2. Review of Literature

*Fusarium* is a diverse and widely distributed fungal genus which is soilborne and consists mainly of saprophytes (Snyder and Hansen, 1981; Burgess, 1981; Katan and Katan, 1988; Gordon *et al.*, 1989; Gordon and Okomoto 1990). Some *Fusarium* are very pathogenic, causing wilts, crown rots and root rots in a wide variety of crops. They often limit crop production (Nelson *et al.*, 1981; Corell *et al.*, 1986; Martyn and Burton 1989; Kim *et al.*, 1991). It consists of species that cause diseases in a wide variety of plants. This makes it one of the most important fungal genera in terms of agriculture and forestry. However, the genus is best known for its plant pathogenic species (Toussoun 1981). As a species, it probably causes more economic damage to agricultural crops than any other pathogen (Corell 1991).

*Fusarium oxysporum* Schlectend. is an anamorphic species circumscribed by different morphological criteria, principally the size and shape of the macroconidium, the presence or absence of microconidia and chlamydospores, colony colour, and conidiophore structure (Nelson *et al.*, 1981; Windellls 1992). This group of cosmopolitan, soilborne filamentous fungi is economically important because many members are the causal agents of vascular wilt or root rot diseases in agricultural and ornamental crops throughout the world (Woo *et al.*, 1998). The typical symptoms caused by *F. oxysporum* include wilting, yellowing and xylem discolouration. However, populations of *F. oxysporum* are also found substantially in many native plant communities, in soils that have never been cultivated (Gordon and Martin 1997). In fact, the near ubiquity of *F. oxysporum* in soils worldwide has led to its inclusion in what has been termed the global mycoflora (Parkinson 1981). *F. oxysporum* is also
implicated in post harvest rots of some tropical crops such as apio (*Arracacia xanthorrhiza*), yams (*Dioscorea* spp.), and taro (*Colocasia esculenta*) (Stover 1981).

Some *Fusarium* species having medical and veterinary importance have also been reported (Rebel 1981; Marasas *et al.*, 1984; Baran *et al.*, 1997; Krcmery *et al.*, 1997; Yera *et al.*, 2003). *F. oxysporum* is among the more commonly isolated fungi from asymptomatic roots of crop plants (Gordon and Martin 1997). It is a causative agent of wilt disease in a wide range of economically important crops (Booth 1984). It has a well documented ability to persist without recourse to pathogenesis. Both pathogenic and nonpathogenic strains of *F. oxysporum* are found in agriculture soils throughout the world and have received a lot of attention from researchers.

*Fusarium* species is commonly identified based on their micro and macroscopic features. But these features are mostly unstable, and render the taxonomy of the group problematic. The presence of different taxonomic systems for the genus also contributes to this problem. *F. oxysporum* is one of the species with inherent morphological instability. This has rendered its taxonomy complicated and controversial (Szecsi and Dobrovolszky 1985). The inherent instability of *F. oxysporum* has enabled the species to occupy a wide range of ecological niches. The difficulty in delineating species based on these features is reported by many (Booth 1971; Gerlach and Nirenberg 1982; Nelson *et al.*, 1983). Many of these taxonomic schemes group the species into sections. Based on morphological criteria, it is sometimes difficult to distinguish *F. oxysporum* from several other species belonging to the sections *Elegans* and *Liseola*. There is further complicacy because plant
pathogenic, saprophytic and biocontrol strains of *F. oxysporum* are morphologically indistinguishable.

When identification of *F. oxysporum* is carried out based on morphological characteristics it can be confused with *F. subglutinans* and *F. solani* (Booth 1971; Nelson *et al.*, 1981). However, the presence of chlamydospores and the short microconidia bearing monophialides in *F. oxysporum* distinguish it from *F. subglutinans* and *F. solani* (Booth 1971; Nelson *et al.*, 1981). *F. oxysporum* mutates frequently. It either become more mycelial or pinnate where aerial mycelium is depressed (Waite and Stover 1960). This causes difficulty in identification of *Fusarium* isolates.

Although pathogenic members of *F. oxysporum* cause destructive vascular wilts in a wide variety of crops, individual pathogenic strains within the species have limited host ranges. Determination of *forma specialis* is carried out by testing the fungus for pathogenicity on various plant species, while race is determined by testing for pathogenicity on cultivars of a single plant species. The *forma specialis* concept was introduced by Snyder and Hansen (1940). The designation was introduced in order to describe the physiological capabilities of the fungi to attack a specific group of plants and was not a part of the formal taxonomic hierarchy. Snyder and Hansen (1940) subdivided the species into *forma specialis* based on the ability of the strains to cause disease in a particular host or group of hosts. Some of the *forma specialis* are further subdivided into races based on pathogenicity to a set of differential cultivars within the same plant species. Over 122 *forma specialis* and races of *F. oxysporum* have been reported (DiPietro *et al.*, 2003). It would therefore be laborious, time taking
to carry out pathogenicity tests for identification of a particular *formae specialis* and race. Unidentified strains would require inoculation to an endless number of different plant species and cultivars (Fravel *et al.*, 2003). Although pathogenicity and bioassays are very useful in verifying pathogenicity, they are highly time consuming.

Pathogenic *F. oxysporum* is very host specific. It attacks only one or a few species of plants and in many cases only certain cultivars of that plant. In other cases, the same pathogen may be pathogenic on a different family of plants. The specificity for a particular host is designated as *formae specialis* and for cultivars it is designated as race of the pathogen. These pathogenic fungi are morphologically indistinguishable from each other as well as from non-pathogens. It has been useful to plant pathologists because it identifies a subset of isolates that are of common concern to the production of crop susceptible to *Fusarium* wilt.

Given the differences among *formae specialis* and races in terms of their pathogenicity to crop species, the ability to distinguish between them assumes economic as well as scientific importance. However, their identification by pathogenicity alone is expensive and labour intensive task (Corell *et al.*, 1987; Ploetz *et al.*, 1990). Moreover, results of pathogenicity tests are often inconclusive as they are affected by a range of factors, such as host genetic composition (Corell 1991), stage of host (Hart and Endo 1981), and mode of inoculation (Kraft and Haglund 1978). Classification solely based on virulence also precludes the classification of non-pathogenic strains, which make up a significant component of the species complex (Corell 1991). These shortcomings in taxonomy of *F. oxysporum* have prompted the search for alternative techniques that are quickly reliable, and that can be used either to
complement or to replace those based on pathogenicity. In addition to morphological identification, pathogenicity tests, vegetative compatibility groups of *Fusarium oxysporum*, DNA-based techniques like ITS sequence information, random amplified polymorphic DNA (RAPD) and development of sequence characterized amplified region (SCAR) markers can lead to rapid identification of plant pathogens in quick time and with less effort.

Numerous pathogens are difficult to identify by morphological characteristics and require extensive, time consuming work with pure cultures and/or pathogenicity tests. It is challenging for most plant pathologists to identify isolates routinely from fungal genera such as *Fusarium* and *Pythium* because each includes a range of plant pathogenic and saprophytic species. Mechanical trapping devices which capture fungal spores are available to plant pathologists, however the usefulness of these tools is limited by the painstaking microscopic sorting and identification required after trapping. Selective media can help but in most cases they go only as far as genus selectivity. Technologies that would enable pathologists to identify from plants, traps, baits or soil samples rapidly and accurately would be very useful in epidemiological studies as well as for detecting initial inoculum in disease forecasting systems. As free trade agreements between countries become the norm, rapid testing for possible food contamination from a wide range of quarantined organisms will be high in demand. For a wide range of disease management applications, there is a need for comprehensive diagnostics kits that can detect the presence of numerous pathogens in a single test. (Levesque 1997).
2.1. Molecular approach

