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AN OVERVIEW OF VARIABILITY IN *FUSARIUM UDUM*, THE INCITANT OF WILT IN PIGEONPEA

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Pigeonpea (*Cajanus cajan* (L) Mills.) is an important pulse crop in semi-arid tropical and sub tropical farming systems adopted by small and marginal farmers, providing high quality vegetable protein to human beings and is one of the animal feed and fire wood. Pigeonpea is commonly known as redgram, arhar and tur and is an important pulse crop grown for its dhal. In India, crop is grown in an area of about 3.73 m ha with annual production of 2.31 m tonnes and productivity about 678 kg/ha (Anonymous, 2010).

Though several factors are known to affect pigeonpea cultivation, the most important being the diseases. Some of the important diseases are *Fusarium* wilt, *Phytophthora* blight, *Cercospora* leaf spot, collar rot, dry root rot, *Alternaria* leaf spot, powdery mildew, sterility mosaic and phyllody. Incidentally, only a few of them causes economic losses in India (Kannaiyan *et al.*, 1984). Among the diseases *Fusarium* wilt caused by *Fusarium udum* is the most important soil borne disease and was first reported from Bihar state in India (Butler, 1906). The disease appears on young seedlings but the highest mortality occurs during flowering and podding stage. Although the disease first appears in patches in a field and can extend to entire field if pigeonpea is repeatedly cultivated in the same field.

The yield loss of pigeonpea depends on the stage at which the plants wilt and it can approach 100, 67 and 30 per cent when wilt occurs at pre-pod, maturity and pre harvest stages, respectively (kannaiyan and Nene,1981) and sometimes it causing upto 100% loss in grain yield (Okiror, 2002). The disease incidence has been increasing year after year and most of the released cultivars became susceptible to the disease indicating the development of more virulent races of the pathogen in major pigeonpea growing areas of the state.

In this chapter we have critically reviewed Disease and the causal organism, Economic importance of disease, Distribution and survey on disease incidence, Pathogenic variability, Biochemical variability and Genetic variability aspects of wilt of pigeonpea.

THE DISEASE AND THE CAUSAL ORGANISM

The genus *Fusarium* was erected by Link in 1809 for the species with fusi form, non-spetate spores borne on a stroma (Booth, 1971).

Butler (1906) published a detailed account on *Fusarium* species and reported pigeonpea wilt for the first time in India from Bihar. Butler (1910) carried out the isolation, identification and established the causal organism *F. udum* as a new species. In the past *F.oxysporum* f.sp. *udum* was frequently used. However, the name *F. udum* has been finally accepted and put in elegance group (Wollenweber and Reinking, 1935).

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Mitra (1934) reported that wilts of sunhemp (*Crotalaria juncea*) and pigeonpea (*Cajanus indicus*) were chiefly caused by similar biological strains of *F. vasinfectum*.

Padwick (1940) tried the ability of the species of *Fusarium* isolated from cotton, pigeonpea and sunhemp to pass from one host to another and cause infection. He also studied cultural characters of *F. udum* and found that it produced abundant spores in sporodchia which were strongly hooked at the apex and proposed the name *F. udum* Butler var. *cajani* and it differed from *F. vasinfectum*.

The fungus *F. udum*, like other *Fusarium* spp, showed a great variation in cultural characters. Butler's description revealed that *F. udum* occured as parasite within the roots of the host plant. Saprophytic culture on agar medium showed deep purple pigmentation, aerial mycelium almost absent and usually with the profuse development of pinnate sporodochia. Microconidia were one celled, hyaline, ovoid/fusoid or curved measuring $6-11 \times 2-3\mu$. Macroconidia were hyaline, typically thin walled, 1-3 septate or occasionally with 5 septa, falcate with a distinct foot cell and an apical cell of decreasing diameter towards the tip which may be curved or hooked, measuring 15-30 x 2.5-3.5 μ . Chlamydospores were globose, intercalary in the mycelium measuring 8-10 μ diameters (Butler, 1910). The macroconidia were distinguished by a prominent hook (Booth, 1971).

The causal organism is a soil borne facultative parasite that enters through roots and then becomes systemic invading tap root, lateral roots, main stem, branches, leaflets, petioles, rachis and pedicel (Nene *et al.*, 1980).

