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Phenotypic Screening Techniques for Fusarium Wilt Disease

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ABSTRACT: Disease attack is a crucial hindrance in agricultural food production. Fusarium wilt disease caused by the fungus *Fusarium* sp. causes worldwide loss in major crops like tobacco, banana, chick pea and cotton. Hence screening techniques are essential to eliminate the diseased plants and develop resistant cultivars. Phenotypic screening for a plant disease refers to testing the performance of a crop under disease stress through various assays. Phenotypic screening offers complete utilization of Host Plant Resistance (HPR) in regional cultivars. The screening assays can be broadly divided into Field based studies and Controlled environment studies. Field environment screening methods provides study of *Fusarium* under practical conditions. The green house screening techniques assure fast and labor-saving experiments where temperature and humidity were under control. *Fusarium* is a soil borne, continuously evolving pathogen, which presents challenges in controlling it. The main challenge faced while screening under field environment is that it is a tedious and laborious process whereas in greenhouse screening, understanding the interaction of the pathogen with other biotic and abiotic factors in field conditions is difficult. Designing screening studies with integration of both techniques would help in observing truly resistant cultivars and better understanding of host-pathogen-environment interactions. The information pertaining to phenotypic screening techniques for *Fusarium* wilt has been carefully reviewed in the present article.

Keywords: Fusarium, wilt, phenotypic screening, root dip, sick plot.

INTRODUCTION

Agricultural food production is frequently hampered by many biotic and abiotic factors, in which loss due to diseases is a concerning aspect. The loss due to diseases caused by pathogens like fungi, bacteria, virus, etc., accounts 10 to 25% around of total crop yield (Strange and Scott 2005; Ranjan *et al*., 2016). *Fusarium* wilt disease caused by *Fusarium* sp. causes worldwide loss in major crops.

Fusarium is a genus under the kingdom Fungi. They are filamentous and belongs to the Class Ascomycetes and Family Hypocreaceae (Okungbowa and Smith 2012). The *Fusarium oxysporum* Species Complex (FOSC) is a collection of strains that cause vascular wilt in economically important crops all over the world (Gordon, 2017). Under the genus *Fusarium*, 14 out of 20 species were highly virulent leading to development of severe wilt symptoms (Suga and Hyakumachi, 2004 and Early, 2009). *Fusarium solani*, *Fusarium chlamydosporum*, and *Fusarium oxysporum* are the most prevalent of these 14 species (De Hoog, 2016).

Fusarium is a soil borne pathogen and can be able to survive in soil for up to twenty years (Shabeer *et al*., 2021). This disease was first appeared and reported in in Panama Canal, Australia as stated by Ploetz and Pegg (1997). In Indian subcontinent, *Fusarium* wilt was first reported in West Bengal (Niwas *et al*., 2021). Most of the *Fusarium* species present in the form of chlamydospores, survival spores with thick walls (Leslie and Summerell 2006) in the soil in the absence of primary host plant (Senthamselvi *et al*., 2019). These chlamydospores cannot be destroyed either by heat or by adverse weather conditions and may stay in the soil further for 20 years or more (Buddenhagen, 2007; Stover, 1962). Fusarium wilt spores will experience fungus stasis, which is the inability of spores to germinate in soil. They require certain substances to aid in the germination process. Different chemical molecules, including as amino acids and carbohydrates, are released by the roots of the host plants, enhancing or stimulating the germination process (Gordan, 2017).

The fungal spores mainly spread from plant to plant within a field and field to field within an area through movement of water, soil, anthropogenic factors and animals (Meldrum *et al*., 2013). The Fusarium wilt fungus thrives in soil and with the help of nematodes penetrates into the root, where it progressively spreads to reach the root's centre, causing the plant to wilt quickly.

