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Current Status of *Fusarium* Wilt Disease of Guava (*Psidium guajava* L.) in India

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Abstract: Guava (*Psidium guajava* L.) is an important fruit crop of subtropical countries. It is grown almost in all the states of India. It is a hardy crop and is cultivated successfully even in neglected soils and is attacked by a large number of pathogens, mainly fungi. Wilt is the most destructive disease for guava plant in India and losses due to this disease are substantial. *Psidium guajava* wilt is known to occur from India, Latin America, Malaysia, Pakistan, South Africa, South Asia and Taiwan. Wilt of guava from India was first reported in 1935 from Allahabad. The disease is soil-borne and is difficult to control. Wilt is predominantly caused by the species of *Fusarium*, of which *F. oxysporum* is generally the main cause. The other species of *Fusarium* i.e., *Fusarium solani* are also dominates in isolation. Since, the disease results in the complete mortality of the affected plants, the loss is total. Although, severe loss is there in the annual crops also, huge monitory losses occur especially in perennial fruit trees as it is a loss of labour of several years. Guava is a crop where this disease is very serious and it can be said that this is the only disease of guava which is threatening guava cultivation in India. It causes monitory as well as nutritional loss. The present communication, deals guava wilt to depict its present status.

Key words: *Psidium guajava* L., wilt, *Fusarium oxysporum* f. sp. *psidii.*, *Fusarium solani*

INTRODUCTION

Das Gupta and Rai (1947) recorded the disease in the severe form the orchards of Lucknow for the first time India. Dey (1948) reported it from Allahabad, Kanpur and Lucknow. During 1949-50, guava trees suffered serious losses in 11 districts of UP (Anonymous, 1949, 1950). Prasad *et al*. (1952) estimated that guava wilt spread rapidly to cover about 20,000 m^2 area in UP. Mathur (1956) reported upto 30% trees affected with wilt in Allahabad, Farrukhabad, Unnao (15-30%), Kanpur and Jaunpur (5-15%) and less than 5% in Gorakhpur, Ballia, Hardoi, Barabanki and Varanasi. Edward and Srivastava (1957) reported wilt as the most serious disease threatening guava cultivation in UP. Later, it was also reported from western parts of UP (Singh and Lal, 1953), Varanasi (Pandey and Dwivedi, 1985), Kaimganj (Farrukhabad), Bithoor (Kanpur), Ganga Ghat (Unnao), Abbubakarpur (Allahabad), Lucknow, Bichpuri (Agra), Sasni (Aligarh) (Misra and Prakash, 1990; Misra, 1987). After the first few reports from U.P., in West Bengal the disease spread in the Gangetic alluvium of Baruipur area in the district of 24 Parganas and in the laterite zone of Jhargram and Midnapur (Chattopadhyay and Sengupta, 1955). Chattopadhyay and Bhattacharjya (1968a, b) reported the disease from Kashakul, Bankura. The disease was also been reported from Haryana (Suhag, 1976; Mehta, 1987), Punjab (Mohan *et al*., 1986), Rajasthan (Katyal, 1972; Bhargava *et al*., 2003), Delhi state (Anonymous, 1953), Jharkhand (Srivastava *et al*., 2001), Andhra Pradesh (Jhooty *et al*., 1984), M.P. in the Hatod area near Indore and Kuthulia farm in Rewa, Faizabad and Darwar district (Gupta *et al*., 2009d; Gupta, 2010), Orissa (Das Gupta and Ghoshal, 1977), Thanjavur district of Tamilnadu (Fig. 1, 2).

Wilted guava plants has also been reported from Florida, USA (Webber, 1928), Taiwan (Hsieh *et al*., 1976; Leu and Kao, 1979), Cuba (Rodriguez and Landa, 1977), South Africa (Grech, 1985; Vos *et al*., 1998), Brazil (Tokeshi *et al*., 1980; Rodriguez *et al*., 1987; Junqueira *et al*., 2001), Pakistan (Ansar *et al*., 1994), Bangladesh (Hamiduzzaman *et al*., 1997) and Canberra, Australia (Lim and Manicom, 2003) (Fig. 1).

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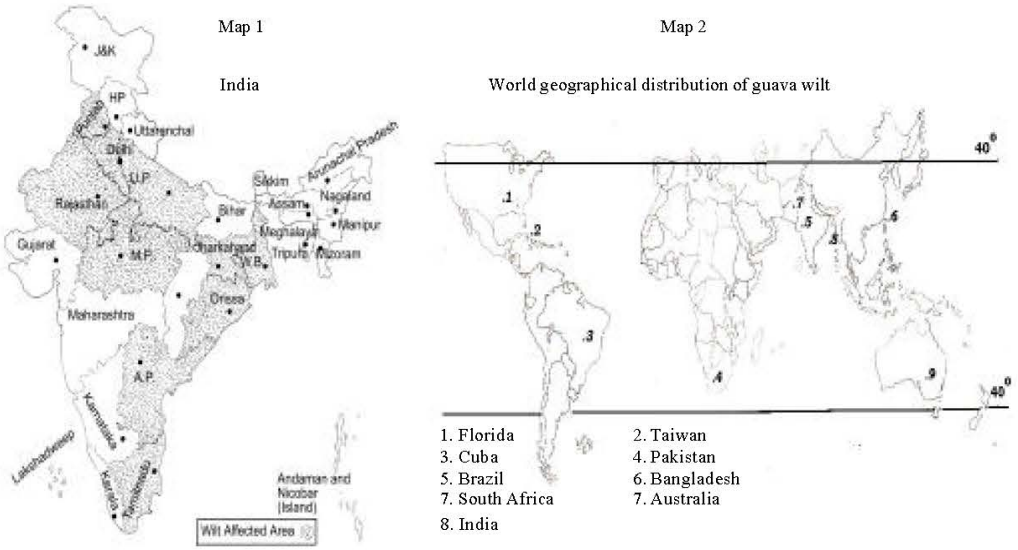

Fig. 1: Present status of guava wilt

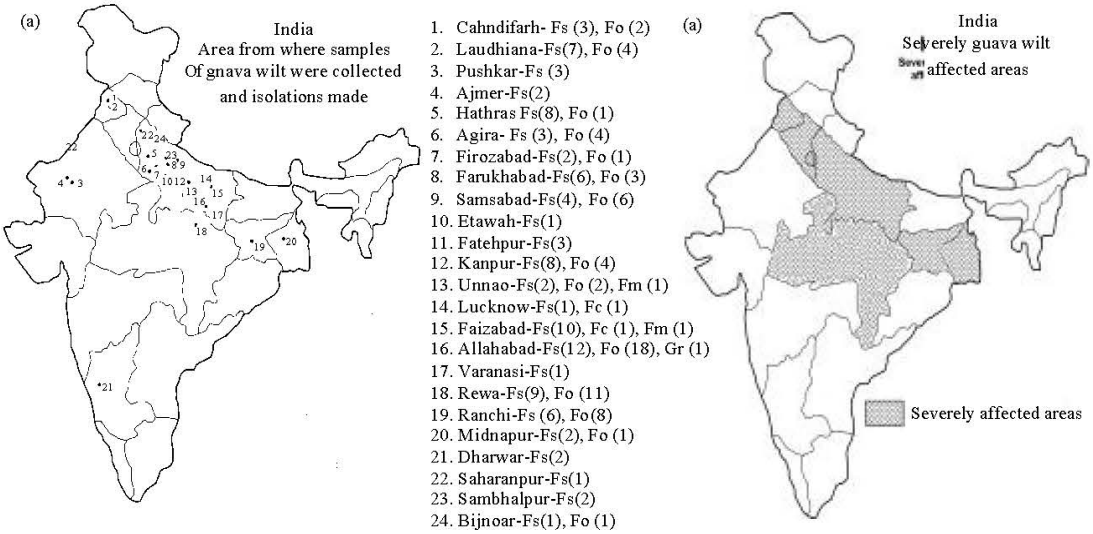

Fig. 2: Sample collection and localization of severely wilt affected area on Indian Map (Gupta *et al*., 2009b) (a) Guava wilt samples were collected from 24 locations and (b) severely affected areas by the diseases

LOSSES

Singh and Lal (1953) estimated 5-15% loss amounting to almost 1 million rupees due to guava wilt every year in 12 districts of U.P. In West Bengal, the disease reduced the yield by 80% i.e., from 113.5 q ha^{-1} in healthy plantations to about 18.16-22.7 q ha^{-1} in affected orchards (Chattopadhyay and Sengupta, 1955). Chattopadhyay and Bhattacharjya (1968a, b) attempted in vein to regenerate the affected trees. The new seedlings, grafted or planted in the affected areas showed stunted growth, flowered rarely and succumbed to wilt within a very short ctime. Seven thousand acres of land in Andhra Pradesh, reduced the land value to half by the presence of the disease (Jhooty *et al*., 1984). About 150 and 300 acres of guava orchards in Punjab and Haryana respectively were uprooted during 1978-81 (Jhooty *et al*., 1984). In general, losses due to wilt in guava around Lucknow area vary from 5-60% (Misra and Shukla, 2002).

