I. INTRODUCTION

Plants have been endowed with multicomponent system of defence to protect themselves against the attack of pathogenic microorganisms. These systems include rapid accumulation of reactive oxygen species (ROS) known as oxidative burst (Sutherland, 1991; Baker and Orlandi, 1995) activation of cell wall cross-linking and lignification thereby strengthening the cell wall and helping confine the pathogen to initial infection site (Bolwell, *et al.,* 1999), synthesis of low molecular weight compounds termed as phytoalexins (Verberne *et al.,* 1999), proteinase inhibitors, (Heitz *et al.,* 1999) antifungal hydrolytic enzymes and several families of pathogenesis related proteins (PR) (Kombrink and Somssich, 1997; Boller, 1987; Van Loon, 1999). A combination of chemical and physical barriers in plants provide an effective defence against the invader.

Antifungal hydrolytic enzymes produced by plants against pathogen ingress are gaining momentum in recent years as they seem to be effective against the entry of fungal pathogens by unleashing their hydrolytic cleavage action on their cell walls. Among the hydrolytic enzymes of plants, chitinases and glucanases have been studied in detail in view of their ability to hydrolyse fungal cell wall components such as chitin and glucan thereby arresting the growth of invading pathogens (Schlumbaum *et al.,* 1986; Boller *et al.,* 1983; Roberts *et al.,* 1988; Mauch *et al.,* 1988b). In addition to chitinases and glucanases there is yet another set of hydrolytic enzymes called chitosanase

(EC 3.2.1.99) (Monaghan *et al.*, 1973) and lysozyme (EC : 3.2.1.17) (Fleming, 1922) which are reported to occur in higher plants (Audy *et al.*, 1988; Grenier & Asselin, 1990; Ouakfaoui & Asselin, 1992b : Audy *et al.*, 1988; Bernasconi *et al.*, 1987).

Chitosanase is one of enzymes of the chitinolytic system which acts on chitosan, a homopolymer of 1-4 linked glucosamine units. Chitosan is present in the cell walls of fungi belonging to zygomycetes (Bartnicki-Garcia, 1968) deuteromycetes (Novaes-Ledieu & Garcia Mendoza, 1981) shellfish and exoskeleton of crustacean (Lotournean *et al.*, 1976; Datema *et al.*, 1977). However, there is no information about the existence of endogenous chitosan or chitosan-like substrate in plants. Chitosan is synthesized from chitin by a process called deacetylation catalysed by the enzyme chitin deacetylase (Araki & Ito, 1975).

Both lower and higher plants do have chitosanases (Ouakfaoui & Asselin, 1992b). Organ specific chitosanase isoforms have been detected in cucumber plant (Ouakfaoui & Asselin 1992). New chitosanase isoforms could be induced in *Allium* and *Pisum* roots following colonization by *Glomus* species (Dumas-Gaudot *et al.*, 1992). Leaves of barley, tomato and cotyledon of cucumber treated with silver nitrate and serine resulted in induction of chitosanase. Such an induced enzyme showed high antifungal activity against

fungal spores of *Verticillium albo-atrum* and *Ophiostoma ulmi* indicating that the enzyme is antifungal (Grenier & Asselin, 1990).

Lysozymes (Muramidase or Peptidoglycan acetylmuramyl hydrolase) are basic bacteriolytic enzymes widely distributed in nature which cleave the peptidoglycan component of bacterial cell wall. The presence of lysozyme was first reported in plant roots and flowers by Fleming (1922). There have been reports on the occurrence of lysozyme in both lower and higher plants (Audy *et al.,* 1990). Lysozyme has been purified and its properties has been studied from the latex of papaya (Howard & Glazer, 1967; 1969), fig (Glazer *et al.,* 1969), cultured *Rubus hispidus* (Bernasconi *et al.,* 1985) and *Parthenocissus quinquifolia* (Bernasconi *et al.,* 1987) and wheat germ (Audy *et al.,* 1988).

Interestingly these purified plant lysozymes exhibit strong chitinolytic activity as well (EC : 3.2.1.14). Like chitinases and chitosanases, lysozymes have no endogenous substrate and its endogenous function remains to be established. However, the presence of lysozymes in plants assumes significance as the soil and the environment in which plants grow contain numerous pathogenic bacteria and a role for lysozymes can be ascribed as lysozymes hydrolyse peptidoglycan of bacterial cell wall.

An attempt has been made in the present investigation to detect whether different organs of groundnut plant such as imbibed seeds, 10 day old seedling and gynophore of *Arachis hypogaea* (cv.TMV-7) possess chitosanase and

lysozyme activities using colorimetric and gel activity staining methods. Both chitosanase and lysozyme activities could be detected in all the organs except seed coat of imbibed seed. In groundnut plant the gynophore is really interesting because after anthesis it enters the soil at certain point of its development and there it faces different environment. In view of its unique development after anthesis, gynophore has been chosen to detect and study the properties of chitosanase and lysozyme after purification. These enzymes have been purified using salting out with ammonium sulphate, gel filtration with sephadex-G100 column, preparative native PAGE and reverse phased high performance liquid chromatography. Physical, biological properties and N-terminal sequencing of purified enzymes of *A.hypogaea* have been studied. Polyclonal antibodies against purified chitosanase isoform 1 and lysozyme of groundnut plant have been raised and their localization in tissues have been attempted with fluorescent antibody technique. Antichitosanase antibodies have been used to detect their relatedness with closely related plants belonging to family fabaceae using western blot technique.

The results of the present investigation are presented in two chapters. The first chapter presents results on the extration, detection, purification, physical and biological properties of chitosanase isoforms N-terminal sequencing of chitosanase isoform 1, immunoserological aspects of purified chitosanase isoform 1 of groundnut such as western analysis and localization of enzyme in groundnut plants using FITC tagged antichitosanase antibody.

The second chapter presents results on detection, purification, physical and biological properties, N-terminal sequencing of lysozyme, western analysis to find out its relatedness with members of Fabaceae and localisation of lysozyme in groundnut (*A. hypogaea*) tissues incubated with fluoresent antibody. Summary common to the two chapters are given at the end of Chapter II.

Results obtained from the experiments have been discussed with available literature