Upadhyaya and Rai (1982) reported the perfect state of *F. udum* on wilted and dead pigeonpea plants near Varanasi in Uttar Pradesh and identified it as a new species of *Gibberella* and named it as *Gibberella indica*.

Synonyms of *F. udum* are *F. butleri* (Wollenweber, 1913), *F. lateritium* var. *uncinatum* (Wollenweber, 1931), *F. oxysporum* f. sp. *udum* (Snyder and Hansen, 1940), *F. lateritium* f. sp. *cajani* (Gordon, 1952), *F. udum* var. *cajani* (Padwick, 1940). At present *F. udum* is widely accepted as a name of imperfect state of wilt pathogen (Subramanian, 1971; Booth *et al.*, 1978; Gerlich and Nerenberg, 1982; Upadhyaya and Rai, 1989).

ECONOMIC IMPORTANCE OF DISEASE

Eshwar Reddy and Basuchoudhary (1985) reported upto 22.5 per cent damage to the pigeonpea crop due to *Fusarium* wilt.

According to Khare *et al.* (1994) wilting symptoms at pre-flowering and podding stage caused 100 per cent, at maturity 67 per cent and at pre harvesting stage 30 per cent loss.

Ranjeet Singh *et al.* (2002) observed a yield loss of 10 to 50 % and in some years up to 90 % in pigeonpea due to *Fusarium* wilt in farmers fields.

DISTRIBUTION AND SURVEY ON DISEASE INCIDENCE

Wilt is the most destructive disease of pigeonpea in India. The disease widely occurs in Asia and Africa. The occurrence and distribution of the disease was earlier doubtful beyond India (Butler, 1906).

Booth (1971) reported the wilt disease from Tanzania, Uganda, Germany, Italy, Vietnam, Kenya, Thailand, Indonesia and Trinidad.

Sharma and Srivastava (1977) conducted a survey on the wilt incidence in 27 districts of Madhya Pradesh at the maturity stage of pigeonpea and reported maximum disease from Shajapur and Baster districts and minimum disease in rest of the districts.

Kannaiyan and Nene (1981) reported the pigeonpea wilt from Uttar Pradesh, Bihar, Madhya Pradesh, Rajasthan, Gujarat, Maharastra, West Bengal, Orissa, Andhra Pradesh, Karnataka and Tamil Nadu. The average incidence varied from 0.1 % in Rajasthan to 22.6 % in Maharastra.

The disease was severe in Maharashtra, Bihar and Uttar Pradesh. In Karnataka, the incidence of wilt varied from 0 to 90 per cent. It was severe in major crop growing areas of Gulbarga, Dharwad, Bidar and Bijapur (Kannaiyan *et al.*, 1981a).

In Africa the disease is quite serious in Malawi, Tanzania and Kenya (Kannaiyan et al., 1984).

Gaur and Sharma (1989) surveyed major pigeonpea growing districts of Rajasthan to determine the prevalence of *Fusarium*wilt (caused by *F. udum*) at seedling, flowering and podding stages and indicated that the disease is an important problem only in Alwar and Dholpur districts.

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A survey on *Fusarium* wilt of pigeonpea in 13 districts in northern and southern Malawi in 1993 showed that *Fusarium* wilt was the most widely distributed disease, with an average incidence of 5.4% (Saka *et al.*, 1994).

Survey on wilt incidence was conducted in 84 fields in different districts of Karnataka, and the study revealed that wilt incidence ranging from 0.05 to 67.0 per cent with an average of 7.65 per cent. The mean incidence of wilt was lowest in Bijapur (4.25 %) when compared to other pigeonpea growing districts. The maximum incidence of 67 % was recorded in Gulbarga followed by 35.70 % in Bidar (Bidari, 1995).

Chauhan and Vinod Kumar (2004) surveyed 15 districts of eastern Uttar Pradesh, India to record the incidence of wilt in pigeonpea caused by *Fusarium udum*. The highest Percent disease incidence was reported from Ghazipur district (14.7%) and that of the lowest was from Pratapgarh district (2.4%). Jaunpur, Varanasi, Goarkhpur, Azamgarh were also affected by wilt, with PDI values ranging from 10.4 to 11.8%.