There is a significant shift in the types of approaches used to identify and characterize plant pathogens with the advent of molecular biology. Advances in molecular biology and genetic modification enable genes and traits to be tracked precisely and via marker based studies. DNA based technologies such as the polymerase chain reaction has been the basis for molecular detection in modern plant pathology. Accurate detection, identification and early detection of pathogens is the cornerstone of disease management in many crops. Many plant pathogens are difficult to identify using morphological criteria, which can be time consuming and challenging and requires extensive knowledge in taxonomy. Molecular detection techniques can generate accurate results rapidly enough to be useful for disease management decisions. (Levesque 2001). Molecular markers based upon sequence variation have proved extremely effective tools for distinguishing between closely related genotypes. With the advent of polymerase chain reaction (PCR) technology, methods have been developed that use amplified DNA sequences as molecular markers. These methods require very little template DNA, which can usually be obtained using a simple mini separation protocol. The production of marker bands is very fast and far less labour intensive than when using restriction fragment length polymorphism (RFLP) technology/(Munthali et al., 1992; Williams et al., 1990; Welsh and McClelland 1990; Caetano-Anolles et al., 1991).
2.2. DNA sequence based phylogenetic identification

Molecular markers may be used for diagnostics or phylogeny. The main difference in diagnostic markers is related to the amount of sample needed. RAPD-PCR based methods require very limited amount of target DNA samples. But in order to perform a diagnostic test using probes and RFLP analysis it requires a higher amount of target DNA samples or it needs to be coupled to PCR. RAPD and RFLP (Restriction–Fragment–Length–Polymorphism) analysis may produce molecular markers to distinguish inter and intra specific level (Woo et al., 1998). Molecular tools have been successfully identified pathogenic strains and in some cases even races of the pathogen. Unfortunately, pathogenicity tests still rely largely on bioassays. Nucleotide sequences from certain genes reflect phylogeny at various taxonomic levels. Identification of eukaryotic organisms is basically done on sequence information from PCR amplification of internal transcribed spacer (ITS) region from the conserved ribosomal RNA genes. Sequences of the intergenic spacer (IGS), translation elongation factor (EF-1α), β-tubulin region and the mitochondrial small subunit (mtSSU) have been valuable in distinguishing species and origins of Fusarium (Baayen et al., 2000; O’Donnell et al., 2000; Baayen et al., 2001; Skovgaard et al., 2001). The sequence information have been widely used in the taxonomy and phylogeny of Fusarium species. This variability which is harboured mainly in the introns provides enough resolution at the sub-species level. Also some of the DNA sequences that have also been used successfully to distinguish Fusarium species include UTP-ammonia ligase, trichothecene 3-O-acetyltransferase, and a putative reductase (O’Donnell et al., 2000), nitrate reductase region (NIR) and phosphate
permease (Skovgaard et al., 2001). O'Donnell and Cigelnik (1997) reported that although ITS regions are useful in distinguishing species in many eukaryotic organisms, they have not been very informative for \textit{Fusarium}.

2.3. Characterization and identification based on protein based methods

Isozyme analysis is a protein based technique. It has also been employed to study the taxonomy of various organisms including \textit{F. oxysporum}. Isozyme analysis involves the detection of antigen-antibody interactions. These antibodies are raised against a protein extract from an isolate. Protein extracts from other isolates or organisms are made to cross react with these antibodies. Also total protein profiling and immunoserology are also widely done. Total protein profile refers to the electrophoretic banding patterns of total soluble proteins, which are revealed using general protein staining systems. Total protein gel profiles that arise as a result of differences in the electrophoretic mobility of proteins reflect differences in the gene sequence encoding these proteins. Thus, if protein patterns of two individual differ, it is assumed that these differences are genetically based and heritable. Chang et al., (1962) first demonstrated that protein extracts obtained from different species of \textit{Neurospora} had distinct electrophoretic patterns. Since then it was increasingly used in fungal taxonomy (Durbin, 1966; Gill and Powell 1968). In 1967, disc electrophoresis was suggested to be useful in characterizing \textit{formae specialis} and races of \textit{Fusarium} species (Macko et al., 1967). However, Hall (1967) showed that culturally and pathogenetically distinct isolates of \textit{F. solani} produced essentially identical protein patterns. Since then, several studies have reported on the merits and limitations of the
technique in the taxonomy of *F. oxysporum*. Glynn and Reid (1969) employed acrylamide gel electrophoresis to study isolates of *F. oxysporum*, other *Fusarium* species, *Verticillium albo-artum* and a *Graphium* sp. They reported that protein profiles do not correlate with virulence.

Saponin detoxification enzymes from pathogenic fungi are involved in the infection process of their host plants (Pareja-Jaime et al., 2008). *F. oxysporum* f. sp *lycopersici* produces the tomatinase enzyme *Tom1*, which degrades *α*-tomatine to less toxic derivates. To study the role of the *Tom1* gene in the virulence of *F. oxysporum*, Pareja-Jaime et al., (2008) performed targeted disruption and overexpression of the gene. The infection process of tomato plants inoculated with transformants constitutively producing *Tom1* resulted in an increase of symptom development. By contrast, tomato plants infected with the knockout mutants showed a delay in the disease process, indicating that *Tom1*, although not essential for pathogenicity, is required for the full virulence of *F. oxysporum*. Total tomatinase activity in the disrupted strains was reduced only 25%, leading to *β*-tomatine as the main hydrolysis product of the saponin *in vitro*. *In silico* analysis of *F. oxysporum* genome revealed the existence of four additional putative tomatinase genes with identities to tomatinases from family 3 of glycosyl hydrolases. These might be responsible for the remaining tomatinase activity in the δ-*Tom1* mutants. Their results indicated that detoxification of *α*-tomatine in *F. oxysporum* is carried out by several tomatinase activities, suggesting the importance of these enzymes during the infection process.
2.4. Random Amplified polymorphic DNA (RAPD)

RAPD (random amplified polymorphic DNA) technique based on PCR has been one of the most commonly used molecular techniques to develop DNA marker. RAPD (Williams et al., 1990) or the arbitrarily primed PCR (AP-PCR, Welsh and McClelland 1990) use single primers of arbitrary sequence to generate DNA fragments. In RAPD, single primers of arbitrary sequence (generally 10 nucleotides) are used to generate DNA fragments. RAPD analysis has been observed to have a high level of variability among many isolates and used for identification of markers in fungi and (Chiocchetti et al., 1999, Pasquali et al., 2003; Wilson et al., 2004; Balmas et al., 2005; Bayraktar et al., 2008). As compared to other molecular techniques such as RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), DAF (DNA Amplified Fingerprinting) and ISSRs (Inter Simple Sequence Repeats), RAPD is simple and relatively faster (Wilson et al., 2004; Guleria et al., 2007).

In RFLP, the use of probes requires high skill. The possibility of human error is quite high. PCR methods allow good sensitivity and also decrease the detection limit. Coddington et al., (1987) used RFLP to distinguish 3 races of *F. oxysporum* f. sp. *pisic* and to distinguish *forme specialis* on *cucurbitaceae* using mitocondrial DNA (Kim et al., 1993). AFLP is based on the selective amplification of restriction fragments from a total digest of DNA. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. (Pieter vos 1995). AFLP analysis has been used for DNA fingerprinting of
microorganisms and was used to evaluate phylogenetic relationships among different *fomae specialis* of *F. oxysporum* (Baayen et al., 2000; Abdel-Satar, Mohamed 2003). DAF differs from RAPD in that it uses primers usually 8-12 nucleotides with a 5'-prime mini hairpin structure to help minimize interactions within and between termini of amplification products during PCR (Caetano-Anolles et al., 1991). Bentley et al., (1998) used DAF to differentiate among VCGs of *F. oxysporum* based only on single representative isolates. Based on their observation, it was concluded that DAF has been inadequate for use in taxonomy of *F. oxysporum*.

Gurjar G et al., (2009) demonstrated the synergistic use of gene-specific markers, ITS-RFLP, ISSR and AFLP for distinguishing Indian *F. oxysporum* f. sp. *ciceris* races. They reported for the first time that *F. oxysporum* f. sp. *ciceris* race 3, a wilt pathogen of chickpea in India, is actually *F. proliferatum* based on phylogenetic analysis with EF-1α sequence data.

