Fungal pathology developments for implementation in plant breeding programs, with emphasis on blackleg and canola

**PROJECT DETAILS**

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<th>PROJECT CODE:</th>
<th>UM151</th>
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<td>PROJECT TITLE:</td>
<td>FUNGAL PATHOLOGY DEVELOPMENTS FOR IMPLEMENTATION IN PLANT BREEDING PROGRAMS, WITH EMPHASIS ON BLACKLEG AND CANOLA</td>
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<td>START DATE:</td>
<td>01.01.2001</td>
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**Summary**

Blackleg resistance is an important trait targeted by canola breeders in the National Brassica Improvement Program (NBIP). The expansion of area sown to canola during the past decade, and the recent breakdown of major gene resistance to blackleg makes it imperative that blackleg be kept in check. Knowledge of the biology and genetics of the blackleg fungus is crucial to ensure that increased blackleg resistance is incorporated into new varieties. This project supported the activities of Dr Barbara Howlett, who leads a multidisciplinary team studying blackleg of canola.

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Conclusions

Blackleg is the major threat to the Australian canola industry.

Strong links between pathologists and breeders are important so that breeders are aware of changes in fungal populations that may overcome disease resistance.

So far only one of the *Leptosphaeria maculans* species complex is predominant in Australia and an assay has been developed to monitor such species.

To minimise the impact of blackleg, growers should, wherever possible, sow their canola crops across their farm rather than adjacent to last season's canola stubble. Furthermore, management practices for destroying canola stubble (raking and burning) will reduce the number of ascospores discharged and thus reduce the likelihood of yield loss caused by blackleg.

The blackleg fungus changes virulence in response to selection pressure from major gene resistance in canola and can overcome this resistance.

Nutrition (availability of readily utilisable carbon (C) source) is important for the ability of the blackleg fungus to infect canola. This finding also applies to the infection of *Stagonospora* of wheat as shown by Richard Oliver.

Recommendations

Links between breeders, field and laboratory pathologists, and molecular biologists are important in ensuring the best outcomes for breeding and deploying genes for resistance to blackleg.

Links also between breeders and pathologists who study other fungal diseases, e.g. rust of cereals, and glume blotch of wheat also will enhance success in beating blackleg, as will strong international links.

GRDC PhD training awards (Grains Research Scholarships) attract high calibre students into the grains industry.

Outcomes

Expected Outcome (Benefits)

Canola provides edible oils and is an essential component of crop rotations in Australia. Blackleg resistance is one of the most important traits targeted by canola breeders in the NBIP. The expansion of area sown to canola during the past decade, and the recent breakdown of major gene resistance to blackleg, makes it imperative that blackleg be kept in check. Knowledge of the biology and genetics of the blackleg fungus is crucial to ensure that increased blackleg resistance is incorporated into new varieties.
This project supported activities and funded Dr Barbara Howlett who supervised a research group studying blackleg. Accordingly, this project provided training of students and staff in molecular plant pathology to enable them to contribute to the Australian grains industry.

Benefits from this project delivered to the NBIP include glasshouse testing of elite germplasm and molecular assays to distinguish between different members of the *L. maculans* 'species' complex. This assay can be used to monitor whether species closely related to *L. maculans* are present in Australia. Further benefits include co-ordination of national meetings of canola pathologists and breeders.

Benefits delivered to growers are the design of crop management strategies minimising the impact of blackleg disease (through PhD training of Steve Marcroft).

**Achievements/Benefits**

**Overview of Project Achievements**

This project partially funded Dr Barbara Howlett (50%) and Anton Cozijnsen (30%), a Senior Research Associate, as well as operating costs (including molecular biology reagents and sequencing of 600 *L. maculans* expressed sequence tags (ESTs)) and travel. As from 01/01/2004, Dr Howlett was funded by the University of Melbourne and Anton Cozijnsen was funded through UM00016.

Dr Howlett’s research activities provided knowledge of the biology and genetics of the blackleg fungus, which is crucial to ensure that increased blackleg resistance is incorporated into new canola varieties and that management strategies are designed to minimise the impact of blackleg. In addition, the project provided training in molecular plant pathology to outstanding students to enable them to become leaders in the Australian grains industry. Two very successful canola pathology workshops have been held at Melbourne University (February 2001 and February 2003). Collaboration with national (e.g. NBIP, Richard Oliver) and international scientists enhances project outcomes.

