Biological Agents in *Fusarium* Wilt (FW) Diagnostic for Sustainable Pigeon Pea Production, Opportunities and Challenges

Deepak Kumar Verma, Shikha Srivastava, Vipul Kumar, and Prem Prakash Srivastav

Introduction

Pulses constitute one of the richest sources including valuable but incompletely balanced protein, particularly in vegetarian's diet (Ghadge et al. 2008) and are consequently known as an important part of the diet in many regions on the earth (Arinathan et al. 2003, 2009). Among the pulses crops, Pigeon pea [*Cajanus cajan*, (L.) Millspaugh] is a diploid (2n = 22, 44, or 66 chromosomes), most widely produced and consumed food legume worldwide. It also known as arhar, congo pea, tur dhal, frijol de árbol, gandul, gandure, gungo pea, no eye pea, poiscajan and red gram (Long and Lakela 1976) and belongs to family *Leguminosae*. The fruit of the pigeon pea are classified as a pod and each pod have three to five seeds with round or lens like shape.

Pigeon pea is an important grain legume crop of rain-fed agriculture in the semi-arid tropics (Mallikarjuna et al. 2011), which have probably originated from India but some people believe that it may have come from Africa. It is evident that pigeon pea had been originated in India and Asia, and moved to African countries

D.K. Verma (✉) • P.P. Srivastav
Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur 721 302, West Bengal, India
e-mail: deepak.verma@agfe.iitkgp.ernet.in; rajadkv@rediffmail.com; pps@agfe.iitkgp.ernet.in

S. Srivastava
Department of Botany, Deen Dyal Upadhyay Gorakhpur University, Gorakhpur 273 009, Uttar Pradesh, India
e-mail: shikha.sriv13@gmail.com

V. Kumar, PhD (✉)
Department of Agriculture, Lovely Professional University, Phagwara 144 411, Punjab, India
e-mail: vipulpathology@gmail.com

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(Onyebuashi 1986). India and Africa both have been the centres of diversity for the genus *Cajanus* (van der Maesen 1990). Nowadays pigeon pea has become prominently second most important pulse crop after chickpea in India and fifth in the world. This pulse crop is widely cultivated between 30° N and 30° S in all tropical and semi-tropical zone of both the old and the new world including about 50 countries of Asia, Africa, and the Americas for a variety of uses in addition of food and fodder. For instance, green manure, soil conservation, rearing lac insects, wind breaks, fuel wood, hedges, roofing, and so on (Long and Lakela 1976; Sharma et al. 2006; Mallikarjuna et al. 2011; Patel and Patel 2012). India is well known as the biggest producer and consumer of pigeon pea. India has been leading producer of pigeon pea since long decades producing about 265,000°MT followed by Myanmar (900,000°MT), Malawi (237,210°MT), United Republic of Tanzania (206,057°MT), Kenya (89,390°MT), Uganda (84,200°MT), Dominican Republic (27,998°MT) and Nepal (14,082°MT) (FAOSTAT 2012).

Pigeon pea is used as proteinaceous food crops as well as nutritional alternative for human consumption and animal feed along with cereals. It is also grown as forage/cover crop which symbiotically fixes about 90 kg nitrogen per hectare (Adu-Gyamfi et al. 1997). It is an economic crop which is considered as major source of proteins for poor communities in many tropical and subtropical parts of the world viz. India, Myanmar, Malawi, United Republic of Tanzania, Kenya, Uganda, Dominican Republic, Nepal etc. (Singh et al. 1984; ICRISAT 1986; FAOSTAT 2012). Many developing countries including India have inadequate availability of proteinaceous foods. This is a global concern because a large number of populations of these developing countries are suffering from protein malnutrition (Arinathan et al. 2009; Soris and Mohan 2011). Only 20–30 % of proteins are estimated to meet the demand of world's population by the total legume production which is similar to wheat and over 50 % more than the rice or corn crop (Rockland and Radke 1981; Gopalan et al. 1985). Researchers are searching the available substitute of proteins for human nutrition that can impart the nutritional demand of pigeon pea in daily diet of human as protein contribute immense health-related benefits and also possess the best alternative due to their high nutritional value. Pigeon pea contain a high level of crude protein ranges from 21 % to 30 % and mostly some important essential amino acid like, methionine, lysine and tryptophan with phenyl alanine+tyrosine found to be of higher in content (110.4 mg/g of protein) (Udedibie and Igwe 1989; Amaefule and Onwudike 2000). Starch store energy and also known as the major constituent of pigeon pea (Ihekoronye and Ngoddy 1985). In addition, pigeon pea contains considerable amount of vitamin-B complex viz. thiamine, riboflavin and niacin (Bressani and Elias 1974; Arora 1977). Thus, pigeon pea is a staple crop because of its nutritional potential.