RAPD has several advantages as a means of characterizing genetic variability. These include speed, low cost, less amount of DNA and lack of radioactivity. Prior sequence information about the target DNA is not required. An advantage of RAPD analysis is that it can be applied to any strain or species of a fungal or bacterial group without previous knowledge of that isolate. It is applicable to large number of isolates and enables analysis of variation at more than one locus (Bentley et al., 1995). RAPD analysis is a fast, PCR-based typing method for study of genomic polymorphisms. RAPD has been found to be efficient in demonstrating the DNA polymorphism as well as in accessing diversity in nature (Bereswill et al.,1994; Dautle et al. 2002). Therefore, this technique has been used extensively in the molecular characterization
of several organisms, microorganisms like bacteria, yeasts, fungi, phytopathogenic fungi and *F. oxysporum* isolates in particular. Many reports on RAPD are available regarding characterization and detection of *F. oxysporum* and other phytopathogenic fungi (Assigbetse *et al.*, 1994; Manulis *et al.*, 1994; Bentley *et al.*, 1995; Migheli *et al.*, 1998; Chiocchetti *et al.*, 2001; Jimenez-Gasco *et al.*, 2004; Bayraktar *et al.*, 2008). Different isolates of *F. oxysporum* infecting basil plants were studied and analyzed by random amplified polymorphic DNA (RAPD-PCR) using random primers and these primers allowed clear differentiation from representatives of other species and from non-pathogenic strains (Chiocchetti *et al.*, 1999b).

The main disadvantage of RAPD technology is low reproducibility and reliability. It is unusually sensitive to changes in reaction conditions and even some of the banding patterns can be DNA polymerase dependent (Li *et al.*, 2010). The high homoplasy in data generated with RAPD primers, the lack of co-dominance, and its reproducibility also pose limitations in the use of the technique. However, development of more specific, sensitive and reproducible markers like RAPD based on sequence characterized arbitrary region (SCAR) can increase application of the molecular techniques. These markers have been used for identification of plant pathogens. SCAR markers are specific as they increase the reproducibility of RAPD markers and have been used for specific amplification of target DNA. PCR methods based on RAPD and SCAR primers analysis have a good sensitivity and are largely used in routine laboratory research. The use of SCAR markers to identify pathogens offers the advantage of low cost and good specificity. Furthermore, these markers are an efficient means of screening large fungal populations and they are economical and
relatively easy to analyze (Chowdhury et al., 2001; Lee et al., 2004; Vakalounakis et al., 2004; Bayraktar et al., 2008; Lievens et al., 2008; Schena et al., 2008). As a result, this technique has been used extensively in the molecular characterization of *F. oxysporum* isolates to distinguish between pathogenic and non-pathogenic isolates of *F. oxysporum* (Grajal-Martin et al., 1993; Assigbetse et al., 1994; Manulis et al., 1994; Kelly et al., 1994; Tantoui et al., 1996; Migheli et al., 1998; Chiocchetti et al., 1999; de Haan et al., 2000; Chiocchetti et al., 2001; Alves-Santos et al., 2002). However, the detection of *F. oxysporum* in tomato wilt has been very less studied in Indian context.

In one study Mutlu et al., (2008) carried out experiments to confirm the monogenic inheritance of *Fusarium* wilt resistance in eggplant and identify molecular markers linked to this resistance. They also developed SCAR markers from most informative markers. They reported the tagging of the gene for resistance to *Fusarium* wilt (FOM) in eggplant using SRAP, RGA, SRAP-RGA and RAPD markers. Analysis of segregation data confirmed the monogenic inheritance of resistance. DNA from F2 and BC1 populations of eggplant segregating for *Fusarium* wilt resistance was screened with 2,316 primer combinations to detect polymorphism. Three markers were linked within 2.6 cM of the gene. The codominant SRAP marker Me8/Em5 and dominant SRAP-RGA marker Em12/GLPL2 were tightly linked to each other and mapped 1.2 cM from the resistance gene, whereas RAPD marker H12 mapped 2.6 cM from the gene and on the same side as the other two markers. The SRAP marker was converted into two dominant SCAR markers that were confirmed to be linked to the resistance gene in the F2, BC1 and F2 of BC3 generations of the same cross. These markers provide a starting point for mapping the eggplant FOM resistance gene in
eggplant and for exploring the synten between solanaceous crops for *Fusarium* wilt resistance genes. The SCAR markers will be useful for identifying *Fusarium* wilt resistant genotypes in marker-assisted selection breeding programs using segregating progenies of the resistant eggplant progenitor used in this study (Mutlu et al., 2008).

Several studies have revealed correspondence between pathogenic groups and VCG and also between pathogenic groups and RAPD fingerprint data. Using RAPD, 24 isolates belonging to 13 *formeae specialis* of *F. oxysporum* were studied (Wang et al., 2001). Seven RAPD primers which were selected from an initial set of 132, revealed RAPD fingerprints unique to each *formeae specialis* and probes were developed from *formeae speciales* specific RAPD bands. It showed different specificity to the 13 *formeae specialis* after Southern hybridization (Southern 1975) with RAPD fingerprints. Based on these findings, the authors concluded that markers based on differences in fingerprints are potentially useful for the identification of *formeae specialis* without the need for pathogenicity tests. Similar studies showed that RAPD fingerprints correspond with VCGs (Grajal-Martin et al., 1993; Bentley et al., 1995; Tantaoui et al., 1996; Nelson et al., 1997; Mes et al., 1999). RAPD fingerprints also reported to correspond with *formeae speciales* (Chiochetti et al., 1999; Harnandez et al., 1999; Vakalounakis and Fragkiadakis 1999; Pasquali et al., 2003), and races (Assigbetse et al., 1994; Manulis et al., 1994; de Hann et al., 2000). However, *formeae speciales* in these studies were represented by only one to three isolates.

Cramer et al., (2003) reported the unsuitability of RAPD to differentiate isolates based on pathogenecity or geographical origin. They studied 34 isolates including *F. oxysporum phaseoli*, *F. oxysporum betae*, non-pathogenic *F. oxysporum*
and an isolate of *F. solani*. They used twelve 10 mer primers for generation of data based on UPGMA analysis which revealed that only *F. oxysporum* collected from the same geographical area clustered together. However, this group also contained members of races 1 and 4. No other grouping by race or geographical area was evident. In fact some *F. oxysporum betae* isolates appeared to be more closely related to non-pathogenic *F. oxysporum*, and the outgroup *F. solani*. Woo *et al.*, (1996) Vakalounakis *et al.*, (2004) also reported similar observations.

The use of RAPD in phylogenetic analysis was evaluated by O’Donnell *et al.*, (1999) employed two 10 mer and one 15 mer provided a better resolution than those inferred from the individual and combined data generated with the 10 mers. RAPD analysis revealed the underlying phylogenetic structure as accurately as DNA sequence data. The authors concluded that it would be better to use longer primers (15 to 20 mer) for phylogenetic studies to reduce the homoplasy associated with shorter RAPD primers. They also suggested that trees inferred from RAPD analysis should be treated cautiously, especially when 10 mer are used.

Woo *et al.*, (1996) reported that RAPD may also fail to differentiate between pathogenic and non-pathogenic isolates. Therefore, it is necessary to screen a large number of primers in order to reach an effective RAPD system, since all primers cannot resolve isolates (Wang *et al.*, 2001).

The fungal species *F. oxysporum* is a ubiquitous inhabitant of soils worldwide that includes pathogenic as well as non-pathogenic or even beneficial strains. Pathogenic strains are characterized by a high degree of host specificity and strains that infect the same host range are organized in so-called *formae speciales*. Strains for
which no host plant has been identified are believed to be non-pathogenic strains. Therefore, identification below the species level is highly desired. However, the genetic basis of host specificity and virulence in \textit{F. oxysporum} is so far unknown (Lievens B \textit{et al.}, 2007).

