

## Two Doctors Theses with Reference to Rapeseed Breeding

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In June 1989 Bao-Yuan Chen from Wuhan, China, defended his doctor thesis titled, "Resynthesized Brassica napus. A potential in breeding and research" at Svalöv. The primary intention was to synthesize a yellow seeded B. napus and for this purpose, crosses were carried out between one light brown-seeded form of B. alboglabra and various yellow seeded forms of B. campestris most of them being yellow seeded sars-son. The embryo rescue technique was compared to seed set in vivo following hybridization between B. alboglabra and B. campestris. The ratio of number of seeds harvested to number of flowers pollinated was 0.013 for seed set in vivo and the ratio of number of embryos survived to number of flowers pollinated was 0.55 for embryo rescue.

The seeds of the resynthesized B. napus were light brown in colour. Crosses between this material and partially yellow seeded natural rape have given some near yellow seeded  $F_2$ -plants but these were not true breeding in  $F_3$ . The material is, however, valuable as the A-genome only has genes for complete yellow seed coat and the improvement can be concentrated on the C-genom. The thesis is based on nine papers.

Male sterile plants were found in one B. napus  $F_2$ -population derived from crosses involving lines from natural breeding material as female and newly resynthesized forms as male. The genetic studies indicated that male sterility was conditioned by the presence of recessive maintainer genes (msms) in the nucleus of the resynthesized B. napus, in combination with the (S) cytoplasm of the breeding lines. In other words, the resynthesized and the natural lines of B. napus functioned as maintainer and restorer lines, respectively. Test crosses with nap maintainer and restorer lines revealed that the observed male sterility is of nap type.

It has been demonstrated that characters such as flower colour and restorer characters as well as flower colour and erucic acid content are independently inherited.

In two crosses between a high-erucic, white-flowered, resynthesized line as the female, and the zero-erucic, yellow-flowered cultivars Topas and Puma as males, 5 of 34  $F_1$ -plants were of androgenetic origin. One of these plants had a diploid chromosome number. In the reciprocal crosses no androgenetic seeds were obtained. Androgenesis is of interest for the plant breeders since it leads to a new combination of nuclues and cytoplasm.

Chens thesis also includes isozyme studies in resynthesized and cultivated Brassica napus, B. campestris and B. alboglabra. The polymorphic enzyme patterns observed in the newly resynthesized B. napus disclosed that the homologous loci contributed by the parental species were expressed in the amphiploid. But analysis of the glucose-phosphate-isomerase enzyme in a line of natural rape (Sv 02372) indicated that, in this case, the gene originated from B. campestris was switched off whereas that of B. oleracea was expressed.

During the last few years the cultivation and somatic hybridization of Brassica protoplasts have developed very quickly. In this work Kristina Glimelius and her group at the Department of Plant Breeding, Swedish University of Agricultural Sciences, Uppsala, have played an important role. One of her students, Christina Sjölin, has this spring defended her doctor's thesis titled: "Transfer of resistance against Phoma lingam to Brassica napus L. via somatic hybridization in combination with in vitro selection".

Toxic compounds have been isolated and purified from the culture medium of Phoma lingam. The main toxic compound was identified as Sirodesmin PL. Fourteen different isolates of Phoma lingam were tested in experiments and it was found that the isolates producing the highest toxin concentration also caused the most disease.

In order to have a better knowledge regarding resistance sources to P. lingam, seedlings from 90 accessions of Brassicaceae were sprayed with a spore suspension from three isolates. After this pre-screening, the accessions expressing the highest level of resistance were subjected to puncture test on cotyledons and adult leaves, and stem base infections. From these more detailed studies of the resistance behavior it was clear that five accessions of B. juncea, two accessions of B. carinata and two accessions of B. nigra could be classified as resistant. In all these accessions, resistance was expressed both at the seedling and at the adult stage. All the resistant forms contain the E-genome of Brassica nigra but there were other accessions with the B-genome that were sensitive.

The selective property of sirodesmin PL were studied. The results revealed a clear correlation between resistance to P. lingam and insensitivity to sirodesmin PL and that the toxin can be used to distinguish resistant plant material from susceptible both in vivo and in vitro.

Complete somatic hybrids were made between B. napus and B. nigra. In these hybrids a successful combination of all three Brassica genomes, A, B, and C, was obtained. From the calli obtained, 32 plants were regenerated. Twenty of these had the sum of the parental chromosome number (54) and 26 plants expressed resistance to P. lingam.

In order to transfer the resistance genes directly into the B. napus genome, asymmetric somatic hybridization was used. Irradiated mesophyll protoplasts from nine different accessions of B. juncea, B. nigra and B. carinata, all resistant to P. lingam, were fused with hypocotyl protoplasts of B. napus susceptible to the pathogen. The experiments were performed both with presence and absence of sirodesmin toxin selection.

When screening for resistance to P. lingam, 19 out of 24 asymmetric hybrids obtained from the toxin-selected cultures were resistant. In contrast, all unselected asymmetric hybrid plants tested were susceptible to the pathogen.

This result shows that by combining asymmetric somatic hybridization with in vitro selection it has been possible to transfer an agronomic character from one species to the other. Whether the resistance gene(s) is incorporated in the recipient genome or not has, however, not yet been demonstrated.