

VIRULENCE ANALYSIS OF DIFFERENT HAPLOTYPES OF *COLLETOTRICHUM LUPINIS* ISOLATES FROM SOUTHERN CHILE

R. Riegel¹, M. Almarza¹, E. Von Baer² and M. Muñoz¹

¹Faculty of Agricultural Sciences, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

²Seeds von Baer, Temuco, Chile

Correspondence to R. Riegel. email: rriegel@uach.cl

ABSTRACT

Anthracnose is the most important and devastating disease in lupins. In order to determine the virulence of different haplotypes of *Colletotrichum lupini* isolates from southern Chile, a bioassay were performed. Based on the genetic distance obtained with RAPD markers, nine representative isolates of *C. lupini* were chosen. With each isolate, inoculation that simulates a primary infection was performed on cultivars Kiev Mutant, Pecosá-Baer and breeding line '246-9'. Plants were rated at three weeks after inoculation based on presence or absence of symptoms. The severity of the disease was very different in dependence of the isolate and the lupin genotype tested, but no significant interaction between them was found. On average, with the low virulent isolates less than 13% of the plants presented symptoms, however, the high virulent isolates affected visibly more than 45% of the seedlings. The highest differences in virulence could be detected within the cultivar 'Kiev Mutant', between isolate A⁻² (15% plants with symptoms) and A⁻¹⁰ (75% plants with symptoms). In average, the cultivar 'Kiev Mutant' was the most susceptible and 'Pecosá-Baer' the most tolerant.

The existence of different molecular phenotypes and the virulence spectrum detected support the existence of different strains of *C. lupini* in Chile. Therefore, when breeding for anthracnose resistance in lupins, emphasis should be placed on the use of diverse resistance sources.

KEY WORDS

Colletotrichum lupini, virulence assay, lupin breeding, pathogen resistance

INTRODUCTION

The further expansion of lupin (*Lupinus* spp.) cultivation depends on the development of cultivars which tolerate anthracnose, the most important and devastating disease in this crop (Cowling *et al.* 1998).

Since the 1990s, the major challenge in lupin improvement has been increasing the resistance towards anthracnose (Cowling *et al.* 1998). Usually the inoculation assays for the detection of anthracnose tolerant genotypes have been carried out only with one

strain, assuming low pathogen diversity (Yang *et al.* 2004; Feiler and Nirenberg, 2004). However, this proceeding can be risky and does not contribute to a perdurable tolerance of the cultivars taking into account the existence of quite diverse haplotypes of *C. lupini*.

The objective of this study was to determine the virulence present in nine representative isolates of *C. lupini* from Chile, which were chosen based on the genetic distance obtained with RAPD markers in previous studies. Such virulence studies may contribute to the development of more efficient genetic improvement programs for anthracnose tolerance in lupin cultivars, which simultaneously operate as preventive control of the disease.

MATERIALS AND METHODS

Based on the genetic distance obtained with RAPD markers, nine representative isolates of *C. lupini* collected in Chile were chosen for virulence assay. Inoculation, that simulates a primary infection, was performed according to Feiler and Nirenberg (2004). In brief, to each litre of a peat-straw-sand (3:1:1) mixture, 2.7 g of lime, 7 g of malt extract and ten pieces of 1 cm² of PDA media with mycelium were added. After the mycelium colonised all the substrate (approx. three days at room temperature) this was mixed with soil at a rate of 1:2 and filled in 1.5 litre pots.

The experiment was conducted in completely randomised block design with 36 treatments consisting of three *Lupinus albus* genotypes (cv. 'Kiev Mutant', cv. 'Pecosá-Baer' and line '246-9') inoculated with nine isolates (A⁻¹, A⁻², A⁻⁴, A⁻⁵, A⁻⁶, A⁻⁷, A⁻¹⁰, A⁻⁴, A⁻¹⁵). Four replications per treatment were used. Each replication consisted of one pot with five seeds per pot. Additional, for each genotype a control without inoculation was used.

Pots with inoculated substrate and seeds were kept in a greenhouse with a day average temperature ranging from 20–25°C and average humidity of 65%. Plants were rated at three weeks after inoculation based on presence or absence of symptoms. The percentage of affected plants in each pot was transformed to arcsosine (Anscombe (1948) cited by Zar (1999)) for doing a factorial ANOVA considering lupin genotypes and

fungus isolate as fixed factors. In cases of significant differences the Tukey HSD test was performed. The interaction of genotypes x isolation was not significant, therefore one way Anova was performed for analysing effects of isolate on survivors of each variety separately.

RESULTS AND DISCUSSION

The pathogenicity of all isolates could be confirmed. Characteristic symptoms of anthracnose were easy to score on diseased plants. The severity of the disease was very different depending on the isolate and the lupin

genotype tested (Table 1). No significant interaction between isolate and lupin genotype was found.

With the low virulence isolates A⁻¹, A⁻² and A⁻⁶ less than 13% of the plants presented symptoms, however, the high virulence of isolates A⁻¹⁰ and A⁻¹⁴ affected visibly more than 45% of the seedlings. The highest differences in virulence could be detected within the cultivar 'Kiev Mutant' and between isolate A⁻² (15% plants with symptoms) and A⁻¹⁰ (75% plants with symptoms). On average, the cultivar 'Kiev Mutant' was the most susceptible and 'Pecosa-Baer' the most tolerant.

Table 1. Virulence analysis of *Colletotrichum lupini* isolates on different white lupin (*Lupinus albus*) genotypes.

Isolates	Lupin genotypes			
	cv. 'Kiev Mutant'	cv. 'Pecosa-Baer'	Line '246-9'	Average
A ⁻¹	20 a	0 a	15 ab	12 a
A ⁻²	15 a	5 a	5 a	8 a
A ⁻⁴	45 abc	15 abc	20 abc	27 ab
A ⁻⁵	55 abc	30 bc	35 bcd	40 b
A ⁻⁶	30 ab	0 a	10 ab	13 a
A ⁻⁷	55 abc	35 c	35 bcd	42 b
A ⁻¹⁰	75 c	10 ab	55 d	45 b
A ⁻¹⁴	70 bc	20 abc	45 cd	47 b
A ⁻¹⁵	60 bc	30 bc	60 d	50 b
Average	47 A	16 C	31 B	

* Percentage of seedlings with symptoms of anthracnose infection.

Values followed by the same small letter within a column and capital letters in a line are not significantly different at $P < 0.05$ according to Tukey's test.

Pecosa-Baer, a cultivar selected in Chile under natural conditions with high anthracnose pressure, had good tolerance for most tested isolates. This cultivar, derivate from TypTop-Baer, is one of the most tolerant cultivar tested by Feiler and Nierenberg (2004). However, if Pecosa-Baer is inoculated with isolate A⁻⁷, as high as 35% of seedlings were affected. A recent study shows that anthracnose resistance in lupin cultivars was temperature dependent (Thomas *et al.* 2008). This ambient factor can also have an interaction with the virulence of different *C. lupini* strains. It is obvious that at least in Chile, no single isolate will suffice for resistance screening. The genetic diversity and level of variability in virulence among isolates identified in this work may help to define the breeding method for effective resistance.

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