SUMMARY

1. A brief review of literature pertaining to the present lines of investigation has been presented which mainly deals with (a) evaluation of the role of phytoalexins in plant disease resistance, (b) plant disease alteration by chemical treatment and (c) serological relationship between host and parasite.

2. Experimental procedure followed and the materials used in this investigation have been described in detail.

3. Pathogenicity of F.graminearum was tested on ten cultivars of soybean (Soymax, KU-254, EC-2575, PK-327, JS-2, EC-95287, Pusa-16, EC-55865, UPSM-19 and R-184). Of the ten cultivars, cvs. Soymax and JS-2 were found to be highly susceptible while UPSM-19 and Pusa-16 were resistant to F. graminearum.

4. The optimum pH, temperature and incubation time required for maximum growth of F.graminearum were determined. The fungus grew at a wide range of pH(5.5-7.0) and temperature (20°C-40°C), the optimum pH and temperature being 6.5 and 30°C respectively. Maximum growth was observed after 15 days of incubation and then rate of growth declined.

5. Phytotoxic effect of metabolic by products in the culture filtrate of F.graminearum on soybean plants (cvs. Soymax and JS-2) was determined. Toxic principle was found to be partially thermolabile and non dialysable.

6. Glyceollin accumulation in the roots of six soybean cultivars (UPSM-19, JS-2, Soymax, KU-254, R-184 and EC-55865) after 24, 48, 72 and 96h of inoculation with F.graminearum were detected. Antifungal activity of glyceollin was determined by "on the Chromatogram inhibition assay" using Bipolaris carbonum as the test organism. Results of both

radial mycelial growth and spore germination tests also confirmed the antifungal nature of glyceollin. The relative antifungal activity of glyceollin was also compared against F.graminearum, F.oxysporum, f.solani, Dreschlera oryzae, Glomerella cingulata, Pestalotiopsis theae and Bipolaris carbonum. UV-spectrophotometric analysis of glyceollin revealed absorptioin peak at 286 nm which was identical with authentic glyceollin.

7. Highest accumulation of glyceollin after 48h of inoculation was noticed. Resistant cultivars (UPSM-19, EC-55865 and R-184) contained more glyceollin (705-896 /ug/g fresh weight of roots) than susceptible cvs. Soymax, JS-2 and KU-254 (285-652 /ug/g fresh weight of roots).

8. A series of experiments were performed in order to study the effects of nine chemicals of three separate groups viz. (a) metal salts (cupric chloride, ferric chloride, mercuric chloride and silver nitrate); (b) reducing agents (sodium selenite and sodium sulphite) and (c) metabolic inhibitors (sodium azide, sodium malonate and sodium molybdate) on disease development of susceptible cultivar (Soymax). Each chemical was also tested for its fungitoxic effect if any on F.graminearum. Sodium selenite and chlorides of mercury, copper and iron totally inhibited spore germination. Cadmium chloride, silver nitrate, sodium sulphite, sodium azide, sodium malonate, sodium fluoride and sodium molybdate also markedly inhibited the spore germination and germ tube growth. Pronounced protective effects were recorded with three chemicals (viz. sodium azide, sodium selenite and sodium sulphite) as evident from the reductions in disease index after 30 days of inoculation with F.graminearum.

9. Susceptible plants (cv. Soymax) treated with sodium azide (10^{-4}M) followed by inoculation with F.graminearum produced high level of glyceollin (545 /ug/g fresh wt. of roots) in relatioin to untreated inoculated plants (256 /ug/g fresh wt. of roots). Production of glyceollin was maximum in sodium azide treated plants inoculated with F.graminearum than sodium selenite treated inoculated plants.

10. Total soluble protein content of healthy and F.graminearum inoculated roots of five cultivars were estimated. Protein contents increased in the susceptible cultivars (JS-2, PK-327 and Soymax) more than resistant cultivars (UPSM-19 and Pusa-16) after 48h of inoculation with F.graminearum.

11. Protein patterns of healthy and infected (with F.graminearum) soybean roots of cvs. Soymax, JS-2, PK-327 and UPSM-19 as well as the mycelia of two isolates of F.graminearum have been evaluated by polyacrylamide gel electrophoresis. Mycelia of two isolates of F.graminearum Fg1 and Fg2 were exhibited 18 and 15 protein bands respectively. Infected roots of cvs. Soymax and JS-2 showed 16 and 18 protein bands while healthy roots exhibited 14 and 15 bands respectively.

12. Plant antigens were prepared from soybean roots of six cultivars (Soymax, JS-2, KU-254, UPSM-19, EC-55865 and R-184). Fungal antigens were prepared from two isolates of F.graminearum (Fg1 and Fg2) and two non pathogens of soybean (viz. G.cingulata, and P.theae). Rabbit antisera were raised against cvs. Soymax (SA), UPSM-19(UA) and isolate Fg1 of F.graminearum (F_1A).

