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# GENETIC DIVERSITY AND PATHOGENICITY OF *FUSARIUM SPECIES* IN PLANTS– A REVIEW

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## ABSTRACT

Plants suffer from many diseases which are caused by various microbes like bacteria, fungi virus etc. Fungi of the genus *Fusarium*, widely distributed saprophyte, are extremely pathogenic to plants causing damage to plants by various vascular wilts, collar rot of seedlings, rot of tubers, bulbs and corms etc. Most of the diversity studies have been done on pathogenic strains of *Fusarium sps* which are split into divisions called host specific *formae speciales* (abbreviated *f.sp.*) .Many *Fusarium sps* produce harmful secondary metabolites called mycotoxins like zearalenones, trichothecenes, fumonisins etc. They reproduce by forming spores viz. microconidia, macroconidia and chlamydospores. Genetic variation studies among these can be performed by various methods using different types of molecular markers. These studies have characterised *Fusarium sps*. in different plants all over the world which can be exploited for developing biocontrol agents for resistance breeding. This would be an environment friendly and economical viable approach to control fungal plant diseases as compared to chemical fungicides.

**Keywords**: Fusarium, mycotoxins, formae speciales, biocontrol agent

## INTRODUCTION

Plants get damaged by various pathogens. Most of which are biotic agents like fungi, bacteria and viruses or abiotic like frost, hail etc. Biotic agents are responsible for parasitic diseases in which they obtain nutrition by growing on or in the plants. On the other hand non parasitic diseases are caused by abiotic factors like moisture and temperature due to unfavourable growing conditions which affect severity of plant diseases. These diseases are generally named on the basis of parts affected viz. Blight (in which leaves, flower or whole plant undergoes browning and dies suddenly)(Yu etal., 2008 ,Bai etal 2004); stem canker (in which any localised organ dies) (Esmailzadeh etal.,2008); wilt (when turgidity is lost) (Muhammad Sibtain etal., 2001); Gall (when cells overgrow) (Armstrong 1995); Stunting (in which plants do not develop fully); and leaf curl, mosaic, and chlorosis(due to lack of chlorophyll) (Athar Nadeem etal.,2001,Sukalpa etal.,2001,Ghosh and Basak 1951,Biswas etal.,1989)Damping Off(where a young seedling collapses and dies)(Erwin etal.,1996) . Among all pathogens, fungi of the genus *Fusarium* (member of Ascomycetes)are widely distributed and extremely pathogenic to plants by causing various vascular wilts, collar rot of seedlings, rot of tubers, bulbs and corms etc(Akinsanmi etal.,2004,H Sarami

etal.,2007,Setti and Bouznad, 1998; Belabid et al., 2000). Many of the species produce a range of secondary metabolites (mycotoxins) that are harmful to human and animal consumers and also contaminate grains (Desjardin 2006, Vesonder 1989).

**Diversity and Life Cycle of *Fusarium sps***

*Fusarium oxysporum* is the most common species worldwide among the plant pathogens.(Agrios 1988,Smith etal 1988). *F. oxysporum* isolates from different agroclimatic areas exhibit considerable diversity(P K Singh et al.,2011)with respect to cultural characteristics (Elwakil and Ghoneem, 1999).It does not reproduce sexually but undergoes asexual reproduction to spread its pathogenecity among plants.It produces three types of spores viz i)**Microconidia**, ii)**Macroconidia** and iii) **Chlamydospores**.(Agrios 1988)Among these most abundant are microconidia which are oval or bean shaped spores produced on aeriel mycelia .Macroconidia have pointed edges found on surface of diseased plants. The toughest spores are chlamydospores which are thick walled spores produced in chains or cluster and can surive in soil for much longer time. Many plants are drastically infected by *Fusarium sps* like *F. graminearum, F. verticillioides*, F. *oxysporum*, *F. subglutinans, F. proliferatum. F.cuminatum, F. avenaceum,F. concolor,F. crookwellense* (*F. cerealis*), *F. equiseti, F. semitectum, F.solani, F.sporotrichioides* and *F. venenatum* which causes significant economic losses. Some of these are further divided into subgroups called races, on the basis of virulence.*Fusarium oxysporum* is split into divisions called *formae speciales* (singular *forma specialis*, abbreviated *f.sp.*) which cause various vascular wilt diseases .Most of diversity studies have been done on pathogenic strains of *Fusarium*.(Kistler.,1997, Baayen etal.,2000, Lattifah etal.,2009).

