DIALLEL ANALYSIS AND CHARACTERISATION OF *VERTICILLIUM* WILT TOLERANCE IN COTTON (*Gossypium hirsutum* L.)

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Declaration

I hereby declare that this dissertation, prepared for the Masters of Science degree in Crop

Sciences, which I submitted to the Faculty of Agriculture and Natural Resource Management of

Midlands State University of Zimbabwe is my original work. All sources of literature and

materials used for this study has been duly acknowledged. I also agree that the Midlands State

University has the sole right to the publication of this dissertation.

Signed on _____ at the Midlands State University,

Gweru, Zimbabwe.

Signature _____

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The undersigned certify that they have read and recommended to the Faculty of Agriculture and

Natural Resource Management for the acceptance of this thesis titled: **Diallel Analysis and**

Characterisation of Verticillium wilt tolerance in Cotton (*Gossypium hirsutum* L.)

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Abstract

Cotton production is generally declining due to several limitations which include climate variations, poor crop management and biotic stress factors. Of the biotic stress, diseases are a major limiting factor of cotton production. *Verticillium* wilt is one of the most important disease and is an economically important fungal disease causing significant losses to seed cotton yield in Zimbabwe. Varietal development for resistance in cotton requires the understanding of the underlying genetic control of *Verticillium* wilt tolerance. Resistant breeding material against the disease is available but further improvement has to be done on other traits of economic importance. Thus, five local varieties with varying tolerance to the disease and different yield potentials were crossed in a half diallel mating design in 2013. This was done in order to elucidate the inheritance pattern and gene action of the resistance traits of the cotton lines. The ten crosses and five parents that were produced by the mating system were screened against *Verticillium* wilt by artificial inoculation at Cotton Research Institute, Kadoma in a glasshouse in 2014. These cotton lines were characterised using agronomic and morphological markers to identify *Verticillium* wilt resistance gene movements from parent lines to offspring. The trial was laid out in a completely randomised design with three replications due to the homogeneity conditions that prevailed in the glasshouse. Screening for *Verticillium* wilt resistance was done using a disease severity scoring on a scale of 1-6 and vascular brown index (VBI) scoring on a scale of 1-4. Morphological and agronomical markers were evaluated and used to group similar lines through cluster analysis. There were significant variations on most of the morphological and agronomical traits. Significant variations in disease severity and the vascular brown index were observed for the cotton lines produced in the half diallel mating system. The general

combining ability (GCA) for the parents and specific combining ability (SCA) for the crosses were also significant. The *Verticillium* wilt severity scores ranged from 1.22 to 3.07 and VBI scores ranged from 0.78 to 2.77. The contribution of GCA to the total sum of squares of crosses for disease scores ranged from 86.9 to 95.2 % compared to SCA that ranged from 4.8 to 14.2 %. Additive gene effects were more important than non-additive effects indicating that *Verticillium* wilt resistance is genetically determined and that selection should be successful to improve resistance. Selection of parents with good GCA effects will be important for success in *Verticillium* wilt resistance breeding in cotton.

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Dedication

This work is dedicated to my wife Gloria, children Chikomborero, Nenyasha and Nokutenda for

standing by my side. It is also dedicated to my mother and late father.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1 : General Introduction

Cotton production has generally been low due to the effect of climatic variations, biotic stress and poor crop management by farmers (ICAC, 2012). Annually in Zimbabwe, 300 000 ha of land is planted with cotton producing about 250 000 tonnes of seed cotton with a national average yield of around 800 kg per hectare (Mudada, 2013) which is too low compared to the expected yield of 2300 kg per hectare (Mudada and Muripira, 2010). Smallholder farmers who account for about 99% of cotton produced in Zimbabwe have contributed to the low yields because of poor cotton production management practices (Mubvekeri and Nobanda, 2012). Cotton diseases including *Verticillium* wilt caused by *Verticillium dahliae* Kleb. have also been cited as a major reason for the reduced yields attained by the smallholder farmers (Mapope, 2001). The diseases particularly *Verticillium* wilt are slowly gaining economic importance status in the major cotton growing areas of Zimbabwe and they are believed to be the major cause of lower yields achieved by farmers (Mubvekeri and Nobanda, 2012).

The smallholder farmers have been blamed for the spread of the diseases into new areas due to poor hygiene, which include using implements with soil particles carrying the disease from infected fields to other areas with no diseases or uncontrolled movement of animals or people across the fields (Gwimbi, 2009; Mapope, 2001). Control of the plant diseases can involve the use of pesticides, biological or cultural methods or varietal tolerance. According to Chapepa *et al*. (2013), the use of pesticides and cultural practise is not effective and development of varietal tolerance is seen as the most viable option. Fradin *et. al*. (2009) also observed that treating *Verticillium* wilt is difficult since many fungicides are unable to reach vascular pathogens and few fungicides exist that can cure plants once infected with fungal diseases.

In the drought prone areas of Zimbabwe like Natural Regions three, four and five, cotton has been the major cash crop for smallholder farmers. Annual rainfall in the sector is predominantly below 650 mm per annum and low yields are attained because of a number of limiting factors like disease incidence and water stress (Mudada, 2013). The need for varieties with superior traits that are able to withstand disease pressure in these regions is imperative. In an effort to meet this demand, cotton plant breeders and agronomists seek varieties with alleles related to disease tolerance which are linked to high yielding cultivars. These traits are quantitative and expression of these traits is influenced by the environment (Yu *et al.*, 2013) Plants, in nature, are generally resistant to most pathogen because they have the innate ability to recognise potential invading pathogens and institute defense mechanisms (Staskawicz, 2001). Plants contain single dominant resistance (R) genes that specifically recognise pathogens that contain complementary avirulence genes. Specific recognition results in the induction of defense gene expression and the inhibition of pathogen growth. The genes can be inherited into the next generations hence conferring resistance to the progenies.

In all plant breeding programs, it is not only the phenotypic composition of a plant that is important but also its breeding value. The success of cotton breeding program is primarily based on the choice and use of the promising parental lines for hybridization, followed by selection for favourable genes and gene complexes in homozygous lines (Khan *et al*., 2009). There are many mating designs used in breeding programmes and crossing in a diallel mating fashion is one of the most dependable and effective technique plant breeders use. This technique is particularly a powerful tool for identification of suitable choice of superior genotypes that can be incorporated in any breeding program as it is effective in estimating genetic variations and the combining abilities of the parental lines.

Furthermore, characterisation of the cotton genotypes with superior traits that show some significant levels of tolerance to *Verticillium* wilt is of paramount importance in choosing promising parental lines and subsequently varieties for commercial use in *Verticillium* wilt prone areas. The traits are employed as markers for the cotton lines for selection purposes during varietal development. Accurate characterisation is an important process in any breeding programme because it facilitates the selection of promising parents for use in the programme (Murtaza *et al.,* 2005). Agronomic and morphological characterisations are the simple techniques that can be employed although molecular characterisation is the current technology used that is quick and reliable. Agronomic characterisation involves the evaluation of the disease and pest resistance, seed cotton yield, lint and fibre quality (Lukonge, 2005). These are heavily influenced by environment and cultivation. Morphological characterisation involves the determination of the leaf shape, hairiness, boll size and shape, root density and plant height

The study will involve genetic analysis of components involving gene action and types of inheritance (genetic components of variation). The project therefore strives to investigate the genetic underpinnings of quantitative traits pertaining to *Verticillium* wilt tolerance and provide information on the prediction of the gene action in a breeding system for the available parental lines. Furthermore it will seek to explore the genetic variation on the phenology level of cotton varieties exhibiting resistance to verticillium wilt by describing the morphological and agronomic descriptors.

1.1 Main Objectives

To identify and characterise candidate cotton lines for breeding programs under *Verticillium* wilt pressure through illustration of the inheritance pattern, gene action and correlation involved in resistance traits to the disease and seed cotton yield

1.2 Specific objectives are:

a) To evaluate the performance of local Zimbabwean cotton lines produced by diallel mating breeding system under *Verticillium* wilt pressure in one season.

b) To estimate general combining ability and specific combing ability for yield and its components of cotton germplasm under *Verticillium* wilt infestation.

c) To correlate the acquired traits of *Verticillium* wilt resistance from diallel crosses with seed cotton yield produced in F2 generation of cotton

d) To assess the genetic variation using agronomic and morphological markers of cotton lines under *Verticillium* wilt pressure

1.3 Hypotheses

a) The F1 offspring respond differently to *Verticillium* wilt infection.

b) The five cotton varieties have different genetic variance components of general and specific combining abilities on resistance to *Verticillium* wilt

c) There is significant linear correlation between the acquired traits of *Verticillium* wilt resistance and seed cotton yield

d) The cotton lines have different agronomical and morphological traits that make them tolerant to the *Verticillium* wilt disease.

Chapter 2 : Literature Review

2.1. Background of cotton production

Cotton is harvested from almost 32.4 million hectares in more than 40 nations of the temperate and tropic regions of the world (ICAC, 2013). In developing countries cotton accounts for nearly 3% of the total crop area and is produced for various purposes (Fortucci, 2001). The crop is grown as far as 47^0 N latitude and 37^0N latitude. In the Southern hemisphere production extends to about 32^0 S latitude (Niles and Feaster (1984) in Ullah-Joy, 2013). It is the third crop on the global trade, with major exporters being countries like United States, China, Brazil, India and Uzbekistan (Michele *et* al., 2008). Cotton grows at an maximum rate in the temperature range of 25-30^{0}C, where 15-25^{0}C is the temperature range for maximum cotton seed germination and growth (Medany *et al*., 2007) and it requires at average around 650 mm of annual rainfall. It is thus a source of livelihood in the most semi-arid regions of the developing nations which are characterised by such conditions and in Zimbabwe these areas include the drought prone areas of Natural Regions three, four and five (Mudada, 2013).

2.2 Trends in cotton breeding in Zimbabwe

Cotton research in Zimbabwe dates back as early as the 1920s where research was initiated in the area of development of insect pest resistance varieties. The major pest that was limiting cotton production during that period was the jassids in which it was controlled by the introduction of the hairy varieties. The source of the breeding material was a variety called U4 which was introduced from a research station called Barberton South Africa Cotton which gave rise to the first ever local variety called 934. This variety, however, had two problems, which were poor lint quality and lack of resistance to blackarm (a bacterial blight disease affecting the stem, leaves and bolls of cotton plants) (Mubvekeri and Nobanda, 2012). Further improvements on the variety were made giving rise to the Albar varieties which had

5

resistance to the blackarm. Cotton varietal development continued and since 1980, 13 commercial varieties have since been released with improvements in the seed cotton yield by the Cotton Research Institute of Zimbabwe. As new varieties were being developed, a new threat was identified in the cotton industry which was the *Verticillium* wilt disease that caused up to 100% yield loss (Mapope, 2005). Deliberate breeding work was done to develop varieties that are resistant to the disease and this gave rise to the variety BC853 in 1995. Ever since no further breeding work has been done to improve the resistance of the varieties to the disease (Mudada, 2013). The current varieties on the market are SZ9314, CRI MS 1, CRI MS 2 and QM301 of which CRI MS 1 has a better tolerance to the disease whilst CRI MS 2 has fair tolerance but in heavy infestation these are heavily affected (Chapepa *et al*., 2013).

