White Mold Resistance in Pea and Lentil through Breeding and Biotechnology

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Summary

Sclerotinia sclerotiorum is an important disease of pea and lentil crops in the US. Complete resistance is lacking, but partial resistance has been found in lentils and, through this research, partial resistance has been identified in pea germplasm. This resistance will be useful in developing improved cultivars. Introduced resistance through biotechnology is an alternative when natural resistance is limiting. Established transformation protocols are being used to transfer the oxalate oxidase gene into pea and lentil to confer resistance. Outcomes of the project are identification of partially resistant pea lines and a twin binary plasmid with the oxalate oxidase and selectable marker genes.

Introduction

Natural genetic resistance
- Germplasm screening
- Field Nurseries
- Artificial inoculation
- Misting for disease development (Figure 1)
- Disease development rated on a 1-9 scale

Introduced resistance
- Genetic transformation (Figs. 4 and 5)
- Oxalate oxidase from barley (*H. vulgare*)
- *Agrobacterium tumefaciens*-mediated transformation
- Twin binary plasmid, pDJW78, for gene segregation
- Pea cvs. Mukta and Joel
- Lentil cvs. Pardina and Pennell

Figure 1. Mist system to promote *Sclerotinia sclerotiorum* growth and disease development at Carrington, ND.

Materials and Methods

Germplasm Screening
- 64 genotypes
- Replicated field trials
- Two locations
- Inoculated with ascospore suspension (2.4 to 3.3 x 10^6 spores/ml)
- Promoted disease development with a misting system (2-4 min each half-hour for 4 wks)
- Scored disease development using a 1-9 scale, where 1=no disease and 9=dead plants.

Genetic Transformation
- Oxalate oxidase gene from barley (*H. vulgare*)
- pDJW78 binary vector
- *Agrobacterium tumefaciens* strain Agl0
- Pea cvs. Mukta and Joel
- Lentil cvs. Pardina and Pennell

