Characterization of the reaction of *Brassica napus*, *B. rapa*, and *B. juncea* plant introductions to isolates of pathogenicity groups 3 and 4 of *Leptosphaeria maculans*

Proposal # 3 - Luis del Rio, $30,545
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Introduction

Blackleg of canola is caused by the fungus *Leptosphaeria maculans* and is, along with Sclerotinia stem rot, one of the most important diseases affecting canola production in North Dakota. Blackleg was first detected in North Dakota in 1991 although it is very likely it had been here for several years already (Lamey and Hershman, 1993). The relative virulence of isolates of *L. maculans* has been characterized in the US on three differential cultivars, Glacier, Quinta, and Westar (Koch et al. 1991). These differentials produce four virulence profiles called pathogenicity groups (PG). PG 1 isolates are currently considered to belong to *L. biglobosa* and are mildly virulent on canola, especially their leaves; isolates belonging to other PGs are considered to be from *L. maculans*. PG 2 isolates are highly virulent on Westar, moderately virulent on Quinta but are unable to attack Glacier; PG 3 isolates are highly virulent on Westar and Glacier and moderately virulent on Quinta, while PG 4 isolates are highly virulent on all three differentials (Koch et al., 1991). More recently, a fifth group, PG T, has been added (Chen and Fernando, 2006). In 1991 isolates retrieved from canola residues were identified as belonging to PG 1 and 2. Field surveys confirmed the geographic expansion of these groups into practically all North Dakota areas planted to canola by 2001 (Lamey et al., 2001).

After epidemics of blackleg broke out in the late 1990s, the canola industry moved quickly to include genetic resistance against the prevalent strains (PG 2) into their cultivars and by 2000 the situation was more or less under control. The discovery in 2004 of strains of pathogenicity groups not previously present in North Dakota (Bradley et al., 2005) has increased the risk of occurrence of severe blackleg epiphytotics tremendously. It is possible that the steady increase in blackleg incidence in our state in recent years (Bradley and Lamey, 2005) is due in part to this change in pathogen population, although other factors could also be at play. Independently of which factors are involved, however, the fact that new pathogenicity profiles are present in our fields is an indication that new and better sources of resistance are needed. The race to identify resistance genes against these strains is on, and it is imperative that efforts be put forth to identify sources of resistance against these new strains.

Current Work

Isolates belonging to all five PGs have been detected in North Dakota. Samples collected in 2003 and 2004 revealed the presence of three new groups, PG 3, 4 and T (Bradley et al., 2005; Chen and Fernando, 2006). However, these groups still have a rather limited geographic distribution in our state. The Canola pathology program at NDSU conducted extensive samplings of blackleg in the summer of 2007 and is in the process of updating the information on the geographic distribution of all known pathogenicity groups.

The USDA curates a large collection of accessions of *Brassicaceae* plants including representatives of *B. napus*, *B. rapa*, *B. juncea*, and *B. nigra*, among others. In the past we have identified plant accessions within the first two collections that showed promising levels of

resistance against *Sclerotinia sclerotiorum*, an important canola pathogen (Khot et al., 2005; Zabala and del Rio, 2007). Other researchers have identified materials with resistance to *L. maculans* within the first three *Brassica* species (Rimmer and van den Berg, 1992; Rimmer, et al., 2002); however, a systematic effort to screen all accessions in search of resistance genes has not been conducted. Thus, the intended work is to search within the USDA collection of accessions of these three species for sources of resistance to blackleg strains of PG 3 and 4 that could be used in a breeding program. The most current work on screening materials for resistance to strains of both PG 3 and 4 are presented in the progress report attached to this proposal.

Objectives

The goals of this project are to identify sources of resistance against blackleg strains belonging to pathogenicity groups 3 and 4 within the USDA collection of *Brassica napus*, *B. rapa*, and *B. juncea* plant introduction collections and to make the resistant materials available to canola breeders. The project will have duration of three years.

Rationale and Significance

Blackleg is a very important disease that has the potential to decimate susceptible canola crops. The identification of sources of resistance to strains of pathogenicity groups 3 and 4 of *Leptosphaeria maculans* is extremely important and urgent for the canola industry in North Dakota. Commercially available canola cultivars (hybrids and open pollinated cultivars) have been bred for resistance against PG 2 strains of blackleg but not against PG 3 and 4 strains. A small survey conducted in 2004 indicated that strains of PG3 and 4 were limited in geographic prevalence to two North Dakota counties; however, it is only a matter of time for these strains to spread to other areas. The identification of sources of resistance against these strains will offer the possibility of developing improved commercial cultivars and will help growers to reduce losses associated with this disease.

Approach

The screening procedure described below will be applied for both pathogenicity groups as well as for all three *Brassica* species (a collection that comprises more than 1,000 accessions collected worldwide). Screening of materials from both collections will be evaluated separately although simultaneously in five stages. The project will have duration of three years.