SYMPTOMLOGY

The affected plants show yellow coloration with slight leaf curling at the terminal branches, becoming reddish at the later stage and subsequently shedding of leaves take place. Twigs become bare and fail to bring forth new leaves or flowers and eventually dry up. Fruits of all the affected branches remain underdeveloped, become hard, black and stony. The entire plant becomes defoliated and eventually dies. It requires almost sixteen days for complete wilting. Some trees affected linger on even up to 252 days and then die (Misra and Pandey, 2000b). Misra and Pandey (2000b) also studied variations in the symptoms during different time of the year. They noticed yellowing of the leaves with inter-venial chlorosis during the month of August, which drop even with the slight shaking of the plants. During September, general drooping of the leaves takes place. During October complete wilting of plants are seen with almost dried leaves and small dried black fruits hanging on the branch. Few plants also show partial wilting, which is a very common symptom of wilt in guava. It is also recorded that some plants show wilting of variable degree (leaf yellowing, drooping of leaves, drying of terminal branches or partial wilting) during different months but later escape/resist wilting. These plants start recovering from December onward. It was recorded that out of total wilting plants, around 17% plants, which initially show some symptoms of wilting, ultimately escape/resist wilting (Neeraj *et al*., 2005; Gupta, 2010).

The finer roots show black streaks, which become prominent on removing the bark (Das Gupta and Rai, 1947). The roots also show rotting at the basal region and the bark is easily detachable from the cortex. The cortical regions of the stem and root show distinct discoloration and damage. Light brown discoloration is noticed in vascular tissues (Chattopadhyay and Bhattacharjya, 1968a, b). Wilted plants later show bark splitting. The pathogen attacks young as well as old fruit bearing trees but older trees are more prone to the disease (Misra and Shukla, 2002). New seedlings and grafts also show disease symptoms (Singh and Lal, 1953; Edward, 1960a). Chakraborty and Singh (1989) identified mainly two types of symptoms i.e., slow wilt (where plant takes several months or even a year or two to wilt after the appearance of initial symptoms) and sudden wilt (where plant takes 15 days to one month to wilt after the appearance of initial symptoms).

CAUSAL ORGANISM

Several plant pathologists have reported that *Fusarium oxysporum* f. sp. *psidii* and *F. solani* are the important causal organism of guava wilt. It have been reported by different workers (Das Gupta and Rai, 1947; Dey, 1948; Prasad *et al*., 1952; Edward and Srivastava, 1957). Edward (1960b, c) explained that *Fusarium oxysporum* f. sp. *psidii* penetrate either directly through the root piliferous layer of the guava seedlings or through openings caused by secondary roots. Hyphae were found in the xylem vessels of the roots of the inoculated plants. He also observed that *Fusarium oxysporum* f. sp. *psidii* existed in a variety of forms, which differ in cultural and morphological characters.

In West Bengal, both *Macrophomina phaseoli* and *F. solani* were reported to incite wilt either individually or in combination. In either case, the fungus first colonizes the surface of roots and then enters in to its epidermal cells. Thereafter, intercellular mycelium establishes first in epidermal cells and then spreads into cortical cells, which get damaged considerably and filled up with the mycelium. *Fusarium solani* enters the xylem vessels, grows inside and blocks them. *Macrophomina phaseoli* first invades the phloem and destroys it. The xylem vessels are also attacked in a few cases (Chattopadhyay and Sengupta, 1955; Chattopadhyay and Bhattacharjya, 1968a, b). Histopathological observations made by various workers in naturally wilted and artificially inoculated plants revealed the presence of *F. solani*, *F. oxysporum* f. sp. *psidii* and *M. phaseolina* in vascular tissues (Edward, 1960c; Chattopadhyay and Bhattacharjya, 1968a, b; Mohan, 1985; Pandey and Dwivedi, 1985). *Gliocladium vermoesenii* Corda, a known saprophytic fungus, is also found associated with diseased plants (Mohan, 1985). From Varanasi, *F. oxysporum* f. sp. *psidii*, *F. solani*, *F. coeruleum*, *F. moniliforme* and *Rhizoctonia solani* were also found on rhizoplane and rhizosphere of guava (Dwivedi, 1991; Dwivedi and Dwivedi, 1999). In *in vitro* studies Misra and Pandey (1992) found *Cylindrocarpon lucidium*, *Gliocladium virens* and *Bartilinia robillardoides* (which were isolated from wilted plants) to cause drooping and subsequent wilting of guava seedlings grown in Hoagland's solution in artificial testing. The recent studies at Central Institute for Subtropical Horticulture, Lucknow by Misra and Pandey (1997, 2000a,b) revealed *Gliocladium roseum* has been the pathogen of guava wilt, which reproduces symptom of wilt on artificial inoculation. They succeeded in reproducing wilt by artificial inoculation in grown up guava plants of 6-7 years by *Gliocladium roseum* by stem cut end wound hole inoculation technique. They earlier also reported *Gliocladium virens*, *G. penicilloides*, *Fusarium oxysporum*, *F. solani* and *Acremonium* sp. to be associated with guava wilt symptoms indicating it's complex nature (Misra and Pandey, 1999a, c). They also

standardized the inoculation technique, i.e., stem hole (stem cut end wound hole) inoculation technique for reproducing wilt symptoms quickly. Compared to soil inoculation and root inoculation techniques their stem inoculation method was superior producing quick wilting symptoms within a month. The former methods produced wilting between 3 to 6 months. Pandit and Samajpati (2002) reported wilt to be caused by *Botyodiplodia theobromae* in Midnapur (W.B.), while Gupta *et al.* (2003) reported association of *Verticillium albo-atrum* with guava wilt from Allahabad. Misra *et al.* (2004) recorded wilting of air layers due to *Gliocladium roseum* in association with *Sclerotium rolfsii* and *Rhizoctonia bataticola.* Khan *et al.* (2001) indicated the role of nematodes as co-factor in guava wilt. They found *Helicotylenchus dihystera* in terms of population frequency and density to be the dominant species in wilted guava plants.

The reports from other parts of the world are different. Webber (1928) reported that *Clitocybe tabescens* killed guava trees in Florida (USA). In Cuba three nematodes viz., *Meloidogyne* sp., *Helicotylenchus* sp. and *Pratylenchus* sp. have been found associated (Rodriguez and Landa, 1977) with guava wilt. In Taiwan, the disease is reported to be caused by *Myxosporium psidii* Corda (Hsieh *et al.*, 1976; Leu *et al.*, 1979; Schroers *et al.*, 2005). Tokeshi *et al.* (1980) isolated *Pseudomonas* sp. from wilt-affected plants. Disease similar to wilt caused by *Erwinia psidii* was also observed at Sao Paulo (Brazil) in 1982 (Rodrigues *et al.*, 1987). In South Africa, *Septofusidium* sp. was found associated with the rapid death of guava plants (Grech, 1985). From Pakistan (Punjab) disease is reported in the name of decline and *Fusarium oxysporum* and *Colletotrichum gloeosporiodes* (*Glomerella cingulata*) are considered associated with the disease and are supposed to act synergistically when present together (Ansar *et al.*, 1994). Hamiduzzaman *et al.* (1997) reported from Bangladesh that wilt incidence was maximum when seedlings were inoculated by *F. oxysporum* f. sp. *psidii* along with the nematodes *H. dihystera* and *H. indicus*. From South Africa, Vos *et al.* (1998) reported Guava Wilt Disease (GWD) caused by *Penicillium vermoensenii*. Recently, Lim and Manicom (2003) from Australia reported wilt of guava by *Fusarium oxysporum* f. sp. *psidii.*

Although several pathogens have been reported for the cause of wilt in guava by different workers but *Fusarium oxysporum* f. sp. *psidii* and *Fusarium solani* were found to most important pathogen associated with this disease (Misra, 2006; Misra and Gupta, 2007; Gupta, 2010). Therefore, in present review the wilt disease of guava has been described in detailed with special reference to *Fusarium* sp. infection in India.

