Rosa hybrida) and rootstock varieties using robust sequence tagged microsatellite markers. Theor. Appl. Genet. 106:277-286.

Huylenbroeck J Van, M.J.M. Smulders, T. Debener, H. Nybom, S. Gudin, P. Cox, L. Crespel, J. De Riek (2005) GENEROSE: Genetic evaluation of European rose resources for conservation and horticultural use. In: ISHS Acta Horticulturae 690: International Rose Hip Conference (ed. H. Nybom, K.Rumpunen).

Martin M, Piola F, Chessel Jay M, Heizmann P (2001) The domestification process of the Modern Rose: genetic structure and allelic composition of the rose complex. Theor Appl Genet 102:398-404

Nybom, H., D. Esselink, G. Werlemark & B. Vosman (2004) Microsatellite DNA marker inheritance indicates preferential pairing between two highly homologous genomes in polyploid and hemisexual dogroses, Rosa L. sect. Caninae. Heredity 92: 139-150.

Rajapakse S, Hubbard M, Kelly JW, Abbott A, Ballard R (1992) Identification of rose culivars by restriction fragment length polymorphism. Sci Hort 52:237-245

Rusanov, K., N. Kovacheva, B. Vosman, L. Zhang, S. Rajapakse, A. Atanassov, I. Atanassov (2005) Microsatellite analysis of Rosa damascena Mill. accessions reveals genetic similarity between genotypes used for rose oil production and old Damask rose varieties. Theor Appl Genet 111: 804–809

Smulders, M.J.M, D. Esselink, R. E. Voorrips & B. Vosman (2005) Analysis of a database of DNA profiles of 734 hybrid tea rose (*Rosa hybrida*) varieties. Document for UPOV Working Group on Biochemical and Molecular Techniques and DNA-profiling in particular (BMT9/12).

Torres AM, Millán T, Cubero JI (1993) Identifying Rose cultivars using random amplified polymorphic DNA markers. Hort Sci 28:333-334

Vainstein A, Ben-Meir H (1994) DNA Fingerprint Analysis of Roses. J Amer Soc Hort Sci 119:1099-1103

Vosman B (1998) The use of molecular markers for the identification of tomato cultivars. In: Molecular tools for screening biodiversity, eds Karp A, Isaac PG, Ingram DS, pp. 382-387

Yan, Z., C. Denneboom, A. Hattendorf, O. Dolstra, T. Debener, P. Stam, P. B. Visser, (2005) Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers, Theor Appl Genet 110: 766 – 777

Appendices

Apendix 1: STMS genotyping of Roses

Sampling

Sample 1-3 very young leafs (often red colored and not fully expanded) in a 2 ml reaction tube. Close the tube with a perforated lid and collect the tubes in liquid nitrogen. Lyophilize all samples overnight according to the manual of the freeze-dryer. Remove the perforated caps, add about 5 glass pearls and close the tube. Grind the material for 2 min. 30/s using the Retsch MM300 mill.

DNeasy 96 protocol for isolation of DNA from rose leafs

Place the samples in order according to the scheme below. Position A1 should be left empty for orientation purposes. For plate 2 position A2 should be left empty etc.

	1	2	3	4	5	6	7	8	9	10	11	12
A		sample1	sample2	sample3	sample4	sample5	sample6	sample7	sample8	sample9	sample10	sample11
B	sample12	sample13	sample14	sample15	sample16	sample17	sample18	sample19	sample20	sample21	sample22	sample23
C	sample24	sample25	sample26	sample27	sample28	sample29	sample30	sample31	sample32	sample33	sample34	sample35
D	sample36	sample37	sample38	sample39	sample40	sample41	sample42	sample43	sample44	sample45	sample46	sample47
E	sample48	sample49	sample50	sample51	sample52	sample53	sample54	sample55	sample56	sample57	sample58	sample59
F	sample60	sample61	sample62	sample63	sample64	sample65	sample66	sample67	sample68	sample69	sample70	sample71
G	sample72	sample73	sample74	sample75	sample76	sample77	sample78	sample79	sample80	sample81	sample82	sample83
H	sample84	sample85	sample86	sample87	sample88	sample89	sample90	sample91	sample92	sample93	sample94	sample95

Preheat buffer AP1 and AE to 65°C

1) Combine buffer AP1, RNAse A (100mg/ml) and Reagent DX

   Mix for 2x96 samples 140 ml AP1

   350 µl RNAse A

   350 µl Reagent DX

   Add 600 µl extraction buffer to each tube and mix

   Incubate for 10-20 min. at 65°C, mix 2-3 times during incubation by inverting the tube.

2) Add 195 µl buffer AP2 to each tube

Mix vigorously and incubate for 10 min. at -20 °C

Centrifuge for 5 min at full speed (14.000 rpm)

3) Transfer 400 µl sample to a 96 deep-well block placed on ice (Square-well block Qiagen cat.no.19573)
4) Add 600 µl buffer AP3/E and mix using a multi-channel pipet
5) Apply 1 ml of each sample to the Dneasy 96 plate
6) Seal the plate with a tape sheet, centrifuge for 4 min. at 5600xg
7) Add 800 µl buffer AW to each well
8) Seal the plate with a tape sheet, centrifuge for 15 min. at 5600xg
9) Place the DNeasy plate on a DNA collecting plate, add 200 µl of buffer AE pre-heated at 65°C and incubate for 5 min.
10) Seal the plate with a tape sheet, centrifuge for 2 min. at 5600xg
11) Check DNA concentration for a number of samples (i.e. row 1) on 0.8% agarose. Typically, a yield of 20 µg is extracted (100 ng/µl)
12) Dilute all samples to 2 ng/µl (10 µl stock DNA + 490 µl water)
13) Check the DNA concentration of all diluted samples (10 µl of 2 ng/µl = 20 ng DNA). If necessary, adjust the dilutions.

