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SOIL & CROP SCIENCES | RESEARCH ARTICLE

Characterisation of upland cotton grown in Zimbabwe using agronomical and morphological markers for *Verticillium* wilt tolerance

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Abstract: Accurate morphological characterisation of plants is used for selecting the best performing varieties. The morphological markers can be visualised without molecular techniques making it the easiest way of identifying variation within varieties. Characterisation of *Verticillium* wilt tolerant lines in cotton variety is important in understanding the underlying genetic control of *Verticillium* wilt tolerance. Five local varieties with varying tolerance to the disease and different yield potentials were crossed in a half diallel mating design in 2013. The ten crosses and five parental lines that were produced by the mating system were subjected to *Verticillium* wilt by artificial inoculation in 2013/14. These cotton lines were characterised using agronomic and morphological markers to identify *Verticillium* wilt resistance. Screening for *Verticillium* wilt resistance was done to identify morphological and agronomical markers which were used to group similar lines through cluster analysis. Three parental lines were identified with good morphological and agronomical performance that could be used in *Verticillium* wilt resistance breeding. These were CRI-MS-1, SZ9314 and BC853 which produced superior offspring in the diallel mating system. Furthermore, qualitative morphological traits and the agronomical traits were used as markers for selection of *Verticillium* wilt resistance cotton germplasm.



Blessing Chapepa

ABOUT THE AUTHORS

Blessing Chapepa is a plant breeder working at Cotton Research Institute of Zimbabwe as a cotton breeder. His research areas are developing, implementation analysing data and information dissemination of the national cotton breeding program in the Department of Research and Specialist Services of the Ministry of Agriculture in the country. Other research interests include crop modelling using remote sensing technology and molecular tool applications.

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PUBLIC INTEREST STATEMENT

Cotton is a very important cash crop in Zimbabwe and it comes third after tobacco and maize. Development of cotton varieties hinges on good identification of suitable parental material which resists against naturally occurring stresses like diseases. The most important disease in Zimbabwe that affects cotton is the *Verticillium* wilt that causes yield losses of up to 100%. The progenies of the resistant parents can be further developed and recommended for production in areas with these problems with minimal yield losses. The cotton farmers in *Verticillium* wilt prone areas like Mazowe in Zimbabwe will be able to go back to growing the crop once again as interest had become low due to the ravaging effects of the disease.

Subjects: Agriculture & Environmental Sciences; Botany; Plant & Animal Ecology

Keywords: morphological markers; resistance; superior lines; Verticillium wilt

1. Introduction

Cotton is a very popular crop that is widely grown in at least 80 countries (Jamshed et al., 2016) and is the second most important source of edible oil and protein (Zhang et al., 2014). In growing the crop, a lot of external factors limit the full potential of the crop and identifying lines that thrive under various conditions become critical in variety development. Characterisation of organisms is an important tool for the selection of potential parents for subsequent crossing and selection of superior progenies (Murtaza et al., 2005). Accurate morphological characterisation of species in the diversity analysis forms a basis for selecting the best varieties with characteristics like resistance to insect pests and diseases in breeding (Lukonge, 2005). Morphological markers can be visualised without special biochemical or molecular techniques making it the easiest way of identifying variation within varieties.

Morphological traits for *Verticillium* wilt tolerance are controlled by dominant or partially dominant genes and can be used as genetic markers as their expression is reproducible over a range of environments (Palanga et al., 2017). Environment and agronomic practices have an effect on most of these morphological characteristics (Pegg & Brady, 2002). In Zimbabwe, variation determination of varietal tolerance has been based on mostly morphological descriptors and partly agronomical descriptors. This has been important in selecting parental material for crossing to improve the available known resistant materials (Mudada, 2013). This has been the pillar of developing resistant varieties and managing *Verticillium* wilt disease in Zimbabwe as the most cost-effective and practical way according to Wang et al. (2014) is to develop cotton cultivars harbouring resistance or tolerance using conventional breeding strategies.

Verticillium wilt symptoms on cotton include reddening of veins, mottling, yellowing of leaf margin, discolouration and death especially between the principal veins and around the leaf margins and brown discolouration of the internal vascular tissue in the stems (Land et al., 2017). Morphological descriptors used for disease resistance include plant shape, plant height, boll size, boll shape, boll prominence and leaf colour whilst agronomic descriptors include adaptability and yield components (boll number, branch number and boll weight). Therefore, the main aim of this study was to characterise five varieties grown in Zimbabwe and ten hybrids from the diallel mating under *Verticillium* wilt pressure using agronomical and morphological markers.

