STUDIES ON THE GENETICS OF COCOA SWOLLEN SHOOT VIRUS DISEASE (CSSVD) RESISTANCE IN COCOA

(*THEOBROMA CACAO L.*)

BY

DANIEL DARE

A THESIS PRESENTED TO THE BOARD OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF M.PHIL DEGREE IN BOTANY.

DECEMBER 1999

DECLARATION

I, Daniel Dare, the author of this work do hereby declare that except for references to works of other researchers duly cited, this work is the result of my own original research and that this thesis, either in whole or in part has not been presented for another degree elsewhere.

DR (MRS) A.H. JUMAH
(SUPERVISOR)

DR YAW ADU-AMPOMAH
(CO-SUPERVISOR)

DANIEL DARE
(STUDENT)

DEDICATION

To

My dearest wife,Betsy

And my son Christopher

Who shared this phase

Of my life with me.

ACKNOWLEDGEMENT

I am very grateful to Dr. (Mrs.) Angelina Jumah for her supervision and suggestions at various stages of the work and painstakingly reading through this manuscript. I am also grateful to Dr. Yaw Adu-Ampomah of the Cocoa Research Institute, Tafo who made it possible for me to carry out my experiment in their cocoa plantation and their gauze house. I am thankful to him for his constructive criticism when he was reading through my manuscript and also for his keen and great interest shown throughout the study.

I owe a debt of gratitude to Dr. I.K. Ofori of the Crop Science Department for his help in the statistical analysis and reading through this manuscript, making useful suggestions.

I acknowledge with gratitude the assistance given me by the staff of the plant breeding division of the Cocoa Research Institute of Ghana, Tafo, Mr. Frimpong and Mr. Antwi during the budding and pollination processes.

I also acknowledge the assistance given me by that transmission staff of pathology division, Cocoa Research Institue of Ghana, Mr. Obimpeh and Mr. Asare Bediako.

I sincerely thank the authority of the Cocoa Research of Ghana, especially Dr. L.A.A. Ollenu (deputy director - coffee) and its present director, Dr. G. K. Owusu, for all assistance given to me during my stay at the Institute.

Finally, I thank God for his grace, strength and peace of my mind to carry on in challenging times as this.

TABLE OF CONTENTS

TITLE	PAGE
DECLARATION	i
DEDICATION	ii
ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	ix
LIST OF PLATES	x
SECTION 1	
ABSTRACT	xi
CHAPTER I	
1.0 INTRODUCTION AND LITERATURE REVIEW	1
1.1 Introduction	
1.2 Literature Review	3
1.2.1 Origin, Classification and Historical Background	3
1.2.2 Uses of Cocoa	5
1.2.3 Floral Biology	6
1.2.4 Pollination	7
1.2.5 Classification of cultivated Cocoa	9
1.2.6 Genetics and Breeding	12
1.2.6.1 Chromosome Number, Fertilization And incompatibility barrier in Cocoa	12
1.2.6.2 Overcoming the incompatibility Barrier in Cocoa	14
1.2.6.3 Wilting of the young fruits	14
1.2.7 Pests affecting Cocoa	15

1.2.8 Diseases affecting Cocoa 16

1.2.8.1 Cocoa swollen shoot virus disease (CSSVD) 17

1.2.8.2 Vectors of the CSSVD 20

1.2.8.3 Spread of the CSSVD 21

1.2.8.4 Alternatives hosts of the CSSV 21

1.2.8.5 Disease symptoms 23

1.2.8.6 Virus Isolates 24

1.2.8.7 Control of CSSV Disease 29

1.2.8.8 The Analysis of Variance of Diallel tables 30

1.2.8.9 Concept of General, Specific Combining Abilities in relation to diallel Crossing systems 31

1.2.9.0 Heritability 34

1.2.9.1 Parent-offspring regression 36

CHAPTER II

2.0 MATERIALS AND METHODS 38

2.1 Base population 38

2.2 Methods 39

2.2.1 Clonal Multiplication of Parents 39

2.2.2 Inoculation 40

2.2.2.1 Collection of mealybugs 40

2.2.2.2 Virus source plant 41

2.2.2.3 Feeding cones and cages 41

2.2.2.4 Acquisition feed 42

2.2.2.5 Removal of mealybugs from Virus source plant 42

2.2.3 Screening of progenies for CSSV resistance 44

2.2.4 Mating Design 45
2.2.5 Experimental design and analysis 45
2.3 Investigating selfing using mentor pollen 47

CHAPTER III
3.0 RESULTS
3.1 Comparative study of healthy and Infected Cocoa seedlings. 48
3.1.1 Percentage resistance/susceptibility 48
3.1.2 Leave symptoms 51
3.1.3 Stem Girth 58
3.1.4 Stem height 58
3.1.5 Analysis of variance of Resistance, Stem height and Stem diameter. 62
3.2 The use of mentor pollen to induce self-fertilization in self-incompatible clones 71
3.2.1 Number of pods obtained 71
3.2.2 Number of viable beans obtained per pod 73
3.2.3 Number of pods obtained in crosses not involving mentor pollen 73
3.2.4 Number of viable beans obtained per pod in crosses not involving mentor pollen 76

CHAPTER IV
DISCUSSION 78

SECTION II - SUMMARY AND CONCLUSION 86
SECTION III - SUGGESTED LINES FOR FURTHER INVESTIGATIONS 88
SECTION IV - REFERENCES 89
SECTION V - APPENDICES 95

LIST OF TABLES

TABLE		PAGE
1.	Distinguishing features of different types of cocoa.	11
2.	Alternative wild hosts for CSSV.	22
3.	Ghanaian Cocoa swollen shoot virus isolates.	27
4.	Combining abilities; estimation of GCA parameters	33
5.	Resistance/Susceptibility levels of five Upper Amazons Clones after inoculation with CSSV 1A from CRIG transmission laboratory	49
6.	Resistance/Susceptibility levels of five Upper Amazons F1 progenies after inoculation with CSSV from CRIG transmission laboratory	50
7.	Leaf symptoms expressed by five Upper Amazon Clones after inoculation with CSSV 1A from CRIG Transmission Laboratory.	53
8.	Leaf symptoms expressed by five Upper Amazon Progenies (F1) after inoculation with CSSV 1A from CRIG Transmission Laboratory.	54
9.	Mean stem girth and height of healthy and infected seedlings of NA33 and PA7.	59
10.	Mean stem girth and height of Five Upper Amazon parents.	60
11.	Mean stem girth of resistant and virus infected F1 seedlings.	61
12.	Mean stem height of resistant and virus infected F1 seedlings.	63
13.	Analysis of variance of diallel results for resistance to CSSV, stem height and stem girth.	65
14.	Genotypic and phenotypic variances and genotypic to phenotypic variance ratio for plant height,	

	stem girth and percentage resistance to CSSVD.	66
15.	Combining ability analysis for plant height, % resistance and stem girth.	
16.	Number of pods obtained from selfing using *Herrania balaensis* as mentor pollen.	72
17.	Number of viable beans obtained per pod after selfing using *Herrania balaensis* as mentor pollen.	74
18.	Number of pods obtained from selfing without Using *Herrania balaensis*.	75
19.	Average number of beans obtained per pod after selfing without using *Herrania balaensis* as mentor pollen.	77

LIST OF FIGURES

FIGURE		PAGE
1.	Graph of Variance/Covariance (Vr/Wr) for resistance to CSSV	67
2.	Graph of (Vr/Wr)for stem height in cocoa	68
3.	Graph of (Vr/Wr) for Stem girth in cocoa	70

LIST OF PLATES

PLATES	PAGE
1. Two different cocoa plantations compared.	19
2. Stem swelling.	26
3. Two feeding cages for mealybugs attached to the stem of a virus infected seedling.	43
4. Symptoms expressed by infected seedlings after inoculation with CSSV 1A isolate.	55

SECTION 1

ABSTRACT

Five Upper Amazons cocoa clones, namely NA33, PA150, PA7, SCA6 and SCA9 were selected for this study. The objective of the study was to ascertain the resistance level of the resistance to the CSSV disease.

Test parents were clonally multiplied and inoculated and the expressed leaf symptoms studied. The difference observed in the parents were used to initiate hybridization. Mentor pollen technique was used to induce self-fertilization.

Clonal multiplication was done by budding and mealybugs (*P. njalensis*) was used for the inoculation. *Herrania balaensis* was also used as mentor pollen to induce self-fertilization in self incompatible clones.

Diallel crosses show that all genetic parameters studied are under polygenic control and all show additive effect. Reistance genes were found to be recessive to susceptibility. The order of resistance among the five clones was NA33 > PA150 > PA7 > SCA6 > SCA9. Comparative studies of the progenies of these

crosses revealed that crosses between NA33 as female parent with PA7 and PA150 as pollen parent scored high percentages of resistance. Crosses between PA7 and PA150 also recorded high percentages of resistance. NA33 had higher general combining ability than the rest.

The expressed leaf symptoms also vary from one clone to the other. The highly resistant ones either showed no symptoms or expressed symptoms associated with mild strain of the virus, while the susceptible ones showed some amount of virulent strain symptoms in addition to the mild ones.

The General Combining Ability (GCA) and Specific Combining Ability (SCA) values for resistance were not significant but the GCA mean square was higher in the analysis than the SCA. The narrow sense heritability was high (79%).

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Cocoa (*Theobroma cacao L.)* is the primary agricultural export commodity of Ghana, where about 765,000 ha of the crop is cultivated in six of the ten regions (Padi, 1997). As an export crop, cocoa has been a major industry in the country since 1910 (Asante, 1995). The industry currently provides employment for about 25% of the population and contributes about 9% of the Gross Domestic Products (GDP), (Asante, 1995). Until recently the industry was responsible for more than 60% of foreign exchange earnings and has contributed substantially to government revenue and the general economic development of the country (Asante, 1995).

Cocoa production in Ghana has declined drastically from an average of 324,000 metric tonnes in 1976/77 to 62 year low of 158,000 metric tonnes in 1983/84. However, due to the cocoa rehabilitation project initiated by the Ghana government with assistance from the world bank, production has increased to 409,000 metric tonnes for the period 1997/98.

One of the most important diseases affecting cocoa in Ghana is the Cocoa Swollen Shoot Virus disease (CSSVD). It has been a continuing

continuing threat to Ghana's prosperity since the economy depends to a large extent on cocoa revenue. The major control measure for the cocoa swollen shoot virus (CSSV) infection has been the removal of infected and contact trees. This control measure has, however, not achieved any lasting result despite the removal of more than 200 million infected and contact trees over the past 50 years (Hughes and Ollenu, 1994). It is therefore necessary that new control measures be sought and it is for this reason that resistant varieties are being sought. If resistant varieties are to be used then the mode of inheritance of resistance to the CSSV must be properly investigated.

Inadequate knowledge of the nature of resistance to CSSV will hamper progress of programs aimed at achieving resistance to the disease. A review of the literature suggests a number of loci with additive gene action (Lockwood, 1981). Results from previous mutation breeding also indicate that resistance might be recessive to susceptibility (Adu-Ampomah *et al.*, 1996). However, the mode of inheritance of resistance to CSSV has not been properly examined. If the type of gene action involved in resistance were determined, more efficient selection techniques could possibly be employed for the genetic improvement of cocoa.

The objectives of this study are to

i. Determine the mode of inheritance of resistance in cocoa to CSSV.

ii Identify and select CSSV resistant parents for hybridization aimed at developing genetically improved varieties in Ghana.

1.2 LITERATURE REVIEW

1.2.1 Origin, Classification and Historical Background

The Cocoa *(Theobroma cacao L)* is indigenous to the forests of Central and South America. *Theobroma* and the genera *Herrania, Guazuma* and *Cola*, which occur in Africa, belong to the family Sterculiaceae (Wood and Lass, 1989).

The genus *Theobroma* consists of a group of small trees that occur in the wild in the Amazon basin and other tropical areas of South and Central America. There are twenty two species in the genus (Mossu, 1992) but *Theobroma cacao* is the only one cultivated widely (Wood and Lass, 1989). Other species in the genus include *T. angustifolia, T. antioquia, T. bicolor, T. calodesmis, T. grandiflorum, T. leiocarpa, T. mammosum, T. microcarpa, T. obovato, T. pentagona, T. simiarum and T. speciosa* (Krug and Quartey-Papafio, 1964). However, among these species *T. bicolor* and *T. grandiflorum* are the better known in the genus (Wood and Lass, 1989). The headwaters of the Amazon basin have been said to be the origin of the cocoa, but it is more correct to describe that area as the primary centre of diversity (Wood and Lass, 1989).

The name *Theobroma cacao* was given to the cultivated cocoa plant by Linnaeus who probably took it from an Indian legend. Theo is a Greek word meaning gods while broma means food, thus Theobroma, meaning food for the gods (Mossu, 1992). It may be assumed that in early times a natural population of *Theobroma cacao* was spread throughout the central part of Amazonia-Guiana, westward and northward to the south of Mexico; that these populations developed into different forms geographically separated by the Panama isthmus; These two original forms, when isolated, had sufficiently consistent characters to be recognised as subspecies (Wood and Lass, 1989). These two subspecies have formed the basis of classifications of *T. cacao* since 1882 when it was distinguished into two great 'classes'; Criollo and Forastero, and the latter divided into several varieties.

The varieties that have been cultivated since pre-historic times in Mexico and Central America must have belonged to the Criollo group. The beans tend to be rounded and are white in cross-section producing cocoa of a weak and special flavour. The trees tend to be susceptible to diseases. (Wood and Lass, 1989).

Cocoa Varieties of the Forastero group came into cultivation in historic times. Their trees are hardy and vigorous. They now form the greater part of all cocoa grown. Compared to Criollo the beans are smaller and flatter and the cotyledons are violet. The beans have a higher fat content than Criollo beans. Of the Forastero varieties Amelonado has been the one most widely grown. It is relatively uniform and has a smooth yellow pod; it has been the major variety planted in West Africa (Wood and Lass, 1989).