The incidence of wilt disease in pigeonpea was studied in 14 districts of Uttar Pradesh, India, from July to March 2005-06 and 2006-07. Wilt caused by a *Fusarium* sp. was present in all the districts surveyed. Disease incidence ranged from 5 to 18% in 2005-06, and from 7 to 23% in 2006-07. Greatest wilt incidence ranging between 13-18% was recorded in Mahsi districts in 2005-06 and that of 9-23% was recorded in 2006-07 (Manju Srivastava*et al.*, 2008).

Muhammad Saifulla and Mahesh (2009) conducted an extensive roving survey and identified hot spots for *Fusarium* wilt of pigeonpea, in different districts of southern Karnataka during three consecutive *Kharif* seasons from 2004-05 to 2006-07. Among the six districts surveyed during *Kharif* 2004-05 (first year) maximum mean wilt incidence of 12.55% was recorded in Kolar district and disease incidence ranged between 0-90%. During the second year (2005-06), among the five districts surveyed, maximum mean wilt incidence of 13.92% was recorded in Chamarajanagar district and disease incidence ranged between 0-65%. During the third year (2006-07), among the seven districts of southern Karnataka surveyed, maximum mean wilt incidence of 8.13% was recorded in Bangalore urban district with a range of 0-96% where as Tumkur district was free from wilt incidence.

PATHOGENIC VARIABILITY

Variation in cultural and morphological characters of Fusarium species

Variation in cultural characters of F. udum was first observed by Butler (1910).

Prasad (1949) studied thirty three strains of *F. solani* and *F. cucurbitarieae* which were found to differ from each other in culture type, growth rate, pigmentation and size of macroconidia.

Subramanian (1955) observed considerable variation in cultural characters of F. udum.

Venkataraman (1955) showed that culture of *Fusarium*wilt of muskmelon produced a fluffy mycelium with spore number and conidia differing with wild type strain having abundant sporulation.

Sharma and Mathur (1971) showed that the monoconidial lines of linseed wilt pathogen isolated from different linseed growing regions differed in their cultural and morphological characters with marked diversity in virulence.

Jeswani*et al.* (1977) demonstrated that, single spore isolates form single strain also very among themselves with regard to growth pattern, segmentation and capacity of selecting metabolic products.

Shit and Sengupta (1978) reported that, seven isolates of *F. oxysporum* f. sp. *udum* Butler when grown on a different media, showed variation in cultural characters like amount of aerial mycelium, texture and also differed in their ability to sporulate.

Eshwar Reddy and Basuchoudhary (1985) demonstrated variation in six isolates of F. *udum*. They also categorized the isolates into three groups based on radial growth and colony characters.

Morphological studies of the six isolates of *F. oxysporum* f.sp *ciceri* revealed variation in size of micro and macroconidia, growth pattern, sporulation and pigmentation of medium which varied from normal white to pale cream, dark brown, crimson and middle buff (Gupta *et al.*, 1986).

Gaur and Sharma (1989) reported that, eleven single spore isolates differed in their cultural and morphological characters and also showed a marked diversity in virulence towards the susceptible variety T 21. Patel (1991) reported variation in 13 isolates of *F.solani* but could not found variation among the isolates of *F* monliformae and *F. oxysporum* f.sp.ciceri.

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Cultural and morphological variability occurred amongst the 9 isolates of *Fusarium oxysporum* f.sp.*cumini*. Six types of mycelial growth characters and two types of margins were recorded in cultures of 9 isolates on 3 different media. Different colours of substrate pigmentation were observed on different media. In all the *Fusarium* isolates, macroconidia were sickle shaped and microconidia were ovoid (Champawat and Pathak, 1989).

Sowmya (1993) studied four isolates *Fusarium oxysporum* var. *cubense*, the panama wilt pathogen of banana on different nutrient media and observed maximum growth and sporulation on Potato Sucrose Agar and Richards's agar media.

Krishna Rao and Krishnappa (1997) reported that *Fusarium* spp. isolated form wilted chickpea plants collected from different locations of Karnataka differed in growth pattern, pigmentation, sporulation and pathogenicity.

