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SCREENING OF SELECTED BRAZILIAN AND TANZANIAN COTTON (*Gossypium* spp.) CULTIVARS FOR *Fusarium* WILT RESISTANCE

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KEYWORDS

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ABSTRACT

Fusarium wilt of cotton caused by *Fusarium oxysporum* f.sp. *vasinfectum* Atk. Sny & Hans is one of the major diseases of cotton in Tanzania. Resistant varieties provide useful tools for management of this disease. Varieties developed at Lake Zone Agricultural Research and Development Institute with previous records of resistance are currently susceptible in areas with high level of inoculum warranting the need to search for additional sources of resistance. Further, cotton varieties of Brazil indicate some level of resistance against *F. oxysporum* f. sp. *vasinfectum* that can be exploited in breeding programmes. In present study, four varieties from Brazil and three from Tanzanian were screened under greenhouse conditions for resistance to *Fusarium* wilt. A population of 40 plants per variety was inoculated with a crude inoculum of *F. oxysporum* f. sp. *vasinfectum* by root-dip method. Randomized complete block design was used with four replications. Resistance was evaluated on basis of foliar symptoms (disease severity index) and plant survival percentage. Further, all plants showing wilting symptoms were examined for vascular discoloration. Among various tested varieties, two cultivars from Brazil (Ipê and Aroeira) had relatively lower disease severity index and higher plant survival, these results suggesting their potential candidature for use in breeding programmes for *Fusarium* wilt resistance. On the other hand, two local cultivars (UK91 and UK08) presented the highest disease severity index and lowest plant survival.

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1 Introduction

Cotton is an annual plant belongs to the family Malvaceae, order Malvales of the genus *Gossypium*. The genus *Gossypium* included about 50 species of which 45 are diploid ($2n = 2x = 26$) and 5 are allotetraploid ($2n = 4x = 52$). Out of these only four species are cultivated, two old world species (*G. arboretum* L. and *G. herbaceum* L.) and two New World species (*G. hirsutum* L. and *G. barbadense* L.) with the former being common to most countries including Tanzania (Iqbal et al., 2001). In Tanzania cotton is a source of income for more than 500,000 smallholder farmers and its contribution to the national export earnings is about US\$ 92 million, which is about 15% of the total national exchange earnings (TCB, 2010).

Cotton production in Tanzania is limited by various factors including diseases (Lukonge et al., 2007). Fusarium wilt is the most important diseases inflicting cotton in Tanzania (Hillocks & Kibani, 2002; Lukonge, pers. Comm.). *Fusarium* wilt of cotton is caused by a soil inhabiting fungus called *F. oxysporum* f. sp. *vasinfectum* that occurs in many cotton-growing areas. Worldwide losses due to *Fusarium* wilt have been estimated to be 1.5 billion bales annually (Gillham et al., 1995). In Tanzania, for the past ten years the disease was not considered significant as the incidence was less than 10 percent in most of infected areas of the WCGA (Hillocks & Kibani, 2002) but it has rapidly spread and causing significant loss to farmers (Lukonge, pers. comm.) and disease incidences reached up to 25 percent in the areas where both *Fusarium* and nematodes simultaneously attacked on cotton plants (DFID, 2000).

F. oxysporum f. sp. *vasinfectum* is a ubiquitous, asexual soil borne fungus, which may live saprophytically or as a destructive pathogen of many crops including cotton. The fungus attacks the cotton plants mostly at seedling stage. The fungus invades the plant through root wounds and then infects the vascular system, resulting in wilt symptoms (Hillocks & Bridge, 1992). The disease is highly destructive and causes great losses in cotton as it affects both yield and fibre quality. Disease incidence was reported in more than five percent of sampled fields (Hillocks & Kibani, 2002). The incidence, rate of development and severity of the disease became more prominent in the presence of the root-knot nematode (*Meloidogyne incognita*) and is known as *Fusarium*-Root-Knot nematode complex (Blasingame, 2006; Wang & Roberts, 2006).

Various races of *F. oxysporum* f. sp. *vasinfectum* have been characterized in different cotton growing countries. The resistance of commercial cotton cultivars to *Fusarium* wilt varies from one race to another and some of cultivars may be resistant to more than one race (Abd-Alsalam et al., 2009). Races of *F. oxysporum* f. sp. *vasinfectum* afflicting are known by numbers viz races 1 and 2 are described to occur in Tanzania and the United States of America while race 3 was

described from Egypt, Israel, China, Sudan and the former USSR. Race 4 was highly prevalent in India; race 5 in Sudan and race 6 in Brazil and Paraguay while races 7 and 8 were described from China (Armstrong & Armstrong, 1980). Additional races of this pathogen have also been identified in the U.S.A and Australia. Evolutionary lineage of races for each country is confirmed by molecular analyses (Kim et al., 2005).