13. In agar gel double diffusion test strong precipitin reactions occurred when antiserum of F.graminearum was

reaction against its homologous antigen (isolate Fg1) but weak precipitation reaction was observed with antigen preparation of isolate Fg2 of F.graminearum.

14. Cross reaction between antiserum of F.graminearum and antigens of susceptible cultivars (Sa, Ja & Ka) developed common precipitin band but no precipitin band was observed with the antigen of resistant cultivar (UPSM-19).

15. Reciprocal cross reaction between antiserum of cv. UPSM-19 (UA) and antigens of F.graminearum isolate Fg1 developed weak precipitin band but no precipitin band could be detected with isolate Fg2. Whereas common precipitin bands were observed in the reciprocal cross reaction between antiserum of cv. Soymax (SA) and antigens of isolates of F.graminearum. Absence of cross reactive antigens were also noted between antisera of soybean cultivars (SA and UA) and antigens of non pathogens of soybean (G. cingulata and P.theae).

16. Effectiveness of each antigen extract of cvs. Soymax(Sa), UPSM-19 (UA) and isolate Fg1 (F_1a) in raising antibodies SA, UA, and F_1A respectively was checked by homologous cross reactions in immunoelectrophoretic test. The homologous patterns formed by cvs. Soymax, UPSM-19 and F. graminearum contained 6, 5 and 6 precipitin lines respectively.

17. In cross reaction with antiserum of Soymax and antigen preparations of roots of five other different cultivars JS-2 gave rise to 5 precipitin arcs and cvs. KU-254 and R-184 exhibited 4 precipitin arcs while cvs. EC-55865 and UPSM-19 showed 3 and 2 arcs respectively. Both the isolates of F.graminearum were antigenically related to cv. Soymax. In

this case, isolate Fg1 and Fg2 shared 3 and 2 common precipitin arcs respectively.

18. In cross reactions with antiserum of UPSM-19 and antigens of soybean cultivars, 4 precipitin arcs each were formed with cvs. EC-55865, JS-2, R-184 and KU-254 while Soymax exhibited 3 precipitin arcs. Antigen prepared from isolate Fg1 developed only one precipitin arc but no such precipitation was observed with isolate Fg2 in cross reaction with antiserum of UPSM-19.

19. Reciprocal cross reactions between antisera of F.graminearum and antigens of cv. Soymax formed 3 precipitin lines while JS-2, KU-254 and R-184 formed 2 precipitin lines only, but antigens of cvs. UPSM-19 and EC-55865 failed to develop any precipitin line.

20. Cross reactive antigens (CRA) in semipurified mycelial preparation from F.graminearum (isolate Fg1) at concentrations ranging from 5-25 µg/ml with antiserum dilutions (1:125 and 1:250) have been detected by indirect immunosorbent assay (A_{405}). CRA were also detected between F.graminearum and cv. Soymax. Antigenic preparations from F.graminearum exhibited higher absorbance value when reacted with antiserum of soymax than when reacted with antiserum of resistant soybean cultivar UPSM-19. There was no cross reactivity with P.theae and G.cingulata (non pathogens).

21. When antigens obtained from F.graminearum (Fg1) and susceptible cultivar (Soymax) before and after treatment with sodium selenite and sodium azide (10^{-4}M) were cross reacted separately with the antisera of Soymax roots developed very faint diffused band in agar-gel double diffusion test.

22. In immunoelectrophoretic tests, antigens of untreated healthy roots of Soymax exhibited 6 precipitin arcs

in homologous reactions while sodium selenite and sodium azide treated roots of Soymax developed 4 and 3 precipitin lines respectively with antiserum of Soymax. Reciprocal cross reaction between antiserum of F.graminearum and antigens of sodium selenite and sodium azide treated roots of Soymax developed 2 and 1 precipitin arc respectively, while untreated Soymax developed 3 precipitin lines.

23. Antibodies indirectly labelled with fluorescein isothiocyanate (FITC) were used to determine the location of CRA in sections of roots of Soymax and fungal cells.

24. Mycelia and conidia of F.graminearum were not autofluorescent nor did they fluores when treated with normal serum followed by FITC. Treatment of mycelia and conidia of F.graminearum with homologous antisera and FITC showed a general fluorescence that was more intense on young hyphae and patch like areas on the conidia.

25. When fungal cells were reacted with antiserum to roots of cv.Soymax and treated with FITC, fluorescence was apparent on young hyphae.

26. Root sections did not exhibited any natural autofluorescence. Sections treated first with normal serum then by FITC also did not exhibited any fluorescence.

27. Root sections of cv. UPSM-19 treated with homologous antiserum and then reacted with FITC developed bright fluorescence which was concentrated mainly on epidermal cells and was distributed throughout the cortical tissue.

28. Strong reaction was evident with root sections of cv. Soymax and antiserum of F. graminearum CRA was

concentrated mainly around xylem elements, the endodermis, epidermal cells and distributed throughout the cortex tissue; cell walls appeared to be the main cellular location of CRA.

29. The implications of the results embodied in Part I-XI have also been discussed.