STMS PCR

All samples will be amplified using 4 multiplexes each containing 3 STMS loci
Transfer 5 µl of a 2 ng/µl DNA dilution to a 96 PCR titerplate.
PCR plates containing DNA can be made in advance and stored for a few days at –20C. Longer storage will result in evaporation of water.
Row H (01-12) of each plate is used for reference samples.
Put together the master mixes of the different multiplexes according to the scheme mentioned below. Add the primers separately to the mixes, so don't mix the primers from each multiplex in advance.

Number of reactions	105X
10x Goldstar PCR buffer	210
$MgCl_2$ (25mM)	126
dNTPs (5mM)	42
primers (see below)	126
MQ	1062.6
Total volume	1566.6 µl

	STMS	pmol/reaction	105 reactions
MP1:	RhAB22	6	63 µl primer (10 pmol/µl)

	RhB303	2	21 µl primer (10 pmol/µl)
	RhO517	3	31.5 µl primer (10 pmol/µl)
	MQ	0.1 µl	10.5 µl MQ
MP2:	RhP519	2	21 µl primer (10 pmol/µl)
	RhAB15	4	42 µl primer (10 pmol/µl)
	RhM405	2	21 µl primer (10 pmol/µl)
	MQ	0.4 µl	42 µl MQ
MP3:	RhEO506	2	21 µl primer (10 pmol/µl)
	RhD221	4	42 µl primer (10 pmol/µl)
	RhE2b	4	42 µl primer (10 pmol/µl)
	MQ	0.2 µl	21 µl MQ
MP4:	RhD201	3	31.5 µl primer (10 pmol/µl)
	RhAB40	3	31.5 µl primer (10 pmol/µl)
	RhE2b	6	63 µl primer (10 pmol/µl)
	MQ	0.0 µl	0 µl MQ

Master mixes without *Taq* polymerase can be made in advance and stored at –20C.

Add 8.4 µl Goldstar *Taq* polymerase (5 U/µl) and mix gently but thoroughly (do not vortex) Place mixture on ice.

Place the PCR plate containing the DNA on ice.
Add 15 µl master mix to each well (total volume PCR =20 µl)
Cover the plate with a rubber cover
Centrifuge the PCR plate briefly before transferring it to the PCR machine.
Place the plate on ice.

Start PCR program STMS on a MJ PTC-200
Wait until LID temp=104°C and the block temp = 60°C or higher
Put PCR plate directly from ice into the PCR machine.

PCR program STMS on MJ PTC-200

94°C	3 min.	
94°C	30 sec.	}
RAMP 1°C/s to 50°C		}
50°C	30 sec.	} 30 cycli
RAMP 1°C/s to 72°C		}
72°C	120 sec.	}
72°C	10 min.	

Check from a few samples 8 µl PCR product on a 2% agarose gel along with 8 µl Multiplex reference DNA and a 1 Kb ladder.

ABI sample preparation

Transfer 5 µl PCR products and 15 µl MQ on a pre-made Multiscreen Sephadex plate.

Centrifuge 5 minutes at 910*g (Sigma Qiagen centrifuge, 2377 rpm/910 rcf (*g)).

Formamide/sizer mix for 96 monsters:	1 ml formamide ultra pure (glass bottle) 30 µl IGD 500 sizer

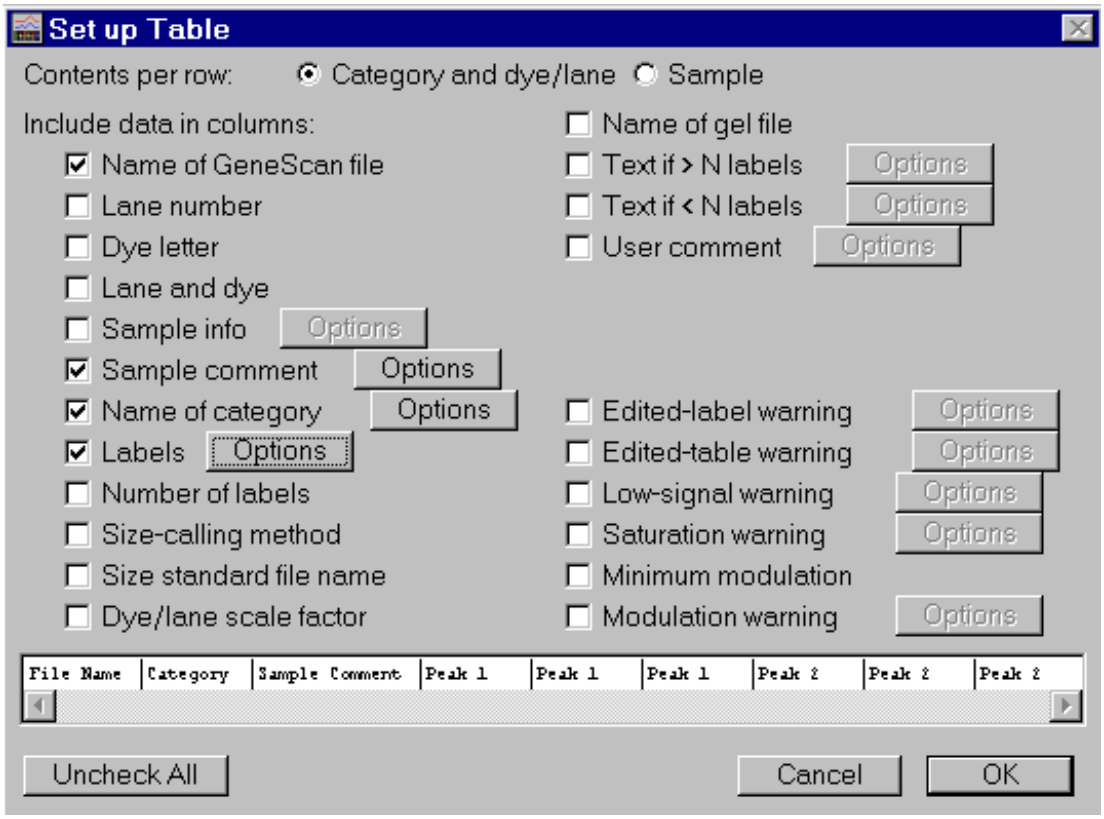
Pipet 10 µl formamide/sizer mix into a 96 wells PCR plate
Add 1 µl purified product (multi-channel pipet 0-10 µl)
Centrifuge the plate briefly

