

AgroSym

# BOOK OF PROCEEDINGS



IX International Scientific Agriculture Symposium  
"Agrosym 2018"  
Jahorina, October 04-07, 2018

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Dusan Kovacevic

### Technical editors

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## FROM THE GREGOR MENDEL'S GARDEN TO A MOLECULAR MARKER LAB: CUTTING EDGE OF BREEDING GRAIN AND FORAGE CRUCIFERS IN SERBIA

Ana MARJANOVIĆ JEROMELA<sup>1\*</sup>, Dragana MILADINOVIĆ<sup>1</sup>, Petar MITROVIĆ<sup>1</sup>, Nada GRAHOVAC<sup>1</sup>, Aleksandra DIMITRIJEVIĆ<sup>1</sup>, Dragana RAJKOVIĆ<sup>1</sup>, Sanja LAZIĆ<sup>2</sup>, Dragana ŠUNJKA<sup>2</sup>, Aleksandar MIKIĆ<sup>1</sup>

<sup>1</sup> Institute of Field and Vegetable Crops, Novi Sad, Serbia

<sup>2</sup> Faculty of Agriculture, University of Novi Sad, Serbia

\*Corresponding author: ana.jeromela@ifvcns.ns.ac.rs

### Abstract

A complex and strategically structured research on oil-rich grain, vegetable and forage crucifers at the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS) is based upon maintaining and sustainably utilising the germplasm collections of each crop. It comprises conventional and molecular breeding, biotechnology, agroecology, physiology, biochemistry, agronomy and seed science as well as commercial production for local and international markets. Here, we shall focus on oil-rich grain and forage crops, namely rapeseed, black and white mustards, forage kale and false flax. All collected accessions of our collection were phenotypically and cytogenetically characterized, including the monitoring and examining flower morphology, pollen features and number of chromosomes. The Mendel's rules are the basis of all the methods in breeding cultivars and hybrids of oil and forage crucifers. Constant and systematic use of these fundamental genetic postulates has led to the development and official registration of 13 autumn-sown rapeseed cultivars and two hybrids, two spring-sown rapeseed, one black mustard, one oil-rich grain white mustard, three autumn-sown forage kale and one spring-sown forage white mustard cultivar, as well as two false flax lines. Today, the conventional breeding methods are closely followed by various molecular genetic tools. So far, the most prominent role in assisting the selection of the genotypes with desirable traits has been played by RAPD and SSR molecular markers. A well-designed and feasibly organized integration of the Mendel's rules, conventional breeding methods and molecular breeding tools are anticipated as significantly improving the existing programmes. It ensures that the future efforts will yield further progress in oil and forage crucifers' research. This will benefit to various aspects of economy, especially the environment friendly production of quality oil for both human consumption and non-food industry and low-input and esteemed forage in ruminant feeding.

**Keywords:** *Breeding, crucifers, forage production, oil production, Serbia.*

### Mendelian rules in breeding of oilseed rape

Two years ago, 150<sup>th</sup> anniversary of Gregor Johann Mendel's paper "Experiments on Plant Hybridization" was celebrated. This famous scientist is recognized nowadays as father of genetics. He was studying inheritance of several traits (seed colour and shape, plant height, flower colour and position on plant, colour of unripe pod and pod shape) of pea (*Pisum sativum* L.). It took him eight years and only 2 hectares of monastery garden to plant around 28 000 of pea plants. Afterwards he deduced three Laws of inheritance (Law of dominance, Law of segregation and Law of independent assortment) that were not appreciated by scientific community at that time. It wasn't until 1900, that his work was rediscovered. His paper was considered premature, but became immortal work of the human spirit infiltrating into the mystery of life (Nemec, 1965).

Today, after century and a half, although a lot of new methods and techniques have developed, use of Mendelian rules is still actual and necessary for plant breeders. First

Mendelian Law of dominance is about trait dominance in offspring of parents that contained two opposite traits. One dominant allele for a trait masks phenotypic effect of recessive allele. This is exactly what happens in F<sub>1</sub> generation during hybrid development. Law of segregation tells about recombination in F<sub>2</sub> generation during meiosis which is of great importance for comprehension of the process of breeding lines. Finally, Law of independent assortment is a tool for combining different desirable properties by selection (Marjanović Jeromela *et al.*, 2016).