Cultural characteristics of *Fusarium oxysporum* f. sp. *lycopersici* were studied in order to establish degrees of variation between isolates obtained from different tomato producing areas in the states of Aragua and North Guarico Venezuela. Sixteen isolations showed great variability in colony colour, as well as in the production of macroconidia, microconidia and chlamydospores. (Lugo and Sanabari, 2001)

Shrivastava*et al.* (2002) collected 71 isolates of *Fusarium oxysporum* f.sp.*ciceri*, chickpea wilt pathogen from 23 locations in Vindhyan plateau. These isolates were categorized into 6 groups on the basis of morphological studies. The size of macro and microconidia and colony characters varied from group to group. The maximum size of 19.48 - 46.62 x $3.33 - 6.66 \mu m$ was observed with group 6 where as the minimum of 16.65 - $36.63 \times 3.33 - 4.99 \mu m$ was exhibited with group 5 in case of macro- and microconidia respectively. Similarly, number of septa in macroconidia also varied. 3-6 septa were found with the groups 2, 3, 5 and 6 where as 2-5 septa with the group 1 and 4.

Kipropet al. (2002) studied 79 single-spore isolates of *Fusarium udum* from Kenya, India and Malawi and characterized them according to their cultural characteristics. They exhibited high variation in mycelial growth and sporulation on Potato Dextrose Agar (PDA) medium. The 79 isolates were categorized into two groups of radial mycelial growth and four groups of sporulation.

ShaliniVerma and Dohroo (2003) found that morphological and cultural variability existed among isolates of *Fusarium oxysporum* f.sp. *pisi* collected from pea. The isolates showed slow to rapid growth, variable pigmentation and morphology of the hyphae, microconidia, macroconidia and chlamydospores.

Anjaneya Reddy *et al.* (2003) observed that the size of macroconidia varied from 13.03 x 3.60 μ m (Bangalore isolate) to 20.60 x 2.10 μ m (ICRISAT isolate), while the size of microconidia variedbetween 5.26 x 1.78 μ m (ICRISAT isolate) to 9.09 x 1.94 μ m (Gulbarga isolate).

Desai *et al.* (2003) recorded variability among 15 isolates of *F. oxysporum* f. spricini collected from different places in India. The study revealed that, six isolates produced moderate to profuse fluffy white mycelium while nine isolates produced thin flat to slight fluffy pinkish mycelium on PDA medium. The colony diameter ranged from 62.67 to 73.67 mm after eight days of incubation at $27 \pm 2^{\circ}$ C on PDA, while the number of spores ranged from 2.18 to 23.82 million / ml on potato dextrose broth medium after 15 days at $27 \pm 2^{\circ}$ C. The size of micro- and macroconidia ranged from 5.25 - 14.00 x 3.50 - 7.00 µm and 17.50 - 70.00 x 3.50 - 5.25 µm respectively.

Variability among the six isolates of *Fusarium oxysporum* f.sp. *ciceris* of chickpea collected from different locations within Ahmednagar district in Maharastra was studied in respect of cultural, morphological characters and pathogenecity. Three isolates produced profuse fluffy white mycelium, while another three isolates produced thin flat to fluffy mycelial growth. Colony diameter ranged from 70.00 mm to 75.30 mm after seven days of incubation at $27\pm2^{\circ}$ C on PDA(Barhate *et al.*, 2006).

Alves Santos *et al.* (2007) reported variation in growth and sporulation of *Fusarium oxysporum* isolates of tobacco. Although daily growth rate seemed to be slightly different with in isolates, no statistical difference was recorded. On the basis of spores per plate ratio, isolates were classified into 3 major groups.

Shantha Laxmi Prasad *et al.* (2008) noted variation in mycelial growth, pigmentation, sporulation, size of conidia and production of micro- and macroconidia of *Fusarium oxysporum* f.sp.*ricini* isolates of castor wilt pathogen. Mycelial growth varied from profuse raised, fluffy to thin and flat. Pigmentation varied from light pink to dark pink, light violet to dark violet, rosy purple to dark purple and orange. Sporulation ranged from scanty to vary high. Average size of microconidia were $9.5 \times 3.2 \mu$ m to $23.4 \times 6.8 \mu$ m and macroconidia 23.2×4.1 to $64.5 \times 5.4 \mu$ m.