Make a sample sheet for Genescan in Excel
Column File name should contain the DNA co-ordinate e.g. Rh03_A01.
Save sample sheet on a floppy disk.
Greenomics will import the column into the Genescan program
Data will be returned on a CD

STMS analysis

Start Genotyper software.
Import all data (red, blue, yellow and green)
Save the file giving it a proper name.
Select red (internal size standard), Label All Peaks and check all lanes for correct sizing of the peaks. If not, make a note.
Select one of the other colors (STMS)
Verify the orientation of the plate by checking the reference well (A1 for plate1, A2 for plate 2 etc, these wells should contain no data or signals below 400 RFU)
Verify the reference varieties for correct allele assignment (position H1 t/m H12)
Genotype all samples (Label Peaks and Filter Labels)
Check all lanes for mistyping, a specific peaks (remove by clicking) or too low signals. Minimum peak high of all peaks in each lane should be 400 RFU (Relative Fluorescent Units). Signals lower than 400 can be the result of contamination of other samples (traces of DNA loaded into a blank sample). These samples should be repeated.
Save the file.
For the sake of accuracy a second person should verify the genotyping.
Append the peak data (name, size, peak area) to the table (Set up Tabel, Append to Tabel) and export the data (Export Tabel) for further analysis in Excel.

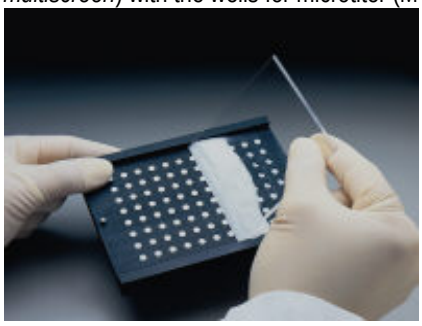
Example Set up Tabel



Sampling of rose leafs

Millipore multiscreen preparation (Sephadex G-50 columns)

Fill the template (*Millipore multiscreen*) with the wells for microtiter (MT) plate with Sephadex G-50 powder.



Place upside down the MT-plate with membranes in wells (columns) onto the template with Sephadex. Turn the two plates together quickly upside down and put onto the table. Knock the template well to drop the Sephadex powder onto membranes. Take the template away.

Add 300 µl water into each well (multichannel pipet of 1200 µl) and incubate for at least 4 hour at room temperature, or overnight at +4°C. Sealed in Saran wrap the plate can be stored for 1 month in a fridge.

Place an empty MT-plate in a centrifuge holder, and set the plate with Sephadex on it. Centrifuge 5 minutes at 910*g (Sigma Qiagen centrifuge, 2377 rpm/910 rcf (*g)). The water will be dropped out of the columns into the MT-plate below.
Place the sepadex plate on a new MT-plate.

The plate is now ready for use.

STMS PCR using Qiagen multiplex kit

All samples will be amplified using 4 multiplexes each containing 3 STMS loci
Transfer 4 µl of a 2 ng/µl DNA dilution to a 96 PCR titerplate.
PCR plates containing DNA can be made in advance and stored for a few days at
–20C. Longer storage will result in evaporation of water.
Row H (01-12) of each plate is used for reference samples.

Put together the master mixes of the different multiplexes according to the scheme mentioned below. Add the primers separately to the mixes, so don't mix the primers from each multiplex in advance.

Number of reactions	100
2x PCR multiplex buffer	500
primers (see below)	126
Total volume	626 µl

	STMS	pmol/reaction	105 reactions
MP1:	RhAB22	6	63 µl primer (10 pmol/µl)

	RhB303	2	21 µl primer (10 pmol/µl)
	RhO517	3	31.5 µl primer (10 pmol/µl)
	MQ	0.1 µl	10.5 µl MQ
MP2:	RhP519	2	21 µl primer (10 pmol/µl)
	RhAB15	4	42 µl primer (10 pmol/µl)
	RhM405	2	21 µl primer (10 pmol/µl)
	MQ	0.4 µl	42 µl MQ
MP3:	RhEO506	2	21 µl primer (10 pmol/µl)
	RhD221	4	42 µl primer (10 pmol/µl)
	RhO507	4	42 µl primer (10 pmol/µl)
	MQ	0.2 µl	21 µl MQ
MP4:	RhD201	3	31.5 µl primer (10 pmol/µl)
	RhAB40	3	31.5 µl primer (10 pmol/µl)
	RhE2b	6	63 µl primer (10 pmol/µl)
	MQ	0.0 µl	0 µl MQ

Add 6µl master mix to each well (total volume PCR =10µl)
Cover the plate with a rubber cover
Centrifuge the PCR plate briefly before transferring it to the PCR machine.