Fusarium wilt disease caused by *Fusarium* sp. effects both sub-tropical and tropical regions, causing loss in major staple crops like banana, tomato, chickpea, lentils, etc., and it also affects commercial crops like cotton (Nelson *et al*., 1983; Zhang *et al*., 1996; Bokshi *et al*., 2003). Stover and Simmonds, (1987) considered FW as one among the destructive plant diseases

recorded in history. In advanced stage of disease, the external leaves turn yellow and then collapse as purplish deeper shade appears in the xylem vessels and they become obstructed (Niwas *et al*., 2021).

Most of the *Fusarium* wilt management methods were commonly followed for all crops that are affected by this pathogen. One of the important management methods is crop rotation with non-host crops. Weed management plays a critical role in controlling Fusarium wilt. As the fungus can stay in the weeds even without the main host crop, regular tillage is required to keep the plots weed free. Other chemical and biological control methods are often employed for limited control of disease.

However, *Fusarium* sp. spreads very rapidly from one place to another and the Chlamydospores resting in soil pose significant layback to these control methods (Joshi *et al*., 2019). Previous studies also showed the evolution of pathogen into different races, showing the evidence of gene for gene hypothesis (Mes *et al*., 1999). This poses further challenge to effectiveness of the management methods. Utilizing the degree of resistance naturally present in the crop varieties paved way to develop resistant cultivars and study the mechanisms of Host-pathogen-environment interaction. In order to study these objectives, phenotypic screening is critical.The importance of phenotypic screening was emphasized by Dekkers and Hospital (2002) who emphasized that laboratory screening based on molecular markers without phenotypic screening in field or glasshouse could not be effective enough in defining the level of resistance. Phenotypic screening helps to identify and conserve the resistant cultivars specific to locations. It is important to develop resistant cultivars that suit well to a particular farming condition. In this review, an attempt has been made to discuss the various techniques utilized in phenotypic screening for Fusarium wilt disease, the advantages of testing methods and the challenges faced while employing these methods.

Host Plant Resistance (HPR): Those characters that enable a plant to tolerate or recover from the pathogen attack that cause great damage to the other plant of same species is called host plant resistance (Painter, 1951). Whether the source of resistance is transgenic or natural host plant resistance, plant resistance to pathogens improves ecologically sustainable and profitable food production and provides food security. Natural resistance that has been bred into commercial types is used in host plant resistance.

Host plant resistance in crops is important in preventing pathogen damage, although it is limited to that disease or pest. Understanding pathogen-plant interactions can help to increase plant resistance durability. Non-host resistance is a sort of resistance to potential diseases found in all plants. Host Plant Resistance is studied by looking at the genes involved in resistance and adopting or improving those genes in commercial cultivars (Yates *et al*., 2018).

Plants have developed a variety of resistance mechanisms. Environmental resistance that is more dependent on environmental factors. Host evasion, induced resistance, and host escape are examples of pseudo resistance (Kumar *et al*., 2018). Structure barriers and allele compounds that disrupt the fungal pathogen's attack process are examples of genetic resistance. Antixenosis, antibiosis, and tolerance are three mechanisms of host plant resistance. Various breeding procedures and genetic engineering approaches can be used to incorporate them.

Techniques for phenotypic screening for *Fusarium* wilt disease

Culturing of Fusarium wilt pathogen:

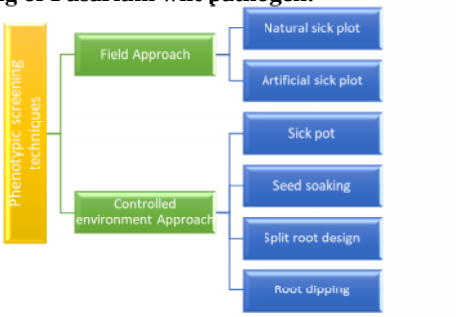

Fig. 1. Techniques for phenotypic screening for *Fusarium* wilt disease.

