Genetics and Breeding

A. DISEASE RESISTANCE

A new source of blackleg resistance from *Brassica sylvestris*

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Blackleg disease of canola caused by the fungus *Leptosphaeria maculans* is a serious disease of both the winter and spring forms of canola (*Brassica napus*). It is a particularly serious disease in Australia because the canola varieties are spring types but are grown throughout the winter/spring in a Mediterranean climate. The combination of a long vegetative period (about 120 days) and virulent races of the fungus has meant that even the most resistant canola genotypes often suffer some reduction in yield.

In 1990 Jonathan Crouch at the John Innes Centre, Norwich, UK began work on transferring blackleg resistance from *Brassica sylvestris* into *B. napus*. This was part of his PhD work. One of his supervisors was Richard Mithen who had collected wild Brassicas and had identified some accessions of *B. sylvestris* as possible new sources of blackleg resistance. In particular, one accession from Sicily proved to have very strong resistance. Crouch crossed this *B. sylvestris* accession with *B. alboglabra* and recovered the hybrid with embryo rescue. (A better classification of this cross would be *B. rapa ssp sylvestris* by *B. oleracea ssp alboglabra*). This material was treated with colchicine to double the chromosome number and so produce the amphidiploid which was a synthetic *B. napus*.

Crouch crossed plants of the synthetic *B. napus* to a number of varieties of *B. napus* – both winter and spring types. Seed of crosses to the spring canola varieties Marnoo and Westar was sent to Australia to be evaluated for disease resistance by Pacific Seeds. This material was sown in a blackleg nursery at Junee, southern New South Wales in 1992. At the late vegetative stage the material in the nursery was scored for blackleg lesions on the leaves. For some of the lines from Crouch it was difficult to find any lesions. When lesions were present they were small and had few or no pycnidia. Not all lines containing

B. sylvestris had lower lesion number. The rest of the material in the blackleg nursery had leaf lesions. This is consistent with the disease pattern in Australia – all varieties whether resistant or susceptible to the stem canker stage of the disease have many leaf lesions in most years. At harvest time the rows of the material that had shown few or no leaf lesions were free of stem canker.

The most resistant lines were from the cross of the synthetic napus to the variety Marnoo. This material was crossed and backcrossed to susceptible varieties to try to incorporate the sylvestris resistance trait into canola quality *B. napus*. Crosses were not made to varieties with other sources of resistance as we wanted to ensure that sylvestris was the only source of resistance in the material.

In 1993 material sown in a blackleg disease nursery in Toowoomba, Queensland included backcross material (BC1 F2) of crosses containing the sylvestris resistance. The disease pressure in that nursery was very high. At harvest time it was clear that plants were either susceptible or resistant to stem canker as the susceptible plants were usually dead and the resistant plants had no stem canker. Resistant plants were selected to continue the breeding process.

The sylvestris resistant material became part of the canola breeding program at Pacific Seeds. At first the material with sylvestris resistance was poor in phenotype and quality. It was selected for canola quality, oil content and agronomic type. By 1997 the material that had good quality and phenotype and was being evaluated in yield trials both as open pollinated (OP) lines and in hybrid combination. The hybrids and OP lines were evaluated for blackleg resistance both in disease nurseries and yield trials. In 2000 the first variety with the sylvestris resistance was released under the name Surpass 400. In 2001 two hybrids were released with this resistance – Hyola 60 (Australia) and Hyola 440 (Canada). As well several OP's were released in Australia – Surpass 402CL, Surpass 603CL and Surpass 501TT.

How strong is the sylvestris resistance? All the varieties tested in Australia with this source of resistance are completely resistant to the stem canker phase of the disease. There are no external symptoms to stem canker. If the stems are cut to inspect the tissue for the black ring associated with the disease then most stems will be found to be without the black ring but some will be found to have it. This shows that the vascular tissue of the plant is infected and therefore the plant has a low level of disease. The resistant varieties often show some leaf lesions but these leaf lesions are small and have fewer pycnidia then normal leaf lesions. The resistant varieties have the highest disease resistance rating possible on both the Australian and Canadian rating scales.

In Australia the stubble from the crop harvested in late spring (November) becomes the source of inoculum for the next crop which is planted in early winter (May). Ascospores are released from the stubble when it rains and these airborne spores fall on the newly sown crop and infect the cotyledons or leaves. A study of the stubble from Surpass 400

has shown that pseudothecia (which release ascospores) are present in similar numbers to that of stubble from other varieties. In other words, it seems that the process of the saprophytic growth of the fungus on the stubble is the same as with less resistant varieties. This may mean that the fungus is not under threat from this resistance and does not need to alter races to survive. Time will tell.

How is this resistance inherited? The F1 hybrids, Hyola 60 and Hyola 440 have only one resistant parent so the trait is dominant. Present work using populations of doubled haploids should determine the number of genes involved and the location of the gene or genes on the genome. Molecular markers are being used to tag the trait as well as to pinpoint the location of the genes.