PCR program STMSMP on MJ PTC-200

95°C	15 min.	
94°C	30 sec.	}
RAMP 1°C/s to 50°C		}
50°C	30 sec.	} 30 cycli
RAMP 1°C/s to 72°C		}
72°C	120 sec.	}
72°C	10 min.	

After PCR add 10 µl water to each well and spin down
Check from a few samples 8 µl PCR product on a 2% agarose gel along with 8 µl Multiplex reference DNA and a 1 Kb ladder
Continue with protocol STMS genotyping of roses.

Appendix 2: Procedure for making photographs of Rose in framework of the CPVO database

CGN protocol and setup

Object:
The photo is made of a flower grown on a well developed shoot that has just fully opened (at first dehiscence of anthers). Also with bunchroses, only a single flower is photographed.

Set up:
The flower is stuck in a holder in front of a middle gray background (Savage background paper color Focus Gray) which is 23 cm below the flower. A size indicator (ruler) is situated at the same level of the flower.
Light source: Two horizontally positioned Kaiser lighting units are used, each of them equipped with two fluorescent lamps of 36W and 5400K (Osram Dulux L 36W/950). The units are positioned at about 45 degrees left and right of the object, at about 50 cm distance.

Camera:
Nikon D1x digital single lens reflex camera with 12 bits RGB-CCD sensor of 23,7 x 15.6 mm and 5.47 Mpixel. Equipped with AF Micro Nikkor 60 mm, 1:2.8 D (for larger objects) or AF Micro Nikkor 105 mm, 1:2.8 D (for smaller objects). The camera is mounted on a heavy duty Cambo UST studio stand.

Lay out:
The photo is made straight from above the flower and the camera is in landscape position. The object fills the viewfinder of camera for about 80%. The size indicator (ruler) is placed on top.

Camera settings:
The lens is manually focussed just below the top of the flower in order to have full profit of depth of field focus. Aperture 22 is standard and time is manually adjusted for lighter or darker flowers. White balance is set on preset which is ajusted to the light source using a Kodak Gray Card.

Storage:
The camera is connected with a computer by a Fire Wire Cable and an IEEE1394 interface. Controlling of exposure is done using Kodak Capture Control software. Pictures are edited with Kodak Capture Editor software and stored as JPG file with low compression and fine quality (0.8 to 1.2 Mb).

NIAB protocol and setup

Object:
The photographs were taken of flowers, grown on well-developed shoots, that had just fully opened (at first dehiscence of anthers). For the 2004 trial, one composite photograph consisting of two flowers, a bud and a leaf was taken of each variety. Following discussion at the project meeting on 17th May 2005, it was agreed to also photograph a single flower in addition to the composite image.

Set up:
The plant material was placed on a middle grey background paper (colour "Real Grey"). A ruler line indicating length is placed on each axis.

Light source:
In 2004, two horizontally positioned lighting units were used, positioned to the left and right of the object, at about 135 cm distance. In 2005, no supplementary lighting was used in order to reduce shadowing.

Camera:
In 2004, a Nikon DIX digital camera with a 55 mm Micro lens was used. The camera was mounted on a heavy duty Cambo UST studio stand. In 2005, an Olympus C4000 digital camera was used.

Lay out:
The photo was made from straight above the flower with the camera in landscape position.

Camera settings:
The lens was manually focussed just below the top of the flower in order to have full profit of depth of field focus.

Storage:
The images were downloaded from the cameras via USB cables to a computer. Pictures were edited with Microsoft Photo Editor Kodak software and stored as JPG files with low compression and fine quality (0.8 to 1.2 Mb).

BSA protocol and setup 2004/2005

Object:
The photographs were taken of flowers, grown on well-developed shoots, that had just fully opened (at first dehiscence of anthers). One composite photograph consisting of two flowers, a bud and a leaf was taken of each variety. Following discussion at the project meeting on 17th May 2005, it was agreed to also photograph a single flower in addition to the composite image. The label includes BSA and CPVO reference number.

Set up:
The plant material was placed on a middle grey background paper (colour "Real Grey"). A ruler line indicating length is existing.

Light source:
The pictures are taken in a glass house, no supplementary lighting. The photos were taken in a slightly shadowed green house with diffuse light conditions.

Camera:
Canon Power shot G 2, 4.0 Mega pixels camera
The pictures are taken in a glass house, no supplementary lighting, the photos are taken in a slightly shadowed green house with diffuse light conditions.

Lay out:
The photo was made from straight above the flower with the camera in landscape position.

Camera settings:
The lens was manually focussed just below the top of the flower in order to have full profit of depth of field focus.

Storage:

The images were downloaded from the cameras via USB cables to a computer. Pictures were edited with Microsoft Photo Editor or Unlead software and stored as JPG files with low compression and fine quality. (0.6 to 1.6 Mb).

