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RESEARCH ARTICLE

Screening of selected cotton genotypes (*Gossypium* spp) for resistance to fusarium wilt disease in Tanzania

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ABSTRACT

Cotton is an important commodity in various industries across the globe; however, cotton yield is adversely affected by the existence of pathogenic fungi including *Fusarium oxysporum* f.sp. *vasinfectum* (FOV), the causative of Fusarium wilt (FW), which is among the major disease-threatening cotton production in Tanzania. This study screened 25 selected varieties resistant to FOV under screen house conditions by inoculating the emerged cotton seedlings with 1.0 x 10^8 conidia/ml of *F. oxysporum* f. sp. *vasinfectum* using the root cut dip method. The fusarium wilt disease was evaluated based on foliar symptoms (disease severity scaling and vascular colouration). Out of 25 evaluated cotton candidates, three candidates F 135, HC-B4-75 and UK 66 were significantly higher resistant with lower disease severity than others, while QUIT122, IL85, PSF2-1-1, and UK91 have significantly lower resistance ($P < 0.05$).

Keywords: cotton genotypes, fusarium wilt, plant disease resistance, screen house experiment.

INTRODUCTION

The perennial shrub cotton is grown in subtropical and tropical areas and it belongs to the Family Malvaceae (Ashokkumar et al., 2013). About 45 of the 50 Gossypium species are diploid (2n = 2x = 26) and 5 are allotetraploid (2n = 4x= 52). Only four species are grown: two old worlds (*G. arboreum L.* and *G. herbaceum L.*) and two new worlds (*G. hirsutum L.* and *G. barbadense L.*). India is the only country to cultivate all four species of cotton (Ashokkumar et al., 2019). Cotton is produced in 80 countries and is worth US$50 billion. China, India, the US, Pakistan, Brazil, Uzbekistan, and Australia grow the most cotton, whereas Mali, Burkina Faso, Benin, Ivory Coast, Cameroon, Nigeria, and Tanzania grow the least (Ashokkumar et al., 2014). Compared to coffee, tobacco, cashew nut, tea, sugar cane, sisal, and

pyrethrum, cotton is Tanzania's fourth most important cash crop based on foreign exchange earnings and the most widely cultivated. Up to 40% of Tanzanians depend on cotton and its value chains. Over 600,000 smallholder farmers in 17 Eastern and Western regions earn their living from cotton: Simiyu, Mwanza, Geita, Shinyanga, Mara, Tabora, Kigoma, Singida, Dodoma, Katavi, Morogoro, Tanga, Kilimanjaro, Iringa, Kagera, Manyara, and Coastal Region.

Biotic and abiotic variables limit cotton productivity (Lukonge et al., 2007; Faustine, 2016; Khaskheli, et al., 2021). Diseases and insect pests including Verticillium wilt, Alternaria, Bacterial blight, and Fusarium Wilt (FW), Tanzania's most harmful cotton disease, are biotic factors. Fusarium wilt disease first appeared in Tanzania in the 1960s (Kibani & Hillocks,1998), and it is spreading rapidly, causing farmers to lose 30% to 60%. Fusarium wilt disease reduces Tanzanian farmers' seed cotton output to 560kg to 750kg per hectare, compared to the potential yield of 2500-3000 kg/ha. A small Tanzanian study related cotton genotype to FOV-resistant genes. The efforts included screening 3 cotton genotypes for FW resistance (Faustine et al., 2016), identifying Tanzanian FOV1 and FOV2 fusarium races, and studying factors affecting the distribution, incidence, and spread of cotton wilt (Kibani and Hillocks, 1998). To identify resistant cotton candidates for FOV breeding efforts, this study screened 25 cotton genotypes by root cut dip inoculation for *Fusarium oxysporum* f.sp. *vasinfectum* resistance.

Pathology lab at Sokoine University of Agriculture (SUA) quantified this modified spore solution, which was held at 4ºC until inoculation.

MATERIALS AND METHODS

Location of the study area

The experiment was conducted at TARI Ukiriguru centre at the latitude of -2.718651^0 and longitude of 33.023956^0 it was established in the screen house.

