# Chapter 11 Management of *Fusarium udum* Causing Wilt of Pigeon Pea



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# 11.1 Introduction

Pigeon pea is an important source of protein and vitamin, and it is the second most edible legume crop after chickpea and contributes about 90% production of the total world production in India (Allen and Lenné 1998; Dhanasekar et al. 2010). Its protein and essential amino acid content makes it an important food in a vegetarian diet, with its seed and pod husk being the sources of feed (Varshney et al. 2010). In addition to protein and amino acid, it also contains carbohydrates, minerals, and fibers. Its plantation covered 4.3 million hectares globally (Anonymous 2007). In India pigeon pea production and productivity are 2.76 metric tons and 762 kg/ha, respectively, coming from an area of about 3.63 million hectare (the Year 2010, ICAR Vision 2030/2010). Thirty-two species belong to the genus Cajanus, and most of them are found in India and Australia, whereas only one species is native from West Africa. Pigeon pea can be grown under drought conditions with significant return and minimum input. In India pigeon pea productivity is low due to the lack of new cultivars and infection by plant pathogens (Nene et al. 1996). It is cultivated with a minimum input of fertilizers and disease management strategies. Pigeon pea production is affected by many biotic and abiotic stresses. Under biotic stress, several pathogens such as fungi bacteria, viruses, nematodes, and

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mycoplasma-like organisms are responsible for the lower yield of pigeon pea (Nene et al. 1989; Kannaiyan et al. 1984). Some important diseases responsible for legume crop loss include Fusarium wilt, sterility mosaic, phytophthora blight, macrophomina root rot, alternaria leaf spot, and cercospora leaf spot caused by Fusarium udum, viruses, Phytophthora drechsleri f. sp. cajani, Macrophomina phaseolina, Alternaria tenuissima, and Cercospora cajani, respectively (Kannaiyan et al. 1984). These diseases and other abiotic factors such as low moisture stress, waterlogging, and salt stress are responsible for a significant reduction in yield of pigeon pea (Varshney et al. 2007; Saxena 2008). The diseases Fusarium wilt and sterility mosaic are economically important in our country. Fusarium wilt is a very severe disease, causing yield loss of about US \$71 million annually in India. Wilt is a soilborne disease that affects the yield of crop significantly especially in wilt-susceptible cultivars (Reddy et al. 1990). Fusarium udum is soil inhabitant in nature and enters the vascular system of the plant through the root system. Because of the soilborne nature of wilt disease, management through cultural practices is very difficult at a significant level. Some chemical fungicides are effectively managing this disease, but the extreme use of chemicals is harmful and noneconomical. Biocontrol strategies are also in use through several antagonistic microorganisms for managing this disease (Chaudhary and Kumar 1999). Many fungal and bacterial commercial products are also developed for soilborne pathogen management (Kumar and Sarma 2016; Kumar et al. 2017). Use of these biocontrol antagonistic microorganisms and their commercial product in plant disease management is economical and risk-free concerning health hazards. In this chapter, we have discussed all the management strategies from conventional to advanced molecular technologies for wilt disease of pigeon pea.

## 11.2 History

In 1809, Link was the first scientist to narrate about the genus *Fusarium* – the pathogen with fusiform, nonseptate spores borne on a stroma. Later, a detailed account of *Fusarium* species and pigeon pea wilt was first reported by Butler (1906). In India, this destructive fungus was first described in 1906 by E.J. Butler in the pigeon pea crop from Bihar and hence named as *Fusarium udum* Butler and later reported in several other countries in Africa, South Asia, and Europe (Karimi et al. 2012). Then, *F. udum* was established as a new species by Butler (1910), and isolation and identification of the fungus were carried out. Previously, *F. oxysporum* f. sp. *udum* was used frequently. Extensive characterization of *Fusarium*-plant interaction in the prospect of its biochemistry and physiology has been already done; however, recognition of vital molecules involved in the pathogenesis of *Fusarium* sp. did not start till convenient molecular genetic techniques for filamentous fungi were available (Timberlake and Marshall 1989; Datta and Lal 2013). Due to the soilborne nature of the pathogen, chemical control is ineffective in many established cases, and managing the disease seems to be very challenging. However, deployment of resistant varieties is unlikely because of its high degree of genetic variability among the pathogenic population (Kumar and Upadhyay 2014). At the present scenario, three fungicides commonly used for the management of *Fusarium* wilt are thiram, benomyl, and bavistin (Vidhyasekaran et al. 1997; Meena et al. 2002; Melent'ev et al. 2006). Moreover, microorganisms producing various types of mycolytic enzymes (chitinases, glucanase, and proteases) have shown a substantial impact on disease development as they can degrade chitin and glucan present in the fungal cell wall (Deshpande 1999; Hillocks et al. 2000; Hoster et al. 2005; Patel et al. 2007).