Appendix 3: Items included in the prototype database

1. Administrative data
    - Application number (CPVO)
    - Application date
    - Number given by testing station
    - Breeders reference.
    - Name of station performing DUS test
    - Year of DUS test (or final year of testing in case of perennial)
    - (proposal) variety denomination
    - Remark field

2. Morphological data (Morphological characters from CPVO/TQ-EN-011 to be included in the database):
    - 4.1 Origin
    - 5.2 Flower: Type
    - 5.3 Flower: diameter
    - 5.4 Flower color group
    - Extra remark field for flower colour group
    - 5.5. Plant Growth Type
    - 7.2.1. Special conditions: Group
    - Remark field

3. A photo; photos (single flower and composite photo) are not stored in the database but in a separate directory
    - A link to the two photos
    - Data that photo was made
    - Remark field

4. Molecular data
    - Data are collected in an excel spreadsheet. An example of how this looks is shown below
    - Remark field

Example for variety Noatraum:

STMS	**1**	**2**	**3**	**4**
RhP50	e	j		
RhP518	d	f		
RhAB73	b	c	e	
RhB303	b			
RhP519	f	g		
RhAB40	a	b	g	
RhO517	d	e		
RhEO506	i	j		
RhD221	d	e		
RhE2b	d	f		
RhD201	g			
RhAB22	b	c	r	
RhM405				
RhAB15				
RhO507				

Appendix 5: Draft minutes of the first meeting of CPVO rose project on September 17, 2004 in Wageningen

Participants:

Burkhard Spellerberg (BSA)
Bob Cooke (NIAB)
Liz Scott (NIAB)
Huw Jones (NIAB)
Lysbeth Hof (CGN)
Henk Stolk (CGN)
Joost Barendrecht (Dutch Board of Breeders' Rights)
Ben Vosman (PRI)

Opening

Ben Vosman opened the meeting and welcomed all participants.

Introduction of participants

Participants introduced themselves

Brief introduction to project

Ben highlighted the main objectives and deliverables of the project.

Investigation of available plant material

Number of candidate varieties under evaluation for CPVO in the different stations:
CGN 84; BSA 80; NIAB 12, total 176. When we assume similar numbers in 2005 it should be no problem to get a database containing the description of 200 varieties. It also means that it is no problem if not all candidates under study in 2004 are included in the database.

Selection of database format

Database used for storage of DUS data in the different stations:

CGN

- Large database in Oracle (Rex)
- All data, including administrative from last 10-15 years, last 6 years including photograph (jpg)
- 8 pictures at a time on screen when searching in photo-database
- Also communication structure with Board for PBR in NL
- Many user system

BSA

- All data in an informix database in JAVA
- Data include administrative data and photo (low and high resolution; jpg)
- and DUS characteristics
- BSA has the intention to put the final database after the project on the web

NIAB

- Oracle based system, shared with PVRO
- Is reviewing the database
- Are considering to switch to Excel and/or Access
- Photo's are linked to the database (digimom project)

CPVO uses also an oracle based database.

All databases allow export/import of data to/from Access. Access looks the most logical framework to build upon. The project aim is to show that a database containing morphological data, photo's and a molecular profile can be used for the applications indicated in the project proposal. We can include recommendations on implementation in the final report (like for instance accessibility via the web, or inclusion in the DUS databases currently in use).

Database searching possibilities will be looked upon in consultation with IT experts. NIAB will contact their IT expert on this and report back to the project group. During the next meeting the IT expert of the BSA will participate in the meeting.

Database will also contain: CPVO referencenumber, national referencenumber, denomination and where appropriate, trade name.

Selection of markers:

At present only CGN/PRI makes a DNA profile of the candidate varieties using microsatellite markers. The varieties characterized are all Hybrid tea varieties and it is unknown whether the primers that are most informative in cut roses are also most informative in garden roses. Therefore a pilot will be performed to evaluate the markers for these roses. To this purpose approximately 25 garden rose varieties will be genotyped.
The pilot will also be used to harmonise scoring and calling of alleles. To this purpose PRI will make available to NIAB: reference DNA for the different alleles found in cut roses and ABI trace files for the different markers. PRI will send information on primers (including pigtail and labels) to be tested to NIAB.
BSA will collect the leaf material and send this to PRI. PRI will send details on sample collection to BSA (incl. Photo's of collection of leaf material). Four samples will be collected for each variety. PRI will freeze dry them and send half of the material to NIAB. Genotyping of the set of 25 varieties will be done by PRI and NIAB.

Selection of photograph format

Current practice:
CGN: one picture from the top.
BSA: deals with different rose and growth types. Makes two pictures,(1) open flower (2) composite photo: buds, open flower from top and button, stem and leaf. It was noted that some of the characters on the photo's can also be seen from the description.
NIAB: single photo from side/top; also field picture to represent the variety, not to describe it.

All photos are in jpg.

An important question is: do we want to automate searching for most similar varieties? If so, then we probably need to make one photo.

Photos should be linked to the database, not included. Information on who made the photo (which testing station) should be included.

Conclusions from discussion:
We will make two photo's

1. flower from the top, on grey background (PRI will send details on background). Ruler should be included, in centimetres. Label photo with CPVO nr and national number. We should also make a recommendation to the breeders on how they should make the pictures in future (part of TQ).
2. a composite photo should be made, similar to BSA, but without the stem. Burkhard to send the standard.

We will need to address questions regarding extra cost of second photo and value of the extra data.