2.2.1 Importance of cotton in Zimbabwe

In 2000, world cotton production amounted to 19 million tonnes but currently it is pegged at 33.1 tonnes (ICAC, 2014). The cotton industry plays a pivotal role in the national economy in most of the African developing countries including Zimbabwe (Baffes, 2004). Around 300 000 ha are put under cotton in Zimbabwe with an average of around 250 000 tonnes national output of seed cotton (Mubvekeri and Nobanda, 2012). According to Sibanda *et al*. (2001), the production of the crop has seen more than 150 000 rural households in the drier parts of the country benefitting from the cash income realised after marketing the crop. More than one million people are involved in its production. The national income being contributed by the crop is significant and it provides significant returns to areas specializing in production (Fortucci, 2001). Currently cotton is one of the five major crops produced commercially in the country and is the second foreign currency earner after tobacco (Sibanda, 2012).

Cotton production contributes to employment particularly in developing countries.

Production of the crop provides additional opportunities for rural employment in cotton ginning, transport and marketing in those countries with textile and clothing manufacturing industries (FAO, 2000). Agrimarket INFO (1998) and Dippenaar-Schoeman (1999) reported that the cotton industry in South Africa is one of the largest employment sectors and it significantly contributes towards social and economic uplifting in the country. Elsewhere in China, 43 million peasants are engaged in cotton production (Hebei Academy of Agricultural Sciences, 2007) whilst more than 500 000 communal farmers are into cotton production in Zimbabwe (Gono, 2005). On average, households use about 35% of their total cash income obtained from cotton to buy food, 10% for clothing, 15% for production inputs and 40% for many other needs such as medical care, communication and education. Households with a school age child use about 40% of the cash income for the child's education (FAO, 2000).

2.3 General cotton development

Four different genus of cotton, upland (*Gossypium hirsutum*), island or sea-land cotton (*G. barbadense*), Asian cotton (*G. aborium*) and *G. harknessii* L., are cultivated for fibre with upland cotton accounting for over 85% of world cotton production (Mudada, 2013) and these are perennial in nature. The *G. hirsutum* varieties, which are day-length neutral plants, yield more than the other varieties from the other genus. Breeders have been successful in developing cultivars that are high yielding and have high quality (Mudada, 2013). Cotton improvement has always targeted yield and yield components like locules, boll size, and number of bolls per plant, seeds per boll, seed size, lint index, seed index and ginning outturn. Work has also been carried out on pest like jassids and bollworms and disease resistant cotton varieties like bacterial blight and verticillium wilt. Zhang (2001) commented that though primarily *G. hirsutum* is cultivated in the world, morphological and cytological studies of cotton lagged behind due to its large genome and small chromosomes. There are

programmes responsible for developing new cultivars adaptable to the environment through gene manipulation, to produce cultivars tolerant to *Verticillium* wilt, nematodes and insects based on morphological characteristics such as hairiness, okra leaf, frego bract and red colour. Development of cotton varieties in Zimbabwe is done at Cotton Research Institute and it involves the use of classical breeding techniques which include the pedigree method, backcross and mass selection. Many traits are considered which include seed cotton yield, fibre quality, fibre length, disease resistance, pest resistance and other abiotic stress tolerance traits like drought tolerance, heat tolerance or salinity tolerance.

2.4 Challenges of cotton production in Zimbabwe

The major cotton production constraints in Zimbabwe include unfavourable weather conditions (mainly drought) in some regions, insect pests (African bollworm, jassids, lygus and aphids), diseases (*Fusarium* wilt, bacterial blight and *Verticillium* wilt), weeds, competition with food crops, declining soil fertility and unsatisfactory marketing and seed distribution systems (Mudada, 2013). These challenges have been exacerbated by the declining producer prices that have made the production of cotton less lucrative. Furthermore manipulation of farmers by contractors who short change them by providing less inputs and yet demanding a lot from them at the end of season has led to more challenges in the industry. This has led to the production of cotton with inferior quality as it lacks the required input levels for high quality cotton production. Ratoon cotton has also become a major challenge in the country as farmers see this as a viable option to these challenges as fewer inputs are used in its production but have a detrimental effect on the quality of the cotton and the outbreak of diseases and pests that become difficult to control (Ephraem, 2014). A threat worth mention is the possible outbreak and proliferation of *Verticillium* wilt disease due to unhygienic practices being observed by the resettled farmers, who are unknowledgeable

about the threat the disease, poses (Mapope, 2001). Control of the disease with pesticides is difficult and uneconomic but use of tolerant varieties reduces the impact of the disease. Hygienic practices to reduce inoculum level in the soil are also important in mitigating the effects of the disease as it is a soil borne disease that thrive in the soil for up to ten years or more in the absence of a host plant (Agrios, 2005; Gomez-Alpizar, 2001).

2.5 The incidence of *Verticillium* wilt disease on cotton

Verticillium wilt disease is caused by the pathogen *Verticillium dahliae* Kleb and was first reported in 1914 on upland cotton (*Gossypium hirsutum* L.) in California, USA (Pegg and Brady, 2002). The disease is spread through infected soil or infected trash and sometimes contaminated seed (Mapope, 2005). In Zimbabwe, the disease normally appears in cotton from six weeks after crop emergence and the problem is made serious around January and February due to the dropping of temperatures caused by the rains received that time (Mapope, 2005). These conditions promote the expression of *Verticillium* wilt (Agrios, 2005). Primary infections of the disease are often caused by microsclerotia, which are capable of surviving in soil, in the absence of the host for many years (Wheeler et al., 2000). In the world *Verticillium* wilt has become a major disease in all major cotton growing countries (the United States of America, China and the former Soviet Union) and in Africa the disease is a problem in countries like South Africa, Sudan and Zimbabwe (Bell, 1992). In Zimbabwe the disease can cause a yield loss of almost 100% in susceptible varieties like the SZ 9314 and FQ 902 (Cotton Research Institute, 2011).

The symptoms of the disease first appear as slight reddening of leaves followed by yellowing at the edge of the leaf. Chinodya (1996) observed that with time the colour of the leaves increases between the veins and more leaves develop symptoms. The leaves then dry between

the veins and this is followed by defoliation. The simplest diagnostic check of the disease is cutting the stem and if infected the characteristic brown discolouration of the vascular tissue is observed (Mapope, 2005). The disease causes stunting, wilting, defoliation, boll shed or total plant death when severe (Agrios, 2005). Extended periods of overcast and rainy periods may result in the severe wilt symptoms. Although plants may recover to some extent during periods of hot weather the yield would have been affected already due to the reduction of photosynthetic area as a result of leaf shedding or death.

2.6 Recommended *Verticillium* wilt control strategies

Control of *Verticillium* wilt is difficult as no single control measure is effective but rather an integrated approach is recommended (Chapepa *et al*., 2013). Preventative approach rather than curative are usually employed. Integrated management system is necessary to minimize losses from the disease. Varietal selection with some degree of resistance to wilt, good agronomic practices and adaptation to the environment of the varieties forms the core of control regime of the disease (Mapope, 2005).

2.6.1. Cultural practices for *Verticillium dahliae* control in cotton

The cultural practices used to control *Verticillium* wilt are aimed at preventing the introduction and establishment of the pathogen in the soil, soil eradicating or reducing inoculum potential in the soil and to optimise the expression and use of resistance (A. Bell in Mapope, 2005). This can be achieved by manipulating the cropping sequence that is crop rotations, nutrients, and soil moisture, planting practices, tillage practices, chemical and biological control. This is done to create an unconducive environment for the pathogen to thrive. Good cultural practices that can attain some level of control depend on the varieties used, inoculum densities of the pathogen in the soil, virulence of the strain in the soil and the environmental conditions (Bell, 1992).

Continuous planting of susceptible crops predispose the cotton to heavier attacks by the disease as the inoculum densities continues to multiply in the soil with every susceptible host planted on the same field. Around 13 to 15 colony forming units per gram of soil increase of the pathogen has been recorded in soil that is continuously grown cotton (Pullman and DeVay, 1984). To break the monoculture practice, rotation with cereals is recommended as a control measure against *V. dahliae* (Mathre, 1989). However, one year rotations do not significantly reduce the inoculum levels and a three to four rotation period is better (Chinodya, 1996). The research by Chinodya (1996) showed that most of the sorghum varieties reduce the density of the pathogen in the soil by stimulating germination of the propagules in the absence of the host. Another predisposing factor to the disease is deficiency of nutrients and deficiency of potassium in particular has been noted to increase the incidence of the disease (Bell, 1989). The deficiency of potassium increases the severity of wilt, presumably by increasing host susceptibility. It was found that fertilising of cotton fields with potassium helped to decrease verticillium wilt (Cotton Research Institute Annual Report, 1993).

2.6.2 Chemical control of *Verticillium dahliae* in cotton
Chemical control of *Verticillium* wilt is possible with the use of systemic fungicides. Research has shown that benzimidazole fungicides gives complete control of the disease when applied as drench in infected cotton (Mapope, 2005). Complete control and improvement of yield by about 50-100kg/ha was achieved by use of Benomyl (Bell, 1992). However, the use of chemical in controlling the disease in cotton is very expensive which have been attributed to the slow adoption of the control strategy in cotton production. The inoculum level in the soil of *Verticillium* wilt can drastically be reduced by fumigation using chemicals like methyl bromide, ethylene bromide or chloropicrin (tear gas) (Mapope, 2005).

2.6.3 Use of resistant cultivars to control *Verticillium* wilt in cotton

An integrated approach in controlling *Verticillium* wilt has been suggested, but the use of resistant or tolerant cultivars in areas with the disease has produced good results as a single strategy (Mapope, 2005). Resistance to *V. dahliae* is associated with the production of some antifungal compounds that take place immediately after infection (Pegg in Mace, 2012). Methylated sesqueterpenoid phytoalexins and ethrel (2-chloro-ethyl phosphonic acid) have been identified as some of the compounds. Antifungal chemicals known as phytoalexins accumulates in the vessels in response to these physical barriers which results in the sealing off of the infected vessels to avoid the upward movement of the fungal pathogens, fungal enzymes or toxins. The rapid formation of gels and tyloses has frequently been associated with the resistance of the plants to vascular pathogens with the susceptibility being correlated with poor or delayed response (Mysore and Ryu, 2004). Another form of inheritable defense mechanism caused by the ability of plants to respond to disease attack by hypersensitive response (HR) is the systemic acquired resistance (SAR). This is whereby the plant limit the access of water and nutrients to the pathogens by sacrificing a few cells to form the characteristic necrosis of the leaves and in the long run causing resistance to a broad range of pathogens (Freeman and Beattie, 2008).