Lievens B \textit{et al.}, (2007) developed a robust RAPD marker based assay to specifically detect and identify the economically important cucumber pathogens \textit{F. oxysporum} f. sp. \textit{cucumerinum} and \textit{F. oxysporum} f. sp. \textit{radicis-cucumerinum}. While the \textit{F. oxysporum} f. sp. \textit{radicis-cucumerinum} strains were found to cluster in a separate clade based on elongation factor \textit{EF-la} phylogeny, strains belonging to \textit{F. oxysporum} f. sp. \textit{cucumerinum} were found to be genetically more diverse. They observed that specificity testing of the identified markers using a broad collection of \textit{F. oxysporum} strains with all known vegetative compatibility groups of the target \textit{forma speciales}, as well as representative strains belonging to other \textit{forma speciales}, resulted in two cross-reactions for the \textit{F. oxysporum} f. sp. \textit{cucumerinum} marker. However, no cross-reactions were observed for the \textit{F. oxysporum} f. sp. \textit{radicis-cucumerinum} marker. This \textit{F. oxysporum} f. sp. \textit{radicis-cucumerinum} marker was observed to show homology to \textit{Folyt1}, a transposable element identified in the tomato pathogen \textit{F. oxysporum} f. sp. \textit{lycopersici} and may possibly play a role in host range specificity in the target \textit{forma speciales}. The markers were implemented in a DNA array that enabled parallel and sensitive detection and identification of the pathogens in complex samples from diverse origins.

In one study, virulence analysis of 64 isolates of \textit{F. oxysporum} f. sp. \textit{ciceris} causing chickpea wilt collected from major chickpea growing states of India on 14
varieties, including 10 international differentials revealed that the isolates from each state were highly variable (Dubey and Singh 2008). Based on the reactions on international differentials, more than one race was found to be prevalent in every state. Majority of the isolates did not match with the race specific reactions. RAPD, ISSR, and simple sequence repeat (SSR) markers were used to assess the genetic diversity of these isolates. Unweighted paired group method with arithmetic average (UPGMA) cluster analysis was used to divide the isolates into distinct clusters. The clusters generated by RAPD grouped all isolates into three categories at 25% genetic similarity and into two major categories at 30% genetic similarity. ISSR and SSR analyses also grouped all the isolates into two major categories. Majority of the isolates from Punjab and a few from Rajasthan were grouped in one category while the isolates from all other states were grouped in another suggesting the existence of diverse genetic populations of the pathogen at the same location.

2.5. Recent trends to study the virulence of *Fusarium*

van der Does HC *et al.*, (2008) showed that, despite their polyphyletic origin, *F. oxysporum* isolates belonging to f.sp. *lycopersici*, contained an identical genomic region of at least 8 kb that is absent in other formae speciales and non-pathogenic isolates, and comprises the genes *SIX1*, *SIX2* and *SHH1*. In addition, *SIX3*, which lies elsewhere on the same chromosome, is also unique for f.sp. *lycopersici*. *SIX1* encodes a virulence factor towards tomato, and the *Six1*, *Six2* and *Six3* proteins are secreted in xylem during colonization of tomato plants. It was speculated that these genes may be part of a larger, dispensable region of the genome that confers the ability to cause
tomato wilt and has spread among clonal lines of *F. oxysporum* through horizontal gene transfer. These findings also have practical implications for the detection and identification of *F. oxysporum* f.sp. *lycopersici*.

Forward genetic screens are efficient tools for the dissection of complex biological processes, such as fungal pathogenicity. Lopez-Berges *et al.*, (2009) developed a transposon tagging system in the vascular wilt fungus *F. oxysporum* f.sp. *lycopersici* by inserting the novel modified impala element *imp160::gfp* upstream of the *Aspergillus nidulans niaD* gene, followed by transactivation with a constitutively expressed transposase. A collection of 2072 Nia*+* revertants was obtained from reporter strain *T12* and screened for alterations in virulence, using a rapid assay for invasive growth on apple slices. Seven strains exhibited reduced virulence on both apple slices and intact tomato plants. Five of these were true revertants showing the re-insertion of *imp160::gfp* within or upstream of predicted coding regions, whereas the other two showed either excision without reinsertion or no excision. Linkage between *imp160::gfp* insertion and virulence phenotype was determined in four transposon tagged loci using targeted deletion in the wild-type strain. Knockout mutants in one of the genes, FOXG_00016, displayed significantly reduced virulence, and complementation of the original revertant with the wild-type FOXG_00016 allele fully restored virulence. FOXG_00016 has homology to the velvet gene family of *A. nidulans*. The high rate of untagged virulence mutations in the *T12* reporter strain appears to be associated with increased genetic instability, possibly as a result of the transactivation of endogenous transposable elements by the constitutively expressed transposase.
The \textit{sti35} gene of the vascular wilt fungus \textit{F. oxysporum} was originally identified based on induced expression under stress conditions. Ruiz-Roldan \textit{et al.}, (2008) examined the transcriptional regulation and biological function of \textit{sti35} in the tomato pathogen \textit{F. oxysporum} f.sp. \textit{lycopersici}. They observed that expression of \textit{sti35} was repressed by thiamine and induced by high temperatures. \textit{Sti35} transcripts were detected both during early and late stages of infection of tomato plants by \textit{F. oxysporum}. Heterologous expression of the \textit{sti35} cDNA restored thiamine prototrophy in a \textit{Saccharomyces cerevisiae thi4} mutant and increased UV tolerance in a \textit{uvr'} mutant of \textit{Escherichia coli}. Targeted \textit{Deltasti35} knockout mutants of \textit{F. oxysporum} exhibited a thiamine auxotrophic phenotype and reduced tolerance to the superoxide-generating agent menadione, indicating that \textit{Sti35} has a dual role in thiamine biosynthesis and oxidative stress response. RT-PCR analysis revealed the presence of differential RNA splicing of the second 5'-UTR intron, suggesting that thiamine may regulate \textit{sti35} expression via a post-transcriptional mechanism. \textit{F. oxysporum} transformants carrying a transcriptional fusion of the \textit{sti35} promoter to the \textit{lacZ} reporter gene produced high levels of \textbeta-galactosidase activity when grown in the absence, but not in the presence of thiamine. Thus, the \textit{sti35} promoter represents a useful tool for the controlled expression of genes of interest in \textit{F. oxysporum}.

To promote host colonization, many plant pathogens secrete effector proteins that either suppress or counteract host defences. However, when these effectors are recognized by the host's innate immune system, they trigger resistance rather than promoting virulence. Effectors are therefore key molecules in determining disease susceptibility or resistance (Houterman PM \textit{et al.}, 2009). It was showed that \textit{Avr2},
secreted by the vascular wilt fungus *F. oxysporum* f. sp. *lycopersici* (*Fol*), shows both activities and it is required for full virulence in a susceptible host and also triggers resistance in tomato plants carrying the resistance gene *I-2* (Houterman PM *et al.*, 2009). Point mutations in *AVR2*, causing single amino acid changes, are associated with *I-2*-breaking *Fol* strains. These point mutations prevent recognition by *I-2*, both in tomato and when both genes are co-expressed in leaves of *Nicotiana benthamiana*. *Fol* strains carrying the *Avr2* variants are equally virulent, showing that virulence and avirulence functions can be uncoupled. Although *Avr2* is secreted into the xylem sap when *Fol* colonizes tomato, the *Avr2* protein can be recognized intracellularly by *I-2*, implying uptake by host cells (Houterman PM *et al.*, 2009).

Martin-Urdiroz *et al.* 2009 identified a new myosin motor-like chitin synthase gene, *chsVb* in the vascular wilt fungus *F. oxysporum* f. sp. *lycopersici*. Phylogenetic analysis of the deduced amino acid sequence of the *chsVb* chitin synthase 2 domain (CS2) revealed that *ChsVb* belongs to class VII chitin synthases. The *ChsVb* myosin motor-like domain (MMD) is shorter than the MMD of class V chitin synthases and does not contain typical ATP-binding motifs. Targeted disrupted single (*DeltachsVb*) and double (*DeltachsV, DeltachsVb*) mutants were unable to infect and colonize tomato plants or grow invasively on tomato fruit tissue. These strains were hypersensitive to compounds that interfere with fungal cell wall assembly, produced lemon like shaped conidia, and showed swollen balloon like structures in hyphal subapical regions, thickened walls, aberrant septa, and intrahyphal hyphae. Their results suggest that the *chsVb* gene is likely to function in polarized growth and
confirm the critical importance of cell wall integrity in the complex infection process of this fungus.