Morphological characters:

Morphological characters from CPVO/TQ-EN-011 to be included in the database:

4.1 Origin
5.2 Flower: Type
5.3 Flower: diameter
5.4 Flower colour group
5.5 Plant Growth Type
7.2.1. Special conditions: Group

Char. 5.4 and 5.5. are not from TG, and should still be recorded by testing station (if possible) for sampled varieties in 2004.
Recommendation: TQ characters should be in TG. DUS rose experts will put this forward during the revision of the TG

Contract & finance details

- Contract between CPVO and PRI signed.
- PRI to check whether CPVO pays VAT

Next meeting - when & where

Next meeting: 13/14 June 2005 at BSA

Any other business

None

Closing of meeting

Ben thanks all participants for their contributions and the constructive discussions.

After the meeting the participants visited the DUS trial of CGN

Appendix 6: Draft minutes of the second meeting of CPVO rose project on May 17, 2005 in Hannover

Participants:

Burkhard Spellerberg (BSA)
Beate Rücker (BSA)
Katrin Siebert (BSA)
Thomas Drobek (BSA)
Peter Ohms (BSA)
Bob Cooke (NIAB)
Liz Scott (NIAB)
Huw Jones (NIAB)
Ton Kwakkenbos (CPVO)
Danny Esselink (PRI)
Joost Barendrecht (Dutch Board of Breeders' Rights)
Ben Vosman (PRI)

Opening

Burkhard Spellerberg opened the meeting and welcomed all participants.

Introduction of participants

Participants introduced themselves

Adoption of the agenda

The agenda was changed slightly. Selection of markers was first discussed among the experts (Danny, Huw and Peter). As last point on the agenda they reported back to the meeting.

Minutes of last meeting

Remarks made:

- Page 1: BSA has the intention to put the final database after the project on the web. The BSA has not this opinion. This issue should be discussed when the database is available.
- Page 2: CPVO reference number should read: application number. Denomination should read breeders reference. Furthermore it was suggested by Ton to include also: authority that made the investigation and data of picture.
- Page 3: Just having flower color group is not enough, more discussion needed

With these remarks made the minutes were adopted.

Investigation of available plant material

Number of candidate varieties under evaluation for CPVO in the different stations in 2004 were CGN 84; BSA 51; NIAB 16, total 151. However not all these varieties could be fully characterized due to the fact that the project started so late in the year. Major problem was obtaining the photographs and sometimes the inclusion of all required TQ characteristics.
The situation for these varieties is as follows:
BSA: all DUS characters available for the 51, not all TQ characters and markers still need to be done. In 2005 BSA will record the TQ characteristics of the 51 "old varieties and of the 41 new varieties.

NIAB: 16 for CPVO, all characterized. CGN for all 84 varieties all morphological observations were made and marker analysis performed. However, only 60 varieties could be photographed. All together this means that from the 2004 trial we will have a complete set of data for 127 varieties supplied by the CPVO to the DUS testing station.
In 2005 we will have the following numbers of varieties in the trials on behalf of the CPVO: CGN 89, NIAB 5 and BSA 41. So there will be enough material available to allow the development of the database containing at least 200 varieties. It was agreed that we would use the same procedure for getting the leaf material for marker analysis to the NIAB (BSA will bring the material on dryice to PRI, they will freeze dry it and sent it to NIAB).

Selection of database format

Beate presented the work developments in the Maize project. This project deals with the creation of a common morphological descriptive database of maize inbred lines only.

The goals are: Management of the reference collection
Exchange of information on candidate varieties between stations
Development of a system for updating

The database is developed in access. The process started with the creation of a data dictionary (description of field to be included and definition of the fields). This resulted in an agreed format and structure. The database contains: administrative data, information on first growing year and a morphological description. To date the dbase contains 6300 maize lines from different countries.

We need to produce a prototype database containing administrative data, morphological data and molecular data. The first thing that needs to be done is making a list of all items to be included. Ben will prepare a draft list and send this around (ASAP). Based on this list the BSA will make a data dictionary, which also will be send around (before july 15).

Data analysis tools:

In the project proposal we have indicated that we will evaluate the database for:

- Selection of reference varieties
- Exchange of data on current candidate varieties between testing stations
- Quality assurance within examination offices (verification of identity/authenticity
- Variety identification and technical verification

To facilitate this we might be able to use several tools.

With respect to Selection of reference varieties we can think of

(1)the tool available at BSA for selecting similar varieties on the basis of morphological data

(2)the use of digimum for selecting the most similar varieties on the basis of photographs. Digimum (NIAB) is a research program for selecting the most similar varieties based on fuzzy logic. It takes the target image and orders all other varieties according to that.

(3)The use of clustering programmes for selecting the most similar varieties on the basis of DNA markers.

Selection of photograph format

No problems were encountered. We will proceed as agreed in the previous meeting, making two pictures:

(1)flower from the top, on grey background (PRI will send details on background).

(2)a composite photo containing open flower from top and bottom, a knob, and a leaf., according to the examples send around.

Ruler should be included, in centimeters. Label photo with CPVO nr and national number. in future (part of TQ).

Morphological characters:

Morphological characters from CPVO/TQ-EN-011 to be included in the database:

4.1 Origin
5.2 Flower: Type
5.3 Flower: diameter
5.4 Flower colour group
5.5 Plant Growth Type
7.2.1. Special conditions: Group

Char. 5.4 and 5.5. will be part of the new TG. No problems encountered, continue as planned.

Selection of markers:

PRI and NIAB genotyped a selected set of 24 garden rose varieties using 20 STMS markers. From this set a subset of 12 markers was selected for garden rose genotyping. Selected markers are 1) polymorphic, 2) robust, 3) show unambiguously scorable patterns and 4) are evenly dispersed on the genome (see table). The set to be used for genotyping the hybrid tea

roses was already selected and overlaps to a great extent. The markers RhP50, RhP518 and RhAB73 proved to be useful for garden roses but do not fit the selection criteria for hybrid teas. Instead the markers RhM405, RhAB15 and RhO507 are included in the core set for genotyping hybrid teas.