***Fusarium* complex:** The genus *Fusarium* contains a number of soilborne species with world wide distribution and known to be important plant pathogens (Nelson, 1991). The anamorph *Fusarium* species belongs to the order *Moniliales* and is placed in the family *Tuberculariaceae*; they include some of the world's most destructive plant pathogens and differ from other fungal groups in many genetic characters. Although most of members of the *Fusarium* species-complex are anamorphs, some produce telemorphic stages, as for example, each of the *Gibberella fujikuroi* and *Necria haematococca* species complexes, once considered by Snyder and Hansen (1940) to be represented by *F. moniliforme* and *F. solani*, respectively, are now known to barbor over 40 phylogenetically distinct species with a bypathesized Gondawanic biogeographical history. All strains of *Fusarium* exist saprophytically, but some are well known for inducing wilt (O'Donnell *et al.*, 2000; Jurgenson *et al.*, 2002; Schroer *et al.*, 2005).

***Fusarium oxysporum*:** *Fusarium oxysporum* is a causative agent of wilt disease in a wide range of economically important crops (Booth, 1984). *Fusarium oxysporum* Schlechtend. Fr. is an anamorphic species circumscribed by different morphological criteria: principally the shape and size of the macroconidium, the presence or absence of microconidia and chlamydospores, colony colour and conidiophore structure (Nelson *et al.*, 1981; Windels, 1992). The difficulty in delineating species based on these features is evidenced by the different systems that have been proposed, recognizing anywhere from 30 to 101 species (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. To further complicate the picture, plant pathogenic, saprophytic and biocontrol strains of *F. oxysporum* are morphologically indistinguishable. *Fusarium oxysporum* is more commonly isolated fungi from asymptomatic roots of crop plants (Gordon and Martyn, 1997). *F. oxysporum* has a well-documented ability to persist without recourse to pathogenesis. Both pathogenic and non-pathogenic strains of *F. oxysporum* are found in agricultural soils throughout the world and have received a lot of attention from researchers. However, substantial populations of *F. oxysporum* are also found in many native plant communities, in soils that have never been cultivated (Gordon and Martyn, 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. This group of cosmopolitan, soil borne

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 *Fusarium oxysporum* include wilting, yellowing and xylem discoloration.

Forma specials with concern to *psidii*: The forma speciales concept was introduced by Snyder and Hansen (1940). This designation was intended 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. It has been useful to plant pathologists because it identifies a subset of isolates that are of concern to the production of a crop susceptible to *Fusarium* wilt. This purpose is served, regardless of whether a forma speciales corresponds to a natural grouping of related strains. Often the same forma speciales has a polyfiletic origin showing genetic heterogeneity (Koenig *et al*., 1997; O'Donnell *et al*., 1998). Pathogenic *F. oxysporum* is very host specific attacking 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 and for cultivars of that host is designated, respectively, as forma speciales. These pathogenic fungi are morphologically indistinguishable from each other as well as from non-pathogens. Forma speciales is determined by testing the fungus for pathogenicity on various plant species (Booth, 1971; Di Pietro *et al*., 2003; Fravel *et al*., 2003). Forma speciales of *Fusarium oxysporum* causing wilt disease in *Psidium guajava* L. is designated as *Fusarium oxysporum* f. sp. *psidii* (Prasad *et al*., 1952; Gupta, 2010).

***Fusarium solani* (Mart.) Sacc.:** The morphological species *Fusarium solani* is also one of the most common species of *Fusarium* found ubiquitously in soil and plant debris. The sexual stage (teleomorph) of *F. solani* (anamorph) is usually known as *Nectria haematococca* Berk. and Br.. *Fusarium solani* can be identified morphologically by the presence of chlamydospores, long unbranched monophialids, predominantly cream mycelia that can vary in pigmentation and the shape and size of microconidia and macroconidia (Gerlach and Nirenberg, 1982).

The 10 Formae speciales of *F. solani* to date are phaseoli, pisi, cucurbitae, batatas, radicicola, robiniae, mori, piperis, eumartii and xanthoxyli (Snyder and Hansen, 1941; Suga *et al*., 2000). Booth (1971) recognized 4 species within section Martiella while Gerlach and Nirenberg (1982) recognized 6 species and there is absence of forma speciales in case of guava wilt isolates of *Fusarium solani* (Chattopadhyay and Sengupta, 1955; Booth, 1971; Nelson *et al*., 1981; Misra and Gupta, 2007).

EPIDEMIOLOGY

Mehta (1951) reported severe incidence of wilt in alkaline soils at pH ranging from 7.5 to 9.0, while Sen and Verma (1954) reported high disease incidence in lateritic soils at pH 6.5. A soil saturation of 60-80% has been reported optimum for disease development in West Bengal. A pH 6.0 has been reported optimum for the development of the disease. Both pH 4.0 and 8.0 reduces the disease. Low incidence of the disease has been reported at 630 ppm N and is more both at higher as well as at lower levels of nitrogen. Moderate to high concentrations of phosphates (207-345 ppm) are effective in reducing the disease (Chattopadhyay and Bhattachrjya, 1968b). Mehta (1987) reported more disease in clay loam and sandy loam compared to heavy soil types.

Guava seedlings are more susceptible to *F. solani* as well as *F. oxysporum* f.sp. *psidii* than older plants of 3 years age. On the other hand researchers reported that *F. solani* could infect guava plants from 1-month-old to more than 4 years old. According to Misra and Shukla (2002) above five-year-old guava plants were more susceptible to the disease. A common practice followed in West Bengal that the growers harvest the fruits upto a 6-year-old plants. They then discard the old plants, take new plantation and their escape the wilt incidence (Misra, 2006).

Infected guava plants start showing sign of wilting right with the onset of rainy season in August with maximum number dying in September and October (Das Gupta and Rai, 1947; Edward, 1960a; Suhag, 1976).

Dwivedi (1990) at Varanasi also found more pathogenic fungi during rainy season. The fungi survived better in association with root bits in adverse climatic conditions in the summer months, while in rainy and winter months they survive on roots. Extensive studies on the progress of natural wilting of guava plants during different months have been made by Misra and Pandey (1999a, b, 2000a) at Lucknow. They found maximum wilting during October. Some plants, which show slight yellowing, started recovering from December onwards. On analyzing the weather data, they found higher rainfall during July-September with maximum temperature ranging from 31.3 to 33.5°C and minimum temperature ranging from 23 to 25°C and humidity of 76%. They further found that generally two-four months are required for the complete wilting of plants after infestation of fungi (Gupta, 2010).

