Genetic variation in *Fusarium oxysporum* f. sp. *conglutinans* strains causing fusarium-wilt of canola in Western Canada

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Abstract

Fusarium wilt of canola (*Brassica napus* L.), caused by *Fusarium oxysporum* f. sp *conglutinans* (Wollenweber) Snyder & Hansen(FOC), is a disease that has recently been discovered in Western Canada. It has already been observed that canola cultivars differ in their susceptibility to this disease, with resistance likely coming predominantly from a monogenic source. Breeders and pathologists have been interested in investigating the variation in the pathogen population to assist breeding efforts. Sequence related amplified polymorphism (SRAP) molecular analysis was used on 89 isolates of the pathogen isolated from fusarium-infected canola plants grown throughout Manitoba, Saskatchewan and Alberta. A very high percentage (99%) of variation was caused by diversity within geographic populations, with the number of polymorphic loci (*r*) within each population ranging from 100 % for Alberta, 97% for Saskatchewan. However the *r*-value for Manitoba was only 30%, probably due to the low # of isolates. On the other hand Nei's genetic distance didn't differentiate the three geographic populations as they clustered as one group on the dendrogram probably due to gene flow among the *Fusarium oxysporum* populations occurring across Western Canada. The isolates from different host species were diverse in genetics. Our data suggests that there are significant polymorphisms between FOC strains implicating diversity among isolates from Western Canada. To our knowledge, this is the first report of the genetic variation determination of the fusarium-wilt pathogen strains from canola.

Key words: Canola, *Brassica napus*, *Fusarium oxysporum* f.sp. *conglutinans*, genetic variation, fusarium-wilt

Introduction

Fusarium oxysporum is a common widespread soil borne fungus (Kistler 1997). It is an anamorphic species with considerable morphological and physiological variation (Alves-Santos et al 1999). Fusarium wilt is a soil borne disease that affects many crops, but displays a high degree of host specificity resulting in the subdivision into formae speciales based on species attacked (Nelson et al 1981). The formae speciales that can attack canola is *conglutinans*, which includes all crucifer infecting isolates. Fusarium wilt was not identified in canola until recently, and was only reported for the first time in Alberta, and then in Saskatchewan and Manitoba of Western Canada in 1999 (Alberta Research Council). Occurrence of this disease is relatively rare; however, when a field is infected yield losses can be high (Alberta Research Council). Alberta and Saskatchewan has reported high incidence of the disease in several locations while it has been quite scattered in Manitoba with only a few locations (i.e Darlingford, MB) showing symptoms of fusarium-wilt on canola. As such, there is very limited information on the host-pathogen relationship of this particular disease. As a result it is important to understand the host-pathogen interaction to prevent occurrence of the disease as much as possible. One key aspect of this is the genetic profile of the fungus infecting the canola. In addition to providing insight into the evolution of FOC, the amount of diversity can have implications on the durability of genetic resistance in the host plant and how resistance should be screened. For example, Alves-Santos et al (1999) recommended new hybrids of common bean in Spain be screened against multiple strains of *F. oxysporum* f. sp. *phaseolis* when they discovered that the pathogen was more genetically diverse than expected, and that single dominant resistance genes may not provide effective resistance. This also concurs with Gordon and Maryn's (1997) conclusion that despite asexual propagation being the dominant influence on populations of *F. oxysporum*, the absence of sexual reproduction is not likely to prevent this pathogen from continuing to inflict significant damage to host crops. The objective of our study was to study the genetic diversity and population structure of this relatively new pathogen to canola using molecular markers.

Material and Methods

Isolates of *F. oxysporum* were collected by the Alberta Research Council from infected canola plants across Manitoba, Saskatchewan and Alberta. In addition, isolates collected from cabbage, radish, mustard, corn, wheat and oat were included as references. All isolates were single spored, and DNA was extracted using an extraction buffer of 100 mM TRIS, 10 mM EDTA, and 1%SDS and quantified using a spectrophotometer. Genetic diversity was analyzed using the PCR-based Sequence Related Amplified Polymorphism (SRAP) technique, with polymorphic bands visually scored on polyacrylamide gels stained with silver as described in Zhang et al (2005). Genetic diversity and population differentiation was determined among and between geographic and host specific populations.

Results and Discussion

To our knowledge, this is the first report of the genetic variation determination of the fusarium-wilt pathogen strains from

canola. There were a total of 89 isolates of *Fusarium oxysporum* collected from three regions: Alberta, Saskatchewan and Manitoba (Table 1). Sixty seven loci were detected from these isolates with two pairs of primers, RP1+EM1 and RP1+EM2. AMOVA analysis (Table 3) revealed that 99% of variation was caused by molecular diversity within geographic populations because the number of polymorphic loci (*r*) within each population was very high ranging from 100 % for Alberta, 97% for Saskatchewan and 30% for Manitoba. The isolates from Alberta and Saskatchewan showed much higher level of heterozygosity than those from Manitoba. Gene flow estimates indicated that much higher rate of gene flow (*Nm*) took place between Alberta and Saskatchewan populations (*Nm*=38.1) than the rate of gene flow either between Alberta and Manitoba (*Nm*=6.9) or between Saskatchewan and Manitoba populations (*Nm*=6.0). However, Nei's (1973) genetic distance didn't differentiate the three geographic populations as they clustered as one group on the dendrogram (data not presented due to space limitations). This might be due to gene flow among the *Fusarium oxysporum* populations occurring across Western Canada.