2. Materials and methods

2.1. Plant material and experimental procedure

Five diverse upland cotton Zimbabwean genotypes (CRI-MS-1, CRI-MS-2, SZ9314, BC 853 and CIM1) in terms of yield, disease resistance and morphological differences and 10 progenies produced after these genotypes were crossed in a half diallel mating design were subjected to *Verticillium* wilt pressure and characterised for morphological and agronomic distinctiveness. The half diallel mating design which included parent lines was carried out in the greenhouse which had conditions adjusted to 24°C and a relative humidity of 65% to promote disease infection and progression at Cotton Research Institute with four pots per plot which had a diameter of 30 cm and depth of 50 cm per cultivar during the winter season of 2013. The 15 lines produced were then grown in the 2013/14 season and laid out in a randomised complete block design to identify morphological and agronomical markers which were used to group similar lines through cluster analysis. Artificial inoculation was carried out on the 15 lines at 8 weeks after crop emergence by injecting 2×10^6 conidia/ml of *Verticillium* inoculum into the stem to initiate infection. The inoculum was isolated using the Andersen sampler following the Melouk's procedure of 1992. The treatments were replicated three times with the plot sizes being three pots per treatment with a single cotton plant in each pot.

3. Data collection and characterisation

The revised International Board of Plant Genetic Resources (IBPGR) (1985) and modified Cotton Research Institute descriptors for cotton were used to describe cotton traits (Table 1). The data that was collected from all the plants in the plots included plant shape, boll shape, boll prominence, leaf colour, number of vegetative and fruiting branches at week six and two weeks thereafter to assess varietal performance. The harvest index (HI), number of seeds per boll and seed weight per boll were determined at harvest. Ten bolls were randomly collected from each plot at boll splitting stage to determine the number of seeds per boll and seed weight per boll.

Coding of morphological data into a binary matrix for cluster analysis was applied to reveal the pattern of genetic similarities in terms of *Verticillium* wilt tolerant genes movement. The characteristics with only two categories for description was scored as present (1) and absent (0) and entered into a matrix (Lukonge, 2005). In this case plant shape was coded in this way, where open was coded as 1 and closed as 0. Plant height, boll size, boll prominence and leaf colour were given a different coding using three classes. For plant height, short, medium and tall categories were used for plant heights of 90–120 cm, 120–150 cm and above 150 cm, respectively, as the cotton plants were grown in the greenhouse. The plants with similar height as the medium class were coded as 1 and those with lower or higher plant heights than the medium class were scored as 0. Boll sizes which were classified as medium were coded as 1 and those smaller or larger than the medium category were coded as 0. The medium class corresponded to boll weight of 5–6 g and those below 5 g were considered as small and those above 6 g as large. Boll prominence was classified based on the fruit retention values where those between 50% and 70% were classified as medium, those below 50% as weak and those above 70% as strong. The plants with medium boll prominence were coded as 1 and those with weak and strong as 0. For the leaf colour, three classes were used which were light green, medium green and dark green. Leaves with medium colour were coded as 1 and those that were light and dark green colour were coded as 0. This system of coding for binary matrix used was done following the procedure of Lukonge (2005).

Table 1. Morphological and agronomical characteristics of cotton

Characteristics	Description
Boll shape	Shape in longitudinal length
Boll prominence	Prominence of tip
Boll size	Size of circumference of boll
Number of bolls per plant	Number of bolls counted at harvest
Leaf colour	Intensity of green colour
Plant shape ^a	Shape of plant at boll splitting stage
Plant height	Height above ground measured in cm at harvest
Number of fruiting branches ^a	Number of boll bearing branches on the main stem at harvest
Number of vegetative branches ^a	Number of vegetative branches at harvest
Harvest index ^a	Proportion of dry matter partitioned to yield (%)
Gin outturn	Content of lint (%)
Number of seeds per boll ^a	Number of mature seeds per boll at harvest
Seed weight per boll	Content of seed (g)
Seed cotton yield	Total weight of seed cotton (kg)

Adapted from IBPGR cotton descriptors

^aAdapted from C.R.I. descriptors

4. Statistical analysis

Analysis of variance (ANOVA) on yield, yield components (boll number, number of fruiting branches, number of vegetative branches and boll size), plant height and number of seeds per boll and seed weight per boll was done using GenStat 14th version. The data that were converted into binary matrix data were subjected to cluster analysis using multivariate analysis of the same software. This was done after constructing the genetic matrices using the Euclidean distance method to reveal the patterns of genetic differences under *Verticillium* wilt pressure. Dendrogram was constructed based on the Euclidean distances using the Hierarchical clustering and measure of goodness of fit carried out for each Dendrogram.