In general, *Fusarium* wilt first appears as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At the seedling stage, plants infected may die soon after appearance of symptoms. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant (Agrios, 1988).*Fusarium sps*. spreads in short distances by water splash or planting equipments while long distances by infected seeds or transplants. It infects a healthy plant through mycelia or germinating spore penetrates in root tip, root wound or lateral roots. (Bhalla et al., 1992).The mycelium develops intracellularly and reaches xylem vessels where it produces microconidia. These microconidia enter cell sap and move by upward transportation. When the sap flow stops they start germinating and block the vascular vessels by triggering the self-defense mechanisms whereby secretion of gel occurs followed by formation of tylose in the vascular vessels. (Bishop and Cooper, 1984).Due to this plant is not able to absorb water and minerals and translocation of nutrients. The movement of water to the upper part of the host plant is blocked and the rate of transpiration becomes more than rate of transportation which causes yellowing, wilting and eventually death to the host plant. After this the fungus penetrates to all tissues and start infecting other neighbouring plants. The pathogen persists in the soil as chlamydospores that can remain viable for several seasons. (Erskine and Bayaa, 1996) As a result of the persistence of the pathogen in the

soil, the disease is best controlled through host plant resistance.

**Diseases caused by *Fusarium species* and symptoms**

There are more than 100 *Fusarium* vascular wilt diseases world-wide.(Machardy 1981) .There are over 100 formae speciales divisions, each with one or two different races. Each forma specialis within the species are host-specific (i.e. specific to a certain plant) and produce different symptoms as pathogenic as well as non pathogenic forms.(Armstrong & Armstrong,1981)It is a cosmopolitan soil-borne fungus which colonizes the vascular system of the host plant. It infects tomato, tobacco, legumes, cucurbits, sweet potatoes and banana alongwith many other herbaceous plants. The symptoms are different in different hosts depending on type of *Fusarium sps*. Early symptoms of diseases caused by *Fusarium sps* causes leaf yellowing, slight wilting during the day and stunting. In hot conditions diseased plants such as tomato and peas can die within a few days. Diseased bananas usually die slowly, taking 1–2 months. Browning of the internal stem (vascular) tissue is a key symptom of pathogens which cause vascular wilt disease. It appears like clearing of vein on younger leaves and drooping of older leaves.Then stunting or yellowing of leaves, defoliation take place and finally plant dies.Various researchers have characterised *Fusarium sp*. in different plants all over the world which have diverse symptoms .Some of them are as follows:

*Fusarium oxysporum f.sp. asparagi* (Fusarium yellows on asparagus)(Elmer & Stephens 1989); f.sp. *callistephi* (wilt on staghorn sumac) (Oellete etal.,2006) ; f.sp. *cubense* (Panama disease/wilt on banana) (Leong etal.,2010,Pegg atal.,1995).; f.sp. *dianthi* (wilt on carnation)(Oellete 2002, Baaen etal.,1996);f.sp. *koae* (on koa)(Gardener 1980); f.sp. *lycopersici* (wilt on tomato) (Jones etal.,1982); f.sp. *melonis* (fusarium wilt on muskmelon) (Gordon etal.,1989,Namiki et al.,1998 ); f.sp. *niveum* (fusarium wilt on watermelon)(Martin and Bruton 1989); f.sp. *pisi* (on edible-podded pea)(Shirley 1963); f.sp. *tracheiphilum* (wilt on Glycine max) (Li and Hartman 2003); and f.sp. *zingiberi* (fusarium yellows on ginger) (Raabe et al., 1981).f.sp.*cumini (*Wilt of cumin) *(*Bardia and Rai 2008*),*f. sp. *vasinfectum (*Wilt of cotton ) (Assigbetse et al., 1994 ) , *f. sp. Phaseoli* **(**Wilt of bean) (Woo etal.,1996,Fernando etal.,1999) ,f. sp. *Lentis (*Wilt of lentil)(Tosi and Capelli 2001) , f.sp. *ciceri(* Wilt of chickpea) *(*Jiménez-Gasco et al., 2001,Bhim Pratap etal.,2006*) f.sp.lagenariae(*Wilt of bottle gourd)(Matio and Yamamoto1956), *f.sp.momordicae(*Wilt of bitter gourd) (Sun and Huang 1983), *f.sp.batatas* (wilt in sweet potato) (Shimizu etal.,2005), *f.sp.curcumerinum*(wilt of Cucumber)( Owen 1956)*F.verticillioides(*Ear rot of Maize)(Eller etal.,2009) *F.graminearum*(Head Blight of Wheat)(Bechtel 1985) *F.fujikuroi (*Bakanae disease of Rice)(Zhang etal.,1998).