Resistance to *Verticillium* wilt is expressed as a delay in the onset of visible symptoms and a decrease in symptom severity. It also involves the ability of the plants to recover after the initial attack and expression of the disease symptoms. The mechanism employed by plants when they recover is that new vessels that supply the upper foliage are formed and by-pass the clogged vessels resulting in no visual symptoms of the disease in the upper parts of the plants. In Zimbabwe, resistant cultivars are those that show a lower percentage of plants with foliar symptoms after the first boll set and when temperature begins to decline usually around February-March period (Mapope, 2005). Cultivars with moderate to high levels of resistance

to *Verticillium* wilt have been developed in several countries, with Zimbabwe having developed G501 and BC853 (Hillocks, 1991). Currently BC 853 is recommended for areas with wilt problems. However its yield and Gin Out Turn (GOT) are slightly lower than conventional varieties like SZ9314 (has GOT of 42% compared to BC853 that has a GOT of 35%, Cotton Research Institute, 2004).

2.7 Important characteristics in cotton improvement for verticillium wilt resistance
Plant defense against any biotic stress can be divided into two categories that is direct and indirect defenses. Where direct defense is aimed directly at the attacking organism and initiate the internal host response mechanisms. On the other hand indirect defense may be morphological that is any method that slows or inhibit the growth of the attacking organism (Van Geem et al., 2013). Different cotton varieties behaves differently in terms of morphological or agronomical characteristic under attack. In developing varieties it is imperative to study how each variety reacts so as to select the outstanding line showing tolerant traits.

2.7.1 Yield
Yield refers to the total biomass accumulated and the economic yield is the harvestable important components of the crop and in the case of cotton at farmer's level it is the total seed cotton. Lint yield is also important to the ginners and this commonly referred to as the Gin-out-turn. Cotton varieties vary in yield potential and this is further compromised by the incidence of *Verticillium* wilt on the cotton. Lint and seed yield are highly positively correlated (Lukonge, 2005). According to Thaxton and El-Zik (1994), selection of high yielding varieties should not be based on seedcotton yield; instead it should be based on lint yield as it depends on seedcotton yield and ginning outturn. *Verticillium* wilt disease greatly reduces the seedcotton yield and quality of the lint. The pathogen blocks of the xylem

vascular tissues of the plant thereby depriving the cotton bolls of the much desired nutrients that results in the bolls realising its full potential (Agrios, 2005) . The resultant seedcotton is under mature and of inferior quality. Tolerant varieties can yield meaningful even under heavy infestation as it tends to develop additional vessels which make the nutrients and water by-pass the infected vessels. The disease can also cause massive defoliation (www.apsnet.org) which results in reduced photosynthetic area and eventually loss of yield.

2.7.2 Number of bolls and boll size

In cotton, the boll is of economic importance as it is the unit package of yield of the plant. The number and size of bolls on a cotton plant determines the potential yield of the plant and thus high yield is achieved when their numbers per plant are maximised. Prolificacy that is the boll number per plant per unit area is an important tool for selecting high yielding cultivars (Mudada, 2013). Selection for boll size and seed size could positively influence lint yield, if a breeder selects for medium boll size, small seeds per boll and maintaining high ginning percentage (Coyle and Smith, 1997). *Verticillium* wilt results in shorter sympodial (fruiting) branches and stunting of the cotton plant generally resulting in fewer bolls per fruiting branch and per plant eventually. This in turn reduces total seedcotton yield of the plant. The disease also cause boll shedding of the bolls already formed and the inhibition of further square production (Pullman and DeVay, 1984).

2.7.3 Height to node ratio

Height to node ratio is an important tool in cotton mapping that is used to assess plant vigour. This ratio indicates the amount of stress that a cotton plant has encountered during its growth. The number of nodes produced by the plant is not influenced by the stress while plant height is greatly influenced by various stresses which include *Verticillium* wilt which when severe causes stunting of the plants. The nodes indicate the age of the plant and height is an

indicator of stress encountered. The lower the ratio the less vigorous the plant and the higher the ratio the more vigorous it is.

2.7.4 Fruit retention

Cotton tends to add more squares (fruiting structures) with few ending up as mature bolls as most are aborted to balance out the demand of the growing boll load. This is physiological square shed and is natural but however several stressing conditions also cause fruit shed. *Verticillium* wilt also cause this unnatural fruit shed as it tends to limit supply of water and nutrients to the increasing boll load demand as it blocks the vascular bundle of the cotton plants. Young bolls are particularly sensitive to severe disease infection as they shed off first as the plants tries to recover from the disease attack and supply some nutrition and moisture to the already mature bolls.

2.7.5 Plant height

Plant height is important as a contributor to yield and can determine vegetative and fruiting branches of the plant. Breeding for plant height variation is influenced by both yield potential and harvesting methods (Niles and Feaster, 1984). Kohel and Benedict (1987) observed plant heights between 0.95-1.07 m while Emeetai-Areke (1999) reported that the plant height of cotton ranged from 1.0-2.0 m. Generally plant height is highly affected by the environment. Stunting is a common symptom of *Verticillium* wilt especially if it occurs early in the growing season. *Verticillium* wilt results in reduced plant growth, epinasty and a slight change in leaf colour (Pullman and DeVay, 1984). In this case main stem nodes number is not altered but elongation is affected by changes in rate of internode elongation.

2.7.6 Ginning outturn (GOT)

Ginning outturn is the percentage of lint fibre obtained from a sample of total seedcotton after removing the seeds. It varies between cotton varieties and for Zimbabwean local varieties it ranges between 40-43% (Mudada, 2013). Christidis and Harrison (1955) and Munro (1987) reported that the range of varieties with regard to ginning percentages was shown to change little from year to year and from place to place. Thus GOT is influenced by genotype by environmental interactions (Mudada, 2013). *Verticillium* wilt reduces the boll sizes as photo-assimilates accumulation in the bolls is greatly reduced due to the blocking of the xylem vascular bundles by the disease. Lint formation is significantly reduced as carbohydrates deposition on the lint fibres becomes reduced resulting in reduced GOT.

2.7.7 Seeds per boll

The number of seeds per boll is a distinct characteristic of a variety and this is dependent on the number of locules per boll (Munro, 1987). In cotton varieties of the species *G. hirsutum,* the number of seeds that occur per locule is eight and the number of locules per boll varies between three to five (Lukonge, 2005). Seeds are the products of fertilization and are the units for reproduction to the next generation. Lint fibres grow from the surface of the seeds and thus the number of seeds determines the amount of lint produced per boll as this increase the surface area for lint production (Culp and Harrel, 1973). The *Verticillium* wilt disease affects the reproductive capacity of the plant and affects fertilization and this reduces the number of seeds set per locule.

2.8 Genetic variance

Survival of organisms depends on their ability to adapt to changes in their habitats and this is due to genetic variation. This is the inherent trait that is characteristic of all organisms that in the population provides the necessary information necessary for selection of the fit organisms

that will continue to exist and pass on their traits to the next generation. It is partitioned into three components which are additive variance, dominance effect and non-allelic interaction or epistatic effect (Meredith, 1984). The additive variance effect is the average effect of genes. The resemblance between parents and offspring is largely due to additive genetic effects and is responsible for determining the response of the population to selection. The dominance effect is the interaction of allelic genes. This represents the deviation of the heterozygote from the average of the parents. Non-allelic interaction or epistatic effect is the interaction of non-allelic genes that influence a particular trait (the interaction deviation is the result of epistatic effect) (Meredith, 1984).

Genetic variation is described in statistical terms as the phenotypic variance of the population is a function of genotypic and environmental variance.

$$VP = VG + VE$$

Where: VP = Phenotypic variance, VG = Genotypic variance, VE = Environmental variance. The genetic variance (VG) is further divided into breeding value (additive variance), dominance variance and interaction variance.

$$VG = VA + VD + VE + VI$$

Where: VA = Additive variance, VD = Dominance variance, VE = Environmental variance, VI = Interaction variance (Falconer, 1989).

The breeding value of a line is a function of the additive gene action. The additive genes are directly transmitted from parents to off-springs and are the source of likeliness and resemblance between relatives (Lukonge, 2005). It can be used to calculate inheritance pattern. The dominance additive ratio indicates the degree of dominance. Dominance:additive ratio of less than one refers to partial dominance, near one indicates complete dominance and

greater than one indicates over-dominance (Falconer, 1989). Gad *et al.* (1974) and Singh and Singh (1980) reported additive genetic variation for seed cotton yield, number of bolls, ginning outturn and lint index. Ahmad *et al.* (1997) observed additive gene action with partial dominance for bolls per plant, boll weight, seed cotton yield and seed index. Epistatic effects were involved in the expression of all the characteristics except for boll weight.

2.9 Diallel analysis

Diallel mating design involves the use of the same parents as females and males in breeding (Isik, 2009). Hayman (1954) defined diallel cross as the set of all possible mating between several genotypes. It relates to crossing of each of several individuals with two or more others in order to determine the genetic contribution of each parent to specific characters in the offspring. The design provides good evaluation of the parents and full-sib families. This mating design also permits estimation of the magnitude of additive and non-additive components of heritable variance (Griffing, 1956; Mather and Jinks, 1977). Four methods of diallel crosses are used which are full/complete diallel mating design which includes all possible crosses including parents, full/complete diallel mating design which includes all possible crosses but no parents, half diallel mating designs with parents and no reciprocals and half diallel mating designs with no reciprocals and parents.

The assumptions of additive-dominance model, genetic mechanism and genetic components of variation which control the various plant characters in genotypes under high disease pressure conditions is taken into account for verticillium wilt tolerant traits. The inheritance patterns of the trait are then subjected to diallel analysis. The cotton cultivars require testing through the use of a test under disease stress conditions to determine the success of such a mating model. Griffing's (1956) half diallel analysis gives a detail of the combining abilities of genotypes which measures the general combining abilities (GCA) and specific combining

abilities (SCA). The two measures provide information on the average behaviour of parents and expression of additive genes and non-additive genes respectively. Data obtained from such cross combinations can be analysed in several ways, but most commonly, analyses are based on the procedure proposed by Hayman (1954) and Griffing (1956). On the basis of these premises, a test for the validity of the additive-dominance model has been suggested. It is possible to obtain estimates of additive and dominance components of heritable components of variation from the mean squares of these mating designs (Hayman, 1954; Mather and Jinks, 1977).

Bolek et al. (2005) showed that verticillium wilt resistance is a quantitative trait with normal distribution of all traits. Diallel analysis and additive dominance models provide the information to conventional breeders to comprehend allelic and non-allelic gene action, nature and magnitude of genetic variance used by genotypes in specific combinations (Batool and Khan, 2012). Gene action is described in statistical terms as additive, dominant and epistatic effects and their interactions with environmental factors (Hussain et al., 1998; Khan and Hassan, 2011).

