Present trends in winter oilseed rape breeding in the Czech Republic

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The present winter oilseed rape breeding research in the Czech Republic is concentrated in the association called „ Czech Oilseed Rape" (COR). Members of the association are Czech research and breeding organizations which are involved in oilseed rape breeding based on the most advanced laboratory instrumentation and technological procedures. The association was set up in the year 1997 and its main goal was to pool personal and material resources of all organizations involved in the process of breeding this strategic crop. A top priority is to develop competitive Czech varieties exhibiting desired yield and quality parameters (high contents of oil and low contents of anti-nutritive substances) which will be suitable for growing in the Czech Republic.

By integrating research and breeding activities of several organizations the association boasts necessary material and technical support and experts on breeding, biotechnological methods and molecular genetics.

The activity of the association focuses not only on the application of the so-called „conventional" breeding methods but also on the introduction and modification of new progressive techniques like molecular and biotechnological methods which make the time-consuming breeding process more rapid and efficient.

► 1) Development of new materials with the desired parameters of yield and quality including dihaploids

In the year 2006 the Oponent variety (developed in OSEVA PRO s.r.o., o.z. Oilseed Crop Research Institute Opava) was registered. During the development of this variety several new procedures in particular workplaces were applied. They included inoculation tests for susceptibility to phoma black stem, infection tests in breeding nurseries, NIRS, liquid and gas chromatography for quality control, freezing tests for frost tolerance, system of inter-station performance tests for yielding capacity of varieties and molecular marker techniques to ensure genetic stability of materials.

Every year members of the COR association register high performance lines and dihaploids for state variety tests.

A member of the association and a coordinator of projects VÚRV Praha – Ruzyně is the main producer of dihaploid (DH) lines of oilseed rape. A technique of DH line development was optimized and the present annual production is about 1000 regenerants. A process of selection for frost tolerance in microspore cultures was tested and optimized by application of hydroxyproline.

Every year promising materials are tested in inter-station performance tests in 7 to 10 localities which are chosen to cover the whole territory of the Czech Republic (Table 1.). Selected breeding materials were also tested in freezing tests and inoculation tests for resistance to phoma black stem.

Table 1: Seed yield in conventionally developed lines and dihaploid lines in the years of testing (inter-station performance testing of the Česká řepka (Czech Oilseed Rape) association:

Year of inter-station performance tests	Yield of line varieties, compared to control (%)	Yield of DH lines, compared to control (%)	Number of materials registered for state variety tests
2001/02	82.0 – 113.5	90.8 – 109.6	4
2002/03	86.1 – 114.4	92.6 – 107.4	3
2003/04	91.4– 110.3	88.5 – 107.3	6
2004/05	85.9 – 113.3	96.0 – 97.9	5

► 2) Hybrid breeding

Members of the COR association are engaged in the hybrid breeding programmes:

A) *Based on sporophytic self-incompatibility (SI).*

Self-incompatibility (SI) is inability of plants to produce seeds after self-pollination. In oilseed rape this involves sporophytic homomorphic SI. The initial SI resources found in the eighties which exhibited high contents of GSL and erucic acid after two crossings with donors of quality and subsequent derivation of DH regenerants produced 20 original SI lines with stable response to self-pollination and satisfactory contents of erucic acid, which became a limiting factor in sporophytic SI (Table 2.). The detection of SI lines is performed in a classical way (self-pollination tests) and also application of molecular methods which are routinely used in the process of breeding SI hybrids (PCR and its modification). Preliminary results with experimental SI hybrids provide opportunities for using the heterosis effect in the system of producing SI hybrids without any negative effect on the double zero quality of seeds. Work on the development of new experimental AI hybrids is now in progress.

