Biochemical bases of resistance in *Brassica juncea* (L.) Czern against *Sclerotinia sclerotiorum* (Lib.) de Bary

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INTRODUCTION

- Stem rot caused by *Sclerotinia sclerotiorum* has become the most wide-spread and destructive disease particularly in the north-west; with losses reported up to 74 per cent (Sharma *et al* 2001).
- Though, a number of partially resistant genotypes have been reported in *B. napus* and few in *B. juncea*; breeding to increase the levels of resistance has been ineffective.
- A study revealed that expression of about 300 genes was altered after fungal inoculation in canola and that resistance is governed by many genes.
- Plants possess diverse mechanisms of defending themselves.
- Amongst various factors, the enzymes SOD, catalase, ascorbate POX, POX, PAL, PPO; soluble phenols and lignin have been highlighted.

OBJECTIVES

- To evaluate the level of resistance in introgressed lines
- The role of defence related biochemicals: SOD, POX, PAL, phenols and lignin in resistance

METHODOLOGY

- A total of 220 introgression lines developed following hybridization of wild crucifers with *B. juncea* were used. Out of these, 24 lines with variable lesion lengths but <10cm were selected.
- The local isolate of *S. sclerotiorum* (Ludhiana) was used in initial screening and 24 isolates were used for next season screening of 24 lines.
- All the inoculations were done using standard stem inoculation technique (Buchwaldt *et al*., 2005) and observations recorded weekly for 3 weeks.
- Further, 12 lines selected for biochemical assay: inoculated with local isolate and sampling was done at 0, 24, 48, 96 and 120 hours for enzymatic and 0 and 120 hours after inoculation for non-enzymatic parameters.
- Biochemical analyses were conducted according to the prescribed protocols: Marklund and Marklund (1974) for SOD, Shannon *et al* (1996) for POX, Burrell and Reis (1974) for PAL, Swain and Hillis (1959) for total phenols and Lee *et al (*2007) for lignin

RESULTS

- The variation of lesion lengths of 220 lines is well depicted by box plot (Fig.1).
- The 24 lines showed great variation and were classified into 3 categories based on disease score according to rating scale by Sansford (1995) (Table 1).
- Enzyme activity and phenol and lignin production increased in all the lines post inoculation but the extent of increase varied significantly.
- The resistant lines showed higher mean SOD, POX and PAL activity, phenol and lignin as compared to the moderately resistant and susceptible (Table 2).
- SOD activity peaked at 24 hours, POX at 96 hours and PAL at 48 hours in resistant and 96 hours after inoculation in mod. resistant and susceptible lines, respectively (Fig.2). Increase in total phenol and lignin content after inoculation was more in resistant lines as compared to others (Fig.3)
- These biochemicals were found to be highly negatively correlated to AUDPC and disease score (Table 3).

Figure 1: The box plot of lesion lengths of 220 introgression lines

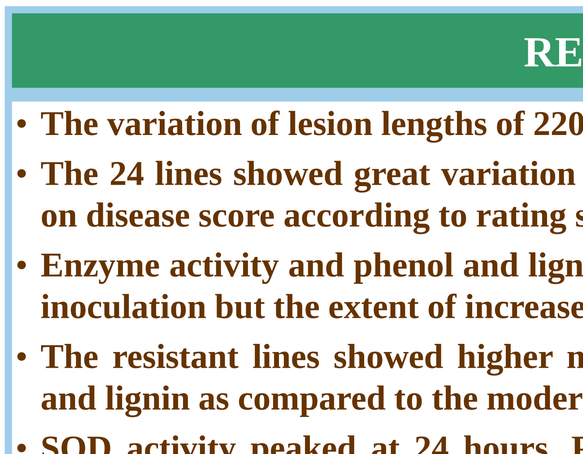

Table 1: Grouping of introgression lines based on average disease score

Category	No. of lines	Introgression lines
Resistant	7	A-825-1, ABR-102, ADJR-8, ARL-33, ARL-42, JL-4, JBN-22
Moderately Resistant	9	ADRL-7, JB-18, M-279, JBR-31, JA-517, AP-4, ARL-41, JBR-106, ADR-28
Susceptible	8	BPB-5, AP-3, ABR-103, JL-18, ARL-131, AV-99, JBN-17, ADR-16

Table 2: Mean activity of enzymes, phenol and lignin content in inoculated samples of introgression lines of different categories

Lines	Reaction of lines	SOD activity 24 hours of inoculation (ΔA/min/g fw)		POX activity 96 hours of inoculation (units/min/g fw)		PAL activity 48 hours of inoculation (µg-t-cinnamic acid formed/h/g fw)		Total phenol 120 hours of inoculation (gallic acid equivalent in mg /g of dry wt)		Lignin content 120 hours of inoculation (mg/g dry wt)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
A-825-1, ADJR-8, ARL-42, JL-4	Resistant	**181.44**	104.68-225.84	**86.85**	68.16-111.27	**397.14**	359.41-479.1	**2.47**	2.05-2.70	**153.16**	149.51-158.51
ADRL-7, AP-4, ARL-41, JBR-106	Moderately Resistant	**85.55**	49.21-145.21	**71.28**	55.1-85.02	**381.68**	215.52-564.67	**2.08**	1.96-2.25	**115.73**	108.14-123.9
ARL-131, AV-99, JBN-17, ADR-16	Susceptible	**36.42**	28.65-43.6	**46.89**	44.02-49.98	**138.01**	116.98-176.9	**1.79**	1.63-1.91	**112.07**	107.48-120.01

Figure 2: SOD, POX and PAL activity in resistant (ADJR-8), mod. resistant (AP-4) and susceptible line (AV99)