Pigeon pea suffers by their natural enemies viz. fungi (83), bacteria (4), viruses and mycoplasma (19) and nematodes (104) over 210 pathogens, reported in 58 countries (Nene et al. 1989, 1996; Reddy et al. 1990). Several fungal pathogens are involved to infect pigeon pea crop such as *Alternaria* spp., *Colletotrichum* spp., *Cercosporaindica*, *Sclerotium rolfsii*, *Rhizoctonia* spp., *Fusarium*spp, *Phytophthora* spp., *Xanthomonas* spp., *Pseudomonas* spp. etc. A list of fungal, bacterial, viral as

Table 1 Major diseases of pigeon pea and their pathogenic agents

Disease	Pathogenic agent
Fungal diseases	
Alternaria blight	*Alternaria* spp., *A. alternata* (Fries) Keissler, *A. tenuissima* (Kunze ex Persoon) Wiltshire
Anthracnose	*Colletotrichum cajani* Rangel, *C. truncatum*, *C. graminicola* (Ces.) Wilson
Botrytis gray mold	*Botrytis cinerea* Persoon ex Fries
Cercospora leaf spot	*Mycovellosiella cajani* (*Cercospora cajani*), *Cercospora indica*, *C. instabilis*, *C. thirumalacharii*
Collar rot	*Sclerotium rolfsii* Saccardo, *Athelia rolfsii* [teleomorph] = *Corticium rolfsii*
Dry root rot	*Macrophomina phaseolina* (Tassi), Goidanich *Rhizoctonia bataticola* (Taub.) Butler
Fusarium leaf blight	*F. pallidoroseum* (*F. semitectum*)
Fusarium wilt	*F. udum* Butler, *Gibberella indica* [teleomorph]
Phoma stem canker	*Phoma cajani* (Rangel)
Phyllosticta leaf spot	*Phyllosticta cajani*Sydow
Phytophthora blight	*Phytophthora drechsleri* Tucker *f. sp. cajani*
Powdery mildew	*Leveillula taurica* [Teleomorph], *Oidiopsis taurica* [Anamorph], *Ovulariopsis ellipsospora*
Rust	*Uredo cajani* Sydow
Bacterial diseases	
Bacterial leaf spot and stem canker	*Xanthomonas campestris* pv. *Cajani*
Halo blight	*Pseudomonas amygdali* pv. *Phaseolicola*
Viral diseases	
Phyllody	Mycoplasma-like organism vector: not known
Sterility mosaic	Vector: Eriophyid mite *Aceria cajani* Channabasavanna
Witches' broom	Mycoplasma-like organism vector: leaf hopper *Empoasca* spp.
Yellow mosaic	Mung bean yellow mosaic virus (MBYMV)
	Vector: India, Jamaica, Nepal, Puerto Rico, and Sri Lanka
Nematode diseases	
Dirty root (reniform nematode)	*Rotylenchulus reniformis* Linford and Oliveira
Pearly root (cyst nematode)	*Heterodera cajani* Koshy
Root-knot (root-knot nematode)	*Meloidogyne acronea* Coetzee, *M. arenaria* (Neal) Chitwood, *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood

Sources: Reddy et al. (1993b)

well as nematode diseases is summarized. (Table 1) (Kannaiyan et al. 1984; Hillocks et al. 2000; Joshi et al. 2001; Maisuria et al. 2008). The diseases of pigeon pea have significant importance including Phytophthora blight (*Phytophthora drechsleri*) (Kannaiyan et al. 1984), powdery mildew (Reddy et al. 1993a), sterility mosaic (Pigeon pea sterility mosaic virus) and wilt. Among them, FW is considered as most destructive disease which is described in detail.

Biological Agents for Plant Health Diagnostic (PHD), Why?

Plant health is a big issue throughout the world to fulfil increasing food demand and balanced food supply. Outbrake of plant diseases and upsurge of insect-pests pose a serious threat to food security. Both of the agents affect plants health leading to significant crop loss and hence productivity worldwide. These agents are needed to be controlled and more emphasis should be given to maintain the quality and abundance of food to mitigate the food demand of world's population. Different approaches are used in disease diagnosis to maintain the plant health. Among them, biological agents have become most promising agents to secure plant health from their pathogens. Biological control is free from use of chemicals, so it is eco-friendly approach which can be helpful to discard some environmental problems like bio-accumulation, bio-magnification and bio-diversity loss.