2.9.1 Combining ability

The mean measurement of a cross is partitioned into major components apart from the general mean and environmental variance. This was put forward by Griffing in 1956. There is the general combining ability (GCA) and specific combining ability (SCA). The general combing ability provides the average performance/contribution of the parents in hybrid combination (Sprague and Tatum, 1942). Falconer and Mackay (1996) defined it as the mean performance of the line in all crosses, when expressed as a deviation from the mean of all crosses. GCA consists of additive and additive epistatic variances (Matzinger, 1963). Specific combining ability provides performance of certain combination or crosses in a hybrid

combination. It is used to designate cases where certain combinations perform better or worse than would be expected relative to the average performance of the line or parent used in the cross (Sprague and Tatum in Lukonge, 2005). It is the deviation to a greater or lesser extent from the sum of the GCA of its two parents. SCA consists of dominance and all types of epistatic variances are regarded as an estimate of effects on non-additive gene actions (Falconer and Mackay, 1996).

The combining abilities are important in breeding work as GCA and SCA effect help to locate the lines/parents and the particular crosses that are responsible for showing a particular type of gene action (Baker, 1978; Meredith, 1984). GCA and SCA effects and variances are effective genetic parameters of direct utility to decide the next phase of the breeding programme (Dabholkar, 1992). It helps selection of parents for construction of synthetics, selection of suitable F1s for a multiple crossing or composite breeding programme and the possibility of employing an appropriate selection technique like modified mass selection, recurrent selection and reciprocal selection (Dabholkar, 1992). Epistasis affects the estimates of GCA and SCA mean squares, variances and other effects in an unpredictable manner (Baker, 1978). El-Adl and Miller (1971) found that GCA of F1s was more important than SCA for lint yield and the components of yield with the exception of lint percentage. Theoretically the presence of significant GCA and SCA in the F1 generation is a consequence of fluctuations in additive and dominance relationships among parents (Tang *et al.,* 1993).

Chapter 3 : Diallel analysis on variation of *Verticillium* wilt tolerance in upland cotton grown in Zimbabwe

3.1 Introduction

The upland cotton (*G. hirsutum*) is the most cultivated fibre crop that provides the world's main supply of raw cotton raw material (above 80%) for further processing (Ashraf and Ahmad, 2000). Cotton has many characteristics that are quantitatively inherited and the most interesting ones being for biotic stress tolerance (Meredith, 1984), especially for disease resistance. This can allow for gene manipulation of the crop in breeding work. Understanding genetic variation and disease tolerance is important for plant breeders and pathologists because the selection of resistant genotypes depends on high heritability of the crop (Ashraf and Ahmad, 2000). Breeders of self-pollinated crops are primarily interested in combining desirable genes from different genotypes into a single genotype to create variability for a characteristic they wish to improve (Dabholkar, 1992). F1 hybrids may exhibit superior performance due to dominance and/or non-allelic interaction.

In plant breeding, identifying suitable parents for the crossing programme is important. Lines or parents in hybrid combinations can be compared on their performance through combining ability analysis (Griffing, 1956). The combining ability is a powerful tool used to determine hybrid vigour in a population and thereby aid in selecting parents for producing crosses and segregating populations (Meredith, 1984). General combining ability and specific combining ability analysis was used in this study to separate good and poor combiners followed by selection of the best cross combination. One of the techniques widely used for this purpose in different crops, including cotton, is diallel analysis (Hayman, 1954).

Diallel mating helps in choosing parents to be used in hybridisation or population breeding

programmes (Murtaza *et al.*, 2005). Prediction of genetic diversity and GCA of parents before crossing reduces the number of crosses and progeny to be screened and leads to a reduction in cost and time (Kumar, 1999). The aims of this study were to study combining ability and identify suitable parents to be used in cotton breeding programme for the improvement of *Verticillium* wilt tolerance and to assess genetic and phenotypic correlation of verticillium wilt tolerance and seed cotton yield using 10 crosses and five parents of a half diallel cross.

3.2 Materials and Methods

3.2.1 Experiment site

The experiment was conducted at Cotton Research Institute (C.R.I) in Zimbabwe which lies at 18° 20′ South and 29° 54′ East at an altitude of 1156 m above sea level. The institute receives an average rainfall of 750 mm per annum and the temperature during the summer season averages $28^{o}C$ maximum and $14^{o}C$ minimum ranges (Mubvekeri and Nobanda, 2012).

3.2.2 Experimental procedure

3.2.2.1 Diallel Mating

Five diverse upland cotton Zimbabwean genotypes (CRI-MS-1, CRI-MS-2, SZ9314, BC 853and CIM1) developed by the Cotton Research Institute varying in year of release, verticillium wilt response and seed cotton yield traits (Table 1) were crossed in a half diallel mating fashion. The half diallel mating design which included parent lines was carried out in the greenhouse where the genotypes were crossed to each other, parent lines selfed but reciprocals excluded in a non-replicated crossing block with four pots per cultivar during the winter season of 2013 as shown in the layout in Table 2.

Table 1: Cotton breeding material for the 5 x 5 half diallel mating design

Cultivars	Breeding Centre	Parentage	Year of Release	Verticillium wilt tolerance
CRI-MS-1	C.R.I	FW-91-3 x TE 93-10	2006	Good
CRI-MS-2	C.R.I	FQ902 x MR-92-5	2006	Fair
SZ9314	C.R.I	STAMF x BC853	1998	Poor
BC853	C.R.I	A72bc3-78-35xDmMs73bc2-79-28	1995	Very good
*CIM1	C.R.I	FQ902 x EU92-16	Pre-released	Fair

(Source: Cotton Research Institute variety catalogue)

* Pre-released variety

Table 1. Continued

Cultivars	Parentage	Boll weight	Plant height	Expected Yield(kg/ha)
CRI-MS-1	FW-91-3 x TE 93-10	Medium	Medium	2600
CRI-MS-2	FQ902 x MR-92-5	Large	Medium-Tall	2300
SZ9314	STAMF x BC853	Very large	Tall	2400
BC853	A72bc3-78-35xDmMs73bc2-79-28	Medium	Medium	1800
*CIM1	FQ902 x EU92-16	Medium	Medium	2300

(Source: Cotton Research Institute variety catalogue)

* Pre-released variety

Table 2: Half diallel mating layout

	V1	V2	V3	V4	V5
V1	O	X	X	X	X
V2		O	X	X	X
V3			O	X	X
V4				O	X
V5					O

Source: Design adopted from Isik F. (2009) in Analysis of Diallel Mating Design

x are the crosses that were done between the male and female rows

O are selfed parents

V1 = CRI MS 1, V2 = CRI MS 2, V3 = SZ9314, V4 = BC 853 and V5 = CIM1

The 10 F_1 hybrids produced and their five selfed parents were evaluated under *Verticillium* wilt pressure in the greenhouse at C.R.I in 2014. The 15 genotypes were laid out in a completely randomised design with three replications each. The plot sizes were composed of three pots containing single cotton plants that were spaced at 1m between rows and 0.3m within rows. Artificial inoculation of *Verticillium* wilt was done on the cotton plants to induce uniform infection in the plants at six weeks after crop emergence as laid out below.

3.2.2.2 Isolation and Inoculation Protocol of *Verticillium dahliae* on Cotton

A collection of five cotton plants showing foliar symptoms of verticillium wilt were collected from the field at Cotton Research Institute. Leaves from these cotton plants were used for isolation of the pathogen and these were surface sterilised using 1% Sodium hypochlorite and cleansed with running distilled water. Twenty small sections of 5 x 5 mm were cut from the margin of the lesions on the leaves such that the leaf piece contained both the healthy and diseased leaf tissue. The five tissues samples were placed in a Petri dish containing Potato Dextrose Agar. Twenty Petri dishes were prepared under aseptic conditions and they were incubated for 48 hours at 26°C. When the pathogen cultures grew on the agar they were sub-cultured using the Czapek Dox Broth media agar three times to get a pure culture (Hillocks, 1991). Microsclerotia developed on the culture and the inoculum for each isolate was adjusted to a concentration of 2×10^{6} conidia/ml using repeated serial dilutions. At six weeks after emergence, plants were stem inoculated at the base of the plant using sewing needles dipped in the conidia suspension of each isolate.

3.2.3 Data Collection

Comparison of varietal performance of the genotypes under verticillium wilt pressure was carried out on disease expression, Vascular Brown Index (VBI), plant height, height to node ratio, fruit retention and bolls per plant, boll weight and seed cotton yield expected per

hectare. The severity of the disease and tolerance was measured by means of scoring and vascular brown index according to the Fusarium and Verticillium Resistance Ranking Protocol for Cotton Varieties (2008), *Australian Cotton CRC*. The Scoring System that was used was as follows:

1   no symptoms

2   Symptoms confined to vein discolouration or slight chlorosis

3   Several leaves showing chlorosis

4   Many leaves showing chlorosis some with necrotic areas

5   Most leaves chlorotic some with severe necrosis usually some defoliation

6   Whole plant affected, top most leaves necrotic and shedding of lower leaves

Severity scoring was done from two weeks after inoculation fortnightly. VBI was determined on plants in the different plots after harvesting where the stems were cut at above ground level of each plant in the plot and average score determined. The rating system used was based on the severity of vascular discolouration (browning) visible in a cross section of the main plant stem cut as practicable to the ground level where:

0   no discolouration

1   discolouration restricted to small spots or an area less than 5% of the stem cross section

2   discolouration of between 5% and 20% of the stem cross section

3   discolouration of between 20% and 40% of the stem cross section

4   greater than 40% vascular discolouration of the stem cross section

The general agronomic practices as recommended in the Cotton handbook (Mubvekeri and Nobanda, 2012) were carried out on the cotton crops. Picking was done at the end of the season on the whole plot.

3.2.4 Traits measurement and statistical analysis

The data recorded on disease score expression, Vascular Brown Index, plant height, height to node ratio, fruit retention and bolls per plant, boll weight and seed cotton yield expected per hectare was subjected to analysis of variance (ANOVA) to test the F-test using Genstat 14th edition statistical package.

3.2.5.1 Genetic analysis

GCA and SCA were estimated based on the fixed model method II as described by Griffing (1956) as used by Machikowa *et al.* (2011) as follows:

$$X_{ij} = \mu + g_i + g_j + s_{ij}$$

Where X_{ij} = the mean phenotypic value; μ = the general mean; g_i g_j, = GCA effects of the ith and j[th] parents respectively; s_{ij} = SCA effects of the cross i x j.

The estimates of GCA and SCA parents and hybrids were obtained as

GCA effects $\quad\quad\quad g_i = 1/n(n-2)[nX_i.-2X..]$

SCA effects $\quad\quad\quad s_{ij} = X_{ij}-1/n(n-2)(X_i+X._j) + 2/(n-1)(n-2)X..$

Where $X_{i.,}$ $X._j$ = means of the ith and j[th] parents respectively; X.. = grand mean; n = number of parent lines

GCA:SCA ratio was calculated using ratios of the mean squares of GCA and SCA.