Table 2: Quality of seed from original and enhanced AI lines after derivation of dihaploids from the first and the second improvement crossing:

Genotype	GSL contents (µmol/ g seed DM at 9% moisture) *Liquid chromatography*	Erucic acid contents (%) *Gas chromatography*
Tandem 6/85 (AI)	**116.31**	**45.78**
OP-2051 (donor of „00“ quality)	**7.96**	**0.53**
Quality of seed in the population of DH regenerants after the first improvement crossing	**84.69**	**22.84**
Quality of seed in the population of DH regenerants after the second improvement crossing	**13.97**	**14.16**

B) *Based on cytoplasmic male sterility Ogu-INRA.*

The association devised an effective system of selection of fertility restorer. Some biochemical and molecular methods of selection are also available. A method for electrophoretic detection of the enzyme PGI from leaves of young oilseed rape plants, which allows searching for lines bearing the fertility restoration gene, was optimized. Another step was the development of molecular analysis by RAPD which enables to detect genes of fertility restorer in a hybrid population. With the aim of obtaining fertility restorer a system of dihaploidization is also used. Other activities are directed at the production of experimental Ogu-INRA hybrids.

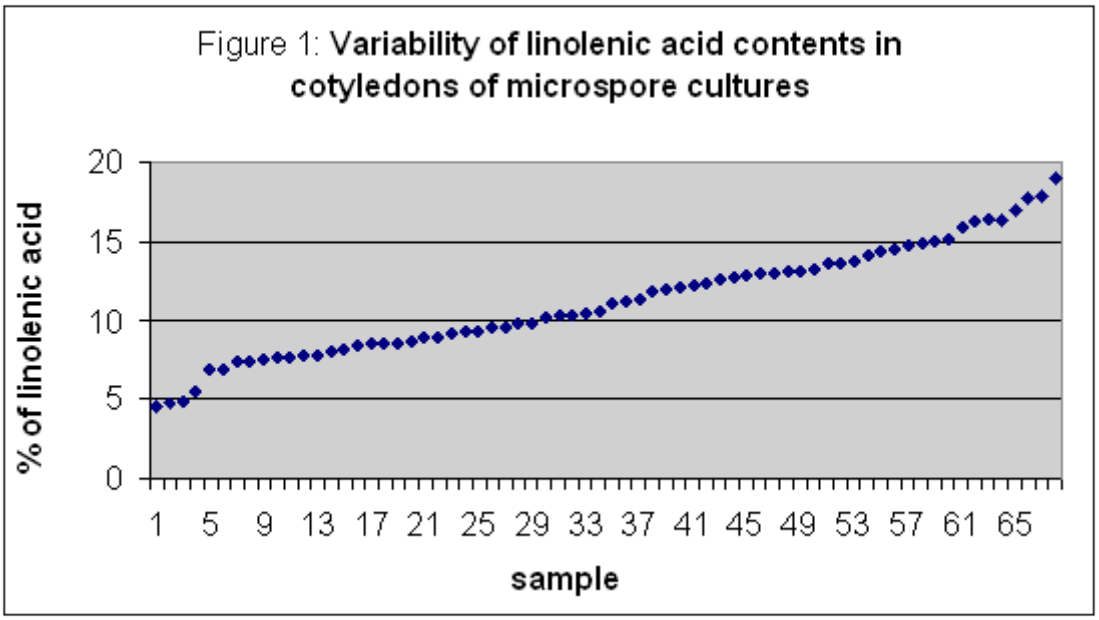
C) *Based on the system of cytoplasmic male sterility SHAAN 2*.

In these materials coming from China quality has been markedly improved and in the future CMS lines and fertility restorers may also be used for hybrid development. So far no hybrid based on this system has been registered in this country.

► 3) Breeding for oil and meal quality

The COR association focuses in the project on the optimum utilization and calibration of available analytical methods for the determination of seed quality but also on the development of low linolenic materials. For this purpose a method which analyses the content of fatty acids in the cotyledons of microspore embryos (cotyledon weight is 0.002 – 0.05 g!) was introduced (Figure 1.). Subsequent selection focuses on final development of desired genotypes. Simultaneously, work is in progress on the introduction and utilization of molecular markering (RAPD) of materials with reduced contents of linolenic acid. The goal of the association is to develop materials with high qualitative indices in oil and meal.

