Revisiting Strategies for Breeding Anthracnose Resistance in Lentil: The Case with Wild Species

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Summary

Breeders at the Crop Development Centre (CDC) have up to now only used germplasm resources available in the cultivated lentil to develop new varieties with resistance to diseases. Based on recent studies, the available cultivated germplasm does not offer sufficient genetic variation for resistance to anthracnose and ascochyta diseases. Lentil crop is attacked by two major diseases (anthracnose and ascochyta) that can cause 100% loss in the worst scenarios. Since anthracnose is only a major lentil disease in North America, no work has been done to improve resistance to this disease elsewhere. Wild species of many crops are known to carry many disease resistance genes lacking in the cultivated crop. We began the search for anthracnose resistance in the six wild species of lentil (world collection), of which two can be easily crossed with the cultivated type. Two strains of anthracnose (race 1 and race 2) with varying degrees of virulence were reported. The 2002 field data suggested that some of the *Lens ervoides* and *Lens lamottei* accessions exhibited no lesions at all when exposed to the combination of the two anthracnose strains. The cultivated types that show resistance to the less virulent strain were severely affected by anthracnose. In the greenhouse study the wild species were inoculated with the two strains separately and results indicate that no accession is immune to the more virulent type. However, some of the *L. ervoides* and *L. lamottei* accessions had good resistance compared to their cultivated counterparts. As a long term strategy, the lentil breeding program at CDC, University of Saskatchewan has a goal of fully utilizing the available resistance sources. However, these two species cannot be easily crossed with the cultivated types using the conventional/manual crossing techniques. A tissue culture procedure involving embryo rescue is used to facilitate crossing. We have been able to successfully rescue some embryos from crosses with *Lens ervoides.* The hybrid plants produce some fertile seeds which will be evaluated for resistance to both anthracnose and ascochyta. The selected resistant lines will then be backcrossed to the adopted backgrounds in order to deploy resistance genes.

Introduction

Lentil is valuable as food and feed and plays an important role in crop rotation and farm economics of the Canadian prairies. Its low fertilizer requirement and ability to fix nitrogen for the following crop makes it environmentally friendly and an important crop in sustainable agriculture. The crop constitutes 29% and 65% of world production and export, respectively, in Western Canada alone (Bi-weekly Bulletin, 2002). However, anthracnose caused by *Colletotrichum truncatum* is one of the most important constraints for production of lentil in North America and can cause up to 100% yield loss in the worst scenarios where frequent rainfall and higher humidity prevail. The isolates of anthracnose vary in their degree of virulence. Some are mild while others are aggressive, making it difficult to breed for resistance. Breeders at CDC continuously search for resistance in the cultivated lentil. Our data showed that sources of resistance in cultivated lentil do not offer sufficient protection against all anthracnose isolates. We began to search for new sources of resistance in six wild species of lentil under both field and greenhouse conditions.

Materials and Methods

Field Experiment:
Lentil accessions (460) received from International Centre for Agricultural Research in the Dry Areas (ICARDA) were planted in a randomized complete block design with two replications at North Seed Farm, University of Saskatchewan, Saskatoon. A mixtures of the two isolates of anthracnose, most prevalent in Saskatchewan was multiplied on autoclaved wheat seeds. The seeds were then spread along the plant rows to infect the wild species and cultivated control lines. The inoculum was manually applied three times at 2 weeks interval. Scoring began when the susceptible checks started showing the symptom.

Greenhouse Experiment:
Eight seeds of each wild lentil germplasm was planted in single 10 cm diameter plastic pots filled with soil. Two seeds of the susceptible cultivar ‘Eston’ were sown in the middle of every pot to ensure that resistant plants were not just escapes. The resistant and susceptible control lines were also included in the experiment. Plants were grown for 3 weeks; each pot with its plantlets was encased in a plastic sheet extending above the height of the plants. The plants were sprayed with aqueous conidial suspension of isolates ‘B36’ and ‘A8’ until run-off using an atomizer. Immediately after inoculation, the plants were incubated in a humidity chamber for 24 hrs and then transferred to greenhouse benches. Host plant reactions were then scored visually when the susceptible parent plants started wilting. Evaluations were performed on individual plants and the average scores were used for further analysis according to the procedure described by Buchwaldt et al. (2001) (1-9 rating scale, where 1= immune and 9= severely diseased or wilted) with little modifications.