As a result of increased demand of cocoa by Europeans, its cultivation spread from its centre of diversity to other parts of South America. By the year 1600, it had crossed the Pacific to the Philippines. Following the independence of Brazil, cocoa of the Amelonado type was taken to Fernando Po and Sao Tomé (Barclays Bank DCO, 1970). Tetteh Quarshie is believed to have introduced cocoa into Ghana, then the Gold Coast, in 1879 from one of these Islands. The type introduced to Ghana, probably as a single Amelonado pod, accounts for the great uniformity of cocoa plant populations of Ghana.

1.2.2 Uses of Cocoa

Cocoa was used as currency among the Mayan Indians especially in Nicaragua (Anonymous).It was also used in many social and official rituals and has various medicinal properties (Wood and Lass, 1989). It was used as a drink by South Americans though unpalatable to Europeans. The Europeans made it more palatable by adding sugar and later on milk. It is also used for making biscuit and confectionery (Mossu, 1992). Other non-traditional products from cocoa include animal feed, potash from cocoa pod husks, pectin, wine and alcohol from cocoa pulp juice, cosmetics and soap from cocoa butter and cocoa powder based foods and pastries (Adomako *et al.*, 1996).

1.2.3 Floral biology

Cocoa shows cauliflory, that is the flower and fruits are produced on the older leafless parts of the trunk and branches. Although the flowers are produced on the old wood, inflorescences arise at cushions, which were originally leaf axils (Urguhart, 1961).

The flower is quite regular, hermaphrodite and with the formula K5C5A5 + 5G(5). The structure of the flower and the stickiness of the pollen preclude wind pollination. The absence of scent or nectar and the structure of the flower, with the anthers hidden in pouched petals and the ring of staminodes hindering access to the stigmas are not features that would normally facilitate insect pollination (Urguhart, 1961).

1.2.4 Pollination

Natural pollination of cocoa, is effected by various small insects. The most important group of pollinating insects is midges belonging to several genera of the family *Ceratopogonidae.* A number of species of the genus *Forcipomyia* are the commonest pollinators (Wood and Lass, 1989).

Other pollinating insects identified by trappings are ants, aphids, fruit flies *(Drosophila spp.)* and thrips. However their role in cocoa pollination is considered to be minimal (Winder, 1977). Pollination by flying insects results in approximately 25-50 percent cross-pollination on self-compatible trees but the proportion of flowers pollinated is comparatively low (Posnette, 1950). The fused gametes form the zygote, from which the seed develops. It has been noted that a certain minimum number of individual fertilization must occur for a pod to be able to grow. This number is about 20 $\pm$ 10, varying according to the cultivar (Toxopeus and Jacob, 1970)

Artificial or hand pollination can also be effected in cocoa. Hand pollination of cocoa is a simple process requiring good eyesight, a steady hand and a pair of forceps. For good results it is also necessary to start early in the morning. The first task is to collect freshly opened flowers from the male parent trees. These are carried to the female parent where freshly opened flowers should also be selected. Pollination is achieved by brushing an anther from a male parent against the stigma of a female parent. In order to avoid pollen contamination through natural pollination, flower buds are bagged one day before they open and after pollination again covered for 48 to 72 hours.

1.2.5 Classification of cultivated Cocoa

The cultivated cocoa is classified into three groups, namely Criollo, Amazonian Forastero and the Trinitario. All these types of cocoa plants are interfertile and by crossing, give fertile hybrids, which today represent most of the cultivars, used in plantations. The origins and characteristics of the three groups are as follows:

a) **Criollo**: This is the original type domesticated by the Mayas. They are found mainly in Venezuela, Mexico, Central America and Colombia. Criollo cocoa is very much sought after for its strong aroma and relatively slight bitterness. It is used in the chocolate industry for luxury production.

b) **Forastero:** Ordinary cocoa from Brazil and Cacao National of Ecuador. The Upper Amazonian cocoa and the Lower

Table 1 Distinguishing features of different types of cocoa

	CACAO TYPES		
Character	CRIOLLO	AMAZONIAN FORASTEROS	TRINITARIOS
1)Fruit colour			
a)Unripe	Red, green	Green	Red or purple
b)Ripe	Yellow, orange	Yellow	Orange
2)Beans per pod	20-30	30 or more	30 or more
3) Apex	Conspicuously pointed	Round ended but with small definite point	Hybrid swarms of Criollo Forastero Stocks. Quite Heterogenous
4) Pod surface	Warty, conspicuously furrowed	-	
5) Fruit wall	Thin, easy to cut	Thick and woody	
6) Seeds	Plump	Flat	
7) Cotyledons	White or pale violet	Dark purple	

Source: Mossu (1992).

Amazonian cocoa (e.g. Amelonado), both of which are common in Ghana, are included in this group. The Upper Amazonian cocoa usually bears the name of the place or of the river in the region in which it has traditionally been harvested; Iquitos, Nanay, Parinari, Scavina, Morona, Moquique, etc.

c) **Trinitario:** This is a population consisting of hybrids between Criollo and Forestero. It is highly heterozygous. The distinguishing characters of the different types of cocoa are listed in Table 1.

1.2.6 Genetics and Breeding

1.2.6.1 Chromosome number, Fertilization and Incompatibility in cocoa

Theobroma cacao L. has a basic chromosome number of n = 10, it is diploid with 2n=2x= 20 (Wood and Lass, 1989; Zadoks, 1996). Successful pollination is considered to have taken place when after three days, the flower is in "swollen ovary" state, which is the first visible sign that the ovules have been fertilized. Incompatibility may however become apparent several weeks after pollination, leading to dropping of the young fruit.

Incompatibility has been defined as the inability of plants with functional gametes to set seed when either self pollinated or crossed with some of their genetic relatives. Usually incompatibility is a stylar or stigmatic reaction. However in cocoa the pollen tubes develop normally in all cases, but when the mating is incompatible the male gamete does not fuse with the female gamete (Wood, 1975).

A genetic mechanism controlling the fusion of gamete has been proposed, consisting of a series of S-factors. Cope (1962), proposed five different S-factors or S-alleles to explain the results of extensive selfing and crossing between many cultivars (in Wood and Lass, 1989). The dominance relations of these alleles are expressed in the following formula:

Sa = Sb = Sc > Sd > Sf

Previously Knight and Rogers (Wood and Lass, 1989) had studied the compatibility relations within a few families of the Amazon material in Ghana. Their study revealed dominance relations as follows:

S1 > S2 = S3 > S4 > S5.

Wood and Lass (1989) suggested that both sets of relationships may exist in different populations and that, further studies may throw more light on the subject.

The degree of incompatibility varies between different populations. Upper Amazonian cultivars are mostly self incompatible but are generally cross-compatible. Trinitario cultivars have a high proportion of self-incompatible trees, which will not cross with other self-incompatible trees, requiring pollen from self-compatible trees for successful pollination. The Amelonado population is entirely self-compatible (Wood and Lass, 1989).

1.2.6.2 Overcoming the incompatibility barrier in Cocoa

The double pollination method has been used to break the incompatibility barrier in cocoa. This method requires the use of two types of pollen mixed and applied together. It is also known as mentor pollen technique.

Adu-Ampomah *et al.* (1991) reported the use of irradiated pollen as mentor pollen to induce self fertilization of two self-incompatible Upper Amazon cocoa clones. Glendening (1960) reported that incompatible pollen from different sources achieved fertilization in several instances when mixed and applied together.

1.2.6.3 Wilting of the young fruit

Irrespective of disease or insect attack, young fruits may be lost due to the cherelle wilting on the tree. This phenomenon generally appears between fifty and seventy days after pollination and can affect 20 percent to 90 percent of the cherelles formed. Several reasons for this have been given, among which are the following: -

a) The phenomenon is the manifestation of a physiological mechanism which regulates fruiting, and which is probably controlled by growth hormones. This is referred to as 'physiological wilt'(Mossu, 1992).

b) Wilting is the result of incompatibility phenomena that can become apparent at a later stage (Mossu, 1992).

1.2.7 Pests affecting cocoa

Over 1,500 different insects are known to feed on cocoa trees (Entwistle, 1972). Though cocoa in West Africa probably suffers more from pests than elsewhere, this is caused largely by two mirids (capsids) and indirectly by a number of mealy bug species involved in spreading cocoa viruses (Wood and Lass, 1989)

Mirids are sap-sucking insects which feed on green shoots (young branches), chupons and pods. The three most important mirid pests of the cocoa industry in Ghana are *Sahlbergella singularis* (the Brown Capsid); *Distantiella theobroma* (the Black Capsid) and

Helopeltis bergrothi (the Cocoa mosquito). Other insects which occur at pest status under natural conditions in Ghana are *Bathycoelia thalasina* (Hemiptera), the moth *Earias biplaga* and the cocoa trunk or stem borer *Eulophonatus myrmelon*(Hepidoptera). Termites and many Aphids (Aphididae) also attack cocoa. Mealy bugs are seldom directly important but are notorious as vectors of cocoa viruses, especially prevalent and injurious in West 5Africa but also present in a number of other cocoa growing areas (Wood and Lass, 1989).

1.2.8 Diseases affecting cocoa

The main diseases of cocoa are *Phytophtora* pod rot (Blackpod disease), Witches Broom *(Crinipelli perniciosa)*, *Moniliopthora* pod rot, cushion gall *(Fusarium/Calonectria rigidiuscula)*, mealy pod rot *(Trachysphaera fruitigena)* Boryodiplodia pod rot *(Botryodiploda theobroma)* Phytophtora canker, vascular streak dieback and the cocoa swollen shoot virus (CSSV) disease (Wood and Lass, 1989). In Ghana the most important diseases affecting cocoa are the blackpod and the CSSV.

Phytophthora pod rot ('Black pod disease') is caused by fungi of the genus *Phytophthora*. The blackpod disease in Ghana is caused by two species, namely, *P. palmivora* and *P. megakarya,* while the

Cocoa Swollen Shoot Virus disease (CSSVD), as the name implies, is caused by a virus.

1.2.8.1 Cocoa Swollen Shoot Virus disease (CSSVD)

The CSSV, a member of the badnavirus group, has been and still is a major problem for the Cocoa Industry of Ghana in particular and the West African sub-region as a whole. It was first reported in 1936 when trees in the Eastern Region of Ghana were found to have developed stem swellings and die back (Steven, 1936). In 1938 Posnette proved that the disease was due to a virus (Posnette, 1940). He recommended that affected trees and those in contact with them should be cut. By 1982 a total of 185.5 million trees had been destroyed since the start of the campaign in 1941 (Wood and Lass, 1989). Estimates of annual yield losses due to CSSV vary from about 20,000 tonnes (Wood and Lass, 1989) to approximately 120,000 tonnes of cocoa from Eastern Region alone (Hale, 1953). Legg (1977) estimated the average annual loss of between 1946 and 1974 to be worth over £3,650,000.

Ghana became the world leading producer with a peak production of 557,000 tonnes in 1964 - 1965 (Bateman, 1974). Owing to a decline in the world market price of cocoa and economic constraints, in addition to pest and disease problems, Ghana is now the world's second largest cocoa producer and account for just over 12% of world production (Asante, 1995). By 1998, Ghana cocoa production is only about 409,359 tonnes (Ghana cocoa board handbook, 2000). Plate 1a shows cocoa plantation devastated by the CSSV disease while Plate 1b shows a healthy cocoa plantation. In addition to CSSV, Brunt and Kenten (Wood and Lass, 1989) identified three other viruses affecting cocoa in West Africa. They are Cocoa mottle leaf virus (CMLV), cocoa yellow mosaic virus (CYMV)and cocoa necrosis virus (CNV).

These viruses are of minor significance in relation to CSSV.

Plate 1
Two Different Cocoa Plantations

(a) Plantation devastated by CSSVD.

(b) Healthy Cocoa plantation

1.2.8.2 Vectors of CSSV

CSSV is transmitted by at least 13 species of mealybugs of the family group *Pseudococcidae* within the *Coccoidae* (Roivainen, 1976). In addition to these at least four incompletely identified mealybug species may also be vectors (Thorold, 1975). Fully identified vectors include *Planococcoides njalensis*, *Planococcoides citri*, *Ferristisia virgata*, *Phenococcae hargreavesi*, *Planococcus kenyae*, *Pseudococcus concavoperrari*, *Pseudococcus longispinus*, *Delococcus tafoensis* and *Paraputo anomalus* (Hughes and Ollennu, 1994).

Many of these are tended by ants in a mutualistic relationship. These ants feed on honeydew secreted by the mealybugs and protect the mealybug colonies by building nests over them. Planococcoides njalensis is the most important vector of the cocoa swollen shoot virus (CSSV) on cocoa in Ghana and other West African countries (Padi and den Hollander, 1996). *Planococcus njalensis* is relatively sedentary while *Planococcus hargreavesi*, *Ferrisia virgata* and *Pseudococcus concavocerarri* and probably *Planococcus citri* are more mobile, often moving between trees (Wood and Lass, 1989).

Mealybugs may be found on almost any part of the cocoa tree, but have a general preference for the unripe pods, the apical region of both chupon (young cocoa shoots) and fan branches, the angles between newer branches and the stem, flower cushions, flower stalks and roots. To a lesser extent they are to be found on the petioles and laminae of leaves and on the main trunk (Wood and Lass, 1989). All these sites are extremely inaccessible to chemical sprays.

1.2.8.3 Spread of CSSVD

The nymphs of both sexes and adult females of mealybugs spread the disease radially between adjacent trees by crawling through the canopy from infected to healthy trees or by being carried by attendant ants (Crematogaster species and Camponotus species) (Hughes and Ollennu, 1994). Occasionally, jump spread may occur when infective mealybugs are blown by the wind and infest trees some distance from the original sites of infection (Strickland, 1950; Cornwell, 1960; Thresh *et al.*, 1988). Adult male mealybugs have very rudimentary non-functional mouth-parts and do not feed.

1.2.8.4 Alternative hosts of CSSV

CSSV has been isolated from several naturally occurring tree hosts widely distributed all over West Africa and which provide ıatural shade for cocoa in local farms. This is why the disease .s thought to be indigenous to West Africa (Are and Gwynne-Jones, .973). A list of alternate wild host is provided in Table 2.

