GENETIC STUDY OF BACTERIAL BLIGHT RESISTANCE IN COTTON (*Gossypium hirsutam* L.)

BY

SAMUEL OLUSEGUN ALABI

B.Sc, M.Sc. (A.B.U.)

DEA, DAA (Rennes, France)

A thesis submitted to the Postgraduate School, Ahmadu Bello University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Breeding.

DEPARTMENT OF PLANT SCIENCE

FACULTY OF AGRICULTURE

AHMADU BELLO UNIVERSITY

ZARIA, NIGERIA.

NOVEMBER, 1994.

DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any previous application for higher degree.

MR. S.O. ALABI — Date: 25/5/95

The above declaration is confirmed.

PROFESSOR C.C. NWASIKE — Date 7/11/95

CERTIFICATION

This thesis entitled "GENETIC STUDY OF BACTERIAL BLIGHT RESISTANCE IN COTTON (Gossypium hirsutum L.)" by Samuel Olusegun Alabi meets the regulations governing the award of the degree of DOCTOR OF PHILOSOPHY of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Professor C.C. Nwasike
(Chairman, Supervisory Committee)

7/11/95
Date

Professor I.D. Erinle
Member, Supervisory Committee

7/11/95
Date

Dr. C.A. Echekwu
Member, Supervisory Committee

25-05-95
Date

Professor C.C. Nwasike
(Head of Department)

7/11/95
Date

Dean, Postgraduate School

25/7/95
Date

DEDICATION

This thesis is dedicated to my Jewel of inestimable value, 'Funmilola and our lovely children, 'Seyi, Bukky, 'Folake and Fisayo.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to my major supervisor, Professor C.C. Nwasike for his untiring efforts, guidance and motivation during the course of this study and for his helpful criticisms and editorial comments, during the preparation of this manuscript. I am also grateful to Professor I.D. Erinle, for his advice, meaningful suggestion, corrections and criticisms rendered during the course of this study and the preparation of this manuscript. The contributions of Drs. C.A. Echekwu and M.A.T. Poswal are also appreciated.

My sincere thanks go to the technical staff of the fibre breeding unit and the fibre testing laboratory, of the Institute for Agricultural Research, Samaru, especially, Alhaji Y.M. Garkida, Mr. A. Maigizo, Mallam Sule Kwarra and Mr. J. Oloye for their assistance in the field trials. I wish to thank Dr. A.A. Adeoti and the technical staff of the Fibre Pathology Laboratory of the Department of Crop Protection, A.B.U. Zaria, especially Messrs S. Negedu and I. Awori, for their assistance in inoculating the plants in the field and in the screenhouse.

The assistance of Mrs. Uwakwe of the Bacteriology Laboratory of the Department of Crop Protection , who prepared the inocula, is also gratefully appreciated.

My sincere appreciation goes to Dr. S.A. Olorunju of the Data Processing Unit of the National Animal, Production and Research Institute, Shika. and Dr. O.E. Asiribo, Messrs M. Otitoju, J. Obemeata and Gideon, all of the Data Processing Unit of the Institute for Agricultural Research, Samaru for their assistance in the analysis of the data.

I am particularly indebted to the Leader, Fibres Research Programme of the Institute for Agricultural Research, Samaru, Zaria, Professor V.B. Ogunlela, for his valuable advice, encouragement and constant support throghout the period of this study. Sincere thanks are also due to the authorities of Ahmadu Bello University and the Director, Institute for Agricultural Research, Samaru, Zaria for placing at my disposal facilities which have made this research project possible.

I am very grateful to Messrs R. Bello, S. Balogun and T.O. Fadiji for typing the initial draft and final copy of this thesis.

I wish to express my deep gratitute to my parents Mr. E. A. Alabi and Mrs. C.E. Alabi for their moral, and financial support and encouragement throughout the period of my education. Sincere thanks also go to my brothers, uncles, aunties and my friends not mentioned here by name.

To my dear wife, Funmilola Alabi and our lovely children. 'Seyi, Bukky, 'Folake and 'Fisayo, whose faith, confidence, understanding and prayers were constant incentives and encouragement, I will forever remain thankful.

Finally, to God be the glory for His grace that has been more than sufficient. Amen.

ABSTRACT

The study was carried out to investigate the nature and mode of inheritance of bacterial blight resistance in cotton; to examine environmental variation, if any, for resistance to bacterial blight and the relationship between resistance at the cotyledon stage with that at the true-leaf stage. The variety S295 was crossed with seven other varieties: TX-CDP37HH-1-83, TAMCOT SP21S, TAMCOT-CAMD-E; SAMCOT-9, SAMCOT-10, SAMCOT-6 and SAMCOT-8. The parents and their crosses were wound-inoculated in the field at three locations and in the screenhouse with separate bacterial suspensions of isolates 1, 2 and a 1:1 mixture of isolates 1 and 2. Disease reaction was evaluated on a scale of 1-10. Disease grades frequency , distributions indicated a single gene with complete dominance for resistance to the two isolates. The analysis of variance indicated highly significant differences among populations and locations. The interaction between populations x locations was also significant. The significance of the interactions between isolates x locations and populations x isolates x locations, indicated that for future screening and testing, materials should be evaluated across several environments and with different isolates. The-' correlation between cotyledon and true-leaf disease grades and phenotypic ratios indicated that resistance

was controlled by the same genetic mechanism at both plant stages.

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CHAPTER 1

INTRODUCTION

Cotton belongs to the family Malvaceae, and the genus Gossypium with about 33 species of annual subshrubs, perennial shrubs or small trees distributed in the tropical and sub-tropical regions of Africa, Asia, Australia and America (Fryxell, 1969). It is the most important vegetative fibre in the world (Purseglove, 1968), and of all the world's economic plants, cotton is perphaps the most widely and completely used (William, 1954).

Until recently, cotton was a major cash crop of the northern states of Nigeria as well as being the largest single employer of labour second to the public sector (Yayock and Kumar, 1988).

Commercially cultivated cottons are restricted to three major types: (i) Gossypium hirsutum L., which includes the group of cotton known as American upland; (ii) Gossypium barbadense L., from which are produced, the Egyptian types and the super quality Sea Island Varieties; (iii) the Asiatic cottons (G. arboreum L. and G. herbaceum L.), which are grown in India, Pakistan, China, Burma etc. (Commonwealth report, 1970). In Nigeria, commercial production utilizes derivations of the upland cotton types.

Although the precise national statistics for cotton hectarage and yields are lacking, it has been estimated that the total area put to cotton is about 0.6 million hectares per annum with an average yield of about 200 kg seed cotton per ha (Kumar and Ogunlela, 1982). This production is largely confined to northern Nigeria, occupying an area of approximately 700,000 square kilometers, that extends from Latitude 7^0 30N to 13^0N and Longitude 4^0E to 13^0E. The production in southern Nigeria is confined to Oyo and Edo States.

The cotton crop has insect and disease problems in every producing region of the world and this can be a constraint to profitable cotton production (Raheja et al., 1982.)

Bacterial blight, caused by Xanthomonas campestris pv malvacearum (Smith) Dye is the most economically important disease of cotton in Nigeria, and other cotton growing regions of the world (Innes, 1983). Different manifestations of the disease have been observed on plants in the three (Northern, Eastern and Southern) cotton growing zones of Nigeria. However, the "angular leaf spot" and "vein-blight" phases are the most predominant (Dransfield, 1965).

Losses to bacterial blight on an annual basis, depending on the disease manifestation and cotton species, have been shown to vary from 5-80% in some parts

of Africa (Innes, 1983). In Nigeria, bacterial blight is estimated to cause an annual yield loss of about 10-20% (Dransfield, 1965).

Management involving exclusion, eradication and sanitation control the disease in areas where it is practical to enforce regulations governing these actions such as California in the United States of America (Presley and Bird, 1969). In most areas of the world, it is more difficult to use management alone for control (Bird et al., 1984). Chemical seed treatment in Nigeria and other African countries is a popular and effective method of minimizing disease loss (Logan, 1960 and Dransfield, 1971).

The development and use of resistant varieties, is considered the ultimate, most economic and most effective approach of controlling the disease irrespective of carefully executed measures of crop sanitation and seed treatment (Verma et al., 1980; Innes, 1983; Bird, 1986). Varieties having some degree of resistance to this disease have been known for many years and it has been shown that a number of varieties will produce individual plants with varying degrees of resistance (Owen, 1965).

Breeding cotton varieties resistant to bacterial blight has been an integral part of the cotton improvement programme in Nigeria. Over the last 60 years, more than a dozen commercial cultivars varying in

levels of resistance to bacterial blight were released. Advanced breeding lines were derived from crosses between Samaru Allen (SA) and different exotic cultivars such as Albar, Reba W296, Reba B50, BJA 592, Acala and Ukiriguru (Mustapha, 1981). The germplasm collection includes lines known to possess the B_2, B_3, B_9l and B_{10}L genes for resistance (Innes, 1983). These genotypes are, however vulnerable to the new and virulent isolates of the pathogen, first reported in Burkina Faso by Follin (1983). The commercial varieties, presently grown in Nigeria have lost resistance to this major disease (Poswal, 1985). The recent introductions of the short-seasoned, multi-adversity resistant (MAR) cotton varieties from Texas, U.S.A., and the cultivar S295 from Chad will be valuable in reinforcing and enhancing the progress in developing cotton cultivars resistant to bacterial blight, other diseases, insect pests and environmental stresses in Nigeria.

Environmental conditions contribute a great deal to the differences in experimental results. Hutchinson (1959), in discussing the differences in results obtained in the Sudan and East Africa stated that the difference between major and minor genes controlling resistance depended more on the uniformity of the environment than on the magnitude of the gene effects, implying that in some environments, a given gene may confer a high degree

of resistance, whereas under a different environment it may have an entirely different reaction. Hutchinson (1959) reported that at locations in Africa other than the Sudan, a failure of uniform incidence of the disease prohibited workers from following the segregation of the major gene. Marked differences in the expression of genetic variation from one location to another have affected both the approach to breeding for resistance and genetical interpretation of results obtained (Arnold and Brown, 1968).

Progressively cotton varieties in recent years have shown a high level of bacterial blight and no genetic studies have been conducted to determine the inheritance of resistance to this disease in Nigeria.

The objectives of the present study are:

1. To determine the nature and mode of inheritance of bacterial blight resistance in cotton varieties developed at the Institute for Agricultural Research, Samaru and elsewhere.
2. To examine environmental effect on resistance to bacterial blight.
3. To examine the relationship between resistance at the cotyledon stage with that at the true-leaf stage.

The information likely to be derived from this study will be useful in planning a more effective cotton breeding programme in order to incorporate durable resistance against bacterial blight.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Resistance Genes and Heritable Resistance.

In cotton two different types of resistance genes have been recognized: .(i) those with no other demonstrable effect on the plant beside conferring resistance (ii) those that affect the plants generally but only serving as incidentals in enhancing resistance. These genes were designated as major and minor genes (Knight, 1948; Knight and Hutchinson, 1950) and were defined as oligogenes and polygenes, respectively (Van der Plank, 1968).

Knight and Clouston (1939) were the first to demonstrate heritable resistance in the Sudan. Knight(1945) screened more than 1000 different wild and cultivated accessions of diploid and tetraploid species of Gossypium. He identified ten major genes, to which he ascribed the symbols B_1 - B_{10} (Knight, 1957, 1963). B_1 and B_2, were found in Gossypium hirsutum L. variety, Uganda B31 (Knight and Clouston, 1939). Knight (1948) suggested that B_2 was the standard gene controlling resistance in American upland cottons. Certain varieties of G. hirsutum race punctatum carry the partially dominant gene, B_3 (Knight, 1944; 1946; 1947). The gene B_4, a strong partially dominant gene was isolated from G. arboreum race bangalense (Knight, 1948) while B_5, a

strong resistance gene fortified by minor genes and possessing an additive effect with, B_1, B_2, B_3 and B_4 gene was isolated from a perennial, G barbadense (Knight, 1950).The B_6m gene from G. arboreum, was originally described by Knight (1953a) as an important modifying gene which conferred no resistance, but greatly intensified resistance to near immunity in combination with B_2 or B_2B_3 in a homozygous condition. Saunders and Innes (1963) found that B_6m was a recessive resistance gene which conferred intermediate resistance in a homozygous state and it was designated B_6. Its value in enhancing resistance to bacterial blight when in combination with other genes in particular B_2 was emphasized. Knight (1953b) identified the major dominant gene B_7 in the upland variety, Stoneville 20. A recessive gene designated B_8 was identified by Knight (1957) in the wild diploid, G. anomalum. Further studies by Khalifa and Saunders (1969) suggested that B_8 has an additive effect with B_2B_3 and anologous to B_6. With the exception of B_8, all the B genes were transferred to G. barbadense stocks (Innes, 1983).In addition, in the Sudan, most of the B-genes described by Knight (1945-1947) were transferred to upland cotton G. hirsutum.It is obvious from Knight's investigations that no B-gene by itself conferred immunity although several gave strong resistance, and digenic and trigenic combinations were

synthesized to produce near immunity and a widely based gene pool (Innes, 1974). Other genes for resistance, given the symbols B_9 and B_{10}, were found in the upland variety Allen (Lagière, 1960). These genes are not homologous with Knight's B_9 and B_{10}, identified in Wagad 8 (G.herbaceum) and Kufra Oasis (G. hirsutum race punctatum), respectively (Knight, 1957). To avoid confusion all four genes were redesignated B_9L, $B_{10}L$ and B_9K, $B_{10}K$ in that order (Innes, 1965a). A gene of low potency transferred by Knight (1945) from G. herbaceum to G. barbadense was identified and described by Innes (1966c) with the symbol B_{11}. In the breeding work with the Albar stocks in the Sudan, it was shown that the key to resistance was the major gene B_2, fortified by genes of small effects (Innes 1963). In the USA, Green and Brinkerhoff (1956) identified and described three major dominant genes which control resistance to bacterial blight in upland cotton strains: these were given the symbols B_In, B_N and B_S (Brinkerhoff, 1963). Polygenic complexes for resistance in the upland varieties Stoneville 2B and Deltapine were found by Bird and Hadley (1958) and were given the symbols B_Sm and B_{DM}, respectively. A dominant gene for resistance was found in a mutant plant from the irradiated susceptible upland variety Westburn 70 (Brinkerhoff et al.,1978)

The interaction of B-genes with each other, the influence of minor and modifier genes and genetic background on the expression of the major genes have been established by a number of workers with evidences indicating that the expression of resistance varied with genetic background and that high levels of resistance could be achieved by the accumulation of polygenes (Arnold, 1963; Innes, 1969 and Innes et al., 1974).

Simpson and Weindling (1946) in a screening experiment involving American upland strains found a selection from Stoneville 2A which was resistant to bacterial blight. The selection was designated Stoneville 20. This strain has been the principal source of resistance used in a number of breeding programmes in the United States (Bird and Hadley, 1958). Blank (1949) reported studies which supported the hypothesis that Stoneville 20 resistance was inherited as a simple recessive character with susceptibility being dominant and that a favorable alignment of modifying or minor gene was necessary for the full expression of resistance. Bird (1950) conducted experiments involving Stoneville 20 resistance in a segregating population following the second backcross (BC_2)to Stoneville 2B. It was shown that heterozygous plants could be distinguished from homozygous ones and that if dominance was present it was in the direction of resistance. Bird and Blank (1951),

after studying segregating populations from third backcross generation (BC_3) in Deltapine and Acala varieties, indicated that resistance was recessive but suggested that minor genes greatly influenced the degree of resistance produced by the major gene. Knight (1953), after studying Stoneville 20 resistance in a Sakel background, concluded that it was inherited as a simple, dominant character and emphasized the importance of minor gene in its expression. Green and Brinkerhoff (1956) reported that stoneville 20 resistance in a cross with Acala 892 was controlled by a major recessive gene but that segregation was obscurred by other genes with less effect. They observed that in the Stoneville 20 x Deltapine cross, resistance appeared to be dominant; whereas in the Stoneville 20 x Acala cross, resistance seemed to be recessive. In the Stoneville 20 x Stoneville 2B cross, no dominance was indicated and when the data from all crosses were analysed statistically there was no evidence of dominance. Bird and Hardley (1958) pointed out that the use of a single genetic background in studying the inheritance of Stoneville 20 resistance could be misleading as to whether resistance was dominant or recessive. They observed in their study that the only component of variance which was highly significant was the one due to the fixable or additive genetic variation. The major and minor genes were

additive with no dominance. Furthermore the results indicated that progress would be better and higher resistance could be obtained with material with two or more components of minor genes.

El-Zik and Bird (1968), in a quantitative genetic study of the inheritance of resistance to bacterial blight, using upland cotton lines known to carry some of Knight's B-genes, revealed that most of the genetic variability for resistance in the lines studied could be attributed to additive and dominance effects of genes. In addtion the Reba gene, B_9L, segregated as a gene of large effect but Mendelian segregation was not discernible in hybrid populations not involving Reba. Studying the effectiveness of specific genes and gene combinations in conferring resistance to races of Xanthomonas malvacearum in upland cotton, El-Zik and Bird (1970) reported that the major genes showed isodirectional dominance towards resistance. Transgressive segregation for the minor genes was indicated. They further emphasized that the genetic background was important in the expression of the major genes. Innes et al., (1974) in another study involving six inbreds with their B-gene content reported that dominance accounted for non-additivity.

Girardot and Follin (1987) and Follin et al., (1988), in their report on the genetic determination of resistance to races 18 and 20 of Xanthomonas malvacearum indicated a probable homology of B_2B_3 and B_9L $B_{10}L$, and concluded that gene combinations tended to be inherited as single factors in the manifestation of total resistance. Wallace and El-Zik (1989) studying the inheritance of resistance in three cotton cultivars to the HVI isolate of bacterial blight reported that resistance in S295 to HVI and the USA race mixture appeared to be controlled by the same gene or two closely linked genes. Resistance to HVI in S295 was inherited as a single gene, designated B_{12}, with complete dominance for resistance. In a quantitative analysis of resistance in cotton to three new isolates of the bacterial blight pathogen, Wallace and El-Zik (1990) reported that dominance when significant was in the direction of resistance. While a diallel cross analysis for resistance under natural infection conditions, showed the existence of significant general and specific combining ability effects, but the role of dominance effects was preponderant in determining resistance (Bachelier et al., 1992).

2.2 Nature of Plant Resistance

Investigations into the biochemistry and physiology of resistant and susceptible plant inoculated with the bacterial blight pathogen have not revealed clear-cut explanations as to the biochemical mechanisms of plant resistance (Innes, 1983). Perry (1966) studying the rate of multiplication of the bacteria in the leaf, found that X. malvacearum multiplied at a similar rate in the leaves of resistant and susceptible varieties for 48h after inoculation, thereafter the rate in the resistant variety was less than in the susceptible. Cason et al., (1977) showed that with large doses of inoculum, damage to immune host tissue occurred 4-6h after inoculation. Essenberg et al., (1979a; 1979b) concluded from a series of studies that in leaves of both susceptible and resistant varieties, infection could be initiated by a single bacterial cell, which then multiplies logarithmically.

Hughes and Fowler (1953) showed that synthesized varieties with B_2 -and B_2 + B_3 -genes had a stronger action of Fehling's solution. They noted that resistance was correlated with a high glucose level in the leaves but did not suggest how that affected the reaction of the plant to the pathogen.

