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Evaluation of new cotton genotypes against Verticillium wilt (*Verticillium dahlia* Kleb)

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Abstract

A study was carried out to determine the response of some new cotton (*Gossypium hirsutum* L.) lines to Verticillium wilt (VW) disease caused by *Verticillium dahliae* Kleb to facilitate the under natural infection. Cotton production faces a lot of choking setbacks especially when still in the field; VW has been noted to be one of the most destructive diseases worldwide. The study was conducted at Cotton Research Institute during the 2020/21 and 2021/22 growing seasons. The field trials were laid out in a Randomized complete block design (RCBD) with three replications where fourteen genotypes were used as plant material, nine new advanced cotton lines and five commercial cultivars. During the cotton growing seasons, foliar disease incidences and vascular browning were observed in addition to seed cotton yield. According to the results, all the cotton cultivars were susceptible to VW except for 562-00-9 which was tolerant and obtained high yields consistently over the two seasons. This variety can be used in the variety development programme to impart VW tolerance.

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Introduction

Cotton (*Gossypium hirsutum*) is an important commodity in the world economy, widely grown in at least 80 countries (Jamshed *et al.*, 2016). It is a cash crop and a source of better-quality natural fibre used as raw materials in the textile industry. Cotton seeds are used in the oil sector, seed cotton cake is used after extracting oil in the livestock sector, and linter is used as raw material in the paper industry (Bolek *et al.,* 2016). Cotton production, Zimbabwe's third most important cash crop after tobacco and sugarcane, contributes significantly to agricultural exports. It is grown by over two hundred thousand (200,000) smallholder farmers (Mukucha and Chari, 2024), with plot sizes of about one hectare on average, during the summer rainfall growing season from November to April (GAIN, 2017). It is such a crucial crop that it is vital that, even as commodity prices dip, the capacity of the industry to produce top-quality export cotton is maintained.

Stress factors (biotic and abiotic) limit the yield in cotton production. Alkher *et al.* (2009) indicated that Verticillium wilt (VW) disease, which is caused by *Verticillium dahliae* Kleb is the most devastating of biotic stress factors. The causal agent can infect about 400 plant species from 40 different families (Joaquim and Rowe, 1990). The disease can cause severe crop losses and adversely affect yield and fibre quality. Pullman and DeVay (1982) noted that the disease causes reduction of biomass, internode length, development of bolls, number of open bolls and fibre production. Bell (1992) also indicated that the length, strength, and fineness of fibres may also be reduced. Yield losses of 20-100% can be realised depending on the variety and severity (CRI, 2012), thus becoming a major hindrance in cotton production especially in the communal areas because of difficulties faced during the disease control processes. The pathogen infects plant roots throughout the growing season and can persist in the soil for 10 to 15 years as melanised microsclerotia, rendering crop rotation strategies for disease control ineffective (Duressa *et al.*, 2013), and seems uneconomic.

Control of VW diseases is complicated by the lack of genetic resistance in many plant hosts, and by the persistence of *V. dahliae* in the soil (Klosterman *et al.,* 2009). According to El-Zik (1985), the disease is best controlled with crop rotation using balanced fertilization, irrigation and tolerant/resistant cultivars as well as weed control against Verticillium wilt that is not effectively and chemically controlled. Bell (1989) cited in Hillocks (1992) also indicated that no single method is highly effective in controlling the disease. The combination of good cultural practices and the use of tolerant cultivars may reduce yield losses. However, Çelik *et al.* (2019) revealed that resistant/tolerant varieties developed against the disease lose their durability over time. For this reason, the evaluation of promising genotypes should be an ongoing process to develop resistant varieties to minimise yield loss and reduce production costs in cotton. Thus, breeding for VW tolerance has become a major goal at the Cotton Research Institute in Zimbabwe (Chapepa *et al.*, 2013). Hence this study seeks to evaluate the performance of promising cotton germplasm from Cotton Breeding programmes under Verticillium wilt naturally infested field for tolerance.