Fusarium wilt of cotton was first time reported in 1954 at Geita district of Tanzania from the Western Cotton Growing Area (WCGA) close to the shores of Lake Victoria (Peat et al., 1955). This disease spread rapidly with the expansion of cotton production from the 1950s to 1960s. By the early 1960s, it had become sufficiently widespread in the WCGA to justify the incorporation of resistance to *Fusarium* wilt as selection criterion in the local breeding program at the Lake Zone Agricultural Research and Development Institute (LZARDI) (Hillocks, 2008). Two cultivars (UK77 and UK91) which were developed at LZARDI had useful levels of resistance against *Fusarium* wilt (Hillocks & Bridge, 1992) but their susceptibility has increased in recent years particularly in areas where the level of inoculum is high or where cotton plants are attacked by the root-knot nematode *M. incognita*.

Some cultivars have shown significant resistance against *F. oxysporum* f. sp. *vasinfectum* and it is possible to introduce such resistance gene into elite germplasm through crossing and subsequent selection (Reid et al., 2004). The resistance of various cultivars also depends on the race of *F. oxysporum* f. sp. *vasinfectum* (Abd-Alsalam et al., 2009). In Australia, Stiller & Reid, (2005) reported that resistance from a cultivar MCU-5, bearing a complex resistance (Becerra Lopez-Lavalle, 2012) was successfully introduced into elite Australian germplasm and the resulting cultivar showed doubled resistance when compared to the previous most resistant Australian cultivars. Identification of resistant germplasm to *Fusarium* wilt is thus a vital tool for the purpose of disease resistance breeding. Some cultivars in Brazil also shown some level of resistance in fields when these were infected by this pathogen (Cia et al., 2008). This study therefore endeavored to screen four selected Brazilian cultivars in parallel with three local cultivars for resistance to *F. oxysporum* f. sp. *vasinfectum* in local growth conditions with the aim of identifying resistant individuals for use in disease resistance breeding programmes.

2 Materials and Methods

2.1 Plant materials

The plant material used in this study were developed at LZARDI and the Embrapa research Centre and all are commercially released varieties (See Table 1).

2.2 Isolation and identification of the pathogen

A mixture of diseased plants were collected from a naturally *Fusarium* infected plot at Nyamasindi-Ukiriguru (it was

located about 5km from LZARDI and established for the purpose of increasing the level of *Fusarium* inoculum for research purposes). Infected plant stems were cut into small pieces of 1 cm by using a sterilized scalpel; these tissue were surface sterilized in 1% sodium hypochlorite (NaOCl) for one minute and rinsed three times using sterile distilled water, and dried with sterile paper napkin. Ends of the surface sterilized tissues were cut to remove sterilitant and plated on a Petri dish containing prepared PCNB-agar medium amended with streptomycin. The plates were incubated at room temperature for seven days. After 7 days of growth, 5mm discs were cut from the edge of fungal culture and placed on freshly prepared Potato Dextrose Agar (PDA) plates, which were sub-cultured and multiplied. The pathogen was identified with reference to Booth (1971).

2.3 Preparation and quantification of inoculums

The inoculum of *F. oxysporum* f. sp. *vasinfectum* was prepared by flooding cultures on PDA plates with distilled water and scraping the hyphae off. The resulting suspension was filtered through four-layer cheesecloth to separate spores from hyphae and spore suspension was quantified and concentration adjusted to 1.0×10^6 conidia/ml using hemocytometer. This adjusted spore suspension was stored at low temperature (about 4° C) ready for inoculation.

2.4 Sowing of seeds and seedling inoculation

Seeds of all seven cultivars were planted in pots containing sand and 14 days after germination the seedlings were removed from the sand by shaking, and then they were washed in water to remove debris and excess sand. Using sterile scissors, 1 cm of the distal end of the root system was cut. The cut plants were dipped in the *Fusarium* inoculum at a concentration of 1.0×10^6 conidial ml⁻¹ for 10 minutes to allow the conidia to enter the wounds created in the root systems. The plants were then carefully transplanted into planting pots (one plant per pot) containing a mixture of sterile soil and sand in the ratio of 3: 1 v/v. The pots were arranged in a randomized complete block design in four replications with 10 plants of each cultivar in each replication in the screen house. Immediately after transplanting, watering was done at an interval of two days. Inoculation process was carried out at late evening to avoid the effect of high day temperature on the newly transplanted seedlings. Temperature of the screen house was not controlled but it never went above 29.7 and never below 18.7°C for the duration of the evaluation.