Blankman et al., (1964) and McBee (1965) indicated that certain amino acids might be associated with

resistance by affecting specific membrane development. Lipke (1968) related disease severity to some free ninhydrin positive compounds (NHPC) of plants with B_4 *gene and the* B_5*m minor genes.*

In considering the role of phenolics in bacterial blight resistance, Jalali et al., (1976) have suggested that leucoanthocyanins may be involved while Verma and Singh (1974a) reported that resistance in cotton appeared to be associated with a low content of citric acid, high carbohydrate and amino acid content such as serine, which inhibits the growth of the pathogen and interferes with its utilization of glutamic acid and alanine. In a study with amino acid extracts from cotyledons of the susceptible variety Acala 44 and the resistant immune 216, Ziegler and Essenberg (1978) found that extracts from the resistant variety were more inhibitory to X. malvacearum than those from Acala 44. In a similar study with these two varieties, Venere (1980) as cited by Innes (1983) postulated that the oxidation of catechin, catalysed by peroxidase, was the mechanism of resistance.

2.3. **Host Tissue and Level of Resistance**

Disease reactions vary with the age and kind of host tissue (Wilkens, 1953; Innes, 1963; Keay et al., 1968). Young succulent tissues tend to be more susceptible

than older tissues (Logan, 1958 and Innes, 1961b).

Knight and Clouston (1939) showed leaf and stem resistance to be correlated when leaf lesion development was used as a basis for assessing resistance. According to Innes and Last (1961), disease reactions of the leaf, stem and boll infection phases usually bear no correlation, especially for different host resistance genes and for different races of the pathogen. Gausi (1980) in assessing the level of resistance of four cotton cultivars, showed some correlation between stem, boll and leaf reactions.

Innes and Brown (1969) studying a five - parent diallel cross of upland cotton varieties in Uganda and the Sudan, found that both leaf and boll resistance were controlled by additive genes and that the proportion of dominant and recessive alleles was the same for both leaf and boll resistance.

Last (1958) investigating disease symptom development in Egyptian and American varieties of cotton showed that the probabilities of leaf symptom development were similar in the two types of cotton; however, when young tissues in young and old plants were inoculated, American cotton was observed to be less affected by stem inoculation than the Egyptian one. In an overview of breeding projects carried out in the Lake province of Tanganyika on the control of bacterial blight in rain-

grown cotton, Arnold (1963) inferred that there is a basic resistance mechanism in all tissues which is under the same genetic control but that results may be biased by genotype - environment interactions and by other factors operating in particular tissues.

2.4 **Variation in the Pathogen.**

In any host - parasite relationship, the development of resistant types almost always results in the evolution of new virulent races of the pathogen especially under favourable disease conditions (Hunter and Blank, 1954; Brinkerhoff, 1963, 1970). For the purposes of identification and designation of new races, two main methods are employed: (i) method based on pathogenicity to differential host lines (ii) method based on the biochemical variation of isolates. Brinkerhoff (1963) identified twelve races of X. malvacearum on the basis of different disease reactions on a set of differential lines of cotton. Subsequently, using an extended set of differentials, Hunter et al., (1968) identified additional three races. Verma and Singh (1970) described two more new races.

Srinivasan and Taneja (1974) found an isolate from cultivar PRS 72 to be pathogenic to Acala 44, stoneville 2B-59, 1-10B and 20-3 corresponding to race 5 in India.

Verma and Singh (1976) identified race 10 in different locations in India. It was found to be virulent for genes B_2, B_1n, B_N and B_7 and different polygenes.

Poswal, (1981) investigating the presence of races of X. malvacearum in Nigeria among twenty isolates, categorized them into twelve provisional race groups. Poswal (1988), reported for the first time the presence of races 6,7 and 10 among the isolates obtained from the cotton zones of Nigeria. In another study, Poswal (1989) investigating the presence of new races of the pathogen among 68 isolates in Nigeria, further confirmed the presence of races 8, 11, 16 and 17 which were pathogenic on both differential line Gregg and DP X P4. None of the races were pathogenic to the B_2 B_3 -gene combination, B_4 - and the B_{12} -genes for resistance.

Hussain and Brinkerhoff (1978) found race 18 at Multan in the Punjab region of Pakistan while Granada and Victoria (1978) reported races 6, 8 and 10 to be widely distributed in Colombia.

Singh et al., (1977) identified 14 races with 48 isolates of X. malvacearum namely race 1, 2, 7, 8, 9,10, 20, 21, 22, 27, 28, 29, 30 and 32.

Verma and Singh (1975b) reported that 59.25% of the isolates tested belonged to race 32, appearing widely in its virulent biotype A (57%). This race is most virulent

attacking B_7, B_2 B_In and B_N major genes. Other frequent races were; race 27 attacking genes B_7, B_2 and B_In and race 28, attacking genes B_7, B_2 and B_N. The less virulent races namely race 2 (attacking only polygenes), 9-attacking B_In and polygenes, race 10 attacking BN and polygenes and race 1-virulent to only Acala 44 with no resistance genes. Nayudu (1964), designated four races A,B,C and D on the basis of their virulence to all four cultivated species of cotton in India.

Crosse (1964) showed increased virulence of an isolate from a resistant breeding line in Tanzania. Arnold and Brown (1968) in Uganda, indicated continuous variability in virulence. Gausi (1980) using five isolates on some cotton cultivars also established virulence variability among them in Malawi.

The continuous variation in virulence in East African isolates of X. malvacearum is possibly a reflection of similar variation in the resistance of the hosts upon which these isolates evolved (Brinkerhoff, 1970).

Bacterial blight resistant varieties such as Acala 1517 BR/Acala 1517 BR-1 and Acala 1517 BR-2 were extensively grown in the United States of America and New Mexico. The resistance of Acala 1517BR and Acala 1517BR-1, depended largely on the B_7 -gene from Stoneville 20. In an extensive survey, Chew et al. (1969) showed that 40

- 60% of all isolates were race 2 at the time the two Acala varieties with B_7-gene were grown. This percentage dropped when race 2 resistant Acala 1517BR-2 with B_2-gene, fortified by a complex of polygenes replaced the other cultivars (Brinkerhoff, 1970).

Hayward (1964) classified 18 isolates of X. malvacearum from Africa, USA and Australia into two biochemical groups based on their capacity to oxidize lactose, colony type on first isolation, proteolytic activity and bacteriophage typing. Verma and Singh (1975a) categorized 133 isolates of the pathogen in India into two biochemical groups - Lactose utilizing (Lac^+) and Lactose non - utilizing (Lac^-). Verma and Singh (1970) indicated that the different procedures for the identification of races bear no relation to each other. They considered that pathogenic reaction of isolates on a selected set of differentials under a set of climatic conditions appears to be quite suitable for race identification.

Recently, highly virulent strains (HVS) of the pathogen (HV-1, HV-3, HV-5, HV-6, HV-7, SU-1 and CH-1) with associated virulence to all the known major and polygenic genes and their combinations for resistance evolved in Chad, Burkina Faso and Sudan (Follin, 1983). All the known international host differential lines for race studies are suceptible to these virulent isolates

Mooseberg (1953) reported that better results were obtained with pure culture inocula. He suggested that the low temperature of the tap water used in preparing his inoculum from infected trash might have been detrimental to the typical development of the disease, but a more probable explanation is that trash inocula contained fewer viable bacteria per unit volume than that prepared from pure culture (Innes and Last, 1961; Gunn and Innes, 1961)

Investigating with two races of the pathogen, Verma et al; (1980) showed that in a mixed inoculation the reaction on a host may be synergistic when the variety inoculated is susceptible to both races or antagonistic (with a hypersensitive reaction) when the variety is resistant to one race and susceptible to the other. Breeders with large field populations to screen have continued to rely on inocula prepared from infected leaves (Brinkerhoff and Hussain, 1978).

In addition to the leaf spraying techniques, a needle inoculation technique for the main veins of cotton leaves was used to advantage by Innes (1961a) and Poswal and Erinle (1984) to assess the level of resistance in some genotypes of cotton.

Under screenhouse conditions various other leaf inoculation techniques, including the use of atomizers, hypodermic syringe and leaf abrasion have been employed

on a limited scale (Weindling, 1948). Last and Dransfield (1959) and Innes (1961a) found needle inoculation to be more reliable with plants in the screenshouse than in the field.

Wickens (1953) reported that seed inoculation to induce primary infection is of limited value, particularly in selecting single plants. Though it may be useful in eliminating susceptible plants from large populations. Various techniques used for seed inoculation have been described by Innes (1961b).

Inducing stem infection by spraying methods have not been too successful however, Weindling (1948) succeeded in infecting stems by using a coarse spray. Typical stem symptoms with little mechanical damage to the plant could be induced with a fine sewing machine needle charged with inoculum (Wickens, 1953, Brown, 1980) or injection of inoculum from a hypodermic syringe (Lagière, 1960).

A number of boll inoculation techniques has been devised, which include the atomizing and brush methods of Wickens (1953) and Logan (1958), needle inoculation (Logan, 1958) or abraison (Weindling, 1948; and Lagière, 1960).

In terms of practical utilization, the use of any particular inoculation technique by a breeder in different countries depend on which technique is most likely to give the most reliable results for the minimum

effort. In the Sudan, (Innes, 1974) reported that stem and boll inoculations do not always produce measurable symptoms while in East Africa the leaf spraying technique as used in the Sudan does not always give reliable results (Arnold et al., 1976). In Tanzania, established field screening techniques have failed to detect small differences between materials with high resistance (Ebbels, 1976). It was suggested that controlled environment tests might be needed for such materials.

In the United States of America and other parts of the world the tooth-pick scratch method of inoculation by Bird (1982), has been popular for green house experiment and small population in the field. Essentially round wooden tooth-picks are dipped into the inoculum and used to scratch the lower epidermis of the cotyledon and true-leaf. One of the main advantages of this technique is that it induces symptoms that can be easily classified on a scale that reflects the degree of resistance/ susceptibility in a plant..

2.6 **Occurrence of Resistance and Immunity in Genetic Stocks.**

Extensive survey for blight resistance of both cultivated and wild species of cotton have been carried out by various workers (Knight and Clouston, 1939, 1941; Knight, 1944, 1947, and 1948; Brinkerhoff, 1970). Their

surveys revealed that immunity and levels of resistance predominated in the races of Gossypium herbaceaum L. and G. arboreaum L. of the commercial diploid cotton of India (Knight and Hutchnison, 1950).

Variability in resistance, has been reported in G. hirsutum at the centre of origin (Central America); and in G. hirsutum var punctatum from the Bahamas. Resistance is rare in G. barbadense; minor genes conditioning resistance have been found in the Sea Island cotton varieties, but the majority are due to introgressive hybridization from the G. hirsutum backgrounds (Knight 1948; Knight and Hutchison, 1950).

Brinkerhoff et al., (1952) found 13 cultivars out of 20 from U.S. having resistance frequencies of 1/414 to 1/24597 plants Bacterial blight immune plants were reported in Russia in three upland cotton strains having frequencies of 212/42,000 (Verderevsky and Viotovich, 1957). High levels of resistance were found in uplands and punctatums from India and Africa as compared to those of the New World (Brinkerhoff, 1970).

CHAPTER 3

MATERIALS AND METHODS

3.1 Genetic Material - Origin and Description

Eight varieties of cotton namely: S295, TX-CDP37HH-1-83, TAMCOT SP21S, TAMCOT-CAMD-E, SAMCOT-9, SAMCOT-10, SAMCOT-6 and SAMCOT-8 were selected as parents for this genetic study based on their differential response to infection by Xanthomonas campestris pv malvacearum. The origin and the description of the varieties are given below.

1. **S295:** A variety bred in Tchad from the cross PAN 575 x $SR1F_4$ x J193. Its pedigree has been reported as a three-way cross of a line derived through panmixia of 34 parents, a line derived from a recurrent selection procedure, and another line related to the recurrent selected line. This variety is highly resistant to all known races and isolates of bacterial blight pathogen (Girardot et al., 1986).

2. **TX-CDP37HH-1-83:** A high yielding multi-adversity resistant cotton variety developed by the Texas Agricultural Experiment station, USA. The variety was bred from the parent strains of TAMCOT SP21S and cotton SP37 Families. The cross (66N, B.V. 65, 418Bgh 66-67)x(52,B.V.65, 437Bgh 66-67) was made and individual

plant selection began in the F_1 of the single cross. Individual selection was based on seed coat resistance to mold and to a reduced rate of germination when held for eight days on 1.5 per cent water agar at 56°F; followed by an evaluation and selection for high seedling cotyledon resistance to a mixed inoculum of races 1, 2, 7 and 14 of bacterial blight pathogen. Selecting to the F_7 gave strains H^2-45-74, H^2-46-74 and H^2-47-74. TX-CDP37HH-1-83 is a composite of the above mentioned strains and it is known to have a high resistance to all known races of bacterial blight pathogen.

3. **TAMCOT SP21S**: Another multi-adversity resistant variety developed from the parent strains as (2) above. The cross (SP21F x SP33F)F_1 x F_1 (SP21v x SP37v) was made and individual plant selection began in the F_1 generation of the double cross. Individual plant selection was based on seed coat resistance
to mold and to a reduced rate of germination when held for eight days on 1.5 per cent water agar at 56°F. This was followed by an evaluation and selection for high seedling cotyledon resistance to a mixed inoculum of races 1, 2, 7 and 14 of the bacterial blight pathogen. Selecting to the F_4 gave strain H^4-14-71 and to F_7, strain H^4-18-72. TAMCOT SP21S is a composite of strains H^4-14-17 and H^4-18-72.

4. **TAMCOT-CAMD-E:** A multi-adversity resistant variety also developed by the Texas Agricultural Experiment Station, USA., from the crosses of parent strains of TAMCOT SP21 and TAMCOT
SP37 families. The cross (MAR SP7-67 x $17M^2$) F_1 x F_1 (SP46-67x$17M^2$) was made and individual plant selection in the F_1 generation followed the same procedure as in (2) an (3) above. TAMCOT CAMD-E is known to be resistant to all US races of bacterial blight pathogen.

5. **SAMCOT-9:** A variety bred by the Institute for Agricultural Research, Samaru, from the cross BJA 592 x (ASA(68)63 x ASA(68)69. BJA 592 is a bacterial blight - resistant variety bred in Tchad. ASA (68)63 is a derivative of SA59 /11 x (Samaru 26J x Albar 59/18). ASA (68)9 originated from the cross, Albar 51/474 x SA55/44. Albar 58/18 and Albar 51/474 are bacterial blight-resistant from Albar 51 in Uganda. Both SA59/11 and SA55/44 are materials derived by selection within the Nigerian Allen at Samaru. The gene pool from which the variety was developed possess the B_2, B_6, B_9L and $B_{10}L$ genes for resistance to bacterial blight disease.

6. **SAMCOT-10:** Another variety developed from the same cross as (5) above and possessing similar morphological characteristics. It was formerly designated as RASA(74)165.

7. **SAMCOT-6:** A variety developed from a cross between an Albar introduction from Uganda and Samaru Allen. Families of crosses between these two varieties were designated ASA (Albar x Samaru Allen). Selection within the ASA family produced a line, ASA (66) 44, which was consequently released as a variety in 1972/73 season. This variety is now being replaced by SAMCOT-9 in the northern cotton growing zone of Nigeria.

8. **SAMCOT-8:** A commercial variety in the eastern cotton growing zone of Nigeria. It is a product of hybridization between (Albar x S.Allen) x (Albar x S. Allen). Formerly designated as AASA (71) 114. It has a higher resistance to bacterial blight disease than SAMCOT-6.

3.2 Collection of Isolates and Preparation of Inoculum.

Bacterial blight infected leaf samples were collected from farmers plots at two different localities namely, Kumo in the eastern cotton growing zone and Faskari, in the northern cotton growing zone, and the pathogen isolated from them on potato carrot dextrose agar (PCDA) media held at 25°C according to standard procedures for the isolation of plant pathogenic bacteria. Pure cultures of the pathogen were obtained by sub-culturing from the original isolates. Inoculum was

prepared from 3 to 4 day-old cultures by suspending a small portion of the bacterial colony in sterile water contained in a small glass vial. The bacterial suspension was adjusted to produce an inoculum density of approximately 1.0 x 10^6 bacteria/ml. Each isolate was handled separately and equal portions of the inoculum of the isolates were combined for the 1:1 mixture. These isolates from Kumo and Faskari have been refered to as isolate 1 and isolate 2, respectively in this study.

3.3 **Field Experiment and Technique.**

Initially, the eight parent materials were planted during the dry season of 1991/92 under irrigation and the following crosses were generated: S295 x TX-CDP37HH-1-83, S295 x TAMCOT SP21S; S295 x TAMCOT-CAMD-E, S295 x SAMCOT-9; S295 x SAMCOT-10; S295 x SAMCOT-6 AND S295 x SAMCOT-8. During the rainy season of 1992/93, the resulting seven F_1s from these series of crosses and their parents were grown in the field. Each F_1 was backcrossed to each of its parents to generate the backcrosses. At the same time fresh F_1s were made and later advanced to the F_2 generation through controlled self-pollination. In the 1992/93 off season, the F_2 plants of each cross were self-pollinated to produce the F_3 generations. During the 1993/94 wet season, the resulting populations inculding eight (8) parents, seven

(7) F_1s, seven (7) F_2s, seven (7) F_3s and fourteen (14) backcrosses ($7BC_1$s and $7BC_2$s) were evaluated for bacterial blight resistance in the field at three locations, Samaru, Daudawa, Maigana and also in the screenhouse at Samaru.

For the field experiment at each location, entries were arranged in a randomized complete block design with four replications. Plots were 10m in length and 0.91m apart with 45cm plant spacing. Parental, F_1 and backcross generations were grown in one - row plots while F_2 and F_3 progenies were in two-row plots. Four to six acid-delinted seeds were sown per hill. The seedlings were thinned to two plants per hill at 4 weeks after sowing. Boronated single superphosphate (with 5% borax as source of Boron) was applied at the rate of 125kg ha^{-1} before ridging, followed by 125kg ha^{-1} of Calcium ammonium nitrate (nitrochalk). Insect pests were controlled with three fortnightly sprays of Karate insecticide (ULV), at the rate of 2.5 litres ha^{-1} starting from nine weeks after sowing. Three hoe weedings were done, followed by earthening up (ridge moulding) using a tractor - mounted ridger.

True leaves were inoculated in the field. For parental and F_1 entries, 10 plants per plot were inoculated; 48 plants per plot for both F_2s and F_3s and 20 plants per plot for the backcross entries.

The top main stem leaves at nodes 7-9 were inoculated using the toothpick scratch method (Bird, 1982). Plants were evaluated for disease reaction 15-21 days after inoculation depending upon disease development. Disease expression was graded using a modified scale of Bird and Hardley (1958) described below.

