Genetics of Resistance in Rutabaga (Brassica napus var. napobrassica) to Plasmodiophora brassicae Pathotype 3

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Clubroot disease caused by Plasmodiophora brassicae is one of the emerging threats to canola (Brassica napus) production in Canada. Several pathotypes of this pathogen have been identified in canola fields of which pathotype 3 is the most prevalent and the virulent one in Canada. Resistance to this pathotype was identified in different Brassica germplasm. The objective of this research is to introgress clubroot resistance in canola from rutabagas and to develop genetic marker(s) for use in marker-assisted breeding. Two rutabaga genotypes, Rutabaga-BF and Rutabaga-PL, inbred for resistance to pathotype 3, were crossed with two clubroot susceptible spring canola lines, A07-29NI and A05-17NI, and F_1 plants were produced. The F_2 and testcross (TC) populations were produced respectively by self-pollination and crossing of the F_1 plants to their susceptible parents. Parents, F_1, F_2 and TC populations were evaluated for resistance to the pathotype 3. In case of the cross Rutabaga-BF × A07-29NI, most of the F_2 families followed a 3:1 and TC families a 1:1 segregation for resistant and susceptible phenotypes. On the other hand, the distribution of the resistant and susceptible plants in F_2 and TC populations of Rutabaga-PL × A05-17NI deviated from simple Mendelian segregation suggesting more complex genetic control of this trait in this population.

Key words: Rutabaga, clubroot, genetics.

Introduction

Clubroot disease of Brassica, caused by Plasmodiophora brassicae Woronin, is an emerging threat to canola production in Canada. In Canada, this disease was first reported in the canola fields in Alberta in 2003 (Tewari et al. 2005) and in Saskatchewan in 2010 (Dokken-Bouchard et al. 2010). Five pathotypes (2, 3, 5, 6 & 8) of this pathogen have been found in Canada (Strelkov et al. 2007, Xue et al. 2008) of which pathotype 3 is the most prevalent and virulent one (Strelkov et al. 2006). According to Tewari et al. (2005) yield loss in canola in Alberta due to this disease could be about 30%. Pageau et al. (2006) reported up to 6.1% reduction in oil content in seeds from the infected plants. The longevity of the resting spores of this pathogen in soil (Wallenhammar 1996) is the major constraint for efficient control of the disease by cultural and/or chemical practices (Voorrips 1995). However, cultivars with genetic resistance in combination with cultural and/or chemical measures can be effective in managing this disease. The durability of genetic resistance in a cultivar can be achieved by pyramiding the resistance genes through marker assisted selection (MAS).

Resistance to clubroot disease, identified in B. rapa genotypes mostly show race-specificity, and controlled by major gene and in B. oleracea often controlled by quantitative trait loci (QTL) (for detailed review, see Piao et al. 2009, Nagaoka et al. 2010). Among B. napus genotypes, the swede or rutabaga (B. napus var. napobrassica) and forage rape (B. napus var. pabularia) were reported to possess race-specific resistance against a wide range of P. brassicae pathotypes (Colhoun 1958, Ayers and Lelacheur 1972, Buczacki et al. 1975, Crute et al. 1983, Gustafsson and Fält 1986). Ayers and Lelacheur (1966) reported that resistance to P. brassicae races 2 and 3 in rutabaga cv. York is controlled by a single dominant gene, whereas the cv. Wilhelmsburger carry one gene for resistance to race 3 and two genes for resistance to race 2. Piao et al. (2009) reviewed that most of the resistant rutabaga genotypes carry one common dominant gene and some genotypes carry an additional dominant gene. The objective of this research was to study the genetic basis of clubroot resistance in two rutabaga genotypes and map the resistance genes for introgression into Canadian canola quality spring B. napus cultivars through MAS.

Materials and Methods

Plant material

Two rutabaga genotypes, Rutabaga-BF and Rutabaga-PL, inbred for resistance to Canadian P. brassicae pathotype 3, were crossed with two clubroot susceptible canola quality B. napus lines A07-29NI (F_7 generation) and A05-17NI (double haploid, DH), respectively. F_2 families were produced by self-pollinating the F_1s on single plant basis and testcross (TC) families were produced by crossing the same F_1 plants to their susceptible parent.