Experimental materials and design

Twenty-five (25) cotton genotypes were selected from the Western cotton growing area (WCGA) at TARI Ukiriguru germplasm and the Eastern cotton growing area (ECGA) at TARI Ilonga germplasm. Sterile sand and soil were used as a growing media and 1.0 x 10^8 conidial/ml was used to inoculate. Each cotton genotype was sown in two pots and arranged by Completely Randomised design (CRD) and replicated three times.

Statistical Analysis

Data collected were subjected to one way of ANOVA to see the significant variation among the genotypes at $p<0.05$ and Duncan multiple tests were used to separate the means, also pivot chat was used to plot the disease severity rate as well as the significant change of foliar wilt after every 7 days of assessment. The software used for statistical analysis was GenStat 16th Edition (VSN International Ltd).

Preparation of inoculum and quantification

FOV inoculum was created by flooding Potato Dextrose Agar (Eur. Pharm.) cultures on a Petri dish with distilled water and scraping the hyphae off at TARI Ukiriguru Centre's Cotton Pathology laboratory. Spores were separated from hyphae by filtering through four layers of cheesecloth. The spore suspension was measured and corrected to 1.0 × 10^8 conidia/ml using a hemocytometer. The Plant

Inoculation of Seedling

The cotton seeds were sowed in sterilized sand media and inoculated using root cut dip. One week following emergence, the seedlings were uprooted and washed in water to remove debris and excess sand. The root system's distal end was severed 1cm with sterile scissors. To introduce conidia into root system wounds, clipped seedlings were dipped in FOV inoculum at 1.0 × 10^8 conidial/ml for 10 minutes. The inoculated plant was put into growth pots with 3:1 sterile soil-sand mixtures, watered immediately, and watered normally after two days. Inoculation was done early in the morning before high temperatures to prevent evapotranspiration from killing the wounded seedlings.

Assessment and rating of the disease severity

After inoculation, plants were examined daily and leaves were appraised and documented for symptoms at 7-day intervals until 35 days after inoculation (DAI) using a 1-5 rating scale. After seven days, some cotton species start to show symptoms, and after 35 days (fifth week), some cotton species recover and the symptoms are hard to spot (Kim et al., 2005). Individual plants were graded for disease severity **(Figure. 1)** using Faustine et al. (2016) modified index: 1 = no symptoms, 2 = cotyledon wilting, 3 = ≤50% true leaves wilted, 4 = >50% but ≤90% true leaves wilted, and 5 = all leaves wilted. The stems were sliced lengthwise and examined for vascular pigmentation 36 days following inoculation **(Figure 2)**. After collecting, calculating, and recording data, foliar disease severity rating,

genotype variation analysis, and genotype severity rating day differences were done.

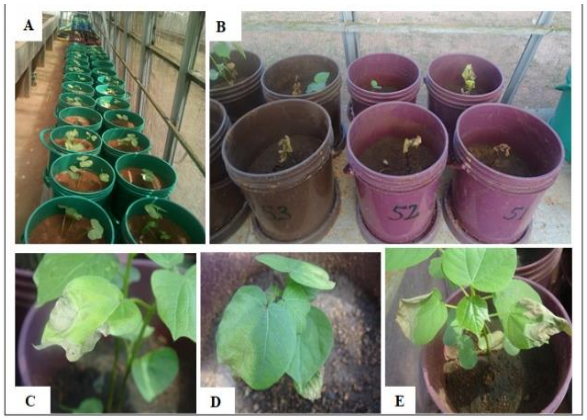
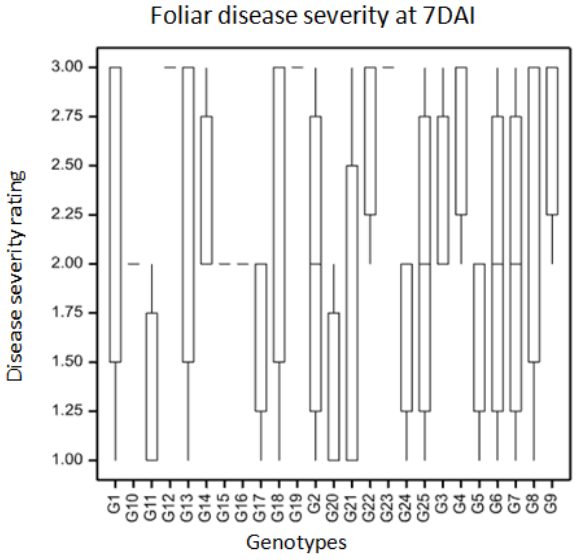


