INOVATIVE USE OF BIOTECHNOLOGY IN GARDEN ROSE BREEDING, IN CENTRAL EUROPE

ABSTRACT;

Breeding of garden roses is a long- term investment. The time range of classical breeding is 8-10 years. Without modern methods, such as bio-informatics, applied statistics and molecular markers techniques it would be not only very strenuous but also time-consuming when it comes to development of modern and healthy roses. **Pheno Geno Roses** team (Serbia & Netherland) have made the decision to invest in marker-assisted breeding as a part of biotechnological tools in rose breeding in a pioneering and innovative way which make us unique as a company in our field of work. On this topic, we have been closely collaborating with the Plant Breeding Department of Wageningen University. First results of this collaboration can be seen in our Winterjewel® Collection which is developed by using MAS, a feature that makes them unique.

Last summer, 58,000 crosses were made in the Netherlands and Serbia. Of that number, we harvested 242,000 seeds in the autumn of 2015 and with an average germination rate of 30% we obtained more than 70,000 new rose seedlings in the spring of 2016. Of that number 5,000 seedlings were chosen for further selection. Each of these beautiful new seedlings is a potential new variety on the market!

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Roses are the most important flowers in today's global flower market and have been the centre of attraction for consumers and breeders for hundreds of years. Modern roses (*Rosa hybrida*) have resulted from extensive hybridization of wild rose species and have various flower colours. Because of the ornamental value of roses, there have been many studies on rose breeding and applied scientific work.

Pheno Geno Roses team, stands as a synonym for expertise and quality where, eight years ago, group of experts brought in their will to learn and their passion for breeding of new varieties of roses by applying state-of-the-art techniques and scientific methods in the area of garden rose breeding (picture 1 breeding scheme). Our vision is to become a leader in the application of modern techniques, combining them with the art of breeding, so we could fashion roses by everyone's liking. Bringing satisfaction to our customers as well as listening to their needs is something we've made a commitment to. In order to achieve all stated above, we must employ knowledge, as well as innovative use of biotechnology in rose breeding.

Innovative projects that we are currently undertaking and developing are: Winter-hardy Rose project, Food project, Polyploidy project, Data Base and Breeding software development.

WINTER-HARDY ROSE PROJECT;

Breeding of garden roses is mostly based on classical breeding. Research related to this project was to enable **m**arker-**a**ssisted **b**reeding **(MAB)** in this auto-tetraploid crop, starting with two essential traits for the booming Central- and Eastern-European markets: winter-hardiness and recurrent flowering. Sufficient levels of these traits are known to exist in Canadian cultivars and wild East European germplasm, while winter hardiness is insufficient in Western European cultivars.

According to literature the current rose breeding is empiric and little is known on garden rose genetics, the aim of our research in **Winter-hardy rose project** was to provide insight into garden rose diversity and differentiation, and develop new methods for quantification of allele dosage in tetraploid cultivars, and develop new strategies for markers. Parallel, the mapping population was made by crossing European and Canadian cultivars and their progeny was exposed to low temperature under controlled (cold chambers) and uncontrolled (trial field) conditions.

All these findings enabled creation of a high density genetic map using SSR and SNP markers and detecting QTLs for winter hardiness. As an outcome, a set of markers enable to distinguish susceptible from for winter hardy cultivars in an early stage (few set of leaves) were proposed, which will help avoid long trial field testing (which depends on the climate) and shorten the breeding period.

As a breeding company, we want to cross in specific traits, like winter hardiness. The selection process of these traits in the bred roses is very time-expensive. Each population has to be observed for years to select properly. Molecular markers offer an improved method for the selection of the bred roses. These markers make it possible to notice indirectly whether a rose has the specific trait that you are looking for if genes that represent these traits are linked to these markers. The selection process will also be much more efficient, because if the gene is not present at first, there is no need to observe the whole population for years.

MAS represents indirect selection of traits using molecular markers that are linked to the genes. MAS comprises a range of molecular methods and approaches that can improve selection methods and might increase the efficiency of breeding by permitting earlier selection and smaller population size during selection. MAS is useful in breeding of traits that are difficult to evaluate (as monitoring is expensive, time-consuming, and/or unreliable), for traits whose selection depends on developmental stage and/or environmental conditions (quality traits, disease, stress, and pest resistance). Furthermore, selection for genotypes resistant to abiotic (cold, drought tolerance, etc.) or biotic (disease resistance) stresses is complicated by the fact that phenotype response often depend on a combination of climatic factors. It is often essential to repeat an experiment for a few years, while correcting for the effects of additional factors. Basically, offspring can be tested at the stage of seedlings, which shortens the period of selection and brings cultivars to the market much earlier.

Pheno Geno Roses is the only company that is using this method for expediting the breeding of roses. The roses from our Winterjewel® Collection are the first roses developed by MAS, which makes them unique. These roses are developed by combining genes for winter hardiness from Canadian roses with beautiful flowers of European roses.

In a future we plan to use MAS for several other breeding goals, for example heat tolerance and disease resistance of roses. Also In our “Food Project” MAS will be used to breed delicious rosees for petals and rose hips.

These results will give a competitive advantage in the breeding of new cultivars, evident from the larger market share in segments with higher prices for involved companies. This project was conducted at WUR-Plant Breeding, represented by Rene Smulders and Paul Arens, as well as Pheno Geno’s R&D Manager Mirjana Vukosavljev - Olujić who completed her PhD thesis on this project.