# 11.3 Distribution

Worldwide, pigeon pea wilt causes considerable devastation to the production of pigeon pea (Kannaiyan et al. 1984). At crop blooming and maturity stages, 30–60% of disease incidence has been recorded; on the other hand, yield losses may increase up to 100% when susceptible cultivars were used (Okiror 2002; Dhar et al. 2005). It is extensively occurring in India, Malawi, and East Africa leading to more than 50% yield losses, and despite these, countries like Indonesia, Mauritius, Bangladesh, Grenada, Myanmar, Venezuela, Trinidad, Nevis, Nepal, and Tobago are well-known for incidence of Fusarium udum (Reddy et al. 2012; Marley and Hillocks 1996). In the Indian context, this disease was reported in most of the pigeon pea-growing states and caused about US\$ 71 million annual production losses (Reddy et al. 2012) except in southern states. However, the heavy incidence was reported in Vidharbha (13.66%) followed by the Marathwada region where maximum severity recorded up to 90% in the state of Maharashtra (Shinde et al. 2014). In other states like Bihar, Jharkhand, Orissa, and West Bengal, Fusarium wilt was effectively found with a substantial range of cultural, morphological, and pathogenic variability in maximum isolates collected from pigeon pea-growing regions (Kumar and Upadhyay 2014). Mesapogu et al. (2012) have reported genetic diversity and pathogenic variability among 30 isolates of Fusarium udum collected from diverse agroclimatic conditions representing 7 states of India, i.e., Andhra Pradesh, Uttar Pradesh, Jharkhand, West Bengal, Haryana, Rajasthan, and Punjab.

#### 11.4 Symptoms

The disease can be diagnosed by visualizing the gradual or sudden wilting of the pigeon pea plant. Similarly, the leaves show interveinal clearing followed by withering, yellowing, and drying of young leaves on the upper portion of the plant. Wilted plant loss their tugidity because off chlorosis and necrosis resulting in premature leaf drop and drooping of apical shoot followed by drying of entire shoot (Upadhyay and Rai 1992). As the pathogen survives in the soil and the nature of the infection is soilborne, it will infect the tap root system of pigeon pea plants resulting in wilting

of the whole plant instead of partial wilting. If the stem of infected plants is split open, browning of vascular tissue mainly the xylem is the most common visible symptom which differentiates it from other diseases. The wilting symptoms are the most common and prominent during the flowering and pod maturation stages (Reddy et al. 1990). Another visible symptom is purple banding, which extends upward from the base of the plants and is easily seen on the stem portion. Purple banding helps in differentiating healthy and infected plants (Sharma et al. 2016).

#### **11.5** Disease Development and Pathogenicity

*Fusarium* wilt of pigeon pea is both a soil-borne and seed-borne disease in which the infection level of untreated seeds may range from 13% to 19% (Kannaiyan et al. 1984). The infected seeds thus serve as a primary vehicle for the spread of this disease over long distances and/or to the newer areas. The pathogen, Fusarium udum, survives in the soil for more than 3 years on the infected plant detritus. The disease incidence and disease severity are principally dependent on the conditions of soil and the genotype of the crop. The incidence of disease in susceptible cultivars is facilitated by a slightly acidic to slightly alkaline soil having sand particles more than half percentage in their soil texture (Singh and Hussain 1964; Upadhyay 1979). A soil temperature of about 20-29 °C and soil moisture of about 6-16% are most suitable for the development of wilt disease in pigeon pea (Upadhyay 1979). As per the reports, disease incidence among different soils depends chiefly on the survival and saprophytic activity of the pathogen in those soils that are ultimately favored by the availability of the host substrate. The severity of the disease is dependent on the duration of the pigeon pea varieties as very short-duration varieties suffer less than the long-duration and medium-duration varieties. Growing of susceptible pigeon pea varieties over the infested soils repeatedly increases the disease severity and disease incidence.