Figure 1. A: Cotton seedling after inoculation, **B**: Seedling shows the total foliar wilting symptoms, **C-E**: Seedling showing the foliar wilting symptoms

RESULTS AND DISCUSSION

Foliar disease severity

Within seven days of post inoculation 22 genotypes out of 25 studied; their plant cotyledons started to show wilting symptoms (**Figure. 3**). The genotype G11=UK66, G20=F135 and G21=J1 (88)14 were not affected and the genotype G1= VM, G8=UK68, G13=UK70 and G18=IL74, were the most affected. Furthermore, in fourteen days post-inoculation, only two genotypes namely G11= UK 66 and G20= F135 maintained the resistance as they did not show wilting symptoms, whereas the one genotype (G21=J1 (88)14) started showing symptoms at 14 days post-inoculation (**Figure 4**).



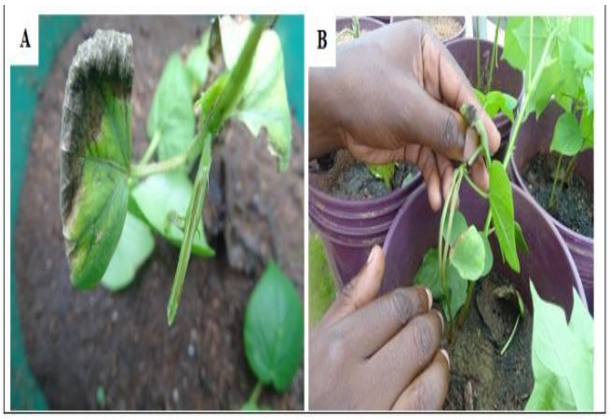


Figure 2A & B. Seedlings showing the vascular colouration after the longitudinal cut, turn to Brownish colour symptoms of FOV.

Figure 3. The rating result of foliar disease severity at 7 DAI.

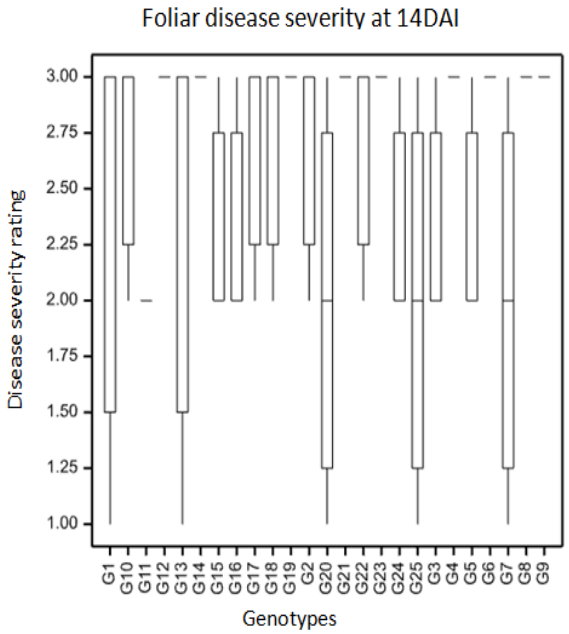


Figure 4. The rating result of foliar disease severity at 14 DAI.

In twenty-one-day post-inoculation, the symptoms progressed to the true leaves, where 22 genotypes were observed to wilt from cotyledon up to the true leaves while three genotypes namely G3= PSF2-1-1, G11= UK66, and G20= F-135 were different from the rest by maintaining symptoms only to the cotyledon. Interestingly, even the three genotypes

namely G7= UK77, G13= UK70 and G25=HC-B4-75 did not show the wilting to the cotyledons (**Figure 5**).

