GENERAL INTRODUCTION

Maize (*Zea mays*. L) is a universally cultivated cereal crop that is used for both human and animal consumption in both processed and unprocessed forms. Archeological and biological evidences indicate that wild maize originated in Mexico for more than 7.500 to 10.000 years ago (Wang et al. 1999, Tenaillon et al. 2001).

Nowadays, maize is considered one of the most widely produced grain in the world; the US and China are the world's largest producers of maize crop, accounting for approximately 60% of the grain produced in the world, followed by Brazil and Mexico (Smith et al. 2004). Although 68% of the land devoted to maize is located in the developing world, however, only 46% of maize production occurs there, indicating the need for improving yields in developing countries where it is a major source of direct human consumption (Pingali and Pandey, 2001). Additionally, numerous varieties of maize have been bred for both food and non-food purposes. The kernels are used for human and livestock consumption, while the corn cob and its extracts are used for a variety of industrial purposes (Sprague et al. 1988). It is estimated that maize yields 4000 industrial products such as maize oil, starch, syrup, gluten, dextrose (used mainly by the pharmaceutical industry for manufacturing vitamin C and penicillin), fructose (used mainly by the soft drink industry), cornmeal, grits, flour, ready-to-eat snack foods, breakfast cereals, ethanol (E85) as automobile biofuels, additives in paint and explosives, biodegradable chemicals and plastics, paper and textiles (Smith et al., 2004; Sprague et al. 1988). However, maize is considered one of the most important feed grains due to its efficient conversion of dry substance to meat, milk or eggs, compared to other grains (Sprague et al. 1988).

On the other side, maize may be attacked by different microorganisms e.g. *Fusarium* spp. that cause enormous economic losses in the crop production and food industry by destroying plants in the field and during storage (Macdonald and Chapman, 1997). In addition, they may also produce a various number of mycotoxins which are often acutely toxic to human and livestock (Sydenham et al. 1990, Ross et al. 1992).

Fusarium moniliforme Sheld. (syn. *F. verticilliodes*; teleomorph: *Gibberella fujikuroi* Sawada) for instance, is the most frequently fungal pathogen which may attack maize kernels before germination causing seeds rots. After germination, *F. moniliforme* may also infect the germlings before emergence above ground resulting in pre-emergence death of germlings or after emergence causing post-emergence damping-off. The fungus is spreading both horizontally to the next plants through infected seeds and plant debris, and vertically in the plant tissues during the endophytic colonization (Bacon et al. 2001). The resulting disease symptoms vary widely and range from asymptomatic infection to severe rotting of all plant parts including

discolouration and non-uniform emergence resulting in large missing plants gaps in the field (Bacon and Hinton, 1996). *F. moniliforme* produces at least three chemically distinct classes of mycotoxins i.e. fumonisins (Bezuidenhout et al. 1988), fusarins (Gelderblom et al. 1984), and fusaric acid (Gäumann 1957). Fusaric acid has not been studied extensively as the fumonisins (Ross et al. 1991) and the fusarins (Gelderblom et al. 1986), perhaps due to its moderate toxicity to mammals compared to other mycotoxins (Wayne et al. 2001). Even fusaric acid inhibited respiration, electrolyte leakage and cytological alterations in root cells of maize (Arias 1985). There are also indications that fusaric acid may act synergistically in animals with other mycotoxins such as fumonisins (Porter et al. 2000).

Plants protection from soil-borne pathogens has so far been largely based on chemical fungicides. A disadvantage of chemical control is that farmers are increasingly often confronted with pathogens resistant to available chemical plant protectants. Further, fungicides application to control soil-borne pathogens seems to be not feasible for both economical and ecological reasons (Buchenauer, 1998). To a certain extent, pathogens can be controlled by proper agricultural practices e.g. removal of plant debris and infected plant parts, use of pathogens-free seeds and suppressive soils, crop rotation, soil fallow, destruction of weeds and appropriate irrigation and fertilization. However, after the infection takes place all these practices often seemed to be of limited consequences. One alternative strategy to maintain populations of soil-borne fungi at low levels is by application of biocontrol methods, i.e. the intentional use of living organisms to suppress the population of a pathogen to an acceptable level. Recently, combating soil-borne pathogens by various microorganisms is possible by using a wide array of antifungal agents that have been launched on the market (Howell, 2003; Nagayama et al. 2007). Among these are species of *Trichoderma*, opportunistic, avirulent plant symbionts, existing in a wide range of climates from the tundra to the tropics (Papavizas, 1985; Harman et al. 2004). This may be attributed to their diverse metabolic capability and aggressive competitive nature (Samuels 1996). The potential of *Trichoderma* spp. as biocontrol agents was first recognized by Weindling in the early 1930s (Weindling 1934) who described the mycoparasitic action of *Trichoderma* spp. on *Rhizoctonia* spp. and *Sclerotinia* spp. This finding however, has stimulated the research on this topic and several species of *Trichoderma* have been commercially marketed for the protection of plants against fungal pathogens and growth enhancement of a varying number of crops as biopesticides, biofertilizers and soil amendments (Harman et al., 2004). Commercially available formulations derived from *Trichoderma* spp. are e.g. Binab-T®, BioTrek 22G, Ecohope®, Gliomix®, Promot®, RootShield®, SoilGard®, Supresivit®, Trichodex®, Trichopel®, Trichojet®, Trichodowels®, Trichoseal®, Trichovab®, Trieco®, Tusal®, T-22G, T-22HB and TRI 002 (Elad,