Plant health diagnostic (PHD) through biological agents is propitious contribution in crop productivity reported over the few decades. Miller et al. (2009) reviewed "Plant Disease Diagnostic, Capabilities and Networks" and stated that diagnostic of plant disease and detection of their pathogen are central to our ability to protect crops and natural plant systems, and are the crucial prelude to undertake management and prevention measures of PHD. According to Miller et al. (2009), Plant disease diagnostic is the determination of the cause of a disease or syndrome in a plant or plant population, whereas detection refers to the identification of microorganisms or their products, e.g., toxins, in any number of substrates including plant tissues, soil, and water.

Recently, biological diagnostic of plant disease (BDPD) have been recognised as swift alternative to chemical fungicides (Fig. 1) and more focused by researches because of the sustainability and eco-friendly. Recently, BDPD has been emerged as a useful technique of organic, eco-friendly and sustainable agriculture involving the use of antagonistic microorganisms to combat the various diseases in most of the crops. BDPD can be proved as best tool to secure pigeon pea from pathogens and control the target organism without being harmful to humans or any beneficial organisms in natural eco-systems. Nowadays the use of promising microorganisms or their formulations have been attracted attention due to increased incidence of disease. These promising microorganisms belong to bacterial as well as fungal genera are registered and commercially available (Table 2).

Fusarium Wilt (FW): Major Disease of Pigeon Pea

Fusarium wilt (FW) caused by soil borne pathogenic fungus *Fusarium udum* (Butler) is one of the major diseases of pigeon pea severely affecting demand, economy, production and seed yield worldwide (Kannaiyan et al. 1984; Reddy et al. 1990; Ajay et al. 2013). The loss of crop begins from pre pod stage. Total loss in

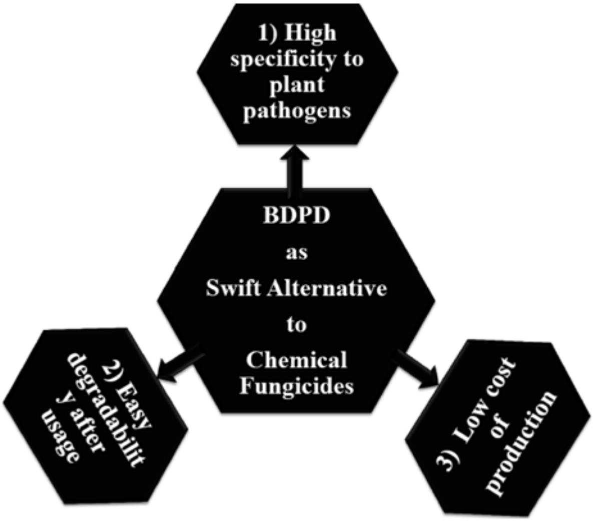

Fig. 1 Biological diagnostic of plant disease (BDPD) as swift alternative to chemical fungicides

Table 2 Promising microorganisms and their formulated products in plant disease diagnostics

Bacterial genera	Fungal genera
Agrobacterium spp.	*Ampelomyces* spp.
Bacillus spp.	*Candida* spp.
Pseudomonas spp.	*Coniothyrium* spp.
Streptomyces spp.	*Gliocladium* spp.
	Trichoderma spp.

Source: Vinale et al. (2008)

yield may ranges from 30 % to 100 % in pre-pod stage, about 67 % and 30 % at crop maturity and pre-harvest stage, respectively and almost 100 % yield losses in susceptible genotypes (Nene 1980; Upadhyay and Rai 1992; Kannaiyan and Nene 1981; Sheldrake et al. 1984; Reddy et al. 1990). The annual loss due to FW disease in India and eastern Africa is estimated to be approximately at US $71 and US $5 million (Reddy et al. 1993a, b; Saxena et al. 2002). The scenario of disease incidence in India is reported maximum in Maharashtra (22.6 %) and minimum in Rajasthan (0.1 %) (Kannaiyan et al. 1984; Upadhyay and Rai 1992). It is reported that the incidence of FW disease have to increased significantly over the time (Gwata et al. 2006) with an average of 10–15 % incidence and 16–47 % of crop loss (Prasad et al. 2003). The global crop loss due to FW disease is reported by Kannaiyan et al. (1984) and it was found to have 15.9 % (0–90 %), 20.4 % (0–60 %) and 36.6 % (0–90 %) in Kenya, Tanzania and Malawi respectively with annual loss estimated at 5 million US$ in each of the countries with 96 % of disease incidence in Tanzania (Mbwaga 1995). This disease poses annual loss by 470,000 and 30,000 ton of total grain production in India and Africa respectively which affects the economy by 71 million US$ (Reddy et al. 1993a; Joshi et al. 2001).