Results and Discussion

Field evaluation:
There was significant difference between species and infection rate between dates suggesting that the level of disease severity was different in different species and the severity increased over time. Cultivated lentil lines with known resistance to isolate 'B36' were affected by the combination of the two isolates of anthracnose. Susceptible controls, namely, 'Eston' and 'Spanish Brown' were severely hit by anthracnose while cultivar 'Indianhead' and germplasm 'PI 320937' had moderate resistance (Table 1). Among the wild species, *Lens ervoides* showed higher level of field resistance followed by *Lens lamottei*. *Lens orientalis* was the most susceptible (Figure 1). Lens ervoides species also showed high level of resistance in field experiments conducted 2 years ago using anthracnose infected lentil residue from the previous years (Tullu et al., 2000). Lens nigricans species was a slow emerging and slow growing type, which made it difficult to score after the first inoculation. The second and third inoculations revealed differences in infection to anthracnose. Most of the resistant ervoides and lamottei accessions flowered and matured similar to cultivar 'Eston', an adapted cultivar in the Prairies.

Greenhouse evaluation:
Of 461 ICARDA accessions planted, 59 lines did not germinate. The wild species were inoculated with the two isolates separately and results indicate that no accession was immune to infection by both isolates. A higher frequency of resistance was exhibited by *Lens ervoides* compared to all other species tested using both isolates (Table 2). Some accessions were uniformly resistant while others exhibited resistant and susceptible plants within an accession. About 4 % of the *Lens orientalis* (the closest relative of cultivated lentil) accessions showed some resistance to isolate 'B36' of anthracnose whereas, none of the *Lens orientalis* accessions had resistance to 'A8' isolate. Apart from *L. ervoides*, few *L. lamottie* and few L. nigricans showed some resistance to the more virulent isolate, 'A8'.

Interspecific hybridization:
Lens ervoides belongs to the secondary gene pool of lentil and it is not crossable to the cultivated lentil. Lens lamottei has not been classified to date in crossability groups. Based on our crossability studies with the cultivated lentil, it may fall under secondary gene pool of lentil. Some of the resistant *Lens ervoides* and *L. lamottei* accessions were crossed to the adapted cultivar by using a tissue culture procedure involving embryo rescue and we have been able to successfully rescue some embryos from crosses with these two species.

The hybrid plants produce some fertile seeds that will be advanced to higher generations and evaluated for anthracnose resistance. The selected resistant lines will also then be backcrossed to the adopted backgrounds in order to deploy resistance genes. The development of genetic populations from the F_1 hybrid seeds is also critical to get information on the mode of inheritance of resistance in these two species and streamline resistance breeding for anthracnose.

Table 1. Field reaction of cultivated (*Lens culinaris*) lines to a mixture of isolates 'B36' and 'A8' of anthracnose in North seed Farm, Saskatoon-Summer of 2002

Lines	Score (1-9)	No of times tested
PI320937	4.3	17
Indianhead	4.5	10
IPK-181	4.8	6
IPK-1254	4.8	6
IPK-214	5.0	6
IPK-153	5.1	6
IPK-1156	5.1	6
IPK-969	5.5	6
IPK-899	6.5	6
IPK-102	6.9	4
Eston	8.0	13
Spanish Brown	8.3	5

Table 2. Evaluation of ICARDA wild germplasm accessions for resistance to isolates '95B36' and 'A8'of anthracnose in the greenhouse

	Resistant	Medium Resistant	Susceptible	Total Number tested
Lens orientalis	7(0)	8(0)	180(189)	195(189)
Lens odemensis	1(0)	0(0)	47(50)	48(50)
Lens nigricans	2(0)	5(0)	25(33)	32(33)
Lens ervoides	32(30)	30(43)	40(37)	102(110)
Lens tomentosus	0(0)	0(0)	6(6)	6(6)
Lens lamottei	6(0)	2(2)	1(7)	9(9)
Total	48(30)	45(45)	299(322)	392(397)