1994; Lumsden et al. 1996; De Vay et al. 1996; Koch, 1999; Howell, 2003; Nagayama et al. 2007, Reino et al. 2008). Despite the fact that some of these products are not registered as biocontrol agents, they are largely marketed as plant protectants, growth promoters, plant strengtheners, or soil conditioners.

From a human point of view, *Trichoderma* spp. may also exert unwanted side effects, i.e. through their high cellulolytic activity; *Trichoderma reesei* may degrade cotton fabrics and yarn (Cavaco-Paulo et al. 1998; Pere et al. 2001), strains of *T. aggressivum* and *T. harzianum* are pathogenic on commercial mushrooms like *Agaricus* spp. and *Pleurotus* spp. (Seaby 1998; Castle et al. 1998). More recently, certain members of the genus may play a role as causative agents of opportunistic infections in humans. For example *T. longibrachiatum* was reported to be an opportunistic pathogen of immunocompromised mammals including humans (Kredics et al. 2003).

The high expectations connected with *Trichoderma* spp. as biocontrol agents depend on multiple factors and their differential expression during cultivation and storage which can combat plants pathogens (Harman, 2006). Modes of action employed by *Trichoderma* species in the biocontrol of phytopathogenic fungi comprise mycoparasitism (Weindling, 1934), production of secondary metabolite(s) with antimicrobial properties (Dennis and Webster, 1971a, b; Howell and Stipanovic 1983), competition with other microorganisms for nutrient, space and even for plant exudates that stimulate the germination of fungal propagules in soil (Howell, 2002), production of different cell wall degrading enzymes that break down the polysaccharides (e.g. chitin and β-glucan) responsible for the rigidity of fungal cell walls (Kubicek, 1992; Metcalf and Wilson, 2001), and, induction of defense responses in plants against pathogens (Bigirimana et al. 1997).

One mechanism proposed by *Trichoderma* spp. is attacking the pathogen by excreting multifarious extracellular lytic enzymes which digest pathogen cell wall ingredients (Goldman et al. 1994; Kaur et al. 2005; Harighi et al. 2007). The extracellular chitinase, cellulase and β-1,3-glucanase have been suggested as the key enzymes in the lysis of cell walls of several phytopathogenic fungi during the mycoparasitic action of *Trichoderma* species (Elad et al. 1982). Through their abilities to produce cellulases, *Trichoderma* spp. were used even industrially with regard to recycling white paper and production of ethanol and textile (Kubicek, 1992; Kuhls, 1997).

The report on the antagonistic properties of *Trichoderma* spp. in terms of production of volatile and non-volatile antibiotics by Dennis and Webster (1971a, b) has stimulated the investigations in this field. Ever since, an increasing number of the antimicrobial metabolites produced by

Trichoderma spp. have been the subject of intensive studies and consequently several compounds with antimicrobial properties have been elucidated (Moffatt et al. 1969, Collins and Halim, 1972, Fujiwara et al. 1982, Almassi et al. 1991). Among the best-known antifungal metabolites produced by *Trichoderma* spp. are e.g. gliotoxin, viridin, gliovirin, glisoprenin, hepteledic acid, 6-pentyl-alpha-pyrone, viridiofungins, koninginins, anthraquinones, trichodermamides, peptaibols, polyketides, terpenoids, polypeptides, trichothecenes, trichodermaides, azaphilones, harzialactones and metabolites derived from alpha-amino acids (Harris et al. 1993; Howell, 1998, Vey et al. 2001, Reino et al. 2008).

6-Pentyl-alpha-pyrone (6PAP), a lactone with coconut-like characteristics, was first isolated from a culture filtrate of *T. viride*, and subsequently it has been shown that the compound is also produced by other *Trichoderma* spp. (Collins and Halim 1972). This lactone is also present in fruits such as peach (Sevenants and Jennings, 1971). 6PAP has been reported to exhibit inhibitory properties towards *Rhizoctonia solani* and *Fusarium oxysporum* (Scarselletti and Faull 1994), *Botrytis* spp. (Poole et al. 1998) and *Athelia rolfsii* (Dodd et al. 2000). This metabolite also displays antifungal volatile activity (Claydon et al. 1987).