Mendel's rules enhance introduction of monogenic traits in rapeseed (*Brassica napus* L.). They are particularly useful in breeding for white flower trait (Huang *et al.*, 2014), genic male sterility (Wang *et al.*, 2007) or characters introduced by interspecific breeding e.g. resistance to clubroot (Diederichsen *et al.*, 2009) and cabbage aphid (Quazi, 1988), pollen fertility gene (Yang *et al.*, 1996), etc. In the last couple of decades, the regular use of Mendelian rules has contributed to the improvement of many rapeseed traits, including tolerance to biotic and abiotic stress, yield and seed quality (Ordon and Friedt, 1998).

### **Oilseed rape breeding in Serbia**

At the beginning of 20<sup>th</sup> century, oilseed rape was main oil crop on Serbian fields. Later on, in the mid-1960s, it was observed that erucic acid can have toxic effect on humans when consumed in higher doses through vegetable oil in salads, cakes etc. Erucic acid is monounsaturated fatty acid that naturally occurs in oil-rich seeds of *Brassicaceae* family. After these findings, rapeseed production decreased significantly and sunflower got leading position as most planted oil crop in Serbia. Great efforts were made to lower erucic acid level from 50% to lower than 2% via selective breeding. Canadians were the first to achieve low content of erucic acid under 2% in spring rapeseed. They named this type of rapeseed Canola. Word canola is coined from "**Canadian oil low acid**" and this oil had also low level of glucosinolates.

Glucosinolates are typically found in crucifers seed. They have high structural diversity due to modifications of side chain. Their taste is sharp bitter and when consumed by animals in higher amounts can be harmful for thyroid gland (Papas *et al.*, 1979). Rapeseed with low erucic acid and low glucosinolates was marked in Europe as double zero canola (00). Breeding programme on oil-rich grain, vegetable and forage crucifers at the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS) has begun in 1984 (Marinković and Marjanović Jeromela, 2006). In 1998, IFVCNS registered Banačanka, first "00" rapeseed in Serbia (Marjanović Jeromela *et al.*, 2007).

Two most important traits for crop breeding are yield and quality. Main goals of oilseed rape breeding include high oil yield in seed, optimal fatty acid composition, low erucic acid level, low glucosinolate content, optimal protein content, and better quality regarding amino acids (Friedt and Snowdon, 2009). When breeding oilseed rape for quality, development of high oleic acid (more than 75%) and low linolenic acid (less than 3%) (so-called HOLL) types is of high importance (Chungu *et al.*, 2001). It was perceived that decreasing of linolenic acid and increasing of oleic acid in canola oil contribute to higher frying stability (Warner *et al.*, 1997). At the beginning of rapeseed breeding programme at the IFVCNS it was necessary to determine level of phenotypic and genetic diversity. Rapeseed has narrow genetic diversity because of intensive breeding for "00" types. Genetic diversity of our collection was assessed using RAPD (Random Amplified Polymorphic DNA) molecular markers. In this study, significant genetic variability between genotypes was recorded on morphological, biochemical and molecular levels, signifying their potential use in further breeding program of rapeseed (Marjanovic-Jeromela *et al.*, 2009).

In order to avoid high temperatures and drought at the beginning of summer, which influence seed filling, rapeseed genotypes with early maturity are selected in breeding programmes.

Nevertheless, it is important to have genotypes resistant to major diseases (Delourme *et al.*, 2012) and insects, to lodging and shattering. Due to adverse effects on bees, number of insecticides is restricted. Some insects have developed partial resistance to insecticides which will raise problems with increase of rapeseed production in Serbia.

In order to enable F<sub>1</sub> hybrid breeding there is need for efficient and trustworthy method for seed production (Yamagishi and Bhat, 2014). Cytoplasmic male sterility (CMS) is stable maternally inherited trait that is used for production of hybrid seed. Plants that have introduced CMS do not produce pollen, while the function of female organs remains unaffected. Ogura CMS and fertility restorer gene were introduced from radish into rapeseed via interspecific crossing (Bannerot *et al.*, 1974) followed by protoplast fusion (Pelletier *et al.*, 1983). Concerning that undesirable (fertile) plants need to be excluded on time, cytogenetical analysis of pollen presence and its viability (Atlagic *et al.*, 2010) are viewed as methods of choice. Moreover, molecular markers are routinely used for evaluation of presence and absence of cms gene in cms lines (Dimitrijević *et al.*, 2015). Establishment of cms into breeding lines has led to first hybrid rapeseed at IFVCNS "NS Ras" that was registered in 2015.