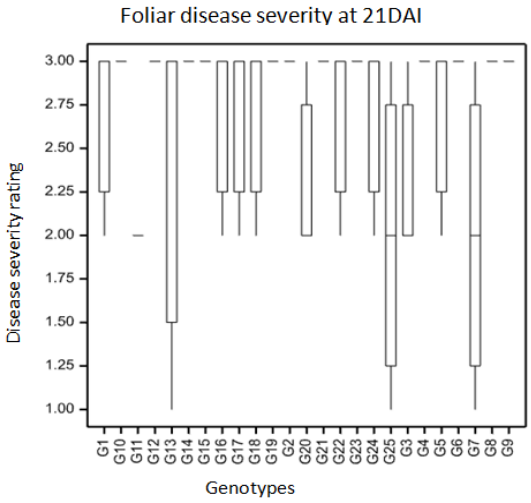


Figure 5. The rating result of foliar disease severity at 21 DAI

After twenty-eight days post-inoculation, the leaves indicated that 50% of the plant wilted to some of the genotypes only one genotype did not reach 27% of the total plant wilting G20= F 135 (**Figure 6**).

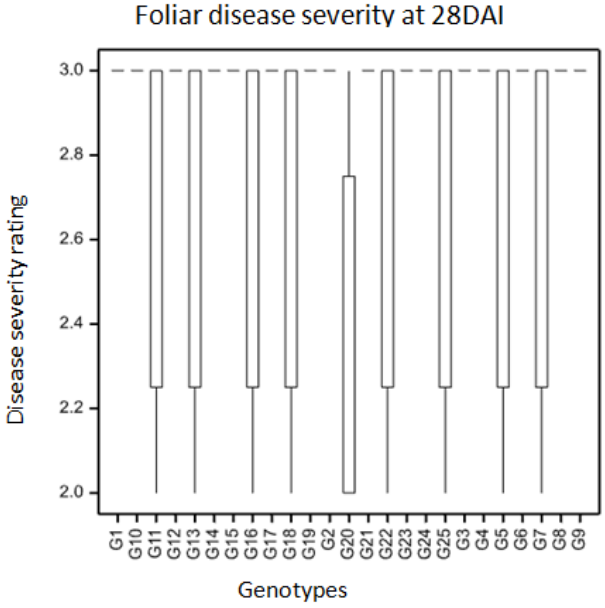


Figure 6. The rating result of foliar disease severity at 28 DAI.

In thirty-five days post-inoculation, the true image was observed in all twenty-five genotypes where only five genotypes were revealed to have a lower rating score below 30% of foliar disease severity G20= F135, G25= HC-B4-75, G5= Local Kigoma, G18= IL74 and G22= UK74 ascending (**Figure 7**)

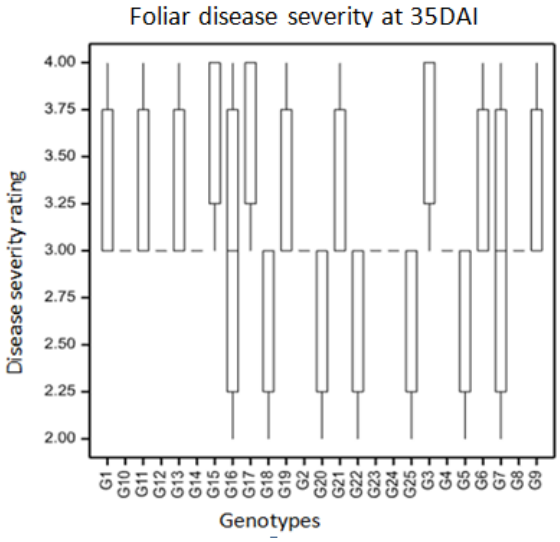


Figure 7. The rating result of foliar disease severity at 35 DAI.

Generally, from the point of view, the foliar disease severity rating indicated the trend of foliar symptoms and three genotypes had performed significant resistance G20= F 135, G11= UK66, and G25= HC-B4-75 compared to other genotypes (**Figure 8**).