Fusarium udum: The Pathogenic Agent

Fusarium udum Butler. (Perfect stage: *Gibberella udum*) is causal organism of wilt disease of pigeon pea. In 1906, Butler firstly reported FW disease of pigeon pea in India (Butler 1906). The pathogenic agent was described as *F. udum* by Butler in 1990 (Butler 1910) and the fungus has subsequent multiple names *F. butleri*, *F. lateritium* f. spp. *cajani*, *F. lateritium* var. *uncinatum*, *F. oxysporum* f. spp. *udum*, *F. udum* f. spp. *cajani* and *F. uncinatum* (Dhar et al. 2005). The name *F. udum* suggests the presence of prominent hook shaped macro-conidia (Booth 1971). *F. udum* is a host specific (pigeon pea) pathogen with consistent pathogenic variability and morphological differences (Padwick 1940; Subramanian 1963; Booth 1971).

The mycelium of this fungus may be parasitic or saprophytic, hyaline, slender and branched. *F. udum* produces different type conidia (like macro and micro) and chlamydospores (Fig. 2). Macroconidia are 1–5 septate (predominantly 3 septate), curved to almost hooked and abundant in sporodochia (Fig. 2a–d) whereas microconidia are fusiform to reniform or oval and 0–1 septate (Fig. 2e–i). Chlamydospores are round or oval, thick walled, hyaline, sometimes in short chains, 5–10 μ in diameter (Fig. 2g–i). Perfect stage of pathogen (*G. udum*) needs further investigations. So far, five variants (strains) of *F. udum* have been identified and documented (Reddy et al. 1996; Mishra 2004).

In 2013, 14 isolates of *F. udum* from pigeon pea collected by Datta and Lal (2013) from major pulse growing parts of India and confirmed the genetic diversity between the races of FW in pigeon pea. A research paper was published in 1983 that revealed five category of *F. udum* on the basis of virulence differences (Pawar and Mayee 1983). Patil (1984) reported 9.4–12.0×3.1–3.3 μm size of conidia, 19.2×3.5–5.0 μm of macro conidia and it was mostly found to be whitish in the basal medium. Six isolates of *F. udum* described by Madhukeshwara and Sheshadri

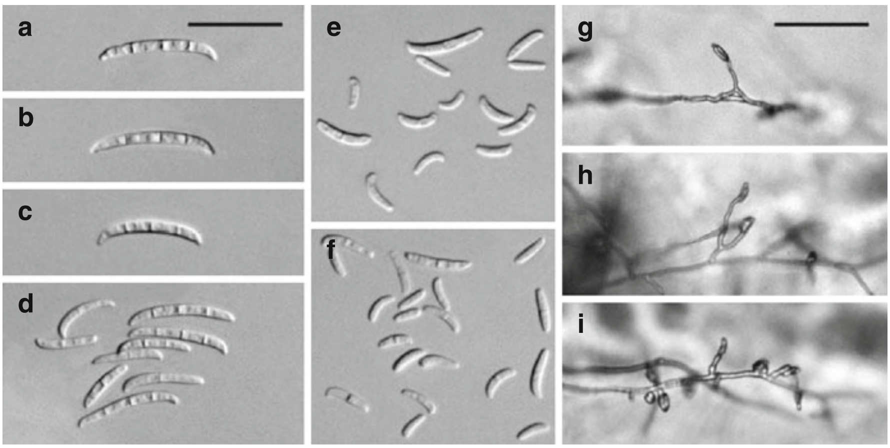

Fig. 2 Type of conidia produces by *F. udum*, they are macroconidia (**a**–**d**) and microconidia (**e**–**i**) (Adapted from Leslie and Summerell (2006))

(2001) with different colony characteristics, pigmentation and sporulation. One hundred ninety-five isolates of *F. udum* has been isolated (IIPR 2007–2008) and revealed that 135 were highly pathogenic (>50 % wilt), 33 moderately pathogenic (30–50 % wilt) and 32 were weak pathogenic (<30 % wilt) agent.

Distribution of Pathogen

Currently, FW diseases is considered as highly destructive (Nene et al. 1989) and distributed in form of both, seed borne as well as soil borne in several countries namely Bangladesh, Ghana, Grenada, Grenada, India, Indonesia, Kenya, Malawi, Mauritius, Myanmar (Burma), Nepal, Nevis, Tanzania, Thailand, Tobago, Trinidad, Uganda, Venezuela, Zambia etc. throughout the globe at where the field loss are widely prevalent (over 50 %) and more common in India, east Africa and Malawi (Kannaiyan and Nene 1981; Kannaiyan et al. 1984; Kimani 1991; Reddy et al. 1993a; Marley and Hillocks 1996; Ajay et al. 2013).