Materials and methods

The field trials were conducted at Cotton Research Institute (CRI) in a research trial block which has a long history of verticillium wilt infections for two seasons 2020/21 and 2021/22. Cotton Research Institute is situated 3km from Kadoma town along Golden Valley Road with coordinates of 18°20′24″S 29°54′00″E (Mubvekeri and Nobanda, 2012). It is in the agro-ecological region III, a traditional cotton growing area that receives an average of 758mm rainfall (October to April) and temperature ranges of 18-38°C (Mugandani *et al.*, 2012). The soils are Fersiallitic (Nyamapfene, 1991) with a pH range of 5.5 to 6.5. The altitude is 1149m above sea level with mean and maximum temperatures of 18°C and 26°C respectively. During the investigation period total rainfall received was 1305.7mm for the 2020/21 season and 772.2mm for the 2021/22 season. The summary of monthly rainfall and temperature records during the investigation term at CRI (Table 1). In the study, five commercial cultivars as control and nine experimental lines were used as material all developed at CRI and are listed in Table 2.

Table 1. Mean temperature, maximum temperature and total rainfall for the period of investigation

Month	Rainfall (mm)		Maximum temperature (°C)		Minimum temperature (°C)	
	2020/21	2021/22	2020/21	2021/22	2020/21	2021/22
October	74.6	21.5	32.3	32.0	13.7	12.0
November	28.0	21.5	34.3	34.3	16.0	15.3
December	340.6	156.9	29.3	33.0	15.7	14.3
January	395.4	326.9	29.0	28.3	16.0	13.7
February	339.6	74.8	28.7	28.7	15.0	12.7
March	75.3	71.6	28.3	30.3	13.7	12.0
April	8.9	68.4	27.3	29.0	9.7	12.0
May	43.0	20.6	27.7	27.0	8.0	7.3
Total Rainfall	1 305.7	772.2				

Source: Kadoma weather station, Zimbabwe.

Table 2. List of CRI cotton lines used in the experiment

Treatment	Attribute
562-00-9	Breeding line
564-00-6	Breeding line
566-99-23	Breeding line
LV96-05-8	Breeding line
97-05-1	Breeding line
816-01-1	Breeding line
833-01-3	Breeding line
913-05-1	Breeding line
931-05-9	Breeding line
BC853	Commercial variety
CRI-MS-1	Commercial variety
CRI-MS-2	Commercial variety
LS9219	Commercial variety
SZ9314	Commercial variety

Experimental design and trial layout

The experiments were implemented in a Randomized Complete Block Design (RCBD) replicated three times with single-row plots of 5.4 m long. The first and the last blocks of 5.4m long each together with the first and last two rows were treated as discard and pathways of one metre separated the replications.

Crop and disease management

Pest management was done based on scouting data results using the general protocol developed at the Cotton Research Institute (CRI, 2012). Scouting started on the fourth week after crop emergence and proceeds once weekly thereafter, where pest control was done only when the pests' threshold levels of infection have been reached. Effective weed control was also done prior to planting and early in the growing seasons to consolidated pest control and all the other agronomic practices were done according to the Cotton Handbook (2000).

Observation of disease incidences was done for each plot once every month from January to April based on the leaf symptoms. Plants exhibiting symptoms were tagged with different colours of knitting wool and the numbers were recorded per plot and disease incidence (%) was calculated at the end of the season using the following formula:

Disease Incidence (DI) = [Number of wilted plants/Total number of plants per plot] *100.

Disease incidence scoring was done using the scoring system shown below (Table 3). Vascular disease rating (VDR) was done based on the intensity of vascular discolouration (browning) visible in a cross-section of the main plant stem cut as practicable to ground level for all plants on each plot after harvesting. The vascular browning scoring system used is as described by Colella *et al.* (2008).

Where,

0 – 1: No discolouration to an area restricted to less than 5% of the stem cross section (R).