In the first stage non-replicated trials will be conducted to evaluate all accessions. Fifteen seeds of each accession will be planted individually in plastic cells (1.5 x 1.5 x 2 inches) filled with greenhouse soil mix and contained in plastic trays. Seedlings will be grown for ten days in growth chambers at 22^{o}C with 16 hours light daily. Four commercial canola cultivars (two hybrids and two open pollinated varieties) will be used as controls. Production of inoculum of each strain and the inoculation method to be used will be adapted from the one described by Chen and Fernando (2006). Briefly, purified colonies of isolates of each pathogenicity group will be produced in individual petri dishes containing clarified V-8 medium. After fourteen days of incubation at 21^{o}C under continuous light conditions the spores will be harvested by adding two ml of distilled sterile water to the medium and gently rubbing the surface with a bent sterile glass rod. The spore concentration in each stock suspension will be calculated using a hemacytometer and then adjusted to 10^{7} spores per ml. Each of the two cotyledons of each seedling will be

gently wounded by pricking them with a needle and the wounds will be covered with a 10 µl aliquot of the spore suspension. Inoculated seedlings will be placed in a moist chamber for the next 21 hours and then returned to the greenhouse room where they will incubate at 22^{o}C and 16 hours of light daily for 12 days. A 0-9 severity scale initially described by Williams will be used to characterize the reaction of all materials evaluated. Williams' scale is described by Chen and Fernando (2006) as follow: " 0= no darkening of tissues around wound, 1= limited blackening around lesion, lesions 0.5-1.5 mm, a faint chlorotic halo may be present, sporulation absent; 3=dark necrotic lesions, a chlorotic halo 1.5-3 mm in diameter may be present, sporulation absent; 5= non-sporulating, by lesions 3-5 mm in diameter, sharply delimited by a dark necrotic margin, may show gray-green tissue collapse; 7= gray-green tissue collapse, lesions 3-5 mm in diameter, sharply delimited by a non-darkened margin; 9= rapid tissue collapse at about 10 days accompanied by profuse sporulation in lesions > 5 mm in diameter with a diffuse margin. Seedlings presenting a 0-2 reaction will be considered resistant; 3-6 intermediate; and 7-9 susceptible."

In the second stage, the best 10% materials of each collection (materials most resistant to each PG strain) will be evaluated in replicated trials to verify reaction of materials to both strains of blackleg. During these trials, four replications will be used. Inoculum production and inoculation procedures will be followed as described. Individual plants scored as resistant will be transplanted and taken to seed production in the greenhouse. At flowering time plants will be covered with pollinating plastic bags to prevent cross pollination. Surviving plants will be scored for canker development when they reach physiological maturity and selections will be made. Canker severity will be evaluated using a 0-5 field scale where 0=no canker; 1= <10% area affected; 2= 11-25% area affected; 3 up to 50% area affected; 4= up to 75% area affected; and 5= dead plant or severely affected with more than 75% of the area affected. This scale is used by Canadian seed companies to rate their breeding materials.

In the third stage, S1 seeds produced in the second stage by elite plants will be planted individually and inoculated as described to fix resistance genes. Plants considered resistant will be taken to seed production as described before.

In the fourth and fifth stages of selection S2 and S3 seeds, respectively, will be planted and screened as described for the second and third stages to continue fixing resistance genes in the selected materials. At the end of the fifth stage S4 seeds with a high degree of homozygosis will be produced. These materials will then be used by the breeder to generate breeding populations and in future studies to characterize the genetics of their resistance.

Expected results

It is expected that materials with promising levels of resistance against PG 3 and 4 will be identified among all accessions evaluated. At the end of the fifth selection cycle seed of elite materials with high degree of homozygocity will be produced.

Pitfalls and limitations

As with many other plant pathogens, the genetic resistance to *L. maculans* has been characterized as being vertical and horizontal. Vertical resistance is conferred by single genes with large effects, whereas the horizontal resistance, also known as adult resistance is composed of multiple

genes each of them making a small contribution to the overall resistance. Vertical resistance is easily appreciated at the seedling stage whereas detection of horizontal resistance is usually done under field conditions. Genetic evidence indicates that these two forms of resistance are controlled by genes that are not necessarily allelic to one another, but in many instances the presence of one set is indication that the other is also present (Rimmer, 2006). In order to overcome this apparent disadvantage plants that are identified as resistant during the inoculation portion of each stage will be allowed to continue growing to produce seed. It is expected that when plants reach maturity they will also be expressing any adult plant resistance they had. The canker severity evaluation will help eliminate plants weaker plants. The methods selected to screen materials have been used by other researchers to characterize reaction of plant materials, thus we do not anticipate major difficulties in conducting the screenings.

Most activities will be conducted in controlled environment to avoid spread of unwanted strains into the fields. Availability of growth chamber and greenhouse spaces will be a factor that will slow down the screening process. During the summer most of our greenhouse rooms have rather poor temperature control, thus activities will be planned to avoid using them for screenings.

Project timeline
This project has duration of three years. In the first year (August 2008 – Dec 2009) initial screenings were conducted, but not all accessions in all collections were evaluated. In the second year (Jan 2010-Dec 2011) we will finish evaluating accessions of all collections. During the second and third year of this project materials with enhanced resistance will be taken to higher levels of homozygocity. Thus, it is anticipated that at the end of the third year materials will be homozygous enough as to facilitate development of breeding lines that could be also be used to study the genetics of their resistance.

Outreach/ Extension activities:
Findings of this project will be shared with the breeder who is a co-PI in this project. Condensed information will be presented to growers to keep them abreast of our progress. Results of our project will also be shared with the scientific community through presentations at professional meetings.

Budget

Student salary and fringe benefits - $3,225
Field Assistant salary and fringe benefits - $21,420
Domestic Travel - $1,000
Foreign Travel Czech Republic - $650
Materials and Supplies - $3,500
Publication Costs - $750
Total - $30,545