Disease Symptoms

The first symptoms of FW disease is usually seen in the field during early developmental stages (Fig. 3) when flowering and podding appears in the crop, sometimes may also be seen at seedling stage (Prasad et al. 2003) but never visible in later

Fig. 3 *Fusarium* wilt symptoms in the pigeon pea field appear during flowering and podding of early developmental stages but may be at seedling stage also

Fig. 4 (**a**) Prominent internal browning and blackening on 1–2 months old plants die from wilt (**b**), Development of *dark purple* bands on the stem surface extending upwards from the base, (**c**) Visible *black* streaks in xylem strands on the main stem or primary branches when it split open, and (**d**) Die-back symptoms with a purple band on branches extending from tip of the plant to downwards and starts drying (Pictures adapted from Reddy et al. (1993b))

developmental stages of pigeon pea (Reddy et al. 1990; Hillocks et al. 2000). The pathogen infects the host via vascularisation of injured root tips causing chlorosis of leaves and branches, wilting and collapsing of root system (Jain and Reddy 1995; Butler 1906). The pioneer symptom of FW is interveinal clearing and loss of turgidity in leaves with slight chlorosis. Leaves appear bright yellow before wilting (Reddy et al. 1990). FW infection is caused via tap root system and results into total wilt. There are many other factors that lead to partial wilting of plants like termite damage, drought and phytophthora blight (Nene 1980; Reddy et al. 1993a, b).

Diagnostic symptoms of FW appear as brown or black streaks on stem surface (Fig. 4a) which turns dark purple extending towards the tip of the main stem (Fig. 4b). The symptoms are more visible in interior section of the main stem or primary branch (Fig. 4c) (Reddy et al. 1990, 1993b). The severity of streaks reduces from base to the tip of the stem. Sometimes the streaks are not visible on main stem but lower branches start becoming non-viable due to die back symptoms which includes appearance of purple bands or streaks extending from upward to downward and blackening of xylem vessels (Fig. 4d) (Reddy et al. 1993b). It is also observed that young plants (1–2 months old) infested with FW may die due to partial wilt without showing characteristic purple bands (Fig. 4b).

Biological Agents in Diagnostic of *Fusarium* Wilt

Biological agents (including bacteria and fungi) have been listed as useful tool for disease diagnostic. Biological agents are devoid of chemical substances and can control target organism efficiently (Romero et al. 2007; Suarez-Estrella et al. 2007; Whipps and McQuilken 2009). These agents are environmental friendly, can be utilized experimentally for the control the enemies of crop plants without causing ill affect to human health or any beneficial organisms (Kaewchai et al. 2009). Some bacterial genera viz. *Bacillus*, *Pseudomonas* and *Rhizobium* and non-pathogenic, non-host *Fusarium* spp. are used as potent biological agent against the pigeon pea disease. Both in field and vitro study inferred significant reduction of disease incidence (Chérif et al. 2007). Biological agent mediated control has been a promising and attractive alternative for PHD and soil borne pathogens as it mitigate the adverse effects of fungicides and pesticides to the farmland.

There are many novel microorganism species viz. *Aspergillus* spp., Bacillus spp., *Pantoea* spp., *Pseudomonas* spp. and *Trichoderma* spp. were evaluated as potential alternative to replace the chemical such as thiram, bavistin and benomyl etc. against fungal pathogen *F. udum* (Upadhyay and Rai 1981; Bhatnagar 1996; Somasekhara et al. 1996, 1998; Gundappagal and Bidari 1997; Biswas and Das 1999; Prasad et al. 2002; Khan and Khan 2002; Anjaiah et al. 2003; Sawant et al. 2003; Roy and Sitansu 2005; Dhar et al. 2006; Maisuria et al. 2008; Ram and Pandey 2011). Many profitable rhizobacteria have been reported by many worker as bio-inoculants (Pusey 1989; Upadhyay and Rai 1992; Bapat and Shar 2000; Siddiqui et al. 2005; Siddiqui 2006; Siddiqui and Shakeel 2007). It has been shown that fungal or bacterial antagonists of pathogen inoculated to soil reduces FW and its pathogenesis (Bapat and Shar 2000; Singh et al. 2002; Anjaiah et al. 2003; Mandhare and Suryawanshi 2005; Maisuria et al. 2008). The mechanism for biological diagnostic of pigeon pea disease is shown in Fig. 5. The mechanisms of biological diagnostic of pigeon pea have different modes of action which are not pathogen specific and many of these mechanisms may be synergistically active and used by the same biological agent (Chérif et al. 2002; Mandeel and Baker 1991) which may not have efficacy to control the major diseases of pigeon pea.