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Sixty nine *F.udum* isolates showed wide variation in sporulation, mycelial colour, conidial length, conidial septation and growth rate. Based on mycelial mat colour, three major colony types viz., whitish, pinkish and yellowish were noted. Growth rate varied from 0.1 to 1.31cm/day. Length and breadth of macroconidia varied from 14.4 to 61.6µm and 1.6to 4.6µm respectively. Based on combining characters like sporulation on PDA, mycelia colour, growth rate (<0.55 or >0.55cm/day), length (<45 or >45mm) and number of septa (<4 or >4) of macroconidia, 69 isolates were categorized into 18 different groups (Sinha *et al.*, 2008)

Dubey *et al.* (2010) reported variability among the isolates of *Fusarium oxysporum* f. sp. *ciceri* from chickpea in colony growth pattern, colony size and pigmentations. The size of microconidia varied from 5.1-12.8x2.5-5.0 μ m, whereas macroconidia ranged from 16.5-37.9x4.0-5.9 μ m with 1-5 septations. One hundred and twelve isolates were grouped into 12 categories on the basis of their radial growth, size of macroconidia and growth pattern.

Variation in pathogenicity

Fusarium spp. one of the most diverse groups of fungi, have worldwide occurrence under the diverse condition of soil and climatic factors. Pathogenicity variation is a well known phenomenon among *Fusarium* spp.

Padwick (1940) categorised 300 isolates of *Fusarium*species from chickpea into three groups on the basis of their pathogenic behaviour and categorised them into pathogenic, non-pathogenic and those causing seed rot.

Armstrong and Armstrong (1950) grouped *Fusarium* wilt isolates collected from soybean and cowpea into three biological races based on their pathogenic reaction to several genotypes of these hosts.

Sharma and Agnihotri (1972) recorded morphological and pathogenic variation among the three isolates of *Fusarium orthocerus*.

Shit and Sengupta (1978) reported that, among the fourteen isolates tested, fourth and sixth isolates were moderate to highly pathogenic to all the four varieties tested including the resistant varieties C-11 and Muktha.

Haware and Nene (1982) reported the occurrence of four races of *F. oxysporum* f.sp.*ciceri* first time from different area in India, *viz.*, Hyderabad (Race-I), Kanpur (Race-2), Gurudaspur (Race-3), Hissar and Jabalpur (Race-4). Among these race-1 was found to be more virulent.

Ramphal and Choudhary (1983) concluded that, the pea wilt pathogen in and around Delhi was a different strain or race and was much more virulent and pathogenic than the strains occuring in other countries.

Gupta *et al.* (1986) reported the existence of races in chickpea wilt pathogen, *Fusarium oxysporum* f. sp.c*iceri*, while Phillips (1988) demonstrated the existence of a new race of the fungus in California and designated it as race-6.

Patel (1991) conducted comparative study of the pathological characters of three isolates of *F. oxysporum* f.sp. *ciceri* and thirteen isolates of *F. solani*. The results clearly revealed pathogenic variation among the isolates of both the species of *Fusarium*.

Kapoor *et al.* (1993) communicated new virulence of the pathogen from Kangra valley of Himachanl Pradesh. They tested fourteen isolates of chickpea wilt pathogen *F. oxysporum* f.sp. *ciceri* collected from distant localities of Himachal Pradesh and observed Kangra valley race as the most virulent race.

Rajendra and Patil (1993) reported the existence of races in pigeonpea wilt pathogen *F. udum* in Maharashtra based on cultural, morphological and genetic variation among the isolates.

Salgado and Schwartz (1993) observed that the Colorado and Colombian strains of common bean wilt fungus produced differential reactions and were considered to be distinct races. However, they also observed that, among the cultivars tested HF 456-63-1 was found highly resistant to all the existing races.

Haware and Nene (1994) reported that pigeonpea variety ICP-8863 was resistant to isolate 1 but highly susceptible to isolate 2 which indicated the existence of pathogenic variability among the isolates of *Fusarium udum*.

Assigbetse*et al.* (1994) assessed genetic diversity among 46 isolates of *F. oxysporum*f. sp. *vasinfectum*of worldwide origin and classified them into three groups on the basis of their virulence on the differentials.