Pathogen isolate

Single-spore derived isolates of P. brassicae, classified as pathotype 3 based on Williams' (1966) differentials, was used. Resting spore suspension (inoculums) was prepared from the preserved gall following the protocol described by Strelkov et al. (2007), and the suspension concentration was adjusted to 10^7 to 10^8 resting spores/ml inoculum.

Resistance test

Four F_2 and their corresponding TC families of Rutabaga-BF × A07-29NI cross and two F_2 and their corresponding TC families of Rutabaga-PC × A05-17NI cross were evaluated. Seeds were germinated on moistened Whatman filter paper No. 1 at room temperature; the seedlings at the age of seven days after seeding were inoculated following two methods, hereafter referred to as Test-1 and Test-2. In Test-1, the seedlings were inoculated by dipping roots in resting spore suspension (Nieuwhof and Wiering 1961). The inoculated seedlings were transplanted in 3 × 3 × 5 cm (L × W × D) cells and were grown in a greenhouse at 21 ± 2°C temperature with 16 hour photoperiod. In case of Test-2, after root dip inoculation the seedlings were transplanted to 3 × 3 × 5 cm cells like Test-1, and 2 mL inoculum was pipetted to each cell immediately after transplantation. The cells were kept saturated with water for first seven days, and HCl solution (10% v/v) was added @ 20 mL/tray (2 × 4 m) /day during this time to ensure acidic condition of soil. From the second week, watering was done once a day. Seedlings were evaluated for clubroot resistance at 42 to 45 days after inoculation and the severity of gall development was rated on a 0 to 3 scale, where 0 = no galling, 1 = one or few small galls on the lateral roots, 2 = moderate galling on the lateral roots and 3 = severe galling on the lateral roots or on the main root. The details of inoculum preparation, inoculation technique and scoring for resistance is described elsewhere (Rahman et al. 2011)

Statistical analysis

Chi-square test for segregation for resistance in F_2 and TC families was done using SAS software version 9.2 (SAS Institute Inc. 2008)

Results

Inheritance of clubroot resistance

Thirty six to 82 F_2 and 33 to 45 TC seedlings from the four families of Rutabaga-BF × A07-29NI cross, and 44 F_2 and 45 to 46 TC plants from the two families of Rutabaga-PC × A05-17NI cross were evaluated in Test-1. In case of Test-2, 129 to 141 F_2 and 68 to 72 TC plants from Rutabaga-BF × A07-29NI, and 137 to 143 F_2 and 69 to 71 TC plants from Rutabaga-PC × A05-17NI were evaluated. Plants with disease score 0 were considered as resistant and those with score 1, 2 and 3 were considered as susceptible.

In most of the F_2 and their corresponding TC families of A07-29NI × Rutabaga-BF, a simple Mendelian segregation for resistance to pathotype 3 was found. For example, among the four families, the F_2 segregation in Family 2 followed a 3:1 ratio and TC segregation followed a 1:1 ratio (Figure 1). However, deviation from this monogenic segregation was also observed in some of the families of this cross, e.g., Family 3 in Test-1.