1. - no infection of angular leaf spot
2. - small pin-point reddish-brown lesions, less than 0.5mm in size, never angular, wet or coalesced.
3. - lesions about 0.5-1mm in size and raised, brown never angular, wet or coalesced.
4. - lesions about 0.5-1.5mm in size, wet slightly angular, with few coalescing.
5. - lesions about 1-1.5mm in size, mostly round to slightly angular and sunken, minimal coalescing.
6. - lesions 1.5-3mm in size, slightly angular, sunken, some coalescing.
7. - lesions 2-3mm in size, angular, sunken, some coalescing
8. - lesions 2-5mm in size, angular with coalescing
9. - lesions 3-7mm in size, angular, frequent coalescing and yellowing of leaves.
10 - lesions 3-10mm in size, extensive coalescing, leaves often yellowish-brown in most cases with some dropping.

3.4 **Screenhouse experiment.**

The Screenhouse trial consisted of the following: Five parents, S295, SAMCOT-9, SAMCOT-10, SAMCOT-6 and SAMCOT-8; their F_1s, S295 x SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8; F_2S and backcross progenies. The twenty-one populations were planted in soil in 150-mm pots. Six seeds were planted in each pot and entries arranged in a randomized complete block design. Parental and F_1 entries consisted of two pots per replication. Four pots represented a single plot for BC_1 and BC_2 entries and eight pots represented a single plot for F_2 entries. After emergence, seedlings were thinned only as required to facilitate a uniform seedling establishment.

Plants were inoculated and evaluated at the cotyledon and true-leaf growth stage. Ten days after planting, fully expanded cotyledons were inoculated with a bacterial suspension of isolates 1 and 2, using the toothpick scratch technique (Bird, 1982). After inoculation and evaluation at the cotyledon growth stage, seedlings in each pot were randomly thinned to four plants. Two main-stem leaves at nodes 7 to 9 were inoculated and evaluated for the true-leaf growth stage.

Plants were evaluated for disease reaction from 15 to 21-days after inoculation depending upon disease development; Disease expression was graded using the

scale of 1(immunity) to 10 (fully susceptible) described by Bird and Hadley (1958).

Disease reactions of the two cotyledons and two true leaves, within a single plant, were examined and the highest disease grade was recorded to represent the disease reaction for that plant tissue.

3.5 **Statistical analyses**

Statistical analyses were used to detect differences among parents and progenies and heterogeneity for number of resistant phenotypes among replications. Frequency histograms of F_2 entries were constructed for each isolate and examined to determine the type of distribution exhibited. Numbers occurring in segregating classes were tested using Chi-square for goodness of fit to classical phenotypic ratios. Pearson product-moment correlation coefficients were calculated using replication means to compare disease reactions of cotyledons and true-leaves, for any association.

CHAPTER 4

RESULTS.

4.1 Field experiment.

The average disease grades of the parental cultivars inoculated with each of the isolates and their mixture in the different locations are presented in Tables 1, 2 and 3. The results indicate definite differential reactions to the isolates. At Samaru, under isolate 1, the cultivar S295 was highly resistant. The multi-adversity (MAR) varieties (TX-CDP37HH-1-83, TAMCOT SP21s and TAMCOT-CAMD-E) were resistant. The highest average disease grade (6.9) was recorded on SAMCOT-6. Although the Samaru varieties showed disease grade in the susceptible range, SAMCOT-9 was comparatively less susceptible.

Considering isolate 2, the cultivar S295 showed immunity, as plants were symptomless. The three MAR varieties gave significantly lower disease grades for this isolate as against isolate 1. SAMCOT-9 and SAMCOT-10 apparently showed disease grades that approached susceptibility while SAMCOT-6 and SAMCOT-8 were susceptible.

Table 1. Mean disease grades of cotton cultivars inoculated in the field with isolates of Xanthomonas campestris pv. malvacearum at Samaru.

Parents	Isolate 1	Isolate 2	1:1 Mixture	Mean of Isolates
S295	1.3 a	1.0 a	1.2 a	1.2 a
TX-CDP37HH-1-83	3.5 b	2.4 b	3.3 c	1.2 a
TAMCOT SP21S	3.8 b	2.8 b	3.7 c	3.4 c
TAMCOT-CAMD-E	3.6 b	2.7 b	2.5 b	2.9 b
SAMCOT-9	4.9 c	4.6 c	4.7 d	4.7 d
SAMCOT-10	5.4 cd	4.5 c	4.9 d	4.9 d
SAMCOT-6	6.9 e	6.2 e	6.4 e	6.5 f
SAMCOT-8	5.6 d	5.5 d	4.9 d	5.3 e

Disease grades are based on a scale of 1 (immunity) to 10 (fully susceptible)
Any two mean grades within a column having a letter in common are not significantly different at P=0.05 according to Duncan's new multiple range test.

Table 2. Mean disease grades of cotton cultivars inoculated in the field with isolates of Xanthomonas campestris pv. malvacearum at Daudawa.

Parents	Isolate 1		Isolate 2		1:1 Mixture		Mean of Isolates	
S295	1.2	a	1.0	a	1.2	a	1.1	a
TX-CDP37HH-1-83	2.8	b	2.2	b	3.3	c	2.8	bc
TAMCOT SP21S	3.4	c	2.3	b	3.7	c	3.1	c
TAMCOT-CAMD-E	2.7	b	2.4	b	2.6	b	2.6	b
SAMCOT-9	4.8	d	4.2	c	4.7	d	4.6	d
SAMCOT-10	5.2	de	4.5	c	4.8	d	4.8	de
SAMCOT-6	6.3	f	5.8	e	6.0	e	6.0	f
SAMCOT-8	5.4	e	5.2	d	4.8	d	5.1	e

Disease grades are based on scale of 1 (immunity) to 10 (fully susceptible)
Any two mean grades within a column having a letter in common are not significantly different at P=0.05 according to Duncan's new multiple range test.

Table 3. Mean disease grades of cotton cultivar inoculated in the field with isolates of Xanthomonas campestris pv. malvacearum at Maigana.

Parents	Isolate 1		Isolate 2		1:1 Mixture		Mean of Isolate	
S295	1.0	a	1.0	a	1.0	a	1.0	a
TX-CDP37HH-1-83	2.6	bc	2.3	b	2.9	bc	2.6	b
TAMCOT SP21S	2.9	c	2.6	bc	3.3	c	2.9	b
TAMCOT-CAMD-E	2.3	b	2.2	b	2.8	b	2.4	b
SAMCOT-9	4.3	d	2.9	c	4.8	d	4.0	c
SAMCOT-10	4.5	de	2.8	c	4.6	d	4.0	c
SAMCOT-6	5.4	f	5.6	e	5.8	e	5.6	d
SAMCOT-8	4.7	e	4.7	d	5.6	e	5.0	d

Disease grades are based on a scale of 1 (immunity) to 10 (fully susceptible)
Any two mean grades within a column having a letter in common are not significantly different at P=0.05 according to Duncan's new multiple range test.

The trend in the reaction of the cultivars to a mixture of isolates 1 and 2 was similar to the reaction observed under isolate 1. The cultivar S295 was highly resistant. The MAR varieties were resistant with TAMCOT-CAMD-E, having an average disease grade of 2.5 being more resistant than Tx-CDP37HH-1-83 with a mean disease grade of 3.3 and TAMCOT SP21s of 3.7. Although the two isolates and their mixture were effective in differentiating resistant parents from susceptible ones (Table.1). Generally the disease grades under isolate 1 and a mixture of isolate 1 and 2 were higher than those under isolate 2.

At Daudawa, differences in resistance between the parental cultivars were also apparent (Table 2). The cultivar S295 was highly resistant/immune to the isolates. The pattern of reaction of the MAR cultivars was similar irrespective of the isolate. However, slightly higher disease grades were recorded for isolate 1 than the 1:1 mixtures of the isolates. Comparatively, SAMCOT-9, SAMCOT-10 and SAMCOT-8 had significantly lower disease grades than SAMCOT-6, though within the susceptible range. The parental disease grades at Maigana (Table 3) were lower than those observed at Samaru and Daudawa (Tables 1, 2). The cultivar S295 was found to be immune to both isolates. The MAR cultivars maitained a high level of resistance.

Averaged over isolates, SAMCOT-9, SAMCOT-10 and SAMCOT-8 were less susceptible when compared to SAMCOT-6. The near immunity of the cultivar S295 at the three locations is striking. It was demonstrably more resistant than the MAR cultivars, which nevertheless were more resistant than the SAMCOT series at all locations.

The combined analysis of variance incorporating populations, isolates, locations and their interactions is presented in Appendix 1. The analysis indicated highly significant differences ($P<0.01$) amongst populations and locations. The interaction between population and location was also highly significant. Significant differences ($P<0.05$) were observed between isolates. Isolates x locations and populations x isolates x locations interactions were also significant. The significance of the interaction between the population and locations suggests that the populations behaved differently in each of the location.

Mode of inheritance

The F_1 populations from the crosses involving the cultivar S295 with each of the MAR varieties were all resistant irrespective of isolates or locations (Tables 4-12). These frequency distribution tables do not show the number of plants in the susceptible range as none were recorded or observed.

Table 4. Frequency distributions of Leaf disease grades in the F_1 and F_2 populations inoculated in the field with isolate 1 of Xanthomanas campestris pv. malvacearum at Samaru.

Progenies	Disease grades											
	1	2	3	4	5	6	7	8	9	10	Total Number of plants	Mean
F_1s S295												
X TX-CDP37HH-1-83	22	18	-	-	-	-	-	-	-	-	40	1.45
X TAMCOT SP21S	20	20	-	-	-	-	-	-	-	-	40	1.50
X TAMCOT-CAMD-E	28	12	-	-	-	-	-	-	-	-	40	1.30
X SAMCOT-9	4	36	-	-	-	-	-	-	-	-	40	1.90
X SAMCOT-10	8	32	-	-	-	-	-	-	-	-	40	1.80
X SAMCOT-6	-	37	3	-	-	-	-	-	-	-	40	2.20
X SAMCOT-8	8	32	-	-	-	-	-	-	-	-	40	1.80
F_2s S295												
X TX-CDP37HH-1-83	36	86	66	4	-	-	-	-	-	-	192	2.20
X TAMCOT SP21S	28	60	64	40	-	-	-	-	-	-	192	2.60
X TAMCOT-CAMD-E	19	52	75	46	-	-	-	-	-	-	192	2.77
X SAMCOT-9	35	73	27	4	12	36	5	-	-	-	192	3.07
X SAMCOT-10	38	77	11	8	11	39	8	-	-	-	192	3.14
X SAMCOT-6	40	86	16	4	6	32	8	-	-	-	192	2.89
X SAMCOT-8	29	100	8	4	4	32	15	-	-	-	192	3.05

Table 5. Frequency distribution of leaf disease grades in the F_1 and F_2 populations inoculated in the field with isolate 2 of Xanthomonas campestris pv. malvacearum at Samaru.

Progenies	Disease grades											
	1	2	3	4	5	6	7	8	9	10	Total Number of plants	Mean
F_1s												
S295												
X TX-CDP37HH-1-83	28	12	-	-	-	-	-	-	-	-	40	1.30
X TAMCOT SP21S	24	16	-	-	-	-	-	-	-	-	40	1.40
X TAMCOT-CAMD-E	32	8	-	-	-	-	-	-	-	-	40	1.20
X SAMCOT-9	8	32	-	-	-	-	-	-	-	-	40	1.80
X SAMCOT-10	4	36	-	-	-	-	-	-	-	-	40	1.90
X SAMCOT-6	8	32	-	-	-	-	-	-	-	-	40	1.80
X SAMCOT-8	16	24	-	-	-	-	-	-	-	-	40	1.60
F_2s												
S295												
X TX-CDP37HH-1-83	42	80	70	-	-	-	-	-	-	-	192	2.15
X TAMCOT SP21S	25	83	84	-	-	-	-	-	-	-	192	2.31
X TAMCOT-CAMD-E	58	134	-	-	-	-	-	-	-	-	192	1.70
X SAMCOT-9	88	45	19	8	10	20	2	-	-	-	192	2.35
X SAMCOT-10	74	48	22	8	8	28	4	-	-	-	192	2.63
X SAMCOT-6	66	36	26	16	12	30	6	-	-	-	192	2.93
X SAMCOT-8	66	48	24	8	8	34	4	-	-	-	192	2.80

Table 6. Frequency distribution of leaf disease grades in the F_1 and F_2 populations inoculated in the field with a mixture of isolates 1 and 2 of Xanthomonas campestris pv. malvacearum at Samaru.

Progenies	Disease grades											
	1	2	3	4	5	6	7	8	9	10	Total Number of plants	Mean
F_1s												
S295												
X TX-CDP37HH-1-83	16	24	-	-	-	-	-	-	-	-	40	1.60
X TAMCOT SP21S	20	20	-	-	-	-	-	-	-	-	40	1.50
X TAMCOT-CAMD-E	24	16	-	-	-	-	-	-	-	-	40	1.40
X SAMCOT-9	8	32	-	-	-	-	-	-	-	-	40	1.80
X SAMCOT-10	-	36	4	-	-	-	-	-	-	-	40	2.10
X SAMCOT-6	-	36	4	-	-	-	-	-	-	-	40	2.10
X SAMCOT-8	-	32	8	-	-	-	-	-	-	-	40	2.20
F_2s												
S295												
X TX-CDP37HH-1-83	20	76	96	-	-	-	-	-	-	-	192	2.40
X TAMCOT SP21S	34	58	70	30	-	-	-	-	-	-	192	2.50
X TAMCOT-CAMD-E	41	65	55	31	-	-	-	-	-	-	192	2.39
X SAMCOT-9	56	80	8	4	4	30	10	-	-	-	192	2.74
X SAMCOT-10	52	76	6	8	3	35	12	-	-	-	192	2.93
X SAMCOT-6	30	72	26	12	10	26	16	-	-	-	192	3.22
X SAMCOT-8	44	76	21	8	8	25	10	-	-	-	192	2.87

Table 7. Frequency distributions of leaf disease grades in the F_1 and F_2 populations inoculated in the field with isolate 1 of *Xanthomonas campestris* pv. *malvacearum* at Daudawa.

Progenies	Disease grades											
F_1s	1	2	3	4	5	6	7	8	9	10	Total Number of plants	Mean
S295												
X TX-CDP37HH-1-83	24	16	-	-	-	-	-	-	-	-	40	1.40
X TAMCOT SP21S	20	20	-	-	-	-	-	-	-	-	40	1.50
X TAMCOT-CAMD-E	24	16	-	-	-	-	-	-	-	-	40	1.40
X SAMCOT-9	28	12	-	-	-	-	-	-	-	-	40	1.30
X SAMCOT-10	20	20	-	-	-	-	-	-	-	-	40	1.50
X SAMCOT-6	12	28	-	-	-	-	-	-	-	-	40	1.70
X SAMCOT-8	16	24	-	-	-	-	-	-	-	-	40	1.60
F_1s												
S295												
X TX-CDP37HH-1-83	13	74	93	12	-	-	-	-	-	-	192	2.54
X TAMCOT SP21S	14	65	96	17	-	-	-	-	-	-	192	2.60
X TAMCOT-CAMD-E	26	83	83	-	-	-	-	-	-	-	192	2.29
X SAMCOT-9	58	75	14	6	2	35	2	-	-	-	192	2.65
X SAMCOT-10	38	90	15	6	7	33	3	-	-	-	192	2.82
X SAMCOT-6	34	87	10	5	9	40	7	-	-	-	192	3.08
X SAMCOT-8	52	78	6	4	4	44	4	-	-	-	192	2.89

Table 8. Frequency distributions of leaf disease grades in the F_1 and F_2 populations inoculated in the field with isolate 2 of Xanthomonas campestris pv. malvacearum at Daudawa.

Progenies	Disease grades											
	1	2	3	4	5	6	7	8	9	10	Total Number of plants	Mean
F_1s S295												
X TX-CDP37HH-1-83	24	16	-	-	-	-	-	-	-	-	40	1.40
X TAMCOT SP21S	28	12	-	-	-	-	-	-	-	-	40	1.30
X TAMCOT-CAMD-E	28	12	-	-	-	-	-	-	-	-	40	1.30
X SAMCOT-9	12	28	-	-	-	-	-	-	-	-	40	1.70
X SAMCOT-10	16	24	-	-	-	-	-	-	-	-	40	1.60
X SAMCOT-6	20	20	-	-	-	-	-	-	-	-	40	1.50
X SAMCOT-8	24	16	-	-	-	-	-	-	-	-	40	1.40
F_2s S295												
X TX-CDP37HH-1-83	35	85	72	-	-	-	-	-	-	-	192	2.19
X TAMCOT SP21S	21	76	96	-	-	-	-	-	-	-	192	2.40
X TAMCOT-CAMD-E	53	86	53	-	-	-	-	-	-	-	192	2.80
X SAMCOT-9	72	48	22	10	5	33	2	-	-	-	192	2.66
X SAMCOT-10	68	48	22	10	10	34	-	-	-	-	192	2.73
X SAMCOT-6	72	44	20	8	5	41	2	-	-	-	192	2.85
X SAMCOT-8	72	43	20	8	6	38	5	-	-	-	192	2.83

Table 9. Frequency distributions of leaf disease grades in the F_1 and F_2 populations inoculated in the field with a mixture of isolates 1 and 2 of Xanthomonas campestris pv. malvacearum at Daudawa.

Progenies	Disease grades											
	1	2	3	4	5	6	7	8	9	10	Total Number of plants	Mean
F_1s												
S295												
X TX-CDP37HH-1-83	16	24	-	-	-	-	-	-	-	-	40	1.60
X TAMCOT SP21S	20	20	-	-	-	-	-	-	-	-	40	1.50
X TAMCOT-CAMD-E	24	16	-	-	-	-	-	-	-	-	40	1.40
X SAMCOT-9	8	32	-	-	-	-	-	-	-	-	40	1.80
X SAMCOT-10	4	36	-	-	-	-	-	-	-	-	40	1.90
X SAMCOT-6	-	36	4	-	-	-	-	-	-	-	40	2.10
X SAMCOT-8	4	36	-	-	-	-	-	-	-	-	40	1.90
F_2s												
S295												
X TX-CDP37HH-1-83	12	67	98	15	-	-	-	-	-	-	192	2.60
X TAMCOT SP21S	12	90	90	-	-	-	-	-	-	-	192	2.41
X TAMCOT-CAMD-E	20	76	96	-	-	-	-	-	-	-	192	2.39
X SAMCOT-9	40	92	14	6	5	33	2	-	-	-	192	2.75
X SAMCOT-10	56	76	14	8	2	34	2	-	-	-	192	2.66
X SAMCOT-6	31	102	11	3	4	31	10	-	-	-	192	2.90
X SAMCOT-8	40	88	12	8	10	32	2	-	-	-	192	2.81

Table 10. Frequency distributions of leaf disease grades in the F_1 and F_2 populations inoculated in the field with isolate 1 of Xanthomonas campestris pv. malvacearum at Maigana.

