Pathogenesis and Varietal Screening for Panama Wilt Disease of Banana in Pakistan

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Abstract: Banana is the world largest fruit crop with an annual production of 114 million tonnes and Pakistan produces 154,800 tons annually. Panama wilt caused *by Fusarium oxysporum* f. sp. *cubense* (Foc) is the most widespread and destructive banana diseases worldwide. In Pakistan Panama disease was first recorded in 2012 in few banana fields of district Thatta and Hyderabad in the Sindh. The infection processes and mechanisms of pathogenesis of Foc in relation to symptoms expression and evaluation of Cavendish varieties for panama wilt of banana were studied. Pathogenesis was confirmed by establishing Koch's postulates. The experiment was to evaluate occurrences of necrotic and wilting symptoms and the disease intensity on banana suckers of Cavendish cultivar, two months plantlets were artificially inoculated with 10^6 conidia/ml suspension of Foc. Five tested Cavendish varieties viz., w11, G9, Basari, Pisang and BT10 showed low to high average of score of wilting and necrotic symptoms. Based on the calculation of disease intensities, all Cavendish varieties tested were identified as susceptible to Foc infection.

Keywords: Panama wilt, *Fusarium oxysporum* f. sp. *cubense,* pathogenesis, screening, Cavendish varieties.

INTRODUCTION

Banana is the world largest fruit crop with an annual production of 114 million tonnes in 2017 and is a staple food for nearly 400 million people worldwide. According to the latest available data approximately 5.6 million hectares of land are dedicated to banana production globally [6]. In Pakistan banana is grown on 34,800 hectares with production of 154,800 tons. It is mainly grown in Sindh province because of favourable soil and climatic conditions for its cultivation. About 87% production comes from the Sindh province. Major conventional variety 95% grown in Sindh is Cavendish Dwarf (BASRAI) and remaining tissue culture varieties Grand nine and William hybrid and three Chinese cultivar viz., Pishang, W10 and W11 [1]. Export of Banana fresh and dried from Pakistan decreased from 75,489 tonnes worth of Rs 2,957 million in 2013-14 to 38,170 tonnes worth of Rs 1,618 million in 2014-15, thus showing decline of 45% in terms of value. Nowadays 25,000 tonnes around 20% of Sindh's production exported to Afghanistan, Iran and Azerbaijan [2].

Major production constraints are poor soil health, nutrients imbalance, diseases and pest. *Fusarium* wilt is also known as Panama wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) is the most widespread and destructive banana diseases worldwide, causes serious threat to banana production [3,13,15,17,20]. The *Fusarium oxysporum* f. sp. *cubense* (Foc) has been diagnosed based on fungal morphology [21], PCR and VCG testing [16] in Pakistan. Panama disease was first recorded in 2012 in few banana fields of district Thatta and Hyderabad in the Sindh, affecting Dwarf Cavendish (Basrai) plantation [21]. It was confirmed on the basis of PCR diagnostics and mating experiments, that the tropical race 4 of Foc is causing Panama disease in Dwarf Cavendish plants in Pakistan [16]. Tropical race 4 (TR4) is able to infect Cavendish in both subtropical and tropical conditions. It caused significant damage, limiting commercial production of the crop in Australia and China [12]. The TR4 has been reported from Jordan [7], Lebanon and Pakistan [16, 21]. Pakistani isolates *F. oxysporum* f. sp. *cubense* belong to previously characterized lineage V of tropical race 4 with 99% bootstrap support and show close relationship with Indonesian and Malaysian Foc TR4 isolates [14]. Foc tropical race 4 is a quarantine pathogen presence in Pakistan has very high economic importance as far as the trade earning of this crop is concern. Therefore, the experimentation was carried out for pathogenesis of *Foc* and evaluation of Cavendish varieties for *Fusarium* wilt of banana.

MATERIALS AND METHODS

The experiment was to evaluate occurrences of necrotic symptoms, the score of wilting symptoms and the disease intensity on banana suckers of Cavendish cultivar were transferred into earthen pots (15 x 30 cm) containing 3 kg of soil medium (soil: sand: compost = 2:1:1 v/v).

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Pure culture of Foc was grown in potato dextrose agar (PDA) medium, conidial suspension made in sterile water, haemocytometer was used to obtained desirable density [8] and was then used to inoculate banana plantlets for the confirmation of Koch's postulates. Two months plantlets were artificially inoculated with freshly made spore suspension (10^6conidia/ml) of Foc for the confirmation of Koch's postulates. For inoculation 50 mL of spore suspension were drench in root zone in both pricked and non-pricked banana plants. For control set 50 ml of distilled water were drench in both pricked and non-pricked banana plants.