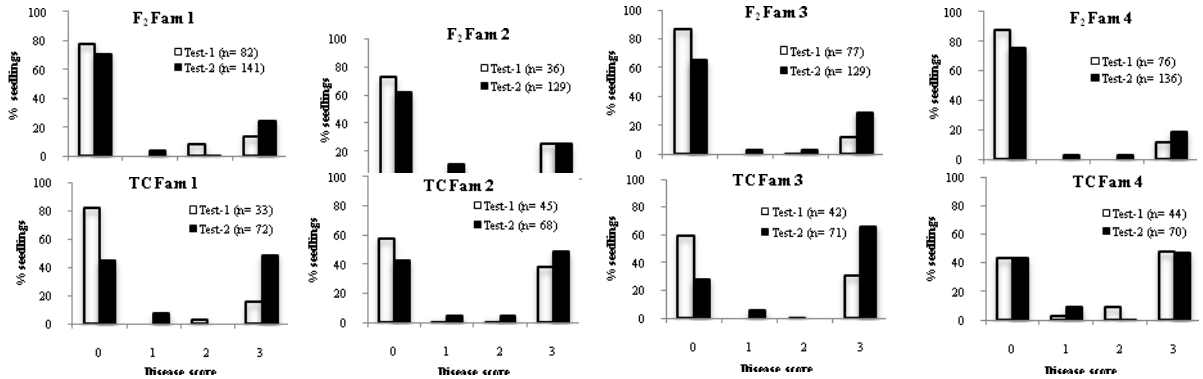

Figure 1: Segregation in F_2 and TC families of Rutabaga-BF × A07-29NI for resistance to Plasmodiophora brassicae pathotype 3 (n = number of seedlings evaluated)

In case of Rutabaga-PC × A05-17NI, though about 75% of the F_2 plants were found to be resistant, however, segregation in its corresponding TC family deviated from 1:1 ratio (Figure 2), where significantly greater number of plants were found to be susceptible than expected.

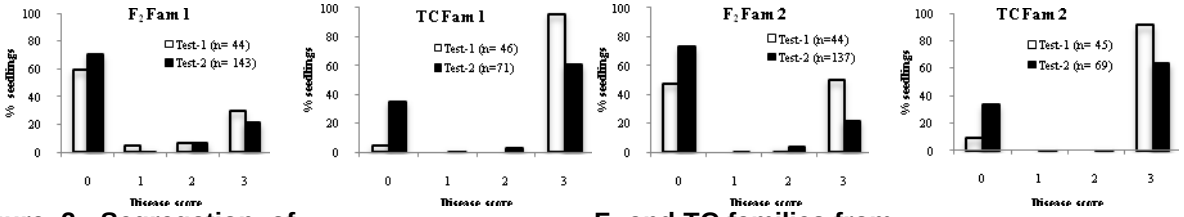

Figure 2: Segregation of F_2 and TC families from Rutabaga-PC × A05-17NI cross for resistance to Plasmodiophora brassicae pathotype 3 (n = number of seedlings evaluated)

Discussion

Root dipping and pipetting inoculation methods have been applied by several researchers to evaluate the Brassica genotypes against P. brassicae pathotypes. In Test-2, in addition to root dip inoculation, 2 ml inoculum suspension was pipetted to each seedling immediately after transplantation to soil. This reduces the chance of escape of any seedlings from infection by the pathogen. Compared to Test-2, in Test-1 increased number of resistant plants was found in most of the F_2 and TC families of Rutabaga-BF × A07-29NI (Figure 1), and deviation from simple Mendelian segregation often found in Test-1 in this population. On the other hand, in case of the segregating families of Rutabaga-PC × A05-17NI, greater number of plants was resistant in Test-2 than Test-1 (Figure 2). This behavior of these two populations could be due to genotypic difference of the populations. Further study would be needed to explain this.

The Rutabaga-BF and Rutabaga-PL plants used in this study were highly resistant to the pathotype 3, and the canola quality plants of A07-29NI and A05-17NI were highly susceptible to this pathotype. The rutabaga genotypes could be considered homozygous for resistance as no segregation for resistance could be found in the self-pollinated progeny of the plants used in crossing. A 3:1 and 1:1 segregation in F_2 and TC families of the Rutabaga-BF × A07-29NI cross indicated that resistance to pathotype 3 in Rutabaga-BF is conferred by a dominant gene. Ayers and Lelacheur (1966) also reported monogenic dominant gene control of resistance to race 3 in B. napus var. napobrassica cvs. York and Wilhelmsburger. The small number of plants with disease score 1 and 2 was observed in all F_2 and TC families could be due to involvement of additional minor genes or QTLs in control of resistance in Rutabaga-BF to P. brassicae pathotype 3. On the other hand, segregation in F_2 and TC families of Rutabaga-PC × A05-17NI indicated involvement of more than one gene and more complex genetic control of resistance in Rutabaga-PC to P. brassicae pathotype 3.

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