3.2.5.2 Correlation

Phenotypic correlation estimates were calculated using the following the formula as used by Lukonge in 2005:

$$rp = covp/\sigma_{p(x)}\sigma_{p(y)}$$

where: rp = phenotypic correlation between characteristics x and y

covp = phenotypic covariance

$\sigma_{p(x)}\sigma_{p(y)}$ = the root of genetic variance of x and y respectively

Negative value of correlation coefficient (r) indicates dominant genes, while if its value was positive then recessive genes were responsible for the phenotypic expression of the trait. Genstat 14th edition statistical package was applied for correlation analysis.

3.2.5.3 Heritability

The narrow sense heritability (h^2) in F1 generation was calculated for each character according to Mather and Jinks (1982) using the F1 narrow sense heritability (h^2) as follows:

$$h^2 = V_G/V_P$$

where h^2 = narrow sense heritability, VG = genetic value, VP = phenotypic value. The heritability values were obtained using GCA and SCA values:

$$h^2 = \sigma^2 g/\sigma^2 p$$

where h^2 = narrow sense heritability, $\sigma^2 g$ total genetic variance = $2\sigma^2 gca + \sigma^2 sca$

$\sigma^2 p$ = phenotypic variance = $\sigma^2 g + \sigma^2 e$

3.3 Results

3.3.1 Morphological traits

Analysis of variance for the cotton lines used in the experiment indicated presence of significant differences on morphological characteristics affected by *Verticillium* wilt infection (Table 3). The plant height was significantly different at P<0.05 among the 15 cotton lines where CRI-MS-2 was taller than all the genotypes (160.3cm) but however was comparable to BC853, the cross between CRI-MS-2 and SZ9314, the cross between SZ9314 and BC853 and the cross between BC853 and CIM1. The cross between SZ9314 and CIM1 yielded the shortest progeny (121.1cm), whilst SZ9314, CRI-MS-1, CIM1, the crosses CRI-MS-1 x CRI-MS-2, CRI-MS-1 x SZ9314, CRI-MS-1 x SZ9314, CRI-MS-1 x BC853, CRI-MS-1 x CIM1 and CRI-MS-2 x BC853 where comparable.

The height to node ratio which indicates the vigour of the plants was highly significantly different (P<0.001). CRI-MS-2, BC853 and the cross between CRI-MS-2 and CIM1 were more vigorous although they were comparable to the crosses CRI-MS-1 x CIM1, SZ9314 x BC853 and BC853 x CIM1. SZ9314 and the cross SZ9314 x CIM1 had the least vigorous genotypes.

Fruit retention among the 15 genotypes was significantly different (P<0.05). The cross between CRI-MS-1 x CIM1 retained most of its fruiting bolls and it was comparable to both parents CRI-MS-1 and CIM1 and also to BC853 and the crosses CRI-MS-1 x SZ9314 and CRI-MS-1 x BC853. CRI-MS-2 shedded most of its bolls as it had the lowest fruit retention percent together with a few other parent lines and the progenies.

Table 3: Means for plant height, height to node ratio and fruit retention of cotton

Genotype	Plant height(cm)	Height to node ratio(cm/node)	Fruit retention(%)
CRI-MS-1	130.7abcd	6.86ab	71.08cd
CRI-MS-2	160.3f	8.02d	53.49a
SZ9314	124.7ab	6.68a	57.83ab
BC853	152.7ef	8.09d	65.49abcd
CIM1	137.2abcde	7.10abc	67.27bcd
CRI-MS-1 x CRI-MS-2	133.3abcde	7.19abc	61.88abc
CRI-MS-1 x SZ9314	133.2abcde	7.31abc	66.33abcd
CRI-MS-1 x BC853	130.8abcd	6.91ab	63.94abcd
CRI-MS-1 x CIM1	126.9abc	7.53bcd	76.06d
CRI-MS-2 x SZ9314	141.9bcdef	7.05abc	61.93abc
CRI-MS-2 x BC853	131.1abcd	7.19abc	61.85abc
CRI-MS-2 x CIM1	148.0def	8.03d	58.26abc
SZ9314 x BC853	145.4cdef	7.71cd	63.77abcd
SZ9314 x CIM1	121.1a	6.65a	68.80bcd
BC853 x CIM1	145.0cdef	7.76cd	62.32abc
Grand Mean	**137.5**	**7.34**	**64.02**
CV%	8.7	6.1	10.1
P-value	0.014	0.002	0.032

In the table above, means in the same column followed by the same letter are not significantly different after separation by Duncan Multiple range Test (P<0.05)

3.3.2 Yield and yield components

Number of vegetative and fruiting branches

Analysis of variance on the number of vegetative and fruiting branches showed that there were no significant differences (P<0.05) among the genotypes as shown in Table 4. 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.

Number of bolls per plant

Table 4 also show varietal performance under the disease pressure in terms of number of bolls produced. The number of bolls per plant also did not vary largely for all the genotypes (P<0.05). The boll number ranged between 12 and 19.

Average boll mass

There were also no noticeable differences on the average boll weight of the different cotton

genotypes which ranged 3 and 6 grams to that were used in the study (Table 4).

Table 4: Means of number of fruiting branches, number of vegetative branches, number of bolls per plant and average boll weight of cotton genotypes

Genotype	Number of fruiting branches	Number of vegetative branches	Number of bolls per plant	Average boll weight (g)
CRI-MS-1	14.56	2.56	17.00	4.33
CRI-MS-2	16.22	2.00	15.67	3.83
SZ9314	15.11	2.11	13.22	4.83
BC853	15.44	2.22	13.78	4.83
CIM1	15.56	1.78	15.11	4.50
CRI-MS-1 x CRI-MS-2	14.56	2.00	15.22	5.00
CRI-MS-1 x SZ9314	13.89	2.44	15.00	5.50
CRI-MS-1 x BC853	15.00	2.00	17.22	4.50
CRI-MS-1 x CIM1	14.00	2.00	17.67	5.00
CRI-MS-2 x SZ9314	15.67	2.11	18.56	4.67
CRI-MS-2 x BC853	14.11	2.11	15.22	4.67
CRI-MS-2 x CIM1	14.22	2.22	15.00	4.67
SZ9314 x BC853	14.78	2.00	13.22	5.00
SZ9314 x CIM1	13.67	2.33	14.00	5.00
BC853 x CIM1	14.22	2.00	12.67	5.50
Grand Mean	**14.73**	**2.13**	**15.24**	**4.79**
CV%	8.8	10.9*	14.8	12.5
P-value	0.484	0.851*	0.086	0.163

Values obtained after transformation of data with the square root transformation

Seed cotton yield

The seed cotton yield of the cotton lines under verticillium wilt pressure varied largely as the

results revealed that the yield means were significantly different at $P<0.05$ (Fig. 1). The

cross CRI-MS-1 x SZ9314 had significantly higher yield (2259kg/ha) although it was

comparable to two parent lines CRI-MS-1 and BC853 and six other crosses. The cross

between CRI-MS-2 and BC853 yielded the least seed cotton (1296kg/ha) but the yield was

comparable to four parent lines (CRI-MS-2, SZ9314, BC853 and CIM1) and four other

crosses.
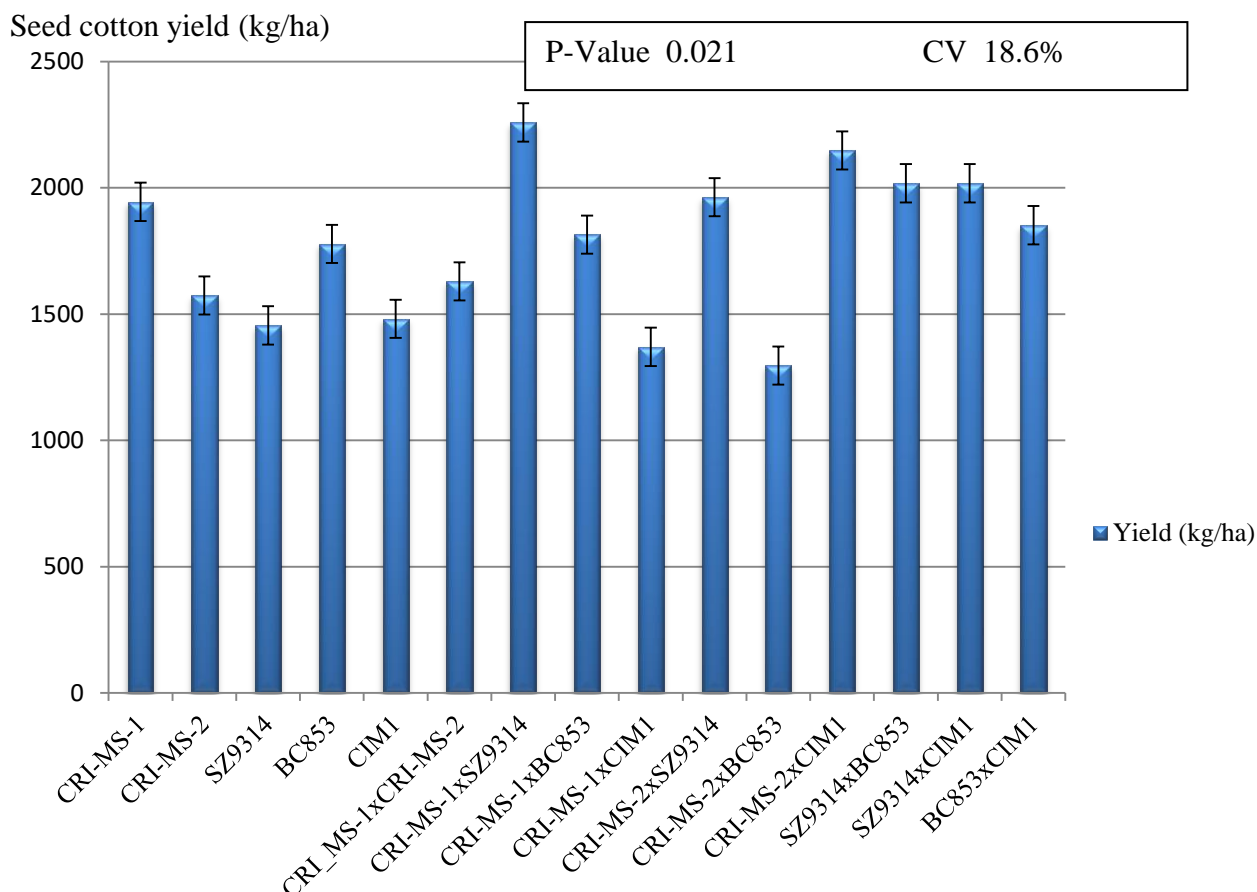
Figure 1: Seed cotton yield (kg/ha) of 15 cotton lines under verticillium wilt conditions

3.3.3 Verticillium wilt infection

Analysis of variance on the 15 cotton genotypes developed in the diallel mating design revealed significance differences among the genotypes when exposed to the same inoculum level of *Verticillium* wilt disease ($P<0.05$) as shown in Table 6. SZ9314 was affected the most with the disease as shown by the high infection score (3.07) and high vascular brown index score (2.67). The genotype was comparable to its cross with CRI-MS-1 (infection score of 2.67 and high vascular brown index score of 2.55) and CIM1 (infection score of 2.56 and high vascular brown index score of 2.77). CRI-MS-2 and BC853 were the most tolerant genotypes as shown by their low infection scores (1.22) and low vascular brown index scores (0.89 and 1.33 respectively). All the genotypes were infected by the disease to some extent

as indicated by varying scores. The *Verticillium* wilt scores ranged from 1.22 to 3.07 and the vascular brown index scores ranged from 0.78 to 2.77.