2 – 3: Discolouration of between 5% and 40% of the stem cross section (T).

4 – 5: >40% vascular discolouration of the stem cross-section, completely black stem and plant death (S).

Total seed cotton yield was measured per plot at the end of the season and the yield in kg/ha was calculated as follows:

Seed cotton yield kg/ha = 10000/yield per plot × plot area /1000

Table 3. Verticillium wilt infection scoring system

Score	% Infection	Leaf symptoms	Rating
0	0	No symptoms	Highly resistant
1	1 – 10	Foliar symptoms (localized leaf yellowing or red, bronze colour of leaves late season), no leaf yellow or necrotic areas	Resistant
2	11 – 50	Widespread leaf discolouration, few plants (<5%) showing any wilting or leaf loss	Tolerant
3	51 – 75	Widespread leaf discolouration (50-75% of plants) with up to 25% of plants showing evidence of leaf yellow and necrotic areas and some indication of leaf wilting or leaf abscission in plants showing more extensive yellowing, necrosis, wilt or leaf loss on up to 50% of original plant stand.	Susceptible
4	>75	Severe foliar damage, including general leaf discolouration on most plants, evidence of yellowing and necrotic areas, wilting leaves, loss of >25% of leaves on >25% of original plant stand - evident defoliated or dead plants within stand	Highly susceptible

Source: University of California Cooperative Extension, 2005.

Data was analysed for variance (ANOVA) using the GenStat 18th edition statistical package. Separation of means was done using the least significant differences (LSD) test at 5 %.

Results and discussion

Significant differences (p>0.016) in disease incidence percentages (%) were observed among the investigated characteristics in 2021. The mean values of disease incidence of genotypes ranged from 27.5% (562-00-9) to 100% (BC853 & 913-05-9) and the average rate of infection was 75.1% (Table 4). The highest disease incidences were observed for the commercial variety BC853 and the test variety 913-05-9, where the lowest infection percentage was noted on the test variety 562-00-9. The result agreed with that of Yaşar (2022), who noted that the susceptibility of cotton cultivars to diseases varies. However, there was no significant difference in terms of vascular browning rating in 2021 as well as for the two attributes (disease incidence and vascular browning) among the tested genotypes (Table 4).

Table 4. Mean verticillium wilt disease incidence and vascular disease rating the evaluated cotton genotypes over the two seasons

Genotype	2021 Season			2022 Season			Average over seasons		
	% incidences	Disease rating	VDR	% incidences	Disease rating	VDR	% incidences	Disease rating	VDR
562-00-9	27.5c	T	1.5	42.9	T	1.5	35.2	T	1.5
564-00-6	85.7ab	HS	1.5	52.5	S	2.1	69.1	S	1.8
666-01-2	59.3bc	S	1.5	52.8	S	2.0	56.1	S	1.8
BC853	100a	HS	1.4	53.7	S	2.1	81.5	HS	1.8
LV96-05-8	62.1abc	S	1.4	62.3	S	2.1	62.2	S	1.8
816-01-1	94.4ab	HS	1.6	53.7	S	2.0	74.1	S	1.8
833-01-3	92.6ab	HS	1.0	63.0	S	1.8	77.8	HS	1.4
913-05-9	100a	HS	1.4	61.1	S	1.9	80.6	HS	1.7
LS9219	-	-	-	63.4	S	1.9	63.4	S	1.9
CRI-MS-1	-	-	-	61.2	S	1.7	61.2	S	1.7
CRI-MS-2	66.0abc	S	1.6	-		-	66.0	S	1.6
SZ9314	63.1abc	S	1.4	-		-	63.1	S	1.4
Grand Mean	75.1		1.44	57.6		1.9			
L.S.D.	32.86		0.604	23.09		0.711			
CV %	28.6		24.5	23.4		21.7			
P-value	0.016		0.578	0.643		0.634			

Means followed by the same letter are not significantly different at p = 0.05.