Miedner*et al.* (1996) tested 42 *F. culmorum* isolates collected from diseased plants from the fields of nine European countries and Australia on a synthetic winter rye population. All the isolates were found pathogenic and differed in their ability to cause diseases.

Of the 69 *Fusarium* isolates tested for pathogencity, 24 were pathogenic to carnation while the remaining 45 were non-pathogenic (Wright *et al.*, 1996).

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Okiror and Kimani (1997) identified great variability among the isolates of *Fusarium udum* in terms of their attack on the various test cultivars. They also cited that, the pathogenic variation is the major drawback in development of pigeonpea varieties resistant to wilt.

Desai *et al.* (2003) reported pathogenic variation among fifteen isolates of castor wilt pathogen *Fusarium oxysporum* f.sp.*ricini* which showed significant difference in their virulence to cause wilt disease in susceptible castor varieties VP-1 and VP-9, while the variety 48-1 showed resistant reaction to all the 15 isolates tested.

Honnareddy and Dubey (2006) studied pathogenic virulence of 25 isolates of *F. oxysporum* f. Sp *ciceris* on a set of differential cultivars and reported the existence of three new races of the pathogen in India.

Shanthalaxmi Prasad *et al.* (2008) revealed the reaction of 29 isolates of *F.oxysporum* f.sp.*ricini* on different castor cultivars and indicated the existence of five pathotypes of the pathogen with different virulence levels.

Twenty-four isolates of *Fusarium oxysporum* f. sp. *ciceri* were used to assess variability in pathogenicity of the populations. Each isolate was tested on 10 different chickpea lines and eight improved chickpea varieties. Isolates showed highly significant variation in wilt severity on the differential lines and improved varieties. Based on the reaction types induced on differential lines, isolates were grouped into four corresponding races (MekiShehabu *et al.*, 2008)

BIOCHEMICAL VARIABILITY

Sadasivan and Subramanian (1963) reported that pectic enzymes are secreted by fungi only in the presence of pectic compound or in other words fungi never produce pectic enzymes on a medium containing carbon source other than pectic compound. Later, Suri and Mandahar (1980) declared their results that, addition of pectic compound repressed production of PG in culture filtrate of *Alternaria brassicae*. It was also reported that PME is induced by the presence of a pectic compound as the carbon source.

Shit and Sengupta (1980) reported the enzymatic variability among the isolates of *F.udum* and have shown correlation between the activity of polymethylgalacturanse and cellulose enzymes produced by isolates and their pathogenicity.

Pectinolytic (PG and PMG), cellolytic, amylase and protease enzymes both *in vitro* and *in vivo* found to be actively associated with cauliflower wilt disease, caused by *Fusariumsolani*. They also confirmed the role of pectinolytic enzymes in pathogenesis of *F.solani* (Rajendra Singh and Sexena, 1989).

Pandey *et al.* (1995) reported the effect of culture filtrate of *F.udum* on wilt susceptible and resistant pigeonpea cultivars. Seedlings of susceptible and resistant cultivar treated with undiluted culture filtrate wilted 8 days after treatment (DAT). Mortality in the susceptible as well as resistant cultivars ranged from 95-100 % at 100 % culture filtrate, 75-85 % at 50 % culture filtrate and 50-75 % at 25 % culture filtrate concentrations. Though there were no differences in the wilting response between the seedlings of resistant and susceptible cultivars at these concentrations, the differences were observed at lower concentrations.

Vishwanath and Kolte (1997) reported that highly virulent isolate showed maximum amount of total carbohydrates content and the least virulent isolates showed least amount of total carbohydrate content of *Alternaria brassicae*. However, isolates containing higher content of total lipids, proteins, RNA and DNA showed moderate virulence.

If tik har *et al.* (2004) studied the effect of the culture filtrates of *Fusarium oxysporum* f. sp. *ciceris* at 1.0 N and 0.5 N concentrations and observed significant reduction in the root length of germinating seeds of all the chickpea varieties tested. They attributed this reduction due to the production of phytotoxins by the pathogen.

Variation in biochemical contents of 14 isolates of *Alternaria brassicae* were determined by Anil Khurana*et al.* (2005). They reported that the amount of reducing sugars, phenol, protein, RNA and amino acids present in different isolates varied significantly.