**Estimation of Genetic Variation**

The genus *Fusarium* consists of variable number of populations (Jelena etal., 2012).Genetic variation studies among these can be performed by various methods using different types of molecular markers which vary in their principles &methodologies. (Mohammadi and Mofrad, 2009, Hill etal.,2011)One can choose according to the requirement of study among these 11 different molecular marker methods: restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeats (ISSRs), sequence characterized regions (SCARs), sequence tag

sites (STSs), cleaved amplified polymorphic sequences (CAPS), microsatellites or simple sequence repeats (SSRs), expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs), and diversity arrays technology (DArT)( Avinash etal.,2011,Semagn etal.,2006, Assigbetse et al., 1994 Woo etal.,2006,Bogale etal.,2006).

Among these widely used method of estimation of genetic variation is Vegetative compatibility grouping(VCG) which is based on the ability of the mycelium to anastomose to form heterokaryon(Chen etal.,2007,Lattifah etal 2008,Kistler etal.,1998,Puhalla 1985).This helps to determine genetic relatedness .This requires special culture media and methods, as well as standard incubation conditions.High variability in species, especially under different environmental conditions, has caused taxonomists to consider some special criteria to be important in the classification of species. For this reason, different methods and keys have been developed.The large variation in some of the characteristics of *Fusarium* isolates such as pathogenicity, colony morphology e.g. form and color, mode of development of the chlamydospores, existence or lack of sporodochia or type of microconidia, have resulted in classification of species into different intra-specific groups. Most of the intra specific classifications are based on pathogenic behavior and vegetative compatibility groups (VCG). Some researchers have placed some isolates of VCG into the same race using the pectic zymogram technique(Balali and Iranpoor 2006, Mukesh etal.,2005, Szekzi 1990 a,b)). Zymogram is an electrophoretic method for measuring protheolithic activity in which the enzymes are separated from one another in a polyacryl-amid gel .The study of the genetic variation among and within species and subspecies using a pectic zymogram marker helps in identifying the relation among genotypes and pathogenic phenotypes. It can also help in understanding evolution of different species through time.

Apart from this, there is another method called RAPD analysis which is a fast, PCR-based method of genetic typing based on genomic polymorphisms. The technique is highly sensitive to nucleotide differences and can assay single nucleotide differences. RAPD produces DNA profiles of varying complexity, depending on the primer and template used. Random markers as products of the PCR-RAPD technique have been developed to differentiate numerous fungi in various plants.(Arif etal.,2011,Arici & Koc 2010) RAPD markers generated with single primers of arbitrary nucleotide sequence have been used in detecting intraspecific polymorphisms among fungi (Pegg etal., 1995). This technique can generate specific DNA fragments that can be used for the identification of isolates, and in molecular ecology (Hadrys et al., 1992). This technique has been applied widely in the detection and genetic characterization of phytopathogenic fungi including race differentiation in several formae speciales of *F. oxysporum*. (El Fadly etal. 2008).A similarity matrix on simple matching co-efficients is calculated from the data based on the RAPD of all isolates. The matrix is used to construct a dendrogram using Unweighted Pair Group Method with Arithmetic Mean Analysis (UPGMA tool of NTSYS) for establishing relatedness.(Rohlf 2000) The dendrogram obtained shows the hierarchical clustering which separated the isolates into different groups according to their similarity co-efficients. (Peter etal., 2009)Sequence-related amplified polymorphism (SRAP) is a novel molecular marker technique based on two-primer amplification that preferentially amplifies open reading frames (ORFs). (Li etal. 2001)The forward primers amplify exonic regions, and the reverse primers preferentially amplify intronic

regions and promoters. With this unique primer design, SRAP markers are more reproducible, more stable, and less complex than RAPD and AFLPs.

Different studies have been done worldwide to understand the diversity of Fusarium species using different molecular markers as per requirement. In 2008 and 2009, samples of weeds and plant debris were collected from 12 locations in eastern Croatia, and 300 *Fusarium* isolates colonizing them were identified. (Jelena etal., 2012).Strains were grouped and identified based on morphology and amplified fragment length polymorphism (AFLP) patterns. Fourteen *Fusarium* species were identified with *F. graminearum* , *F. verticillioides* , *F. oxysporum* , *F. subglutinans, F. proliferatum, F.cuminatum, F. avenaceum, F. concolor, F. crookwellense* (*F. cerealis*), *F. equiseti, F. semitectum, F. solani, F.sporotrichioides* and *F. venenatum*.Their study indicated that economically important *Fusarium sps.* may be isolated from numerous alternative hosts during the off season and that weeds and plant debris can serve as a reservoir of genetically diverse inoculum.

