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GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR ROSE

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PROPOSAL FOLLOW UP OF R&D PROJECT:
A EUROPEAN COLLECTION OF ROSE VARIETIES

Document prepared by experts from the European Community

INTRODUCTION

1. DUS testing of roses is currently carried out by 3 examination offices, Bundessortenamt (garden and pot roses), the Raad voor Plantenrassen (greenhouse roses) and NIAB (garden roses). The objective of the project is to create a unified database of the most important rose varieties within the European Union (EU) member States, which can assist in the management of reference collections, help in verifying the identity of reference varieties supplied for DUS testing and can be used to help breeders tracing potential infringements. A copy of the final report of the project can be found at http://www.cpvo.europa.eu/documents/techreports/RD_rose_project_final_report.pdf.

OBJECTIVES OF THE PROJECT

2. The first objective was the construction of an integrated database containing:

(a) key morphological descriptors;

(b) one or more photographs of the variety; and

(c) molecular profile (based on DNA microsatellites).

3. The second objective was the evaluation of the database for:

(a) selection of suitable reference varieties;

(b) exchange of data on current candidate varieties between testing stations;

(c) quality assurance within examination offices (verification of the variety identity); and

(d) variety identification and technical verification.

SUMMARY OF THE FINAL REPORT OF THE R&D PROJECT

4. The project has delivered a pilot database (Access format) containing the following information:

(a) administrative data of approximately 400 varieties – 314 Community Plant Variety Office (CPVO) candidates;

(b) morphological data (set of standard descriptors);

(c) pictures (193 varieties with single pictures – 184 with double pictures); and

(d) molecular data of 364 varieties (only from PRI)

5. Final report: evaluation of the database:

(i) Morphological data:

- Origin: seedling/mutant: was felt to be useful.
- Flower type: single/semi-double/double was felt to be useful. However, for greenhouse roses this characteristic is less useful because over 90% of the varieties are double flower type.
- Flower color (group): needs careful examination: 77 out of 220 varieties needed changes in the data scored by the applicant
- Plant growth type: more useful for garden roses because all greenhouse roses belong in the same group.
- Group: an essential characteristic, because the type is decisive for the test (garden, cut-flower or pot-rose).

(ii) Photo: was judged to be very useful. However, for greenhouse roses it was felt to be less useful to have the composite photo.

(iii) Molecular data:

- The database contains molecular data for 275 greenhouse roses. The data are complete and there are no missing data.
- For garden roses the database contains molecular data for 145 varieties, 14 data are missing.
- Duplicates in case of greenhouse roses: 35 varieties were tested in duplo. 34 were identical.

- Mutants in case of greenhouse roses: 37 varieties (mutants + parent varieties) were tested. All mutant/parent groups showed identical patterns.
- Mutants in case of garden roses: 10 varieties (mutants + parent varieties) were tested. Eight (8) mutant/parent varieties had identical patterns.
- The molecular data obtained by NIAB could not be used for the database. The main explanation given in the report is the fact that DNA of garden roses is more difficult to clean and analyze. Finally, only the data obtained by PRI were used for inclusion in the database.

6. Discussion molecular data ←→ morphological data: Questions for discussion:

(1) Do mutants and parents come out together in molecular data?

7. For greenhouse roses parent and mutant varieties group clearly together. Some varieties that are claimed by the breeder to be a variety coming from crossing, appeared to be a mutant. In one case a non-similarity of a mutant with its parent could be traced back to a mistake in the sampling in the greenhouse.

(2) Would it be possible with the knowledge of the morphological and administrative data to explain the varieties that show similarity in the molecular data?

8. NIAB – garden roses: in general the molecular data of varieties, resulting from crossing was very different. Bundessortenamt – garden roses: grouping of molecular data gave similar varieties or varieties that are genetically close. The similarity of seedlings was found to be always lower compared to the similarity of mutants.

(3) Check whether the selected reference varieties group together in the molecular data?

9. As can be concluded from (2), candidates and reference varieties usually do not group together very closely unless they are genetically, closely-related.

10. During the meeting the discussions were focused on the outcome of the project and the possible use in our DUS system.