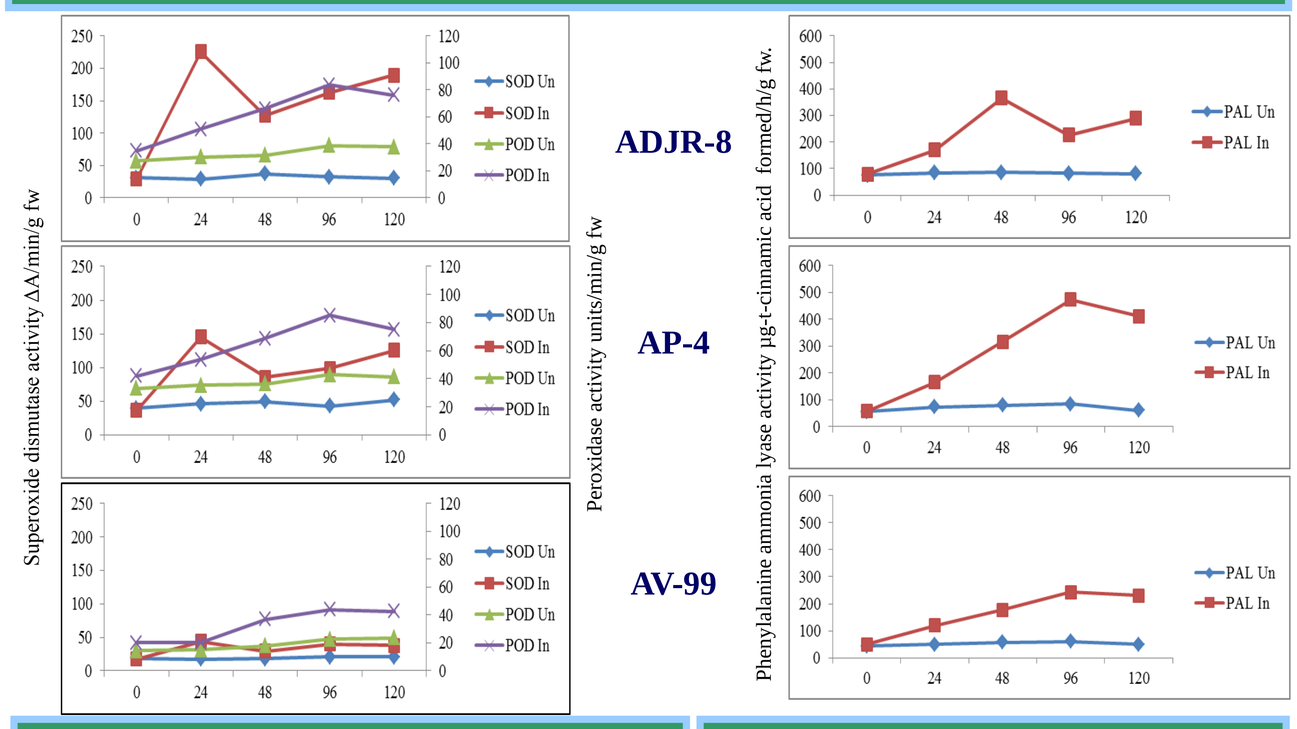

Figure 3: Total phenols and lignin content in resistant, mod. resistant and susceptible line

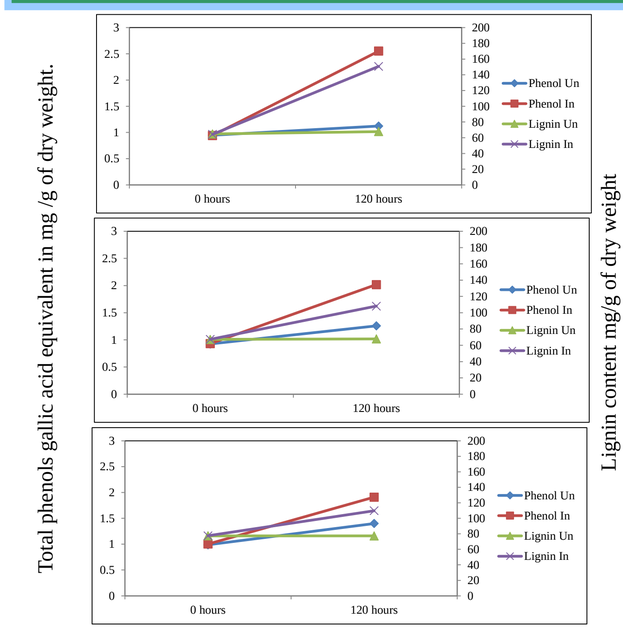

Table 3: Correlation coefficients for enzyme activities, phenols, lignin with the AUDPC and disease score

	AUDPC	Disease score
Superoxide dismutase	-0.85	-0.85
Peroxidase	-0.88	-0.89
Phenylalanine ammonia lyase	-0.83	-0.86
Total phenols	-0.87	-0.88
Lignin	-0.82	-0.81

CONCLUSION

- The introgression lines varied considerably in their reactions to the pathogen challenge inoculation, as evident from disease scores and AUDPC values.
- The lines of resistant, moderately resistant and susceptible category varied significantly amongst them with respect to the activity of defence-related enzymes: SOD, POX, PAL and phenol and lignin content.
- It is very much evident from the data obtained that these biochemicals play a significant role in imparting resistant to *B. juncea* against *S. sclerotiorum*.
- The lines exhibiting higher levels of resistance to this pathogen also showed greater activity of defence related enzymes and phenol and lignin content.
- These biochemical parameters have potentiality in assisting the selection of resistant genotypes, along with phenotypic evaluation and genetic markers.