Table 5: Means of Verticillium wilt infection scores and vascular brown index (VBI) scores on cotton

Genotype	Verticillium wilt infection scores	Vascular Brown Index scores
CRI-MS-1	1.44ab	0.78a
CRI-MS-2	1.22a	0.89ab
SZ9314	3.07g	2.67c
BC853	1.22a	1.33ab
CIM1	1.67abcd	1.33ab
CRI-MS-1 x CRI-MS-2	1.89abcdef	1.22ab
CRI-MS-1 x SZ9314	2.67fg	2.55c
CRI-MS-1 x BC853	1.33ab	1.33ab
CRI-MS-1 x CIM1	2.33cdefg	1.33ab
CRI-MS-2 x SZ9314	1.67abcd	1.11ab
CRI-MS-2 x BC853	2.11bcdef	1.44b
CRI-MS-2 x CIM1	1.78abcde	0.89ab
SZ9314 x BC853	1.56abc	1.44b
SZ9314 x CIM1	2.56efg	2.77c
BC853 x CIM1	2.44defg	1.22ab
Grand Mean	**1.93**	**1.49**
CV%	10.7*	11.7*
P-value	<.001*	<.001*

* Values obtained after transformation of data with the square root transformation

In the table above, means in the same column followed by the same letter are not significantly different after separation by Duncan Multiple range Test (P<0.05)

3.3.4 Combining ability

Table 6: Mean squares for morphological traits, yield components and the GCA, SCA and GCA:SCA ratio for cotton genotypes used in the half diallel design

Source of variation	DF	Mean Squares				
		Plant height (cm)	Height to node ratio	Fruit retention (%)	Number of fruiting branches	Number of vegetative branches
Genotypes	14	367.4**	0.72**	93.10*	1.68	0.12
GCA	4					
SCA	9					
Residual	30	141.9	0.20	41.70	1.69	0.20
GCA:SCA						

Where DF =degrees of freedom, n = number of parents, r = replications, GCA = general combining ability, SCA = specific combining ability, * = p ≤ 0.05, ** = p ≤ 0.01

Table 7: Mean squares for Verticillium wilt infection scores, vascular brown index scores, yield components, seed cotton yield and the GCA, SCA and GCA:SCA ratio for cotton genotypes used in the half diallel design

Source of variation	DF	Mean Square				
		Verticillium wilt infection scores	Vascular brown index scores	Bolls/plant	Average boll mass (g)	Seed cotton yield (kg/ha)
Genotype	14	0.98***	1.24***	9.14	0.54	253220*
GCA	4					
SCA	9					
Residual	30	0.17	0.11	5.07	0.36	104476
GCA:SCA						

Where DF =degrees of freedom, GCA = general combining ability, SCA = specific combining ability, * = p ≤ 0.05, *** = p ≤ 0.001

Table 8: General combining ability (GCA) effects for Verticillium wilt scores, vascular brown index scores, morphological traits and yield components

Genotype	Verticillium wilt scores	Vascular brown index scores	Fruit retention (%)	Fruiting branches	Vegetative branches
CRI-MS-1	0.001	-0.04	3.84	-0.33	0.07
CRI-MS-2	-0.24	-0.40	-5.14	0.32	-0.02
SZ9314	1.66	1.95	31.18	7.05	1.09
BC853	-0.10	-0.21	-0.11	0.10	-0.02
CIM1	-0.26	-0.16	3.25	0.83	-0.35

Table 8. Continued

Genotype	Plant height (cm)	Height to node ratio	Bolls per plant	Average boll mass (g)	Seed cotton yield (kg/ha)
CRI-MS-1	-6.51	-0.18	1.18	0.08	101.20
CRI-MS-2	7.84	0.23	0.88	-0.33	-58.99
SZ9314	58.12	3.18	4.98	2.63	788.50
BC853	11.35	0.59	-2.01	0.38	75.27
CIM1	-0.27	-0.24	-0.13	-0.29	-258.05

Table 9: Specific combining ability (SCA) effects for Verticillium wilt scores, vascular brown index scores, morphological traits, yield and yield components

Genotype	Verticillium wilt scores	Vascular brown index scores	Fruit retention (%)	Fruiting branches	Vegetative branches
CRI-MS-1xCRI-MS-2	-0.04	-0.22	-5.98	0.16	-0.2
CRI-MS-1xSZ9314	0.74	1.11	-1.53	-0.51	0.24
CRI-MS-1xBC853	-0.60	-0.11	-3.92	0.60	-0.2
CRI-MS-1xCIM1	0.40	-0.11	8.20	-0.40	-0.2
CRI-MS-2xSZ9314	-0.03	0.03	3.05	0.62	0
CRI-MS-2xBC853	0.42	0.36	2.97	-0.95	0
CRI-MS-2xCIM1	0.09	-0.19	-0.62	-0.84	0.11
SZ9314xBC853	-0.84	-0.85	0.30	0.26	-0.15
SZ9314xCIM1	0.16	0.48	5.33	-0.85	0.18
BC853xCIM1	0.61	-0.06	-1.59	-0.61	-0.11

Table 9. Continued

Genotype	Plant height (cm)	Height to node ratio	Bolls per plant	Average boll mass (g)	Seed cotton yield (kg/ha)
CRI-MS-1xCRI-MS-2	2.35	0.03	-1.20	0.13	-211.11
CRI-MS-1xSZ9314	2.24	0.15	-1.42	0.63	418.52
CRI-MS-1xBC853	-0.20	-0.25	0.80	-0.37	159.27
CRI-MS-1xCIM1	-4.09	0.37	1.25	0.13	-470.36
CRI-MS-2xSZ9314	-3.44	-0.52	2.45	0.21	282.40
CRI-MS-2xBC853	-14.22	-0.38	-0.90	0.21	384.26
CRI-MS-2xCIM1	2.67	0.46	-1.11	0.21	208.33
SZ9314xBC853	15.03	0.70	-0.26	0.06	347.01

| | SZ9314xCIM1 | -9.30 | -0.36 | 0.52 | 0.06 | 35.34 |
| BC853xCIM1 | -3.84 | -0.17 | -0.56 | 0.34 | 37.04 |

3.3.5 Correlation

Phenotypic correlations for most of the characteristics were positive and but not significant as shown in Table 11. Most of the characteristics that were negatively correlated were significant. Height to node ratio had high significant positive correlation with height (0.80). Fruiting branches were also strongly positively correlated to height (0.58) whilst vascular brown index scores was also significantly correlated to *Verticillium* wilt infection scores (0.55). Seed cotton yield had significant correlation with average boll mass. This indicate that average boll mass is important to cotton yield. *Verticillium* wilt infection scores had a high significant negative correlation with height (-0.42), height to node ratio (-0.33) and number of fruiting branches (-0.23). Fruiting retention was not highly associated with the number of fruiting branches (-0.27) and height (-0.38). the seed cotton yield had an insignificant negative association with the *Verticillium* wilt infection scores.

Table 10: Phenotypic correlation coefficients for *Verticillium* wilt infection scores, vascular brown index scores, morphological traits, yield and yield components in cotton

	Yield	VBR	VBI	VIS	H:N ratio	FR (%)	FB	Height (cm)	Bolls/ plant	ABM
Yield		0.03	0.04	-0.05	0.08	0.17	0.01	0.11	0.23*	0.40***
VBR			-0.002	-0.02	-0.15	0.03	-0.21*	-0.14	-0.16	0.01
VBI				0.55***	-0.36**	0.14	-0.21*	-0.41***	-0.15	0.38**
VIS					-0.33**	0.10	-0.23*	-0.42***	-0.17	0.46***
H:N ratio						-0.20*	0.14	0.80***	-0.14	0.17
FR (%)							-0.27*	-0.38**	0.26*	0.14
FB								0.58***	0.12	-0.16
Height									-0.04	-0.02
Bolls/plant										-0.20*
ABM										

*VIS = Verticillium wilt infection scores, VBR = vegetative branches, VBI = vascular brown index, H:N = height to node ratio, FR = fruit retention, FB = fruiting branches, ABM = average boll mass, * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001*

3.3.6 Heritability

3.5 Discussion

3.5 Conclusion and Recommendation

Chapter 4 : Characterisation of upland cotton grown in Zimbabwe using agronomical and morphological markers for *Verticillium* wilt tolerance

4.1 Introduction

Characterisation of organisms is an important tool for selection of potential parents for subsequent crossing and selection of progenies up to the final product of a breeding program (Murtaza *et al.*, 2005). Analysis of the diversity of species and maintenance of genotypes are important in identification of genetic kinship which form the pool for selection. 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. Agronomical characterisation can also be used although this is subject to environmental influences and must be assessed during a fixed vegetative phase of the crop (Swanepoel, 1999).

Morphological traits for *Verticillium* wilt tolerance are controlled by a single locus and can be used as genetic markers as their expression is reproducible over a range of environments. Besides environment, expression of morphological markers is altered by epistatic and pleiotropic interactions (Rana and Bhat, 2004). In Zimbabwe, variation determination of varietal tolerance has been based on morphological descriptors and partly agronomical. This has been important in selecting parental material for crossing to improve the available known resistant materials (Mudada, 2013). 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). Environment and agronomic practices have an effect on most of

these morphological characteristics and most of all level of pathogen inoculum density in the soil (Pegg and Brady, 2002). Therefore the main aim of this study was to characterise five varieties grown in Zimbabwe and the ten hybrids from the diallel mating under *Verticillium* wilt pressure using agronomical and morphological markers.

4.2 Materials and Methods

4.2.1 Plant Material and Experimental Procedure

The five parental lines and the 10 progenies developed from the crosses in a diallel mating scheme were characterised at the Cotton Research Institute situated in Kadoma. The lines and progenies were planted in greenhouse as 15 treatments during the growing season of 2013/14 following the recommended agronomic procedures. Artificial inoculation was carried out on the 15 lines of potted cotton using $2x10^6$ conidia/ml of *Verticillium* inoculum to initiate infection. The treatments were laid out in a completely randomised design replicated three times. Plot sizes were three pots per treatment with a single cotton plant in each pot.