11. Summary discussion in the final report:

(i) Morphological data: useful, but some Technical Questionnaire (TQ) data will need careful checking by the examination office (e.g. color groups)

(ii) Pictures: important; however, the composite photo was felt to be less useful for greenhouse roses. To take into account:

- Effect of the screen, used to display pictures
- Labor costs
- Database would be useful when it contained a large number of varieties (several thousand varieties: protected + on the market)
- Such a database would need a selection tool

(iii) Molecular data:

- Highly discriminating
- Mutants are easily detected
- Difficult to merge data from 2 laboratories - ? caused by the type of rose?
- DNA can be stored – needs further elaboration

Five possible uses for the database

(1) Characterization and cataloging of reference collections

(2) Pre-screening and selection of reference varieties

(3) Exchange of data between examination offices

(4) Strong reduction of number of varieties in living reference collections

(5) Quality assurance: verification of identity of reference varieties/authenticity check

DISCUSSION OF THE RESULTS AND POSSIBLE FOLLOW UP OF THE PROJECT

12. In this part, the possible use of the outcome of the project will be discussed in relation to: I Use for DUS testing; and the other use: II Possible use for variety identification. The discussions on the possible use for variety identification will be focused on the use of molecular marker profiles.

13. The objectives of the project were:

Firstly, the construction of an integrated database containing:

- Key morphological descriptors
- One or more photographs of the variety
- Molecular profile (based on DNA microsatellites)

Secondly, the evaluation of the database for:

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

I. Discussions in relation to DUS testing

14. The project has succeeded in creating an integrated database with the foreseen content. Key morphological descriptors have been chosen. A harmonized approach in relation to the pictures in the database has been achieved and molecular profiles of approximately 380 varieties have been included in the database.

15. In relation to the continuation of the current database, two situations should be taken into account:

(1) In relation to the present system of DUS testing (centralized testing)

(2) In relation to a possible future system of DUS testing (depending on the consequences of the strategic discussion: could be a non-centralized testing situation for roses)

(1) In relation to the present system of DUS testing with centralized testing for most rose types

16. In order to be able to decide on the implementation of such a database, the following questions should be raised and reflected upon in the light of the results of the project as presented in the final report:

Added value for the system?

17. All test stations have a database to select reference varieties. Since the different types of rose varieties are separated, the need/added value for a centralized database for DUS testing might not be in balance with the work to update and maintain the database, taking into account that thousands of rose varieties are considered to be of common knowledge. It can be concluded that under these circumstances the database does not provide an added value to the DUS testing of roses.

Risks of overlapping of different types of roses?

18. The risk of overlapping of the different rose types is present (in particular some garden types can also be cultivated for cut flowers), but practice demonstrates that up to now and with the knowledge we have, this overlap has not happened or caused major problems in DUS testing. In any case the database would not help because the variety description of the same variety grown as an outdoor or as an indoor plant is expected to give different expressions for some characteristics and would, therefore, give a (partly) different variety description indoor and outdoor.

Which should be the size of the database in order to have a useful tool?

19. A centralized database would only be of value as a basis to test distinctness if it was as complete as possible. The database should, therefore, as a minimum include all the varieties that are protected in Europe and all varieties that are marketed in Europe. A rough estimation leads to 4,000 varieties that should be included.

Would the data help for the selection of suitable reference varieties (pre-screening)?

20. Although the aim of the project was not to study the genetic distances of varieties and the relation between morphological characteristics and the DNA fingerprint, it can be concluded

that (within the scope of the project) mutant varieties have the same fingerprint. Further studies seem necessary to study the relation between morphological differences and the genetic distance. With the present knowledge of this subject, it can be expected that this correlation would be low. With the present knowledge, it can be concluded that the molecular data would be of low value in relation to prescreening for rose.