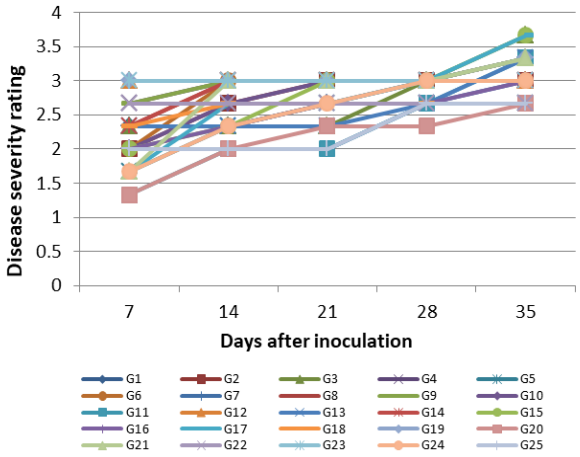


Figure 8. The summary rating result of foliar disease severity is from 7 to 35 DAI.

Genotypic differences

By One-way ANOVA, the result indicated that there is no significant difference among the genotypes within the day (7, 14, 21, 28, and 35) of assessing

the foliar disease symptoms at P<0.05, rather there are differences made from the scare. Six genotypes were scaled to 1 which is no foliar symptoms were recorded in 7 days after inoculation (DAI) these were G5, G11, G17, G20, G21 and G24 **(Table. 1)**. In days 14 to 35 after inoculation the foliar symptoms were recorded to all 25 genotypes although the severe were differences and the highest scared genotypes were G3, G15 and G17 to the 35 days after inoculation.

Genotypic difference from the bar graph also indicated that 35 days after inoculation, the higher-scaled genotypes showed more than 80% foliar wilting to the plant at G3, G15, and G17 (**Figure 9**).

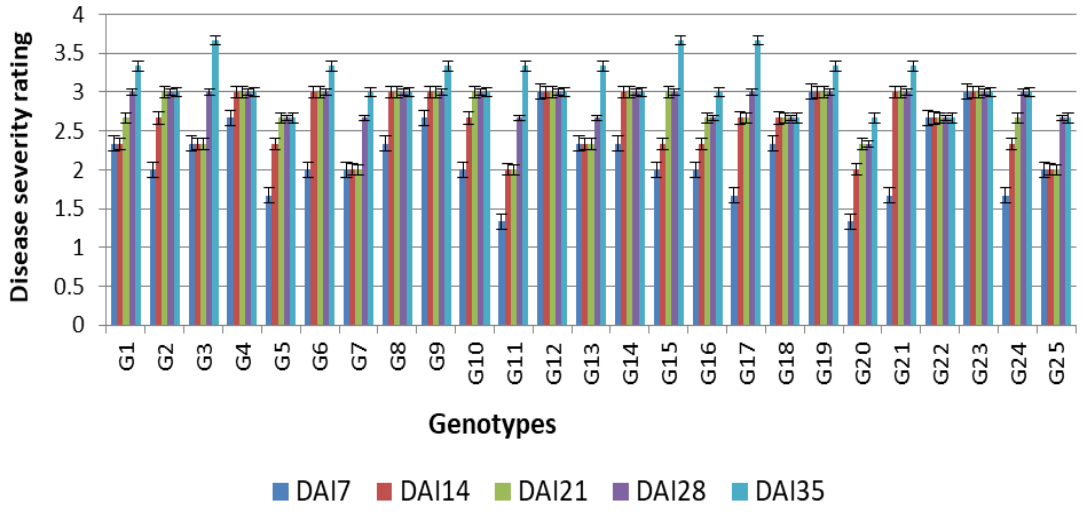


Figure 9. Summary of rating result for 25 genotypes of foliar disease severity of fusarium wilt symptoms from day 7 to 35 days after inoculation

