EVALUATION OF ANTHRACNOSE RESISTANCE ON A TARWI (*LUPINUS MUTABILIS*) GERMPLASM COLLECTION

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INTRODUCTION

Anthracnose caused by *Colletotrichum lupini* (Bondar) Nirenberg, Feiler & Hagedorn is the most important disease in *Lupinus,* is known since the first half of the twentieth century by causing significant yield losses and to be a major limiting factor for lupin production. Most *Colletotrichum* pathogens are polyphagous, with the same genetic entity found on multiple hosts. Moreover, frequently the same host is affected by multiple *Colletotrichum* spp., with no clear differences on the symptoms caused. However, the lupin anthracnose pathosystem seems to be an exception to this common trend, as lupin anthracnose is caused solely by *C. lupini* and *C. lupini* seems to prefer *Lupinus spp*. Additionally, very little genetic diversity is recognized among *C. lupini* populations, with only two groups reported: one corresponding to the North American outbreak in the first half of the 20th century, and currently not occurring in the nature; the other corresponding to the contemporary outbreak, found across the world (Talhinhas *et al.,* 2016a). In a context of near-clonality of the pathogen, finding resistant germplasm seems a promising strategy for sustainable disease control. Previous studies on tarwi showed little success, prompting the need for further studies on this species with a relatively limited genetic diversity pool. In this work we report the characterization of anthracnose response in a tarwi germplasm collection that is being studied for its adaptability to Mediterranean climate growing conditions, in Portugal. For such, a contemporary and local *C. lupini* strain was used.

MATERIALS AND METHODS

Two isolates of *Colletotrichum lupini* were obtained one in Portugal "NG001-local" and other in France "RB221-contemporary" and placed to grown on PDA in dark condition at 25ºC. DNA of these isolates was extracted for sequencing ApnMat1 gene to verify that the causal agent of disease in Portugal is the same of the founded across the world. The ApnMat1 gene was chosen in this study for being much informative than ITS, ß-tub2 and GS (Silva *et al*., 2017). For resistance essays were selected 10 accessions of *Lupinus mutabilis* and 6 of *Lupinus albus* with resistance known (Talhinhas *et al.,* 2016b). The plants were cultivated in trays containing sterilized sand within of greenhouse with temperature around 25ºC. Ten days after cultivation of the fungus a spore suspension was obtained by inundation the petri dish with sterile water and the spores was released and separated from the mycelia by mesh filter nr.1. The spores suspension obtained was applied in sixteen accessions when the plants had 7-8 leaves and incubated in humid chamber. Each accession was composed by fifteen plants and to guarantee the adherence of spores drops in the plants was applied gelatin at 2% of concentration in a ratio of 1:1 with spore suspension. The evaluate of severity of disease was made using the method proposed by Talhinhas *et al.* (2016b), with some improvements.

$$S=n_{Si}*S_i+n_{Pi}*P_i*f_i+n_{Li}*L_i*f_i$$

n_{Si}-number of occurrences in the stem;
Si-Stem of plant $_i$
n_{pi}-number of occurrences in the petiole;
Pi-Petiole of plant $_i$
n_{Fi}-number of occurrences in the leaves;
Li-Leaves of plant $_i$
f_i-Weight factors; were used the following values: 0.7, 1 and 1.3 as weight factores.

Table 1: Symptoms scale used to assess anthracnose resistance in plants of *Lupinus mutabilis* adapted of (Talhinhas *et al.,* 2016b).

Notation		Symptoms
R	0	no symptoms
	5	torsion without necrosis in the main stem
S	8	torsion with lateral necrosis in the main stem
	10	torsion with fatal necrosis in the main stem
	1	presence of symptoms in the petiole
	0.2	presence of symptoms in the leaflet
	0.7	Weight factor for torsion without necrosis
	1	Weight factor torsion with lateral necrosis
	1.3	Weight factor torsion with fatal necrosis

The final concentration of spore was adjusted for $1x10^6$ calculated using hemacytometer. The data analysis was done using Kruskal Wallis for the means comparison in Studio Program Version 1.1.456.

RESULTS

Sequencing results

The results of sequencing of ApnMat1 gene for the two isolates reveals an similarity of 100% between them. The Portuguese isolate does not differ from the isolated reported to the rest of the world. This result reinforce the results founded by Nirenberg *et al*. (2002), Lotter & Berger (2005) and more recently validated by Damm *et al*. (2012) that attribute *Colletotricum lupini* as casual agent of disease. In Chile was reported similar with our in diferente samples collected and only *C. lupini* was detected as the causal agent of anthracnose (Riegel *et al*., 2010).