4.2.2 Data collection and characterisation

Data was collected from all the plants on size of bolls, number of bolls per plant, plant height, and plant shape, number of vegetative and fruiting branches, harvest index (HI), ginning outturn (GOT), number of seeds per boll, seed weight per boll and yield were collected on the three plants in the plot at week six and two weeks thereafter to assess varietal performance. Ten bolls were randomly collected from each plot at boll splitting stage to determine ginning outturn, 100 seed weight, and number of seeds per boll, seed weight per boll, lint weight per boll and boll weight. Seed cotton yield (tonnes/ha) was estimated after two pickings.

Modified International Board of Plant Genetic Resources (IBPGR) and modified Cotton Research Institute descriptors for cotton were used to describe cotton traits under *Verticillium* wilt conditions (Table 6). 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. 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 90-120cm, 120-150cm and above 150cm 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 5-6g and those below 5g were considered as small and those above 6g 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 11: List of morphological and agronomical characteristics measured

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	Shape of plant at boll splitting stage
Plant height	Height above ground measured in cm at harvest
*Number of fruiting branches	Number of boll bearing branches on the main stem at harvest
*Number of vegetative branches	Number of vegetative branches at harvest
*Harvest index	Proportion of dry matter partitioned to yield (%)
Gin outturn	Content of lint (%)
*Number of seeds per boll	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

* Adapted from CRI descriptors

4.2.3 Statistical analysis

Analysis of variance (ANOVA) and correlation coefficients on yield, yield components (boll number, number of fruiting branches, number of vegetative branches and boll size), plant height and ginning outturn (GOT) was done using Genstat 14th version.   The data that was converted into binary matrix data was subjected to cluster analysis using multivariate analysis of Genstat 14th version 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. Dendrograms were constructed based on the Euclidean distances using the Hierarchical clustering and measure of goodness of fit carried out for each dendrogram.

4.3 Results

Morphological characteristics

There were significant differences on the plant height on the cotton used to characterise for tolerance against verticillium wilt disease as shown in Table 3 at P<0.05. There was variation among the genotypes in terms of number of seeds per boll at $P \leq 0.05$ (Table 12). The parental line CRI-MS-1 had the highest number of seeds per boll (24.67 seeds) with its cross with CRI-MS-2 having the least number of seeds per boll (17 seeds). CRI-MS-l was comparable to the parental lines BC853 and CIM1 and the crosses CRI-MS-2 x CIM1, SZ9314 x BC853.

The seed weight per boll of the different genotypes used in this experiment were not significantly different as the cotton lines produced statistically similar weight of seeds of the cotton from the cotton bolls as shown in Table 12.

Table 12: Means of number of seeds per boll and seed weight per boll

Genotype	Number of seeds per boll	Seed weight per boll
CRI-MS-1	24.67c	1.91
CRI-MS-2	19.00ab	1.64
SZ9314	17.33ab	1.78
BC853	20.33abc	1.93
CIM1	20.67abc	1.88
CRI-MS-1 x CRI-MS-2	17.00a	1.74
CRI-MS-1 x SZ9314	22.00bc	2.19
CRI-MS-1 x BC853	17.67ab	1.71
CRI-MS-1 x CIM1	18.67ab	1.90
CRI-MS-2 x SZ9314	19.33ab	1.79
CRI-MS-2 x BC853	19.00ab	1.84
CRI-MS-2 x CIM1	21.00abc	1.96
SZ9314 x BC853	22.00bc	1.99
SZ9314 x CIM1	19.00ab	1.98
BC853 x CIM1	18.67ab	2.00
Grand Mean	**19.76**	**1.88**
CV%	12.4	9.7
P-value	0.042	0.104

In the table above, means in the same column followed by the same letter are not significantly different after separation by Duncan Multiple range Test (P<0.05)

Agronomical characteristics

The agronomical characteristics had no significant differences among the cotton genotypes characterised. These were number of bolls per plant, boll weight, number of vegetative and fruiting branches and seed cotton yield (Table 4 and 5).

The fruiting efficiency of the cotton genotypes used in this experiment revealed significant differences among varieties as they had statistically different harvesting indexes ($P<0.05$) as shown in Table 8. 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.

Table 13: Means of ginning outturn (GOT) of cotton and harvest index (HI)

Genotype	Gin outturn (%)	Harvest Index (%)
CRI-MS-1		43.91abcd
CRI-MS-2		35.98a
SZ9314		40.81abcd
BC853		43.35abcd
CIM1		43.08abcd
CRI-MS-1 x CRI-MS-2		38.09abc
CRI-MS-1 x SZ9314		45.63cde
CRI-MS-1 x BC853		48.88de
CRI-MS-1 x CIM1		38.33abc
CRI-MS-2 x SZ9314		44.69bcde
CRI-MS-2 x BC853		37.00ab
CRI-MS-2 x CIM1		49.29de
SZ9314 x BC853		52.90e
SZ9314 x CIM1		47.71de
BC853 x CIM1		44.36abcd
Grand Mean		**43.60**
CV%		10.2
P-value		0.001

In the table above, means in the same column followed by the same letter are not significantly different after separation by Duncan Multiple range Test (P<0.05)

Cluster analysis

Cluster analysis on the morphological characteristics of plant shape, plant height, boll size, boll prominence and leaf colour (Appendix 7.3), 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 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.

Main group B had a single cotton line CRI-MS-1 x CIM1. The cross in this cluster was distinguishable from other cluster because it had the only strongest peduncle which gave it a 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 apart from having a medium boll size, they had a different leaf colour from other lines which was caused by the presence of the verticillium wilt disease in their system.

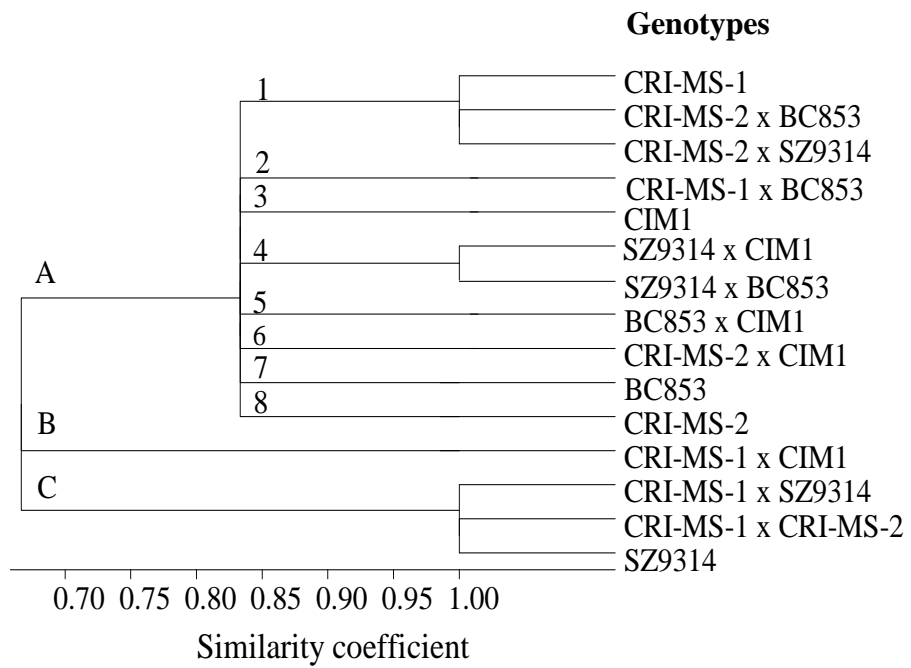**Figure 2: Dendrogram based on Euclidean distance and Hierarchical Clustering method for morphological data**

4.4 Discussion

4.5 Conclusion and Recommendation

Chapter 5 : General Discussion and Conclusion

Chapter 6 : General Recommendations

REFERENCES

Adams S.S., Rouse D.I. and Bowden R.L. 1987. Performance of the alternative versions of POTWIL: A computer model that simulates the seasonal growth of Verticillium infected potato. AmPot.J. 64:429-431.

Agrios G.N. 2005. Plant Pathology. 5th Ed. Department of Plant Pathology, University of Florida. Elsevier Academic Press.

Agrimarket INFO. 1998. Cotton in South Africa. Agricultural News. Newsletter of the National Department of Agriculture (R.S.A).

Baffes J. 2002. Tanzania's cotton sector: Constraints and challenges in a global environment. African Region Working Paper Series No. 42.

Batool S. and Khan N. U. 2002. Diallel studies and heritability estimates using Hayman's approach in upland cotton. Department of Plant Breeding and Genetics, PMAS Arid Agric. Uni., Rawalpindi, Pakistan.

Bell A.A. 1989. Role of nutrition in the diseases of cotton. In Engelhard A.W. (Ed.) Soil borne plant pathogens: Management of diseases with macro and micronutrients. American Phytopathological Society Press, St Paul, M.N. 167-204.

Bell A.A. 1992. Verticillium wilt in R.J Hillocks (Ed), Cotton Diseases. CAB In., London.

Freeman C.B. and Beattie G.A. 2008. An overview of Plant Defenses against Pathogens and Herbivores. The Plant Health Instructor. DOI: 10.1094/PHI-1-2008-0226-01

Bolek Y., El-Zik K.M., Pepper A. E., Bell A.A., Magill C.W., Thaxton P.M and Reddy O.U.K 2005. Mapping of verticillium wilt resistance genes in cotton. Plant Science 168:1581 - 1590. Science Direct (Elsevier).

Chapepa B., Chitamba J., Manjeru P. and Mudada N. 2013. Screening of *Gossypium hirsutum* germplasm against Verticillium dahliae K. by natural infection. IJAAR Vol. 3, No 1:26-31

Chinodya R. 1996. Verticillium wilt screening in the Cotton Research Institute Annual Report, Department of Research and Specialist Services, Ministry of Agriculture, Zimbabwe.

Cotton Research Institute, Annual Report, 1993. Department of Research and Extension, Ministry of Agriculture, Zimbabwe.

Cotton Research Institute, 2011 Pathology Annual Report. Department of Research and Extension, Ministry of Agriculture, Zimbabwe

Dippenaar-Schoeman, A. 1999. Spiders as biochemical agents. ARC-news. In: Agricultural Newsletter pp 12-16. National department of Agriculture Directorate Communications (Pub).

Endrizzi J.E., Turcotte L. and Kohel R.J. 1985. Genetics, cytology and evolution of *Gossypium. Advances in Genetics* 23:271-375.

Ephraem S. 2014. Ratoon threatens cotton industry. Manica Post http://zw.manicapost.com/index.php?option=com_content&view=article&id=29529:ratoon-threatens-cotton-industry&catid=38:local-news&Itemid=131#.U0J_taiSxCY

FAO. 2000. FAO Statistical Database.