PATHOGENICITY

Das Gupta and Rai (1947) also reported that wilt starts in the month of August with the largest number of plant dying during September and October. Later, Edward (1960b) observed that the disease appears in the beginning of June but the intensity of infection and spread accelerates with the onset of rains. Suhag (1976) indicated that soon after rainy season, guava plants die in 3-4 weeks and some plants take 6-8 month period for complete wilting. Pathogenicity testing in guava using stem cut end wound hole technique has been reported by Misra and Pandey (2000a). It requires almost sixteen days for initiation of wilting in guava. Some trees affected longer on even up to 252 days and then die (Misra, 2006). Misra and Pandey (2000b) also studied variations in the symptoms during different time of the year. They noticed yellowing of the leaves with inter-venial chlorosis during the month of August, which drop even with the slight shaking of the plants. During September, general drooping of the leaves takes place. During October complete wilting of plants are seen with almost dried leaves and small dried black fruits hanging on the branch. Few plants also show partial wilting, which is a very common symptom of wilt in guava. In a recent study made by Gupta (2010) it was observed that among 89 *Fusarium* sp. isolates of guava wilt pathogens viz. *F. oxysporium* f. sp. *psidii* anf *Fusarium solani*, tested for pathogenicity. In the present investigation it was found that during experiment wilting symptoms starts from the month of August. During September-October fast wilting was recorded. Maximun wilting was recorded during the month of October. In the present investigation further quantification was done and October was identified as the most favorable month for wilt incidence. Das Gupta and Rai (1947) investigated that wilting was observed during rainy season.

Stem cut end wound hole technique as described by Misra and Pandey (2000a) was proved to be a good technique for reproduction of guava wilt. Studies of Misra and Pandey (2000b) supports our findings which showed that when healthy guava plants were inoculated with *F. oxysporium* f. sp. *psidii* anf *Fusarium solani* isolates, wilting starts from August which increases during September-October. Maximum wilting takes place during the month of October and maximum recovery of plants were seen during January and April with a total of 76% wilting during their experiment. Maximum time taken for complete wilting was 240 days in the study of Gupta (2010).

DISEASE MANAGEMENT

Time to time recommendations for the control of guava wilt has been suggested by different workers. These are summarized below:

Disease management through chemicals: During 1949, control of wilt was suggested with Chaubatia paste (Anonymous, 1949) but this control measure is not considered valid, as guava wilt is a soil borne disease. Jain (1956) found chemotherapeutic action of 0.1% water-soluble 8-Quinolinol sulphate against the wilt pathogen (*Fusarium oxysporum* f. sp. *psidii*). It's Injection in apparently healthy guava plants in a diseased area provided protection against wilt at least for one year and when injected into slightly wilted plants, it was beneficial for their partial recovery. Suhag (1976) reported control of wilt by severe pruning and then drenching with 0.2% either Benlate or Bavistin 4 times in a year and spraying twice with Metasystox and Zinc sulphate. But due to soil borne nature of the disease, pruning does not seem to control wilt. Misra and Pandey (1999b) reported that though different fungicides viz. bavistin, topsin M, indofil M-45, thiram, blitox check the various wilt pathogens in laboratory effectively but these pathogens increases it's aggressiveness with profuse spore mass production in the soil, once the effect of these fungicides diminishes in soil. Bhargava *et al.* (2003) also found control with thiophanate methyle in lab. In Taiwan, Carbendazim, Captafol and Thiabendazole proved effective against wilt pathogen under laboratory experiments but failed *in vivo* (Leu *et al.*, 1979). In South Africa tebuconazole, propiconazole, prochoraz, triforine and carbendazim + flusilazole were effective *in vitro* evaluation (Joubert and Frean, 1993). Antibiotic actidion (Dwivedi, 1990) and heavy metals Hg, Cd and Cu (Dwivedi, 1991) were found effective for control of wilt. Nematodes are reported to aggravate the wilt incidence in guava. Disinfection of soil with DBCP at 52.8 mL/10 m^2 or Metham sodium at 252.5 mL/10 m^2 was achieved to control nematodes (Rodriguez and Landa, 1977).

Besides fungicides some soil amendment chemicals/cakes/fertilizers were also evaluated for control of wilt. Mathur *et al.* (1964) and Mathur and Jain (1960) found wilt control by soil treatment with 1.82 kg lime or gypsum/tree, although the control mechanism was not well understood. At CISH, Lucknow also wilt was controlled by application of 6 kg. neem cake + 2 kg. gypsum per plant (Misra and Pandey, 1994). Oil cakes like neem cake, mahua cake, kusum cake supplemented with urea at 10 and 1 kg, respectively also check the disease (Das Gupta and Ghoshal, 1977). Suhag and

Khera (1986) advocated that spread of wilt could be checked by judicious amendments of N and Zn.

Disease management through cultural practices: Mathur (1956) advocated that wilt could be controlled by proper sanitation in the orchard. Wilted trees should be uprooted, burnt and trench should be dug around the tree trunk. Edward (1960a) suggested that while transplanting, roots of plants should not be severely damaged. Maintenance of proper tree vigour by timely and adequately manuring, inter-culture and irrigation enable them to withstand infection. The pits may be treated with formalin and kept covered for about 3 days and then transplanting should be done after two weeks. Symptoms of the disease do not appear under green manuring and the disease development is less when organic sources of nitrogen are used (Chattopadhyay and Bhattacharjya, 1968b). Soil solarization with 30fm transparent polyethylene sheet during May-June (Dwivedi, 1993) have been suggested for the control of wilt pathogens. Prasad *et al.* (2003) and Misra *et al.* (2004) reported intercropping with turmeric or Marigold to check the wilting of guava. These cultural practices are useful and should be adopted to escape wilt.

Disease management through varietal resistance: None of the guava varieties in India is reported free from wilt incidence and hence these cannot be recommended directly for cultivation in wilt infested areas. No information is available in literature regarding breeding varieties for wilt resistance except the information on relative resistance on the natural incidence, which is provided under heading varietal reaction. To combat the disease, option of resistant rootstock seems to be of great use and some work was done on this aspect and is summarized here. Since interspecies and intergeneric graft compatibility is possible, Edward (1961) suggested guava species *Psidium cattleianum* var. *lucidium* and *Syzigium cuminii* (Jamun), which seldom get attacked with wilt, may be an effective way for the control of wilt disease. Edward and Gaurishanker (1964) in their further studies found *Psidium cattleianum* (*Psidium molle*), *P. quianense*, Chinese guava (*P. friedrichsthalianum*) and Philippine guava compatible and suggested them for the use of rootstock. A local variety Pei-pa In Taiwan was reported resistant and *Psidium friedrichsthlianum* has been recommended as possible rootstock (Leu and Kao, 1979). Misra *et al.* (2003b) identified F1 population of *Psidium molle* X *Psidium guajava* free from wilt, when grown in wilt sick plot and artificially inoculated repeatedly with *Gliocladium roseum*, *Fusarium solani* and *Fusarium oxysporum*. As graft compatibility is very successful, this resistant rootstock is very useful for the control of wilt.

Integrated eco-friendly approach: Considering the complexity of the problem integrated eco-friendly approach for the control of guava wilt was suggested by Misra *et al.* (2003b, 2004) and Misra (2005) utilizing bioagent *Aspergillus niger* strain AN17, using resistant root stock (*P. molle x P. guajava*), intercropping of guava with turmeric or Marigold as well proper cultural practices and can be integrated to minimize losses due to the disease.

Management of *Fusarium* wilt is not possible when we go for a single control measure. So, an integrated approach based on biocontrol agents, plant products and cultural methods are being adopted for the successful management of *Fusarium* wilt.