[ABLE 2 Alternate wild hosts for cssv

[ree species	Family	Reference
[ola caricaefolia	Sterculiaceae	Are and Gwynne - Jones, 1973
[ola chlamydantha		
[ola cordifolia		
[ola gigantea		
[ola heterophylla		
[ola lateritia		
Erythropsis barteri		
Pterygota macrocarpa		
Sterculia hinopetala		
Sterculia ragocantha		
Adansonia digitata	Bombacaceae	Are and Gwynne - Jones, 1973
Bombax buonopozense		
Ceiba pentadra		
Corchorus stridens	Tiliaccae	Posnette, 1950; Attafuah, 1965

A morphologically similar virus has been isolated from the herbaceous plants *Asystesia* sp and *Commelina* sp in the Eastern Region of Ghana (Hughes and Ollennu, 1994). With the exception of *Cola chlamydantha*, it has proved difficult to transmit virus from these species to cocoa in the field (Posnette, 1981).

1.2.8.5 Disease Symptoms

There are many strains of cocoa swollen shoot virus which differ in the symptoms they produce, the vectors that transmit them and range of their alternate hosts (Wood and Lass, 1989). The virulent strains predominate and cause various types of leaf chlorosis, root necrosis, root and stem swellings and die-back. Die-back is common in the Amelonado variety which is the most susceptible to CSSV in Ghana. Avirulent strains are not lethal and rarely induce leaf symptoms although swellings are often pronounced (Legg, 1972), but they have only a small effect if any on yield.

CSSV isolates may induce both leaf symptoms and swellings on the stem and roots. Leaf symptoms may include red vein banding (Posnette, 1947) where the red coloration is attributed to accumulation of anthocyanins along the veins and veinlets (Knight and Tinsley, 1958). This is followed by chlorotic vein flecking or banding which may extend along larger veins giving angular flecks (Posnette, 1947). The banding may be confined to the secondary vein to form a 'fern leaf' pattern. Stem swellings may develop at the nodes, internodes or tips of shoots (Posnette, 1941; 1947)(Plate 2). Many isolates of CSSV also induce swellings on the roots (Attafuah, 1957). The most apparent symptom exhibited by pods is a change in shape although some green mottling may occur. The pods become rounder and often spherical in shape (Posnette, 1943). In addition, infected pods are smaller than healthy pods and their surface is smoother. Some isolates of CSSV induce only leaf symptoms for example, Kpeve and Enchi (Hughes and Ollennu, 1994). Others induce stem and root symptoms with only very mild leaf symptoms, for example Bakukrom, while many other isolates induce both types of symptoms for example, Kofi Pare and New Juaben (Hughes and Ollennu, 1994). In some cases the leaf symptoms are transient while in other cases severe symptoms recur and the infection leads to the death of the plant.

1.2.8.6 Virus Isolates

Table 3 shows Ghanaian cocoa swollen shoot virus isolates which have been maintained at the Cocoa Research Institute of Ghana. The isolates from each region are listed alphabetically with the natural host and region from which they were isolated, any synonyms and where available a brief description of their symptoms in Amelonado cocoa.

Plate 2

Stem Swelling (Swollen portion is near the base of stem)

Table 3. Ghanaian cocoa Swollen Shoot Virus Isolates

Isolate	Synonyms	Source (Region)	Natural host	Symptoms I	L	S	R	P
AD 75	CMLV[a]	Ashanti	*Adansonia digitata*	+	+		-	
Amakom/Bosomtwi		Ashanti	*Theobroma cacao*	+	+	+	+	+
Amanchia		Ashanti	*T. cacao*	+	+	+	+	
Bobriso/Juaso[b]		Ashanti	*T. cacao*	+	+	+	-	
Bosomtwi	IJ	Ashanti	*T. cacao*	+	+	+	+	
Koben		Ashanti	*T. cacao*	+	+	+	+	
Konongo	IK	Ashanti	*T. cacao*	+	+	+	+	+
Krofa/Juansa		Ashanti	*T. cacao*	+	+	+	+	
Kwakoko/Juansa		Ashanti	*T. cacao*	+	+	+	+	
Madjeda/Nkwanta		Ashanti	*T. cacao*	+	+	+	+	
Morso		Ashanti	*T. cacao*	+	+	+	+	
Onyimso/Agogo		Ashanti	*T. cacao*	+	+	+	+	
Sedi-Nkawie		Ashanti	*T. cacao*	+	+	+	+	-
Bechem	F1	Brong Ahafo	*T. cacao*	+	+	+	+	-
Kwadwokumkrom		Brong Ahafo	*T. cacao*	+	+	+	+	-
Kwaku Anyan	T1	Brong Ahafo	*T. cacao*	+	+	+	+	
Nkrankwanta	T1	Brong Ahafo	*T. cacao*	+	+	+	+	
Nkwanta/Dorma Ahenkro		Brong Ahafo	*T. cacao*		+	+	+	
Oterikrom		Brong Ahafo	*T. cacao*	+	+	+	+	
Sankore		Brong Ahafo	*T. cacao*	+	+	+	+	
Techimantia		Brong Ahafo	*T. cacao*	+	+	+	+	
Nsaba		Central	*T. cacao*	+	+	+	+	
Group A								
Achiasi		Eastern	*T. cacao*		+	+	+	-
Agyepomaa		Eastern	*T. cacao*	+	+	+	+	-
Asamankese		Eastern	*T. cacao*		+	+	+	-
Dawa[b]	IH	Eastern	*T. cacao*	+	+	+	+	+
Dochi	IG	Eastern	*T. cacao*	+	+		-	
Kofi Pare	1A	Eastern	*T. cacao*	+	+	+	+	+
Mampong	1M	Eastern	*T. cacao*	+	+	+	+	+
Miaso		Eastern	*T. cacao*	(+)	+	+	+	
New Juaben	Tafo yellows, 1A	Eastern	*T. cacao*	+	+	+	+	+
Nkawkaw	ID	Eastern	*T. cacao*	+	+	+	+	+
Pamen	IE	Eastern	*T. cacao*	(+)	+	+		-
Tease Atomsu Aboum		Eastern	*T. cacao*		+	+	+	-

(Table 3 continued)

Isolate	Synonyms	Source (Region)	Natural host	Symptoms I	L	S	R	P
Group B								
N1b		Eastern	*T. cacao*	(+)	(+)	+		−
SS 70[b]	MI	Eastern	*T. cacao*	(+)	(+)		−	−
SS 90[b]	MI	Eastern	*T. cacao*	(+)	(+)		−	−
SS 167[b]	MI	Eastern	*T. cacao*	(+)	(+)	−	−	
SS 365B[b]	MI	Eastern	*T. cacao*	(+)	(+)	−	−	
Group C								
Bisa[b]	IB	Eastern	*T. cacao*	(+)	−	+	+	
Domkorkrom[b]		Eastern	*T. cacao*	(+)	(+)	+	+	−
Tease Aduadan		Eastern	*T. cacao*	(+)	(+)	+	+	−
Tease Adeakyi		Eastern	*T. cacao*	(+)	(+)	+	+	−
Group D								
AD 7	CMLV	Eastern	*A. digitata*	+	+			
AD 36	CMLV	Eastern	*A. digitata*	+	+			
AD 49	CMLV	Eastern	*A. digitata*		+	(+)		−
AD 111	CMLV	Eastern	*A. digitata*		+	(+)		−
AD 135	CMLV	Eastern	*A. digitata*	+	+	−		
AD 196	CMLV	Eastern	*A. digitata*	+	+	−		−
Group A								
Goviepe Todzi		Volta	*T. cacao*	+	+	−		
Kpeve	IC	Volta	*T. cacao*	+	+	−		
Wusuta		Volta	*T. cacao*	+	+	−		
Group B								
Djinji		Volta	*T. cacao*	(+)	+	+	+	+
Peki[b]		Volta	*T. cacao*	−		+	+	
Peki Tsame[b]		Volta	*T. cacao*	−		+	+	
Worawora[b]	IW	Volta	*T. cacao*	(+)		+	+	
Group A								
Aduakaa/Enchi		Western	*T. cacao*	+	−		−	
Enchib		Western	*T. cacao*	+	−		−	
Group B								
Aboboya		Western	*Cola chlamydantha*	(+)	+	+	+	
Achechere		Western	*C. chlamydantha*	(+)	(+)	+	+	−
Adiembra		Western	*C. chlamydantha*	(+)	+	+	+	
Aiyim		Western	*C. chlamydantha*	(+)	+	+	+	−
Anibil		Western	C. chlamydantha	+	+	+	+	−
CC644		Western	*C. chlamydantha*		+	+	+	−

(Table 3 continued)

Isolate	Synonyms	Source (Region)	Natural host	Symptoms I	L	S	R	P
Group C								
Amafie	IF	Western	*T. cacao*	-	+	+	+	+
Bakukrom		Western	*T. cacao*	+	+	+	+	
Bosomuoso	IF	Western	*T. cacao*	-	+	+	+	+
Datano		Western	*T. cacao*		+	+	+	+
Janesi		Western	*T. cacao*	+	+	+	+	+
Punekrom	IF	Western	*T. cacao*	+	+	+	+	+
Surawno	IF	Western	*T. cacao*	+	+	+	+	+
Suhuma[b]	IF	Western	*T. cacao*	+	-	+	+	-
Wiawso	IF	Western	*T. cacao*		+	+	+	+
Yiboso		Western	*T. cacao*		+	+	+	

[a]Cocoa mottle leaf virus
[b]Mild isolates.

Key: I, Symptoms on immature leaves, typically red vein banding; L, symptoms on mature leaves, typically mosaic, chlorotic vein clearing and flecking; S, Stem (fan/chupon) swellings; R, root swellings; P, pod symptoms.
+, symptoms present; - , symptoms absent; ( ), symptoms rare or very mild.

Source: Hughes and Ollennu, (1994).

1.2.8.7 Control of CSSV Disease

Attempts have been made to control the mealybug vectors by the use of insecticides and by biological control, but the results have been unsuccessful (Ollenu *et al.*, 1989). The most effective means of control is as described by Posnette (1943), which involves the removal of infected and contact trees. However if one considers that official statistics of trees removed by this method from the early 1940s to 1988 was more than 190 million trees with an estimated 5.15 million still infected in 1989 and over 10 million in 1990 (Ollennu *et al.*, 1989), then a better alternative to control or to contain the disease must be found. This would involve breeding for tolerant or resistant varieties.

1.2.8.8 The Analysis of Variance of Diallel tables

A diallel cross is the set of all possible matings between several genotypes. The genotypes may be defined as individual clones, homozygous lines, etc., and if there are n of them, there are n^2 mating combinations, counting reciprocals separately. A diallel table is an arrangement in a square of n^2 measurements corresponding to offspring with a common parental genotype. A summary of a method of describing the genetical situation generating a diallel table has already appeared (Zinks, 1954) and (Hayman, 1954 a & b).

1.2.8.9 Concept of general and specific combining ability in relation to diallel crossing systems

The term 'general combining ability' (GCA) is used to designate the average performance of a line hybrid combination. The term 'specific combining ability' (SCA) is used to designate those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved.

The essential idea is to consider a systematic set of crosses between a number of parents and enquire to what extent variation among crosses can be interpreted as due to statistically additive features of the parents and what must be attributed to residual interactions. In effect, this means writing, for each cross (parents A, B), as equation:

$X_{AB} = X + G_A + G_B + S_{AB}$

Where X is the general mean, G_A and G_B are the general combining abilities (hereafter GCA) of the parents (the additive bits) and S_{AB} is the statistically unaccounted for residual, or specific combining ability (SCA). Evidently, the G here are measured as deviations from the general mean; some authors omit the X, thus in effect, estimating the G each increased by 1/2X (Simmonds, 1979).

ven though this discussion is restricted to diallel crosses, here are possible variations in the diallel crossing system itself and in the assumptions concerning the sampling nature of the experimental material.

iallel crossing techniques may vary depending upon whether or ot the parental inbreeds or the reciprocal F_1's are included or oth. With this as a basis for classification, there are four ossible experimental methods: (1) parents, one set of F_1's and eciprocal F_1's are included (all p^2 combinations); (2) parents nd one set of F_1's are included but reciprocal F_1's are not (1/2p p + 1] combinations); (3) one set of F_1's and reciprocals are ncluded but not the parents (p [p - 1] combination); and (4) one set of F_1's but neither parents nor reciprocal F_1's is included (1/2p [p - 1] combinations). Each method necessitates a different form of analysis (Griffing, 1956b)

For the purpose of this study, we shall restrict our analysis to conform to only one design, namely half diallel, with parents but no reciprocals. Table 4 shows the estimation of combining abilities.

Table 4 Combining abilities; estimation of GCA parameters

	Type of cross structure and GCA
DIALLEL	N parents in all combinations. Parents, A, B C etc with gametes a, b, c, and combining abilities G_A, G_B, etc; measured from general mean so that (G_A + G_B + ...) = 0 and column T_A distinguished as T_A and $T^1{}_A$ where necessary.
(a) Complete N x N With reciprocals and selfs	$G_A \quad \left(T_A + T^1{}_A/2N\right) - \quad T/N^2$
(b) half diallel, with parents, no reciprocals	$G_A = (T_A + aa/N + 2) - 2T/N(N + 2)$
(c) reciprocals but no parents (diagonal missing)	$G_A = (T_A + T^1{}_A/2(N - 2)) - 2T/N(N + 2)$

(d) half diallel $G_A = (Tn/(N - 2)) - 2T/N(N -2)$

no parents, no reciprocals

(Source: Simmonds, 1979)

For the purpose of this study, formula (b) is applicable for the estimation of the general combining abilities.

1.2.9.0 Heritability

The conventional symbol for heritability is h^2 even though it is a ratio rather than the square of a quantity. Conventionally also, h is written for the square root of h^2 when necessary. Narrow sense heritability (i.e. V_A/V_p) is usually understood to be implied unless the context states otherwise. [V_A is the expression for the expectations of mean squares that can plausibly be attributed to additive effects and V_p = phenotype].