Next to this research, we are about to start a new project that will focus on genetic analysis of polyploid roses together with 10 breeding companies of other polyploid crops.

POLYPLOID PROJECT;

In order to enable a more successful hybridization, and considering the presence of polyploidy in *Rose sp.*, germplasm, polyploidy of selected genotypes was measured. We use that information in our breeding plan every year. Based on these findings hybrid combinations from parents of

different levels of polyploidy were selected and crosses between genotypes with different ploidy levels were made.

The effect of crosses between cultivars of different ploidy level often depends on direction in which cross was made (4n x 3n is not the same as 3n x 4n). Also, the final expression of quantitative traits (size for instance) depends on ploidy level. To estimate the effect of ploidy level on success of cross and size of morphological traits we will conduct a test, and make diallel crosses between cultivars of different ploidy level.

Based on literature, phenotyping and earlier testing PGR implement a **pedigree based analysis (PBA)** on most promising varieties used as parent line. We are making family tree for 5 generation to reduce the negative effect of inbreeding depression. Additionally, the **ploidy level** of all promising cultivars are tested in order to improve pollination and germination success.

In diploid crops, we see a large development of Marker Assistance Breeding. In polyploid crops we see much more difficulties in the inheritance of traits. In future, we want to develop both methods and software for genetic analysis of polyploidy crops.

USE OF ROSE-HIPS AND PETALS AS A FOOD PRODUCT

Roses as salads are already long time part of our food. It is eaten for over 1000's of years already in China and we can still find them on every corner of the streets in India. We know now, why they are eaten so often. Some of our rose cultivars have 20 times more antioxidants as cultivated blue berries and 5 times more than wild blue berries.

In 2015 Pheno Geno Roses has started a food project in cooperation with Frank Coenders, a rose grower in the Netherlands. The aim of this study is to identify rose cultivars of which the petals or hips are suitable for eating.

Eating roses is very healthy, because they contain a lot of antioxidants. This is especially true for the hips, which not only contain a large amount of antioxidants, but also have a high concentration of vitamin C. Additionally, composition of rose petals (vitamins, antioxidants, phenols, carotenoids, etc.) candidates them as a new potential source of food. Till now rose petals are used in wine and jam production, while their usage for fresh salads is not considered widely. At the moment, mainly wild rose cultivars are used for food. That is a trend we are hoping to change by highlighting the importance of domesticated cultivars. During this large-scale study around 340 cultivars in total were tested.

The aim of this project is to detect which rose cultivars are the most valuable for the nutrition (from biochemical aspect), which are the most appropriate for human intake (taste characteristics), to detect which parents are good donors of characteristics related to nutrition, and to detect tools for MAB (QTLs).

To be a good rose for the food industry the flowers and hips should not only have a nice taste, but should also have a good nutritional value, look good and have a nice fragrance suitable for food.

During the study, we map all these different traits. Study is conducted that will show us how the flowers and hips look, taste, smell and grow. Next to these phenotypic values, attention is given to the nutritional value of the flowers and hips. We are currently looking at the content of malic acid, citric acid, vitamin C, total antioxidants and sugars. In addition to these measurements, we will also look at the DNA to find genes for these traits. These genes can be used in marker assisted breeding.

Using the results following this study, we can open new market possibilities. Based on the results, we can start breeding new cultivars which are perfectly suited for the use as a food ingredient. Since this kind of large scale study has never been previously performed and no other rose breeder is currently breeding for the food industry, this gives us some unique possibilities.

DATA BASE AND BREEDING SOFTWARE DEVELOPMENT

The key features of this project are focused on building a database with breeding information, as well as providing useful output which increases the efficiency of breeding. It covers the most important breeding projects (from pollination to selection of commercial varieties) and has a goal to store breeding data, to use algorithms for planning specific projects and to provide an overview of breeding results in different reports.

IMPROVING GERMINATION OF SEEDS FROM HYBRID ROSE VARIETIES

Aim of this study was to provide a better understanding of the causes of low seed germination of roses (roughly 30%). Rose seeds are subject to two types of seed dormancy (physical and physiological) due to the stony endocarp and physiological barriers within the seed, which inhibit germination. In addition, low seed quality can be a major cause of low germination. In order to improve rose seed germination, dormancy has to be broken, while sustaining a high seed quality. Therefore, five main experiments were performed focusing on sustaining a high seed quality and breaking seed dormancy of cultivated roses. Part of this research was conducted at HAS Den Bosch in the Netherlands. (picture 2)

Breeding will always remain an art, where the most important step is to select properly (picture 3,4,5,6,7). There are currently more than 50,000 roses budded on the trial fields in Serbia. These are monitored weekly and all the properties such as flower colour, fragrance and disease susceptibility are recorded. All these data will be used again in a new breeding program the following year. This is how our Serbian breeding company got its name: **Pheno Geno Roses.**

The breeding of roses means knowing what consumers and growers want. To meet this need, many investments, researches and patience, but also the great love for the roses are essential!

Picture 1. Pheno Geno Rose Breeding scheme

Picture 2. Germination of rose seeds and seedlings

Picture 3. Rose seedlings


Picture 4,5,6,7, Selected seedlings for further selection



Picture 8,9 Pheno Geno Roses from Frayla and Winterjewel collection