Progenies	Disease grades											
	1	2	3	4	5	6	7	8	9	10	Total Number of plants	Mean
F_1s												
S295												
X TX-CDP37HH-1-83	28	12	-	-	-	-	-	-	-	-	40	1.30
X TAMCOT SP21S	24	16	-	-	-	-	-	-	-	-	40	1.40
X TAMCOT-CAMD-E	32	8	-	-	-	-	-	-	-	-	40	1.20
X SAMCOT-9	16	24	-	-	-	-	-	-	-	-	40	1.60
X SAMCOT-10	20	20	-	-	-	-	-	-	-	-	40	1.50
X SAMCOT-6	20	20	-	-	-	-	-	-	-	-	40	1.50
X SAMCOT-8	24	16	-	-	-	-	-	-	-	-	40	1.40
F_2s												
S295												
X TX-CDP37HH-1-83	20	76	96	-	-	-	-	-	-	-	192	2.39
X TAMCOT SP21S	4	68	120	-	-	-	-	-	-	-	192	2.60
X TAMCOT-CAMD-E	26	88	80	-	-	-	-	-	-	-	192	2.30
X SAMCOT-9	80	47	15	8	4	38	-	-	-	-	192	2.59
X SAMCOT-10	73	44	20	10	5	38	2	-	-	-	192	2.75
X SAMCOT-6	70	45	26	13	9	25	4	-	-	-	192	2.72
X SAMCOT-8	80	36	19	17	10	30	-	-	-	-	192	2.64

Table 11. Frequency distributions of disease grades in the F_1 and F_2 populations inoculated in the field with isolate 2 of Xanthomonas campestris pv. malvacearum at Maigana.

Progenies	Disease grades											
	1	2	3	4	5	6	7	8	9	10	Total Number of plants	Mean
F_1s												
S295												
X TX-CDP37HH-1-83	24	16	-	-	-	-	-	-	-	-	40	1.40
X TAMCOT SP21S	28	12	-	-	-	-	-	-	-	-	40	1.30
X TAMCOT-CAMD-E	32	8	-	-	-	-	-	-	-	-	40	1.20
X SAMCOT-9	20	20	-	-	-	-	-	-	-	-	40	1.50
X SAMCOT-10	24	16	-	-	-	-	-	-	-	-	40	1.40
X SAMCOT-6	16	24	-	-	-	-	-	-	-	-	40	1.60
X SAMCOT-8	20	20	-	-	-	-	-	-	-	-	40	1.50
F_2s												
S295												
X TX-CDP37HH-1-83	34	86	72	-	-	-	-	-	-	-	192	2.20
X TAMCOT SP21S	21	76	96	-	-	-	-	-	-	-	192	2.40
X TAMCOT-CAMD-E	48	84	62	-	-	-	-	-	-	-	192	2.09
X SAMCOT-9	77	46	21	13	3	30	2	-	-	-	192	2.57
X SAMCOT-10	72	48	20	8	4	38	2	-	-	-	192	2.72
X SAMCOT-6	72	44	20	8	8	30	10	-	-	-	192	2.82
X SAMCOT-8	88	40	16	8	12	28	-	-	-	-	192	2.27

Table 12. Frequency distributions of leaf disease grades in the F_1 and F_2 populations inoculated in the field with a mixture of isolates 1 and 2 of Xanthomonas campestris pv. malvacearum at Maigana.

Progenies	Disease grades										Total Number of plants	Mean
	1	2	3	4	5	6	7	8	9	10		
F_1s												
S295												
X TX-CDP37HH-1-83	24	16	-	-	-	-	-	-	-	-	40	1.40
X TAMCOT SP21S	24	16	-	-	-	-	-	-	-	-	40	1.40
X TAMCOT-CAMD-E	28	12	-	-	-	-	-	-	-	-	40	1.30
X SAMCOT-9	16	24	-	-	-	-	-	-	-	-	40	1.60
X SAMCOT-10	12	28	-	-	-	-	-	-	-	-	40	1.70
X SAMCOT-6	12	28	-	-	-	-	-	-	-	-	40	1.70
X SAMCOT-8	16	24	-	-	-	-	-	-	-	-	40	1.60
F_2s												
S295												
X TX-CDP37HH-1-83	19	77	96	-	-	-	-	-	-	-	192	2.40
X TAMCOT-SP21S	3	69	120	-	-	-	-	-	-	-	192	2.61
X TAMCOT-CAMD-E	32	70	90	-	-	-	-	-	-	-	192	2.30
X SAMCOT-9	80	45	18	9	5	35	-	-	-	-	192	2.58
X SAMCOT-10	76	53	16	8	3	36	-	-	-	-	192	2.57
X SAMCOT-6	64	48	28	10	8	34	-	-	-	-	192	2.75
X SAMCOT-8	84	39	18	12	7	30	2	-	-	-	192	2.57

The F_2 populations of these crosses were also resistant and showed no segregation for susceptibility. The F_2 distribution was skewed to resistant types only. This tends to suggest that the cultivars probably have the same genetic composition .

In the cross between S295 x SAMCOT-9, inoculated with isolate 1 at Samaru, all the F_1s were resistant. (Table 13), indicating that dominant gene or genes govern resistance in this cross. Bimodal F_2 disease grade frequency distributions expected of a monogenic resistance were observed (Fig.1). Parental disease grades and F_2 distributions indicated two classes of disease reactions, with Grade 1 to 4 being resistant and Grade 5 to 10 susceptible. Chi-square (X^2) values were calculated assuming that resistance was determined by a single dominant gene, thus distributed 3:1 for the F_2 and 1:1 for the test crosses. The Chi-square test on the F_2 population gave a low value of 0.69 and with 1 degree of freedom, the calculated value is below the $p=0.05$ threshold indicating that the deviation between the observed and the expected ratios was not significant, therefore the pattern of segregation in the F_2 fits a 3 resistant: 1 susceptible phenotypic ratio (observed numbers, 139 resistant and 53 susceptible). A perfect fit to a 3:1 phenotypic ratio should give, 144 resistant and 48 susceptible plants). The close fit to a 3:1

Table 13. Segregation for leaf disease reactions to isolate 1 of Xanthomonas campestris pv. malvacearum among F_1, F_2, BC_1 and BC_2 generations inoculated in the field at Samaru.

Progenies	Expected segregation	Observed segregation Number: Resistance	Susceptible	X^2 value	P
		Number of plants			
SAMCOT-9 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-9)	1:1	42	38	0.20	0.75-0.50
F_2	3:1	139	53	0.69	0.50-0.25
SAMCOT-10 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295		80	0	-	-
$BC_2(F_1$ X SAMCOT-10)	1:1	44	36	0.80	0.50-0.25
F_2	3:1	135	57	2.25	0.25-0.10
SAMCOT-6 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-6)	1:1	40	40	0	1
F_2	3:1	146	46	0.11	0.75-0.50
SAMCOT-8 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-8)	1:1	81	39	0.05	0.90-0.75
F_2	3:1	141	51	0.25	0.75-0.50

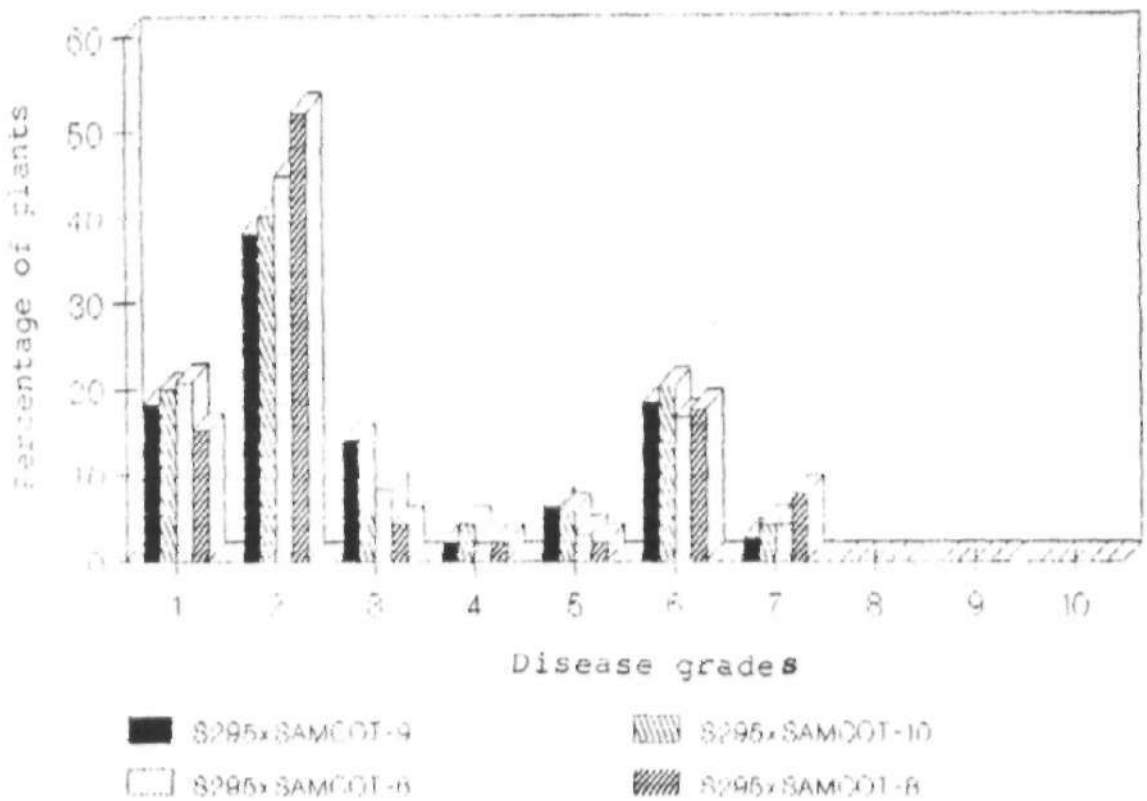


Fig. 1. F_2 frequency histograms of leaf disease grades for the cotton crosses S295 x SAMCOT-9, S295 x SAMCOT-1[illegible] S295 x SAMCOT-6 and S295 x SAMCOT-8, inoculated with isolate 1 of *Xanthomonas campestris* pv *malvacearum* at Samaru.

phenotypic ratio suggests that resistance is governed by a single gene.

The F_1S backcrossed to the resistant parent (S295) gave progenies that were all resistant (Table 13) while the backcross to the susceptible parent segregated for a 1:1 ratio which would be expected of resistance under monogenic control.

In the cross, S295 x SAMCOT-10, the F_1s were also resistant (Tables 4 and 13). The observed segregating ratio in the F_2 gave, 135 resistant and 57 susceptible plants. The F_2 frequency distribution for the cross was bimodal (Fig. 1). Calculated X^2 value was 2.25 and this was not significant at p=0.05 indicating that the F_2 data fits a 3:1 phenotypic ratio. The backcross of the F_1 to the resistant parents that were all resistant while the backcross to the susceptible parent segregated for a 1:1 resistant: susceptible ratio.

Data on F_1 population of the cross, S295 x SAMCOT-6 (the most susceptible parent), indicated resistance. The grading distribution of the F_2 fits a 3:1 segregating ratio while the backcross to the susceptible parent fitted perfectly a 1:1 phenotypic ratio. Similar segregating patterns of the F_2 and BC_2 generations were also observed in the Cross (S295 x SAMCOT-8) (Table 13).

Grouping the 4 F_2 populations together, S295 x SAMCOT-9; S295 X SAMCOT-10; S295 X SAMCOT-6 and S295 X SAMCOT-8) F_2s gave 561 resistant to 207 susceptible plants, that is 73.1 and 26.9%, resistant and susceptible plants, respectively. The calculated, X^2 value of 1.6, with 1 degree of freedom is below the p=0.05 threshold, hence the segregation observed in the F_2 suggests a simple inheritance of the disease reaction, with resistance determined by a single dominant gene. A verification of the pattern of resistance in the backcross progenies gave 167 resistant to 165 susceptible plants. The segregating ratio in the backcross generations confirms the fact that resistance is governed by a single dominant gene in the cultivar S295.

Table 14 contains data on crosses involving S295 and SAMCOT-9, SAMCOT-10, SAMCOT-6 AND SAMCOT-8, respectively inoculated with isolate 2 at Samaru. Plants of the F_1 generations were classified as resistant. The frequency distributions in the F_2s were bimodal (Fig.2). The F_2 array of the cross (S295 x SAMCOT-9) gave 160 resistant and 32 susceptible plants. The X^2 value assuming the hypothesis of a single dominant gene inheritance was 7.11. This value is significant both at P=0.05 and P=0.01. The X^2 value rejects segregating ratio of 3:1 due to excess number of plants appearing in the resistant classes.

Table 14. Segregation for leaf disease reactions to isolate 2 of Xanthomonas campestris pv. malvacearum among F_1, F_2, BC_1 and BC_2 generations inoculted in the field at Samaru.

Progenies	Expected segregation	Observed segregation Number		X^2value	P
		Resistance	Susceptible		
		Number of plants			
SAMCOT-9 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-9)	1:1	50	30	5.0	0.05-0.01
F_2	3:1	160	32	7.11	< 0.01
SAMCOT - 10 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-10)	1:1	48	32	3.20	0.06-0.08
F_2	3:1	152	40	1.77	0.25-0.10
SAMCOT-6 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-6)	1:1	42	38	0.20	0.75-0.50
F_2	3:1	144	48	0	1
SAMCOT-8 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-8)	1:1	44	36	0.80	0.50-0.25
F_2	3:1	146	46	0.11	0.75-0.50

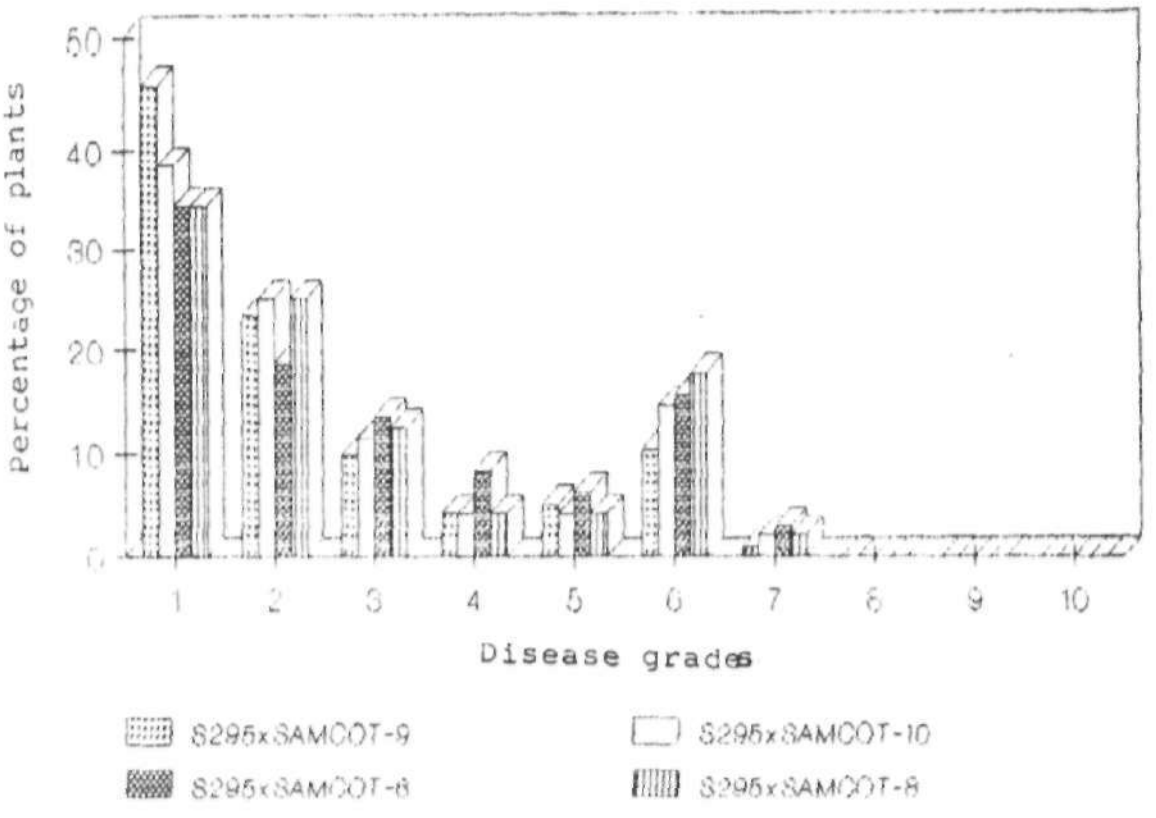


Fig. 2. F_2 frequency histograms ofleaf disease grades for the cotton crosses S295 x SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8, inoculated with isolate 2 of Xanthomonas campestris pv malvacearum at Samaru.

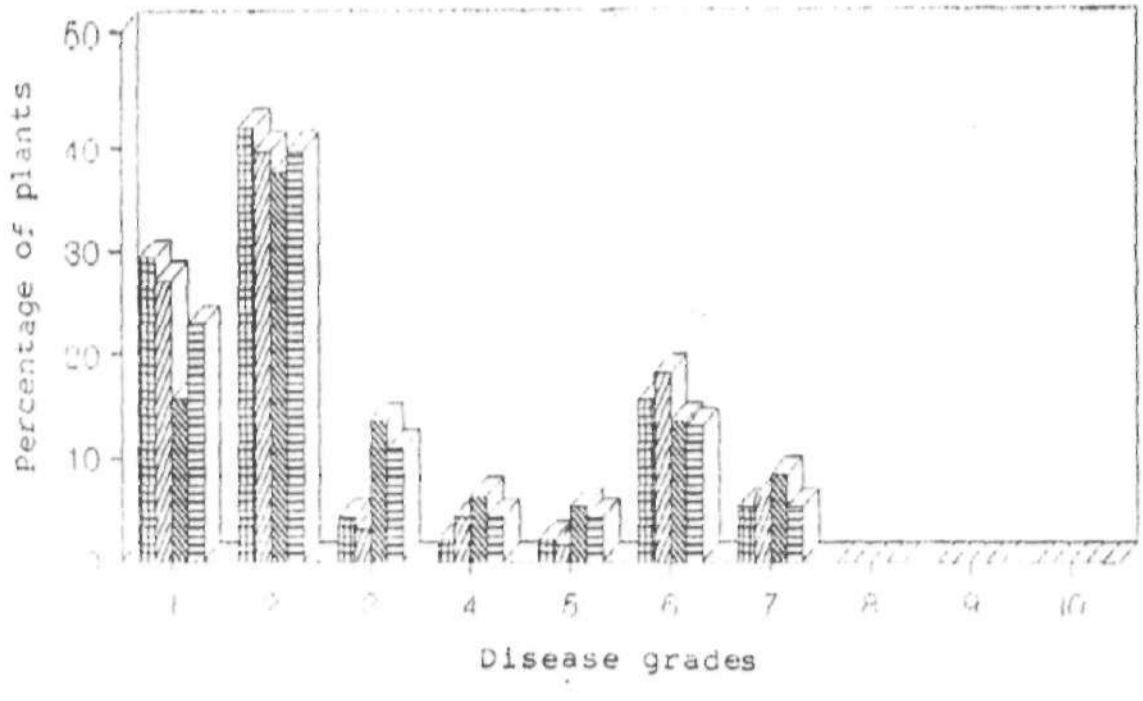


Fig. 3. F_2 frquency histograms of leaf disease grades for the cotton crosses S295 x SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8, inoculated with a 1:1 mixture of isolate 1 and 2 of *Xanthomonas campestris* pv *malvacearum* at Samaru.

The disease reaction of (S295 x SAMCOT-10; S295 x SAMCOT-6; S295 x SAMCOT-8) F_2 plants indicate that the segregation in the BC_2 progenies gave a close fit to a 1:1 phenotypic ratio suggesting a single dominant gene inheritance in the cultivar S295.