Heritability often estimates the fraction of total variance among lines attributable to genetic differences between them.

An experiment that distinguished an additive component (V_A) from total genetic variance (V_G) would yield two heritabilities as follows.

First, there is the ratio:

An experiment that distinguished an additive component (V_A) from total genetic variance (V_G) would yield two heritabilities as follows.

First, there is the ratio:

$$V_G/V_G + V_{GE} + V_E = V_G/V_p$$

And second, the ratio

$$V_A/V_G + V_{GE} + V_E = V_A/V_p$$

The latter ratio, since V_A is at most equal to V_G, is smaller than the former: it may be called the 'narrow sense' heritability in contrast to the 'broad sense' type V_G/V_p.

V_{GE} = genotype x environmental effect

V_E = variance due to environment

V_G = variance due to genotype.

1.2.9.1 Parent - offspring regression

This is an alternative approach to heritability. Consider a reasonably diverse array of parents measured for whatever character the experimenter is interested in. If samples of progeny from these parents are measured, they will themselves tend to be high but will diverge from their parents as a result of genetic segregation and of environmental effects on both generations. At the low extreme, the same will apply; the progeny sample will again tend to diverge towards the general mean. This was the basis of Galton's use of the word 'regression': progeny of extreme parents tend to regress towards the mean. If parents and progeny agreed perfectly, the regression would have a slope of unity but there would be no regression in Galton's original sense (Simmonds, 1979).

At the other extreme, if there were no similarity between parents and offspring, the result would be a random scatter of points on the diagram, with no evident regression. This would only happen if there were environmental effects, but no heritable genetic ones. So, close parents-offspring (high regression) suggest a large genetic effect and a small environmental one. In fact it can be shown that b, the regression coefficient itself estimates the narrow sense heritability, V_A/V_P. This is true if parental values are mean of both parents (i.e. are mid-parents); if only one parent is known or relevant (as in some animal experimentation or polycrosses) then b = 1/2 (V_A/V_p).

Regressions of this kind are essentially empirical: they describe parent-offspring relationships directly. Thus they do not depend upon genetic assumptions, though interpretation of b as a narrow-sense heritability does involve the idea of separating V_A from the rest (Simmonds, 1979).

2.0 MATERIALS AND METHODS

2.1 Base Population

A single population described as base population was used for the crossing.

This population was derived from five Upper Amazon cocoa clones that had shown varying levels of resistance/susceptibility to CSSV in various field trials over the years at CRIG and elsewhere. They were used in a diallel crossing scheme. The five clones are

1	PA 7
2	PA 150
3	NA 33
4	SCA 6
5	SCA 9

These parents/clones were obtained from plots Q6 and M6 belonging to the Plant Breeding Division of CRIG.

2.2 Methods

2.2.1 Clonal Multiplication of parents

In order to obtain 10 genetically identical clones per parent for the inoculation test from each of the clones, the test parents were multiplied vegetatively by budding.

Parental clones for these test parent were also raised by budding. Budwoods from the branches of the trees for each clone were collected from the plots in the field. The budwoods were wrapped in moistened cotton wool and labeled accordingly. Budding was done within 24 hours of cutting of the budwood. The T-bud method as described by Hardy (1960) was used. The procedure is described as follows:

Two small cuts at right angles were made in the stock and the bud shield inserted in it under the flaps made by slightly lifting the edges. After insertion, the bud was wrapped tightly using a transparent plastic tape. This was done so as to exclude air and prevent infection by fungus. These plants were then transferred to the nursery where they were left for 14 days. The stem plant was then cut back 14 days after budding, leaving about 5cm of stem above the bud. The fan bud shoots begun to grow after about 14 days at an inclined angle. They were allowed to grow to the age of 3 months.

2.2.2 Inoculation

Ten buddlings from the test parents/clones were inoculated using mealybugs as vectors, to ascertain their resistance/susceptibility levels in the gauzehouse. The details of the procedure is described below:

2.2.2.1 Collection of mealybugs

All of the virus transmission tests were done with *P. njalensis*. The mealybugs were collected in the field as colonies on cocoa pods or young cocoa shoots (chupons). When these parts of the plants were tapped and left for a few minutes, most of the insects withdrew their stylets and were then collected with a fine brush without damaging them. The collection was done by a team of 'bug-hunters' which brought in the daily catch from various cocoa plantations of the Cocoa Research Institute. A collection brought into the laboratory consisted of mealybugs at various stages of development. In the inoculation process both nymphs and adults were used.

2.2.2.2 Virus source plant

Young cocoa seedlings of the Amelonado type with prominent symptoms of virus infection were used as standard virus source plants. Source plants were produced, according to standard procedure at the Cocoa Research Institute (Roivainen, 1976), by putting infectious mealybugs on cocoa beans for 24 hours after which the beans were planted into ordinary soil in wooden boxes. Germinated seedlings usually produced prominent leaf symptoms in about four to six weeks after planting.

2.2.2.3 Feeding Cones and Cages

In the inoculation process the mealybugs were fed on cocoa seedlings with known symptoms (virus source plants) by using the paper cone technique of Posnette and Strickland (1948). In this technique, a piece of paper formed into a cone is fixed near the terminal bud of the seedling and the mealybugs carrying the virus are placed inside the cone. As many mealybugs disappeared into the soil or otherwise escaped from the cones, this technique was replaced by confining the mealybugs into feeding cages, which were fixed on the seedlings (Plate 3). These cages had several advantages; the mealybugs could not escape, they were not exposed to external hazards and they readily settled down to feed inside the cages (Plate 3).

2.2.2.4 Acquisition feed

During acquisition feed virus is taken up by the vectors, which may subsequently transmit the virus unto other plants (the test plants), during the next feed.

2.2.2.5 Removal of mealybugs from virus source plant

At the end of acquisition feed the mealybugs were removed from the source plant for transmission of the acquired virus to the test parents. The standard procedure of removal at the

Plate 3

Two feeding cages for mealybugs attached to the stem of a virus infected seedling

Cocoa Research Institute is to tap the source plant gently over a sheet of glass. Feeding mealybugs are disturbed to the extent that they withdraw their stylets and are all removed in about fifteen minutes.

2.2.2.6 Inoculation feed

The final stage, the transmission of the virus by mealybugs into a healthy plant, thus takes place when an infectious mealybug settles down to feed on the test plant, inoculating it with the virus. In this study ten cocoa buddlings from each of the five test plants/parents were used.

2.2.3 Screening of Progenies for CSSV resistance

The process here was similar to the inoculation of the parental clones except that beans instead of seedlings were inoculated with the infectious mealybugs. The procedure was as follows: Beans of the progenies were dusted with saw dust so as to make their handling easier because of the mucilage surrounding them. With the use of a sharp knife, the coat surrounding the beans was removed. The beans were then collected in a beaker, rinsed with water and soaked for 24 hours. The soaked beans were then dried on a filter paper and membranes around them removed to reveal cracks around the bean. After drying, the seeds were placed in a polypot and infective nymphs placed on them using fine transmission brush. About ten nymphs per seed were deposited. The pots were then covered and left to stand for 24 hours to allow for effective feeding. The beans were then disinfected by washing with nicotine sulphate. The beans were then planted in wooden boxes and the leaf symptoms expressed recorded and scored.

2.2.4 Mating design

The five test parents/clones were crossed in all possible combinations.

2.2.5 Experimental design and analysis

Randomised complete block design was used. A parent offspring regression was also conducted using data collected on parental clones and their progenies.

The following characters were scored.

a. Resistance/susceptibility levels of clones to CSSV

b. Expressed leave symptoms

c. Morphological characters

I Height of seedlings

II Girth of seedlings

III Analysis of variance of Percentage Resistance, Stem height and stem girth.

In the Hayman (1954a) analysis of variance the partitioning of the variances primarily test the significance of the following effects:

a = the additive effect of the genes

b = the dominant effect of the genes

b_1 = directional dominance

b_2 = assymetry of the distribution of dominant genes in the parent

b_3 = dominance interaction between specific genotypes (same as the specific combining ability of Griffing 1956)

c = reciprocal effects due to maternal effects

d = reciprocal effects other than to maternal effects

B = blocks

The error variance was the blocks total (BT) as Bartlett's test shows that heterogeneity of block interaction variances was negligeable. The correlation between wr + vr and parental means indicates the direction of dominance for a character within the population. A negative correlation coefficient approaching -1 indicates that dominant alleles act to increase the expression of the character, a positive value approaching +1 indicates that recessive alleles increase the expression and value approximating 0 indicate ambi-directional dominance.

2.3 Investigating into selfing using mentor pollen

Since the program was going to involve some selfing and within group crosses, an efficient method for investigating selfing was also undertaken with eleven Upper Amazon clones.

The following parameters were studied.

I Number of pods obtained in pollination using mentor pollen.

II Number of beans obtained in pollination when mentor pollen was used.

III Number of pods obtained in pollination without mentor pollen.

IV Number of beans obtained in pollination without the use of mentor pollen

3.0 RESULTS

3.1 Comparative study of healthy and infected cocoa seedlings

3.1.1 Percentage resistance

Table 5 shows the results obtained from inoculation of five (5) diallel parents in the transmission laboratory. The order of resistance is depicted as follows

NA33 > PA150 > PA7 > SCA6 > SCA9.

It means that NA33 shows the highest level of resistance followed by PA150, PA7, SCA6 with SCA9 being the most susceptible.

Comparative studies of the progenies of these crosses as shown in Table 6 reveal the following;

1 The crosses between NA33 as female parent with PA7 and PA150 as pollen parent scored high percentages of resistance. However the reciprocals ie PA7 x NA33 and PA150 x NA33 had lower percentages of resistance.

2 Crosses between PA7 and PA150 also recorded high percentages of resistance. This is more true with PA150 x PA7.

3 NA33 had higher general combining ability than the rest. SCA6, PA150, PA7 and SCA9 follow in that order.

TABLE 5

RESISTANCE/SUSCEPTIBILITY LEVELS OF FIVE UPPER AMAZONS CLONES AFTER INOCULATION WITH CSSV 1A FROM CRIG TRANSMISSION LABORATORY

CLONE	PERCENTAGE SCORES	
	R	S
PA7	50	50
PA150	70	30
NA33	100	0
SCA6	40	60
SCA9	20	80

R - Resistance

S - Susceptibility

10 Buddlings per clone were used. 50% resistance means half (or five seedlings) showed no symptoms.

TABLE 6

RESISTANCE/SUSCEPTIBILITY LEVELS OF FIVE UPPER AMAZONS F1 PROGENIES (F1) AFTER INOCULATION WITH CSSV FROM CRIG TRANSMISSION LABORATORY

			PERCENTAGE SCORES	
			R	S
PA7	X	PA150	47	53
PA7	X	NA33	40	60
PA7	X	SCA6	27	73
PA7	X	SCA9	22	78
PA150	X	PA7	58	42
PA150	X	NA33	35	65
PA150	X	SCA6	28	72
PA150	X	SCA9	36	63
NA33	X	PA7	70	30
NA33	X	PA150	50	50
NA33	X	SCA6	33	67
NA33	X	SCA9	37	62
SCA6	X	PA7	43	57
SCA6	X	PA150	53	47
SCA6	X	NA33	41	58
SCA6	X	SCA9	27	73
SCA9	X	PA7	35	65
SCA9	X	PA150	34	65
SCA9	X	NA33	48	52
SCA9	X	SCA6	18	82

R - Resistant seedlings
S - Infected seedlings
60 Buddlings per cross were used. 50% resistance means 30 seedlings showed no resistance.

followed by Chlorotic Vein Flecking (CFL)(Plate 4c), Diffused Flecking (DF)(Plate 4d), and Green Vein Banding (GVB) (Plate 4f) respectively. Symptoms such as RVB, CFL and GVB are often associated with the avirulent strains of the virus, while diffused Flecking (DF) and Fern Pattern (FP) are characteristics of the virulent strain. NA33 and PA150 the most resistant clones had no virulent symptoms. PA7 which is the third in rank had mostly the avirulent symptoms but only 10% of the virulent. SCA6 and SCA9 the relatively less resistant clones recorded higher symptoms of the severe strain than the most resistant clones.

The trend for the symptoms exhibited by the F1 progenies is shown in Table 8. The comparison done here is based on the total virulent symptoms expressed. PA150 performed better as female parent in that the total virulent symptoms expressed is 48 with two crosses namely PA150 x PA7 and PA150 x NA33 showing no seedling death. NA33 ranked second with two crosses namely NA33 x SCA6 and NA33 x SCA9 showing no seedling death. SCA6, PA7 and SCA9 were respectively third, fourth and fifth. Even though none of the parent showed fern pattern symptom during inoculation, crosses between them showed this symptom associated with the virulent strain of the virus in their progenies (Plate 4e). The only exceptions were NA33 X PA7, PA7 X SCA6 and NA33 X SCA9.

SCA9 were respectively third, fourth and fifth. Even though none of the parent showed fern pattern symptom during inoculation, crosses between them showed this symptom associated with the virulent strain of the virus in their progenies (Plate 4e). The only exceptions were NA33 X PA7, PA7 X SCA6 and NA33 X SCA9.

TABLE 7

LEAF SYMPTOMS EXPRESSED BY FIVE UPPER AMAZON CLONES AFTER INOCULATION WITH CSSV 1A FROM CRIG TRANSMISSION LABORATORY

CLONE	COMPONENT OF SUSCEPTIBILITY							TOTAL VIRULENT SYMPTOM EXPRESSED
	0	1	2	3	4	5	-	
	HP	RVB	CFL	CVC/GVB	DF	FP	DEAD	
PA7	50	10	30	-	10	-	-	10
PA150	70	20	10	-	-	-	-	0
NA33	100	-	-	-	-	-	-	0
SCA6	40	30	10	-	20	-	-	20
SCA9	20	20	20	10	30	-	-	30

HP - Healthy Plant
RVB - Red Vein Banding
CFL - Chlorotic Vein Flecking
CVC - Chlorotic Vein Clearing
GVB - Green Vein Banding
DF - Diffused Flecking
FP - Fern Pattern

THE SCORES ARE FROM 0-5 DEPENDING ON THE SEVERITY OF THE SYMPTOM 1 BEING THE LOWEST SYMPTOM AND 5 BEING THE SEVEREST SYMPTOM.