Disease management through botanicals: The indiscriminate and inappropriate uses of synthetic fungicides lead to the development of resistance in pathogens, environmental pollution and food contamination by toxic residues. An alternative approach in disease management is therefore, the use of botanical fungicides. Plants are the reservoirs of biodegradable secondary metabolites that are reported to inhibit various phytopathogenic fungi. Botanical fungicides developed from plant products are eco-friendly and found to control many plant diseases (Oros and Ujvary, 1999; Mamatha and Rai, 2004). Patel and Vala (2004) studied that extract of garlic produced maximum inhibition of *F. solani in vitro* condition. Mamatha and Rai (2004) reported antifungal activity in leaf extract of *Lantana* and *Azadirachta indica A. Juss.* against *Fusarium solani*, which causes leaf blight of *Terminalia catappa*. Bhatnagar *et al.* (2004) also tested 17 plant species including *Azadirachta indica* A. Juss., *Curcuma longa* L., *Osimum sanctum* along with Dathura and Isabgol against wilt of Cumin caused by *Fusarium oxysporum* f.sp. *cumini.* and resulted that extract of Dathura and Isabgol were more effective fallowed by *Azadirachta indica* A. Juss., *Ocimum sanctum* and *Curcuma longa* L. 23 plant extract were tested *in vitro* for their biocontrol efficacy to inhibit the growth of *Fusarium oxysporum* f. sp. *psidii* and *Fusarium solani* isolates. Percent of inhibition of fungal growth was calculated as compared to growth in control. Highest percentage of inhibition for all the five isolates of *Fusarium oxysporum* f. sp. *psidii* was achieved by extracts from *Achyranthes roses*, *Curcuma longa* L. and *Calotropis gigantea* L. R.Br. and *Tagetes erecta* L. It was also found that F24 and F30 isolate were comparatively more aggressive among all the isolates of *Fusarium oxysporum* f. sp. *psidii.* Leaf extract of *Calotropis gigantea* L. R.Br and *Cannabis sativa* L. showed comparatively maximum inhibitory effect to *Fusarium solani* (Gupta *et al.*, 2007).

Disease management through bioagents: Biocontrol is the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than inoculum. It is the use of natural or modified organisms, genes or gene products to reduce the effects of pests and diseases (Cook and Baker, 1983).

Being the soil borne nature of wilt pathogen, it is unpractical to control it with any chemical. The effects of chemicals are also hazardous for the soil and environment, moreover when the effect of chemicals diminishes, the pathogen become more virulent and aggressive (Misra and Pandey, 1999b). Hence, considering the above facts, it was considered more desirable to use the bio-agents for the control the wilt disease.

Dwivedi (1992) from Varanasi advocated *Trichoderma* sp. and *Streptomyces chibaensis* for the control of wilt. Seed oil of *Foeniculum vulgare* were also reported to control wilt (Dwivedi, 1993). In Pakistan combined use of Topsin M sprays and the antagonists *Trichoderma harzianum* and *Arachniotus* sp. added in soil amended with wheat straw controlled decline of guava (Ansar *et al*., 1994). Logani *et al.* (2002) reported application of wiltnema (seven plant extract - *Allium cepa*, *Allium sativum*, *Ocimum sanctum*, *Azadirachata indica*, *Datura stramonium*, *Cannabis sativa* and *Nicotiana tabacum*) for prevention of wilt and better growth of guava plants. Srivastava *et al.* (2001) found that by use of VAM symbiont at the rate of 5 kg tree^{-1} is beneficial for the control of wilt.

Bioagents like *Aspergillus niger*, *Trichoderma* sp. *Penicillium citrinum* and some bio-dynamic antagonists have shown their effectiveness towards the control of wilt pathogens of guava (Singh *et al*., 2003; Misra, 2006; Gupta *et al*., 2009a). When these fungi were tested for the control of wilt pathogen in laboratory conditions, these were found quite effective (Misra *et al*., 2004). When relative growth of the three bioagents was studied, it was found that *Aspergillus niger* was fastest growing and most effective (Misra and Prasad, 2003). These can be grown easily on any substrate like maize/bajra seeds etc. and can also be multiplied on cheap substrates like *Sacchrum* sp. (grass) and dry and green leaves of *Psidium guajava* (Shukla *et al*., 2003). It was also found that at village level these bioagent can be multiplied in earthen pots (Misra and Prasad, 2004). Among these, *Aspergillus niger* was found very fast growing, easy to propagate and most effective in controlling the wilt disease in field. Besides this quality, it is also growth enhancer and the plants treated with *Aspergillus niger* developed faster with more height, more thickness and more numbers of leaves (Misra *et al*., 2000). Dwivedi and Shukla (2002) reported that out of three bioagents *Trichoderma harzianum*, *T. viride* and *Gliocladium virens*, *T. viride* is best for the control of wilt. Singh *et al.* (2003) reported bioagent *Aspergillus niger* most effective in controlling the wilt disease followed by *Trichoderma viride*.

Isolates of bioagents, comprising *Aspergillus niger*, *Trichoderma* sp. (*T. virens, T. harzianum* and *T. viride*) and *Penicillium* citrinum, their culture filtrates and volatile compounds were evaluated against isolates of *F. oxysporum* f sp. *psidii* and *F. solani* (Gupta and Misra, 2009; Misra and Gupta, 2009). All isolates of bioagents significantly checked the growth of *F. oxysporum* f. sp. *psidii* and *F. solani.* Per cent inhibition was maximum by direct use of *Trichoderma* sp. in dual culture *A. niger* expressed moderate efficacy against *F. oxysporum* f. sp. *psidii* and *F. solani.* However, *P. citrinum* isolate showed lesser effect. They also observed that in overall, *T. virens* and *T. viride* were superior in inhibiting the growth of both *Fusarium* sp. and *Trichoderma* and *Aspergillus* isolates were evaluated under field conditions for the management of guava wilt disease. These bioagents were effective in complete suppression of wilt incidence.

HISTOPATHOLOGY

All fungi most often interact in order to survive in their habitat. This interaction can be achieved by the development of different mechanisms, which could be direct or indirect, including: neutral, commensalisms, mutualism, competition, parasitism and synergism. The organisms and the physico-chemical conditions present in an ecological niche will delimit the type of interactions that can be observed.

In West Bengal, *F. solani* was reported to incite wilt. It was reported that *Fusarium solani* enters the xylem vessels, grows inside and blocks them (Chattopadhyay and Bhattacharjya, 1968a, b; Chattopadhyay and Sengupta, 1955). *F. oxysporum* f. sp. *psidii* penetrate either directly through the root piliferous layer of the guava seedlings or through openings caused by secondary roots. Hyphae are found in the xylem vessels of the roots of the inoculated plants (Edward, 1960b, c). Histopathological observations made by various workers in naturally wilted and artificially inoculated plants revealed the presence of *F. solani* and *F. oxysporum* in vascular tissues (Chattopadhyay and Bhattacharjya, 1968a, b; Edward, 1960a-c; Mohan, 1985; Pandey and Dwivedi, 1985). *Gliocladium vermoesenii* Corda., a known saprophytic fungus, is also found associated with diseased plants (Mohan, 1985). *F. oxysporum* f. sp. *psidii*, *F. solani*, *F. coeruleum*, *F. moniliforme* and *Rhizoctonia*

solani were also reported from rhizoplane as well as from the soil from Varanasi (Dwivedi, 1991). *Cylindrocarpon lucidium*, *Gliocladium virens* and *Bartilinia robillardoides* caused drooping and subsequent wilting of guava seedlings grown in Hoagland's solution on artificial testing (Misra and Pandey, 1992). In a recent study Misra and Pandey (1997) and Misra and Pandey (2000a) reported that *Gliocladium roseum* as a most potent pathogen, which reproduces symptom of wilt on artificial inoculation. They also developed an inoculation technique i.e., stem cut end wound hole inoculation technique, which reproduce the wilt symptom very quickly. Misra *et al*. (2003a) further reported pathogenic diversity in the cause of wilt disease of guava. Histopathological of wilted root was studied using microtomy. Light microscope studies of naturally infected or inoculated plants by *Fusarium oxysporum* f. sp. *psidii* and *F. solani* are reported. The observations of wilted guava root showed disintegration/necrosis of the cells. No trace of mycelium was observed in any of the wilted guava root samples so far. In T.S. of root of wilted plant the normal shape of the epidermis was disturbed and breaken through which pathogen can enter in the host tissue. These results confirm the observations made by Chattopadhyay and Bhattacharjya (1968a). Wilting of the plant may also be due to production of toxin by the *Fusarium* sp. pathogen. In the xylem, they produce toxic materials that pass throughout the plant bringing about yellowing and wilting of leaves and stem and eventually resulting in the plant's death (Beckman and Roberts, 1995).

The finer roots show black streaks, which become prominent on removing the bark. The roots also show rotting at the basal region and the bark is easily detachable from the cortex. The cortical regions of the stem and root show distinct discoloration and damage. Light brown discoloration is noticed in vascular tissues (Das Gupta and Rai, 1947; Chattopadhyay and Bhattacharjya, 1968a, b; Gupta, 2010).

