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Screening of *Gossypium hirsutum* germplasm against *Verticillium dahlia* Kleb by natural infection

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Abstract

An experiment on screening of cotton germplasm against Verticillium wilt caused by *Verticillium dahlia* Kleb was carried out at Cotton Research Institute in 2008, 2009 and 2010 seasons in Zimbabwe. The objective of the study was to identify cotton germplasm with considerable resistance to the disease under natural infection for use in cotton variety development programmes. Twelve varieties were laid in a randomised complete block design replicated three times. Eight were new elite varieties and three were commercial cultivars. Infection percentages were calculated for infected plants in each plot. Percentage infection were Arcsine transformed and varieties with scores of 0-9% rated highly resistant, 10-19% rated resistant, 20-29% rated tolerant and above 29% rated susceptible. Three test varieties LS96-05-1, 937-05-8 and 97-05-1 were identified as resistant to the disease and were recommended for use in cotton breeding programmes.

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Introduction

Diseases are a major limiting factor in cotton production in Zimbabwe and are one of the major reasons accounting for the low yields of 800 kg/ha currently being realised mainly by smallholder farmers compared to potential yields of 4000 kg/ha for the current cultivars (Mudada *et al*, 2007). Cotton diseases reduce seed cotton yields and farmers suffer the blunt as they fail to properly recuperate from such losses. Control of the diseases using conventional methods is more expensive and in some instances ineffective. Effective control by conventional methods of using chemicals has proved difficult and costly therefore justifying varietal resistance as the major source of the disease control method in commercially grown cultivars (Mudada and Chapepa, *unpublished*). Thus screening for disease tolerance is a priority at Cotton Research Institute for varieties recommended for commercial production. Currently, cotton prices are on the decline globally (ICAC, 2012) and production costs are spiralling out of control and so diseases have a big impact on the income generated by farmers. Most farmers are contemplating quitting the production of cotton as a result of these challenges.

Verticillium dahliae Kleb is a major disease affecting cotton production and productivity in Zimbabwe (Mapope, 2001). Verticillium wilt diseases were shown to be spreading into new areas where it was not previously reported in the country. Apart from the old areas like Mazowe, Chinhoyi, Glendale, Bindura and Chisumbanje where the disease is prevalent, more inoculum has been noted in new areas like Chakari, Chegutu and Matebeleland. It was also noted that 54% of cotton farmers reported the Verticillium wilt disease (Mapope, 2004); an increase from the 41% reported by Chinodya (1994). The spread, though it was assumed to be caused by poor hygiene during the land reform of 2000 (Gwimbi, 2009), requires address.

V. dahliae diseases can cause yield loss of 20-100% depending on the variety and severity (Hillocks, 1992). Considerable progress has been made in improving resistance or tolerance to the pathogen, but even the most resistant cultivars can become severely diseased by defoliating strains of the fungus if mean soil temperatures fall below 25˚C (Bell, 1992). An integrated disease management system is employed in Zimbabwe, employing all possible control techniques but varietal tolerance and sanitation are the pillars.

Promising germplasm coming out of the breeding programmes that includes strains, lines and varieties are screened for resistance and be recommended for Verticillium wilt infested soils. The objective of this study was therefore to identify new elite cotton germplasm resistant to *V. dahliae* under natural infections for recommendation for use in the cotton breeding programmes.

Materials and methods

The trial was conducted in 2008, 2009 and 2010 seasons at Cotton Research Institute situated in Kadoma, Zimbabwe under dry land production system. Kadoma is in Region 2 of the Natural Farming Regions of Zimbabwe. Kadoma is located at 18° 19' south and 29° 53' east with an altitude of 1156 m above sea level. A good deal of high day and low night temperatures, accompanied by high relative humidity characterised the three seasons. The details of activities and general information of the site is given in Table 1. The site experienced long overcast conditions in the latter half of all the seasons. The long overcast conditions created conducive conditions for the development of Verticillium wilt disease in the field (Chinodya and Hillocks, 1988).

The land used had a long history of Verticillium wilt pathogens after inoculation with *V. dahlia* isolates. The Andersen sampler Melouk's procedure of 1992 was used to check the *V. dahliae* inoculum level of the pathogen in the soil. 32 Colony Forming Units per gram (CFU/g) were noted. The germplasm that was screened is shown in Table 2.

The experimental design was a randomised complete block design replicated three times. The treatments were laid in single rows of 5.4 m long at the general

recommended spacing of 1 m x 0.3 m in all seasons and a discard variety SZ9314 was grown as a border strip crop to avoid foreign infections entering into the plots. Infected plants were tagged with cotton wool for the purpose of differentiating infected plants from those that die from other causes other than the disease. Tagging was done from January of every season as plants are mainly affected from 6 weeks after emergence and also visual symptoms are more defined. In subsequent months, tagging was done using different colours of cotton wool for easier identification. Infected plants in each plot were recorded once every month thus enabling the calculations of infection percentages at the end of the season. Scoring of the disease expression was done and transformation using the Arcsine Transformation (Karman, 1971) was carried out. Data were subjected to general analysis of variance using the Genstat 8th edition statistical package (Hempstead, 2005).

Table 1. General information and schedule of activities for the three seasons at Cotton Research Institute in Kadoma, Zimbabwe.

	Season		
Item	2008-09	2009-10	2010-11
Planting date	12-12-08	14-12-09	06-12-10
Fertilizer quantities	Comp L -200kg/ha AN – 100kg/ha	Comp L - 150kg/ha AN – nil	Comp L -250kg/ha AN – 150kg/ha
Harvesting date	14-07-09	14-06-10	14-06-11
Rainfall (mm/annum)	884.4 mm	792.8 mm	1019.3mm
Mean Temperature (°C)	27.8 °C	28 °C	27.2 °C
Soil type	Brown sand clayey loam		
Altitude	1183 m		

Table 2. Sources of germplasm used in this project.