21. In the case of roses (except for a limited range of garden roses), the examination offices do not have a living reference collection. Usually, the reference varieties are ordered. Molecular data can be used for a variety identity check of the ordered reference variety in this case. It needs to be kept in mind that with this identity check it cannot be fully verified that the reference variety exists unaltered with regards to its phenotypical expression. In particular, in the case of mutants it cannot be verified with 100% reliability, because all mutants of the same mutation group are expected to have the same fingerprint. Taking into account the necessary costs to put in place such a procedure, the maintenance of a living reference collection of varieties of most frequent use as a reference might be more efficient.

Financial aspects?

22. A centralized database would only be of value as a basis to test distinctness when it was as complete as possible. This would bring enormous costs when administrative-, morphological- and molecular data and pictures of all varieties should be included. Furthermore, the method of DNA extraction and fingerprinting should be further studied in relation to garden and pot roses, with additional costs.

(2) In relation to a possible future system of DUS testing:

23. Since the definitive future system of DUS testing for roses is not clear for the moment it is difficult to anticipate the possible need for a centralized database in the future.

Conclusion

24. Having put the added value and the financial aspects together, it is clear that we <u>do not recommend</u> the implementation of such a database for the present system of DUS testing for roses.

For discussion with the breeders/examination offices:

25. What is the opinion of the applicants in relation to the continuation with the database in the frame of the DUS testing in the present situation?

II <u>Discussion in relation to other use of the results of the project, here focusing on the use of molecular marker profiles only</u>

26. In the view of the CPVO, the results of the project open the possibility to use molecular profiles of varieties for variety identification in relation to enforcement of Community Plant Variety Rights.

Conditions of using molecular techniques

Technical conditions

(a) discriminating power

The discriminating power of the markers used should be sufficient. Although a limited number of varieties were used, the project has shown, for the moment, sufficient discriminating power.

(b) standardization of the protocol that is used

A full protocol for sampling, DNA extraction and fingerprinting should be developed. Elements from the present project can be used as basis for such a protocol, in particular for cut flower roses, although some fine tuning might be necessary: the reproducibility of molecular genotyping was good, but not 100% reliable. If considered necessary, further research should be done in order to improve the DNA quality of the sample of garden roses. If relevant, because pot rose types were not included in the project, the DNA extraction and fingerprinting of this type of roses should be studied.

(c) reproducibility of data between laboratories

The current project demonstrated problems in reproducibility between the 2 laboratories that performed the DNA fingerprinting. Should we further investigate reproducibility or aim for a centralized analysis of the DNA samples?

(d) repeatability over time

The repeatability over time should be investigated. The current project does not answer this question. Furthermore, the stability of the DNA sample over time should be investigated.

(e) accessibility of the methodology and markers

The methodology and markers were available for the project. Availability for future use should be studied.

(f) protocol for keeping a standard DNA sample

Since the project opens the possibility to keep DNA samples of the standard samples which were used for the DUS test, a protocol of keeping of the samples should be developed.

(g) Breeders' role: maintenance of the variety

In order to have a reliable system, breeders would need to maintain their varieties uniform and stable in the expression of their "fingerprint".

(h) Costs aspects

It is expected that a DNA profiling will cost 150€ - 200€ per sample for the 9 markers selected in the project, when more than 30 samples are supplied in one batch.

Legal conditions

(a) What would be the legal status of fingerprints attached to the variety description? Should the fingerprint be an ‘official’ part of the variety description or as an annex only?

(b) Procedure and responsibility for conservation of the standard DNA sample.

(c) How to handle requests for using the standard sample by third parties?

In order to have a clear view on the legal conditions, a further study of the subject in the light of the Community regulations would be necessary. Elements of the legal conditions should be taken into account if protocols for fingerprinting and DNA sample-keeping are developed.

For discussion with the breeders/examination offices

(a) Would you support the development of a system where a DNA fingerprint is attached to the variety description (as annex) of varieties applied for Community plant variety rights?

(b) If there is an interest, should fingerprints be attached as a standard procedure or only on request of the applicant?

(c) Is there an interest for all rose types (cut flower, garden roses, pot roses)?

(d) Would you be in favor to keep a standard DNA sample and/or a standard living sample of your variety?

(e) Should the procedure to keep a DNA sample be dependant or independent from the request for a molecular profiling of the variety in question?

(f) Are you willing to pay the extra costs?

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