From the infected plants, the fungi are isolated and cultured on Potato Dextrose Agar (PDA) medium followed by pure culture preparation using the single spore isolation technique (Ranjan & Singh 2021). The purified culture was further sub-cultured for multiplication of pathogen which is used for inoculation in field or in controlled conditions. The amount of spore load in a inoculation varies depending upon the crop, pathogenic strain and environmental factors. After inoculation, usually 24 hrs-2 weeks' time period is required for incubation of the pathogen. Under the disease inoculated environment, the cultivars were assessed for various qualitative and quantitative traits and the best performing cultivars were designated into various categories of resistance based on the values of Disease Incidence (DI), Severity Index and Disease Progress Curve (AUDPC).

Phenotypic screening by Field Approach

Field Sick Plot method. Field sick plots can be divided into two types. Natural field sick plots for a particular disease (Bohn & Tucker 1939), where the field area was naturally contaminated with pathogen and Artificial sick plots which the name itself depicts that pathogen culture were isolated, cultured, multiplied and drenched into soil of the field that was located in the area of "hot spot" for the particular pathogen (Shirani *et al*., 2021). This method is the most effective and commonly used for identifying wilt-resistant castor genotypes (Prasad *et al*., 2019). This method was used in a number of studies for phenotypic screening of Fusarium disease in different crops (Sinha *et al*., 2021; Reddy *et al.*, 2016; Manjunatha and Saifulla, 2018; Pande *et al*., 2012). Pratap *et al*. (2017) incorporated 5–6 × 10^6 conidia/ml/g of soil of *Fusarium oxysporum* f. sp. *ciceris* for screening introgressed lines in chickpeas. Jyothi *et al*., (2011) evaluated 35 accessions of sesame against *Fusarium* wilt under field conditions and in pots in greenhouse, all the cultivars showed certain

percentage of disease infection and none of the cultivar reported to be immune. Garcia *et al*. (2021) reported that biocontrol agents have tremendous possibilities for the control of *Fusarium* wilt in Gooseberry based on their study under field conditions in artificial sick plots. Sarwar *et al*. (2012) identified two resistant lines in chickpea using this method. Yadav *et al*. (2019) concluded significant variation between field screening and pot screening. A main advantage of field sick plots is that screening can be done at real field condition, assuring the resistance of any cultivar under farming conditions while the disadvantages of field sick plots lies in its nature of being laborious to maintain germplasm at field condition. Environmental variations might influence the disease incidence in that particular season. Also, there may be a high chance for other pathogens in soil to interrupt in the screening process (Chitwood-Brown *et al*., 2021). Zhang *et al*. (2020) evaluated 3258 lines of cotton for screening of resistant plants for *Fusarium* wilt of cotton and reported high disease severity in low temperature conditions.

Phenotypic screening by controlled environment approach

Wellman root dip method. This is one of the earliest methods introduced by Wellman in 1939 initially to study *Fusarium* wilt of tomato. Later, it was widely adopted for other crops like cucumber, chickpea, cotton, etc.,. In this technique, roots of 8-10 days old seedlings were immersed in mixtures of cultured *Fusarium* spores. Thus, the direct contact of pathogen with the host root was made. The seedlings were then grown in completely sterilized soil at controlled atmosphere at 20 to 30°C, favorable for growth and multiplication of pathogen inside the host. After two to three weeks, seedlings were evaluated. The presence of *Fusarium* wilt symptoms were thoroughly checked. Thus, the lines were screened for susceptible and resistant cultivars (Chitwood-Brown *et al*., 2021).

In root dip method, development of plant is harmed by colonisation of plant roots, which leads to systemic plant invasion (Wang *et al*., 2017). Hirai *et al*., (2002) developed two root stocks resistant to *Fusarium oxysporum* f.sp. *melonis* in melon with the help of Willman root dipping technique. Scott *et al*. (2004) used 6×10^7 spores/ml for root dipping in tomato for RFLP analysis whereas Faustine *et al*. (2016) used 1×10^6 conidia spores/ml of *Fusarium oxysporum* f.sp. *vasinfectum* for screening in cotton.