Genotype	Source	Attribute
LV96-05-08	Cotton Research Institute.	Breeding line
1100-05-4	Cotton Research Institute.	Breeding line
930-05-3	Cotton Research Institute.	Breeding line
931-05-9	Cotton Research Institute.	Breeding line
CRIMS1	Cotton Research Institute.	Commercial variety
CRIMS2	Cotton Research Institute.	Commercial variety
LS96-05-1	Cotton Research Institute.	Breeding line
SZ9314	Cotton Research Institute	Commercial variety
BC 853	Cotton Research Institute.	Commercial variety
97-05-1	Cotton Research Institute.	Breeding line
937-05-8	Cotton Research Institute.	Breeding line
930-05-3	Cotton Research Institute.	Breeding line

Results and discussion

Lines responded differently to Verticillium wilt attack over the three seasons. There were no significant infection differences ($P > 0.05$) in 2008 and 2009. However, in 2008, LS96-05-1 and 97-05-1 showed high resistance against the disease whilst 937-05-8 showed high resistance scores in 2009 (Table 4). In 2010 season, significant scores were noted ($P<0.05$), (Table 4). 931-05-9 was the experimental line that had tolerance to the disease when compared to all the test varieties. Results for the three seasons indicated that 97-05-1 was ranked

from tolerant to highly resistant in the three seasons. Varieties LS96-05-1 and 937-05-8 were in the tolerant to highly resistant categories in 2008 and 2009 seasons (Table 4). The standard variety BC853 showed resistance to Verticillium wilt (Table 4). Variety 931-05-9, 97-05-1 and SZ 9314 were comparable to BC853. The rest of the test varieties had high infection percentages.

Table 3. The scoring system used in the study.

% Arcsine transformed range	Disease rating	Symbol
1% - 9%	Highly Resistant	HR
10% - 19%	Resistant	R
20% -29%	Tolerant	T
>30%	Susceptible	S

Source: Cotton Research Institute Annual Report (2009).

Table 4. *Verticillium* Wilt scores of cotton genotypes over three seasons at Cotton Research Institute in Zimbabwe.

	2008 season		**2009 season**		**2010 season**		**Average over three seasons**	
Varieties	% Arcsine transformed	Disease Rating	% Arcsine transformed	Disease Rating	% Arcsine transformed	Disease Rating	% Arcsine transformed	Disease Rating
1100-05-4	45.1	S	16.2	R	50.7[a]	S	37.3	S
LV96-05-8	21.7	T	32.0	S	33.0[b]	S	28.9	T
930-05-3	10.5	R	21.4	T	41.9[a]	S	24.6	T
LS96-05-1	5.6	HR	22.4	T	31.8[b]	S	19.9	R
931-05-9	26.4	T	28.7	T	27.9[bc]	T	27.7	T
930-05-3	10.5	R	21.4	T	38.4[b]	S	23.4	T
937-05-8	17.2	R	9.6	HR	30.2[b]	S	19.0	R
97-05-1	5.4	HR	13.3	R	27.4[bc]	T	15.4	R
SZ9314	25.9	T	19.5	R	24.6[bc]	T	23.3	T
BC853	18.3	R	26.5	T	16.6[c]	R	20.5	T
CRI-MS-1	-#		-#		35.2[b]	S	35.2	S
CRI-MS-2	-#		-#		40.3[b]	S	40.3	S
Grand Mean	23.6		24.6		34.9			
Cv%	-		4.7		12.0			
Significance Level	NS		NS		***			
SED	7.96		8.3		9.2			

*** = shows highly significance difference -# indicates a different variety was used.

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

The screening procedure revealed that the promising cotton germplasm reacted similarly to the disease in the soil because there were no significant differences for disease response among genotypes in 2008 and 2009. In 2010, there was significant difference to disease response amongst the experimental lines and the controls. This can be attributed to the high levels of infection in some experimental lines which were seriously attacked by the disease showing disintegration of the tolerant traits in the cultivars when compared to the controls. All lines were affected to some extend and this is consistent with assertion by Bell (1992) that *Gossypium* species lack high resistance against the pathogen *Verticillium*

dahlia. According to Bell (1992), cotton varieties at most exhibit tolerance and not complete resistance. However, some of the test varieties delayed expression of visual symptoms and have shown that they are able to continue surviving by the high number of plants that remained in the experimental plots. Such experimental lines are the most preferred germplasm for advancement in breeding programmes and when combined with other agronomic traits and yield further evaluations can be made of their performance. This forms the basis for releasing new varieties. There is suggestive evidence that the pathogen *V. dahliae* thrives in the soil for as long as there is a host plant for their nutrition.

Infection in the 2009 season was very subdued mainly because of unfavourable climatic conditions that prevailed during the year (Table 2). The variations of infection in different seasons show the differences in disease development caused by dynamic environmental conditions. This enables cotton breeders to come up with a resistant variety that is adaptable to different climatic conditions (Kang, 1998). However, excessive disease pressure exhibited by known resistant varieties like CRI-MS-1 could be caused by different races of the *V. dahliae* isolates around the country (Mapope, 2004).

Conclusion

The screening trial showed that the varieties had different tolerance levels to the *V. dahlia*. disease and a few test varieties proved to be able to withstand the pressure of the disease and will be very useful in breeding programmes. Generally all test varieties except 1100-05-4 were in the tolerant to resistant category. These varieties can be used in the variety development programme to impart tolerance to *V. dahliae*. The varieties LS96-05-1, 937-05-8 and 97-05-1 were recommended for agronomic, yield and fibre quality evaluation programmes.

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