Earlier the wilt of pigeon pea was known to be caused only by the imperfect state of the pathogen (*Fusarium udum*), but the discovery of its perfect state, i.e., *Gibberella indica* (Upadhayay and Rai 1983), is known to occur through both the stages. As the perfect state is not known to be present frequently under natural conditions, the imperfect state is most common to incur the disease. In both the states, the pathogen is known to grow externally and internally through the production of a mycelial mass and conidia on the host's surface, majorly on the collar region and roots (Upadhyay and Rai 1982). After the surface colonization, the fungal hyphae invade the fine branches of roots that grow laterally and continue to proliferate in the vessels of xylem. Even though the infection may take place in the seedling stage of the plant, but the expression of disease is maximum during flowering and the podding stage of plants (Reddy et al. 1998), which can be due to the longer time required by the pathogen for colonization in the plants. It takes approximately about 3–4 months for the fungus to cause wilting in the infected plants which are when the basal half of the main stem is colonized by the pathogen (Reddy et al. 1998). This

is the reason that can be understood as to why the short-duration crops have low levels of wilt infestation when compared to long-duration crops as the former ones are escaping the wilt incidence.

Once the infected plants wilt and die, the pathogen continues to live and survive as a saprophyte for many years, mainly on the dead plant parts in its perfect form (Upadhayay and Rai 1983) or imperfect form (Nene et al. 1980). Both the states of the fungus survive simultaneously on the host plant. In addition to the confinement of pathogen survival mainly on the dead roots and debris of infected plants, it may survive on the other organic matter for a limited period. Apart from these, the fungus Fusarium udum also survives on other fungi in the soil as mycoparasite as well as on the bodies of termites that feed on the wilted host roots (Upadhyay and Rai 1982, 1983). The chlamydospores are also known to be formed in both the phases of the fungus, i.e., the parasitic and the saprophytic phases, depending on the environmental conditions from the hypha and the conidia (Sinha 1975). The fungus has been also observed to produce a large number of dark violet perithecia on the exposed roots and collar region of the host plant which also serves as resting structures. These Fusarium udum perithecia produce ascospores in large numbers which remain physiologically inactive in the soil for a limited period and after which they produce either conidia or somatic hyphae on germination leading to infection of the pigeon pea plants (Rai and Upadhyay 1982).

In recent years, many of the studies on morphological, cultural characterization and the rate of reaction of the pathogen *Fusarium udum* have provided enough evidence for the existence of different virulence groups (Harlapur et al. 2007; Mahesh et al. 2010; Karimi et al. 2010). The variable reactions of various tested resistant pigeon pea varieties show the possibility of the presence of different physiological forms of the pathogen (Muhammad et al. 2011). In a study, Reddy et al. (1998) reported three strains of the pathogen which showed sensitivity/or resistance against several pigeon pea differentials.

# 11.6 Mechanism of Host Plant Resistance

The employment and use of resistant varieties of the crop is the most economical, effective, and eco-friendly strategy for the control of diseases even though their response to the cultivating conditions will be a subject of concern (Saxena et al. 2012). To come up with a sound breeding program for the development of disease-resistant crop varieties, we need to understand the mechanism of host plant resistance and what mechanism to strengthen up in plants to restrict pathogen invasion. There are mainly two mechanisms that constitute host plant resistance, viz., constitutive and induced defense mechanisms. The constitutive resistance mechanisms contain all the preformed chemical factors and physical barriers that are present in the host plant in advance to the attack of phytopathogens (Dangl and Jones 2001). The physical barriers consist of the thick and/or hard cuticle, wax deposition in the epidermal cells, stomatal shape and size, and the pericycle of the root (Keen 1992).