Data presented in Table 15 suggest also that S295 has a single gene for resistance. The differences in the grade distributions of the various populations could be due to differences in the parents used or different physiologocal responses to environmental conditions.

Daudawa.

The results of the inoculations of the F_1, F_2, BC_1 and BC_2 populations inocuated with isolate 1 are presented in Table 16. The F_1 of the crosses between S295 x SAMCOT-9, SAMCOT-10, SAMCOT-6 and SAMCOT-8, were all resistant to the isolate. Disease grade frequecy distributions of the F_2 array indicate a bimodal distribution (Fig. 4). The disease grades distribution approximated a 3:1 ratio. When the F_1s were backcrossed to the susceptible parents, the BC_2 populations exhibited a bimodal distribution, giving a close fit to a 1:1 ratio. The X^2 values obtained for the F_2s and backcross populations using the hypothesis of dominant single gene factor resistance, were all lower than the table values

Table 15. Segregation for leaf disease reactions to a mixture of isolates 1 and 2 of Xanthomonas campestris pv. malvacearum among F_1, F_2, BC_1 and BC_2 generations inoculated in the field at Samaru.

Progenies	Expected segregation	Observed segregation Number		X^2value	P
		Resistance	Susceptible		
		Number of plants			
SAMCOT-9 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-9)	1:1	44	36	0.80	0.50-0.25
F_2	3:1	148	44	0.44	0.75-0.50
SAMCOT-10 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-10)	1:1	44	36	0.80	0.50-0.25
F_2	3:1	142	50	0.11	0.75-0.50
SAMCOT-6 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-6)	1:1	41	39	0.05	0.90-0.75
F_2	3:1	140	52	0.44	0.75-0.50
SAMCOT-8 X S295					
F_1		40	0	-	-
$BC_2(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-8)	1:1	48	32	3.20	0.06-0.08
F_2	3:1	149	43	0.69	0.50-0.25

Table 16. Segregation for leaf disease reactions to isolate 1 of Xanthomonas campestris pv. malvacearum among F_1, F_2, BC_1 and BC_2 generations inoculated in the field at Daudawa.

Progenies	Expected segregation	Observed segregation Number: Resistance	Susceptible	X^2value	P
		Number of plants			
SAMCOT-9 X S295					
F_1		40	0	-	-
BC_1(F_1 X S295)		80	0	-	-
BC_2(F_1 X SAMCOT-9)	1:1	48	32	3.20	0.06-0.08
F_2	3:1	153	39	2.25	0.25-0.10
SAMCOT-10 X S295					
F_1		40	0	-	-
BC_1(F_1 X S295)		80	0	-	-
BC_2(F_1 X SAMCOT-10)	1:1	45	35	1.25	0.50-0.25
F_2	3:1	149	43	0.69	0.50-0.25
SAMCOT-6 X S295					
F_1		40	0	-	-
BC_1(F_1 X S295)		80	0	-	-
BC_2(F_1 X SAMCOT-6)	1:1	42	38	0.20	0.75-0.50
F_2	3:1	136	56	1.77	0.25-0.10
SAMCOT-8 S295					
F_1		40	0	-	-
BC_1(F_1 X S295)		80	0	-	-
BC_2(F_1 X SAMCOT-8)	1:1	44	36	0.80	0.50-0.25
F_2	3:1	140	52	0.44	0.75-0.50

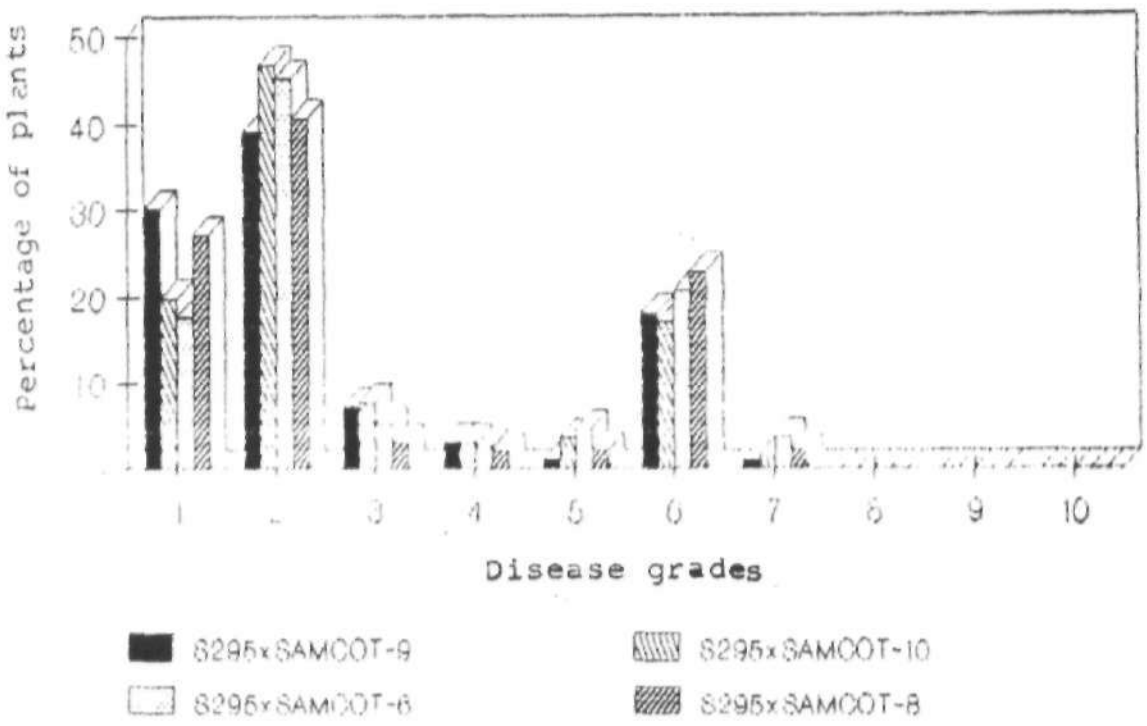


Fig. 4. F_2 frequency histograms of leaf disease grades for the cotton crosses S295 x SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8, inoculated with isolate 1 of Xanthomonas campestris pv malvacearum at Daudawa.

at P=0.05 chosen. Thus the F_2 and BC distribution, suggest major gene effects with dominance in the direction of resistance . as opposed to continuous variation.

The disease grades distributions of (S295 x SAMCOT-9)F_2; (S295 x SAMCOT-10) F_2; (S295 x SAMCOT-6) F_2 and S295 x SAMCOT-8) F_2 and their backcrosses inoculated with isolate 2 also agree with the hypothesis of a dominant single gene inheritance. The results are presented in Table 17 and Figure 5. The F_2 population of the Cross (S295 x SAMCOT-9) inoculated with a mixture of isolates 1 and 2 gave 152 resistant to 40 susceptible plants (Table 18). The calculated X^2 value 1.77 was not significant (P=0.25-0.10) indicating that the data fit a 3:1 segregating ratio expected for a single gene inheritance. The backcross to the resistant parent (S295) produced resistant progenies, while the backcross to the susceptible parent produced 49 resistant to 31 susceptible plants. The X^2 value of 4.05 was significant (P=0.05-0.025), indicating that the distribution in the testcross population did not adequately fit a 1:1 ratio. The disease grades distribution of the F_2 and backcross population of (S295 X SAMCOT-10); (S295 X SAMCOT-6); (S295 X SAMCOT-8) fitted a 3:1 and a 1:1 phenotypic ratios, respectively as the X^2 value was not significant at P=0.05 (Table 18 and Figure 6).

Table 17. Segregation for leaf disease reactions to isolate 2 of Xanthomonas campestris pv. malvacearum among F_1, F_2, BC_1 and BC_2 generations inoculated in the field at Daudawa.

Progenies	Expected segregation	Observed segregation Number: Resistance	Susceptible	X^2value	P
		Number of plants			
SAMCOT-9 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-9)	1:1	47	33	2.46	0.25-0.10
F_2	3:1	152	40	1.77	0.25-0.10
SAMCOT-10 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-10)	1:1	42	38	0.20	0.75-0.50
F_2	3:1	148	44	0.19	0.75-0.50
SAMCOT-6 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-6)	1:1	44	36	0.80	0.50-0.25
F_2	3:1	144	48	0	1
SAMCOT-8 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_2$ X SAMCOT-8)	1:1	43	37	0.45	0.75-0.50
F_2	3:1	143	49	0.03	0.90-0.75

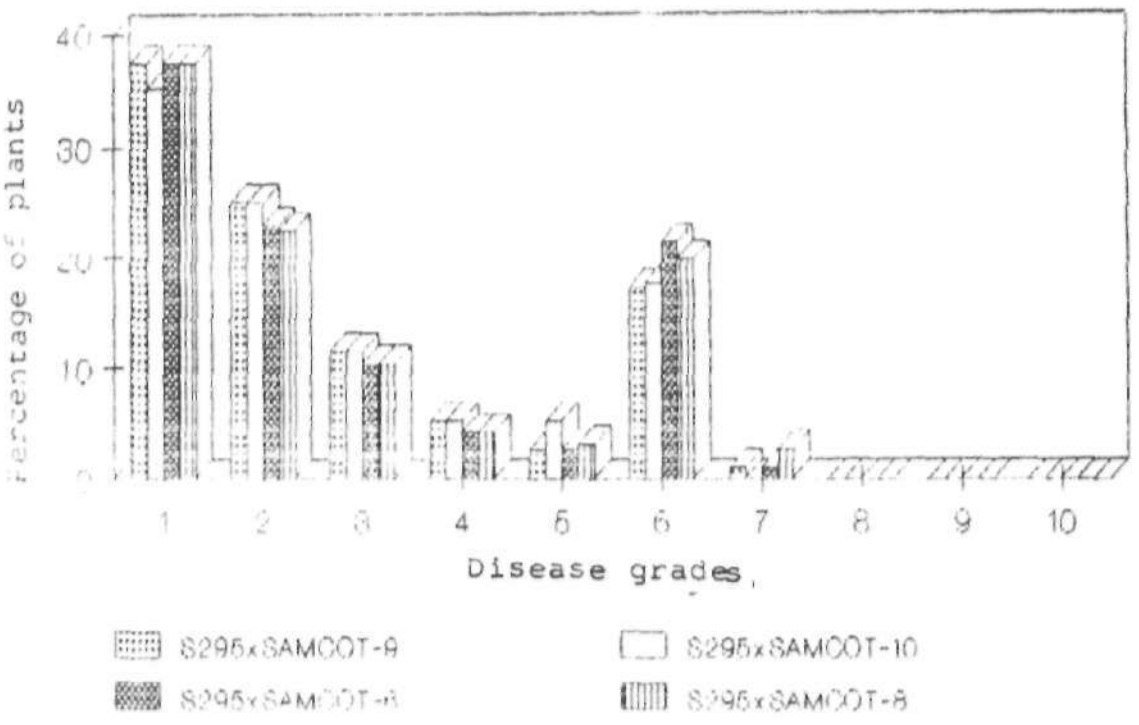


Fig. 5. F_2 frequency histograms of leaf disease grades for the cotton crosses S295 x SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8, inoculated with isolate 2 of Xanthomonas campestris pv malvacearu at Daudawa.

Table 18. Segregation for leaf disease reactions to a mixture of isolates 1 and of Xanthomonas campestries pv. malvacearum among F_1, F_2, BC_1 and BC_2 generations inoculated in the field at Daudawa.

Progenies	Expected segregation	Observed segregation Number: Resistance	Susceptible	X^2value	P
		Number of plants			
SAMCOT-9 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-9)	1:1	49	31	4.05	0.05-0.025
F_2	3:1	152	40	1.77	0.25-0.10
SAMCOT-10 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-10)	1:1	47	33	2.45	0.25-0.10
F_2	3:1	154	38	2.77	0.10-0.05
SAMCOT-6 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-6)	1:1	44	36	0.80	0.50-0.25
F_2	3:1	147	45	0.24	0.75-0.50
SAMCOT-8 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-8)	1:1	48	32	3.20	0.06-0.08
F_2	3:1	148	44	0.44	0.75-0.50

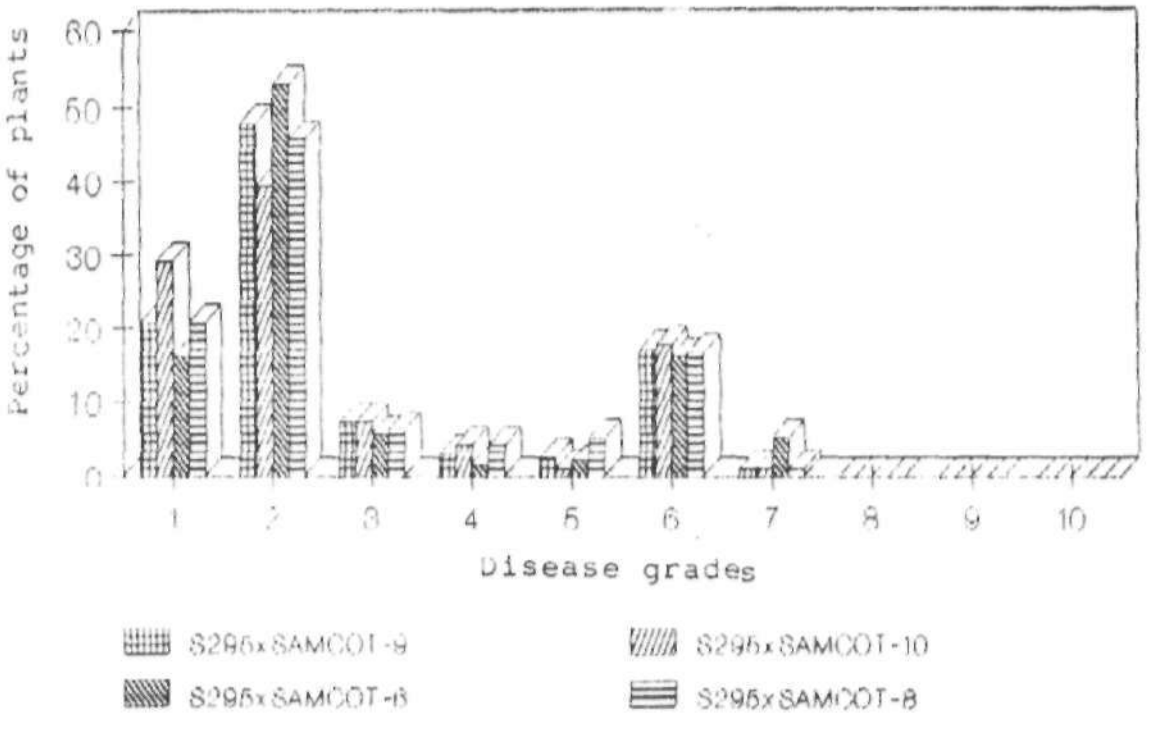


Fig. 6. F_2 frequency histograms of leaf disease grades for the cotton crosses S295 x SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8, inoculated with a 1:1 mixture of isolate 1 and 2 of Xanthomonas campestris pv malvacearum at Daudawa.

Maigana.

Table 19 contains data on crosses of the cultivar S295 with SAMCOT-9, SAMCOT-10, SAMCOT-6 and SAMCOT -8 inoculated with isolate 1. These cultivars gave resistant F_1 populations. Bimodal F_2 disease grade frequency distribution were observed (Fig 7). In the F_2 of the cross (S295 x SAMCOT -9), there were 150 resistant to 42 susceptible plants gave a ratio approaching 3:1. Backcrossing of the F_1 to the resistant parent, produced resistant progenies. However, the backcross of the F_1 to the susceptible parent gave 48 resistant to 32 susceptible plants with X^2 of 3.20 which was not significant (P=0.06-0.08). The data fits a 1:1 segregation which confirmed the nypothesis of a single dominant gene inheritance.

The disease grades distribution of the F_2 generations of the cross (S295 x SAMCOT-10) fit closely a 3:1 ratio, (X^2 = 0.25, P=0.75-0.05) Table 19. The backcross population produced 50 resistant: 30 susceptible plants, approximating 62.5 and 37.5% resistant to susceptible progenies respectively. More resistant plants then would be expected for a single gene inheritance. The X^2 value 5.00 was significant (P=0.025) suggesting that the data did not adequately fit a 1:1 ratio.The lack of fit may be as a result of the effect of the environment or differential virulence of the isolates

Table 19. Segregation for leaf disease reactions to isolate 1 of Xanthomonas campestris pv. malvacearum among F_1, F_2, BC_1 and BC_2 generations inoculated in the field at Maigana.

Progenies	Expected segregation	Observed segregation Number		X^2value	P
		Resistance	Susceptible		
		Number of plants			
SAMCOT-9 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-9)	1:1	48	32	3.20	0.06-0.08
F_2	3:1	150	42	1.00	0.50-0.25
SAMCOT-10 X 5295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-10)	1:1	50	30	5.00	0.05-0.025
F_2	3:1	147	45	0.25	0.75-0.50
SAMCOT-6 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-6)	1:1	47	33	2.50	0.25-0.10
F_2	3:1	154	38	2.78	0.10-0.05
SAMCOT-8 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-8)	1:1	46	34	0.80	0.50-0.25
F_2	3:1	152	40	1.77	0.25-0.10

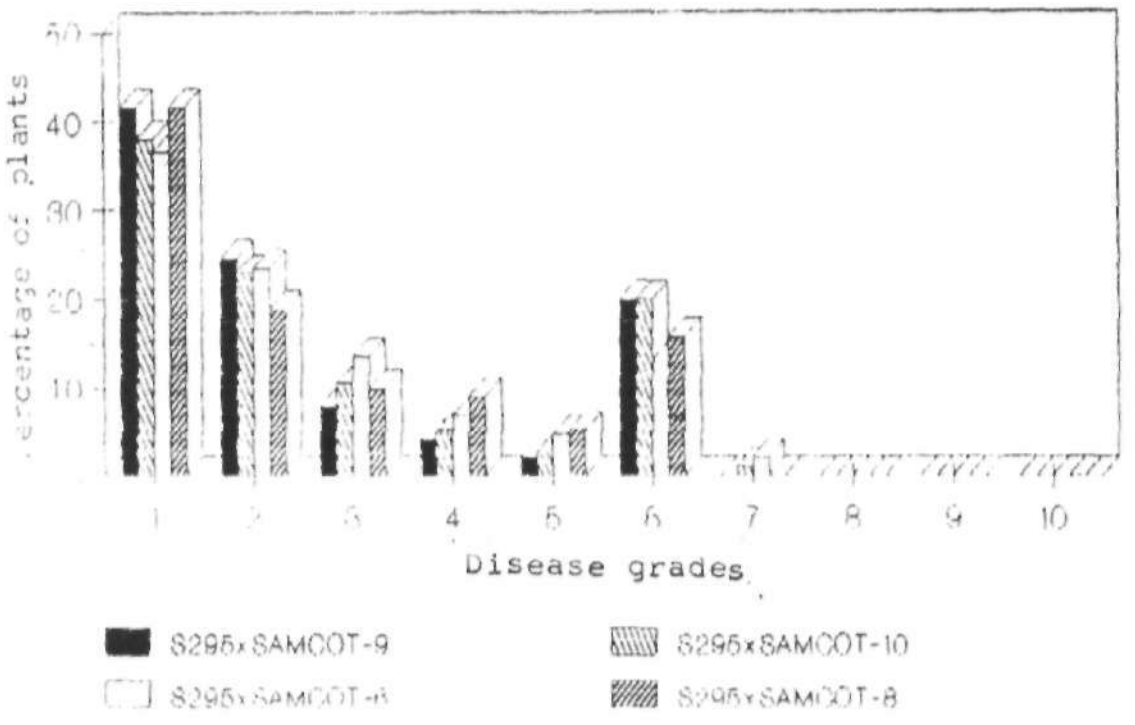


Fig. 7. F_2 frequency histograms of leaf disease grades for the cotton crosses, S295 SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8, inoculated with isolate 1 of Xanthomonas campestris pv malvacearum at Maigana.

used.