So far, Institute of Field and Vegetable Crops has produced 11 varieties and 20 lines of spring type, and 56 varieties and 980 lines of winter type rapeseed. In Serbia and abroad 14 varieties of winter oilseed rape, two hybrids and two spring varieties have been registered so far.

#### **False flax collection**

Oil crops are cultivated world-wide and are important part of country economy. They are used for food production and non-food products. Oil of traditional crops and especially of alternative oil crops is very valuable and appreciated on market. False flax (*Camelina sativa* (L.) Crantz) is oil crop from *Brassicaceae* family used as food, feed and in industry. It is very adaptable species to different environmental terms and requires modest conditions for cultivation. Camelina is often planted on less productive and marginal fields. False flax is quite resistant to diseases, has low needs for inputs e.g. watering and nitrogen fertilizers. Joint false flax collection of IFVCNS and BOKU was phenotypically characterized to get better insight of false flax germplasm as potential source of desirable genes (Marjanović Jeromela *et al.*, 2018b). Chemical analyses of oil and protein content as well as research of entomofauna of camelina were also carried out. Significant variability was found between tested genotypes regarding oil and protein content (Marjanović Jeromela *et al.*, 2018a). Average oil content ranged from 30% to 50% (Marjanović *et al.*, 2018b) which indicates possibility for further breeding on specific fatty acid and amino acid composition. This alternative oil crop has high value of meal with protein content that resembles soybean (Zubr and Matthäus, 2002). That is why it is considered as ideal meal for poultry and other livestock (Frame *et al.*, 2007; Zubr and Matthäus, 2002). There is renewed interest in camelina because it has unique fatty acid profile with 30-40% of  $\alpha$ -linolenic acid and around 15% of eicosanoic acid (Marjanović Jeromela *et al.*, 2018b). Collected results confirmed that use of new methods and techniques for traits of interest and introducing new variability into collection are essential for effective breeding of this re-emerging crop.

#### **Forage crucifers collection**

Forage kale (*Brassica oleracea* L. var. *viridis* L.), turnip rape (*Brassica rapa* subsp. *oleifera* (DC.) Metzg.), brown (*Brassica juncea* (L.) Czern.), black (*Brassica nigra* L.) and white (*Sinapis alba* L.) mustards are crucifers mostly used as first fresh spring feed. Of all mentioned brassicas, forage kale is the most cultivated forage crucifer in Serbia. It is usually autumn-sown, with earliness and cold tolerance as the most important agronomic characteristics in breeding programmes (Mikić *et al.* 2014). Both black and white mustard seeds have lower level of oil content (28-42%) relating to other crucifer species. White

mustard has lower level of volatile oils and milder taste comparing to black mustard. It also has better tolerance to drought and heat in contrast to rapeseed which makes it better for cultivation on drylands (Slominski *et al.*, 1999). White mustard has very shatter-resistant pods. Although black and white mustards have lower yield of fresh forage, their early maturity is what makes them advantageous for planting in early spring. Forage crucifers have lower dry matter content than forage legumes, ranging from 0.08% to 0.11% of forage dry matter (Mihailović *et al.* 2008), while, due to a high crude protein content in forage dry matter, they have moderately high crude protein yield (Ćupina *et al.* 2014) and thus may be successfully used not only in animal feeding, but also as green manure and other environment-friendly and ecological services (Antanasović *et al.* 2012). All forage crucifers are suitable for reliable forage production intercropped with legumes (Marjanović Jeromela 2017). The accessions of mustard germplasm collection at IFVCNS are being routinely examined for following traits: flower morphology, pollen viability and number of chromosomes. So far, IFVCNS has registered three autumn-sown forage kale varieties, namely NS-Bikovo, Perast and NS Rani, one spring variety of forage white mustard "NS Gorica", one spring variety of grain white mustard "Bela" and one spring variety of black mustard "Crna".

### Conclusions

Complex and strategically structured research at the IFVCNS was based upon maintaining and sustainable use of germplasm collections of each crop. It comprised conventional and molecular breeding, biotechnology, agroecology, physiology, biochemistry, agronomy and seed science as well as commercial production for local and international markets. Nowadays, when genome of oilseed rape is sequenced it is expected that new findings on genome organization and structure will benefit to breeders and enhance genome assisted selection. Mendelian rules will continue to assist in development of new *Brassicaceae* cultivars.

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