Plant Genomics in Lentil breeding: Development of a cDNA based rapid screening method for *Ascochyta* blight resistance in Lentil (*Lens culinaris* L.)

Perumal Vijayan , T. Warkentin , S. Banniza and A. Vandenberg
Department of Plant Sciences/Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8

Abstract

Ascochyta blight causes severe crop losses in temperate lentil production areas around the world, including Saskatchewan. At present there is no reliable system available to breeders for identifying blight resistant lentil lines. We are trying to develop a reliable, high throughput and low cost cDNA based system for screening lentil germplasm for polygenic resistance to *Ascochyta* blight. We have chosen the *Ascochyta* blight susceptible lentil variety Richlea and the closely related resistant breeding line 1156-2-17A for this study. Lentil plants were inoculated with spore suspensions of *Ascochyta* under conditions that clearly showed the difference in disease resistance between these lines. Tissue collected from resistant and susceptible lines at different times after inoculation will be used for extraction of total RNA - representing all the genes expressed by the plants in response to *Ascochyta* infection. Complementary DNA (cDNA) made from these RNA samples along with mock-inoculated controls will be visualized on poly acrylamide gels using the technique of 'differential display'. We intend to identify the sequences of lentil genes expressed only by the resistant lines in response to *Ascochyta* infection and use them for developing molecular markers for the resistance trait. cDNA samples made from these plants will also be useful in developing a cDNA library of lentil tissue for future EST projects.

Materials and Methods

Standardization of plant growth and inoculation conditions:

In order to isolate genes expressed by the resistant line it is necessary to compare the pathogen-induced genes in both the resistant and susceptible lines of lentil. To achieve this, the ascochyta blight resistant lentil line 1156-2-17A and the susceptible variety Richlea were grown (5-6 plants per pot) in disease free pots and potting medium in controlled environment growth chambers. Cultures of *A. lentis* (isolate No. 80982 obtained from the pulse pathology lab, CDC, University of Saskatchewan) were grown on Potato Dextrose Agar plates and used for harvesting conidiospores for inoculation. Inoculation of both lines was carried out on 14 to 18 day old plants, by spraying 1.5 ml spore suspension per plant using an atomizer. Un-inoculated controls were sprayed with an equal volume of sterile distilled water. Inoculation was carried out in illuminated mist chambers and the inoculated plants were allowed to remain in the mist chambers for 24 hours after inoculation. The plants were subsequently transferred to growth chambers and pots were individually enclosed in transparent plastic collars and periodically sprayed with sterile distilled water to maintain humidity and encourage maximum infection. The

inoculated plants were scored for disease symptoms (lesions) on the 14th day after inoculation.

While sufficient infection pressure must be applied to elicit a defense response from both the resistant line 1156-2-17A and the susceptible variety Richlea, excessively high infection of tissues may destroy too many host cells and reduce the ability of the affected tissue to produce the messenger RNA s responsible for resistance. Therefore three standardization experiments were conducted to determine the optimal conditions for inoculation and disease development so that lentil tissue could be harvested from plants that were responding robustly to *Ascochyta*. mRNA isolated from such tissue is expected to contain maximum expressed copies of resistance related genes. With this aim, different spore concentrations were used for inoculation to test if differential resistance and susceptibility by the respective lines was optimally expressed.

Isolation of high quality mRNA from control and infected lentil plants

High quality total RNA was isolated from the tissue collected 10, 24 and 72 Hrs after inoculation using the RNAeasy kit manufactured by Qaiagen Inc., Mississauga, Ontario. All the RNA Samples were tested for integrity by electrophoresis on Agarose formaldehyde gels.

Results

Disease development in Ascochyta inoculated lentil

The inoculated plants were scored for disease symptoms (lesions) on the 14th day after inoculation. The number and relative size of the lesions on each plant was recorded the data for 15 plants were averaged to arrive at a disease score for each treatment. It was observed that the differential response of the two lines to Ascochyta was maintained under inoculum concentrations ranging from 6,250 to 500,000 spores /mL (Table 1). All replicates showed similar results. The results showed that the resistant line 1156-2-17A exhibited a high degree of resistance to Ascochyta blight at all spore concentrations. The susceptible variety Richlea was highly susceptible at even the lowest concentration of spores used (See table:1). Visual observation showed that none of the spore concentrations seriously damaged the plant tissue upto 72 Hrs in either of the lines.

Yield of mRNA from control and infected lentil plants

While the yields from the 72 and 24-hour samples varied between 75 µg and 200 µg per 20 mg of tissue, the RNA form 10 hr samples were between 10 µg and 60 µg per 20 mg. tissue. All the RNA Samples were tested for integrity by electrophoresis on Agarose formaldehyde gels and found to be of good quality.

Table: 1 Severity of Ascochyta blight disease in lentil lines 1156-2-17A and Richlea 14 days after inoculation with spore suspensions of different concentrations.

Inoculum 1000 spores/ml	diseased leaves/ plant								Stem lesions/plant		Total lesions per plant	
	mild		moderate		severe		dead					
	1156	Richlea	1156	Richlea	1156	Richlea	1156	Richlea	1156	Richlea	1156	Richlea
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.3	2.0	0.3	0.0	2.0	1.5	6.1	3.1	3.4	0.0	0.5	3.5	8.9
12.5	1.3	0.5	1.1	2.4	2.0	4.0	0.5	2.6	0.1	1.2	4.5	8.1
25.0	2.4	0.3	1.8	3.0	1.9	3.8	0.1	4.4	0.2	2.4	6.3	9.5
50.0	2.4	0.0	1.1	2.4	1.3	5.0	0.0	4.1	0.0	1.8	4.7	9.2
All treatments	8.1	1.0	3.9	9.8	6.7	18.9	3.7	14.4	0.3	5.8	19.0	35.6

Discussion of results and conclusions:

Conditions for inoculation and harvesting of lentil tissue from Ascochyta blight resistant and susceptible lines has been standardized and sufficient tissue collected and stored under appropriate conditions for extraction of RNA. RNA has been isolated from inoculated and control tissue and the quality of extraction have been confirmed to be high. Differential display experiments will be conducted using the RNAimage kit supplied by GenHunter Corporation.

The genes differentially expressed in response to pathogen inoculation by the resistant lentil line but not by the susceptible line will identified and sequenced. Oligonucleotide primers based on these sequences can be used to detect the expression of resistance by Reverse Transcriptase PCR on RNA isolated from inoculated plants. Two different sources of resistance to Ascochyta have been identified in Lentil. They have been originally derived from line ILL 5588 and the variety Indianhead. Lentil line 1156-2-17 used in this project carries the ILL 5588 source of resistance. The expression of resistance related genes identified in this line could be compared with that carrying Indianhead source of resistance, to find out if different sources of resistance to *Ascochyta* blight respond by activating different sets of downstream genes. If this is true, the RT PCR based detection may be suitable to identify lines in which resistance genes have been pyramided.