This project is linked to several GRDC projects and the achievements are listed:

1. **Genetic variation of Australian blackleg isolates (UM149 ends June 2004).**
   Four hundred and fifty blackleg isolates have been cultured from canola in Victoria (VIC), New South Wales (NSW), and Western Australia (WA) and their mating type, virulence and genetic variation have been determined. Changes in virulence of populations have been monitored, particularly with respect to Surpass varieties with major gene resistance, which has been overcome in 2003. The finding that the reference isolate M1 (which was cultured 14 years ago) causes stem cankers on Surpass 400 indicates that even before the blackleg population was exposed to this variety, isolates existed that were able to overcome resistance. Neutral markers have been developed to assess genetic variation. Due to the low level of restriction fragment length polymorphism (RFLP), mini and microsatellites are now being exploited. Sequence data from the many genes of *L. maculans* characterised within Dr Howlett’s laboratory have been used to design primers for 15 microsatellites. Profiles are currently being analysed. An assay for mating type has been developed and applied to 200 isolates to show that equal ratios of both mating types are present in Australia. These data are being analysed and technical reports and publications are being prepared. The final report of UM149 will discuss these findings in detail.

2. **Application of a diagnostic test for members of the *L. maculans* species complex.**
   *L. maculans* is not a single species, but comprises a species complex of ‘A’ (*L. maculans*) and ‘B’ types (NA1 *L. biglobosa*, NA2 *Phoma wasabiae*, NA3, and weed infecting spp.). Within Australia, the ‘A’ type isolates predominate - only three ‘B’ type isolates have ever been found and these cause only very small leaf lesions and no cankering. In 2002, a breeder suggested that, based on lesion morphology, *L. maculans* ‘B’ type isolates were present in NSW, a claim that concerned growers. To examine this possibility, Dr Howlett’s group developed a polymerase chain reaction (PCR) assay that distinguishes between closely related species of *L. maculans*. Representative isolates for each of the ‘B’ sub-groups were taken from the International Blackleg of Crucifers Network (IBCN) collection for comparison. Each of the subgroups (which have now been designated as different species) has a different DNA amplification pattern. This PCR diagnostic test showed that all isolates proposed to be *L. maculans* ‘B’ type were in fact ‘A’ type isolates. This finding was significant as it shows that there is no evidence to classify *L. maculans* isolates with altered lesion morphology as a new species. Protocols for this simple test were distributed to Tamrika Hind (Wagga Wagga), Martin Barbetti (AgWest) and Wallace.
Cowling (University of WA (UWA)) and pathologists will now be readily able to classify isolates in an objective manner.

Two PhD students and a post doctoral fellow have been trained in this aspect. Alex Idnurm (GRS13) discovered two genes crucial for pathogenicity. One of these, isocitrate lyase, is involved in nutrition of the fungus in the plant, while the other has an unknown function. He also discovered a novel mutation process used by the fungus to avoid damage by transposable elements (jumping genes). Alex’s thesis led to six papers. Don Gardiner (GRS38) focused on identifying L. maculans genes involved in disease by analysis of ESTs. This sequencing has led to characterisation of the biosynthetic pathway for a toxin, sirodesmin. In addition, Dr Candace Elliott (UM005 for one year, now an Australian Research Council (ARC) post doctoral fellow) is using random insertional mutagenesis to identify genes crucial to pathogenicity and to uncover the biology of the infection process, as a basis for discovery of anti-fungal targets. So far, three genes are characterised, alcohol dehydrogenase, histone H4 and a transcriptional regulator. These findings highlight the significance of fungal nutrition in blackleg disease.

4. Role of stubble in blackleg disease (with Marcroft and Salisbury UM142 ended June 2003).
Steve Marcroft has shown that stubble and blackleg inoculum load decreases dramatically from 6-18 months after harvest. This information has been disseminated widely in GRDC updates and information sheets. Growers are now advised to, wherever possible, sow their canola crops across their farm rather than adjacent to last season's canola stubble. Furthermore, management practices for destroying canola stubble (raking and burning) will reduce the number of ascospores discharged and thus reduce the likelihood of yield loss caused by blackleg. Dr Howlett supported by helping with experimental design, interpretation and publication of results and supervising S Marcroft’s PhD.

5. Alternative sources for blackleg resistance (with Marcroft and Salisbury DAV452, ends June 2004).
Sources of blackleg resistance are being sought for incorporation into Australian canola lines for use by the NBIP. Dr Howlett has tested 30 lines in glasshouse trials and has identified resistance to well characterised virulent L. maculans isolates.

6. Training of postgraduate and undergraduate students in molecular plant pathology.
Two students (Leanne Wilson, Jasmine Rickards) graduated with B.Sc. (Hons), three students (Alex Idnurm, Joe Barrins, Kate Griffiths) graduated with PhDs, another (Steve Marcroft) submitted his PhD thesis. Currently, four PhD students (Don Gardiner, Sue Sprague, Harjono, Ellen Fox) are being trained. During 2001-3, 22 refereed papers and 12 unrefereed papers on blackleg have been published. In addition, numerous posters, seminars and field day presentations have been made by members of Dr Howlett’s group.

Intellectual property summary
All intellectual property (IP) generated in this project so far is in the public domain. If any genes which could be used as fungicide targets are discovered, they will be protected by patents and used in the development of proprietary gene technologies. This will be done in partnership by GRDC and the University of Melbourne.

Additional information
Refereed publications (2001-2004)