5. Results

The results of foliar infection and vascular browning index which indicated the response of the cotton lines to the *Verticillium* wilt infection were presented in the paper published in 2015 (Chapepa et al., 2015).

6. Agronomical characteristics

The cotton agronomical traits that were characterised in this study showed no significant variations among the 15 cotton genotypes except for the number of seeds per boll and fruiting efficiency of the cotton genotypes which was denoted by the statistical difference on the harvesting index ($P < 0.05$) as shown in Table 2. CRI-MS-1 had the highest number of seeds per boll of 24.67 whilst the cross between CRI-MS-1 x CRI-MS-2 had the least of 17 seeds per boll. The cross SZ9314 x BC853 had most of its dry matter partitioned to the yield than to the plant residues when infected with *Verticillium* wilt disease and it was comparable to most of the crosses used in the study. The parental line CRI-MS-2 partitioned less of its dry matter to the yield under *Verticillium* wilt pressure and was like all of the parent lines used in the study. It was also comparable to four crosses which included CRI-MS-1 x CRI-MS-2, CRI-MS-1 x CIM1, CRI-MS-2 x BC853 and BC853 x CIM1. CRI-MS-1 was comparable to the parental lines BC853 and CIM1 and the crosses CRI-MS-2 x CIM1, SZ9314 x BC853 in terms of the harvest index. The seed weight per boll of the different genotypes used in this experiment was not significantly different as the cotton lines produced a statistically similar weight of seeds of the cotton from the cotton bolls as shown in Table 2. Analysis of variance on the number of vegetative and fruiting branches showed that there were no significant differences ($P < 0.05$) among the genotypes. The number of fruiting branches produced by the cotton plants exposed to *Verticillium* wilt disease in this study did not differ significantly as well as the vegetative branches. The plants produced fruiting branches that ranged between 13 and 17 and a range between 3 and 6 for the vegetative branches under the *Verticillium* wilt pressure.

7. Morphological characteristics

The lines showed a variation on morphological characteristics as shown in Table 3. The parental lines ranged from medium height to tall, open shaped to close shaped with small to medium boll size. The bolls were mainly conical with BC853 and CIM1 being rounded. Boll prominence was medium whilst leaf colour was medium coloured to light coloured for SZ9314. The progenies produced from these parental lines revealed differences in their morphological characteristics in response to *Verticillium* wilt pressure.

8. Cluster analysis

Cluster analysis on the morphological characteristics of plant shape, plant height, boll size, boll prominence and leaf colour (Table 3) revealed three main groups A, B and C as shown in Figure 1. The main cluster group A consisted of eight sub-clusters having 83% similarities in 11 of the genotypes on most of the morphological characteristics which include plant height, boll prominence and leaf colour. Sub-cluster 1 had three genotypes CRI-MS-1, the cross CRI-MS-2 x BC853, the cross CRI-MS-2 x SZ9314 which were different from other genotypes on plant shape, boll size and boll prominence at similarity coefficient of 1. Sub-cluster 2 consisted of one cross of CRI-MS-1

Table 2. Means of agronomical parameters of cotton under Verticillium wilt pressure

Genotype	Number of seeds per boll	Seed weight per boll	Number of fruiting branches	Number of vegetative branches	Harvest index (%)
CRI-MS-1	24.67 c	1.91	14.56	2.56	43.91abcd
CRI-MS-2	19.00ab	1.64	16.22	2.00	35.98a
SZ9314	17.33ab	1.78	15.11	2.11	40.81abcd
BC853	20.33abc	1.93	15.44	2.22	43.35abcd
CIM1	20.67abc	1.88	15.56	1.78	43.08abcd
CRI-MS-1 x CRI-MS-2	17.00a	1.74	14.56	2.00	38.09abc
CRI-MS-1 x SZ9314	22.00bc	2.19	13.89	2.44	45.63cde
CRI-MS-1 x BC853	17.67ab	1.71	15.00	2.00	48.88de
CRI-MS-1 x CIM1	18.67ab	1.90	14.00	2.00	38.33abc
CRI-MS-2 x SZ9314	19.33ab	1.79	15.67	2.11	44.69bcde
CRI-MS-2 x BC853	19.00ab	1.84	14.11	2.11	37.00ab
CRI-MS-2 x CIM1	21.00abc	1.96	14.22	2.22	49.29de
SZ9314 x BC853	22.00bc	1.99	14.78	2.00	52.90e
SZ9314 x CIM1	19.00ab	1.98	13.67	2.33	47.71de
BC853 x CIM1	18.67ab	2.00	14.22	2.00	44.36abcd
Grand Mean	19.76	1.88	14.73	2.13	43.60
CV%	11.8	9.8	8.5	10.1*	10.1
P-value	0.028	0.121	0.414	0.772*	0.002