Figure 1: Variability of linolenic acid contents in cotyledons of microspore cultures (gas chromatography – VÚOl Opava)



► 4) **Breeding for disease and pest resistance**

A) Breeding for disease resistance

In the Czech Republic the most serious disease of oilseed rape is phoma stem canker (*Leptosphaeria maculans*) and white mould (*Sclerotinia sclerotiorum*). A greater incidence of *Peronospora parasitica* is reported. Disputable is the occurrence of verticillium wilt (*Verticillium longisporum*) and cylindrosporiosis (*Cylindrosporium concentricum*). The occurrence of the last two pathogens predominantly verticillium wilt was recorded but it was not diagnostically confirmed. The aim of monitoring is to map the actual occurrence and harmfulness of verticillium wilt, cylindrosporiosis and causal agents of leaf blotches such as e.g. *Mycosphaerella spp*.

The occurrence of phoma stem canker and white mould in the Czech Republic is closely dependent on the season, locality and crop rotation. High incidence of phoma stem canker was recorded in the growing season 2001/2002, when up to 70 % of plants were infected and infection involved severe damage to roots and root collars, the plants were prematurely dead. In the year 2002/2003 nearly 80 % of plants were infected. Plant infection was not as severe as in the year 2001/2002 but root infection was combined with frost damage and *Delia radicum* infection. In the year 2003/2004 and 2004/2005 fewer than 50 % of plants were infected. Depending on the genotype, the injury was mostly surface damage to the stem base. White mould infection in the year 2002 was in less than 30 % of plants, in the year 2003 the infection was almost zero, in the year 2004 it was less than 39 %, in the year 2005 less than 51 %. Since the year 2000 there has been a higher proportion of plants infected with white mould from soil through plant roots – mycelium infection from sclerotia in soil.

Oilseed rape breeding for disease resistance is targeted at phoma stem canker and white moult. When evaluating the resistance of breeding materials the following methods are applied: methods of natural infection, infection field and methods of artificial inoculation under field and laboratory conditions.

The evaluation of resistance to *Leptosphaeria maculans* is based on methods of artificial inoculation under laboratory conditions. This is a method of soil inoculation by infected and crushed rape straw before seeding and a method of inoculation of emerged oilseed rape plants by foliar spraying with suspension of pathogen spores. Under laboratory conditions a method of artificial inoculation at the stage of cotyledons by application of pyknospore suspension was used. For this reason a collection of *Leptosphaeria maculans* isolates is made. The isolates originate in the Czech Republic. The isolates have mostly properties of non-aggressive isolates Tox^0 – *L.biglobosa*. Sporadically, they have properties of an aggressive isolate Tox^+ - *L.maculans*. Under laboratory conditions the isolate Tox^+ is often used but also the response of genotypes to non-aggressive isolates is being tested. A molecular method of markering whose aim is to characterize individual phome isolates was introduced. The reliability of results from laboratory tests is examined under field conditions.

When evaluating resistance to *Sclerotinia sclerotiorum* natural infection is often used. Methods of artificial inoculation under field conditions are also verified. Methods of artificial inoculation are based on inoculation of oilseed rape plants at the start of blooming by material which is penetrated by pathogen mycelium. The material is kept in the place of inoculation. The material is wood (wooden pegs – clips, toothpicks) or wheat grains. In toothpicks and wheat grains inoculation is conducted by injecting into the stem at the point of branching approximately in the middle of the plant. In wooden clips inoculation is made by clipping the stem. In the wooden material it is difficult to ensure higher moisture at the point of inoculation under adverse conditions. As for inoculation by wheat grains which are on the pin, higher moisture at the point of inoculation is ensured by putting damped cotton wool and wrapping the point of inoculation using aluminium foil.

B) Breeding for resistance to some pests

Serious pests of winter oilseed rape are the stem ceutorhynchid beetles – rape stem weevil (*Ceutorhynchus napi*) and cabbage stem weevil (*Ceutorhynchus pallidactylus*). Their harmfulness especially in weak stands and stands under stress is considerable. One of the activities of the COR association is to examine natural plant resistance to these beetles. One of the possibilities of natural protection which are under discussion is glucosinolate production in herbage. The first trials showed some differences between varieties both in infection and contents of these substances and also showed that some groups of glucosinolates act as repellents whereas others act as attractants (Figure 2, Table 3). The findings obtained will be used in the process of developing varieties with improved resistance to stem ceutorhynchid beetles.

The following relationships were revealed:
- Negative correlations between relative contents of aliphatic GSL and cabbage stem weevil infection (significance level of P = 0.01)
- Positive correlation between relative contents of indolic GSL and cabbage stem weevil infection (significance level of P = 0.05)
- Positive correlation between relative contents of desulfoglucobrassicin (from the group of indolic GSL) and cabbage stem weevil (significance level of P = 0.05).