Michielse CB et al., (2009) have identified a homolog of a master regulator of this morphological switch in the plant pathogenic fungus *F. oxysporum* f. sp. *lycopersici*. This non dimorphic fungus causes vascular wilt disease in tomato by penetrating the plant roots and colonizing the vascular tissue. Gene knock-out and complementation studies established that the gene for this putative regulator, *SGE1* (*SIX* Gene Expression 1), is essential for pathogenicity. In addition, microscopic analysis using fluorescent proteins revealed that *Sgel* is localized in the nucleus, is not required for root colonization and penetration, but is required for parasitic growth. Furthermore, *Sgel* is required for expression of genes encoding effectors that are secreted during infection. Michielse CB et al., (2009) proposed that *Sgel* is required in *F. oxysporum* and other non-dimorphic (plant) pathogenic fungi for parasitic growth.

During infection of tomato, the fungus *F. oxysporum* f. sp. *lycopersici* secretes several unique proteins, called 'secreted in xylem' (Six) proteins, into the xylem sap. At least some of these proteins promote virulence towards tomato and among them, all predicted avirulence proteins that can trigger disease resistance in tomato have been found (Lievens et al., 2009). Lievens et al., (2009) screened a large, worldwide collection of *F. oxysporum* isolates for the presence of seven *SIX* genes (*SIX1*-*SIX7*). Their results convincingly showed that identification of *F. oxysporum* f. sp. *formeae speciales* and races based on host-specific virulence genes can be very robust. *SIX1*, *SIX2*, *SIX3* and *SIX5* can be used for unambiguous identification of the *formeae specialis lycopersici*. In addition, *SIX4* can be used for the identification of race 1
strains, while polymorphisms in *SIX3* can be exploited to differentiate race 2 from race 3 strains. For *SIX6* and *SIX7*, close homologs were found in a few other *formeae speciales*, suggesting that these genes may play a more general role in pathogenicity. Host specificity may be determined by the unique *SIX* genes, possibly in combination with the absence of genes that trigger resistance in the host.

Houterman *et al.*, 2008 showed that a plant pathogenic fungus secretes an effector that can both trigger and suppress *R* gene-based immunity. This effector, *AvrI*, was secreted by the xylem-invading fungus *F. oxysporum* f.sp. *lycopersici* (*Fol*) and triggers disease resistance when the host plant, tomato, carries a matching *R* gene (1 or I-1). At the same time, *AvrI* suppressed the protective effect of two other *R* genes, *I-2* and *I-3*. Based on these observations, they tentatively reconstruct the evolutionary arms race that has taken place between tomato *R* genes and effectors of *Fol*. This molecular analysis had revealed a hitherto unpredicted strategy for durable disease control based on resistance gene combinations.

*F. oxysporum* f. sp. *phaseoli* strains isolated from runner bean plants showing *Fusarium* wilt symptoms were characterized. The analysis of the genetic diversity of these strains and the comparison with strains formerly isolated from diseased common bean plants indicated a close genetic similarity among them. Pathogenicity assays carried out on runner bean plants showed virulence differences that allowed the classification of these strains into three groups: super-virulent, highly-virulent and weakly-virulent. However, all the analyzed strains behaved as highly-virulent when inoculated on common bean plants, indicating that virulence is specific of the host-pathogen interaction. We also analyzed the number of copies and expression of the
gene encoding the transcription factor ftfl, which has been shown to be specific of virulent *F. oxysporum* strains and highly up-regulated during plant infection. *In planta* real-time qPCR expression analysis showed that expression of ftfl was correlated with the degree of virulence. The comparative analysis of the polymorphic copies of ftfl detected in the strains here characterized and those detected in the genome sequence of *F. oxysporum* f. sp. *lycopersici* strain 4287 indicates that some of the copies are likely non-functional.

During infection, fungal pathogens activate virulence mechanisms, such as host adhesion, penetration and invasive growth. In the vascular wilt fungus *F. oxysporum*, the mitogen-activated protein kinase *Fmkl* is required for plant infection and controls processes such as cellophane penetration, vegetative hyphal fusion, or root adhesion. Lopez-Berges *et al.*, (2010) showed that these virulence-related functions are repressed by the preferred nitrogen source ammonium and restored by treatment with L-methionine sulfoximine or rapamycin, two specific inhibitors of *Gln* synthetase and the protein kinase *TOR*, respectively. Deletion of the bZIP protein *MeaB* also resulted in nitrogen source-independent activation of virulence mechanisms. Activation of these functions did not require the global nitrogen regulator *AreA*, suggesting that *MeaB*-mediated repression of virulence functions does not act through inhibition of *AreA*. Tomato plants (*Solanum lycopersicum*) supplied with ammonium rather than nitrate showed a significant reduction in vascular wilt symptoms when infected with the wild type but not with the DeltameaB strain. Nitrogen source also affected invasive growth in the rice blast fungus *Magnaporthe oryzae* and the wheat head blight pathogen *F. graminearum*. They proposed that a conserved nitrogen-responsive
pathway might operate via TOR and MeaB to control virulence in plant pathogenic fungi.

Virulence in plant pathogenic fungi is controlled through a variety of cellular pathways in response to the host environment. Nitrogen limitation has been proposed to act as a key signal to trigger the \textit{in planta} expression of virulence genes. Moreover, a conserved Pathogenicity mitogen activated protein kinase (MAPK) cascade is strictly required for plant infection in a wide range of pathogens. Lopez-Berges \textit{et al.}, (2010) investigated the relationship between nitrogen signaling and the Pathogenicity MAPK cascade in controlling infectious growth of the vascular wilt fungus \textit{Fusarium oxysporum}. Several MAPK-activated virulence functions such as invasive growth, vegetative hyphal fusion and host adhesion were strongly repressed in the presence of the preferred nitrogen source ammonium. Repression of these functions by ammonium was abolished by L-Methionine sulfoximine (\textit{MSX}) or rapamycin, two specific inhibitors of \textit{Gln} synthetase and the protein kinase \textit{TOR} (Target of Rapamycin), respectively, and was dependent on the \textit{bZIP} protein \textit{MeaB}. Supplying tomato plants with ammonium rather than nitrate resulted in a significant delay of vascular wilt symptoms caused by the \textit{F. oxysporum} wild type strain, but not by the \textit{MeaB} mutant. Ammonium also repressed invasive growth in two other pathogens, the rice blast fungus \textit{Magnaporthe oryzae} and the wheat head blight pathogen \textit{Fusarium graminearum}. Their results suggest the presence of a conserved nitrogen-responsive pathway that operates via \textit{TOR} and \textit{MeaB} to control infectious growth in plant pathogenic fungi.
Cutinolytic enzymes are secreted by fungal pathogens attacking the aerial parts of the plant, to facilitate penetration of the outermost cuticular barrier of the host. The role of cutinases in soilborne root pathogens has not been studied thus far and so Rocha AL et al., 2008 reported the characterization of the zinc finger transcription factor Ctfl from the vascular wilt fungus *F. oxysporum*, a functional orthologue of *CTF1alpha* that controls expression of cutinase genes and virulence in the pea stem pathogen *F. solani* f. sp. *pisi*. Mutants carrying a *Deletactfl* loss-of-function allele grown on inducing substrates failed to activate extracellular cutinolytic activity and expression of the *cutl* and *lipl* genes, encoding a putative cutinase and lipase, respectively, whereas strains harbouring a *ctfl(C)* allele in which the *ctfl* coding region was fused to the strong constitutive *Aspergillus nidulans gpdA* promoter showed increased induction of cutinase activity and gene expression. These results suggest that *F. oxysporum* Ctfl mediates expression of genes involved in fatty acid hydrolysis. However, expression of *lipl* during root infection was not dependent on Ctfl, and virulence of the *ctfl* mutants on tomato plants and fruits was indistinguishable from that of the wild-type. Thus, in contrast to the stem pathogen *F. solani*, Ctfl is not essential for virulence in the root pathogen *F. oxysporum*.