The disease grades recorded at Maigana were lower than those at Samaru and Daudawa. In the crosses (S295 x SAMCOT-6) and (S295 x SAMCOT-8), the disease grades distribution in their F_2 population closely fitted a 3:1 ratio; X^2 values were not significant (P=0.25-0.10). The segregation in the backcross population also fitted a 1:1 ratio.

For the inoculation with isolate 2, the F_2 array from the cross (S295 x SAMCOT-9) segregated into 157 resistant: 35 susceptible plants (Table 20) with X^2 of 4.69 which was significant (P=0.055-0.025) indicating that the data did not fit adequately a 3:1 ratio. There were 13 more resistant plants than would be expected under the hypothesis of a 3:1 ratio. The backcross population (BC_2) produced 49 resistant to 31 susceptible plants. The distributions in the F_2 and backcross generations of the crosses (S295 x SAMCOT-6) and (S295 x SAMCOT-8) satisfactorily fit a 3:1 and 1:1 phenotypic ratio, respectively.

The F_1 populations of the crosses (S295 x SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8) were all resistant to the 1:1 mixture of isolates 1 and 2 (Table 21), suggesting that dominant genes govern resistance. The frequency distribution of the F_2 array approached a 3:1 ratio, X^2=1.77 for (S295 x SAMCOT-9) F_2;

Table 20. Segregation for leaf disease reactions to isolate 2 of Xanthomonas campestris pv. malvacearum among F_1, F_2, BC_1 and BC_2 generations inoculated in the field at Maigana.

Progenies	Expected segregation	Observed segregation Number		X^2value	P
		Resistance	Susceptible		
		Number of plants			
SAMCOT-9 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-9)	1:1	49	31	2.05	0.25-0.10
F_2	3:1	157	35	4.69	0.05-0.025
SAMCOT-10 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-10)	1:1	48	32	3.20	0.06-0.08
F_2	3:1	148	44	0.44	0.75-0.50
SAMCOT-6 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295		80	0	-	-
$BC_2(F_1$ X SAMCOT-6)	1:1	46	34	1.80	0.25-0.10
F_2	3:1	144	48	0	1
SAMCOT-8 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-8)	1:1	43	37	0.45	0.75-0.50
F_2	3:1	152	40	1.77	0.25-0.10

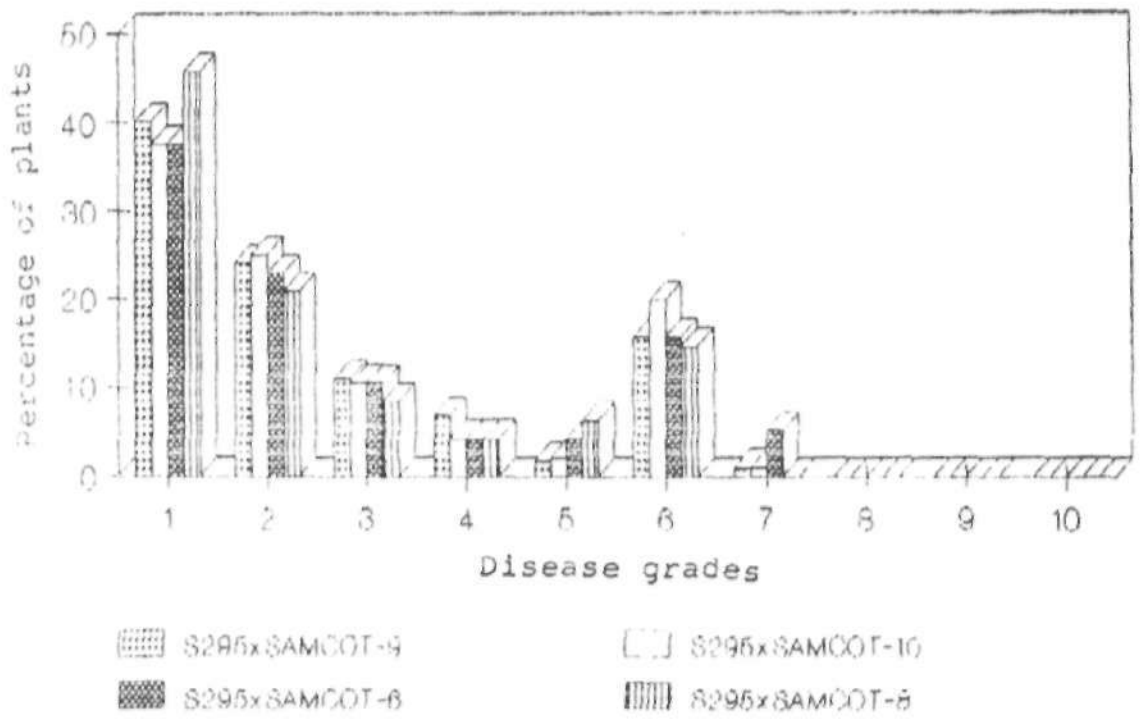


Fig. 8. F_2 frequency histograms of leaf disease grades for the cotton crosses, S295 x SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8, inoculated with isolate 2 of Xanthomonas campestris pv malvacearum at Malyana.

X^2=1.36 for (S295 x SAMCOT-10)F_2, X^2=1.00 for (S295 x SAMCOT-6)F_2 and X^2 = 2.25 for (S295 x SAMCOT-8)F_2, respectively. These X^2 values with 1 degree of freedom were not significant (Table 21). In the backcross population, the observed segregation fitted a 1:1 resistant:susceptible ratio for all the crosses except for the BC_2 (F_1 x SAMCOT-10) where the X^2 = 4.05 and this was significant (P=0.05-0.025). Apparently there were more resistant plants than expected for a 1:1 segregating ratio.

Considering the total number of plants for the four F_2 segregating population, the observed segregation was 608 resistant: 160 susceptible plants. The calculated X^2 of 6.89, which is significant (P=0.015-0.008), suggests that the data did not adequately fit the segregating ratio for a single gene hypothesis. Two crosses, (S295 x SAMCOT-10) and (S295 x SAMCOT-8) contributed more than 65% of this X^2 value.

Table 21. Segregation for leaf disease reactions to a mixture of isolates 1 and 2 of Xanthomonas campestris pv. malvacearum among F_1, F_2, BC_1 and BC_2 generations inoculated in the field at Maigana.

Progenies	Expected segregation	Observed segregation Number		X^2value	P
		Resistance	Susceptible		
		Number of plants			
SAMCOT-9 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-9)	1:1	47	33	2.46	0.25-0.10
F_2	3:1	152	40	1.77	0.25-0.10
SAMCOT-10 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-10)	1:1	49	31	4.06	0.50-0.025
F_2	3:1	153	39	2.25	0.25-0.10
SAMCOT-6 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-6)	1:1	48	32	3.20	0.06-0.08
F_2	3:1	150	42	1.00	0.50-0.25
SAMCOT-8 X S295					
F_1		40	0	-	-
$BC_1(F_1$ X S295)		80	0	-	-
$BC_2(F_1$ X SAMCOT-8)	1:1	44	36	0.80	0.50-0.25
F_2	3:1	153	39	2.25	0.25-0.10

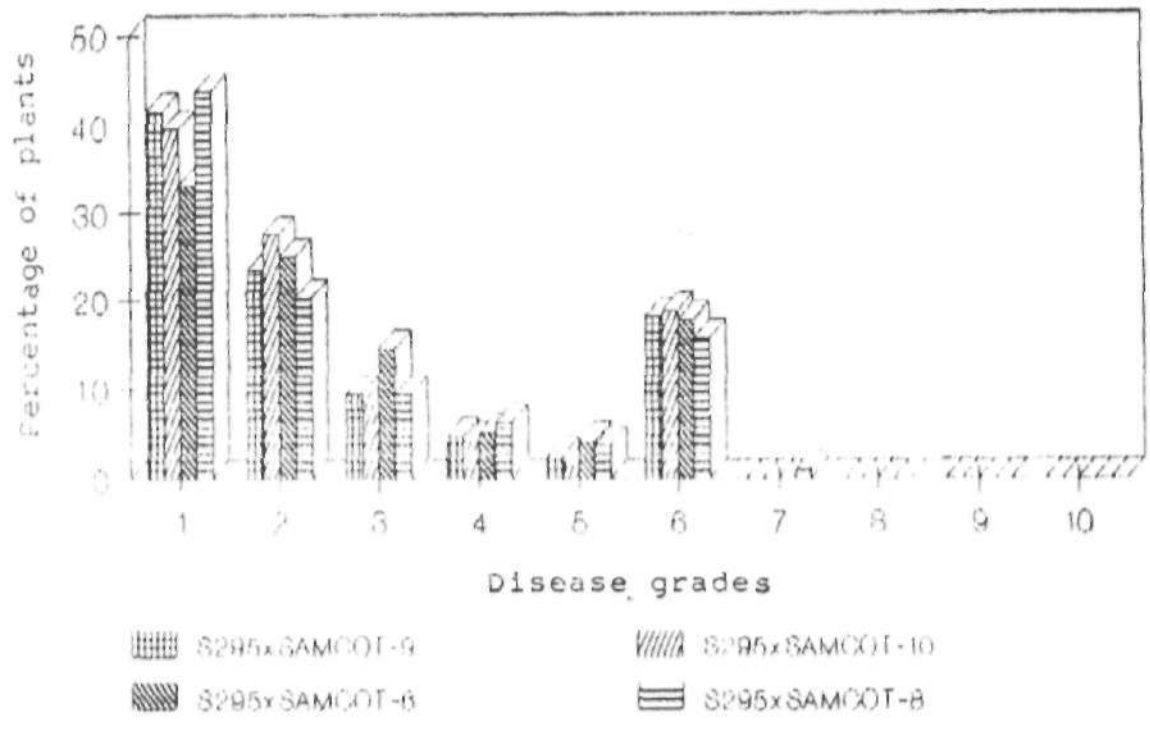


Fig. 9. F_2 frequency histograms of leaf disease grades for the cotton crosses, S295 x SAMCOT-9, S295 x SAMCOT-10, S295 x SAMCOT-6 and S295 x SAMCOT-8, inoculated with a 1:1 mixture of isolate 1 and 2 of Xanthomonas campestris pv malvacearum at Malgana.

4.2 Screenhouse experiment

Cotyledon and true-leaf disease grades of the parental population inoculated with isolate 1 ranged from 1.0 to 5.7 and 1.0 to 5.1, respectively (Table 22.) while for isolate 2, the range was 1.0 to 5.2 and 1.0 to 5.0 for cotyledon and true-leaf, respectively. The cultivar S295 showed immunity at both growth stages to the two isolates.

The Multi-adversity resistant cultivars also maintained high resistance irrespective of isolate. It is interesting to note that SAMCOT-9 and SAMCOT-10 showed disease reaction in the resistant range while the disease grades of SAMCOT-8 and SAMCOT-6 at the two growth stages were in the susceptible range, although SAMCOT-8 was less susceptible than SAMCOT-6. Analysis of parental and backcross means for all crosses indicated that there were differences among the parents for resistance to either of the isolates. Cotyledon and true-leaf disease grades were similar for any parent, however, true-leaf disease grades were slightly lower than the cotyledon grades for any of the isolates. The mature true-leaves were less susceptible than the cotyledons.

Bimodal F_2 disease grade frequency distributions were obtained for the growth stages of the four crosses (S295 x SAMCOT-9) F_2; (S295 x SAMCOT-10) F_2; (S295 x SAMCOT-6) F_2 and (S295 x SAMCOT-8) F_2. (Fig 10-13).

Table 22. Cotyledon and true-leaf disease grades of cotton cultivars inoculated with isolates of Xanthomonas campestris pv. malvacearum in the screenhouse.

Parents	Isolate 1 cotyledon	Isolate 1 true-leaf	Isolate 2 cotyledon	Isolate 2 true-leaf	Mean of isolates
S295	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a
TX-CDP37HH-1-83	2.7 b	2.4 b	2.5 b	2.3 b	2.5 b
TAMCOT SP21S	2.8 b	2.5 b	2.6 b	2.4 b	2.6 b
TAMCOT-CAMD-E	3.3 c	2.4 b	2.9 bc	2.4 b	2.8 b
SAMCOT-9	3.6 c	3.4 c	3.2 c	3.0 c	3.3 c
SAMCOT-10	3.7 c	3.2 c	3.4 c	3.1 c	3.4 c
SAMCOT-6	5.7 e	5.1 e	5.2 e	5.0 e	5.3 e
SAMCOT-8	4.9 d	4.5 d	4.4 d	4.3 d	4.5 d

Disease grades are based on a scale of 1 (immunity) to 10 (fully susceptible). Any two mean grades within a column having a letter in common are not significantly different at P = 0.05 according to Duncan's new multiple range test.

Table 23. Segregation for cotyledon and true-leaf disease reactions to isolate 1 of *Xanthomonas campestris* pv. *malvacearum* among F_1, F_2, BC_1 and BC_2 generations inoculated in the screenhouse.

Progenies	Expected segregation	Cotyledons				True-leaves			
		Observed segregation				Observed segregation			
		R	S	X^2 value	P	R	S	X^2 value	P
		Number of plants				Number of plants			
SAMCOT-9 X S295									
F_1		47	0	-	-	30	0	-	-
$BC_1(F_1$ X S295)		96	0	-	-	64	0	-	-
$BC_2(F_1$ X SAMCOT-9)	1:1	55	41	2.40	0.25-0.10	38	26	2.26	0.25-0.10
F_2	3:1	147	45	0.25	0.75-0.50	98	30	0.17	0.75-0.50
SAMCOT-10 X S295									
F_1		48	0	-	-	32	0	-	-
$BC_1(F_1$ X S295)		96	0	-	-	64	0	-	-
BC_2(SAMCOT-10)	1:1	57	39	3.38	0.10-0.05	34	30	0.25	0.75-0.50
F_2	3:1	142	50	0.11	0.75-0.50	100	28	0.67	0.50-0.25
SAMCOT-6 X S295									
F_1		45	0	-	-	32	0	-	-
$BC_1(F_1$ X S295)		94	2	-	-	64	0	-	-
$BC_2(F_1$ X SAMCOT-6)	1:1	52	44	0.67	0.50-0.25	36	28	1.00	0.30-0.35
F_2	3:1	138	54	1.00	0.30-0.35	96	32	0	1
SAMCOT-8 X S295									
F_1		45	0	-	-	32	0	-	-
$BC_1(F_1$ X S295)		96	0	-	-	64	0	-	-
$BC_2(F_1$ X SAMCOT-8)	1:1	50	46	0.16	0.75-0.50	35	29	0.56	0.50-0.25
F_2	3:1	143	49	0.03	0.90-0.75	93	35	0.38	0.75-0.50

R= resistant, S= susceptibility.

Table 24: Segregation for cotyledon and true-leaf disease reactions to isolate 2 of *Xanthomonas campestris* pv. *malvacearum* among F_1, F_2, BC_1 and BC_2 generations inoculated in the screenhouse.

Parents and Progenies	Expected segregation	Cotyledons: Observed segregation R	S	X^2value	P	True-leaves: Observed segregation R	S	X^2value	P
		Number of plants				Number of plants			
S295		48	0	-	-	32	0	-	-
SAMCOT-9									
SAMCOT-10									
SAMCOT-6		0	48	-	-	0	32	-	-
SAMCOT-8		4	44	-	-	0	32	-	-
SAMCOT-9 X S295									
F_1		48	0	-	-	31	0	-	-
BC_1(F, X S295)		96	0	-	-	64	0	-	-
BC_2(F, X SAMCOT-9)	1:1	53	43	1.04	0.50-0.25	37	27	1.6	0.25-0.10
F_2	3:1	152	40	1.78	0.25-0.10	101	27	1.04	0.50-0.25
SAMCOT-10 X S295									
F_1		48	0	-	-	30	0	-	-
BC_1(F, X S295)		96	0	-	-	64	0	-	-
BC_2(SAMCOT-10)	1:1	51	45	0.38	0.75-0.50	35	29	0.56	0.50-0.25
F_2	3:1	149	43	0.69	0.50-0.25	97	31	0.04	0.90-0.75
SAMCOT-6 X S295									
F_1		48	0	-	-	32	0	-	-
BC_1(F, X S295)		96	0	-	-	64	0	-	-
BC_2(F, X SAMCOT-6)	1:1	49	47	0.04	0.90-0.75	33	31	0.06	0.90-0.75
F_2	3:1	150	42	1.00	0.30-0.35	94	34	0.17	0.75-0.50
SAMCOT-8 X S295									
F_1		48	0	-	-	32	0	-	-
BC_1(F, X S295)		96	0	-	-	64	0	-	-
BC_2(F, X SAMCOT-8)	1:1	54	42	1.50	0.25-0.10	30	34	0.25	0.75-0.50
F_2	3:1	147	45	0.25	0.75-0.50	92	36	0.67	0.50-0.25

R = resistant, S = susceptibility.

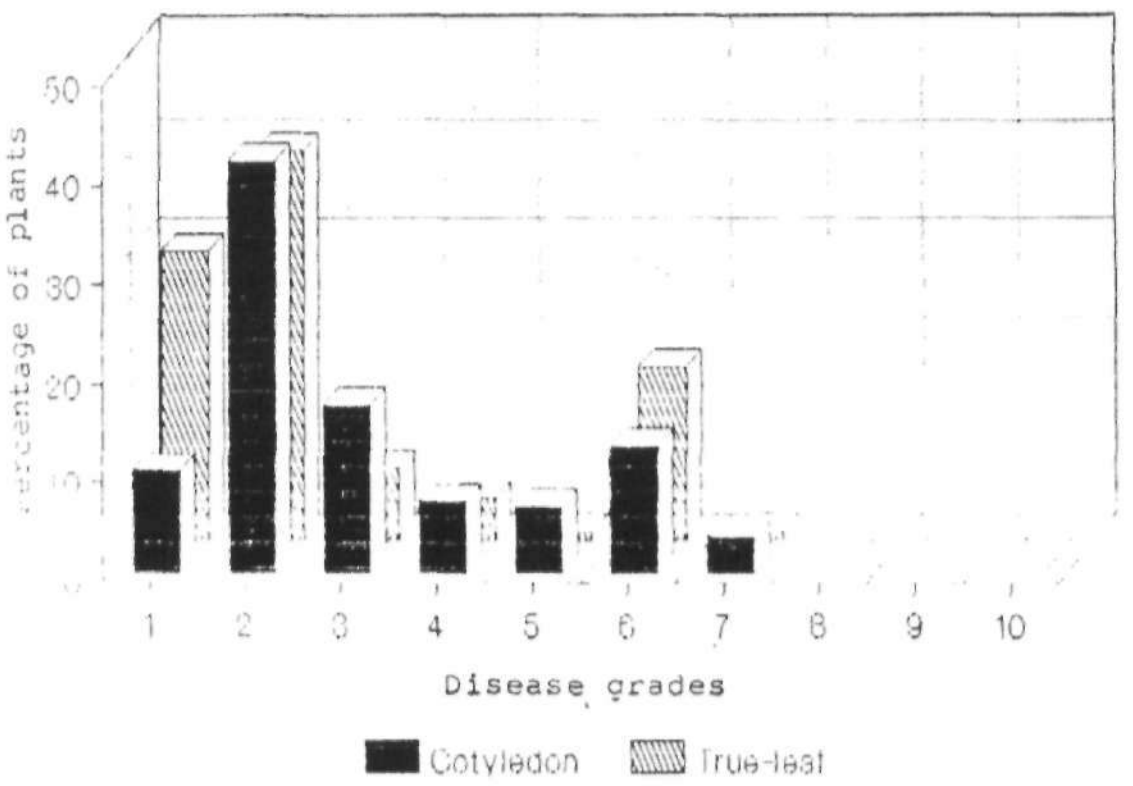


Fig. 10. F_2 frequency histograms of cotyledon and true-leaf disease grades for the cotton cross S295 x SAMCOT- 9, inoculated with isolate 1 of Xanthomonas campestris pv malvacearum in the screenhouse.