Upadhyay and Rai (1981) reported many species of fungi viz. *A. niger*, *A. flavus*, and *A terreus* could be used for suppression of the population of *F. udum*. Soil antagonistic bacteria are well known to suppress the wilt through inducing resistance (Upadhyay and Rai 1981, 1992). Isolation of indigenous *Bacillus* spp. from the disease suppressive soil of the same environment may increase the probability of disease suppression (Cook and Baker 1983; Weller et al. 1985). Harman et al. (1989) studied combined effective strains of *T. harzianum* and solid matrix priming for biological seed treatment. The production of antibiotics by *P. cepacia* was used as biological control agent for soil borne plant pathogens (Homma et al. 1989). Bhatnagar (1996) studied the antifungal activity of three *Trichoderma* spp. as multiple action bio-inoculants and to control variable pathogenesis against wilt pathogen at different pH, temperatures and C/N ratios and found that all of them were equally efficient and showed maximum antagonistic properties at 35 ± 2 °C temperature and about of 6.5 pH.

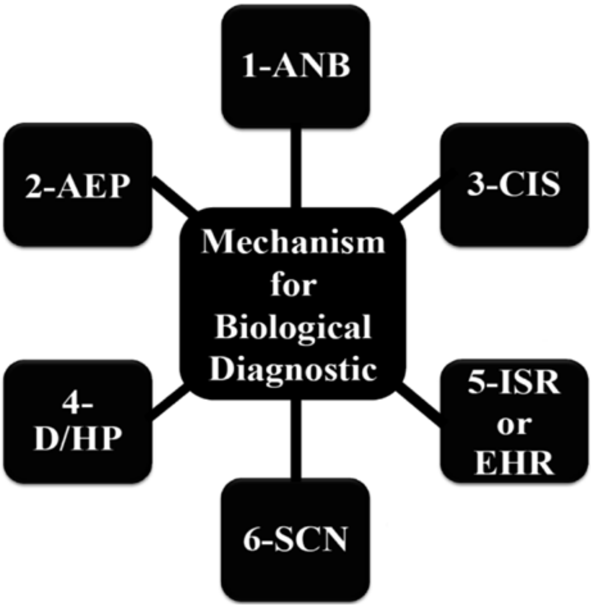

Fig. 5 Biological diagnostic mechanism for pigeon pea disease. (*1*) ANB (Antibiosis), (*2*) AEP (Antifungal enzyme production), (*3*) CIS (Competition for infection sites), (*4*) D/HP (Direct/Hyper parasitism), (*5*) ISR or HER (Induced systemic resistance or Enhanced host resistance, and (*6*) SCN (Saprophytic competition for nutrients) (Concept adapted from Chérif and Benhamou (1990), Fuchs et al. (1997), and Chérif et al. (2002, 2003))

Apparently, Somasekhara et al. (1996) worked on two delivery systems (seed treatment and foliar application) by using six isolates of *Trichoderma* spp. and studied their efficacy which was found to be extreme on the 35 days of inoculation. As the plant is resistance to dry period, Gundappagal and Bidari (1997) used *T. viride* for seed treatment to resistant cultivar that can be effective in integrated disease management of pigeon pea under dry land cultivation. *Trichoderma* spp. are well known producer of extracellular volatile compound, which was found to be potent fungi toxic to wilt pathogen (Pandey and Upadhaya 1997). Somasekhara et al. (1998) evaluated *Trichoderma* isolates and their antifungal extracts as potential bio-control agents against pigeon pea wilt pathogen, *F. udum*. Butler observed that non-volatile antibiotics of *T. viride* was highly toxic followed by *T. harzianum*, *T. harzianum* and *T. koningii*.