The chemical factors of the constitutive defense mechanism consist of peptides, proteins, protein inhibitors, preformed secondary metabolites, alkaloids, phenols, phytoanticipins, etc., which add up to the early barriers of defense being a part of plant's natural growth and development (Heath 2000; Dixon 2001; Grayer and Kokubun 2001). The plants are also reported to exudate some fungi toxic substances that restrict and/or inhibit the spore germination of the phytopathogen (Agrios 2004).

The induced defense mechanisms are the ones which get triggered on after the attack of phytopathogen and involve both chemical and physical factors (Agrios 2004). The most important step of induced defense mechanism is the recognition of the phytopathogen by the host plant so that it can conjure the defense reactions (Dixon et al. 1994; Schenk et al. 2000). The process of reaction starts with the recognition of the molecular pattern of the pathogen and is termed as pathogenassociated molecular patterns (PAMP) (Nürnberger and Lipka 2005). This recognition of the pathogen leads to signal transduction involving a cascade of biochemical events which leads to incitation of defense responses (Keen 1992; Dixon et al. 1994; Baron and Zambryski 1995). The most frequent defense response is the hypersensitive response (De Wit 1992) which is a form of programmed cell death (Greenberg and Yao 2004). The hypersensitive reaction restricts the growth of the fungus to newer plant cells (Tomiyama 1982; Keen 1992; Schenk et al. 2000). In addition to this, the other induced reactions include rapid oxidative burst, ion fluxes, and strengthening of the cell wall by increased synthesis of cellulose, lignin, phenolic compounds, and hydroxyproline-rich glycoproteins (Bowels 1990; Agrios 2004). The rapid oxidative burst is mainly through the production of hydroxyl radical (OH), hydrogen peroxide ( $H_2O_2$ ), and superoxide ( $O_2^-$ ), and these reactive oxygen species impart cross-linkage of the proteins present in the cell wall of the plant resistant to fungal enzyme attack (Bradley et al. 1992; Keen 1999). These reactive oxygen species are also known to induce hypersensitive cell death while working as an agent in the cell signaling process (Levine et al. 1994; Alvarez et al. 1998).

There are other defense mechanisms which constitute in host plant resistance, and it comprises of production of vascular occlusions such as tyloses and gels (Mace 1963) and defense-related gene expression involving the production of suberin and lignin, signal transduction proteins, phytoalexins, and pathogenesis-related proteins (Reymond and Farmer 1998; Greenberg and Yao 2004). The production of the signaling compounds in the host plant after the recognition of the phytopathogen attack leads to the enactment of defense reactions systemically throughout the plant and is termed systemic resistance (Ryals et al. 1994).

## 11.7 Management of Fusarium Wilt Disease

There are different methods for the control and management of *Fusarium udum* followed in agricultural technology with its positive and negative impacts. For complete resistance, single, race-specific resistance genes (R genes) could be used. For incomplete resistance, a bunch of minor genes work together for broad-spectrum effect. Complete management of fungal disease is difficult due to lack of knowledge regarding plant-pathogen interaction at genetic, histological, and molecular levels. Thus, to protect pigeon pea from *Fusarium* in a sustainable way, it is necessary to build a novel and potential approach by investigating the existing technologies. Some of the important control methods are discussed here.

#### 11.7.1 Cultural Management

For the formation of barrier in pigeon pea against *Fusarium* wilt, numerous cultural practices are used. Among them, crop rotation is one of the best control measures. Crops like tobacco (*Nicotiana tabacum* L.), sorghum (*Sorghum bicolor* (L.) Moench), or castor (*Ricinus communis* L.) are rotated with pigeon pea for 3 years to wipe out the pathogen completely from the field. To reduce the infestation percentage below 20%, cultivation of the main crop could be followed with a year break with sorghum, or the land could be left fallow. The application of farmyard manure or *Crotalaria juncea* as green manure also reduces the incidence of wilt to a significant level (Ingole et al. 2005). Another method is reducing *Fusarium* inoculums from the field by solarization technique during the summer season (Reddy et al. 2012). Intercropping of sorghum with pigeon pea reduces incidences to 24% as compared to the sole crop which gets 85% incidence (Natarajan et al. 1985). Mixed cropping of *Crotalaria medicaginea* also has a positive impact on reducing wilt (Upadhyay and Rai 1981).