TABLE 8

LEAF SYMPTOMS EXPRESSED BY FIVE UPPER AMAZON PROGENIES (F1) AFTER INOCULATION WITH CSSV 1A FROM CRIG TRANSMISSION LABORATORY

			COMPONENT OF SUSCEPTIBILITY							
			0	1	2	3	4	5	-	TOTAL VIRULENT
			HP	RVB	CFL	CVC/GVB	DF	FP	DEAD	SYMPTOM EXPRESSED
PA7	X	PA150	47	5	42	-	5	2	-	
PA7	X	NA33	40	2	38	-	15	4	1	
PA7	X	SCA6	27	2	32	1	31	-	9	90
PA7	X	SCA9	22	5	40	-	27	3	3	
PA150	X	PA7	58	8	25	2	5	2	-	
PA150	X	NA33	37	8	42	-	11	3	-	
PA150	X	SCA6	28	10	52	-	8	2	1	48
PA150	X	SCA9	37	6	42	-	12	1	3	
NA33	X	PA7	70	3	13	-	10	-	5	
NA33	X	PA150	50	3	30	-	12	2	3	
NA33	X	SCA6	33	7	50	-	7	3	-	57
NA33	X	SCA9	38	7	40	-	15	-	-	
SCA6	X	PA7	43	3	30	-	8	6	9	
SCA6	X	PA150	53	5	30	-	7	1	5	
SCA6	X	NA33	42	13	30	-	8	2	5	74
SCA6	X	SCA9	27	5	46	-	20	1	2	
SCA9	X	PA7	35	5	33	-	25	2	1	
SCA9	X	PA150	35	1	40	-	16	3	6	
SCA9	X	NA33	48	12	26	-	4	3	6	97
SCA9	X	SCA6	18	2	49	-	27	2	2	

HP - Healthy Plant
RVB - Red Vein Banding
CFL - Chlorotic Vein Flecking
CVC - Chlorotic Vein Clearing
GVB - Green Vein Banding
DF - Diffused Flecking
FP - Fern Pattern

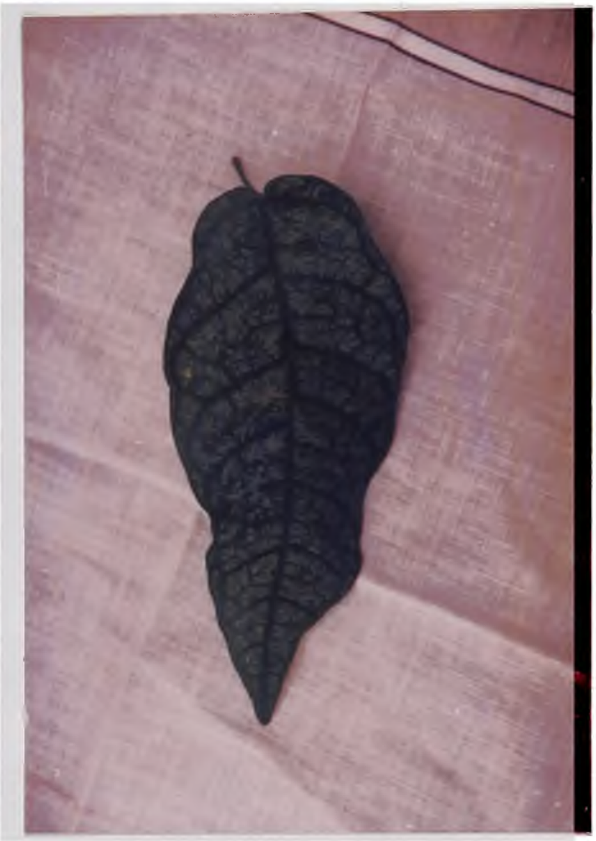
THE SCORES ARE FROM 0-5 DEPENDING ON THE SEVERITY OF THE SYMPTOM 1 BEING THE LOWEST AND 5 BEING THE SEVEREST SYMPTOM.

Plate 4:
Symptoms expressed by infected seedlings after inoculation with CSSV 1A isolate

(a) Healthy Leaf

(b) Red Vein Banding

(c) Chlorotic Vein Flecking



(d) Diffused Flecking

Plate 4 (continued)

(e) Fern Pattern

(f) Green Vein Banding

Plate 4 (continued)

3.1.3 Stem girth

Table 9 shows the results obtained for girth measurement of the stem for the F1 progeny of the cross i.e. NA33 x PA7. The resistant seedlings were constantly broader than the virus infected seedlings throughout the period of observation. In the four week old seedlings, there was a difference of 1.4% between the resistant seedling and the virus infected one. This difference increased to 11.4% between the two types of seedlings at age 9 weeks. By the fourteenth week there was 17% difference between the resistant seedlings and the infected ones.

A comparison of the healthy and virus infected parents (Table 10) also reveals that PA150 has the largest stem diameter. The rest follow in the order NA33, SCA9, PA7 and SCA6. Table 10 also shows the result of their progeny crosses. Similarly the healthy ones are constantly larger than the virus infected ones. PA150 x NA33 and PA150 x SCA6 have the largest girth. From these results there is no correlation between resistance and stem girth.

3.1.4 Stem height

Table 9 also shows results obtained for height measurement of the stem for the same F1 progeny of the cross i.e. NA33 X PA7.

TABLE 9

MEAN STEM GIRTH AND HEIGHT OF HEALTHY AND INFECTED SEEDLINGS OF NA33 X PA7

GROWTH MEASUREMENT	PERIOD (WEEKS)	TREATMENT		S.E. OF MEANS
		F1 SYMPTOMLESS SEEDLINGS	F1 INFECTED SEEDLINGS	
Stem Girth (mm)	4	3.50	3.45	±0.04
	9	7.00	6.20	±0.10***
	14	9.40	7.80	±0.12***
Stem Height (m)	4	18.50	17.0	±0.19***
	9	21.00	18.40	±0.21***
	14	33.40	24.80	±1.12***

Measurement is for five seedlings.

TABLE 10

MEAN STEM GIRTH AND HEIGHT OF FIVE UPPER AMAZON PARENTS

CLONE ITEM	RANKING IN TERMS OF RESISTANT	PERIOD (WEEKS)	STEM GIRTH (CM)	STEM HEIGHT (CM)
PA7	3	35	6.68	41.26
PA150	2	35	8.01	52.10
NA33	1	35	7.27	54.30
SCA6	4	35	6.47	58.20
SCA9	5	35	7.15	49.33

Measurement is for five seedlings per clone

1- Highly resistant

5- Susceptible

TABLE 11
MEAN STEM GIRTH OF RESISTANT AND VIRUS INFECTED F1 SEEDLINGS

F1 PROGENIES			PERIOD (WEEKS)	TREATMENT	
				R (cm)	S (cm)
PA7	X	PA150	35	6.86	5.60
PA7	X	NA33	"	6.62	5.68
PA7	X	SCA6	"	6.56	5.56
PA7	X	SCA9	"	6.68	6.38
PA150	X	PA7	"	7.96	5.56
PA150	X	NA33	"	9.40	8.32
PA150	X	SCA6	"	8.62	8.12
PA150	X	SCA9	"	6.06	5.94
NA33	X	PA7	"	6.68	5.76
NA33	X	PA150	"	7.64	7.16
NA33	X	SCA6	"	7.20	6.60
NA33	X	SCA9	"	7.56	7.04
SCA6	X	PA7	"	6.60	5.16
SCA6	X	PA150	"	7.18	5.82
SCA6	X	NA33	"	6.04	5.20
SCA6	X	SCA9	"	6.04	4.88
SCA9	X	PA7	"	7.26	7.06
SCA9	X	PA150	"	7.52	7.16
SCA9	X	NA33	"	7.60	7.02
SCA9	X	SCA6	"	7.44	7.36

R - F1 symptoms seedlings
S - F1 infected seedlings

Meaurement is for five seedlings.

The pattern was similar to that obtained for measurement of the girth. The resistant seedlings were consistently significantly taller than the virus infected ones from week 4 to week 14.

The results of height measurement for the five diallel parents in Table 9 show that PA150 was the fastest growing buddling. The rest follow in the order NA33, SCA6, SCA9 and PA7. Studies of the F1 progenies in Table 12, reveal that PA150 x NA33 has the fastest growing buddling. Other buddlings that may be selected for fast growth are SCA6 x PA7, NA33 x SCA6, and NA33 x SCA9. Resistant F1 progenies were consistently taller than the susceptible ones. Again there was no relationship between resistance and growth rate.

3.1.5 Analysis of variance for Resistance, Stem height and Stem Girth

Table 13 presents the analysis of variance of the diallel table. While Table 14 shows the heritability estimates and Table 15 estimates the general and specific combining abilities. Regression was significant for resistance to CSSVD (R) and height of seedlings (H) but not for girth of seedling.

TABLE 12

MEAN STEM HEIGHT OF RESISTANT AND VIRUS INFECTED F1 SEEDLINGS

F1 PROGENIES			PERIOD (WEEKS)	TREATMENT	
				R (cm)	S (cm)
PA7	X	PA150	35	33.10	30.00
PA7	X	NA33	"	44.80	32.40
PA7	X	SCA6	"	47.80	37.60
PA7	X	SCA9	"	39.20	37.60
PA150	X	PA7	"	47.80	35.60
PA150	X	NA33	"	68.20	67.60
PA150	X	SCA6	"	58.40	45.80
PA150	X	SCA9	"	34.20	24.40
NA33	X	PA7	"	33.00	33.40
NA33	X	PA150	"	62.60	53.20
NA33	X	SCA6	"	60.90	55.50
NA33	X	SCA9	"	60.80	58.20
SCA6	X	PA7	"	61.40	44.80
SCA6	X	PA150	"	58.60	42.60
SCA6	X	NA33	"	59.80	50.00
SCA6	X	SCA9	"	52.80	48.60
SCA9	X	PA7	"	42.80	38.60
SCA9	X	PA150	"	52.00	48.80
SCA9	X	NA33	"	52.50	48.00
SCA9	X	SCA6	"	50.00	49.60

R - F1 symptomless seedlings
S - F1 infected seedlings

Measurement is for five seedlings.

The graph of Wr/Vr (Fig 1) suggested that dominant alleles were in excess. However, NA33, which has the highest resistance, lie in the recessive area. Most of the variance was attributed to addictive effect as indicated by the significant value of a in Table 13. There was no evidence of maternal effect. Earlier work had provided no evidence of maternal effect (Legg *et al.*, 1973). GCA and SCA mean square values for general combining ability(GCA) and specific combining ability (SCA) (Table 15) were not significant but the GCA mean square was higher in the analysis than the SCA. Lockwood (1981) also found that the SCA effects were smaller than GCA effect in all his analysis. There was also much less evidence of specific reciprocal differences. The narrow sense heritability was as much as 79% (Table 13). The heritability value shows that resistance is largely controlled by additive effect.

(b) Stem Height (H)

The analysis of the diallel table (Table 13) shows that only the additive effect of the genes was significant. Dominant alleles were however ambi-directional, (Fig 2). Narrow sense heritability estimates was 51%. This also supports the fact that stem height is controlled by additive effect. Analysis of variance for

TABLE 13

ANALYSIS OF VARIANCE OF THE DIALLEL RESULTS FOR RESISTANCE TO CSSV - STEM HEIGHT AND STEM GIRTH

ITEM	DF	R	H	G
a =	4	1047.63*	401.5982*	2.1906*
b_1 =	1	1115.5600NS	66.7489NS	0.4045NS
b_2 =	4	222.0867NS	34.6908NS	0.0497NS
b_3 =	5	79.2667NS	95.7885NS	0.6888NS
b =	10	240.0240NS	68.4455NS	0.4047NS
c =	4	248.2500NS	20.4910NS	0.9841NS
d =	6	48.0000NS	67.9585NS	0.3574NS
t =	24	327.9900NS	115.8568NS	0.7871NS

NS - Not significant

* - Heterogeneity over arrays significant at 5% level

* - Items are defined on page 46

TABLE 14
GENOTYPIC AND PHENOTYPIC VARIANCES AND GENOTYPIC TO PHENOTYPIC VARIANCE RATIO FOR PLANT HEIGHT, STEM GIRTH AND PERCENTAGE RESISTANCE TO CSSVD