2.5 Assessment and rating of the disease severity

Disease severity for foliar symptoms was measured five weeks after inoculation, since it has been reported that leaf symptoms in some resistant plants become harder to recognize with continued plant growth beyond 5 weeks after inoculation (Kim et al., 2005). Plants were rated for disease severity based on the 1-5 scale as proposed by Wickens (1964) with minor modifications according to foliar wilt symptoms as follows: 1 = healthy, 2 = only cotyledons wilted, 3 = ≤50% true leaves wilted, 4 = >50% but ≤90% true leaves wilted and 5 = all leaves wilted and plant was dead. The disease severity index was calculated by using the formula:

$$\text{Disease index} = \sum_{\text{rating}=1}^4 \frac{(\text{rating} \times \text{no. of plants with rating})}{\text{total no. of plants}}$$

All plants showing wilting symptoms were examined for vascular discoloration and the pathogen re-isolated from such diseased plants. Plant survival percentage was computed using the formula:

$$\text{Survival percentage} = \frac{\text{number of surviving plants}}{\text{initial number of plants after establishment}} \times 100\%$$

3 Results

All diseased plants showed wilting symptoms and when dissected had brown colouration in their stems (Figure 2). On basis of foliar symptoms, the cultivars UK91, UK08, UKM08, Cedro and Araça were found more susceptible to *F. oxysporum* f. sp. *vasinfectum* as they presented relatively higher disease severity index (Figure 3). However, two of the Brazilian germplasm viz Ipê and Aroeira exhibited relatively low disease severity indices. These two cultivars also had higher plant survival percentages compared to the others. Low disease severity index was generally seen to be associated with relatively higher plant survival and vice versa. There was an extensive range of symptoms observed on other parts of the plants in addition to foliar symptoms. These included vascular browning (Figure 2), chlorosis and abscission of cotyledons, chlorosis and necrosis of individual or multiple leaves, plant wilting, stunted growth and death of plants.



Figure 1 1-5 *Fusarium* wilt infection severity scaling on basis of foliar symptoms. U- Uninoculated

Table 1 Plant materials used in the study with their origins.

S. No	Country of Origin	Name of Cultivar	Characteristics of Cultivar
1	Tanzania	UK 91	Yield 1200– 1600kg/ha; Ginning percentages - 39.6%; Resistant to Jassids; Resistant to Fusarium wilt and Bacterial Blight; Fibre length 1.151in; Fibre strength 27.9g/tex; Fibre micronaire 4.16mic/in; Rainfall requirement 600 – 1200mm; Grown at Low land, Middle land to high land
2	Tanzania	UK 08	Yield 1600 – 2000kg/ha; Ginning percentages 40.2%; Resistant to Jassids; Resistant to <i>Fusarium</i> wilt and Bacterial Blight; Fibre length 1.276 in ; Fibre strength 28.2 g/tex; Fibre micronaire 4.20mic/in; Rainfall requirement 600 – 1200mm; Grown at Low land, Middle land to high land
3	Tanzania	UKM 08	Yield 2000 – 2500kg/ha; Ginning percentages 42.3%; Resistant to Jassids; Resistant to <i>Fusarium</i> wilt and Bacterial Blight; Fibre length 1.275 in ; Fibre strength 30.2g/tex; Fibre micronaire 4.03mic/in; Rainfall requirement 600 – 1200mm; Grown at Low land, Middle land to high land
4	Brazil	BRS Cedro	Yield 3695 kg/ha; Ginning percentages 40.0 to 42.5%; Moderately resistant to Blue disease virose, ramulosis, <i>Ramularia areola</i> , Bacterial Blight; Moderately Susceptible to <i>Meloydogyne</i> + <i>Fusarium</i> complex; <i>Alternaria</i> + <i>Stemphylium</i> ; Fibre length 30.4mm (1.197in); Fibre strength 28.5 gf/tex; Fibre micronaire 4.3
5	Brazil	BRS Araca	Yield 3972 kg/ha; Ginning percentages 37.5 to 38.30%; Moderately resistant to Blue disease virose, ramulosis, <i>Ramularia areola</i> , Bacterial Blight, <i>Meloydogyne</i> + <i>Fusarium</i> complex; Susceptible to <i>Alternaria</i> + <i>Stemphylium</i> ; Fibre length 30.2 mm (1.189 in); Fibre strength = 30.1 gf/tex; Fibre micronaire 4.2
6	Brazil	BRS Ipe	Yield 3149 kg/ha; Ginning percentages 40.5 %; Moderately resistant to <i>Fusarium</i> , <i>Stemphylium</i> , <i>Alternaria</i> ; Resistant to <i>Ramularia areola</i> ; Fibre length 26.7mm (1.051 in); Fibre strength 27.7 g/tex; Fibre micronaire 4.40
7	Brazil	BRS Aroeira	Yield 3958 kg/ha; Ginning percentages 38.8%; Moderately resistant to <i>Fusarium</i> , <i>Meloidogyne</i> , <i>Ramulosis</i> ; Resistant to <i>Stemphylium</i> , <i>Alternaria</i> ; Fibre length 27.9 mm (1.098 in) ; Fibre strength 30.6 g/tex; Fibre micronaire 4.20 mic/in