Biswas and Das (1999) performed in-vitro experiments to reduce pathogenesis and tested five *Trichoderma* spp. *T. harzianum* was found to be most effective antagonist followed by *T. hamatum*, *T. longiconis* and *T. koningii*. They also reported that by giving seed treatment of *T. harzianum* to pigeon pea, inoculants spores failed to reduce pathogen growth while soil amendment with *T. harzianum* in maize meal: sand applied at 40–60 g/kg soil resulted a significant reduction of wilt up to 90 %. Under field conditions, Prasad et al. (2002) studied the effect of soil and seed application of *T. harzianum* on pigeon pea wilt caused by *F. udum* and inoculation with *T. harzianum* controls the disease by 22–61.5 %. Khan and Khan (2002) confirmed differential behavior of multiple bio-control agents (*Trichoderma*, *Bacillus*, *Pseudomonas*) controlling FW and recorded 17–48 % of decrease disease incidence. Khan and Khan (2002) also observed that rhizospheric application of *B. subtilis*, *P. fluorescens*, *A. awamori*, *A. niger* and *Penicillium digitatum* resulted in significant decline of *F. oxysporum*.

Biological control of FW of pigeon pea had been reported by Vaidya et al. (2001, 2003) with chitinolytic activity of *Alcaligenes xylosoxydans*. Vaidya et al. (2003) conducted a pot experiment and field trials. *A. xylosoxydans* was used to treat pigeon pea seeds because it has antifungal activity due to chitinase production. The treated seeds were sown in *Fusarium* infested soil. He found that the incidence of wilt was reduced by 43.5 % and grain yield was increased by 58 %. Anjaiah et al. (2003) studied bio-control experiment to investigate the effect of genotype and root colonization in biological control of FW and reported that disease incidence of wilt was drastically reduced after inoculation of *P. aeruginosa* (PNA1) to both chickpea and pigeon pea in naturally infested soil. de Boer et al. (2003) experimented on combined *P. putida* strains to control of FW as it has different disease-suppressive mechanisms. Siddiqui et al. (2008) studied biological control of wilt disease of pigeon pea by fluorescent pseudomonads under pot and field conditions. He isolated a *Pseudomonas* strain Pa324, known as strong antagonist of *F. udum* and reported that this strain had an ability to produce hydrogen cyanide (HCN) and siderophore in excessive amount. Sometimes HCN is called as prussic acid (Gail et al. 2005). These bacterial origin volatile compounds produced by many fluorescent pseudomonads in the exponential growth phase in media containing $FeCl_3$ or inorganic phosphate may also influence plant root pathogen (Voisard et al. 1989) and suppresses the diseases (Glick 1995).

The efficacy and comparison of different biological control agents and their products studied by Sawant et al. (2003) against wilt of pigeon pea showed reduced wilt incidence by *Trichoderma* spp., and seed treatment with its formulated cell mass at 8 g/kg seed recorded the lowest wilt incidence. Many mutational and recombinant bio-inoculants have been experimented in this field to reduce the wilt incidence and found to be successful.

Roy and Sitansu (2005) published a research paper on biological control potential of some mutants of *T. harzianum* against wilt of pigeon pea and reported that recombinant *T. harzianum*, 50Th3II and 125Th4I reduced the wilt disease in non-sterilized soil, while 75Th4IV reduced the wilt disease in sterilized soil with a percentage of 36.51 %, 33.86 % and 33.33 % respectively. The application of *Trichoderma* spp. for managing FW of pigeon pea has been recommended by Mandhare and Suryawanshi (2005) as a seed treatment and soil application. The efficacy of *Trichoderma* spp. against pigeon pea wilt caused by *F. udum* was studied by Jayalakshmi et al. (2003). The observation of the study suggested that the seed of pigeon pea treated with *T. viride* followed by *T. harzianum* was found to be effective in reduction of the wilt disease by controlling *F. udum* effectively, when compared with individual treatments. In 2006, differential efficacy of bioagents namely *T. viride*, *T. harzianum* and *Gliocladium virens were* combined used by Dhar et al. (2006) against *F. udum* isolates and showed up to 35.5–57.3 % of reduction in disease incidence in FW of pigeon pea.

Burkholderia spp. reported as potential biological control agent (Heydari and Misaghi 1998; Zaki et al. 1998). Pandey and Maheshwari (2007) studied on bioformulation of *Burkholderia* spp. and reported antifungal properties against *F. udum*. These properties were due to an antibiotic 2-hydroxymethyl-chroman-4-one produced by *Burkholderia* spp.