HERITABILITY ESTIMATES

CHARACTER	Vg	Vp	Vg/Vp or h^2
PLANT HEIGHT	40.35	79.10	0.51
STEM DIAMETER	0.49	0.80	0.61
% RESISTANCE	100.40	927.70	0.79

TABLE 15
COMBINING ABILITY ANALYSIS FOR PLANT HEIGHT, % RESISTANCE AND STEM GIRTH

SOURCES OF VARIATION	MEAN SQUARE			
	DF	PLANT HEIGHT	% RESISTANCE	STEM GIRTH
GCA	4	257.65^xx	517.50	10.00^xx
SCA	10	150.01^xx	175.50	15.10^xx
ERROR	10	24.98	404.44	1.46
GCA/SCA	-	1.72	2.95	0.66

xx - SIGNIFICANT AT 1% LEVEL

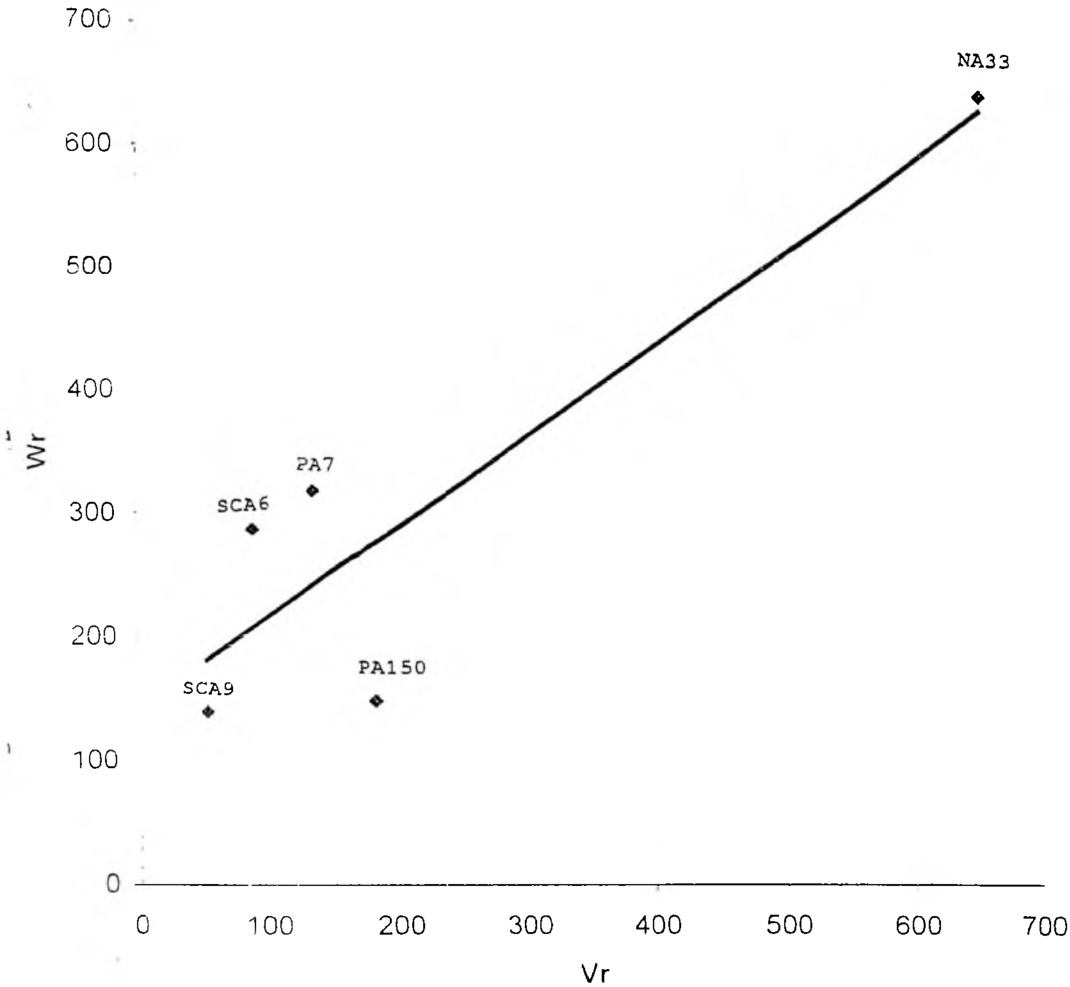


Fig 1: Graph of Variance/Covariance (Vr/Wr) for Resistance to CSSV.

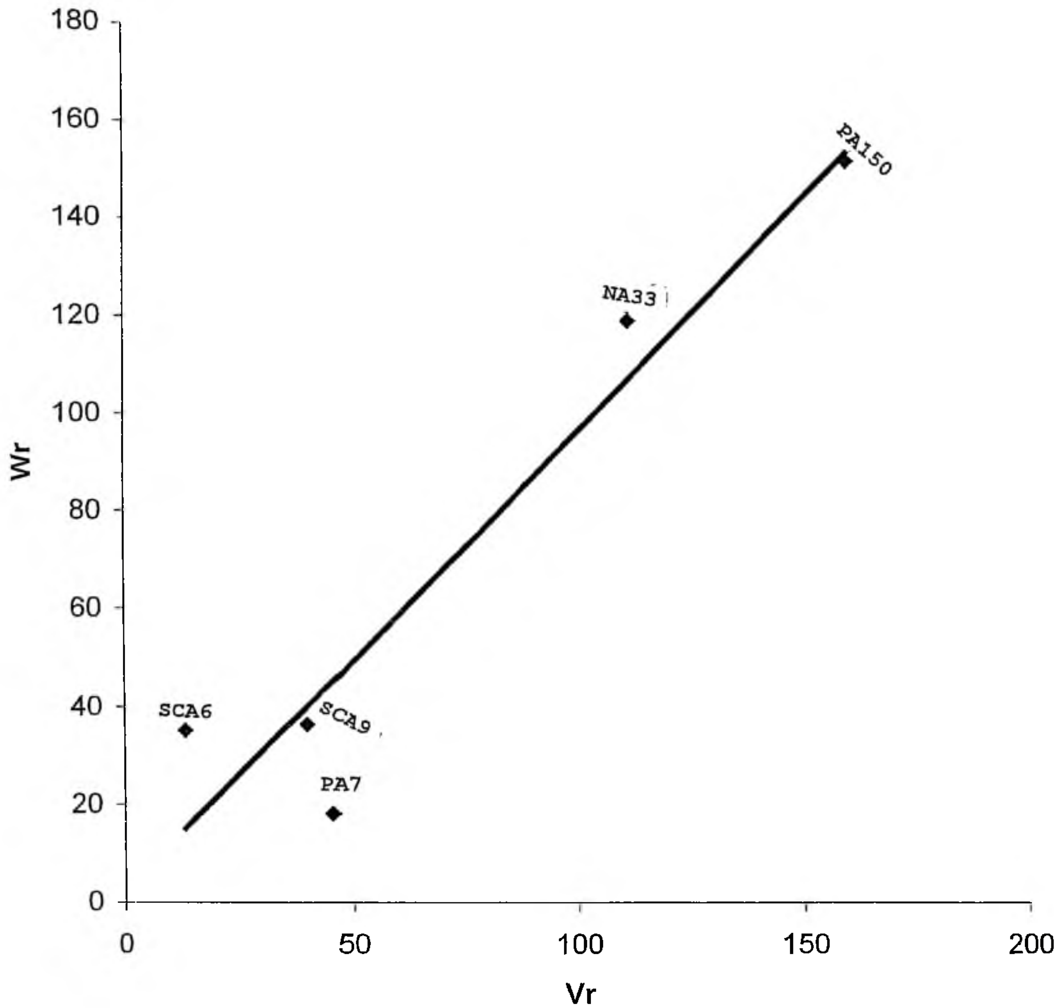


Fig. 2: **Graph of Vr/Wr for stem height in cocoa.**

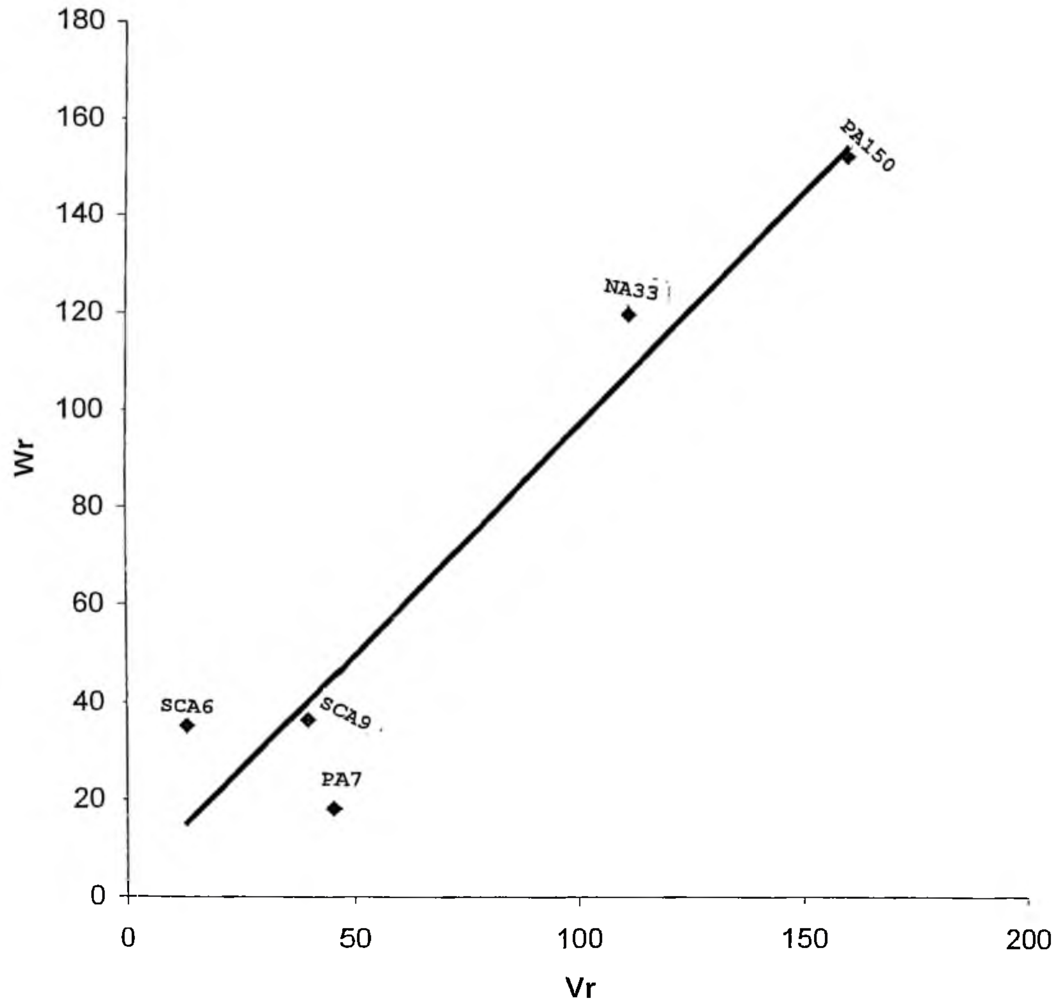


Fig. 2: **Graph of Vr/Wr for stem height in cocoa.**

combining ability reveals that variance due to GCA and SCA were highly significant. This also indicates the importance of both additive and non-additive genetic variances. The relative magnitude of σ^2GCA was less than σ^2SCA as revealed by σ^2GCA/σ^2SCA ratio for stem height.

(c) Stem Girth

The only genetic parameter that was significant is the additive effect of the genes. Fig 3 suggested that dominant alleles were in excess. PA150 and NA33 lie in the recessive area. Heritability obtained was 61% supporting the fact that stem girth is controlled by additive effect. Although variance due to GCA and SCA were highly significant, the GCA/SCA ratio was low meaning that much of the variation was due to SCA effect rather than GCA.

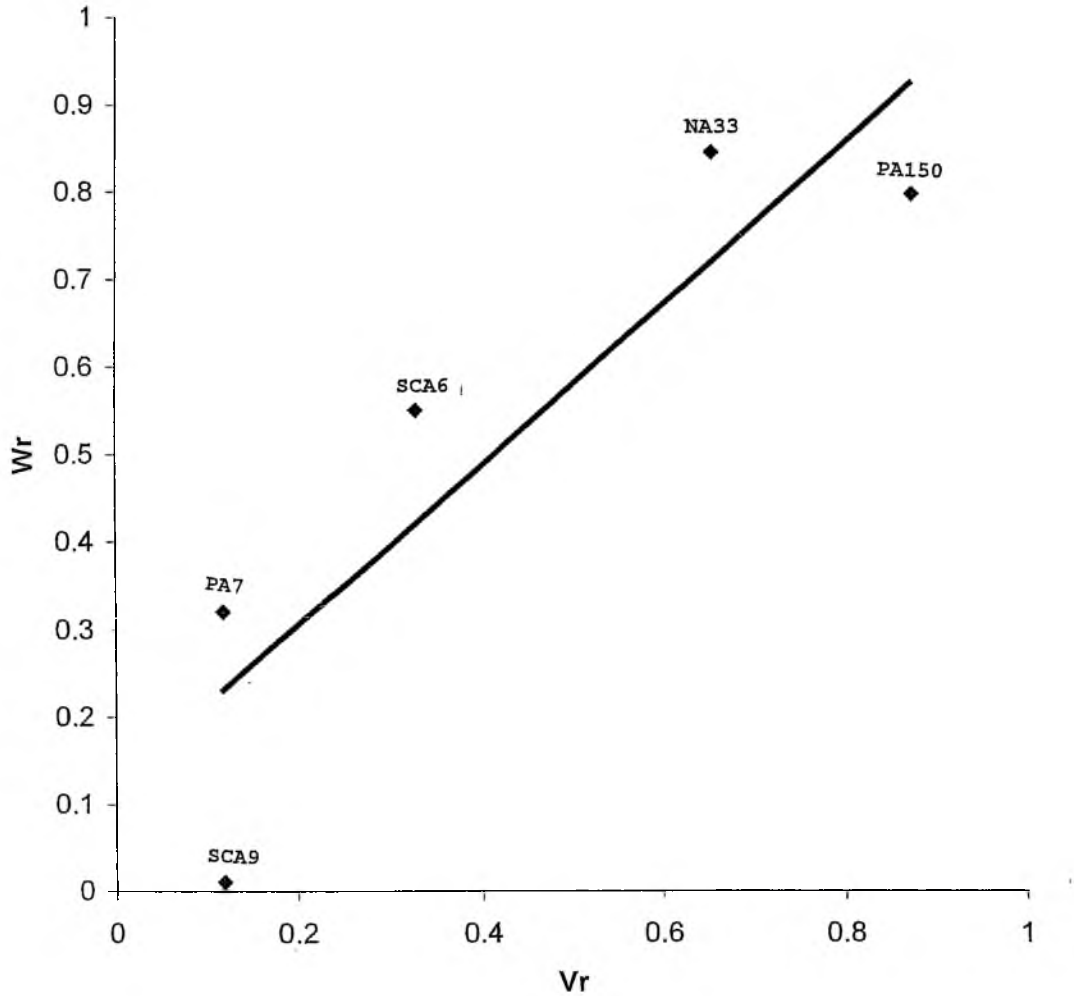


Fig. 3: Graph of Vr/Wr for stem girth in cocoa.

