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A DNA DATABASE FOR ROSE: DEVELOPMENT AND VALIDATION OF A SNP MARKER SET

Document prepared by experts from the Netherlands, the International Rose Breeders Association (IRBA) and the International Community of Breeders of Asexually Reproduced Ornamental and Fruit-Tree Varieties (CIOPORA)

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INTRODUCTION

1. The ultimate goal is the construction of an internationally harmonized Rose DNA database with varieties of common knowledge and the implementation in the Rose DUS testing system. The construction and use of the potato database will be followed as an example (see documents BMT/17/11 and BMT/17/12). For the potato database, the genotypes are generated using 9 SSR markers. For Rose also, SSR markers are available (Esselink *et al*, 2003). However, in the light of the rapid technological developments, it is generally acknowledged that SNP markers have many advantages over SSR and are therefore the preferred marker type. The first step towards a database for rose is the development and validation of a SNP marker set for rose. This is the scope of the current research project.

2. Project partners: Naktuinbouw (project coordinator) and Rose Breeders joined in the International Rose Breeders Association (IRBA) which is the CIOPORA Crop Section Cut Rose (10 companies)

ROSE AS A MODEL FOR ORNAMENTAL CROPS WITH A COMPLEX-POLYPLOID GENOME

3. Rose is an important ornamental crop and the largest grown in many countries. Also in the scientific world, rose is studied intensively by academic groups around the world stressing the importance of this genus. Recently, two papers were published to describe a reference genome sequence for Rose (Hibrand Saint-Oyant *et al*, 2018; Raymond *et al*, 2018). It makes Rose a model crop for ornamentals with a complex genome (as most commercial varieties are tetraploids).

4. The importance of this crop is also reflected in the high number of applications for Community Plant Variety Rights each year. These increased numbers of applications each year is the result of breeding activities that take place all over the world and produces a broad diversity of types and varieties. Three examination offices are involved in DUS testing of the new applications: National Institute of Agricultural Botany (NIAB) in the United Kingdom is responsible for rootstock and outdoor garden types, Bundessortenamt (BSA) in Germany for outdoor types and pot roses and at Naktuinbouw in the Netherlands, the DUS examinations of all greenhouse, cut flower applications are performed. In this project we focus on the cut flower types since the challenges and risks in the DUS system are predominantly for this type.

CHALLENGES AND RISKS IN THE CURRENT DUS SYSTEM

5. One of the major challenges in DUS testing of rose is to ensure that new applications are clearly distinct from varieties of common knowledge. The number of existing varieties (common knowledge) is high. Over 25.000 varieties of modern roses have been described by Cairns (Cairns, 2000; Vosman, 2006). It is not realistic to compare all new application to the complete collection of common knowledge varieties. There is no full overview of these varieties due to the rapid development of new varieties and the global character of breeding activities. In addition, it is not possible to maintain all varieties vegetatively in the living reference collection. Due to phytosanitary restrictions it is very difficult and costly to import living reference materials from

other collections. Moreover, morphological variety descriptions that are generated in different climate zones with various environments, by different institutes and DUS experts cannot be compared directly. The quality of the DUS decision therefore depends largely on the management of the reference collection and design of the DUS trials that contain an acceptable and workable number of comparing varieties without missing any relevant variety of common knowledge as a reference.

BENEFITS AND APPLICATIONS OF A DATABASE FOR ROSE

6. DNA profiles available in well-organized databases with DNA data of varieties of common knowledge, are considered as not influenced by environment and are a valuable tool to overcome above mentioned challenges and risks and guarantee the efficiency and quality of DUS tests in the near future.

- No restrictions on the number of varieties of common knowledge in such DNA database.
- No restrictions on phytosanitary regulations as DNA can be exchanged instead of whole plants/seeds. Exchange of plants/seeds can be limited to similar varieties.
- DNA profiles are not sensitive to external environmental factors and are considered as an objective description of the genotype.

DEVELOPMENT AND VALIDATION OF A SNP MARKER SET FOR ROSE – PROJECT OBJECTIVES AND TASKS

7. The first step in the construction of a database for rose is the development and validation of a SNP marker set. Although we focus on the cut flower type, the final SNP marker set should also be applicable for DUS testing of other types (outdoor, garden and pot rose types). Several tasks can be identified:

- Identification of rose-specific SNP markers based on screening the 68k Axiom Rose Genotyping Array (Affymetrix)
- Selection of SNP markers: *in silico* selection based on quality criteria and *in vivo* selection of SNP markers based on quality criteria and performance testing SNP detection methods based on a 'multiplex targeted amplicon sequencing' approach
- Optimizing the detection method to improve SNP calling quality
- Validation/evaluation of SNP markers and final selection
- Publication of the results

MEMORANDUM OF UNDERSTANDING (MOU) AND PLANT MATERIAL

8. By signing a detailed MoU, all project partners agreed on the conditions of the cooperation and content of the project performed by Naktuinbouw. It was agreed that the breeding companies of the IRBA group of CIOFORA supplied Naktuinbouw with plant material for both the training set (representative for the diversity of modern rose and required for the identification of SNPs) and the test set (needed for the validation/evaluation of the SNPs). The MoU provides for both legal and practical guidance during the project.

STATE OF AFFAIRS

9. At this moment, the training set of 164 varieties and 28 control sample are being genotyped using the Axiom Rose Genotyping Array by an external partner. The training set contains commercial varieties, breeding stock, parental lines and sibs from 9 different breeding companies. The samples represent a broad genetic diversity. The focus is on cut flower type but 10-15% of the samples represent other types except for rootstock species. All samples are processed under code to guarantee anonymity and objective observation of the results.

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