Numbers in parenthesis represent values for 'A8' anthracnose isolate

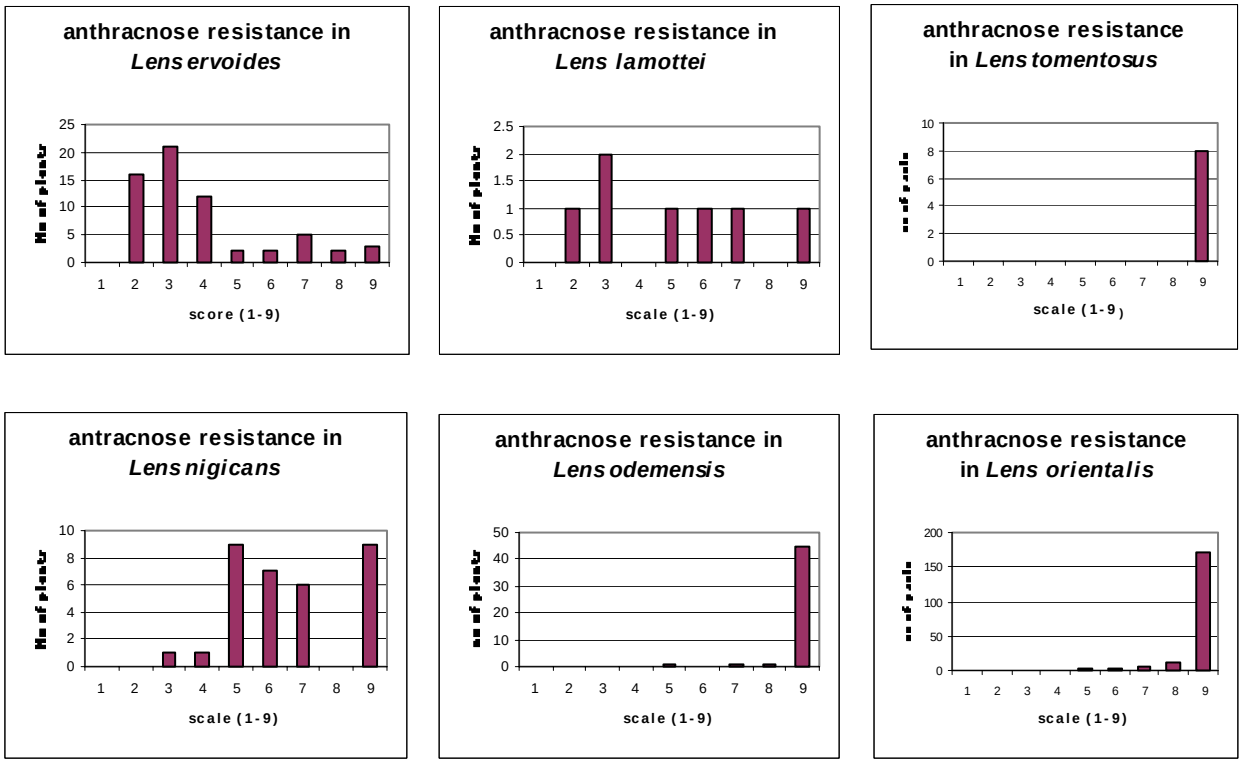

Figure 1. Evaluation of ICARDA germplasm for resistance to a mixture of isolates B36 and A8 of anthracnose in North Seed Farm, Saskatoon, summer of 2002

Reference:

Agriculture and Agri-Food Canada, February 2002. Canadian pulse and special crops industry: Situation and outlook. Bi-weekly Bulletin. 15(3).

Buchwaldt, L., A. Vandenberg, A. Tullu and C.C. Bernier. 2001. Genetics of resistance to anthracnose (Colletotrichum truncatum) in lentil. In:AEP (ed) Proc 4th European Conference on Grain Legume Research, Cracow, Poland, pp. 242. (Poster).