2 The use of mentor pollen to induce self-fertilization in self-incompatible clones.

3.2.1 Number of pods obtained

Eleven parental clones were selfed using *Herrania balaensis* as mentor pollen. The eleven parents are PA7, PA150, NA33, SCA6, SCA9, IMC53, IMC67, IMC76, T60/887,T63/971 and T85/799.

After twenty pollinations all the eleven parental clones set fruits, however some of the fruits wilted before reaching maturity.

Selfing with these clones almost always resulted in zero pod formation (Table 18). However, with *Herrania balaensis* pollen as mentor pollen some selfing could be achieved as shown in Table 16. With PA7, SCA9 and T60/887 selfing could not be achieved but the rest of the clones could be selfed at varying degrees of success. T85/799 recorded the highest number of selfing (40%) whiles SCA6 and IMC67 recorded the relatively low number of selfing (10% each). IMC67 is noted to be one of the clones which is difficult to effect selfing (Adu-Ampomah *et al*., 1991)

TABLE 16

NUMBER OF PODS OBTAINED FROM SELFING USING *Herrania balaensis* AS MENTOR POLLEN

NUMBER OF FLOWERS POLLINATED PER CLONE = 20

CLONE	NO FRUITED TO MATURITY	% FRUITING
*PA7	0	0
PA150	4	20.00
NA33	6	30.00
SCA6	2	10.00
SCA9	0	0
IMC53	4	20.00
IMC67	2	10.00
IMC76	4	20.00
*T60/887	0	0
T63/971	3	15.00
T85/799	8	40.00

* Pods wilted at age 3 months

3.2.2 Number of viable beans obtained per pod

The number of beans obtained per pod is presented in Table 17. The number of viable beans obtained per pod ranges between 4 and 8. Majority of the beans were flat. NA33 and T85/799 had the highest number of viable beans followed by PA150,IMC53, IMC67, IMC76 and T66/971. The lowest number of viable beans was recorded with SCA6. Even though these figures are low, they seem to suggest that selfing which is difficult to achieve in Upper Amazon cocoa could be achieved by the mentor pollen technique.

3.2.3 Number of pods obtained in crosses not involving mentor pollen

Table 18 represents the results obtained when pollen from incompatible clones were dusted on their own stigma. Fruiting was unsuccessful in almost all the clones except PA7 and SCA9 which yielded one pod each. This proves that without mentor pollen all the clones are sufficiently self-incompatible.

TABLE 17

NUMBER OF VIABLE BEANS OBTAINED PER POD

AFTER SELFING USING *Herrania balaensis* AS MENTOR POLLEN

AVERAGE NO OF BEANS PER POD = 30

CLONE	NO. OF VIABLE BEANS PER POD	% NO. OF BEANS
PA7	0	0
PA150	6	15
NA33	8	20
SCA6	4	10
SCA9	0	0
IMC53	6	15
IMC67	6	15
IMC76	6	15
T60/887	0	0
T63/971	6	15
T85/799	8	20

TABLE 18

NUMBER OF PODS OBTAINED FROM SELFING WITHOUT USING *Herrania balaensis* OR ANY TECHNIQUE AS MENTOR POLLEN

NUMBER OF FLOWER POLLINATED PER CLONE = 20

CLONE	NO. OF PODS OBTAINED	% FRUITING
PA7	1	5
PA150	0	0
NA33	0	0
SCA6	0	0
SCA9	1	5
IMC53	0	0
IMC67	0	0
IMC76	0	0
T60/887	0	0
T63/971	0	0
T85/799	0	0

3.2.4 Number of viable beans obtained in crosses not involving mentor pollen.

Table 19 represents the number of beans obtained from pods in crosses not involving mentor pollens. The two clones, PA7 and SCA9 had 28 and 30 viable beans respectively. These numbers are comparable to the number of viable beans obtained in outcrossing (about 30). However, selfing without mentor pollen technique often results in zero pod formation.

TABLE 19

AVERAGE NUMBER OF BEANS OBTAINED PER POD AFTER SELFING WITHOUT USING *Herrania balaensis* AS MENTOR POLLEN

AVERAGE NUMBER BEANS PER POD = 40

CLONE	NO OF BEANS OBTAINED PER POD	% NO. OF BEANS
PA7	28	70
PA150	0	0
NA33	0	0
SCA6	0	0
SCA9	30	75
IMC53	0	0
IMC67	0	0
IMC76	0	0
T60/887	0	0
T63/971	0	0
T85/799	0	0

DISCUSSION

In earlier attempts to find immunity, resistance or tolerance to the virulent New Juaben strain of CSSV(Posnette and Todd, 1951), clones were established from trees found surviving in devastated farms and showing only mild symptoms or none. In subsequent tests certain Trinitario varieties showed a low degree of tolerance but no detectable resistance, while a measure of resistance and more, though variable, tolerance was exhibited by some of the Upper Amazon cocoa type introduced via Trinidad in 1944 (Knight and Rogers, 1955).

The five Upper Amazon cocoa clones selected for this study show varying levels of resistance. NA33 was the most resistant (100% resistant) while SCA9 was the least resistant (20% resistant). This result agrees with findings by Posnette and Todd (1951) that plants of Nanay origin were the most promising as regards resistance and tolerance. Subsequent studies by Dale (1957. 1958), Blencowe and Attafuah (1959), suggested that material from the nearby amazon island of Iquitos may be also equally resistant.

In this experiment parents of plants which have proved the most resistant in standardized test (within the limits of incompatibility) were crossed with others of varying levels of resistance in the hope that some of the progeny will prove even more resistant.

The F1 progenies (Table 6) which could be selected with better than average resistance are NA33 x PA7 and SCA6 x PA150.

Whether uniformly tolerant or resistant planting material will be obtainable by simply using seed of known parentage, or only by selection and subsequent vegetative propagation, has an important practical implication. The fact that some resistance has been encountered in a very limited range of material is a promising indication that more resistant plants may occur amongst the cocoa of the Upper Amazon region.

More than fifty distinct isolates of CSSV have been distinguished in Ghana by the sequence of symptoms produced in seedlings of West Africa Amelonado cocoa (Posnette 1947; Thresh and Tinsley, 1959). Some isolates cause severe symptoms and death in cocoa while others produce indistinct symptoms and have little overall effect on the trees.

The symptoms produced in Upper Amazons by the virulent New Juaben are similar in nature to those of Amelonado but differ in degree being usually milder (Blencowe and Attafuah, 1959).

The results of the inoculation test indicate that four of the five Upper Amazon parents selected for the study show prominent leaf symptoms induced by both avirulent strain and virulent types. NA33 did not express any disease leaf symptoms because it was completely resistant. PA7 and PA150 exhibited symptoms of the mild strains only, while SCA6 and SCA9 exhibited some mild symptoms of severe strain.

Taking the totality of the expressed symptoms expressed in the parents, RVB is the most frequently occurring symptom followed by CFL, DF and GVB. Since the highest percentage of the symptoms produced (namely RVB and CFL) are associated with the mild or avirulent strain, the effect of the virus on two parents, namely, NA33 and PA150 may not be lethal. Lethality of plants is associated with severe symptoms, namely, 'fern leaf' pattern and diffuse flecking especially in Amelonado cocoa. However, with PA7, SCA6 and SCA9 which produced some of the severe symptoms and are the more susceptible, seedling death could have occurred. The trend is generally true for their F1 progenies. The pattern of the leaf symptoms exhibited by the cross NA33 x PA7 which show some hybrid vigour in terms of resistance, is also very promising considering the fact that the 'fern pattern' associated with advanced stage of infection was completely absent in this progeny. This is a further confirmation that the progeny of the cross

NA33 x PA7 is a good source of resistant material. It is worth noting that most mild strain symptoms occur in the resistant clones while relatively severe symptoms occur mostly in susceptible ones.

The CSSV disease has a significant effect on the growth of young cocoa seedlings. The effect of the virus isolate on stem girth was less severe than on shoot extension, causing stunted growth.

Viruses in cocoa have been shown to affect photosynthetic efficiency. Hutcheon (1975) demonstrated that the photosynthetic rate (PR) for healthy seedling was twice that of virus infected seedlings. This difference is attributed to either chlorosis of the leaves by infection as severely chlorotic areas had low values of PR or to accumulation of carbohydrates as has been shown to occur in virus infected plants (Adomako and Hutcheon, 1974).

The inheritance of three characters (i.e. resistance, plant height and stem girth) was studied in diallel experiments involving clones from a single base population. The use of the Hayman-Jinks diallel analysis rather than the analysis of Griffing (1956) or Comstock and Robinson (1952) meant that relatively few parents could be examined per population and that the result could not necessarily be related to the overall population from which the studied plants were taken. It did, however, provide a complete analysis including for non-allelic interaction and thereby produced a greater amount of information for these parents than would otherwise have been obtained. Data in Tables 13,14,15 and Figs 1-3 may be used to identify those parent for crosses to produce progeny that can be selected rapidly and effectively for enhancement of a desired character. Those characters most likely to respond to selection can also be recognized. Such genetic information may therefore be used to augment the success of the largely empirical techniques of the plant breeder.

Breese (1972) states that the way in which a polygenic, economic character is inherited (via additivity, dominance or epistasis) can determine breeding methods and that strategy and tactics in plant breeding depend on the knowledge of the genetic and environmental components of the variation. Inbred varieties exploit additive effect and non-allelic interaction, depending on the method of selection employed.

In the diallel cross, resistance to swollen shoot virus is found to be largely additive and so it should be possible to increase resistance by selection within crosses between parents with different resistance factors. Successful breeding for enhanced resistance depends on there being different resistance factors in the parents of the progenies used for selection as well as the availability of suitable selection technique. Positive and significant additive X additive effects prevailing in all the five crosses showed that selection will be advantageous in further generations.

From Fig 1 it can be deduced that resistance is recessive to susceptibility. This is because NA33, which is 100 % resistant, lies in the recessive area and SCA9 the most susceptible, lie in the dominant area. Adu-Ampomah *et al.,* (1996) had suggested earlier on that susceptibility might be dominant to resistance as most induced mutations go from dominance to recessiveness. For stem height and diameter, NA33 and PA150, which are the most resistant clones, lie in the recessive area. The implication is that resistance to CSSV, stem height and girth are factors that will be inherited together.

Self-incompatibilty is a genetic character of general occurrence in cocoa. It is explained by the fact that the presence of certain genes inhibits the fertilization of the ovules by the pollen produced by the same plant or the same genotype (Soria,1960). Since inbred varieties exploit additive gene action, an effective means of inducing self- fertilization in self-incompatible clones must be found.

The use of *Herrania balaensis* as compatible pollen for pollination resulted in the pod containing a mixture of both fully formed viable beans and flat beans. A greater percentage of the beans were flat. From previous work done by Glendening (1960), NA33 was selfed using compatible pollen from a tree with the incompatible one and an axil spot marker used to detect selfing. He proposed a theory in which he explained that " if the proportion of ovules in which dominant allele was combined was sufficiently low the flower may remain on the tree and selfed ovules in which different alleles, or alleles recessive to others present, had come together could develop into seed."

Furthermore, in the situation in which pollen mixture consists of compatible pollen and self-incompatible pollen, Glendening (1960) proposed that the low number of self fertilization is likely due to two types of pollen arriving at the ovules at the same time and that competition for ovules obviously favour the compatible pollen tube.

Pandey (1977) in an experiment in which mentor pollen was used to overcome intra-and interspecific incompatibility in *Nicotiana*, suggested from his experimental results that a role of mentor pollen in overcoming incompatibility may be to provide extra free pollen growth promoting substances (PGS). These substances are apparently able to promote just sufficient further growth to allow some fertilization by certain pollen tubes with inherent weak expression of incompatibility.

From the present result in cocoa it may be concluded that the mentor pollen effect at least under certain circumstances, promoted pollen tube growth. In this particular experiment, the use of *Herrania balaensis* as compatible pollen for pollination caused the development in an increasing percentage of flat beans. A possible explanation may be that syngamy may have occurred but is reminiscent of the sort of "pseudo fertilization" reported by Grant *et al.*, (1980) after irradiating *Nicotiana* pollen. However, an interaction of the compatible pollen tubes and pistil tissue might have induced some fruit set signals causing haploid parthenogenesis in the ovules.

In these studies selfing the incompatible clones without using mentor pollen technique did not yield any appreciable success. Since selfing in Upper Amazons often results in no pod formation, mentor pollen will always be deployed to ensure selfing in these clones.

SECTION 11
SUMMARY AND CONCLUSION

1. The mode of inheritance of resistance to CSSV shows that Inheritance of CSSV is via additivity.

2. The results of the inoculation test revealed that the five clones show varying levels of resistance with NA33 being the most resistant and SCA9 the most susceptible.Similarly, the following Fi progenies scored high percentage of resistance, these are NA33 as female parent with PA7 and PA150 as pollen parent. Hence, NA33, PA150 and possibly PA7 could be selected as resistant material for cocoa breeding in Ghana. In addition, progenies of the following crosses: NA33 as female parent with PA7 and PA150 as pollen parent and PA7 as female parent with PA150 can also be selected for trial in search for resistant material.

3. Symptoms observed consisted of red vein banding, chlorotic vein flecking and clearing, green vein banding, diffused flecking, fern pattern and seedling death. The most resistant clones/progenies show mild strain symptoms whiles the susceptible plants had more severe strain symptoms than the resistant plants. NA33 (100% resistant) showed no symptom while PA150 (70% resistant) showed 0% virulent symptom and 30% mild strain symptom. PA7 (50% resistant) showed 10% virulent symptom and 40% mild strain symptom.

SCA6 (40% resistant) showed 20% virulent symptom and 40% mild strain with SCA9.

4 The effect of the virus infection on the growth of young seedling was studied. The virus infection reduced growth in stem height and stem girth but the reduction is more noticeable in stem height. There was no correlation between resistance and growth rate.

5 *Herrania balaensis* was used as mentor pollen to induce self-fertilization in self-incompatible clones. A mixture of viable and flat beans were obtained with the greater percentage of the beans being flat.