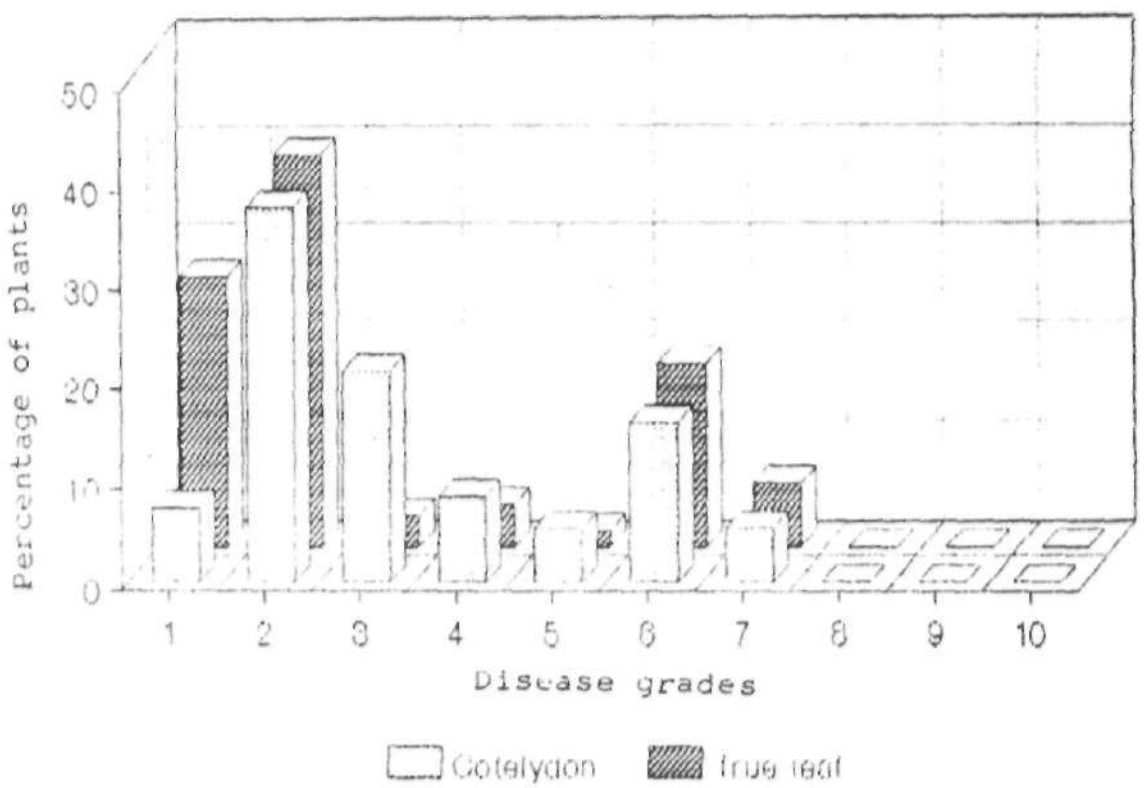


Fig. 11. F_2 frequency histograms of cotyledon and true-leaf disease grades for the cotton cross S295 x SAMCOT-10, inoculated with isolate 1 of Xanthomonas campestris pv malvacearum in the screenhouse.

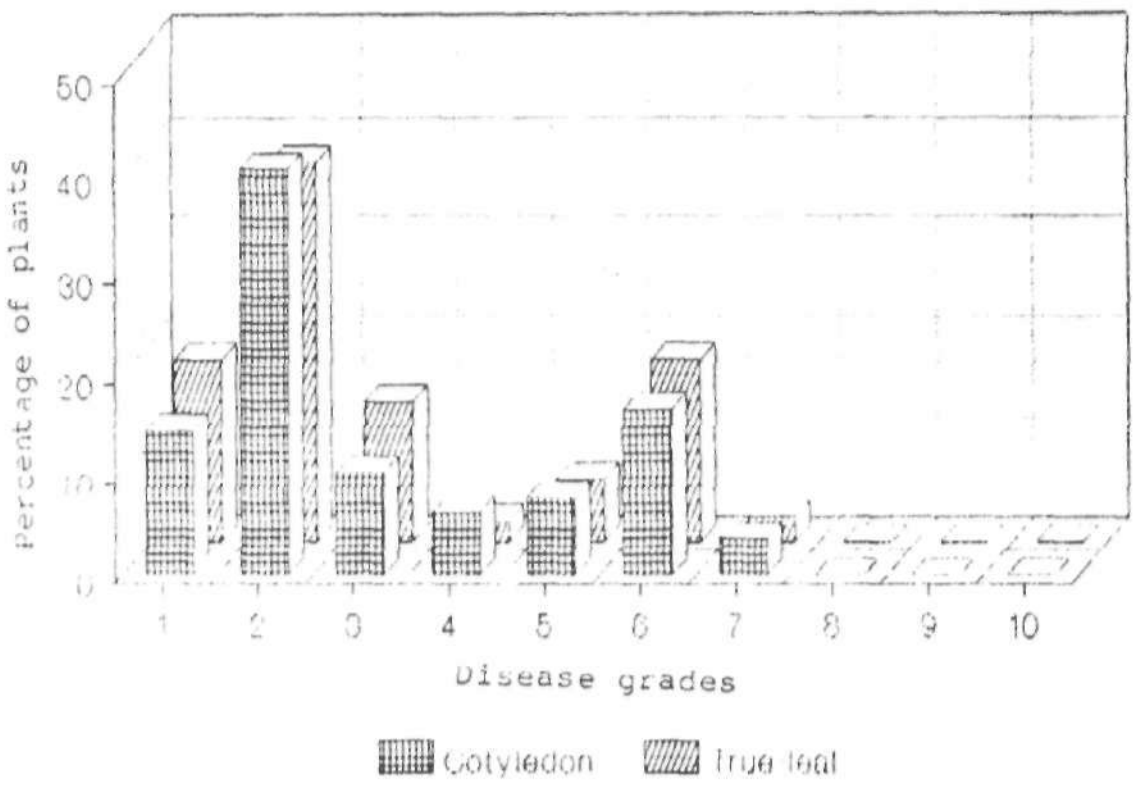


Fig. 12. F_2 frequency histograms of cotyledon and true-leaf disease grades for the cotton cross S295 x SAMCOT-6, inoculated with isolate 1 of Xanthomonas campestris pv malvacearum in the screenhouse.

Disease grades distributions indicated a resistant and susceptible class represented by Grades 1-4 and 5-10, respectively. Chi-square test for goodness of fit to a 3:1 resistant: susceptible phenotypic ratio was not significant. When the F_1s were backcrossed to the susceptible parent, the BC_2 populations exhibited a bimodal distribution. The data gave a close fit to a 1:1 ratio. The backcross to the resistant parent gave resistant progenies.

When cotyledons and true leaves were inoculated with isolate 2, a bimodal F_2 disease grades distributions were observed (Figures 14-17) and a 3:1 phenotypic ratio was the only acceptable fit. The backcross population, BC_1 yielded resistant progenies while the BC_2 gave a closs fit to a 1:1 ratio. The distribution in the F_2 and backcross populations suggested major gene effects with dominance in the direction of resistance.

Cotyledon and True-leaf correlation.

Correlation coefficients using replication means of disease grades revealed a positive correlation between cotyledon and true-leaf disease grades for isolate 1 (r=0.73), and isolate 2 (r=0.68), respectively (Table 25). The age of the cotyledon or true-leaf appeared to influence the degree of disease expression. In general, younger cotyledons and true-leaves of susceptible plants

Table 25: Correlation coefficients of cotyledon and true-leaf growth stages for isolates 1 and 2 of Xanthomonas campestris pv. malvacearum.

Isolate 1

	Cotyledon	Leaf
Cotyledon	1.000	
Leaf	0.731	1.000

Isolate 2

	Cotyledon	Leaf
Cotyledon	1.000	
Leaf	0.680	1.000

scored higher on the disease grading scale than did older tissues.

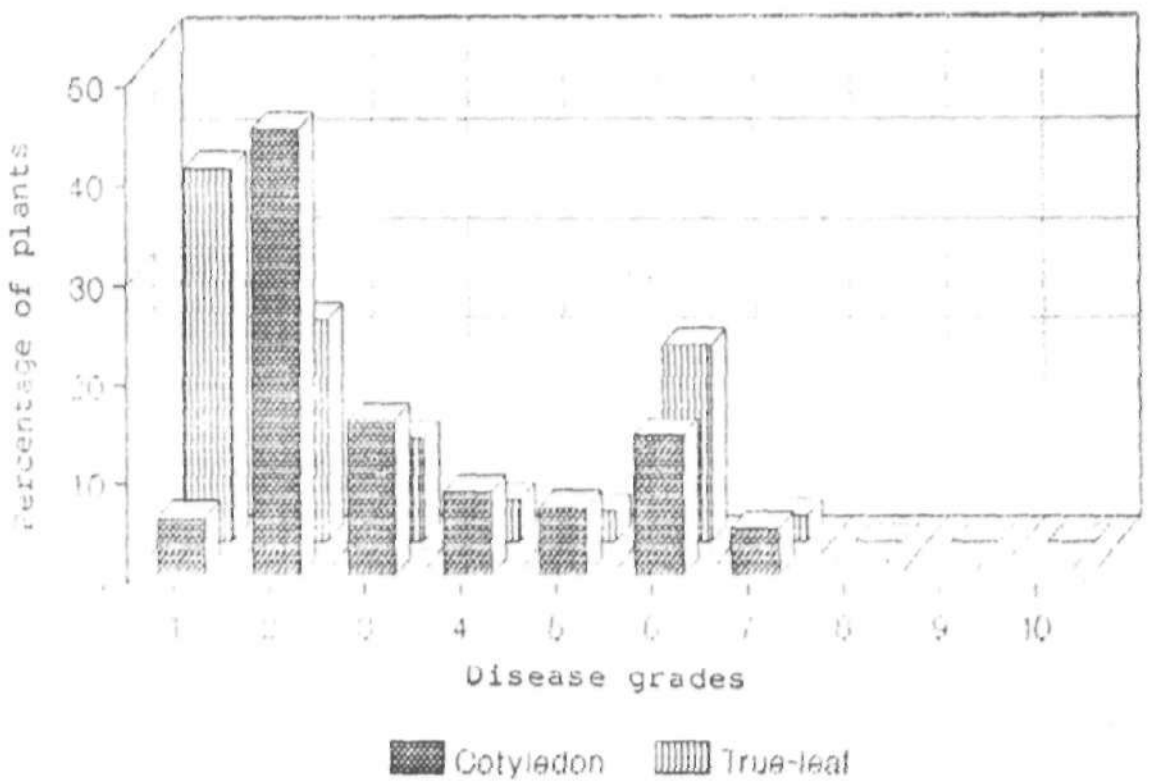


Fig. 13. F_2 frequency histograms of cotyledon and true-leaf disease grades for the cotton cross S295 x SAMCOT-8, inoculated with isolate 1 of Xanthomonas campestris pv malvacearum in the screenhouse.

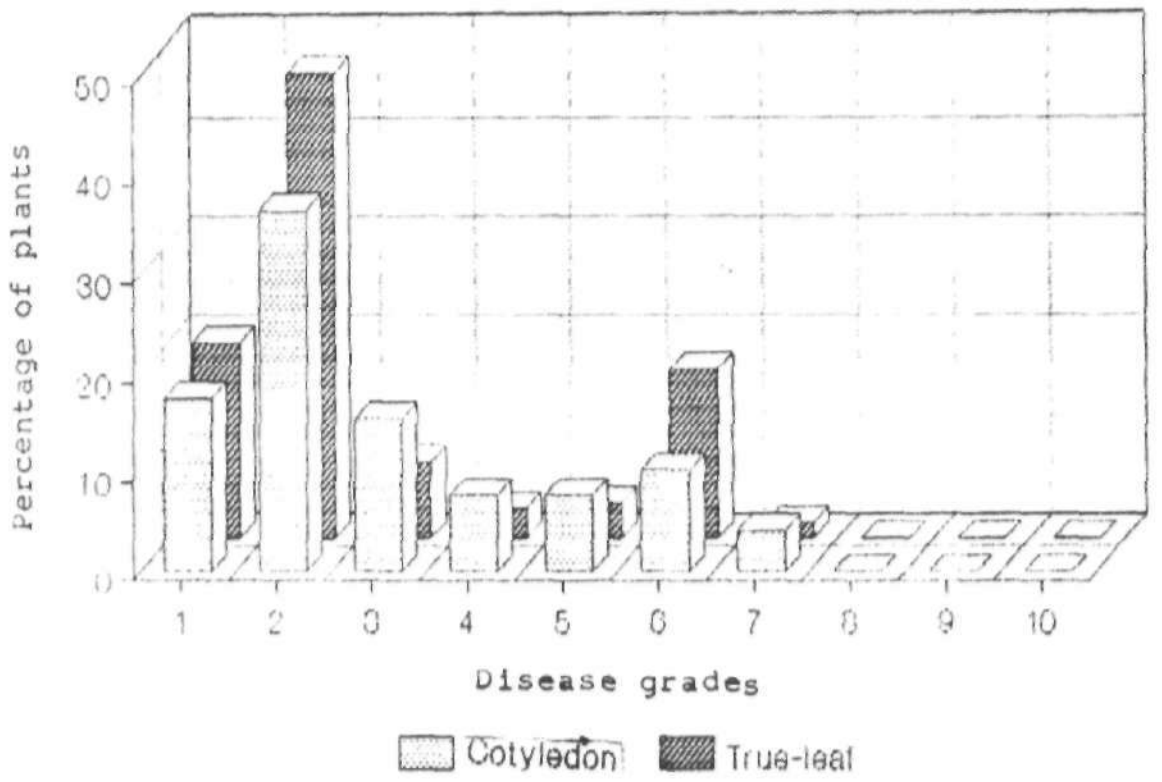


Fig. 15. F_2 frequency histograms of cotyledon and true-leaf disease grades for the cotton cross S295 x SAMCOT-10, inoculated with isolate 2 of Xanthomonas campestris pv malvacearum in the screenhouse.

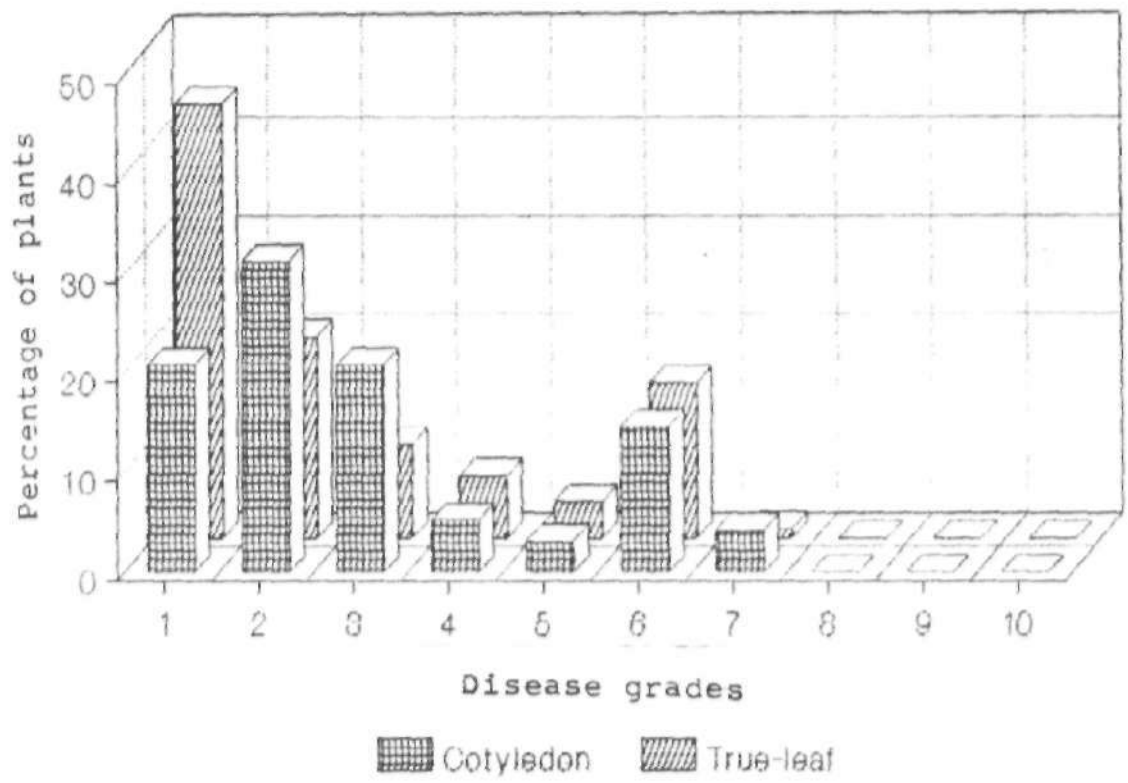


Fig. 16. F_2 frequency histograms of cotyledon and true-leaf disease grades for the cotton cross S295 x SAMCOT-6, inoculated with isolate 2 of Xanthomonas campestris pv malvacearum in the screenhouse.