*F. oxysporum* is an asexual, soil inhabiting fungus that comprises many different *formae speciales*, each pathogenic towards a different host plant. In absence of a suitable host all *F. oxysporum* isolates appear to have a very similar lifestyle, feeding on plant debris and colonizing the rhizosphere of living plants. Upon infection *F. oxysporum* switches from a saprophytic to an infectious lifestyle, which probably includes the reprogramming of gene expression (van der Does et al., 2008b). van der
Does et al., 2008 showed that the expression of the known effector gene SIX1 of *F. oxysporum* f. sp. *lycopersici* is strongly upregulated during colonization of the host plant. Using GFP (green fluorescent protein) as reporter, they showed that induction of SIX1 expression starts immediately upon penetration of the root cortex. Induction requires living plant cells, but is not host specific and does not depend on morphological features of roots, since plant cells in culture can also induce SIX1 expression. Taken together, *F. oxysporum* seems to be able to distinguish between living and dead plant material, preventing unnecessary switches from a saprophytic to an infectious lifestyle.

2.6. Management and Control of phytopathogen

Soil borne plant pathogens are a major problem in many agricultural and greenhouse crops. Pathogens are often able to survive for several years in the soil as dormant, environmentally persisting resting structures, until a susceptible crop is introduced (Vurro and Gressel 2006). *Fusarium oxysporum*, is ubiquitous phytopathogen causing root rot, vascular wilt and damping off in many plant species. Management of *Fusarium* wilt is mainly through chemical soil fumigation and use of resistant cultivars. The broad spectrum of biocides used to fumigate soil before planting, particularly methyl bromide, is environmentally damaging. The most cost effective environmentally safe method of control is the use of resistant cultivars, when these are available (Katan 1996; Fravel et al., 2003). Unfortunately chemicalization of agriculture in both the undeveloped world with aims to achieving maximum production has led to irreversible depletion of the environment making certain
agricultural practices unsustainable in the longer term. New disease management strategies have also been devised using biotechnology in the development of biological disease control, inoculum production, formulation and application procedures (Geoffrey Hewitt 2004). Several attempts have been made through conventional breeding and the molecular biological approaches to understand the biology of host-pathogen interaction so that the disease can be managed and crop loss prevented (Panthee and Chen 2010).

Variability of pathotypes of *F. oxysporum* and breakdown of natural resistance are the main hindrances to developing resistant plants by applying resistant breeding strategies. Additionally, lack of information of potential resistant genes limits gene transfer technology. A thorough understanding of *Fusarium* spp. host interaction at a cellular and molecular level is essential for isolation of potential genes involved in counteracting disease progression. Gupta *et al.,* (2009) designed experiments to trigger the pathogen challenged disease responses in both susceptible and resistant plants and monitor the expression of stress induced genes or gene fragments at the transcript level. cDNA amplified fragment length polymorphism followed by homology search helped in differentiating and analyzing the up and downregulated gene fragments. Several detected DNA fragments appeared to have relevance with pathogen mediated defense. Some of the important transcript derived fragments were homologous to genes for sucrose synthase, isoflavonoid biosynthesis, drought stress response, serine threonine kinases, cystatins, arginase.

There have been growing consumer concerns about food quality and safety over the last few decades. Other uneasiness about the short and long term...
susceptibility of the environment to pesticide usage has raised the question of the sustainability of natural resources. Each of the major crops is susceptible to a thankfully limited and often specific complement of fungi and it is their control by synthetic fungicides that has helped to underpin the success of modern agriculture. However, the fungicide market with over 6 billion in annual sales in Europe and the Far East faces an uncertain future. (Geoffrey Hewitt H 2004).

Synthetic pesticides have, of course played a major significant role in restricting many pest problems. However, their indiscriminate use has taken its toll by creating several problems like pesticide-resistance insects, contamination of food by toxic residues, resurgence of pests and determines effects on non-target organisms. The number of effective pesticides available is dwindling day by day, further leaving less choice among which to choose. These ill effects of synthetic pesticides have aroused interest in alternate methods of plant production. In recent years there has been a gradual revival of interest in the use of medicinal plants in developed as well as in developing countries, because herbal have been reported to be safe and without any adverse side effects. Thus, a search for new drugs with better and cheaper substitutes from plant resources is a natural choice. Botanical pesticides are good alternatives to chemical pesticides. They are eco-friendly, economic, target-specific and biodegradable. For example, neem based botanical pesticides have been used traditionally for many years. There are many other trees (besides herbs and shrubs) which are also useful as sources of botanical pesticides (Ignacimuthu S 2004). Phyto-extract of plants is noted to contain phytochemicals which inhibit the growth of phytopathogens and these may be thermolabile in nature. (Parveen et al., 2000).
Antifungal activity of locally available plants (mostly medicinal) has been tried in vitro and in pot culture against *Pythium aphanidermatum* (Bhat and Shukla 2001).

Nascimento et al. 2009 reported *Piper tuberculatum* which is an exotic *Piper* from the Amazon region to show resistance to infection by *F. solani* f. sp. *piperis*, causal agent of *Fusarium* disease in black pepper (*Piper nigrum* L.). They studied the interaction between *P. tuberculatum* and *F. solani* f. sp. *piperis* at a molecular level, using suppression subtractive hybridization to identify genes potentially related to *Fusarium* disease resistance. Comparative sequence analysis confirmed that clones isolated showed a high identity with genes coding for proteins that have a known role in plant defense response mechanisms, such as peroxidase, hydroxyproline-rich glycoprotein and CBL-interacting protein kinase. Their study constitutes the first effort to understand the molecular basis of this plant-pathogen interaction, identifying genes which may be used in the future genetic improvement of black pepper.

Yoon et al., (2010) investigated antifungal and antioomycete activities against various plant pathogens using from the methanol extract of *Prunella vulgaris*. Two polyacetylenic acids were isolated from *P. vulgaris* as active principles and identified as octadeca-9,11,13-triynoic acid and trans-octadec-13-ene-9,11-diynoic acid. These two compounds inhibited the growth of *Magnaporthe oryzae*, *Rhizoctonia solani*, *Phytophthora infestans*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum* f. sp. *raphani*, and *Phytophthora capsici*. In addition, these two compounds and the wettable powder-type formulation of an n-hexane fraction of *P. vulgaris* significantly suppressed the development of rice blast, tomato late blight, wheat leaf rust, and red pepper anthracnose. Their data showed that the extract of *P. vulgaris* and two polyacetylenic
acids possess antifungal and antioomycete activities against a broad spectrum of tested plant pathogens. Yoon et al., (2010) first reported the occurrence of octadeca-9,11,13-triynoic acid and trans-octadec-13-ene-9,11-diynoic acid in P. vulgaris and their efficacy against plant diseases. They suggested that the crude extract containing the two polyacetylenic acids can be used as a natural fungicide for the control of various plant diseases.

Mandal et al., 2009 demonstrated that exogenous application of 200 μM salicylic acid through root feeding and foliar spray could induce resistance against F. oxysporum f. sp. lycopersici in tomato. Endogenous accumulation of free salicylic acid in tomato roots was detected by HPLC and identification was confirmed by LC-MS/MS analysis. The salicylic acid-treated tomato plants challenged with F. oxysporum f. sp. lycopersici exhibited significantly reduced vascular browning and leaf yellowing wilting. The mycelial growth of F. oxysporum f. sp. lycopersici was not significantly affected by salicylic acid. Significant increase in basal level of salicylic acid in noninoculated plants indicated that tomato root system might have the capacity to assimilate and distribute salicylic acid throughout the plant. The results indicated that the induced resistance observed in tomato against F. oxysporum f. sp. lycopersici might be a case of salicylic acid-dependent systemic acquired resistance.