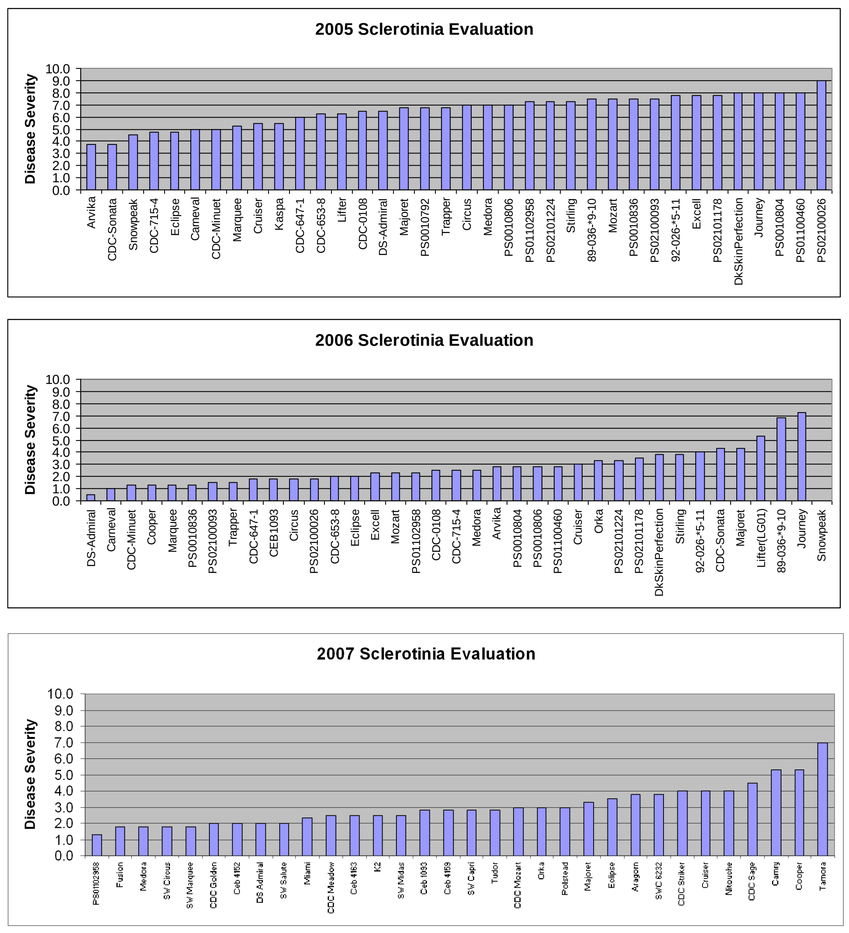

Figure 2. Summary of disease incidence scores for 64 cultivars or breeding lines screened for resistance to *Sclerotinia sclerotiorum* in 2005, 2006 and 2007 at Carrington, ND.

Results – Germplasm Screening

Varieties and breeding lines
- Disease incidence and intensity across the three years varied at Carrington, ND (Figure 2.)
- The 2005 trial produced higher average disease intensity scores (mean = 6.6) while the 2006 and 2007 trials were similar with mean scores of 2.8 and 3.0, respectively.
- 'Journey', 'Lifter' and 89-036-*9-10 were among the most susceptible in 2005 and 2006 with average scores of 7.7, 6.4, and 5.8, respectively, across the two years.
- Six entries were evaluated in all three years (Table 1).
- DS-Admiral had the lowest mean score over the three years and would serve as an acceptable parent for breeding resistant germplasm.
- Majoret had the highest disease score among the six lines.

PI Accessions
- 292 PI Accessions were evaluated; 150 in 2006 and 142 in 2007 (Figure 3).
- Disease was more severe in 2007 compared to 2006 as evidenced by a maximum disease severity score of 5 in 2006 compared to 9.0 in 2007.
- All accessions were evaluated in a single replicate plot and should be tested again to verify the disease reaction.

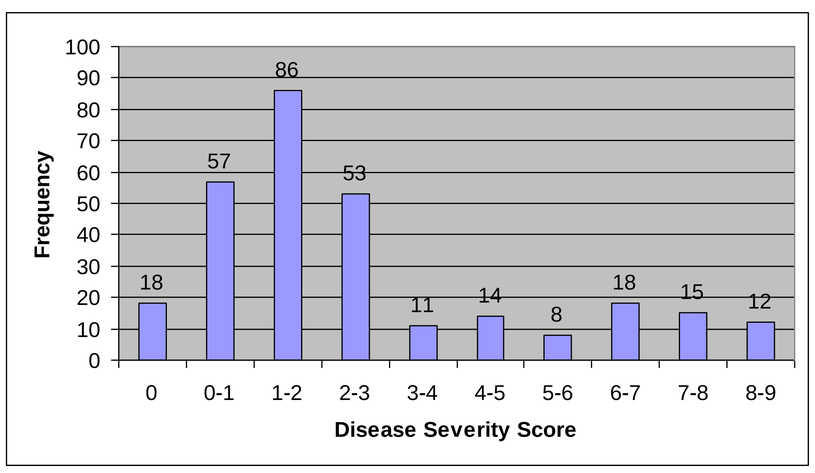

Figure 3. Histogram of disease scores for 292 PI accessions screened for resistance to *Sclerotinia sclerotiorum* in 2006 and 2007 at Carrington, ND.

Table 1. Summary of disease severity for six lines tested in all three years (2005-2007).