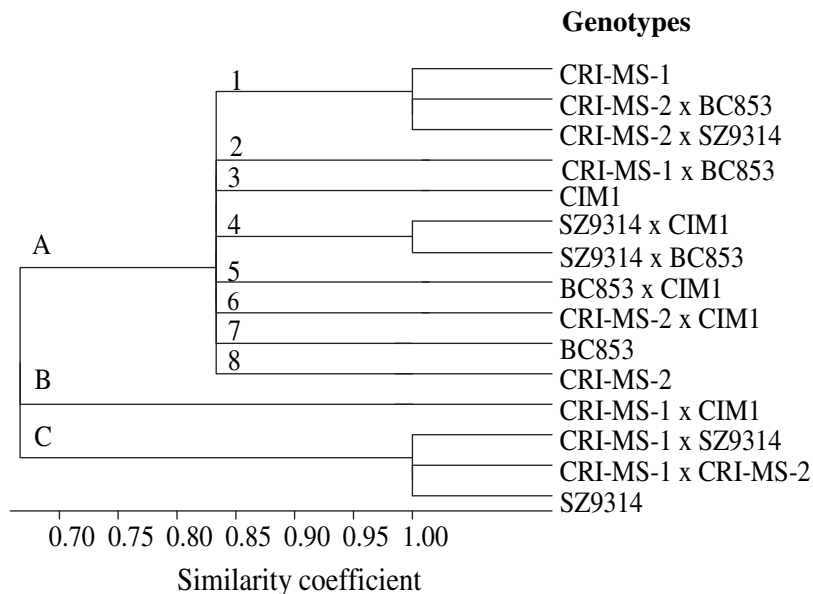
Means in the same column followed by the same letter are not significantly different after separation by Duncan Multiple range Test ($P < 0.05$) *Data transformed using square root transformation

Table 3. Morphological traits for different genotypes

GENOTYPE	Plant height	Plant shape	Boll size	Boll shape	Boll prominence	Leaf colour
CRI-MS-1	Medium	Open	Small	Conical	Medium	Medium
CRI-MS-2	Tall	Open	Small	Conical	Medium	Medium
SZ9314	Medium	Open	Medium	Conical	Medium	Light
BC853	Tall	Closed	Medium	Rounded	Medium	Medium
CIM1	Medium	Closed	Small	Rounded	Medium	Medium
CRI-MS-1 x CRI-MS-2	Medium	Open	Medium	Conical	Medium	Light
CRI-MS-1 x SZ9314	Medium	Open	Medium	Conical	Medium	Dark
CRI-MS-1 x BC853	Medium	Closed	Small	Conical	Medium	Medium
CRI-MS-1 x CIM1	Medium	Closed	Medium	Conical	Strong	Medium
CRI-MS-2 x SZ9314	Medium	Open	Small	Conical	Medium	Medium
CRI-MS-2 x BC853	Medium	Open	Small	Conical	Medium	Medium
CRI-MS-2 x CIM1	Medium	Open	Medium	Rounded	Medium	Medium
SZ9314 x BC853	Medium	Closed	Medium	Rounded	Medium	Medium
SZ9314 x CIM1	Medium	Closed	Medium	Rounded	Medium	Medium
BC853 x CIM1	Medium	Closed	Medium	Rounded	Medium	Dark

x BC853 whilst sub-cluster 3 consisted of CIM1. Sub-cluster 4 had two cotton lines SZ9314 x CIM1 and SZ9314 x BC853. Sub-clusters 5, 6, 7 and 8 had single cotton lines each.

Figure 1. Dendrogram based on euclidean distance and hierarchical clustering method for morphological data.



Main group B had a single cotton line CRI-MS-1 x CIM1. The cross in this cluster was distinguishable from other clusters because it had superior boll prominence. The main cluster group C consisted of three cotton lines CRI-MS-1 x SZ9314, CRI-MS-1 x CRI-MS-2 and SZ9314. These were unique in that they had a different leaf colour from other lines apart from having a medium boll size which was caused by the presence of the *Verticillium* wilt disease in their system.