Kavroulakis N et al., (2007) observed that strain Fs-K, an endophytic fungal isolate (Fs-K), identified as a Fusarium solani was able to colonize root tissues and subsequently protect plants against the root pathogen F. oxysporum f.sp. radicis-lycopersici (FORL), and elicit induced systemic resistance against the tomato foliar pathogen Septoria lycopersici. Interestingly, they detected attenuated expression of
certain pathogenesis-related genes, i.e. \textit{PR5} and \textit{PR7} in tomato roots inoculated with strain \textit{Fs-K} compared with non-inoculated plants. The expression pattern of \textit{PR} genes was either not affected or aberrant in leaves. A genetic approach, using mutant tomato plant lines, was used to determine the role of ethylene and jasmonic acid in the plant's response to infection by the soil-borne pathogen \textit{F. oxysporum f.sp. radicis-lycopersici} (FORL), in the presence or absence of isolate \textit{Fs-K}. Mutant tomato lines \textit{Never ripe (Nr)} and \textit{epinastic (epil)}, both impaired in ethylene-mediated plant responses, inoculated with \textit{FORL} are not protected by isolate \textit{Fs-K}, indicating that the ethylene signalling pathway is required for the mode of action used by the endophyte to confer resistance. On the contrary, \textit{defl} mutants, affected in jasmonate biosynthesis, show reduced susceptibility to \textit{FORL}, in the presence \textit{Fs-K}, which suggests that jasmonic acid is not essential for the mediation of biocontrol activity of isolate \textit{Fs-K}.

Tomato wilt, caused by the soilborne fungus \textit{Fusarium oxysporum f. sp. lycopersici}, is reported to be effectively controlled by a foliar spray of validamycin A (\textit{VMA}) or validoxylamine A (\textit{VAA}) (≥10 \textmu gml}^{-1}) (Ishikawa R \textit{et al.}, 2007). However, neither \textit{VMA} nor \textit{VAA} is antifungal \textit{in vitro}. In pot tests, the effect of a foliar application of \textit{VMA} or \textit{VAA} at 100 \textmu gml}^{-1} lasted for 64 days. Plants sprayed with \textit{VMA} or \textit{VAA} accumulated salicylic acid and had elevated expression of the systemic acquired resistance (\textit{SAR}) marker genes \textit{P4 (PR-1)}, \textit{Tag (PR-2)}, and \textit{NP24 (PR-5)}. Foliar spray of \textit{VMA} also controlled late blight and powdery mildew of tomato. The disease control by \textit{VMA} and \textit{VAA} lasted up to 64 days after treatment, was broad spectrum, and induced the expression of \textit{PR} genes, all essential indicators of \textit{SAR}, suggesting that \textit{VMA} and \textit{VAA} are plant activators. The foliar application of plant
activators is a novel control method for soilborne diseases and may provide an economically feasible alternative to soil fumigants such as methyl bromide (Ishikawa R et al., 2007).

Corrales Escobosa et al., (2010) studied the potential role of the phytopathogenic fungus \( F. \ oxysporum \) f. sp. \( lycopersici \) in the translocation of metals and metalloids from soil to plant roots in tomato (\( Lycopersicum \ esculentum \)). Two varieties of tomato (one susceptible and another resistant to infection by \( F. \ oxysporum \) f. sp. \( lycopersici \)) were challenged with the fungus for different periods of time, and several elements (V, Cr, Mn, Co, Cu, Zn, As, Se, Mo, Ag, Cd, Pb) were determined in roots and in soil substrate. Additionally, phenolic plant products were also analyzed for the evaluation of the plant response to biotic stress. In order to obtain representative results for plants cultivated in noncontaminated environments, the infected and control plants were grown in commercial soil with natural, relatively low metal concentrations, partly associated with humic substances. Using such an experimental design, a specific role of the fungus could be observed, while possible effects of plant exposure to elevated concentrations of heavy metals were avoided. In the infected plants of two varieties, the root concentrations of several metals/metalloids were increased compared to control plants; however, the results obtained for elements and for phenolic compounds were significantly different in the two plant varieties. It is proposed that both \( Lycopersicum \ esculentum \) colonization by \( F. \ oxysporum \) f. sp. \( lycopersici \) and the increase of metal bioavailability due to fungus-assisted solubilization of soil humic substances contribute to element traffic from soil to roots in tomato plant (Corrales Escobosa et al., 2010).
Wu HS et al., (2008) evaluated the allelopathic effect of artificially applied cinnamic acid on *Fusarium oxysporum* f.sp. *niveum*. Their results showed that hyphal growth of *F. oxysporum* f.sp. *niveum* was strongly inhibited by cinnamic acid. At the highest concentration of cinnamic acid, the biomass in liquid culture was decreased by 63.3%, while colony diameter, conidial germination on plates, and conidial production in liquid culture were completely inhibited. However, mycotoxin production and activity of phytopathogenic enzymes were greatly stimulated. Mycotoxin yield, pectinase activity, proteinase activity, cellulase activity, and amylase activity were increased by 490, 590, 760, 2006, and 27.0%, respectively. It was concluded that cinnamic acid dramatically stimulated mycotoxin production and activities of hydrolytic enzymes by *FON* but inhibited growth and germination of *F. oxysporum* f.sp. *niveum*. Their findings indicated that cinnamic acid is involved in promoting watermelon *Fusarium* wilt.

Fungal secondary metabolites are chemical compounds identified in a limited number of species. They consist of toxins, antibiotics and antifungal agents. Ait Kettout and Rahmania (2010) could isolate these secondary metabolites from the pathogen grown in a liquid medium, and then identified them by gas chromatography coupled with mass spectrometry (GC-MS), phenylacetic acid has been distinguished. This compound is widely described in the literature as having antimicrobial, antifungal, phytotoxic properties and also endowed with hormonal activity similar to that of indole acetic acid (*IAA*). To date, this metabolite has never been reported in *F. oxysporum* f. sp. *albedinis*, the causal agent of bayoud, the wilt disease of the date palm (*Phoenix dactylifera* L.).
Minerdi et al., (2009) demonstrated that small volatile organic compounds (VOCs) emitted from the wild type (WT) strain negatively influence the mycelial growth of different formae speciales of *F. oxysporum*. Furthermore, these VOCs repress gene expression of two putative virulence genes in *F. oxysporum* f. sp. *lactucae* strain Fuslat10, a fungus against which the WT strain MSA 35 has antagonistic activity. The VOC profile of the WT and CU fungus shows different compositions. Sesquiterpenes, mainly caryophyllene, were present in the headspace only of WT MSA 35. No sesquiterpenes were found in the volatiles of ectosymbiotic *Serratia* sp. strain DM1 and *Achromobacter* sp. strain MM1. Bacterial volatiles had no effects on the growth of the different formae speciales of *F. oxysporum* examined. Hyphae grown with VOC from WT *F. oxysporum* f. sp. *lactucae* strain MSA 35 were hydrophobic whereas those grown without VOCs were not, suggesting a correlation between the presence of volatiles in the atmosphere and the phenotype of the mycelium. Minerdi et al., (2009) first reported that VOC production by antagonistic *F. oxysporum* MSA35 and their effects on pathogenic *F. oxysporum*. The results obtained in their work led us to propose a new potential direct long-distance mechanism for antagonism by *F. oxysporum* MSA 35 mediated by VOCs. Antagonism could be the consequence of both reduction of pathogen mycelial growth and inhibition of pathogen virulence gene expression.