One way-ANOVA revealed a significant difference ($p < 0.0001$) among the cultivars for foliar/wilting symptoms. Highest mean disease severity index was recorded from the cultivar UK91 (85.91) and Ipê recorded the lowest index (37.69) (Figure 3). Similarly, the percentage of plant survival varied significantly ($p = 0.0001$) across the cultivars with Ipê recording the highest plant survival percentage and UK91 the least (Figure 3).



Figure 2 Vascular discoloration symptoms for infected plants

4 Discussions

Several sources of resistance to *F. oxysporum f. sp. vasinfectum* have been established and the resistance trait has been successfully introgressed into susceptible locally adapted elite cotton cultivars (Stiller & Reid, 2005).

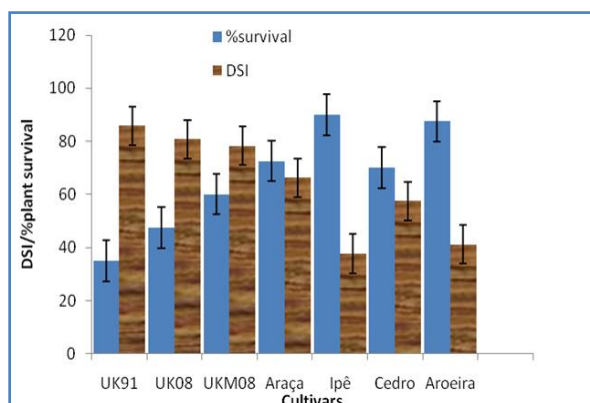


Figure 3 Disease severity index and plant survival percentage of seven cultivars

In this study, seven cultivars were screened for *Fusarium* wilt resistance and among these two cultivars Ipê and Aroeira showed relatively lower disease severity index and higher survival percentage suggesting some resistance against *F. oxysporum* f. sp. *vasinfectum* race 1 and/or race 2 as described by Armstrong & Armstrong (1980) to be found in Tanzania. Further, Cia et al. (2008) also studied the performance of 12 genotypes in different cotton growing regions in the state of Sao Paulo and found that the cultivars Ipê and Aroeira were moderately resistant to race 6 of *F. oxysporum* f. sp. *vasinfectum* as described by Armstrong & Armstrong (1980). Resistance characteristics of these two cultivars suggested that they had resistance for a range of races of *F. oxysporum* f. sp. *vasinfectum* and therefore the germplasm present in these two is a potential candidate for breeding programmes in different regions where different races of the pathogen occur.

The Tanzanian cultivar UK91, previously known to be resistant to Fusarium wilt (ICAC, 2003; Ruzo, Pers. com) presented in this study the lowest plant survival percentage and the highest disease severity index compared to other cultivars suggesting that either the earlier observed resistance had broken down and/ or that the resistance scoring was not on an adequate level of infection. Further, Ulloa (1990) reported that increasing the inoculum concentration had a direct effect on disease index of cultivars in seedling screening techniques. Likewise Stiller & Wilson (2014) observed that most resistant cultivars can have only 10% survival if the fields with high level of inoculum, this is suggesting that some cultivars can only resist at certain level of inoculum above which they develop wilting symptoms while Chawla et al. (2012) suggested that *F. oxysporum* f. sp. *vasinfectum* race 1 can cause severe symptoms even in absence of nematodes at 6.5×10^5 cfu/ml. These observation suggesting that the cultivar UK91 may resist up to a certain level of inoculum (less than 1.0×10^6 conidia/ml) and above this it start showing wilt symptoms.

The study was conducted under the greenhouse conditions, the results may not reflect the field responses of cotton cultivars since field grown plants are frequently under environmental or biotic stresses which was not present in the greenhouse. Despite the differences between this experimental and field conditions, disease development in the field possibly follows the general trends observed in this study.

Further studies are required for a broader understanding of disease development and distribution, which depends on climate, soil conditions, race of the pathogen, as well as the resistance level of cotton plants. Breeding for Fusarium wilt resistant cotton varieties has been a main goal of cotton breeders and it has been established that resistance trait can be introgressed into elite cultivars through crossing (Stiller & Reid, 2005). Due to general adaptability presented by the Brazilian cultivars in local growth conditions (data not shown) and the resistance to Fusarium wilt reported in this study, the two cultivars (Ipê and Aroeira) can be considered for use in

local breeding program after further screening under field conditions.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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