To identify *Fusarium sps* associated with diseases of root and basal plate of onion, surveys were conducted in seven provinces of Turkey in 2007(Harun etal. 2011). Samplings were performed in 223 fields, and 332 isolates belonging to 7 *Fusarium spp*. were obtained. The isolates were identified as *F. oxysporum, F. solani, F. acuminatum,F. equiseti, F. proliferatum, F. redolens, and F. culmorum* based on morphological and cultural characteristics. Also, species-specific primers were used to confirm the identity of *Fusarium sps*.

In India many crops are affected by *Fusarium sps* but the research on genetic diversity is in intial stages.

A study was conducted in 1995-2006 in which 48 isolates of *F. oxysporum* f.sp.*ciceris (FOC)* collected from the diverse agro-climatic chickpea growing regions of India was done using AFLP markers. Out of these 41 were found pathogenic showing characteristic wilt symptoms and seven isolates were non-pathogenic which did not show any symptoms at all. (Sharma etal, 2009).

Not only these but much more research has been conducted to understand the pathogenecity of *Fusarium sps* in plants worldwide. It's not possible to quote all of them but the objective is same that variability in *Fusarium sps* in differents areas is found which affect their pathogenecity to different degrees.

**Types of Toxins released from Fusarium species**

*Fusarium* toxins are categorised in different groups based on international regulation. First group consists of those which are under international regulation like deoxynivalenol (DON), zearalenone and fumonisins . Other group comprises of new emerging varieties, such as beauvericin, enniatins and culmorins. Nivalenol (NIV), T-2 toxin and HT-2 toxin produced by *Fusarium* species, are under evaluation by European regulation. The zearalenones are biosynthesized through a polyketide pathway by *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium equiseti*, and *Fusarium crookwellense*. All these species are regular contaminants of cereal crops worldwide.(Hagler etal.,2001) Fumonisins are synthesized by condensation of the amino acid alanine into an acetate-derived precursor (Sweeney & Dobson 1999).They are produced by a number of *Fusarium* sps like *Fusarium verticillioides* , *Fusarium proliferatum,* and *Fusarium nygamai,* as well as *Alternaria alternata* f. sp. *lycopersici (*Rheeder etal.,2002,Marasas etal.,2001)*)*.The trichothecenes are a family of more than sixty

sesquiterpenoid metabolites produced by a number of fungal genera, including *Fusarium, Myrothecium*, *Phomopsis, Stachybotrys, Trichoderma*, *Trichothecium etc. (*Uneno 1983,Scott 1989*).* They can be classified into macrocylic or nonmacrocyclic, depending on the presence of a macrocylic ester or an ester-ether bridge between C-4 and C-15 (Chu 1998). Non macrocyclic trichothecenes are produced by *Fusarium* sps while macrocyclic by *Myrothecium, Stachybotrys*, and *Trichothecium* species (Marasas etal.,1984). The nonmacrocylic trichothecenes can be subclassified into two groups: type A, which have a hydrogen or ester type side chain at the C-8 position, and include T-2 toxin, neosolaniol, and diacetoxyscirpenol, while the type B group contain a ketone and include fusarenon-x, nivalenol, and deoxynivalenol. Diacetoxyscirpenol, deoxynivalenol(DON), and T-2 are the best studied of the trichothecenes produced by *Fusarium* species.(Desjardin and Proctor., 2007,Kristensen etal.,2007)

## CONCLUSION

*Fusarium sps* is widespread in many crops so it poses a significant problem. Certain difficulties have been encountered for the management of various fungal diseases in plants because there is lack of proper knowledge of recommended fungicides in the market. Different chemicals are used in form of pesticides to protect plants from pathogens. These cause a lot of harm to the environment through pollution. In addition, these chemicals are costly and not fully efficacious in some cases. Not only this but many plants have become resistant against these chemical bactericides and fungicides. (Nene et al., 2000) and some new pathogenic strain have also been identified and documented (Mamatha et al., 2004). In such situation, wilt can be managed by resistance breeding using biocontrol agents which is an economically viable and ecologically desirable strategy. (Reid et al., 2002; Jegathambigai et al., 2009). Knowledge of diversity and relatedness among the pathogen populations is, however, a prerequisite for exploitation of resistance breeding. Several nonpathogenic strains of *F. oxysporum* have been selected as potential biological control agents they can protect plants against the pathogenic strains (Biles & Martin 1989,; Postma and Luttikholt 1996, Bao et al. 2000). These can be introduced in infected crops .They take nutrients from those pathogens and thus help in defending plant by creating tough competition for food and nutrition among pathogens Better screening and breeding procedures are required to access the host plant resistance in new cultivars.