6 The analysis of the variance of the diallel results reveals that the inheritance of resistance, stem height and stem girth is via additivity. Resistance is recessive to susceptibility and therefore appropriate breeding techniques can be employed in cocoa breeding for a good resistant hybrid material.

SECTION 111

SUGGESTED LINES FOR FURTHER INVESTIGATION

Cocoa production in the country continues to decline as a result of the devastating effects of diseases especially, the CSSV. The search for hybrid materials that will confer resistance is more urgent now than ever. This search should be widened to include all genotypes of cocoa. Since resistance is recessive to susceptibility, mutation breeding may be employed to obtain more resistant material. Individual F1 progenies, which are resistant, must be isolated and backcrossed to highly resistant parents. They may also be selfed or crossed to other individual F1 resistant materials and their progenies (F2) studied.

References

Adomako, D. and Hutcheon, W. V. (1974). Carbohydrate metabolism and translocation in healthy and cocoa swollen shoot virus-infected cocoa plants. *Physiol. Plant* 30; 90-96.

Adomako, D; Osei-Amaning, E.O. (1996). Livestock feeding trials with cocoa pod husks in Ghana; *Cocoa Research Institute, Ghana, Technical bulletin No. 13* pp. 23.

Adomako, D.; Amaning, E.O.; Agyente-Badu, C.K.; Takrama, J. ; Oppong, H.; Gyedu, E.(1996). Production of cocoa by-products from fresh cocoa pod waste and non-traditional products from cocoa beans. *Cocoa Research Institute, Ghana; Technical Bulletin No.14*, pp,34

Adu-Ampomah, Y.; Novak, F.J.; Klu, G.Y.P. and Lamptey, T.V.O. (1991). Use of irradiated pollen as mentor pollen to induce self-fertilization of two self-incompatible Upper Amazon cacao clones. *Euphytica 51*:219-225.

Adu-Ampomah, Y.; Owusu, G.K.; Sackey, S.; Padi, B. and Abdul-Karimu, A (1996). Use of gamma rays to induce mutants resistant to cocoa swollen shoot disease in Theobroma cacao L. *Plant breeding 115*, 74-76.

Are, L.A. and Gwyne-Jones, D.R.G. (1974). *Cocoa in West Africa.* Oxford University Press, Ibadan.

Asante, E.G. (1995). The economic relevance of plant diseases and pest management in the Ghana Cocoa Industry. *First international cocoa pest and disease management* pp.288-299.

Attafuah, A. and Brunt, A.A.F. (1959). Virus research: the host range of cacao viruses. Rep. *West Africa Cocoa Res. Int.* (1957-58). Pp. 22-3.

Barclays Bank DCO (1970). *Cocoa.* Well hall Press Ltd., London pp.94.

Bateman, M.J., (1974). *An econometric analysis of cocoa supply.* In kotey RA, Okali C, Rouke B.E. eds. Economics of cocoa production and marketing, Accra, Ghana. University of Ghana.

Blencowe, J.W. and Attafuah A. (1959). Virus research: resistance and tolerance. *Ibid. 1957-58*. pp. 24-5.

Breese, E. L. (1972). Biometrical genetics and its application in: The way ahead in plant breeding (eds. Lupton. F. G. H.,Jenkins, G.;Johnson. R.) pp 135-146. *Proc. 6th Euphytica*.

Comstock, R. E; Robinson, H. F.(1952). Estimation of avereage dominance of genes. In *: Heterosis* (ed. Gowen J. W.). pp 494-516.Iowa: Iowa state college press.

Cope, F. W. (1962). The mechanism of pollen incompatibility in Theobroma cacao L., *Hereditary 17*:157

Cornwell, P.B. (1960). Movements of the vectors of virus disease of cacao in Ghana 11. Wind movements and aerial dispersal. *Bulletin of Entomological Research 51*:175-201.

Dale, W.T. (1957). Virus research: resistance and tolerance.*Ibid. 1955-56*. pp. 33-5; 41-5

Dale, W.T. (1958). Virus research: resistance and tolerance. *Ibid. 1956-57*. pp. 27-30

Dale W.T. (1962). *Diseases and pest of cocoa.* A virus disease Agriculture and land use in Ghana (Ed. J.B. Wills) Oxford University press London pp 286-316.

Entwistle, P.F. (1972). *Pests of cocoa*. Longman group limited, London pp. 779.

Jinks, J. L. (1954): The analysis of continuos variation in a diallel cross of nicotiana rustica. *Genetics 39*, 767-788.

Ghana Cocoa Board Handbook. 8th edition, 2000. pp. 62.

Glendening, D. R.(1960). Selfing of self-incompatible cocoa. *Nature 187*; 170.

Grant, J. E., Pandey, K. K. and Williams, G. E. (1980). *Pollen nuclei after ironizing*

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

Hobbs, S.L. A. and Burnett, J. H. (1982). The genetic control of morphological and yield character in Vicia faba .*Theor. Appl. Genetical* 62: 9-15.

Hale, S.L. (1953). *World production and consumption 1951 to 1953.* Report and proceedings of the 1953 London Cocoa Conference pp.3-11.

Hardy, F. (1960). *Cocoa Manual, English Edition.* Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica pp.395.

Hayman, B. I. (1954a). The analysis of variance of diallel tables. *Biometrics 10.* 235-234.

Hayman, B. I. (1954b). The theory and analysis of diallel tables. *Genetics* 39. 769-788.

Hughes, J. d'A and Ollennu L.A.A. (1994). Mild strain protection of cocoa in Ghana against swollen shoot virus - a review. *Plant Pathology 43*:442-457.

Hutcheon, W. V. (1975).Growth and photosynthesis in relation to enviromental and internal factors. *Proc. 5th Int. Cocoa Res. Conf., Ibadan, Nigeria, 1975*

Knight, R.and Tinsley, T.W. (1958). Some histological observations on virus-infested Theobroma Cacao L. *Annals of Applied Biology 46*:7-10

Knight, R. and Rogers, H.H. (1955). Incompatibility in *Theobroma Cacao. Hereditary 9*:69-77.

Knight, R. and Rogers, H.H. (1955). Recent introduction to West Africa of *Theobroma Cacao* and related species: 1, A review of the first 10 years. *Emp. J. Exp. Agric. 23*, 113-125.

Krug, C.A. and Quartey-Papafio, E. (1964). *World cocoa survey FAO Agricutural Studies No.63* pp 241.

Legg, J.T. (1972). Measures to control spread of cocoa swollen shoot diseases in Ghana. *Pest articles and news summary 18*:57-60.

Legg, J.T. (1977). Cocoa swollen shoot disease know your enemy. *Proceedings of the Fifth International Cocoa Research Conference Ibadan 1975*. 397-402.

Lockwood, G. (1981). Genetic aspects of resistance to cocoa swollen shoot virus in Ghana. *Ann Appl. Biol. 98*:131-141.

Mossu, G. (1992). Cocoa. *The Tropical Agriculturalist. English Edition*. The macmillan press Ltd. pp 103

Ollennu, L.A.A.; Hughes, J. d'A and Owusu, G.K. (1996). Mild strain cross protection of cocoa against cocoa swollen shoot badnavirus. *Trop. Sci 36*:116-128.

Padi, B. (1995). Parthenogenesis in mealybugs occuring on cocoa. *First International cocoa Pests and Diseases Seminar, Accra, Ghana,* 242-248.

Padi, B. and den Hollander, J. (1996). Morphological variation in planococcoides njalensis occuring on cocoa in Ghana. *Entomologia Experimentalis et Applicats 79*:317-228.

Padi, B. (1997). Prospect for the control of cocoa mealybugs. *First International Cocoa Pest and Disease Seminar, Accra, Ghana* 249-263.

Pandey, K.K. (1977). Mentor pollen: Possible role of wall-held pollen growth promoting substances in overcoming inter-and interspecific incompatibility. *Genetica 47*, 3:219-229.

Posnette, A.F. (1940). Transmission of 'swollen shoot' disease of cacao. *Tropical Agriculture, Trinidad 17*:98.

Posnette, A.F. (1941). Swollen shoot virus disease of cocoa. *Ibid. 18*, 87-90.

Posnette, A.F. (1943). The diagnosis of swollen shoot disease of cacao. *Tropical Agriculture, Trinidad 21*:156-158.

Posnette, A.F. (1947). Virus IA, IB, IC and ID. *Annals of Applied Biology 34*: 388-402.

Posnette, A.F.; Robertson, N.F. and Todd, J.M. (1950). Virus disease of cocoa in West Africa V. Alternative host plants. *Ann. Appl. Biol. 37*:229-240.

Posnette, A.F and Strickland, A. H. (1951). Virus disease in cacao in West Africa 8, the search for virus resistant cacao. *Ann. Appl. Biol. 38*, 785-800

Posnette, A.F. and Todd, J.M. (1951). Virus disease of cacao in West Africa: 8, The search for virus reistant cacao. *Ibid.* 38, 785-800.

Posnette, A.F. (1981). The role of wild hosts in cocoa swollen shoot diseases. In Thresh J.M. ed. Pest Pathogens and vegetation. London, U.K. *Pitman 71-78*.

Roivainen, O. (1976). Transmission of cocoa virus by mealybugs (Homoptera: Pseudo coccidae). *Journal of the Scientific Agricultural Society of Finland 48*:203-304.

Simmonds, N.W. (1979). *Principles of crop improvement..* Longman group limited. pp. 408.

Soria,J. (1960). *The genetics and breeding of cacao.* Hardy,F. ed. Cacao manual IICA, Turrialba, Costa Rica. pp325-344

Steven, W.F. (1936). A new disease of cocoa in the Gold Coast. *Tropical Agriculture, Trinidad, 14*:84.

Steven, W.F. (1936). A new disease of cocoa in the Gold coast, *Gold Coast farmer* 5: 122-144.

Strickland, A.H. (1950). The disposal of Pseudoccocidae (Hemiptera-Homoptera) by air current in the Gold Coast. *Proceedings of the Royal Entomological society*, London, 25; 1-9.

Thresh, J.M.; Owusu, G.K.; Boamah, A.; Lockwood, G. (1988a). Ghanaian cocoa varieties and swollen shoot virus. *Crop protection* 7:219-231.

Thorold, C.A. (1975). *Diseases of cocoa.* Oxford, U.K. Clarendon Press.

Toxopeus, H. and Jacob, V. J. (1970). Studies on pod and bean values of *Theobroma cacao* L. in Nigeria: 11 Number of bean per pod, special reference to the natural pollination process. *Neth J. Agric Sci* 18: 188-94

Urguhart, D.H. (1961). *Cocoa*. Second Edition, Western printing services Ltd., Bristol pp.293.

Wills, J. B. (1962). *Agriculture and land use in Ghana*, Oxford university press, pp 504.

Winder, J. A (1977). Recent reserch on insect pollination of cocoa. *Cocoa Grower's Bull.* 26: 11-19

Wood, G.A.R. and Lass, R.A. (1989). *Cocoa*, Fourth edition. Tropical Agriculture Series pp. 620.

Wood, G.A.R. (1975). *Cocoa*. Third edition. Longman group limited. pp. 291.

Zadoks, J.C. (1996). *Disease resistance testing in cocoa*. A review on behalf of FAO/Ingenic pp.110.

APPENDICES

APPENDIX 1(a)

Diallel table for resistance to CSSV

	PA7	PA150	NA33	SCA6	SCA9
PA7	50.00	47.00	40.00	27.00	22.00
PA150	58.00	70.00	35.00	28.00	37.00
NA33	70.00	50.00	100.00	33.00	38.00
SCA6	43.00	53.00	42.00	40.00	27.00
SCA9	35.00	35.00	48.00	18.00	20.00

APPENDIX 1(b)

Vr, Wr, Wr-Vr and Wr+Vr calculated from current table for resistance to CSSV

Array	Mean	Vr	Wr	Wr-Vr	Wr+Vr
PA7	44.2000	137.5750	317.2500	179.6750	454.8250
PA150	48.3000	183.5750	147.7500	-35.8250	331.3250
NA33	55.6000	657.4250	629.2500	-28.1750	1286.6750
SCA6	35.1000	54.4250	139.2500	84.8250	193.6750
SCA9	30.0000	90.8750	286.2500	195.3750	377.1250

APPENDIX 2(a)

Diallel table for stem height

	PA7	PA150	NA33	SCA6	SCA9
PA7	38.40	33.10	44.80	47.80	39.20
P150	47.80	68.00	68.20	58.40	34.20
NA33	33.00	62.60	63.20	60.90	60.80
SCA6	61.40	58.60	59.80	59.00	52.80
SCA9	42.80	52.00	52.50	50.00	47.00

APPENDIX 2(b)

Vr, Wr, Wr-Vr and Wr +Vr calculated from current table for stem height

Array	Mean	Vr	Wr	Wr-Vr	Wr+Vr
PA7	42.6700	45.6220	18.0470	-27.5750	63.6690
PA150	55.0900	160.6705	151.2390	-9.4315	311.9095
NA33	56.9000	111.9763	119.1900	7.2137	231.1663
SCA6	56.7700	13.5820	34.9370	21.3550	48.5190
SCA9	47.8300	40.0620	36.2830	-3.7790	76.3450

APPENDIX 3(a)

Diallel table for stem diameter

	PA7	PA150	NA33	SCA6	SCA9
PA7	6.640	6.860	6.620	6.560	6.680
PA150	7.960	9.200	9.400	8.620	6.060
NA33	6.680	7.640	7.840	7.200	7.560
SCA6	6.600	7.180	6.040	6.590	6.040
SCA9	7.260	7.520	7.600	7.440	7.200

APPENDIX 3(b)

Vr, Wr, Wr-Vr and Wr+Vr calculated from current table for stem girth

Array	Mean	Vr	Wr	Wr-Vr	Wr+Vr
PA7	6.8500	0.1213	0.3186	0.1973	0.4398
PA150	7.9640	0.8815	0.7943	-0.0872	1.6758
NA33	7.4420	0.6606	0.8389	0.1783	1.4995
SCA6	6.8860	0.3254	0.5524	0.2270	0.8778
SCA9	7.0560	0.1183	0.0111	-0.1073	0.1294