# 11.7.2 Chemical Management

Chemical management is one of the most effective and common measures. An equivalent mixture of benomyl and thiram is used for seed treatment and considered effective (Reddy et al. 2012). Use of biocontrol agent like formulation of *Trichoderma viride* and farmyard manure (2 kg and 125 kg, respectively) for one square measure is also found to be very successful in reducing *Fusarium* wilt (Perchedpied and Pitrat 2004). Addition of mineral in the soil like boron (Bo), zinc (Zn), manganese (Mn), and methyl bromide (CH<sub>3</sub>Br) diminishes the disease event of *Fusarium* wilt (Maisuria et al. 2008). For effective management of this disease, antibiotics like bulbiformin and griseofulvin have also been accounted.

#### 11.7.3 Biological Management

As chemicals lead to undesirable and harmful effects on various living entities, moreover it also causes an imbalance in the ecosystem. Thus, it creates a need for a healthy control measure. The use of biological agents is thus a significant measure

as it is a member of the ecosystem and a potential antagonist to pathogens. According to a few reports, addition of antagonists in the soil diminishes the *Fusarium udum* incidence (Maisuria et al. 2008; Bapat and Shar 2000; Singh et al. 2002; Anjaiah et al. 2003). Various rhizobacteria as biocontrol agents are used for its management (Siddiqui 2006; Siddiqui and Shakeel 2007; Pusey 1989; Bapat and Shar 2000; Siddiqui et al. 2005). The addition of *T. harzianum* provides disease control of 22–61.5% at all pathogen levels (Prasad et al. 2002). According to reports population of *F. udum* is drastically reduced by antagonism of *Aspergillus terreus*, *Aspergillus niger*, *Micromonospora globosa*, and *Aspergillus flavus* (Upadhyay and Rai 1981) in a biocontrol experiment. In naturally infested soil, the addition of *Pseudomonas aeruginosa* PAN1 significantly suppresses the incidence of *Fusarium* in pigeon pea and chickpea (Anjaiah et al. 2003). A graphical representation of direct and indirect mechanisms of biocontrol is presented in Fig. 11.1.

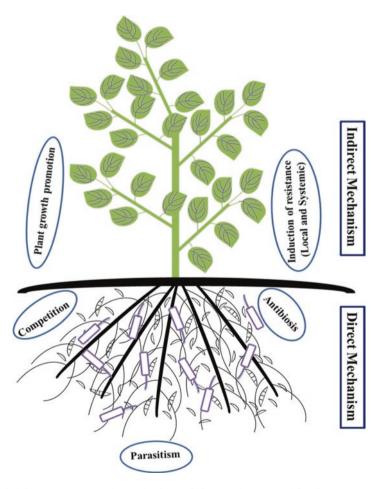
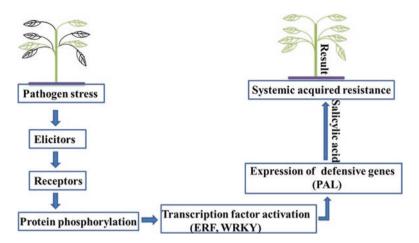


Fig. 11.1 Diagram represents the mechanisms of biocontrol agent used for disease management