Variety	2005	2006	2007	Mean Score
Cruiser	5.5	3.0	4.0	4.2
DS-Admiral	6.5	0.5	2.0	3.0
Eclipse	4.8	2.0	3.5	3.4
Majoret	6.8	4.3	3.3	4.8
Medora	7.0	2.5	1.8	3.8
PS01102958	7.3	2.3	1.3	3.6
Trial Mean (n)	6.6 (36)	2.8 (36)	3.0 (32)	

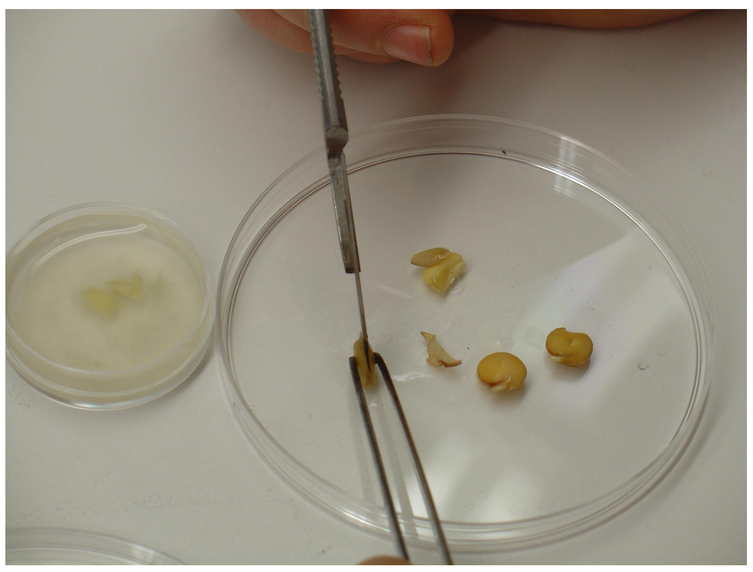

Figure 4. Preparation of lentil explants for genetic transformation.

Results – Genetic Transformation

- The oxalate oxidase gene was cloned from *Hordeum vulgare* cDNA.
- A twin binary plasmid pDJW78 was created with the oxalate oxidase gene from barley and *nptII* as the selectable marker in independent T-DNAs.
- *Agrobacterium tumefaciens* strain Agl0 was used to transform pea and lentil.
- 1900 and 1342 explants were generated for each of two pea cultivars, Mukta and Joel, respectively.
- To date no shoots have survived selection.
- 1159 and 1080 explants were generated for Pardina and Pennell, respectively.

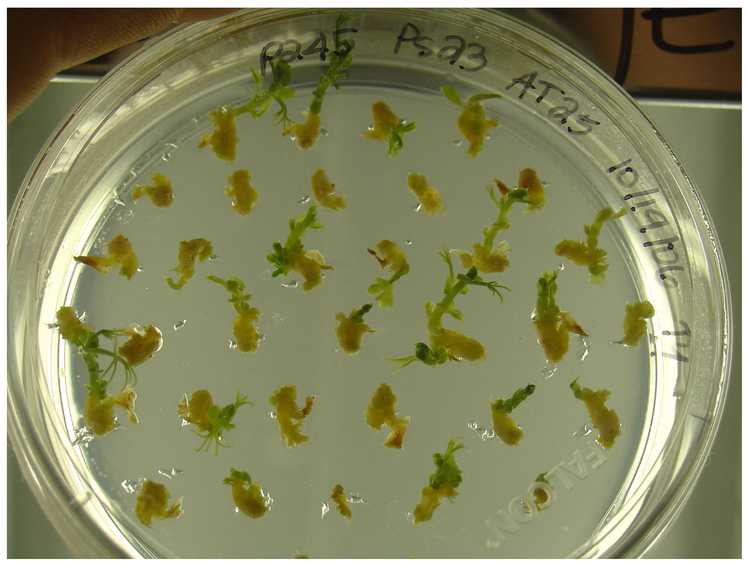

Figure 5. Pea explants on kanamycin selection media (100 mg/l).

Reference

Matthews, P.R., M-B. Wang, P.M. Waterhouse, S. Thronton, S.J. Fieg, F. Gubler, J.V. Jacobsen. 2001. Marker gene elimination from transgenic barley, using co-transformation with adjacent 'twin T-DNAs' on a standard transformation vector. Molecular Breeding, 7:195-202.