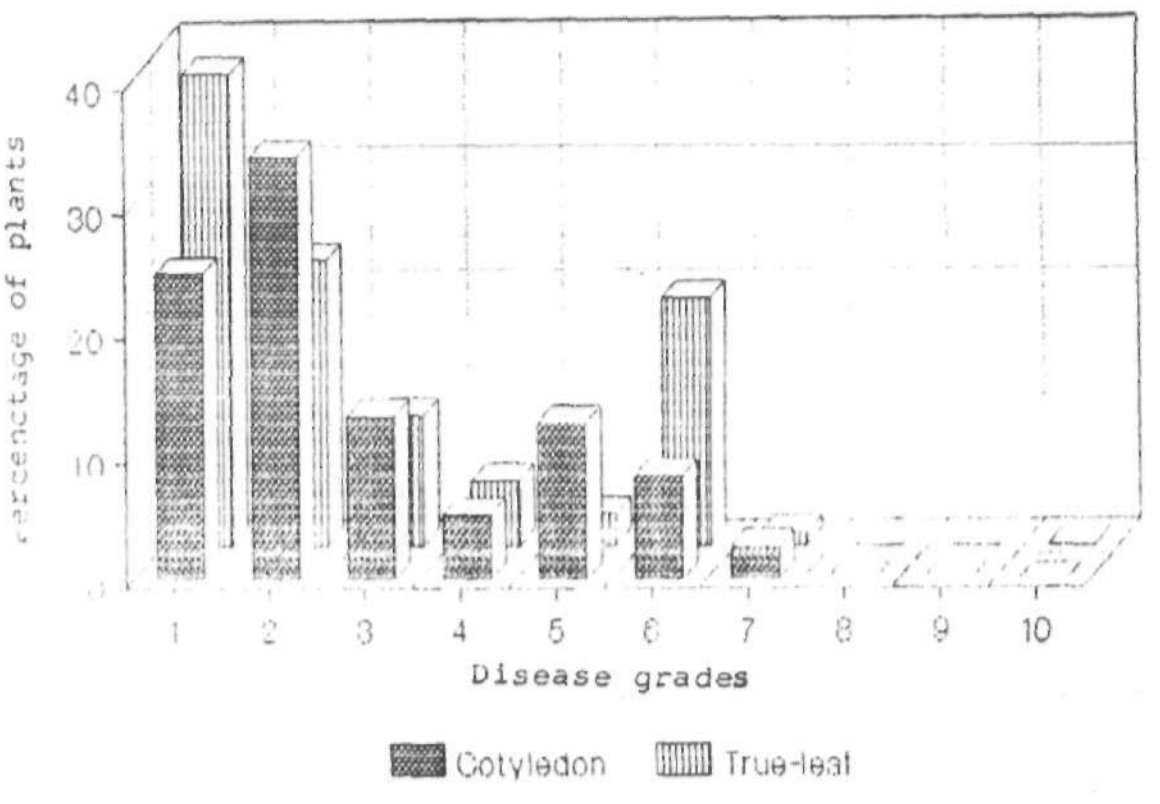


Fig. 17. F_2 frequency histograms of cotyledon and true-leaf disease grades for the cotton cross S295 x SAMCOT-8, inoculated with isolate 2 of Xanthomonas campestris pv malvacearum in the screenhouse.

CHAPTER 5

DISCUSSION

The differential response of the parental cultivars and their crosses to the different isolates in the different locations could partly be attribited to the influence of the environment as portrayed by the significance of the population x location interaction. These results are in agreement with those obtained by Bird and Hadley (1958), Arnold (1963), Arnold and Brown (1968) and Innes et al., (1974), who have also reported significant genotype x environment interaction in various studies on bacterial blight resistance in cotton. Such interactions of genotype with environment might partly explain the differences in relative resistance shown by a range of host varieties when tested under different conditions, such as those observed from one country to another or from one tissue to another on the same host variety (Arnold and Brown, 1968). These differences are associated both with the mean expression of resistance in a host population and with its variance. The differences in the expression of genetic variation from one country to another have affected both the approach to breeding for resistance and the genetical interpretation of results obtained.

In the present investigation, although the relative resistance shown by host varieties differed both with the

isolate of the pathogen used for inoculation and with environmental conditions, over-riding patterns of host resistance could be detected, showing that certain varieties maintained their resistance over a wide range of conditions. The near immunity of the cultivar S295 at all locations is striking. It was demonstrably more resistant than the multi-adversity resistant cultivars which nevertheless, maintained high resistance at all locations as well.

In addtion to the influence of the environment, the genetic variation in the parasite is also an important factor that affects disease expression. The results of the present study show that the disease grades under isolate 1 were higher than those observed under isolate 2 while the disease reaction under isolate 1 and a 1:1 mixture of isolates 1 and 2 were comparable. This trend tends to suggest variation in virulence or aggressiveness of the pathogen. As has been noted by Arnold (1963), variation in the severity of disease expression with respect to lesion development after inoculation, is not only markedly affected by environmental conditions but that these effects differed with the genotype of the variety and pathogenicity of the isolates under test.

Arnold and Brown (1968) have postulated that the host parasite relationship is a dynamic system in which disease expression is a function of the interaction of

suggests that resistance to all isolates of the pathogen tested would be multigenic in nature. However, observation from the field and screenhouse experiments clearly indicated monogenic inheritance of disease resistance.

The monogenic inheritance of disease resistance indicates that the backcross breeding method would be appropriate in transferring resistance, if the cultivar S295 is used as a source of total resistance. Nevertheless, there remains the question of durability of this single gene resistance. Theoretically, polygenes are thought to confer stable resistance, and qualitative genes confer non-stable resistance (Nelson, 1978). In contrast to immunity as conditioned by multiple genes, resistance to bacterial blight of cotton as conferred by single genes has repeatedly proved to be ineffective because of the appearance of new races of the pathogen (Brinkerhoff and Verhalen, 1976). Exceptions exist however in which single-gene resistance has remained stable for considerable period of time (Robinson, 1976).

There has been conflicting evidence for a basic mechanism controlling resistance to bacterial blight in different plant parts and at different stages of plant growth. In the present study, cotyledon and true-leaf disease grades were positively correlated and when monogenic inheritance was observed at the cotyledon stage

of growth, the same was observed in the true-leaf.Similar observation was reported by Wallace and El-Zik (1989). While Bird (1950) and Lagière (1960) have also reported positive correlation for the seedling and adult plant resistance. Wickens (1953) and Last (1959) did not find any relationship between these growth stages. The results obtained in the present study support the concept of a common basic mechanism controlling resistance in the two different stages of plant growth and suggest that selection for resistance could be accomplished in either growth stage.

Arnold (1963) discussed correlations between stem, boll and leaf resistance in cotton and inferred that there was a basic resistance mechanism in all tissues which is under the same genetic control but results may become confused by genotype x environment interactions and by other factors operating in particular tissues. When considering, for example, the relationship between resistance in leaves and bolls on the same plant inoculated at different times even with the same bacterial strain the infected organs reacted in different ways because they are subjected to different conditions.

To conclusively prove the hypothesis of a common basic resistance mechanism in all tissues, it would be necessary to select for resistance to one phase of the disease and study the effect of such selection on

resistance to the other phases. Arnold and Brown (1968) have stressed on the desirability in cotton breeding programmes to screen for resistance at different stages of plant growth and in a range of environments. Programmes based on alternate testing sites are more likely to produce plants with well-balanced resistance mechanisms than are those in which genotypes evolve in isolation.

Implications of results and approach to resistance breeding.

The monogenic inheritance observed in the study suggests, from a practical point of view that the backcross method could be used to transfer the major gene resistance to susceptible types.

Knight (1946) produced a series of resistant varieties by repeatedly backcrossing to commericially acceptable varieties. However, a major criticism of this method has been that such a system does not exploit genetic variability for characters other than resistance, and is unlikely, within a reasonable working period, to restore a critical balance between genotype and environment, which has been built up by continued selection in modern varieties of rain-grown cotton (Arnold. 1963). Moreover, it may prove impossible to retain the factors for resistance as a genetic entity through succession of backcross generations.

An alternative approach to this problem is to establish recombinant lines by selection amongst F_2 population or early backcross progenies with the objective of producing new balanced genotypes by prolonged selection. These anticipated new types may exceed in economic worth the sum of the different contributions of their parents. Another alternative is to exploit genetic variability for resistance, detected by critical examination of locally adapted stocks (Arnold, 1963).

As a result of the uncertainty of the durability of a major gene resistance, Follin et al., (1988) have suggested that rather than breed for total resistance, it might just be encouraging for breeders to develop cultivars with as much horizontal resistance as possible through recurrent selection method, which encourages the accumulation of minor genes which can lead to the creation of new linkages which behave like major genes.

Genetic studies have indicated that the combination of two or more major genes and a modifier complex is responsible for the observed immunity in commercial cotton varieties grown in the USA for more than two decades (El-zik and Bird, 1970; Innes, 1974 and Bird, 1982).

Brinkerhoff et al., (1984) described a breeding procedure which may be useful in deriving long term immunity to bacterial blight of cotton. This involves combining several single gene resistance factors preferrably two or more genes with large effect unto a polygenic resistance background. The initial breeding procedure involves backcrossing, with the recurrent parent possessing the polygenic resistance, screening the segregating progeny after each backcross with a compatible mixture of virulent races of the pathogen and selfing of the selected plants follow thereafter. Pedigree breeding with continued screening and selfing is employed in later generations. The success of this approach depends greatly on the availability and use of pathogen genotypes that permit consistent and rigorous screening of plant genotypes in segregating generations (Nelson, 1978).

The broad aim of the cotton breeding programme in Nigeria is to develop new varieties of cotton having higher yields, improved resistance to pests and diseases and lint quality acceptable to the Nigerian textile industry. Most of the varieties grown in Nigeria are selections from crosses involving Samaru Allen which was developed from an Allen stock introduced into the country in 1912. Other types such as Albar, Bar, Reba and BJA some of which are resistant to

bacterial blight were also introduced and tested in Nigeria. Most of the introductions were found to be unsuitable for direct utilization but various crosses were made between Samaru Allen, (SA) and the exotics which resulted in several families having a high degree of resistance as well as good yield and quality characteristics. Among these families, the ASA (Albar x Samaru Allen), RSA (Reba x Samaru Allen) and RASA (Reba or BJA x (ASA x ASA) were found to be promising, with the RASA being the most outstanding in terms of blight resistance.

It is of interest to note, however, that at the time of initial crossing and subsequent selection involving the exotics and the local varieties, the most predominant race of Xanthomonas campestris pv. malvacearum, present in Nigeria was race 2. It is therefore possible that the impressive level of resistance shown by the RASA family is to one race of the pathogen. This might probably explain the suspected break down of resistance in the Samaru varieties as new and more virulent races of the bacterial blight pathogen have been identified by Powal (1981 and 1988).

The relative resistance shown by host varieties and their cross progenies in the present investigation showed some differences both with the isolates and environments; these differences were generally not too obvious. The

isolates used in the study were isolated from infected leaf samples collected from localities where the pathogen have been known to evolve; although the isolates were not tested on any host differential lines for proper categorization into the race group that they belong. This is probably a major draw back in this study, as the two isolates could be said to be narrow-based and not truely representative of the variation in levels of virulence and aggresiveness within the Nigerian population of Xanthomonas campestris pv. malvacearum.

The use of a mixture of the two isolates for inoculation was conceived as a way to further increase the level of virulence and broad spectrum of the inocula, as the development of immunity in cotton to bacterial blight has been successful, utilising a mixture of races for screening germplasm (Bird, 1982). Arnold and Brown (1968) indicated that the bacterial pathogen expresses a continous variation in virulence and suggested that variants should not be tested separately but in a mixture when screening for host resistance while Verma and Bokar (1984) concluded from their study that inoculation with a mixture composed of a virulent and avirulent race of X. campestris pv. malvacearum on a susceptible cultivar results in a reduction in disease severity compared to inoculation with the virulent isolate alone.

From the results of the present investigation, it is suggested that for future screening and testing in the breeding programme an inoculum with a broad range of virulence should be used. In addition, testing of materials for resistance to bacterial blight should be done across locations (environments) under conditions which favour the expression of variation. This will allow a critical analysis of population variances. A population which showed little variation for resistance under one set of conditions might show considerable variation in a different environment or when inoculated with a different isolate.

Evaluation for resistance at both cotyledon and true-leaf stages would give an added measure of assurance in correctly identifying resistant genotypes. These suggestions would be valuable in selection and breeding for stable resistance to bacterial blight of cotton in Nigeria.

CHAPTER 6

SUMMARY AND CONCLUSIONS

A genetic study of bacterial blight resistance in cotton which hitherto has received little or no attention in Nigeria was conducted. The major objectives of the study were; to determine the nature and mode of inheritance of bacterial blight resistance in cotton varieties developed at the Institute for Agricultural Research, A.B.U., Samaru, Zaria and elsewhere; to examine environmental effects on resistance to bacterial blight; and to examine the relationship between resistance at the cotyledon stage with that at the true-leaf stage.

Eight parent materials varying in their differential response to Xanthomonas campestris pv malvacearum were selected for this study with the variety S295 as the major source of resistance.

Initial crossing between the variety S295 and the other seven varieties started during the off-season of 1991/92. The resulting populations including eight parents, seven F_1s, Seven F_2s, seven F_3s and 14 backcrosses were evaluated for bacterial blight resistance in the field at Samaru, Daudawa, Maigana and also in the screenhouse at Samaru.

Data were analysed in a randomized complete block design with four replications and differences among

parents and their cross progenies were detected. Frequency histograms of F_2 entries were examined to determine the type of distribution exhibited.

The average disease grades of the parental populations indicated differential reaction to the isolates used irrespective of location or environment. The importance of the influence of the environment in a study of this nature has been emphasized and it has been suggested that future screening and testing should be carried out across several environments and with different isolates in order to breed for stable resistance.

The genetics of resistance was not the same for all crosses in this study. In the crosses between the variety S295 and the Multi-adversity resistant (MAR) varieties, their F_2 grade distributions did not indicate a 3:1 phenotypic ratio as resistance was conspicuously skewed towards the resistant parents. The results of this study indicate that caution is necessary when studying different sources of resistance to bacterial blight. The failure of a cross between two resistant lines to produce susceptible plants in the F_2 generation might tend to suggest that the materials are of the same genetic composition but this might not necessarily be true especially when resistance ranges from immunity to tolerance. If inheritance of resistance is quantitative

and gene action is additive in conferring increased resistance, then susceptible plants would not be expected unless large populations were grown.

In the crosses between the variety S295 and SAMCOT-9, SAMCOT-10, SAMCOT-6 and SAMCOT-8, their disease grades distributions in the F_2 and backcross generations indicated that resistance in S295 was dominant and monogenic. It is therefore recommended that the backcross method would be appropriate in transferring the resistant gene to susceptible commercial varieties.

On the mechanism of resistance, the results revealed that the same genetic mechanism was controlling resistance at both the cotyledon and true-leaf growth stages. It is suggested that evaluation at both stages would give an added measure of assurance in identifying resistant genotypes.

Since the time Knight (1945) first reported that genes for resistance to bacterial blight in cotton were accumulative in their effects, cotton breeders have been striving to find combinations of those genes that give the greatest degree of resistance. It is therefore suggested for further research, that a hybridization programme between the multi-adversity resistant (MAR) varieties and the Samaru varieties be embarked upon.

The present investigation is the first conscious attempt to study the genetics of bacterial blight

resistance in cotton in Nigeria. It is therefore concluded that the variety S295 could be used to enhance the level of resistance in Samaru varieties.

The segregating populations that have been developed through this study, could form a base for a breeding programme for resistance to bacterial blight. These populations would be adequately screened to identify resistant materials. In addition, new sources of resistance could be introduced from time to time in order to broaden resistance.

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APPENDIX

Appendix 1. Sources of variation, degrees of freedom and mean square values in the combined analysis of variance incorporating populations, isolates, locations and their interactions.

Source of Variation	DF	MS
Replications	3	0.1065
Populations	5	316.1966 **
Isolates	2	0.8143 *
Locations	2	2.6646 **
Populations x Isolate	10	0.2748 NS
Population x Locations	10	3.4928 **
Isolates x Locations	4	0.7988 *
Populations x Isolates x Locations	20	0.4337 *
Error	1455	0.2674

*, ** significant at 0.05 and 0.01 probability levels, respectively.
NS = Not significant.

Appendix 2. Sources of variation, degrees of freedom and mean square values in the analysis of variance incorporating populations, isolates and their interactions at Samaru.

Sources of Variation	DF	MS
Replications	3	0.0643 NS
Populations	5	129.0487 **
Isolates	2	1.7237 *
Population x Isolates	10	0.8657 *
Error	483	0.4398

*, ** significant at 0.05 and 0.01 probability levels, respectively.
NS = Not significant.

Appendix 3. Sources of variation, degrees of freedom and mean square values in the analysis of variance incorporating populations, isolates and their interactions at Daudawa.

Source of Variation	DF	MS
Replications	3	0.0360
Populations	5	103.4710 **
Isolates	2	0.4045 NS
Populations x Isolates	10	0.1351 NS
Error	483	0.1884

*, ** Significant at 0.05 and 0.01 probability levels, respectively
NS = Not significant.

Appendix 4. Sources of variation, degrees of freedom and mean square values in the analysis of variance incorporating populations, isolates and their interactions at Maigana.

Source of Variation	DF	MS
Replications	3	0.0319
Populations	5	90.6625 **
Isolates	2	0.2839 NS
Population x Isolates	10	0.1414 NS
Error	483	0.1772

*, ** Significant at 0.05 and 0.01 probability levels, respectively
NS = Not significant.

Appendix 5. Sources of variation, degrees of freedom and mean square values in the analysis of variance incorporating populations, locations and their interaction considering isolate 1.

Source of Variation	DF	MS
Replications	3	0.0314
Populations	5	110.3523 **
Locations	2	3.7751 **
Populations x Locations	10	1.8880 **
Error	483	0.2547

*, ** Significant at 0.05 and 0.01 probability levels respectively.
NS = Not Significant.

Appendix 6. Sources of variation, degrees of freedom and mean square values in the analysis of variance incorporating populations, locations and their interaction considering isolates 2.

Source of Variation	DF	MS
Replications	3	0.0603
Populations	5	98.4580 **
Locations	2	0.1975 NS
Populations x Locations	10	0.5943 *
Error	483	0.2499

*, ** Significant at 0.05 and 0.01 probability levels, respectively.
NS = Not significant.

Appendix 7. Sources of variation, degrees of freedom and mean square values in the analysis of variance incorporating populations, locations and their interaction considering mixture of isolates 1 and 2.

Source of Variation	DF	MS
Replications	3	0.0287
Populations	5	107.9359 **
Locations	2	0.2899 NS
Populations x Locations	10	1.8779 **
Error	483	0.3010

*, ** Significant at 0.05 and 0.01 probability levels respectively.
NS = Not significant.

BIOGRAPHY

Name : SAMUEL OLUSEGUN ALABI
Date of Birth : 17th August, 1955.
Business Address : Plant Science Department, Institute for Agricultural Research, P.M.B. 1044, A.B.U., Zaria.
Home Address : No. 60 Area G. A.B.U., Residential Area, A.B.U., Zaria.

Previous Qualification.

Institution attended	from	to	Certificate obtained
Université de Rennes/ ENSAR	1990	1991	DEA, DAA
Ahmadu Bello University Zaria	1980	1982	M.Sc. (Crop breeding)
Ahmadu Bello UNiversity	1975	1978	B.Sc.(Bot.) Hons
College of Arts and Science. Zaria	1973	1975	I.J.M.B. Passed
St. John's College, Kaduna	1969	1973	WASC. Grade 1
Baptist Primary School, Makera, Kaduna South.	1962	1968	primary six.

Post Held since the Award of First Degree.

Organization	From	To	Position
IAR/A.B.U., Zaria.	1979	1980	Graduate Assistant
"	1981	1982	Asst. Reseach Fellow
"	1982	1984	Research Fellow II
"	1987	date	Research Fellow I

Year of Admission for Postgraduate Studies: 1985
Admission Number Ph.D/Agric./12956/1985-1986.