## 11.7.4 Transcriptomics Approaches

Plant receptor protein recognizes the pathogen-derived molecule which is the initial step in defense response by activation of signal transduction cascades which triggers expression of various plant defense genes (Barilli et al. 2014). The study of gene expression provides a detailed knowledge regarding genes which were differentially expressed and various metabolic conduits at the time of host-pathogen interfaces. It can jointly help to unveil candidate resistant genes collaborating in every step of plant defense response (Ichinose et al. 2001). In the era of molecular plant breeding, marker-assisted selection (MAS) could be highly useful by applying the knowledge of the defense-responsive genes in legumes against fungal pathogen attack to legume plants, and under transformation event, any change in expression of such candidate genes could be linked with improved resistance. There are certain techniques used in transcriptomics like enhancing the potential number of defense-related genes by generating cDNA (complementary DNA) libraries from plants under stress against pathogens inoculation or elicitor-treated tissues or cells. The second one is the application of macro- or microarray designed by using orthologue sequences from other legumes in the format of unigenes, cDNA, expressed sequence tags (ESTs), or resistance gene analogs (RGAs) in the query legumes like pigeon pea under specific fungal stress conditions. These methods help to identify transcripts that are induced under pathogenic attacks and majorly associated with candidate resistant genes with a certain level of expression. Transcriptomics also helps to explore the information of genome sequence information with the aid of new less expensive sequencing platforms (Illumina (Solexa) sequencing, Roche 454 sequencing, Ion Torrent (Proton/PGM sequencing), and SOLiD sequencing). NGS technologies decrease the complexity of transcriptome techniques like SSH, cDNA-AFLP, SuperSAGE (serial analysis of gene expression), or MPSS (massive parallel signature sequencing), thereby increasing the identified transcript amount devoid of cloning and Sanger sequencing. Now, RNAseq technique allows building de novo transcriptomics that generates the transition of the transcript in expression form of both plant host and the inoculated fungal pathogen for examining plant-pathogen interactions, in addition to its basic work of studying all expressed transcript's sequencing at that particular time (Tadege et al. 2009). With the help of transcriptome profiling techniques, numerous diverse expressed genes population across the genome can be easily generated under pathogen attack. It is difficult to differentiate such a transcript associated with defense response and resistant phenotypes. This can be resolved by studying their co-localization with quantitative trait loci (QTLs) and exploring their functional analysis. Different advanced molecular techniques like gene silencing via RNA interference (RNAi) and virus-induced gene silencing (VIGS) are also used nowadays for knowing functional activities of PR proteins and biotic stress-induced genes (Tadege et al. 2009). A generalized presentation of phases showing the involvement of transcription factor in the induction of systemic acquired resistance against pathogen stress is presented in Fig. 11.2.



**Fig. 11.2** A generalized presentation of phases showing involvement of transcription factor in induction of systemic acquired resistance against pathogen stress. Protein phosphorylation occurs early with the recognition of pathogen elicitor by host receptor. Further transcription factor activation induces expression of defense genes such as PAL. Salicylic acid biosynthesis and defense gene activate systemic acquired resistance during plant pathogen interaction

# 11.7.5 Proteomics Approaches

Protein expression and its functional activity rely on the extent of expression of genes and posttranscriptional and posttranslational regulations. Therefore there could be a large chance that all transcripts derived from the successful expression of mRNA do not form successful protein accumulation and function. Thus, it is also significant to study protein accumulation to get a clear picture of the mechanisms of plant-pathogen interaction. Recent proteomic technologies provide opportunities for large-scale protein profiling via quantitative and qualitative methods (Qin et al. 2013). In comparative proteomics, protein is separated by electrophoresis based on their mass and isoelectric points followed by spectrometry techniques based on protein identification like de novo sequencing or peptide mass fingerprinting. Another technique is a separation of chromatography-based peptide mixtures continuing their detection through mass spectrometry (Nautrup-Pedersen et al. 2010) and shotgun proteomics which analyzes direct tandem mass spectrometric analysis that includes chromatographic separation based on cell lysis (Qin et al. 2013). All these techniques are practiced in legume particularly in the establishment of subcellular localization of target proteins, thus forming reference protein maps (Salavati et al. 2012). But, in legumes after pathogen attack, the study of proteomics is quiet far lacking behind as compared to other molecular advancements. But there is an example of a proteome study in chickpea – Fusarium oxysporum (Bourgeois et al. 2011). To detect protein variation under biotic stresses, comparative proteomic approaches are highly significant. Thus, there is a huge expectation from proteomic techniques that might unveil endogenous elements that provide resistance to fungal diseases.

# 11.8 Conclusion

The use of resistant variety is the most effective way to restrict the incidence of a disease. At present in the molecular biology and biotechnology era, it is possible to know about the genes, enzymes, proteins, and transcription factors that show a highly active defense response against pathogen attack. The study of resistances sources (Genes, protein etc.) can be beneficial for developing resistace in crop plant. For this purpose the current biotechnological and molecular biology techniques provide knowledge on transcription factors to detect stress-responsive genes of the plant. Further proteomics and genomics information is mandatory to know all cellular processes under stress response for better crop improvement.

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