Fortucci, P., 2001. The contributions of cotton to economy and food security in developing countries. *http://www.ICAC.org/icac/meetings.*

Fradin E.F., Zhang Z., Ayala J.C.J, Castroverde C.D.M., Nazar N.R., Robb J., Liu C. and Thomma B.P.H.J. 2009. Genetic dissection of *Verticillium* wilt resistance mediated by tomato. Ve1. *Plant Physiol*. 150: 320-332

Gomez-Alpizar L. 2001. *Verticillium dahliae.* NC State University. http://www.cals.ncsu.edu/course/pp728/Verticillium/Vertifin.htm

Gono L.T., 2005. Cotton research institute*: an overview of research highlights, current status and future strategies,* CRI, Bag 765, Kadoma, Zimbabwe.

Griffing B. 1956. Concept of general and specific combining ability in relation to diallel crossing system. *Aust. J. Biol. Sci*. 9:463-493.

Gwimbi P. 2009. Cotton farmers' vulnerability to climate change in Gokwe District, Zimbabwe: Impact and influencing factors. Department of Environmental Health, National University of Lesotho. JAMBA: Journal of Disaster Risk Studies 2(2), November 2009

Hayman B.I 1954. The theory and analysis of diallel crosses. Journal of Genetics 39:789

Hebei Academy of Agricultiral Sciences. 2007. Training course on Cotton Breeding Technology for developing countries. National Cotton Improvement Center, Hbei Branch. Hebei Province, China.

Hillocks R.J. 1992. Cotton Diseases. London, UK: CAB International

http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/Verticillium wilt.as.aspx

Sibanda S., Barton A., Ellis-Jones J., Nyakudya E., Mashingaidze A.B., Chivinge O. and Riches C. 2001. Weed Management in Cotton Production: A Case Study from the Zambezi Valley, Zimbabwe. Paper presented at the World Congress on Conservation Agriculture, Madrid, Spain 1 - 5 October 2001. 467 – 472 pp.

Hussain B., Amin M.A., Khan M.A. 1998. Assessment of genetic mechanism in some important quantitative parameters in G. hirsutum L. J. Agric. Res. 36: 313-319.

International Cotton Advisory Committee (ICAC). 2014. World Prices High Despite Excess Production. 1629 K Street NW, Suite 702, Washington, DC 20006 USA.

International Cotton Advisory Committee (ICAC). 2012. World cotton situation. 1629 K Street NW, Suite 702, Washington, DC 20006 USA

Isik F. 2009. Analysis of Diallel Mating Designs. North Carolina State University, Raleigh, USA

Khan N.U., Hassan G. 2011. Genetic effects on morphological and yield traits in cotton (G. hirsutum L.). Span. J. Agric. Res . 9(2): 460-472.

Khan N.U., Hassan G., Marwat K.B, Farhatullah, Kumbhar M.B, Parveen A., Umm-E-Aiman,

Khan M.Z and Soomro Z.A. 2009. Diallel Analysis of Some Quantitative Traits in *Gossypium hirsutum* L. Pakistan Journal of Botanics 41(6).

Lukonge E. P. 2005. Characterisation and Diallel Analysis of Commercially Planted Cotton (*Gossypium hirsutum* L.) Germplasm in Tanzania. Phd Dissertation, University of the Free State, South Africa.

Lukonge E. P. and Ramadhani O.S. 1999. Review of plant breeding and agro-biodiversity in the Lake Zone. Breeding and Agro-biodiversity workshop proceedings pp 6-11. Agricultural Research and Development Ukiriguru, Mwanza, Tanzania.

Mace M. 2012. Fungal Wilt Disease of Plants. Elsevier Copyright. http://books.google.co.zw/books?id

Machikowa T., Saeteng C. and Funpeng K. 2011. General and Specific Combining Ability for Quantitative Characters in Sunflower. Journal of Agricultural Science. Vol 3, No. 1.

Mapope N. 2001. Verticillium Wilt Survey in Cotton Research Institute Annual Report, Department of Research and Specialist Services, Ministry of Agriculture, Zimbabwe

Mapope N. 2005. Molecular characterisation of *Verticillium dahliae* Kleb isolates and comparison of their virulence on selected cotton varieties in Zimbabwe. MSc dissertation, University of Zimbabwe. Published in 2012.

Mather K., Jinks J.L. 1982. Introduction to Biometrical Genetics. Chapman & Hall Ltd. London. McCarty JC, Jenkins JN

Mathre D.E. 1989. Pathogenicity of an isolate of Verticillium dahliae from Barley. Plant Disease, Vol. 73, No.2:164-167.

Medany M.A., Hegazy A.K., Kabiel H.F. and Maez M.M. 2007. Prediction of seed germination and seedling growth of four crop plants as affected by root zone temperature. World J. Agric. Sci., 3(6): 714-720

Michele G., Ceddia U.K., Manuel Gomez-Barbero and Cerezo E.R 2008. An ex-ante evaluation of the economic impact of Bt cotton adoption by Spanish farmers facing the EU cotton sector reform. European Commission, DG JRC, Institute for Prospective Technological Studies. AgBioForum, 11(2):82-92.

Mubvekeri, W. and Nobanda, S. 2012. Cotton Agronomy. CRI, Bag 765, Kadoma, Zimbabwe.

Mudada N. 2013. Genotype by Environmental Interaction on Yield, Yield Components and Fibre Quality Parameters of *Gossypium hirsutum* varieties. MSc Dissertation, Midlands State University, Zimbabwe. *Unpublished*.

Mudada N. and Muripira V. 2010. Cotton Variety Catalogue. *Unpublished.* Cotton Research Institute, P. Bag 765, Kadoma, Zimbabwe.

Murtaza N., Kitaoka M. and G.M. Ali. 2005. Genetic differentiation of cotton cultivars by polyacrylamide gel electrophoresis. *Journal of Central Europe Agriculture* 6:69-76

Mysore K.S. and Ryu C.M. 2004. Non host resistance: how much do we know ? Trends in Plant Science 9(2):97-104.

Niles, G.A. and C.V. Feaster, 1984. Breeding. In: R.J. Kohel and C.F. Lewis (Eds.), Cotton Agronomy Monograph pp 201-231. ASA, CSSA, SSSA, South Segoe, Madison, WI

Pegg G.F. and Brady B.L. 2002. Morphological traits for *Verticillium* wilt tolerance in cotton. Verticillium Wilts. CABI Publishing, Wallingford, UK. .

Pullman G.S. and DeVay J.E. 1984. Epidemiology of Verticillium Wilt of Cotton: Effects of Disease on Plant Phenology and Lint Yield. Phytopathology 72:554-559.

Saeed I.A.M., MacGuidin A.E. and Rouse D.I. 1997. Synegism of *Pratylenchus crenatus* and *Verticillium dahliae* manifested by reduced gas exchange in potato. Phytopathology 87:435-439a

Sibanda S. 2012. ir.uz.ac.zw/jspui/bitstream/10646/882/5/02Thesis-bodySipho.pdf.tx

Staskawicz B.J. 2001. Genetics of Plant-Pathogen Interactions Specifying Plant Disease Resistance. Plant Physiology Journal, Vol 125 No 1: 73-76

Steel R.G.D., Torrie J.B. 1980. Principles and procedures of statistics, a biological approach, 2nd ed. McGraw Hill, Inc. New York.

Ullah-Joy R. 2013. Cotton Fiber: Cultivation to Production http://textilelearner.blogspot.com/2013/09/cotton-fiber-cultivation-to-production.html

Van Geem M., Gols R., van Dam N. M., van der Putten W.H. Fortuna T. and Harvey J.A. 2013. The importance of aboveground-belowground interactions on the evolution and maintenance of variation in plant defense traits. Front Plant Science 2013; 4: 431

Verhalen L.M., Morrison R.C., Al-Rawi B.A., Fein K.C., Murray J.C. 1971. A diallel analysis of several agronomic traits in upland cotton for various characters of cotton (G hirsutum L.). Crop Sci. 11: 92-96.

Wheeler T.A., Gannaaway J.R. and Kaufman H.W. 2000. The relationship between incidence of wilt and reflectance in a wilt nursery. Texas A&M University Systems Lubbock, TX, USA

Yu J., Kohel R.J. and Smith C.W. 2013. The construction of a tetraploid cotton genome wide comprehensive reference map.Genomics Journals Vol 95, Issue 4:230-240

Zhang T.G.W. 2001. Present status and prospect on cotton genomic studies. Department of Agronomy, Nanjing Agricultural University, Nanjing.

APPENDIX

Appendices 1: Skeleton ANOVA table for general analysis of variance for variety performance

Source of variation	Degrees of freedom
Treatments	t-1 = 14
Error	rt-t = 30
Total	rt-1 = 44

**Appendices 2: Skeleton ANOVA table of diallel analysis method II, model 1**

Source of variation	Degrees of freedom	Mean Square
Genotype	[n(n+1)/2)-1] = 14	M1
GCA	n-1 = 4	M2
SCA	[n(n-1)/2]-1 = 9	M3
Residual	(r-1)[n(n+1)/2] = 30	M4
Total	r[n(n+)1] – 1 = 44	

Where DF =degrees of freedom, n = number of parents, r = replications, GCA = general combining ability, SCA = specific combining ability

Appendices 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

Appendices 4: Vascular Browning Index for Assessing Verticillium Wilt Infection

0	1	2	3	4

0%   5%   20%   40%
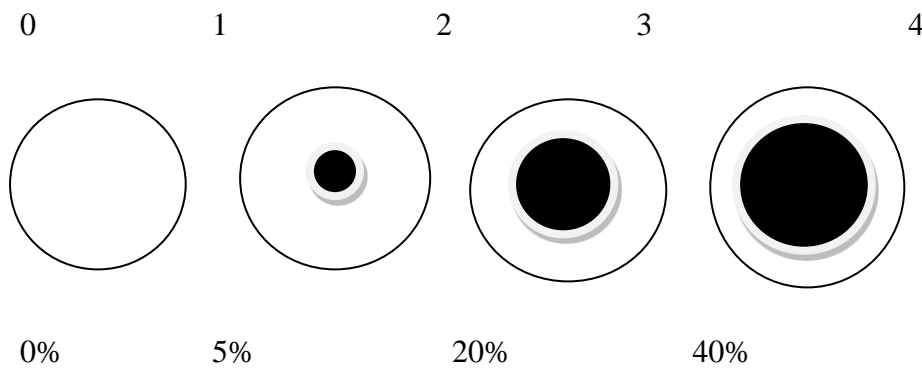
Note: The discoloured areas show the upper limit for each category

This rating system is based on the severity of vascular discolouration (browning) visible in a cross section of the main plant stem cut as practicable to ground level where:

0 = no discolouration

1 = discolouration restricted to small spots or an area less than 5% of the stem cross section

2 = discolouration of between 5% and 20% of the stem cross section

3 = discolouration of between 20% and 40% of the stem cross section

4 = greater than 40% vascular discolouration of the stem cross section

*Source – Fusarium and Verticillium Resistance Ranking Protocol for Cotton Varieties, 2008 Australian Cotton CRC.