>CluNG001_ApnMAT1
CAACGTCGCTGCATTAGTTCCTACCATGCTAGGTCTCAATGACTGTGCAAGAATTTTCGCGACTACAAGTTGCCGCGAAGGAGCACCTTCGTCTGCTGCCT
TAGGCCGAGTATGCCTCGTCTTCCCTCAGAGCCATGTCCCAGCTCGCGCCGAGAAAGTCGAAAAAACACCAACACTTGATGTTTGGGCCAGAACTAAGCA
GACATGCCGTGCCCGGCACTCACCCTTACCATGCTCAGCCGGAGCAAAAGAGACTTCTTCTGCTAACTTAGGGGCCTGTCGAGACCAGGAAAGGCTCGAA
TTCGTGTAAGGCTCCAAAGCCACTTCGTGGAGTTTTACCTGCTCTACTCACAAGATGGGCGAATTCCGGACGCATCATTGGGCGGAGACGGTGGCGACGC
TCTTGATGGGGTTCAAGCAAAGAGTGAGCTACTTCAAGCAGAGCAAGACTCGAAAGGATAGAGCAATTCGCGTCGTCGCTGCCGAAGAATCGGATGTCTA
ACTCTGCCATGATGCGAAGATGAGCCACCACAAGACCGATATAGAATGCTTGATAGATTTACTAAAACGATAATGGAATCATAATGTTCATGTCTCATTGCC
TTAATCTTCTTCTTCTGCACGACTAAACTACCTAGCTGTCTCTTTTCGATAGTAACGGTTACAGCTCGTCACACTGTTCGTCGAATCTCGCGTGACTCCACAT
CGCCCCTTAGAAATGTATAGACTACGACGCTTGAGACTGGCTCTTTTTCCAATCACTGCTCCATATAAAAGTACCGCACCTCCACTCCGTCCCCTTTTCCTT
TTCTCCGGATTTCCCCAGAGGACGGGCGCAGATGaAAAACGACCTGCCTATGTGGATCATCAGCTATGAATGGGGGTCGAGGGGGGGCGGGGGCAGAAA
GATCGGGATATGTCGGGGGACGAGAGAGAGGAGGGTaGGACAGAGGCCTCGGTGGACGCAT

>CluRB221_ApnMAT1
CAACGTCGCTGCATTAGTTCCTACCATGCTAGGTCTCAATGACTGTGCAAGAATTTTCGCGACTACAAGTTGCCGCGAAGGAGCACCTTCGTCTGCTGCCT
TAGGCCGAGTATGCCTCGTCTTCCCTCAGAGCCATGTCCCAGCTCGCGCCGAGAAAGTCGAAAAAACACCAACACTTGATGTTTGGGCCAGAACTAAGCA
GACATGCCGTGCCCGGCACTCACCCTTACCATGCTCAGCCGGAGCAAAAGAGACTTCTTCTGCTAACTTAGGGGCCTGTCGAGACCAGGAAAGGCTCGAA
TTCGTGTAAGGCTCCAAAGCCACTTCGTGGAGTTTTACCTGCTCTACTCACAAGATGGGCGAATTCCGGACGCATCATTGGGCGGAGACGGTGGCGACGC
TCTTGATGGGGTTCAAGCAAAGAGTGAGCTACTTCAAGCAGAGCAAGACTCGAAAGGATAGAGCAATTCGCGTCGTCGCTGCCGAAGAATCGGATGTCTA
ACTCTGCCATGATGCGAAGATGAGCCACCACAAGACCGATATAGAATGCTTGATAGATTTACTAAAACGATAATGGAATCATAATGTTCATGTCTCATTGCC
TTAATCTTCTTCTTCTGCACGACTAAACTACCTAGCTGTCTCTTTTCGATAGTAACGGTTACAGCTCGTCACACTGTTCGTCGAATCTCGCGTGACTCCACAT
CGCCCCTTAGAAATGTATAGACTACGACGCTTGAGACTGGCTCTTTTTCCAATCACTGCTCCATATAAAAGTACCGCACCTCCACTCCGTCCCCTTTTCCTT
TTCTCCGGATTTCCCCAGAGGACGGGCGCAGATGAAAAACGACCTGCCTATGTGGATCATCAGCTATGAATGGGGGTCGAGGGGGGGCGGGGGCAGAAA
GATCGGGATATGTCGGGGGACGAGAGAGAGGAGGGTAGGACAGAGGCCTCGGTGGACGCAT

Figure 1: DNA sequence comparison of the intergenic regions between Portuguese (NG001) and France (RB221) isolates obtained by sequencing ApnMat1 gene.

Assessment of severity

Table 2: Means and homogeny groups for the severity of infection caused by anthracnose in 16 *lupin* accessions.

