

# ***Introduction***

Soybean [ *Glycine max* (L.) Merrill ] is one of the most important legume crops of the world and is a primary source of vegetable oil and protein. Each year soybean products become more important in formulating new, low cost nutritionally balanced high protein foods and beverages for human consumption. As soybean acreage has expanded throughout the world, diseases have increased in number and severity. Root rot of soybean caused by *Fusarium oxysporum* Schlecht is considered a potentially destructive disease in the tropics and subtropics. In this disease, infection is generally confined to root and lower stem region and when the disease is severe seedling emergence is slow and poor and seedling growth is stunted and weak. The pathogen is usually confined to the cortex but vascular elements may be invaded in advanced stages of the disease. Although the pathogen is confined to the root, nearly mature pods may be invaded under prolonged humid and wet conditions. The pathogen may cause latent infection of the seeds which remain apparently healthy (Sinclair, 1991).

Rhizosphere of plants contain a large number of microorganisms and one of the most intriguing and exciting problems is the study of complex interactions occurring among soil microorganisms and their effects on disease reaction in plants. The legume rhizosphere contains a large population of rhizobia, most of which are highly specific to their host. It has been clearly established that sustained high levels of nodulation by contrasting *Bradyrhizobium japonicum* strain can alter the productivity of the *Bradyrhizobium*-soybean symbiosis under green house and field conditions. (Israil, 1981; Fuhrmann, 1990; Miller and May, 1991) Since nodulation effectively increases the growth and yield of soybean plants, there is a common agricultural practice of inoculating soybean seeds or soil with *Bradyrhizobium japonicum* prior to sowing (Plate-I). Other than increasing yeild and growth, there are several reports that different species of *Rhizobium* / *Bradyrhizobium* are capable of protecting their hosts against fungal pathogens (Tu, 1978 ; Purkayastha, 1981; Chakraborty and Purkayastha, 1984; Chakraborty and Chakraborty, 1989).

Introduction of biocontrol agents into the soil is also becoming a common practice in recent years, of which *Trichoderma sp*, *Pseudomonas sp*, *Bacillus sp. etc* are generally used (Dunne et al, 1996; Hessemuller and Zeller, 1996 and Mandeel, 1996). In certain cases combined effect of one or more antagonist in reducing disease severity has also been reported (Novicova, 1995 ; Dubey, 1996 ; Craft and Nelson, 1996). The reduction in disease intensity of a particular plant due to the activity of one or more microorganisms in the soil may be attributed to biochemical changes induced in the host. Most important biochemical changes in plants following pathogen infection are production of phytoalexins, changes in enzymes and in pathogenesis related proteins (Ji and Kuc, 1996 ; Wibber et. al. 1996; Urbanek et. al 1996; Kristensen et. al. 1997).

However, biochemical changes in host resulting from combined inoculations of antagonist and pathogen have been relatively less investigated though increased phytoalexin production has been implicated in certain cases (Skipp and Deveral, 1973; Chakraborty & Chakraborty, 1989). Closely related to the biochemical changes in host pathogen interactions is the phenomenon of common antigen sharing between host and pathogen. Close serological similarity between host and pathogen is considered to be one of the prerequisites for the successful establishment of a pathogen within the host. Little information is available on the serological changes in host due to microbial interactions among biocontrol agents and pathogens (Troxler et al ; 1997).

Considering that soybean is a very important legume crop which is generally preinoculated with *Bradyrhizobium* and that information on the biochemical and serological changes in soybean due to *B. japonicum* - *F. oxysporum* interaction are lacking, the following study was undertaken, the main objectives which are as follows : (a) selection of soybean cultivars resistant or susceptible to *F. oxysporum*, (b) determination of the effect of preinoculation of soybean seeds by *B. japonicum* on disease development, (c) determination of the effect of soil infestation with *Trichoderma harzianum* on disease development (d) *invitro* interaction studies between *F. oxysporum*, *B. japonicum* and *T. harzianum*, (e) estimation of phenylalanine ammonia lyase and peroxidase, from soybean roots inoculated with *F. oxysporum* and *B. japonicum* and evaluation of the quantitative differences between them, (f) extraction and

estimation of glyceollin in roots of soybean cultivars challenged with *F. oxysporum* and subsequent changes following interaction with *B. japonicum*; (g) analysis of protein changes by SDS-PAGE after inoculation with *F. oxysporum* and *B. japonicum*; (h) raising of antisera against antigen preparation from soybean roots and mycelium of *F. oxysporum*; (i) detection of serological cross reactivity between *F. oxysporum* and soybean varieties following agar gel double diffusion, immunoelectrophoresis and enzyme linked immunosorbent assay, (j) detection of *F. oxysporum* in infected tissues and in roots preinoculated with *B. japonicum*, (k) determination of the cellular location of cross reactive antigens in soybean root tissues as well as of mycelia, conidia and chlamydo-spores of *F. oxysporum*.

Before going into details of the present investigation, a brief review in confirmity with this study has been presented in the following pages.