AMOVA performed on *Fusarium oxysporum* populations collected from different host species (Table 3) showed that 32.6% of total variance was due to differences among host crop, and 67.4% of total variance was caused by differences within crop species. It means that the isolates living on different host species are diverse in genetics. The number of polymorphic loci (*r*) within each population collected from different crops was not high (lower than 30%) except on canola (100%) (Table 2). Gene flow estimates indicated that it only took place (NM > 1) between the populations obtained from cabbage and mustard, cabbage and canola, radish and canola, and mustard and canola. Nei's genetic distance showed that the populations isolated from different species were mainly clustered as two groups (Fig. 1), in which the isolates from mustard, canola, cabbage, radish and corn were grouped together and totally different from wheat and oat. Mustard, canola, cabbage and radish belong to the same genus *Brassica* except corn; they are different from cereals wheat and oat in genetics. Species *Fusarium oxysporum* evolved along with different host species and developed diversity in genetics. It is believed sexual recombination does not play a role in the development of genetic diversity of *Fusarium oxysporum*, although it has not been proven. However, host selection pressure does increase the speed of the genetic evolution among the *Fusarium oxysporum* because it was observed that high heterozygosity existed among the isolates collected from canola. Determination of the degree of variation within the pathogen population is important not only in understanding the basic biology and epidemiology of this disease, but may also have implications in how resistance is screened for in the host and how durable that resistance could be in the long term.

Table 1. Genetic diversity among FOC populations sampled from three different regions

	SRAP data by region[a]		
Population	*n*	*g*	*r* (%)
Alberta	64	52	100
Saskatchewan	21	20	97.1
Manitoba	4	4	29.9

[a] n = Population size, g = number of genotypes, r = percentage of polymorphic loci (99% criterion)

Table 2. Genetic diversity among *F. oxysporum* populations obtained from seven host crops

	SRAP data by host crop[a]		
Population	*n*	*g*	*r* (%)
Cabbage	3	3	14.93
Corn	2	2	7.46
Radish	2	2	2.99
Wheat	2	2	28.36
Oat	1	1	0
Mustard	2	2	20.9
Canola	89	69	100

[a] n = Population size, g = number of genotypes, r = percentage of polymorphic loci (99% criterion)

Table 3. Analysis of molecular variance (AMOVA) of *F. oxysporum* populations among and within regions and by host species.

	AMOVA Analysis			
		Sum of	Variance	Total
Source of variation	d.f.	squares	components	Variance
Among regions	2	16.186	0.11062	1.1
Within regions	86	875.994	10.1859	98.9
Among crops	6	152.206	4.38926	32.6
Within crops	92	834.138	9.06672	67.38

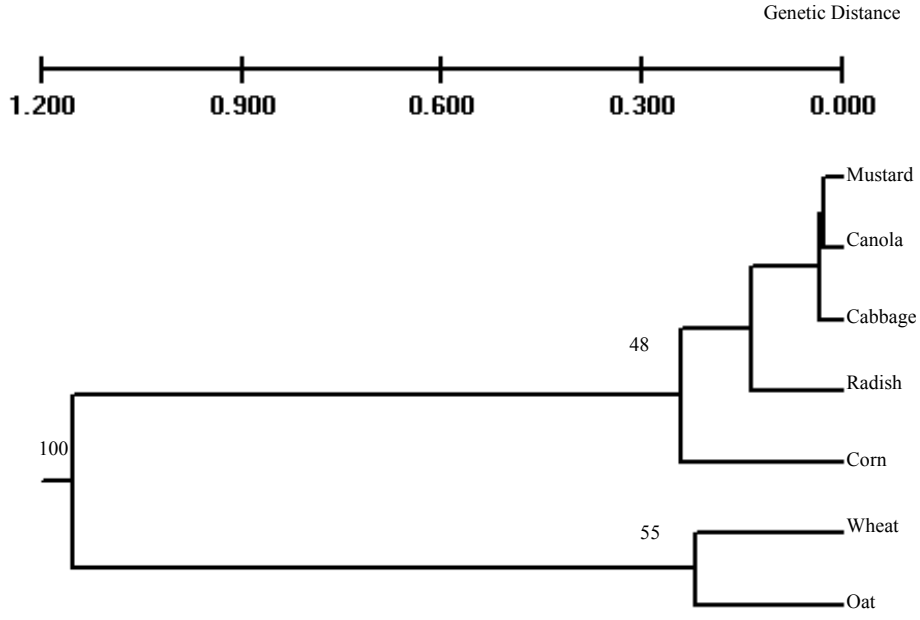

Fig. 1 Nei's genetic distance between populations isolated from canola, mustard, cabbage, radish, corn, wheat and oat.

References

Alberta Research Council. 12 April, (2004). ⩽http://www.arc.ab.ca/extranet/fusarium/>.

Alves-Santos F.M., Benito E.P., Eslava A.P., Diaz-Minguez J.M. (1999). Genetic Diversity of *Fusarium oxysporum* Strains from Common Bean Fields in Spain. Appl. Environ. Microbiol. 65:3335-3340.

Gordon T.R, Martyn R.D. (1997). The Evolutionary Biology of *Fusarium oxysporum*. Annu. Rev. Phytopathol. 35:111-128.

Kistler H.C. (1997). Genetic Diversity in the Plant-Pathogenic Fungus *Fusarium oxysporum.* Phytopathology 87:474-479.

Nei, M. (1973). Analysis of genetic diversity in sub-divided populations. Proc. Natl. Acad. Sci. USA. 70:3321-3323.

Nelson P.E., Toussoun T.A. and Cook R.J. (1981). *Fusarium*: Disease, Biology and Taxonomy. Pennsylvania State University Press, University Park, PA.

Zhang, J.X., Fernando, W.G.D., and Remphrey, W.R. (2005). The genetic structure of populations of *Apiosporina morbosa* in Canada. Phytopathology 95:859-866.