MOLECULAR CHARACTERIZATION OF *Fusarium* sp. ISOLATES OF GUAVA

At present, molecular tools have been successfully employed in the characterization of pathogenic isolates of plant pathogens. Unfortunately, determining pathogenicity still relies largely on bioassays. In the recent past encouraging success has been achieved in developing wilt resistant varieties in some crops. However, instability of resistance in the newly developed varieties develops because of high pathogenic variability in the pathogen (fungus). This is an area of major concern for pathologists and breeders engaged in crop improvement programme. It is, therefore, of utmost importance that the extent of variability prevalent in the pathogen(s) across the locations is clearly understood to streamline the resistance breeding programme. *In vitro* screening of wilt resistant rootstock is reported (Vos *et al*., 1998; Bajpai *et al*., 2007). Recent studies on genetic variability using DNA based markers in these pathogens have opened up new avenues to use molecular markers as a fast and accurate method for diagnosis of the pathogen. Pathogenicity tests is a very cumbersome and time consuming (50-300 days) process and is not sure as several factors influence the disease development and requires extensive facilities. Differential line tests of *F. oxysporum* can take over 40 days to complete. Therefore, it would be desirable to have more rapid methods with molecular techniques of distinguishing different variants. Reports reveal wide variations in cultural, morphological characteristics and pathogenicity of isolates of *F. oxysporum* and *F. solani* across the location. Existence of variability in *F. solani* and *F. oxysporum* appears to be one of the reasons influencing the instability of wilt resistance in newly bred genotypes and this is a matter of great concern. Efforts are needed to elucidate the extent of variability in order to streamline the resistance-breeding programme. Better understanding of genetics of pathogenic diversity through 'DNA finger printing' is now possible with the advent of molecular tools.

Random amplification of polymorphic DNA (RAPD) technique: The disease is caused by a complex of several *Fusarium* species in most areas, differing in ecological and biological features. Recent epidemics throughout the world have made researchers to focus on *Fusarium* sp. pathogens and their epidemiology. The existence of high variability in fungal community at ecological niches indicates interactions for nutrients and space. These interactions can broadly be classified in 9 categories based on the effects or mechanisms of the interaction between the two species and range from neutral through beneficial to harmful for one or the two species. Quantification of fungal DNA in different plant matrices is becoming more common in the epidemiology of plant pathogens (Schaad and Frederick, 2002; Bogale *et al*., 2007).

Detection using real-time PCR can be easily done, in less than 1 day compared to the 2-3 weeks required for the microbiological detection. Molecular tools make facility to study interactions among different strains within one species that is not possible using of the micro and macro-morphological features. Effective, reliable and rapid

species identification and detection of *Fusarium* pathogens are essential keys and a milestone work for basic and practical research in different disciplines. Many species-specific primers for detection *Fusarium* species have been designed (Jurado *et al*., 2005; Bogale *et al*., 2007). Using additional sets of primers in a multiplex PCR, enables the simultaneous detection more than one species in every PCR reaction.

RAPD analysis has been currently used to distinguish pathogenic isolates of *F.oxysporum* f.sp. *dianthi* (Migheli *et al*., 1998), to distinguish *F. oxysporum* f. sp. *vasinfectum* (Assigbetse *et al*., 1994), two pathotype of f.sp. *ciceris* (Kelly *et al*., 1994) and race 2 of f. sp. *pisi* (Grajal-Martin *et al*., 1993), the f.sp. *albedinis* (Tantaoui *et al*., 1996), the f.sp. *basilici* (Chiocchetti *et al*., 1999) at genetic level. RAPD technique showed to be a useful tool in providing sources of sequences to develop the SCAR technique in order to create a simple PCR test based on specific sequences (De Haan *et al*., 2000). In present study, the application of RAPD analysis was to evaluate genetic diversity/genetic similarity of *F. oxysporum* f. sp *psidii* and *F. solani* isolates, causal agents of *Fusarium* wilt on guava. Genetic diversity of *Fusarium oxysporum* isolates, causing wilt in cucumber has been done by RAPD fingerprinting (Vakalounakis and Fragkiadakis, 2008). A total of 106 isolates of *Fusarium oxysporum* obtained from diseased cucumber plants showing typical root and stem rot or wilt were characterized by RAPD and it was effective in distinguishing isolates of *Fusarium oxysporum* f. sp. *radisis-cucumarium* from those of *Fusarium oxysporum* f. sp. *cucumarium*. Taxon specific band is also possible by RAPD analysis. Khalil *et al*. (2003) did RAPD to study the kinships among 5 *Fusarium* species. Out of 10 primers tested, four primers produced polymorphic amplification patterns with taxon specific bands, in addition to individual specific band.

Mishra (2006) studied four RAPD primers (decamer; BMFUS-2, 3, 13, 14) in the genome of *Fusarium oxysporum*, causing guava wilt in Lucknow region and found that the larger amplified products were 2000 bp and smallest amplified products were 200 bp. The number of scorable bands for corresponding primers ranging from 1 to 6 with an average of 3 bands with polymorphism banding pattern among the six isolates of *F. oxysporum*. Based on similarity index and dendogram, Mishra (2006) also concluded that the six isolates were similar with good enough genetic diversity. Molecular diversity study of *Fusarium solani* isolates of *Dalbergia sissoo* wilt assessed through RAPD markers by Arif *et al*. (2008) revealed the presence of highest genetic similarity (97%) and categorization of *Fusarium solani* isolates into many sub cluster with RAPD-PCR amplified product size of 210 to 3200 bp. Results obtained by these workers correlate with the results of present investigation in case of *Fusarium* sp. isolates, as there is high genetic variability among the isolates of Fop due to chances of occurrence of mutation and high genetic similarity in Fs isolates due to absence of any genetic mutation.

In the recent study made by Gupta (2010) on use of RAPD marker for genetic diversity study of *Fusarium oxysporum* f.sp. *psidii* and *Fusarium solani,* he observed that 0.21% polymorphism was found in individual isolates of *Fusarium solani*, which showed per cent polymorphism is not significant (p=0.05) among the 42 tested isolates of *Fusarium solani*. This means that the 42 isolates of *Fusarium solani* are almost similar and there is no mutation occurred among these isolates. However, among 42 isolates of *Fusarium oxysporum* f. sp. *psidii*, a significant result (p = 0.05) showing 2.58% polymorphism in individual isolate was obtained. It indicates that per cent polymorphism is statically significant (p = 0.05) among the 42 tested isolates of *Fusarium oxysporum* f.sp. *psidii*. Hence, there is molecular variability among the isolates of *Fusarium oxysporum* f.sp. *psidii* due to chances of occurrence of mutation among the isolates of *Fusarium oxysporum* f. sp. *psidii*. The similarity values shown by the *Fusarium solani* isolates from the same or different region were very high. It emphasizes the close relationship among the isolates and may be concluded that there is no occurrence of mutation. However, in case of Fop it clearly indicates that there is lesser level of similarity among the isolates showing moderate value of close relationship due to occurrence of mutation among the tested isolates.

Such diversity of population within same geographical region and similarity of the population between different geographical regions might also be due to factors like heterogeneity, genetic architecture of population history of selection and/or developmental traits and has been reported in different fungi including *Fusarium* sp. (Prasad *et al*., 2008; Gupta *et al*., 2009d). Gupta (2010) also concluded that RAPD markers OPA01, OPA03, OPA09, OPA11, OPA15, OPA19, OPC02, OPC05 and OPC08 were amplified with reproducible allelic banding pattern and can be efficiently used as DNA marker for genetic diversity.