STMS	linkage group	No. of alleles in 23 varieties	No. of allele phenotypes	scoring quality
RhO517	1	5	14	1
RhEO506	2	12	19	1
RhP50	3	11	13	1
RhD221	4	8	12	1
RhP518	5	7	15	1
RhE2b	6	7	12	1
RhAB73	7	9	18	1
RhB303	unknown	6	14	1
RhP519	unknown	7	15	1
RhAB40	4	11	18	1
RhD201	unknown	7	10	1
RhAB22	6	12	15	1

In a Powerpoint presentation the results were presented by Danny Esselink. This Powerpoint will be sent to all partners.

First report to CPVO

- An interim report needs to be produced before August 1 and a cost statement. CPVO does not have formats for this.
- Ben will make a first draft of the interim report and then circulate to partners.
- Regarding cost statement; Ben will check with CPVO whether we can use the EU format for this. If so, Ben will sent it around.

Report to BMT and TWO

- It was considered important to inform the BMT and TWO about our project. No results can be communicated but we can outline the objectives. Ben will participate in the BMT. For TWO not sure yet. Ben will inform CPVO on the intention to present the project in both meetings.

Next meeting - when & where

Next meeting: 23/24 Febr. 2006 at NIAB, Cambridge

Any other business

- Report to breeders. Ton will check whether the CPVO would like a meeting together with the breeders. Perhaps at the end of the project, summer 2006.
- Ton raised the question about keeping the DNA sample for reference. This might be a good idea but issues like costs involved, reliability and legal basis need to be addressed first.

Closing of meeting

Ben thanks all participants for their contributions and the constructive discussions. He also thanks BSA and Burkhard for the excellent organization of the meeting.

Participants visited the DUS trial of BSA

Appendix 7: Draft minutes of the third meeting of CPVO rose project on March 1, 2006 in Cambridge

Participants:

Burkhard Spellerberg (BSA)
Svenja Tams (BSA)
Ton Kwakkenbos (CPVO)
John Austin (Defra-PVRO)
Liz Scott (NIAB)
Huw Jones (NIAB)
Richard Horsnell (NIAB)
Adrian Pickett (NIAB)
Joost Barendrecht (Dutch Board of Plant Varieties)
Ben Vosman (PRI)

Opening

Liz Scott opened the meeting and welcomed all participants. She also informed the participants on changes at NIAB and changes in the rose DUS work

Adoption of the agenda

The agenda was changed slightly. Following points raised by Ton Kwakkenbos were put on the agenda:

- how examination offices think they could use the results of the project for their rose crop group
- assessment of the costs of a DNA profiling
- keeping DNA samples

Minutes of last meeting

Remarks made:

- Reports to BMT and TWO have been brief. Only the fact that the project is ongoing has been mentioned.
- Report to breeders is on the agenda of this meeting.

With these remarks made the minutes were adopted.

Final discussion on demo version of the database.

The current state of the database was discussed by Burkhard. All data are collected in 4 excel files (administrative data, morphological data, images and molecular data). The link between the different files is made by a 'leading number'. The management of the database is done for the moment by the Bundessortenamt. All files are brought together in an Access database. The following additions/changes were made to the data dictionary:

- Appl_nr_CPVO should be indicated without slash
- Appl_nr_nation is a text field
- Breeders reference should be added as a text field
- Denomination should be the approved one.

The final version of the files will be send by the BSA to PRI and NIAB before March 10. In this mail they will include also a file in which data have been entered as an example.
All participants will enter all data in the database and send the files to Burkhard before March 31. This includes a CD containing the photos of the candidate varieties. Burkhard will give a serial number to all entries and send the final version of the database to all participants before April 30.

Marker analysis:

PRI: Ben presented a short powerpoint showing the data. 12 STMS markers (4 multiplexes of 3 markers) were used to genotype the hybrid tea roses. Allele assignments were made according to the reference alleles, which were included in each run (5 to 7 varieties). From the 2004 trail 123 samples (95 applicants, 6 varieties for comparison, 12 standard varieties, 10 duplicate plant samples) were genotyped. Of the 2005 trail 152 samples (114 applicants, 14 varieties for comparison, 12 standard varieties, 12 duplicate plant samples) were genotyped. The applicants include also some national applications. The results were as follows:

- All varieties showed unique profiles, except the mutants. There was one known mutant that did not show a pattern identical to the original variety, In this case one of the alleles was just below the threshold level. All other mutants showed profiles identical to the original variety.
- All duplicate samples except one (sampling error or variety impurity)
- All standard varieties between the years

Varieties with a similarity coefficient between 0.90 – 1.0 are considered as having identical profiles, unless difference is based on 3 STMS markers. In this way problems with alleles just below the threshold level are avoided.
At present Joost is analyzing the marker results of PRI

NIAB: NIAB encountered some problems with DNA extraction from garden roses. An extra DNA purification step was necessary to get DNA profiles from the material. Due to these problems they have not completed the full analysis yet. At this moment they have a full dataset for approx. 150 lines including reference varieties.

It was decided that Danny and Huw should get together ASAP to solve the problems and to agree on the final allele designations. Huw proposed to visit PRI on March 27. However this might already be too late. Ben will discus ASAP with Danny and get back to Huw regarding another suitable date.