Figure 2: Differences in resistance to stem weevils in selected registered varieties

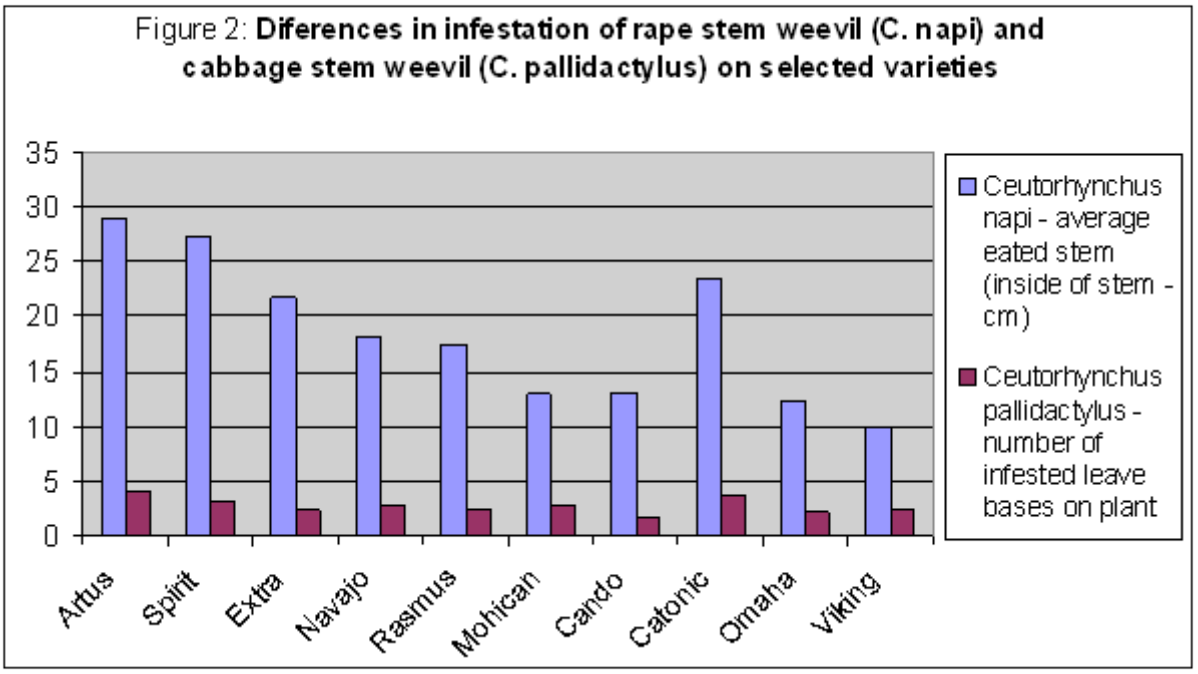


Table 3: Results of GSL analysis in oilseed rape leaves (collected on 3 May 2005 – the method was HPLC VÚOl Opava)

VARIETY	GLUCOSINOLATE (GSL) CONTENTS IN LEAVES [µmol/g of herbage DM]						
	ALIPHATIC		AROMATIC		INDOLIC		∑
	µmol/g	%	µmol/g	%	µmol/g	%	µmol/g
Artus	1.06	84.8	0.07	5.6	0.12	9.6	1.25

Spirit	1.97	91.6	0.04	1.9	0.14	6.5	2.15
Extra	2.12	92.2	0.03	1.3	0.15	6.5	2.30
Navajo	1.47	89.6	0.04	2.4	0.13	7.9	1.64
Rasmus	0.63	87.5	0.02	2.8	0.07	9.7	0.72
Mohican	1.93	91.5	0.03	1.4	0.15	7.1	2.11
Cando	2.29	97.5	0.00	0.0	0.06	2.6	2.35
Catonic	1.54	83.2	0.00	0.0	0.31	16.8	1.85
Omaha	1.87	90.8	0.09	4.4	0.10	4.9	2.06
Viking	1.60	88.4	0.03	1.7	0.18	9.9	1.81

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