Microsatellite marker based detection technique: The high variability in cultural characteristics exhibited by *Fusarium* species (Nelson *et al*., 1983; Nirenberg, 1989) poses a challenge to efforts aimed to breeding for resistance to wilt diseases. Moreover, host specificity (Li *et al*., 2000) as well as a ribosomal DNA nucleotide

sequence (O'Donnel and Gray, 1995; Suga *et al*., 2000) has shown that *Fusarium* species has a very diverse form, almost indiscernible from other related form or forma speciales. High variability has generally been observed in the genus *Fusarium* using dominant AFLP markers and cultural characteristics (Kiprop *et al*., 2002). However, dominant markers and morphology may be under selection and can provide biased inferences of genetic relationships. Analysis using a neutrally evolving and co-dominant marker such as microsatellite could shed insight into the genetic identification within the *Fusarium* species isolates of guava, to aid breeding programmes aimed at developing resistance to this fungus. PCR method (Microsatellite) has a good sensitivity and is largely used in laboratory research routine (Schaad and Frederick, 2002). The microsatellite marker system is best over than RAPD-PCR for diagnostic purpose and allows a higher sensitivity. It offers moreover the opportunity of quantification of the target sequence opening new perspectives of research.

Microsatellite marker or Simple Sequence Repeats (SSRs) provide a powerful tool for taxonomic and population genetic studies. SSRs are suitable for studies involving deep divergences as well as very closely related taxa as an identifying marker (Avise, 1994). Specifically, the use of SSRs in fungal diversity and identification studies is prosperous since these markers are fairly well characterized (Bergemann and Miller, 2002; Fournier *et al*., 2002; Sirjusingh and Kohn, 2002; Kretzer *et al*., 2000) and their transferability across *Fusarium* species (Sirjusingh and Kohn, 2002; Naef *et al*., 2006) and *Fusarium* genera (Mishra *et al*., 2003; Mwangombe *et al*., 2007) has been demonstrated. The present study tested the level of phenotypic and microsatellite DNA variation and relationship among the *Fusarium* species isolates of guava viz. *Fusarium oxysporum* f. sp. *psidii* and *F. solani*.

Gupta (2010) successfully amplified microsatellite marker MB 13 in both *Fusarium oxysporum* f.sp. *psidii* and *Fusarium solani* with product size of 296 bps and 1018 bps, respectively. Therefore, it reveals that this marker can be used for genetic identification of both *Fusarium oxysporum* f.sp. *psidii* and *Fusarium solani* from a given numbers of unidentified samples of *Fusarium* species simultaneously. Also, microsatellite markers viz. MB 17, RE 102 and AY212027 were also exactly amplified with a single banding pattern. MB 17 was amplified in *Fusarium oxysporum* f.sp. *psidii* isolates with a product size of 300 bps. RE 102 and AY212027 were amplified in *Fusarium solani* isolates with the product size of 153 and 300 bps, respectively. Therefore, MB 17 can also be used as genetic identifying marker for

Table 1: Amplification product size of microsatellite marker in the genome of *Fusarium* sp. isolates of guava

		Product size (bp)	
Primer name	Gene bank accession No. (NCBI)	Fop	Fs
MB 13	AY931026	296	1018
MB 17	AY931023	300	-
RE102	RE102	-	153
AY212027	AY212027.1	-	300

-: Not amplified

Fusarium oxysporum f.sp. *psidii* isolates with respective amplification size and RE 102 and AY212027 as identifying microsatellite marker for *Fusarium solani* isolates with resulted product size (Table 1). He also compared SSR amplicon sequences with the results of *Fusarium* sp. complex as reported by Bogale *et al*. (2005, 2006) and Mwangombe *et al*. (2008).

The successful amplification of these microsatellite markers of *Fusarium* sp. complex in the genome of *Fusarium oxysporum* f.sp. *psidii* and *Fusarium solani* revealed with these primers should be sufficient for studies of genetic identification. He also concluded that amplified microsatellite marker can be used as universal identifying marker for *Fusarium oxysporum* f. sp. *psidii* and *Fusarium solani* isolates occasionally. Hence, these primers would be particularly useful because the fungus is one of the most common *Fusarium* sp. residing in the soil environment in India.

Virulence factor gene related microsatellite locus technique: *Fusarium oxysporum Schlecht* causes vascular wilt diseases in a wide variety of crops (Beckman, 1987). Based on host specificity, more To enter plant cells directly, *F. oxysporum* has to produce a wide variety of extracellular Cell Wall Degrading Enzymes (CWDEs), including endo- and exopolygalacturonases (PGs), xylanases, cellulases, proteases and pectate lyases. These enzymes may contribute to the degradation of the structural barriers constituted by plant cell walls (Christakopoulos *et al*., 1995; Di Pietro and Roncero, 1998; Ruiz Roldán *et al*., 1999; García-Maceira *et al*., 2000). Comparative analysis of an endoPG gene, PG1, in isolates of seven *Fusarium* species indicated that this region would be very useful for phylogenetic analysis in the genus *Fusarium* (Posada *et al*., 2000). Targeted genes encoding for the endopolygalacturonase (endoPGs) secreted by *F. oxysporum*, PG1 and PG5, has shown that these mutants having capability in virulence (Di Pietro and Roncero, 1998). Endo--1,4-xylanases are produced by a number of plant pathogenic fungi and it has been suggested that they may play a role during infection (Walton, 1994). Xylanases act on xylan, which makes up a significant part of the hemicellulose fraction of the plant cell wall (Wong *et al*., 1988; Woo *et al*., 1998;

Ruiz-Roldán *et al*., 1999). The presence of xylanases in *F. oxysporum* and *F. solani* was previously reported (Ondrej *et al*., 2008; Gupta *et al*., 2009b, c). In *F. oxysporum* f.sp. *lycopersici,* four xylanase genes (Xyl2, Xyl3, Xyl4 and Xyl5) have recently been identified (Ruiz-Roldán *et al*., 1999; Gómez- Gómez *et al*., 2002). Seventeen virulence genes have already been characterized in *F. oxysporum*. One of these genes produces proteins that proved to be of great importance in disease development. These proteins are the Mitogen-Activated Protein Kinase (MAPK) (Di Pietro *et al*., 2001). MAPKs are involved in transducing a variety of extracellular signals and for regulating growth and differentiation processes in eukaryotic cells (Schaeffer and Webber, 1999). Di Pietro *et al*. (2001) identified the gene fmk1 that encodes a MAPK in *F. oxysporum.* This gene is related to formation of infection hyphae, root attachment and invasive growth by *F. oxysporum* (Di Pietro *et al*., 2001). Chitin synthases (CHS) are the enzymes implicated in chitin synthesis and in fungi, they constitute a great family of isozymes (Munro and Gow, 2001; Ruiz-Herrera *et al*., 2002). Furthermore, each fungal species contains a number of CHS belonging to different divisions and classes (Ruiz-Herrera *et al*., 2002). In the pathogen *Fusarium oxysporum*, five genes implicated in chitin synthesis, CHS1, CHS2, CHS3, CHS7 and CHS V, have been isolated and characterized (Udiroz *et al.,* 2004). In *F. oxysporum*, an intact cell wall structure has been implicated in the plant-host interaction is related to these genes (Schoffelmeer *et al*., 1999).

Previously, the comparative analysis of a PG1 gene in seven *Fusarium* species proved to be valuable (Posada *et al*., 2000). In this study, both coding and two intron sequences contained in the endoPG fragment were compared to evaluate their potential use in phylogenetic studies. It was concluded that this PG1 region would be very useful for virulence analysis in the genus *Fusarium*.

Mishra (2006) successfully amplified six virulence factor genes such a PG, KHS, PDA, PelA, CHS and FMK1 of *F. oxysporum* associated with vascular wilt in tomato, pea and bean tested in the genome of guava wilt causing *F. oxysporum* isolates of Lucknow region, India. It has been observed that four microsatellite of virulence factor genes such as PG 1 and 2, FMK1, PDA and KHS were amplified with 714, 765; 1221, 514; 308, 441, 809; 1359, 2297 and 900, 1361 bp respectively and concluded that these markers are highly conserved in *F. oxysporum* causing guava wilt in Lucknow. Simultaneously, Groenewald (2006) resulted that the virulence-associated gene relate marker viz. FMK1, PG1 and Xyl were present in all of *pathogenic* isolates *F. oxysporum* f. sp. *cubense*, with PCR amplified gene regions product of 1244, 740, 260 bp, respectively.