Calcium cyanamide (CaCN₂) has been one of the potential candidates as soil disinfectant since the restriction of methyl bromide in soil fumigation due to its ecological risk (Shi K et al., 2009). However, little information is available on effects of CaCN₂ on soil microbial community. In one study, the soil microbial communities
and the fate of pathogen *F. oxysporum* (Schlechtend, Fr) f. sp. *cucumerinum* (Owen) Snyder and Hansen (*F. oxysporum* f. sp. *cucumerinum*) in response to CaCN₂ treatment was evaluated. *F. oxysporum* f. sp. *cucumerinum* population in soil treated with CaCN₂ at rates of 80 and 200 gm⁻¹ was suppressed by 88.7 and 92.2% after 15 days of CaCN₂ application (Shi K *et al.*, 2009). Bacterial, fungal, and actinomycete populations were also greatly decreased after 3 days of CaCN₂ application, but they recovered to the control level by 15 days. The variation in functional diversity of soil microbes characterized by principal component analysis, diversity and evenness indices followed a similar trend. Meanwhile, the band number from the DGGE of soil 16S rDNA fragments increased from 9 for the non CaCN₂ treated soil to 10 or 12 after different rates of CaCN₂ application at 15 days, indicating the increase of abundant rDNA types in the community. The results suggested that CaCN₂ application had only a short-term and transitory impact on the indigenous soil microbial community in contrast to the long term suppression of the *F. oxysporum* f. sp. *cucumerinum* population. It is feasible to reduce *Fusarium* wilt without significant impact on microbial community by application of CaCN₂ at reasonable doses.

According to the works carried out by Saikia *et al.*, 2009, *Pseudomonas fluorescens* 4-92 (Pf4-92) strain can suppress the *Fusarium* wilt of chickpea. They observed that amendment of zinc EDTA and copper EDTA could not suppress the disease significantly when used alone. However, they significantly suppressed the disease in presence of Pf4-92. *In vitro* observation showed that at 40, 30 and 20μgml⁻¹ concentrations of these minerals, i.e. Zn, Cu and Zn plus Cu, respectively, completely repressed the production of the phytotoxin, fusaric acid (FA). FA concentration (0.5
μgml⁻¹) has been shown to suppress the production of 2,4-diacetylphloroglucinol (DAPG) by Pf4-92, and DAPG, salicylic acid, pyochelin and pyoluteorin production was enhanced by these mineral amendments. In rockwool bioassays, Zn, Cu and Zn plus Cu amendments reduced FA production and enhanced DAPG production. Their study demonstrated that Zn and Cu enhance biocontrol activity by reducing FA produced by the pathogen, *F. oxysporum* f. sp. *ciceri*.

The allelopathic potential of an artificially applied allelochemical, benzoic acid, on *in vitro* *F. oxysporum* f.sp. *niveum* (a soil-borne pathogen causing watermelon wilt) was evaluated by Wu *et al.*, (2009). They observed that benzoic acid strongly inhibited its growth, sporulation and conidia germination, whereas it stimulated virulence factors of this pathogen. The biomass was reduced by 83-96 % and the conidia germinating rate and conidia production rate were decreased by 100 % at a concentration of >200 mg/L. However, phytopathogenic enzyme activities and mycotoxin production were stimulated with an increase of 10.2-1250 % for enzyme activities and 610-2630 % for mycotoxin yield.

The importance of integrating existing control practices, partially effective by themselves, with other control measures to achieve appropriate management of *Fusarium* wilt and increase of seed yield in chickpea in Mediterranean type environments is demonstrated by the results of Landa *et al.*, (2004). A 3 year experiment was conducted by Landa *et al.*, (2004) in field microplots infested with *F. oxysporum* f. sp. *ciceris* race 5 at Cordoba, Spain, in order to assess efficacy of an integrated management strategy for *Fusarium* wilt of chickpea that combined the choice of sowing date, use of partially resistant chickpea genotypes, and seed and soil
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treatments with biocontrol agents *Bacillus megaterium* RGAF 51, *B. subtilis* GB03, nonpathogenic *F. oxysporum* Fo 90105, and *Pseudomonas fluorescens* RG 26. Advancing the sowing date from early spring to winter significantly delayed disease onset, reduced the final disease intensity and increased chickpea seed yield. A significant linear relationship was found between disease development over time and weather variables at the experimental site, with epidemics developing earlier and faster as mean temperature increased and accumulated rainfall decreased. Under conditions highly conducive for *Fusarium* wilt development, the degree of disease control depended primarily on choice of sowing date, and to a lesser extent on level of resistance of chickpea genotypes to *F. oxysporum* f. sp. *ciceris* race 5, and the biocontrol treatments. The main effects of sowing date, partially resistant genotypes, and biocontrol agents were a reduction in the rate of epidemic development over time, a reduction of disease intensity, and an increase in chickpea seedling emergence, respectively. Chickpea seed yield was influenced by all three factors in the study. The increase in chickpea seed yield was the most consistent effect of the biocontrol agents. However, that effect was primarily influenced by sowing date, which also determined disease development. Effectiveness of biocontrol treatments in disease management was lowest in January sowings, which were least favourable for *Fusarium* wilt. Sowing in February, which was moderately favourable for wilt development, resulted in the greatest increase in seed yield by the biocontrol agents. In March sowings, which were most conducive for the disease, the biocontrol agents delayed disease onset and increased seedling emergence. *B. subtilis* GB03 and *P. fluorescens* RG 26, applied either alone or each in combination with nonpathogenic *F. oxysporum* Fo
90105, were the most effective treatments at suppressing *Fusarium* wilt, or delaying disease onset and increasing seed yield, respectively.

Volatile emissions from the leaves of *Lycopersicon esculentum* at the two, ten leaf and anthesis periods were collected by a gas absorbing method and analyzed by gas chromatography (GC) mass spectrometry (Zhang *et al.*, 2008). In total, 33 compounds of volatile emissions from three developmental stage plants were separated and identified, and quantitatively analyzed by the internal standard addition method. All of the samples of volatile were found to be rich in monoterpenes and sesquiterpenes. β-phellandrene and caryophyllene predominated in the volatiles of the leaves of plants at the two and ten leaf stages. Furthermore, β-2-hexenal were the dominant components in the volatiles emitted from anthesis plants. The results of volatile emissions showed that the compositions varied depending on the developmental stages. The volatiles emitted from crushed tomato leaves of plants at the anthesis stage had the most strongly inhibitory activity against the spore germination and hyphal growth of *Botrytis cinerea* and *F. oxysporum*, followed by ten and two leaf plants. However, the activity of volatiles, emitted from the leaves of plants at the two leaf stage, in inhibiting *F. oxysporum* was reported to be greater than *B. cinerea*.

Peroxidase activity towards phenolic substrates, i.e. pyrogallol, syringaldazine and guaiacol, and ascorbate peroxidase activity were analyzed in embryo axes of *Lupinus luteus* L. cv. Polo cultured on Heller medium for 96 hours after inoculation with the necrotrophic fungus *F. oxysporum* f.sp. Schlecht lupini. Four variants were compared: inoculated embryo axes cultured with 60mM sucrose (+Si) or without it (-
Si), and non-inoculated embryo axes cultured with 60mM sucrose (+Sn) or without it (-Sn). Between 0 and 96 hours of culture, peroxidase activity towards the phenolic substrates increased in all variants except -Si, where a decrease was noted in peroxidase activity towards syringaldazine and guaiacol, but not towards pyrogallol. In +Si tissues, a considerable increase in enzyme activity towards these substrates was recorded starting from 72 hours of culture. Lignin content of +Si tissues increased already at the first stage of infection, i.e. 24 hours after inoculation. Additionally, in +Sn tissues, high ascorbate peroxidase activity was observed during the culture. Its activity increased in +Si tissues, beginning at 72 hours after inoculation. However, this was lower than in +Sn tissues. At 72 hours after inoculation, a considerably stronger development of the infection was observed in -Si than in +Si tissues during their earlier research (Morkunas I et al., 2005). Both peroxidases assayed towards phenolic substrates and ascorbate peroxidase was less active in -Si tissues than in -Sn tissues. Hydrogen peroxide concentration was much higher in -Si than in +Si tissues. Their results indicated that peroxidases may be some of the elements of the defense system that are stimulated by sucrose in yellow lupine embryo axes in response to infection caused by *F. oxysporum*.