Several *Bacillus* spp. have been proved to be used as bio-control agents for reduction of pathogen growth and disease incidence across the world (Siddiqui 2006). Many scientific evidences are available in literature, which have been reported that *Bacillus* species, most commonly found soil bacteria are excellent biocontrol agent (Dal-Soo et al. 1997; Bacon et al. 2001; Basha and Ulaganathan 2002; Chaurasia et al. 2005). Bapat and Shar (2000) used *B. brevis* as biological control agent of FW of pigeon pea as it produce antibiotic substance, which inhibit the growth of *F. oxysporum* and F. *udum* pathogen. Pandey et al. (2006) isolated HCN producing *Bacillus* spp. under in-vitro conditions. This inorganic compound reduces the radial growth of *F. udum*. Siddiqui and Shakeel (2007) screened *Bacillus* strains (B603, B613, B615) which had biological control potential against wilt disease of pigeon pea (*C. cajan*) under greenhouse and small-Scale field conditions. He found these agents can be used against *F. udum*, in both pot and field experiments and reported to be effective in terms of reduction in fungal growth and disease incidence. In 2008, Maisuria et al. (2008) reported *Pantoea dispersa* as biological control agent for FW of pigeon pea in field assessment.

Integrated management was recommended by Mahesh et al. (2010) in a combined way such as systemic fungicide, biological control agent and farmyard management as one of the most effective treatment of *F. udum* to control its infestation globally. The study showed considerable efficacy in controlling wilt incidence and increasing yield compared to untreated control with mean wilt incidence of 63.53 % and an yield of 362.72 kg/ha. Recent reports (Ram and Pandey 2011) suggested the combined use of *T. viride* and *P. fluorescens* for reduction of growth of *F. udum*. In 2011, by Gopalakrishnan et al. (2011) isolated five strains of *Streptomyces* spp. (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) from herbal vermicomposting and reported that they have potential for biological control of FW.

Challenges Raised to Biological Agents in Disease Diagnostics

Field Application

Antagonistic microorganism and its formulation application influences the success of field trials, they are; (1) seed inoculation, (2) vegetative part inoculation, and (3) soil inoculation Several factors like, organic matter (%), pH, nutrient level, and moisture level of the soil influences the potential of antagonists from in vitro tests and efficacy of biological control agents and they often fail to work effectively (Lee et al. 1999).

Mixtures of Multiple Antagonists and Their Efficacy

Several microorganisms and its association are needed to control most pathogens in field. The appropriate combination of the microbial strains and their efficacy against pathogen can be significantly achieved with a higher level of protection (Becker et al. 1997; Raupach and Kloepper 1998; de Boer et al. 2003; Davelos et al. 2004).

Genetic Manipulation

The molecular techniques have been employed for strains modification to improve their ability against the soil borne pathogens. Advanced technologies in molecular genetics and genomics are been introduced to enhance new possibilities for improving the characterization, selection and management of biological control. The development in functional genomics-proteomics can give us the expression of crucial genes of biological control agents during mass production, application and mechanism of action. The major challenges in genetic manipulation of biological agents for disease diagnostic are the insertion of appropriate genes that express their antagonists to achieve the efficient control over plant pathogen (Baker 1989).

Whole-Genome Analysis

The revolutionary high throughput DNA sequencing of whole genomes have resulted tremendous success for understanding the mechanism of action of biological agents. The construction of artificial chromosome viz. bacterial artificial chromosome (BAC) and yeast artificial chromosome (YAC) libraries gene expression study and identification of genes of interest is of great value, especially in bacteria whose genome has not been sequenced, but having promising disease diagnostic potential (Rondon et al. 1999).

Formulation and Methods of Application

A correct formulation and right method of application of biological agents and its formulation are the major challenges. There is a lack of best alternative to come out of these challenges because formulations are being carried out without methodology. Greater efficacy, increased safety, lower production costs, ease of handling and compatibility with agricultural practices are major advantages of formulation.

Opportunities for Future Research

Significant efforts to broaden the genetic base and introduction of various traits for desirable biotic and abiotic stress are one of the important aspects of "Biological Agents in *Fusarium* Wilt (FW) Diagnostic for Sustainable Pigeon Pea Production, Opportunities and Challenges". Currently, fundamental knowledge in computing, molecular biology, biotechnology, statistics and chemistry have led to new research aimed at characterizing the functions of biological agents, pathogens, and host plants at sub-cellular and ecological levels. Biological agents in disease diagnostic are of supreme importance in the present crop production scenario, but its potential is still to be utilized and needs attention to produce the commercial formulations. Biological agents and their formulations are commercially available in market. But not getting adequate attention due to lack of information regarding its importance and use for sustainable production. Many research challenges are raised in this area to explore the biological agents for diagnostic of plant disease and have already been discussed above in five major points (see sections "Field Application", "Mixtures of Multiple Antagonists and Their Efficacy", "Genetic Manipulation", "Whole-Genome Analysis", and "Formulation and Methods of Application"). The challenges need to be addressed by the scientific community to solve the issue of use of multiple biological agents, their combined action on diagnostic of plan disease by controlling the plant pathogen.

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