As not all molecular data are available it was not possible to draw any further conclusions at this moment. A proposal was made to evaluate the dendrograms in the following way:

- Do all original varieties and mutants derived from them come out together
- Try to explain all similarities with values larger than 0.8-0.85
- Check whether varieties that were used for comparison with a particular variety also group with that variety

Evaluation of database

A discussion was held on how we shall approach this next project phase. In the project proposal it was stated that we would like to evaluate the database for the following objectives

- Selection of suitable reference varieties
- Quality assurance within examination offices (verification of variety identity)
- Variety identification and technical verification

These points are still very valid and the discussion overlaps with the question raised by Ton on how the different testing stations will use the database.
The database will contain administrative data, TQ information and a photograph, so in principle all information needed to select varieties for comparison in the traditional way is there. In addition, the database will contain molecular data. Having all this information in one database was felt to be very valuable. Prescreening could be carried out using TQ data and the photo. The molecular data could be used for identification, e.g. to check the identity of material that was ordered from breeders and should be used for comparison (Quality control). In addition the data could be used to spot mutants.
Points raised in relation to the follow up of the pilot project:

- Should CPVO enter the administrative data, TQ information and the photograph into the database before the request for analysis is send to one of the testing stations? The advantage of this is that all testing stations have information on what is under study in the other station as well. The CPVO is not sure that it can add the picture, provided by the applicant, to the database in relation to restrictions of use of the pictures/publication of pictures of candidates that could influence the novelty of a candidate. Would be nice when CPVO enters the data, but some concerns were raised regarding the scanning in of the photograph, as colors might change. Recently it was decided

with the ornamental experts that the CPVO will ask for a printed photo submitted by the breeder to send to the station that will carry out the DUS test of the candidate variety.

- What would be the minimum content of a future data base.Should the reference collection be included? More than 3000 varieties should be included for the garden roses. It was felt necessary that at least all EU protected varieties and all varieties that are still in trade should be included.
- Management of the database needs to be very strict. Who should do this? CPVO?
- Advantages of the database:
  - Improvement of quality of DUS testing
  - Improvement of quality of protection
  - Database will reduce the work to find similar varieties
  - Higher reliability as all information for the whole of Europe is contained within one database
- Advantages should be looked at from the point of all users
  - Should breeders have access? Yes, register of CPVO is also public
  - Breeders should be able to use the dbase to enforce their rights
- Maintaining the database
  - Should CPVO maintain the database? NL, UK and DE were in favour to put the database on a central place, preferably at the CPVO,, testing stations should be able to read/write
  - It should be studied what data, to include in the database, are already available on national level at the test stations. Costs for sending information (data) on varieties to the central database (at CPVO) should be compensated
  - PVRO and board for BVR in the Netherlands should also have access to central database
  - Reumeration of test offices for delivering of data/photos for the data base

Blindtest

It was decided that the blind test will be carried out by PRI and NIAB. PRI will analyse a minimum of 12 samples from the outdoor roses. As PRI still has leaf material from BSA in store, Huw will indicate which samples should be analysed. He will include a number of samples that gave problems with DNA extraction. NIAB will receive 12 DNA samples from PRI. All data are to be send to Burkhard after completion of the analysis.

Points raised by Ton

- how examination offices think they could use the results of the project for their rose crop group
- assessment of the costs of a DNA profiling
- keeping DNA samples

First point is covered by previous item
Regarding costs: Ben indicates that cost would be around 150€ per sample for 9 markers, when number of samples is more than 30.
Keeping DNA samples: cost include deprecation of equipment and running cost of freezer. Until now no clear picture of what the cost would be. In addition problems regarding liability need to sorted out. CGN for the moment stores leaf samples. It was questioned whether this is the best solution. Probably storing clean, freeze dried DNA samples is better. DNA samples could be used to screen reference varieties, used in the case of technical verification and for the enforcement of rights. It seems that database information of DNA profiles is not sufficient. Analysis need to be run again. Storing DNA has the advantage that it can be used to obtain DNA profiles in future with newly developed tools.

Deliverables

This item was not discussed in detail. It looks we are well on track with the deliverables.

Final report to CPVO

The final report should be send to the CPVO before the first of September. As this is also holiday time we should aim at completing the first draft of the final report around the end of June. Ben will make a first draft and then circulate to partners. The final report will be delivered to the CPVO on a CD, which also includes the database.

Report to BMT and TWO

It was considered important to inform the BMT and TWO about the results of our project. The TWO meeting is in Brasil August 2006. Liz, Ton and Joost will participate in that meeting. They could present the project. The BMT is in Seoul in November 2006. Ben would like to go there to present the project. However, the meeting is after the end of the project. Additional funding will be needed. Ton will check with CPVO if there would be funds available from CPVO, to finance the participation of Ben in the BMT meeting.

Contract & finance details

Financial report will be made in the same way as the interim financial report. Ton will check with the CPVO office whether this is OK.

Next meeting. Presenting the final result to the CPVO?

For the moment a new project meeting was not felt necessary. Presenting the results to the rose breeders was felt very necessary. During that meeting also the cost aspect of the DNA fingerprinting should be discussed. The CPVO will organize the meeting with the rose breeders and professional organizations in a similar way as the start up meeting in Angers. Project partners feel that cost associated with the meeting should be covered by the CPVO. The following dates were suggested: September 19/20 or 28/29, with a strong preference for the latter one.

Any other business

None

Closing of meeting

Ben thanks all participants for their contributions and the constructive discussions. He also thanks Liz Scott for the excellent organization of the meeting.