

# *Summary*

- (1) A review of literature has been presented in connection with biochemical changes following chemical treatment and serological relationship between host and parasite.
- (2) Materials used in this investigation and experimental procedures have been described in detail.
- (3) Pathogenicity test of *Sclerotium rolfsii* on different soybean varieties (e.g. PK-262, Bragg, NRC-7, J-80, Pusa-16 and Macs-58) was done. Among the six soybean varieties, Macs-58 was found to be highly susceptible which was selected for induction of resistance.
- (4) Accumulation of glyceollin from six soybean varieties were detected after 24 and 48h of inoculation with *S. rolfsii*. Higher accumulation of glyceollin was noticed after 48h of inoculation. PK-262 and Bragg contained more glyceollin ( 377-392  $\mu\text{g/g}$  fresh wt. tissue ) than Macs-58 ( 189  $\mu\text{g/g}$  fresh wt. tissue ).
- (5) A series of experiments have been performed using twelve chemicals belonging to three separate groups, viz. metal salts ( cupric chloride, ferric chloride, lithium sulphate, manganese sulphate, sodium molybdate, magnesium sulphate, zinc chloride and barium sulphate ); growth regulators ( IAA, 2,4-D, and 2,4,5-T) and biological compound (chitosan) in order to induce resistance in soybean plants ( highly susceptible variety – Macs58 ). Among the tested chemicals cupric chloride and ferric chloride were found to be highly effective in reducing disease intensity.
- (6) Fungitoxicity assay using selected metal salts, growth regulators and chitosan, on sclerotial germination of *S.rolfsii* were done.
- (7) Biochemical changes associated with induction of disease resistance in soybean plants by non-conventional chemicals with special reference to phenol content, enzyme activities such as peroxidase, polyphenol oxidase, pectolytic enzymes, phenylalanine ammonia lyase as well as calcium and magnesium levels were determined.

- (8) Maximum and minimum increase in phenol level ( total phenol and orthodihydroxy phenol ) was recorded in cupric chloride and sodium molybdate treated plants respectively.
- (9) Polyphenol oxidase and peroxidase activities were higher in treated inoculated plants than untreated inoculated plants. However, pectolytic enzyme activity in treated inoculated plants was significantly less than untreated inoculated plants.  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  content were also related to pectolytic enzyme activity. Reduction of pectolytic enzyme reduced its activity in rotting tissue, was linked with greater accumulation of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ .
- (10) The plants treated with cupric chloride, the most effective treatment, elicited maximum increase in PAL activity following inoculation with *S.rolfsii*.
- (11) Accumulation of glyceollin in susceptible soybean variety – Macs58 before and after alteration of disease reaction by cupric chloride and ferric chloride were detected. Both the chemicals induced glyceollin synthesis in uninoculated soybean plants.  $\text{CuCl}_2$  and  $\text{FeCl}_3$  induced high level of glyceollin (485 and 332  $\mu\text{g/g}$  fresh wt. tissue respectively) after challenge inoculation with the pathogen (*S.rolfsii*) in comparison to the untreated inoculated plants.
- (12) Mycelial protein of *S. rolfsii* and different parts of inoculated soybean plants were estimated and analysed by SDS-PAGE. Old culture of mycelia contained more protein than the young. Leaves contain more protein than roots. Soluble proteins prepared from collar region of soybean plants (Macs-58) inoculated with *S.rolfsii* exhibited 2-3 additional bands, in comparison to their healthy control.
- (13) Polyclonal antisera were raised against antigen preparations from mycelia of *S. rolfsii* and soybean roots (Macs-58).
- (14) In agar gel double diffusion tests antiserum of *S. rolfsii* and Macs-58 reacted with antigens of different isolates of *S. rolfsii* and soybean varieties. Strong precipitin reaction occurs between pathogen antigen and host antisera as well as host antigen and pathogen antisera.

- (15) Antigenic comparison among six soybean varieties and six isolates of the pathogen and two non pathogens using anti- *S.rolfsii* antiserum were done following conventional set up for immunoelectrophoresis. Susceptible soybean varieties shared the common antigens with the different isolates tested.
- (16) Optimum conditions for ELISA reactions with anti-*S.rolfsii* were determined. An antiserum dilution 1:125 and an enzyme (alkaline phosphatase) dilution of 1: 10,000 were optimum. Antigen upto a concentration of 10µg/ml were detected in homologous reaction by ELISA.
- (17) Detection of pathogen ( *S. rolfsii* ) in artificially inoculated soybean root tissues using DAC-ELISA formats, were developed.
- (18) Cellular location of cross reactive antigens(CRA) shared by host(*Glycine max* ) and parasite ( *S.rolfsii* ) using fluorescein isothiocyanate (FITC) was determined. Cellular location of CRA in mycelia and sclerotia of *S.rolfsii* were also studied. Major CRA was concentrated on the epidermal and cortical tissues of soybean roots and young hyphal tips of the pathogen.
- (19) Alteration in antigenic patterns after chemical induction of resistance by cupric chloride in susceptible soybean plants were detected using immunodiffusion and immunoelectrophoretic tests. These observed antigenic changes owing to  $\text{CuCl}_2$  treatment have some significance in the resistance of soybean to *S.rolfsii*.
- (20) Implication of the results have been discussed.