T-Tolerant, S-Susceptible, HS-Highly Susceptible

LSD – Least significance difference, CV – coefficient of variation

Table 5. Mean seed cotton yield (kg/ha) for the two seasons

Genotype	2021 Season	2022 Season	Mean
562-00-9	1252	2031	1642
564-00-6	506	1793	1150
666-01-2	624	2506	1565
BC853	699	1519	1109
LV96-05-8	836	1509	1173
816-01-1	655	1241	948
833-01-3	527	1324	925
913-05-9	470	1336	903
LS9219	-	1130	1130
CRI-MS-1	-	2867	2867
CRI-MS-2	793	-	793
SZ9314	690	-	690
Grand Mean	706	1726	
L.S.D.	1.236	0.4576	
CV %	27.3	8.4	
P-value	0.714	0.539	

The vascular browning scores varied from 1.0 to 2.1 during the period of investigation although there was variation of the rating from one season to the other. During the 2020/21 season, the lowest score of 1.0 was recorded on the test variety 833-01-3 although it recorded 1.8 in the 2021/22 season while in 2022 the lowest score was recorded on the test variety 562-00-9 variety 562-00-9 which was the same for 2021. In terms of disease vascular discolouration, all the genotypes were tolerant to VW. Our findings agree with that of Yaşar (2022), who noted that disease observation can be investigated from both leaf symptoms and vascular browning. He also indicated that the diversified reaction of genotypes to VW also shows that some genotypes are tolerant although they may show high levels of disease incidence. McCarville and Ayres (2018) ascertain that tolerance promotes plant host health while having a neutral to positive impact on pathogen fitness. However, these results contradict Karademir *et al.* (2010) who revealed that foliar symptoms are usually less common than vascular browning but are the major determinants in losses caused by VW. No significant differences were recorded among genotypes for seed cotton yield (Table 5). However, the influence of the disease epidemics was noticed on the yield of the tested cultivar. A huge gap was noticed in the season differences for seed cotton yield. In the 2021/22 season higher rainfalls, a total of 1305.7mm were experienced leading to higher rates of disease incidence on genotypes which recorded up to 100% and lower seed cotton yield (range from 470 to 1252kg/ha). During the 2020/21 season, a total rainfall of 772.2mm was recorded and higher yields of 1130 to 2867kg/ha were realised. This observation is in agreement with the findings noted by Land *et al.* (2017) that moist soils enhance pathogen virulence and increase infection rates. Mean seed cotton yields for the test genotypes varied between 903kg/ha to 1642kg/ha, the highest obtained from 562-00-9 genotype followed by 666-01-2 with 1563kg/ha and lowest obtained from 913-05-9. This result is consistence with our previous studies at CRI Annual Report of 2020 and that of Chipuriro (2018) where the test genotype 562-00-9 was reported to have been performing better than the commercial varieties. This is an indication that the usefulness of some resistant types has been restricted by evolution disease strains. The commercial varieties BC853 proved to still have tolerance to VW as is shown by the seed cotton yield obtained in the 2021/22 season (1109kg/ha) although it was ranked as one of the highly susceptible genotypes. The ability of a crop to maintain yield in the presence of disease is not an easy characteristic to measure (Newton, 2016). This observation is consistent with previous studies reported by Mukoyi *et al.* (2014) indicating that BC853 obtained higher yields of 1117kg/ha and Chapepa *et al.* (2013) who stated that some varieties delay the expression of visual symptoms and were able to withstand disease pressure.

Conclusion

The study revealed that the investigated characteristics responded differently to Verticillium severity parameters and seed cotton yield. The most tolerant genotype to Verticillium wilt disease was 562-00-9 having the lowest disease incidence rate and high yielding capacity and can be used in the variety development programme to impart VW tolerance. The susceptible variety 666-01-2 and the highly susceptible BC853 had high yields, indicating the impact of the disease's occurrence time. Hence, there is need to determine the relationship between the earliness of crop maturity and Verticillium wilt.

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