9. Genotypes

10. Discussion

The study revealed a great deal of variation of the cotton lines under *Verticillium* wilt infection and selection of resistant cotton lines could be done based on the extent of disease infection on the foliar parts, vascular infection, plant height, fruit retention and seed cotton yield (Chapepa et al., 2015). Furthermore, results of morphological and agronomical characterisation indicated that differences existed on the phenological level on different varieties of cotton exposed to *Verticillium* wilt disease and could be used as additional tools to select the resistant lines. These differences were as a result of defence mechanism response triggered within the cotton plants and this agrees with statements made by Du et al. (2004) where it was noted that *Verticillium* wilt resistance was under the control of dominant or partially dominant genes. Morphological traits that changed in the presence of the disease included plant height which was in agreement with Mapope (2005) who highlighted that *Verticillium* wilt caused stunting in cotton plants if infection occurred at an early stage of growth. This was confirmed in this study as SZ9314, a variety with an indeterminate growth habit but very susceptible to the disease was the shortest in the study due to the effects of the disease. The number of seeds per boll was also greatly reduced as the variety which had the highest number of seeds, CRI-MS-1, failed to reach the expected number of seeds which is 32 seeds per boll. Again parental varieties that were affected more by the disease produced fewer seeds as would be expected and this was observed in their progenies produced in the diallel mating. This was due to the effect the disease on the fruiting efficiency where dry matter partitioning to yield components was reduced due to the effect of blocking of the vascular system by the pathogen (Klosterman et al., 2009).

The disease did affect the performance of varieties on agronomical characteristics but, however, influenced the seed cotton yield outputs. This was revealed by the significant variation of the harvest index among the experimental cotton genotypes under *Verticillium* wilt pressure. According to Gutierrez et al. (1983), the disease causes gross defoliation once established in the

plant by changing the ethylene metabolism. This reduces the full expression of yield components such as boll numbers, boll weight and hence low harvest index. The cross between SZ9314 and BC853 partitioned most of its dry matter to the yield components showing that it can withstand the effect of the disease. This can also be attributed to the low vascular brown index score indicating that low levels of the pathogen were lodged in the vascular bundles which inhibits movement of photo-assimilates to the sink from the source. The harvest index is a better tool to assess the performance of the genotypes in terms of seed cotton yield under *Verticillium* wilt pressure as noted by Hillocks (1992). This can be useful in breeding for varietal tolerance against the disease as the cotton varieties will yield significant yields under the disease stress.

The cluster analysis revealed the relatedness of the cotton genotypes by the use of the qualitative inherited morphological and quantitative inherited agronomical characteristics which is important for the selection of potential parents for subsequent crossing and selection of progenies up to the final utilisation of cultivars in production schemes (Murtaza et al., 2005). The study revealed that genes from the parents were transferred to their progenies as observed in the Dendrogram where parental lines fell in the same main cluster groups with their offspring. In a similar study by Lukonge (2005), grouping of genetically similar cotton lines was observed using qualitatively inherited morphological traits. CRI-MS-1 and BC853 were in the same main cluster group A with most of their offspring showing that they transferred most of their qualitative morphological traits involved in conferring resistance against *Verticillium* wilt with relative ease. The same was also observed for SZ9314 which was also grouped together in the same main cluster with one of its offspring CRI-MS-1xSZ9314. This study showed that morphological characteristics controlled by qualitative inherited traits and agronomical characteristics controlled by quantitative inherited traits were useful in characterising cotton under *Verticillium* wilt pressure and most of these traits are inheritable. Clustering based on qualitative morphological characteristics depends on the origin of the material and on the traits involved.

11. Conclusion and recommendation

The agronomical and morphological characterisation of cotton under *Verticillium* wilt pressure revealed the distinguishable traits that were useful for identifying lines resistant to the disease. These lines proved to be the most ideal lines that could be grown under *Verticillium* wilt infected fields and the parental lines identified could be used as a base for disease resistance breeding. The parental lines identified were CRI-MS-1, SZ9314 and BC853 which were able to transfer most of their inheritable traits of disease resistance to their offspring. These traits could be used to identify and select ideal genotypes with resistance or tolerance to the disease in varietal development programmes in Zimbabwe. The morphological and agronomical traits could assist the breeders in selecting the best varieties that are likely to withstand the *Verticillium* wilt pressure. The study revealed that the qualitative and quantitative traits are the first level of characterising the cotton varieties; however, further elucidation of resistance can be revealed using molecular markers and so further studies have to be carried out using these. From the study, it was therefore recommended that the three cotton lines be used in all *Verticillium* wilt disease resistance breeding in Zimbabwe.

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Competing Interests

The authors declare no competing interests.

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