Vinod Kumar *et al.* (2007) conducted a study on pathogenic and biochemical variability among the isolates of *Fusarium udum* and the isolates were grouped into three clusters based on morphological characters and wilt incidence. The study also revealed variability in enzyme production and cell bio-molecular composition viz., total sugar, total protein and amino acids and it was noticed that, most aggressive isolates were rich in sugar content. Enzymes were more in highly aggressive isolates and less in low aggressive isolates.

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Nahed Z. Haikal (2008) conducted investigation on phytotoxic effect of cell free culture filtrate of certain soybean fungi *viz., Aspergillus niger, Fusarium culmorium, Penicillium* sp. and *Rhizoctonia solani* on seed germination and seedling development of soybean by soaking in 25, 50, 75 and 100% concentrations of 4, 8 and 12 day- old cell-free culture filtrates for 2, 4, 6, 8 and 24 h. The results indicated that that all the fungal filtrates irrespective of filtrate concentrations, filtrate age and soaking period significantly reduced seed germination and seedling development when compared to control. However, the percentage seed germination and seedling growth decreased with the increase in filtrate concentration, filtrate age and soaking time in all the fungal culture filtrates.

Culture filtrates of the isolates of *F oxysporum* f.sp.*ricini* reduced the germination of castor seeds. The germination is directly correlated with the concentration of the culture filtrate in comparison to check. Maximum inhibition in germination was observed with undiluted culture filtrate followed by 100 and 75 ppm concentration. The isolates also differed in degree of inhibition in root growth. The entire seedlings wilted in undiluted culture filtrate of all the isolates where as no wilting was observed in seedlings kept at 25ppm concentration (Shantha Laxmi Prasad *et al.*, 2008)

Rajput *et al.* (2008) reported maximum reduction in root and shoot length in plants injected by spore suspension of *F. Solani*ca using dieback of Shisham (*Dalbergiasissoo*).

GENETIC VARIBILTY

Assigbetse *et al.* (1994) used 46 isolates of *F. oxysporum* f.sp. *vasinfectum*, for analysis of genetic variability and evaluated by Polymerase Chain Reaction (PCR) amplification with a set of 11 random 10-mer primers. All amplifications revealed scorable polymorphisms among the isolates, and a total of 83 band positions were scored (1/10) for the 11 primers tested. Cluster analysis was done to generate a dendrogram showing relationships between them. Isolates clustered into three groups corresponding to their pathological reactions.

Sivaramkrishnan *et al.* (2002) analysed the genetic variability in 36 isolates of *Fusarium udum* using Random Amplified Polymorphic DNA (RAPD) and amplification fragment length polymorphism (AFLP) techniques. Though the two molecular markers detected high levels of polymorphism among the fungal pathogen isolates, the degree of polymorphism varied depending on markers selected. Cluster analysis of the similarity index data from the two DNA markers classified the isolates into 3 major groups suggesting that existence of a minimum of 3 specific races of the pathogen.

The amount of genetic variation from thirty-two isolates of *Fusarium oxysporum* f. sp. *lentis* was evaluated by PCR amplification with a set of 6 RAPD primers and 3 AFLP selective nucleotide primer pairs. All amplifications revealed scorable polymorphisms among the isolates, and a total of 8 polymorphic fragments were scored for the RAPD primers and 93 for the AFLP primers. Results obtained indicated that there was little genetic variability among a subpopulation of *Fol* as identified by RAPD and AFLP marker and also the data suggested that, *Fol* isolates were derived from two genetically distinct clonal lineages. (LakhdarBelabid *et al.*, 2004)

Pooja Sharma *et al.* (2006) studied the genetic diversity in 24 isolates of *Fusarium oxysporum* f.sp. *pisi* using ten 10-mer primers which generated 134 polymorphic markers.

Noher Mahmoud *et al.* (2009) used Random Amplified Polymorphic DNA (RAPD) technique to assess genetic variability within the *Fusarium oxysporum* f. sp. *Ciceris* isolates, and to determine the relationships between pathological characterization and molecular characterization. The genetic variation was evaluated with a set of-5 random-10 mer-primers to determine whether the tested isolates could be distinguished at the molecular level. No correlation between RAPD patterns and pathogenicity of the tested isolates was found.

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