Scoring of wilting symptoms in tested banana suckers after inoculation with *Foc* (score 0-4) was followed where score 0 – inoculated plants were healthy and did not show wilting symptoms; score 1 – some leaves at lower part of plants yellowing; score 2 – number of yellowing leaves increased and suckers or plant showing symptoms of wilting; score 3 – necrosis occur in all plant parts except the new and unopened leaves; score 4 – all banana suckers or plants died [5]. Scoring of necrotic symptoms at the base of banana plants inoculated with *Foc* was conducted following (score 0-5), where transversal cut of banana rhizomes showed score 0 – no tissue colors change; score 1 –0-5% tissue colors change; score 2 – 5-35% tissue colors change; score 3 –35-50% tissue colors change; score 4 – 50-75% tissue colors change; score 5 – > 75% tissue colors change [11]. Disease intensity (DI) based on the wilting or the necrotic symptoms were calculated using the equation given DI = [(ni x si)/(N x S)] x 100% (where ni: number of plants with ith score of symptoms, si: the value of the ith score of symptoms, N: total number of tested plants, and S: the highest value of score of symptoms) [4]. Response of different banana Cavendish varieties after *Foc* inoculation was noted by using criteria where immune (Im) having 0% disease intensities (DI), resistant (Rs) if > 5% DI; moderately resistant (Mr) if 5–10% DI; moderately susceptible (Ms) if 10– 25% DI; susceptible (Sc) if 25– 50% DI; and very susceptible (Vs) if > 50% DI [22]. The data were analysed by using Duncan multiple ranges test and compared means of SPSS version 19.

RESULTS AND DISCUSSIONS

The inoculated banana suckers or plants showed various degrees of wilting after 15 to 60 days of artificial inoculation, while control one did not show wilting symptoms (Figure **1**). The transversal sections of banana corm and longitudinal section of pseudostem of symptomatic suckers or plants showed dark brown colour on epidermal, cortex and vascular tissues due to *Foc* infection (Figure **2**). The symptomatic corm and pseudostem of banana suckers or plants were plated on PDA plates; colonies of *Foc* were successfully re-

Figure 1: Cavendish banana plants with various degrees of wilting symptoms at 60 days after inoculation with *F. oxysporum* f.sp. *cubense*.

a

b

Figure 2: a) Longitudinal section of pseudostem and **b**) transversal sections of banana corm showed dark brown colour on epidermal, cortex and vascular tissues due to *Foc* infection.

isolated. Foc infection in banana is of a complex processes involving recognition, attachment to surface of banana roots, penetration of hyphae into cortex tissues, degradation of physical defense system of root tissues, proliferation of *Foc* hyphae and production of conidia in xylem tissues, and secretion of fungal toxin and hydrolytic enzymes resulting tissue damages of the infected roots [18]. There is a similar finding that high density of *Foc* conidia has effectively increased the intensity of the wilting and necrotic symptoms after 60 days of inoculation banana plants [19]. The use of *Foc* conidia at high densities (5 x 10^6) conidia/ml resulted in severe disease incidence on *Musa* species [11]. The root exudates inhibited the germination and growth of *Foc* in highly-resistant cultivars whereas no affect fungal germination and growth in susceptible cultivars [10].

Five tested Cavendish varieties viz., W11, G9, Basrai, Pisang and BT10 showed low to high average of score of wilting and necrotic symptoms but disease intensities is fall into categories of susceptible (Table **1**). TR4 seriously attacked the popular local variety 'Fenjiao' (ABB, subgroup Pisang awak) in China [12]. These findings are in conformity with the findings of other researcher stated that crops species such as

Table 1: Effect of Inoculation of *F. Oxysporum* f.sp. *cubense* Isolate in Disease Intensities of Five Banana Cavendish Varieties

Cavendish varieties	Score of Symptoms with disease intensities (DI)								Response against *Foc*
	Wilting		DI%		Necrotic		DI%		
	I	NI	I	NI	I	NI	I	NI	
W-11	3.5_c	0.6_a	43.7_c	10.5	2.5_b	0.9_a	41.6_c	20.0_c	Susceptible
G-9	2.5_b	0.8_a	37.5_b	18.7	1.5_a	0.8_a	33.5_a	16.5_b	Susceptible
Basari	3.5_c	0.4_a	43.5_c	8.5	3.5_c	0.6_a	43.7_c	10.5_a	Susceptible
Pisaung	1.5_a	0.8_a	30.5_a	17.7	2.0_b	0.6_a	37.3_b	10.5_a	Susceptible
BT10.	1.0_a	0.6_a	25.0_a	11.0	1.0_a	0.7_a	31.5_a	15.5_{ba}	Susceptible

Mean followed by the same letter within a column are not significantly different at (P=0.05) according to Duncan's multiple range test. I: inoculated; NI: without inoculation.

banana (*Musa* sp.) and cotton (*Gossypium* sp.) did not have resistance mechanism against *Fusarium* wilt. It was reported that similar responses of banana cultivars against *Foc* infection might be due to the absence of resistance gene to *Foc* infection and to the limited genetic diversity of the Indonesian abaca germplasm collections [9, 19] Development of resistant cultivars an integration of other disease management technologies involving bio-agents, botanicals and plant activators is the only solution of this devastating disease of banana.

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