Table 1. Summary of Foliar Disease Severity Means At 7-35days after inoculation

Genotypes ID	7DAI	14DAI	21DAI	28DAI	35DAI
G1	2.333	2.333	2.667	3	3.333
G2	2	2.667	3	3	3
G3	2.333	2.333	2.333	3	3.667
G4	2.667	3	3	3	3
G5	1.667	2.333	2.667	2.667	2.667
G6	2	3	3	3	3.333
G7	2	2	2	2.667	3
G8	2.333	3	3	3	3
G9	2.667	3	3	3	3.333
G10	2	2.667	3	3	3
G11	1.333	2	2	2.667	3.333
G12	3	3	3	3	3
G13	2.333	2.333	2.333	2.667	3.333
G14	2.333	3	3	3	3
G15	2	2.333	3	3	3.667
G16	2	2.333	2.667	2.667	3
G17	1.667	2.667	2.667	3	3.667
G18	2.333	2.667	2.667	2.667	2.667

G19	3	3	3	3	3.333
G20	1.333	2	2.333	2.333	2.667
G21	1.667	3	3	3	3.333
G22	2.667	2.667	2.667	2.667	2.667
G23	3	3	3	3	3
G24	1.667	2.333	2.667	3	3
G25	2	2	2	2.667	2.667
Cv%	12	6.2	2.3	0.8	2
F Pr.	0.179	0.273	0.147	0.609	0.446
S.E.d.	0.5847	0.4815	0.4163	0.288	0.438

Note: The scores from the table within the day of assessment were not significant across the date of scores according to Duncan's multiple tests (p<0.05).

DISCUSSION

Resistant cotton materials to *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) have been enacted and the resistant genes were recognized (Ulloa et al., 2013 and Wang et al. 2014) and it is reported that the introgression of a resistant gene(s) to susceptible cultivars was made and resistance traits transfer was successfully made (Ulloa et al., 2016). In this experiment, twenty-five genotypes were screened for resistance to FOV about three genotypes were recorded to have relatively lower disease severity with higher survival rates, this revealed the findings of resistant genotypes F 135, HC-B4-75 and UK 66 after vascular evaluation, resistant to FOV race 1 and/or race 2 as described by Armstrong & Armstrong (1980) to be found in Tanzania.

Among these 25 genotypes, three were previously screened for FOV, Two genotypes UK77 and UK91 by Kibani & Hillocks (2002), were reported to have traits for resistance even in this study observed to have those traits but not as better as the three recognized genotypes; Other genotype UKM 08 were screed by Faustine (2016) and reported that the cultivar had a resistance traits, the resistant level may be due to 1.0 x 10^6 concentration of conidial per ml for higher concentration will lead to susceptible to FOV. Also, was reported by Stiller & Wilson (2014) that the resistant genotypes can be susceptible to higher concentrations of conidial.

This study collected the cotton materials from the Western cotton growing area as well as the Eastern cotton grown area in Tanzania plus the outsourced materials and found that three genotypes were relatively resistant compared to the UK 77 and UK 91 screen by Kibani & Hillocks (1998) and UKM08 screened by Faustine (2016). Due to the findings from only screen house results of this study purposively conidial introgressed to the cotton genotypes by root cut dip method, these results may somehow not reflect the natural field infection and knowing the susceptibility and resistance of the genotypes. As reported race 1 or/and 2 can enter the cotton root through the injuries made by the nematode.

Further studies are required to be made to survey if there are newly introduced FOV races from the two cotton growing areas WCGA and ECGA, as the study for on-field screening of these cotton materials to assess the response under field conditions, as well as to screen the materials by using molecular markers as reported by Yu, J. et al (2021) having the QTL primers resistance to FOV; Cotton breeders' main goal has been to develop Fusarium wilt-resistant cotton varieties, and it has been demonstrated that resistance traits can be introgressed into elite cultivars by crossing (Stiller et al., 2005; Zhang et al., 2015).

CONCLUSION

The three cotton genotypes recorded in this study, F 135, HC-B4-75, and UK66, which are resistant to Fusarium wilt disease by foliar disease severity and vascular brownish colouration showing Fusarium wilt disease can be considered for use in the local breeding program after further screening under field conditions and with the assistance of molecular markers.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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