Sick Pot method. This method is employed to screen cultivars at green house in pots. The recombinant Inbred Lines (RILs) of Castor were phenotypically screened for *Fusarium* wilt disease (Shaw *et al.,* 2022). Following the isolation of *Fusarium oxysporium* f.sp. *ricini*, the initial inoculum was prepared by using PDA medium and multiplied by using sorghum grains as substrate. The seeds were sown 24 hrs after inoculation of spores. The disease reaction is recorded up to 75 days from sowing and the data was analyzed (Ranjan *et al*., 2016). Kumar *et al*. (2021) incubated the soil for 20 days before planting in sick pots. In sick pots, the roots should be protected from any injury as this may leads to mixing of spore load is the soil.

Naik *et al*. (2008) evaluated thirty genotypes of chillies for *Fusarium* wilt caused by *Fusarium solani* (Mart.) Sacc by sick pot method. Out of the thirty genotypes screened, only two cultivars showed moderate resistance to *Fusarium* wilt. The authors concluded that both seeds and plants were subjected to infection through potting culture, owing to high mortality of plants. Among the other methods utilized to screen, sick pot method was considered more suitable because it was possible to differentiate between moderate and highly resistant lines (Ranjan *et al*., 2016). Lal *et al*. (2022) used this method for validating molecular markers in RILs of chickpea, where 23 markers recorded polymorphism among the parental lines and can be used for further genotyping of RILs. Fatima *et al*. (2022) employed this method to assess the loss due to *Fusarium* sp. in chickpea and reported three predominant strains of *Fusarium* sp.

Split Root Design. Akram *et al*. (2013) followed greenhouse screening of tomato cultivar for *Bacillus* induced resistance to *Fusarium* wilt. A 30-day-old tomato seedling's roots were split in half and a single seedling was transplanted into two combined pots. Each treatment included 50 mL of bacterial inoculum for the inducer side and 50 mL of pathogen inoculum for the responder side. The inducer side received distilled sterilized water and the responder side received pathogen inoculums for pathogen control. Both sides of the untreated control received distilled sterilized water. For incubation, pots were maintained in a greenhouse environment. 30 days after inoculation, the disease index and control effects were examined. Two Bacillus strains *B. fortis* IAGS162 and *B. subtilis* IAGS174 showed reduction of disease symptoms in tomato plants.

Seed soaking technique. Ranjan *et al*. (2016) followed this method in which surface sterilized seeds were immersed in 200 mL of spore (1×10^6 spores/ml) suspension for 2 hours. Seeds that have been treated were planted in plastic containers. As a control, seeds treated in sterile water were employed. This approach provides an easy screening of huge number of germplasms at one place. Further, the sterile growth medium is inoculated with specific pathogen spores under study, leading to no contamination of soil by other pathogens. Screening done at seedling stage itself saves cost and time. Although, phenotypic screening at final stage is also very crucial before releasing a resistant cultivar (Koebner and Summers 2003).

CONCLUSION

Identification of resistant cultivars against *Fusarium* wilt primarily depends on phenotypic screening following various methods. Techniques like natural and artificial sick plot screening are considered advantageous as they are performed under real field conditions but they are little bit tedious in nature. Other techniques like Wellman root dip method and its modifications, sick pot method and split root design were employed for screening at green houses, where zero to very low interaction by other pathogens were accounted. Thus, these techniques represent true level

of resistance against the pathogen. In recent years, a number of innovative approaches like Marker Assisted Breeding, Marker Assisted Selection, Genome editing, etc. have been developed and used by scientists to develop resistant cultivars. However, none of the techniques can be directly implemented or validated without a strong phenotypic screening for wilt resistance. This indicates that phenotypic screening techniques for *Fusarium* wilt is indispensable. Further, it is an evident fact that the *Fusarium* sp. continuously evolve and may increase their disease severity thus periodical screening of available resistant cultivars is desirable to maintain durable resistance against the pathogen.

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Conflict of Interest. None.

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