Nr.	Accession name	Type[a]	Means	H.G[b]	Resistance
1	**MUTAL**	**BL**	**10.00**	**a**	**S**
2	BUNHEIRO MURTOSA	BL	6.88	a b c d	-
3	**I82**	**BL**	**6.83**	**a b c d**	**S**
4	**XM139**	**BL**	**6.75**	**a b**	**S**
5	**JKI377**	**BL**	**5.44**	**a b c d**	**S**
6	**LM13**	**BL**	**5.30**	**b c d**	**S**
7	PRIMA	BL	4.95	a b c d	-
8	**LM231**	**BL**	**4.89**	**c d e**	**R**
9	**LM268**	**BL**	**4.70**	**c d e**	**R**
10	**LM34**	**BL**	**4.57**	**b c d e**	**R**
11	RIO MAIOR	BL	4.57	b c d e	-
12	**LM18**	**BL**	**4.39**	**a b c**	**R**
13	**XM5**	**BL**	**3.81**	**b c d e**	**R**
14	ESTORIL	BL	3.01	d e	-
15	LUBLANC	BL	1.57	c d e	-
16	MISAK	BL	1.36	e	-

[a]Type of germplasm: Breeder lines
[b]H.G-Homogeny Groups: accessions with one or more letter in common are not different statistically for 95% of significance.
S-Susceptible
R-Resistant

The accessions of Lupinus albus marked with trace have their resistance characterized por Talhinhas et al. (2016). This accessions was used only as reference in this study.

Figure 2: Symptoms and signal of the disease visible in the petiole and main stem. Torsion and formation dark injury in depression. Productions of conidium aggregate of mucilaginous mass of yellow color.

Figure 3: Typical symptoms of anthracnose visible in the main stem torsion by bending.

Figure 4: Plant exhibit anthocyanin pigmentation visible in main stem and petiole. Used as a criterion for selection of accessions for the assays.

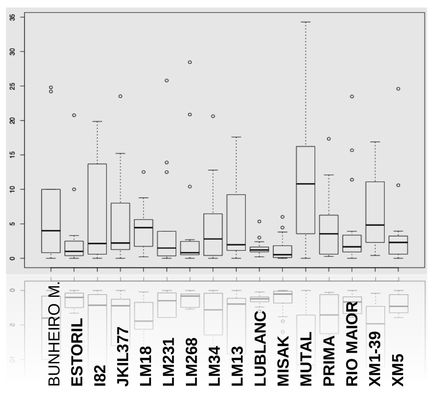

Figure 5: Box-plot representing the average, s.d. and extreme values observed in 16 lupin accessions for resistance to *C. lupini.*

The results show that 50% of the accessions of *Lupinus mutabilis* are susceptible and other 50% resistant for *Colletotrichum lupini*. Although some accessions belong to two groups do not present statistically significant differences. The susceptible accessions are distributed in the range of 5.3 even 10 and the resistant from 3.81 until 4.89. In the susceptible group stands out Mutal line as most susceptible (Fig. 3). The Mutal line just like XM1-39 are characterized by absence of anthocyanin pigmentation in plants. The accessions I82, JKIL377 and LM13 although present anthocyanin pigmentation the plants of this accessions are less robust with thin main stem, unlike LM231, LM268, LM34, LM18 and XM5 accessions classified as resistant also exhibit anthocyanin pigment and robust main stem. These accessions present low values for standard deviation and extreme values (Figure 5). Talhinhas *et al.* (2016b); Falconi (2012) and Falconi *et al.,* (2015) studying the anthracnose resistance in the same germplasm collection, verified that all *Lupinus mutabilis* accessions were susceptible. The present results differ from those found by this author and the probable cause of this difference may be related with anthocyanin pigmentation, criterion used to define the accessions to be integrated in the assays. Anthocyanins are the most well-distributed and can be found in many species under various forms, with multiple biological functions such as fungitoxic, antibacterial and antiviral (Lo & Nicholson, 2008). Several studies report the role of anthocyanins in the resistance to fungi of the genus *Colletotrichum* and other species in different cultures (Hammerschmidt, 1977; Wegener & Jansen, 2007). Talhinhas *et al.* (2006) reported that the resistance for *Colletotrichum lupini* in *L. angustifolius* was related with vegetative development organs of plants. Our results corroborate with these authors where the robust plants are resistant against lupin anthracnose.

CONCLUSIONS

In the 10 accessions of *Lupinus mutabilis* under evaluation, we conclude that some accessions has some resistance to lupine anthracnose. The accessions considered as resistant should be used in breeder program to integrate resistance in other accessions with agronomic interest but susceptible to lupin anthracnose and for the improvement the germplasm banks of *Lupinus mutabilis*.

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