Fusarium genera hold a prominent role in agro biological research with rich gene pool. It was observed that the virulence factor genes viz. Xyl, KHS1, PelA1, PG6/7, CHS1/2 and FMK1/MAPK1 causing vascular wilt in many plants viz. bean (Li *et al*., 1995; Mishra, 2006), pea (Rogers *et al*., 2000; Crowhurst *et al*., 1997; Mishra, 2006), tomato plant (García-Maceira *et al*., 2000; Udiroz *et al*., 2004; Mishra, 2006) banana (Groenewald, 2006) and woody crops (Christakopoulus *et al*., 1995; Ruiz-Roldán *et al*., 1999), respectively were highly conserved in *Fusarium* sp. isolates of guava. The allelic pattern obtained by cross species microsatellite markers of virulence factor gene of *Fusarium* sp. can serve many fundamental objectives against *F. oxysporum* f. sp. *psidii* and *F. solani* isolates of guava wilt. Since above amplified microsatellite marker for virulence factor genes are related to disease related locus of *Fusarium* sp. isolates of Fop and Fs isolates. Hence, it can be said that these locus may play an important role in initiate expression of the wilt symptoms and infection in guava plant. Chalvet *et al*. (2003) worked on active DNA transposon in the genome of *Fusarium oxysporum* and identified niaD target gene and revealed that the virulence related sequence are present in different species of *Fusarium.*

Gupta (2010) successful amplified virulence factor gene related microsatellite marker viz. Xyl, KHS1, PelA1, PG6/7, CHS1/2 and FMK1/MAPK1 with specific amplicon size indicates the presence of virulence gene locus in the genome of *Fusarium* sp. isolates causing wilt disease of guava (Table 2). Microsatellite marker for virulence factor genes of Xyl loci was amplified in both *F. oxysporum* f. sp. *psidii* and *F. solani* isolates with product size of 281 bps in all the isolates of *F. oxysporum* f. sp. *psidii* and *F. solani*. PG6/7, CHS1/2 and FMK1/MAPK1 were amplified in *F. oxysporum* f. sp. *psidii* isolates with a product size of 765, 1566, 1010 and 1244 bps. PelA1 and KHS1 were amplified only in *F. solani* isolates with the product size of 586 bps and 1359 bps, respectively. Therefore, it was resulted that these disease related virulent microsatellite loci are also present in the genome of *F. oxysporum* f. sp. *psidii* and *F. solani* isolates,

Table 2: Amplification product size of virulence factor gene related microsatellite marker in the genome of *Fusarium* sp. isolates of guava

Locus symbol	Gene Bank Locus Name (NCBI)	Product size (bp) Fop	Fs
CHS1/2	Class V chitin synthase	1010	-
FMK1/MAPK1	FMK1 encoding mitogen activated protein kinase	1244	-
PelA1	Pectate lyase	-	586
PG6/7	Endo-polygalacturonase	765,1566	-
KHS1/2	Kievitone hydratase	-	1359
Xyl	Family 10-Endoxylanase	281	281

-: Not amplified

respectively and could be responsible for wilt producing character and expression in host plant when these *Fusarium* species infect the healthy plant.

Therefore, it can be said that these disease related loci are present in the genome of *F. oxysporum* f. sp. *psidii* and *F. solani* isolates are responsible for wilt producing character/expression in host plant. This study represents the first record where sequences of virulence genes were applied for analysis within *Fusarium* sp. isolates of guava wilt. This result is in confirmation with observations made by Ruiz-Roldan *et al.* (1999), Udiroz *et al.* (2004) for *Fusarium oxysporum* and Suga *et al.* (2000) for *Fusarium solani*.

Sequence analyses of virulence locus demonstrated that they are unique to pathogenic forms of this vascular wilt pathogen. It implies that they play a role in pathogenesis. Differential expression of virulence genes upon infection of plant hosts, therefore, should in future be investigated. Such specific virulence proteins produced by pathogens can be bound for developing transgenic plants resistant to disease. For example, plant polygalacturonase inhibitor proteins (PGIPs) can bind fungal PGs. Inhibition specificities and kinetics, however, might vary within and among species. Bean PGIP, for instance, inhibited fungal PGs from *Fusarium moniliforme* and *Botrytis cinerea Pers. ex. Fr.*, while pear PGIP inhibited only *B. cinerea* and tomato PGIP inhibited *B. cinerea* (Stotz *et al.*, 2000). The conserved structure among MAPK members suggests a high degree of virulence function relatedness (Di Pietro *et al.*, 2001).

CONCLUSIONS

Inoculating the fungal mycelia using stem end cut wound hole inoculation technique is seems to be good fot pathogenicity test. Wilt symptoms start from 28-30 days after inoculation and during September-October fast wilting occurs, while maximum wilting occurs in the month of October. Quantification clearly indicates that October is the most favorable month for wilt incidence. which indicates that the increased disease incidence on guava in the subtropics is primarily a function of the guava plant being more vulnerable to infection under rainy/winter temperatures, rather than the pathogen becoming more competitive. In general maximum plants takes three month period for typical wilting after appearance of first visible symptoms though maximum time taken for complete wilting was 240 days.

Histopathology study of wilted root of guava tree was studied using microtome. Wilted guava roots showed disintegration/necrosis of the cells. No trace of mycelium was observed in any of the wilted guava root samples. Transverse section of root also showed that the shape of the epidermis was disturbed in the wilted plants. It may be distributed by broken epidermis, through which pathogen can enter in the host tissue. Therefore, it may be concluded that wilting of the guava plant could be due to production of toxin by the *Fusarium* sp. pathogen. As they produce toxic materials that can pass throughout the plant through vascular system bringing about yellowing and wilting of leaves and stem and eventually resulting in the plant's death.

The aqueous extracts/leaf from *Curcuma longa* L., *Achyranthes roses*, *Calotropis gigantea* L. R. Br. *Cannabis sativa* L. may be more useful against *Fusarium* wilt pathogens of guava. The extracts/leaf of these plants can be mixed to the soil near root zone of wilted guava plant to control the wilt problem. Meanwhile consortiums of *Trichoderma* sp. as biocontrol agent guava wilt pathogen viz. *F. oxysporum* f. sp. *psidii and F. solani* may be further tested and used for effective management of the disease.

It has been shown that different tools are valuable in investigating the variability of this fungus and molecular techniques can increase the level of detection of pathogenic group of *Fusarium* sp. isolates facilitating a preventive approach to the disease. In particular Random Amplified Polymorphism of DNA is useful in describing the origin and the phylogeny of the isolates like those from different origin/region. It is possible to show they have a clonal origin. These tools integrate the knowledge obtained by classical methods such as pathogenicity tests and it make possible to propose hypothesis on the phylogenetic relations between isolates.

RAPD marker can be used as most informative marker for genetic diversity and genetic distance related study of *Fusarium* sp. isolates of guava. As microsatellite marker technology is based on identifying highly conserved gene sequence of a concerned organism and in the present investigation due to presence of specific single band product size these amplified microsatellite marker found to best for genetic identification. Virulence factor gene related microsatellite marker promise to be a valuable tool to get the information that the virulence gene sequence are highly conserved region these virulence marker are associated with the virulence/pathogenic nature of the *F. oxysporum* f. sp. *psidii* and *F. solani* in reference to produce vascular wilt expression. RAPD marker, microsatellite marker and Virulence factor gene related microsatellite marker showed that it is a promising technology for the fast diversity, detection and diagnosis of *Fusarium* sp. isolates pathogenic to guava, respectively.

Introduction and evaluation of new technology like RAPD marker, microsatellite marker and Virulence factor gene related microsatellite marker techniques in agricultural system will certainly influence the biotechnological way and will be performed in the near future for assessing the intra- and interspecific identification of *Fusarium* wilt pathogens of guava. In addition to increase the understanding of the diseases for improving crop productivity these results can be explored for developing integrated strategies for disease management. Further, it may help to researchers of agro-biotechnology for developing a genetic map of *Fusarium* sp. isolates of guava with reference to resistant breeding line programme of guava cultivar.

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