**Future prospects**

*Fusarium species* have vast morphological and physiological variations. Most of the research focuses on this fungus due to its ability to cause diseases to economically important plants. But this is not enough as its presence in soils in different areas worldwide have reveal wider and diversified ecological activities.The study of relationships between pathogenic and nonpathogenic forms may shed new light on the evolution of pathogenic *F. oxysporum* strains (Corelle 1991, Gordon & Martin 1997). Most research has been focused on strains capable of causing disease and nonpathogenic strains have been neglected, except using as biocontrol agents. For better understanding of biological significance *Fusarium species* should be studied in their natural habitats. They can be used as a model for studying soil borne pathogens. Molecular-biotechnological tools can be promoted for disease characterisation and long-term local monitoring which can lead to regional capacity strengthening.

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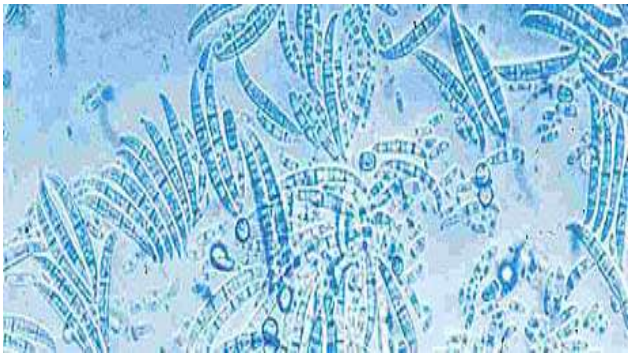
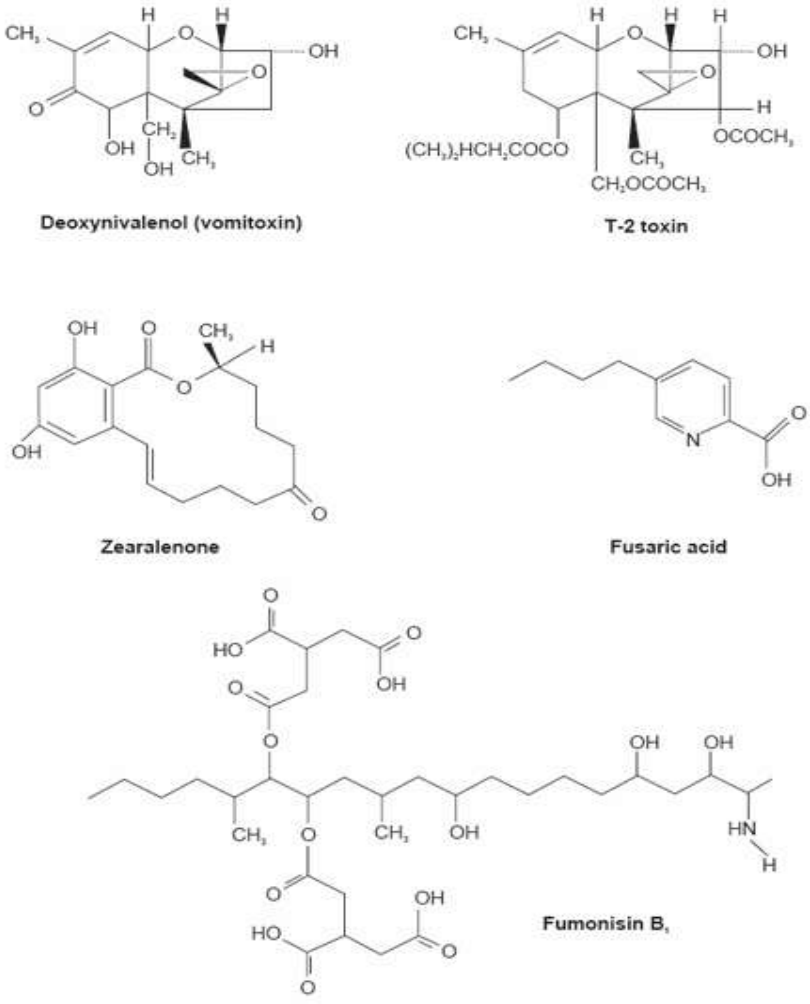


**Asexual stages (Microconidia, Macroconidia,Chlamydospore) in life cycle of *Fusarium***




**Types of toxins produced by Fusarium Species**