INTRODUCTION

Soybean [*Glycine max* (L.) Merill] is a major world crop. It is an ancient crop with hundreds of food, feed and industrial uses. Today, soybeans are grown to some extent in most parts of the world and are a primary source of protein and vegetable oil. Soybean products have 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. One or more diseases can generally be found in fields wherever soybeans are grown. Fusarium root rot occurs in most soybean growing areas of the world and is considered potentially destructive in the tropics and subtropics. Root rot of soybean caused by *Fusarium graminearum* Schwabe has been reported by Agarwal and Sarbhoy (1978). The disease usually develops on seedlings and young plants. Older plants generally are less susceptible than younger ones. When the disease is severe, seedlings are stunted and weak. Infection is generally confined to the roots and lower stem. Cotyledons of diseased seedlings are chlorotic and later become necrotic. The lower part of the tap root system may be destroyed. The pathogen is usually confined to the cortex but vascular elements are invaded in advanced stages of disease. When soil moisture is low, infected seedlings or plants may wilt and in some instances, plants in an entire field may be wilted.

Increasing movement of plant material in world-wide trade coupled with restrictions on the use of plant protection chemicals has emphasized the need for phytosanitary measures for controlling the spread of economically important pathogenic organisms. Routine testing of large numbers of samples will, however, only be possible when specific sensitive and easy handle methods of diagnosis are available. Recent trends in detection of plant pathogenic fungi include the development of more rapid diagnostic techniques with high specificity for the target organism. These techniques can be used to detect fungi present in low

amounts in and on plant tissue and, therefore, in many cases the pathogen can be detected at an early stage of disease development than was previously possible (Hansen and Wick, 1993; Werres and Steffens, 1994; Chakraborty and Chakraborty, 2002).

One of the most difficult and intriguing aspects in the study of biology is an understanding of the significant events of the interaction between plants and micro-organism at the cellular and subcellular level. The success or failure of infection is determined by dynamic competition and the final outcome is determined by the sum of favourable and unfavourable conditions for both pathogen and host cells. It is generally accepted that the cells recognize one another through pairs of complimentary structures on their surfaces. There is evidence that host-parasite compatibility is related to their antigenic similarly (Devay and Adler, 1976). The presence of cross-reactive antigens (CRA) between plant hosts and their parasites and the concept that these antigens might be involved in determining the degree of compatibility in such interactions have been demonstrated by several authors (Chakraborty, 1988; Purkayastha, 1994). Besides, recent trends in detection of plant pathogens include the development of more rapid diagnostic techniques with high specificity for the target organism. These techniques can be used to detect fungi, bacteria, and viruses present in low amounts in and on plant tissue and, therefore, in many cases the pathogen can be detected at an earlier stage of disease development than was previously possible. Some of these rapid, sensitive techniques are enzyme linked immunosorbent assay (ELISA), immunofluorescence (IF) and the polymerase chain reaction (PCR). A limited number of immunoassays have been developed for the detection of organisms in soil employing polyclonal, immunological reagents. An assay of detecting the cavity spot pathogen, *Pythium violea* has been developed as a practical method for determining the pre-sowing disease risk in fields (Manocha and Su, 1992). Of the assays tested by Wakeham and White (1996), indirect immunofluorescence appeared to be the most rapid and amenable assay for the detection in soil of low levels of resting spores of *Plasmodiophora brassicae*.

Although most plant pathogenic fungi can be detected by microscopy or other conventional means, serological techniques have advantage where (a) the fungus in question is not readily identified by morphological characteristics, (b) species identification is important and difficult by conventional means, (c) detection of root pathogens prior to development of foliar symptoms is necessary, (d) large numbers of samples must be processed for a particular disease for which conventional methods are time-consuming, (e) rapid, on-site detection is necessary for making disease management decisions for high-value crops, (f) regulations governing the use of pesticides require demonstration of the presence of a particular pathogen, (g) the fungus causes disease at low, difficult-to-detect populations in plant tissue, or (h) plant material is subject to quarantine regulation.

With the advent of immunoassay detection systems and their successful adaptation to the detection of root pathogen, the present investigation has been undertaken:

(a) to screen soybean varieties resistant to *Fusarium graminearum*,

(b) to prepare mycelial antigens from *F. graminearum*, *T. harzianum* and *T. viride* and raise polyclonal antibody against these mycelial antigen preparations;

(c) purification of antigen and antisera and analysis by immunoblotting;

(d) to determine serological cross reactivity between soybean roots and *F. graminearum* using ELISA

(e) to detect *F. graminearum* in soil and in soybean root tissues at an early stage of disease development using immunoassays.

(f) *In vitro* interaction studies of *F. graminearum* with *T.harzianum* and *T. viride*

(g) to apply T. harzianum and T. viride in soil for biological control of root rot of soybean

(h) to determine the changes in the population of the root pathogen after Trichoderma infestation in soil by ELISA and immunoblotting