6PAP can be prepared both synthetically in the laboratory and naturally by fermentation of *Trichoderma* spp. (Pittet and Klaiber, 1975; Dieter and Fishpaugh, 1988). Synthesis of 6PAP was found to be costly because it required many reactions steps at high temperature (490°C) and expensive reagents (Bengtson et al. 1992), therefore, its cost is an apparent obstacle for further development. Moreover, the toxicity of 6PAP to plant pathogens has been proposed to be related to its ability to adsorb onto hydrophobic cell membranes forming a water-impermeable barrier on hyphal cells (Scarselletti and Faull, 1994).

On the other hand, studies on other *Trichoderma* metabolites possessing biological properties such as viridiofungins are extremely limited (Harris et al. 1993). Viridiofungin A (VFA) and a number of structurally related derivatives are secondary metabolites of the alkyl citrate family that have been originally isolated from culture filtrates of *Trichoderma viride* (Harris et al. 1993). These compounds attracted attention due to their antifungal activity, but, they lack antibacterial activity (Onishi et al. 1997). It was shown that VFA inhibits the squalene synthase of *Candida albicans*. Inhibitors of squalene synthase have received concern as potential cholesterol lowering agents and antifungal compounds (Harris et al. 1993). Recent studies revealed that the antifungal activity is based primarily on the inhibition of the serine palmitoyltransferase, a key enzyme in *de novo* synthesis of sphingolipids (Mandala et al. 1997). The ability of viridiofungins to inhibit the farnesyltransferase might make it as potentially anticancer drug (Wittinghofer and Waldmann, 2000).

Contrary to the previous statments on potential biocontrol mechanisms by *Trichoderma* spp., direct effects on plant pathogens are only one mechanism of biocontrol, and are perhaps less important than indirect effects initiated by induced resistance. Promotion of plant growth and induction of defense responses in host plants against pathogens following treatment with the biocontrol agent represent mechanisms that have been put forward by several authors to explain the biocontrol activity by *Trichoderma* spp. (Bigirimana et al. 1997; Yedidia et al. 2000; Harman, 2006). The first clear demonstration of induced resistance by *Trichoderma* spp. was published in 1997 by Bigirimana et al. describing that treating soil with *T. harzianum* made leaves of bean plants resistant to diseases incited by *Botrytis cinerea* and *Colletotrichum lindemuthianum*.

General defense responses of plants to infections by pathogens may be recognized by the induction of pathogenesis related (PR)-proteins such as chitinases and β-1,3-glucanases, or phenol-oxidizing enzymes such as peroxidases and polyphenoloxidases that may lead to the accumulation of antifungal compounds e.g. phytoalexins, and the deposition of structural polymers, such as callose and lignin (Benhamou, 1995; Dalisay and Kuc, 1995).

The investigations in these studies are primarily focused on the interactions between the soil-borne pathogen *F. moniliforme*, the cause of seedling blight in maize, and the multiple mechanisms of the biocontrol agent *T. harzianum*, *in vitro* and in the host plant. Hence, the research objectives in this thesis were based mainly on the following topics:

(1) Selection of the biologically active *Trichoderma* isolates against the maize pathogen *Fusarium moniliforme*, the causal agent of seedling blight on maize *in vitro.*
(2) Molecular identification of the biologically active isolates of *Trichoderma* spp. and describing the differential expression of enzymes with cell wall lytic activities of biocontrol agents in the presence of different carbon sources.
(3) Studying the antibiosis as an important mechanism employed by the *Trichoderma* isolates. i.e. Purification, identification and structure elucidation of the most active secondary metabolites produced by *Trichoderma harzianum* isolate T23 and T16 e.g. 6-pentyl-alpha-pyrone, viridiofungin A and other antimicrobial compounds.
(4) Investigating the antagonistic potential of *T. harzianum* isolates and its metabolite 6PAP in both non-volatile and volatile phases on suppressing *F. moniliforme in vitro*.
(5) Examining the capability of *T. harzianum* and its metabolite 6PAP in suppressing the mycotoxin fusaric acid synthesized by *F. moniliforme in vitro*.

(6) Evaluating the antifungal potential of VFA and some other secondary metabolites purified from cultures of *T. harzianum* isolates T23 and T16 in the non-volatile and volatile phases against *F. moniliforme*.

(7) Supplying evidence on the antifungal spectrum of VFA towards various important plant pathogens.

(8) Describing the pathogenicity of ten *F. moniliforme* isolates and the susceptibility of different commercial maize cultivars to *F. moniliforme* infections.

(9) Studying the phytotoxic effects of 6PAP in maize plants and investigating the mutual effects of *T. harzianum* isolates (T23, T16) and the metabolite 6PAP in controlling seedling blight incited by *F. moniliforme* under greenhouse conditions.

(10) Determining the efficiency of *T. harzianum* isolate T23 on suppressing fusaric acid synthesized by *F. moniliforme* under greenhouse conditions.

(11) Providing evidences on the potential of 6PAP to generate defense responses in maize plants against *F. moniliforme*.