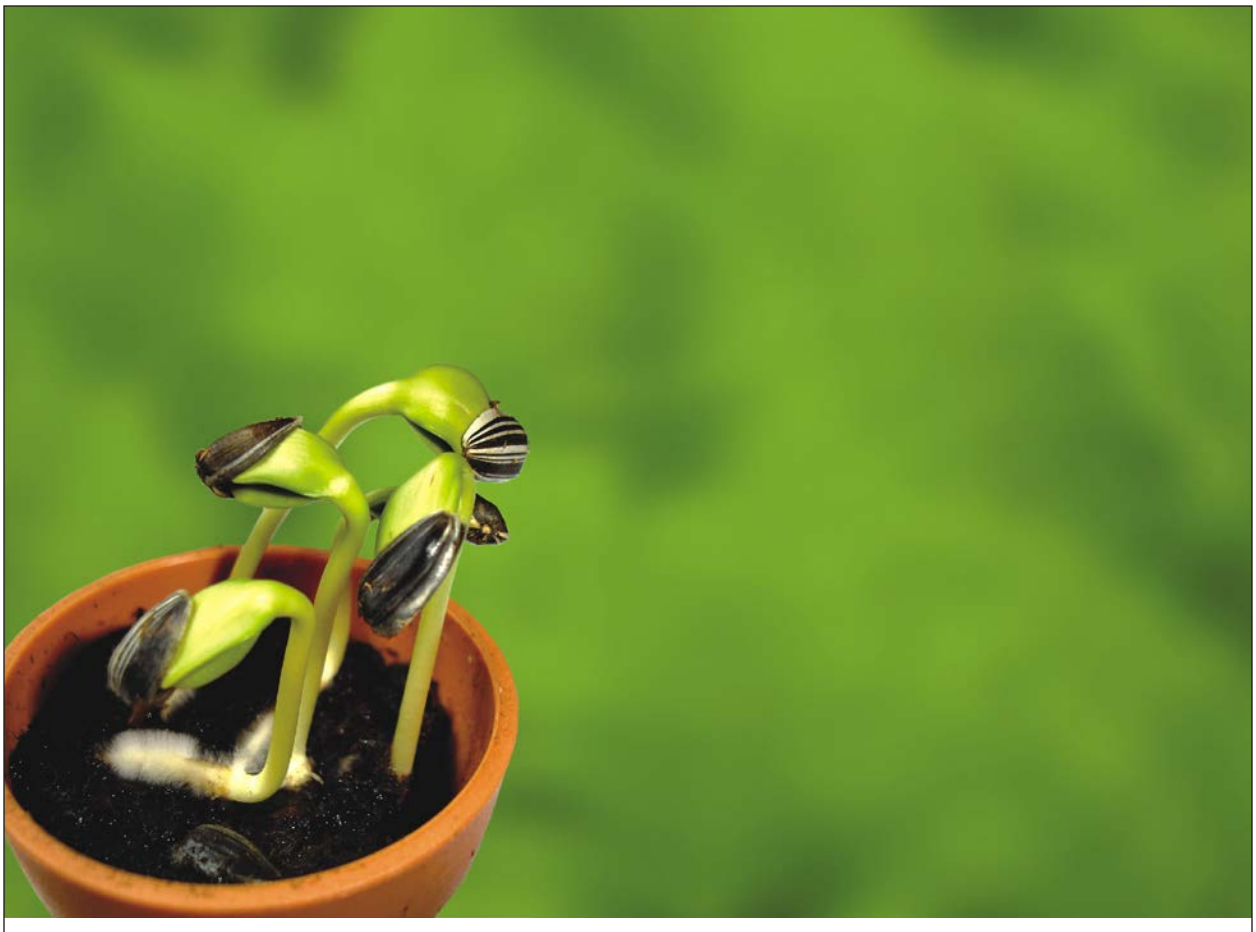

International Journal of Plant Breeding and Genetics

ISSN 1819-3595

Academic Journals Inc.

www.academicjournals.com

International Journal of Plant Breeding and Genetics 6 (4): 182-194, 2012
ISSN 1819-3595 / DOI: 10.3923/ijpbg.2012.182.194

Breeding for Multiple Disease Resistance in Cocoa (*Theobroma cacao* L.)

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ABSTRACT

Black pod and Canker caused by *Phytopthora palmivora* and *Phytophthora megakarya* and Cocoa Swollen Shoot Virus Disease (CSSVD) caused by cocoa swollen shoot virus are important diseases of cocoa in Ghana. Host plant resistance has been considered the most effective method of controlling these diseases. This study was initiated to determine whether multiple forms of resistance to these diseases could be identified in hybrids of cocoa. Thirty six crosses of 6×6 diallel mating design of cocoa were screened for resistance to black pod (*P. palmivora* and *P. megakarya*), CSSVD (severe New Juaben CSSV Strain 1A and Nsaba CSSV strain) and *Phytophthora* canker (*P. palmivora* and *P. megakarya*) under a controlled environment. Of the thirty six crosses, 12, 6 and 7 were resistant to black pod, cocoa swollen shoot virus disease and *Phytophthora* canker, respectively. No cross was found to have combined resistance to the three diseases. This indicates that selection and breeding of cocoa genotypes for multiple disease resistance based on phenotypic data alone could be difficult. Marker assisted selection using tightly linked gene-specific molecular markers will play a larger role in future studies and could be an asset in working with quantitative resistance systems. Cocoa hybrids Alpha B36×Pa7/808, Pa7/808×Pound 7 and Alpha B36×T65/326 which possess high levels of resistance to more than one disease were identified through this study and would be useful in cocoa multiple disease resistance breeding programmes.

Key words: *Theobroma cacao* L., multiple disease resistance, black pod disease, cocoa swollen shoot virus disease, phytophthora canker

INTRODUCTION

Theobroma cacao L. (Cacao; Malvaceae *sensu lato*) is a small tree endemic to the lowland rainforests of the Amazon basin (Wood and Lass, 1985; Bartley, 2005). Cocoa is the major export commodity in Ghana and other countries in West Africa (68% of world production). Cocoa diseases reduce the potential crop yield by an estimated 810,000 tons annually (30% of world production) and individual farm losses can approach 100% (Keane, 1992; Bowers *et al*., 2001).

Cocoa diseases of most economic importance in Ghana include, black pod disease, Cocoa Swollen Shoot Virus Disease (CSSVD) and trunk canker (Opoku *et al*., 2007).

Cocoa Swollen Shoot Disease (CSSD), in particular poses a serious threat to Ghana's cocoa industry. The disease has caused enormous devastation of cocoa farms in Ghana since its discovery

in 1936 and over 200 million visibly infected and 'contact' trees have been cut-out from about 130,000 hectares of land during the past 50 years as a control measure (Ampofo, 1997). However, CSSVD is still prevalent and has spread to all cocoa growing regions of Ghana (Ollennu *et al.*, 2002). Infection with severe strains reduced the yield of mature trees by 25% after 1year, by 50% after 2 years and almost totally after 3 years, by which time most infected trees were dead or dying (Posnette, 1941; Brunt, 1975). Millions of cocoa trees have been killed as a result of the CSSVD since then and the spread of the disease has been largely unimpeded in the area of mass infection in the Eastern Region of Ghana.

The practical method of control of CSSVD has been identification and destruction of infected and neighboring contact trees and subsequent re-inspection of treated farms. Cutting out, however, seems unable to prevent new outbreaks which continue to occur due to the difficulty of controlling the mealybug vectors of CSSV, the severity of the disease in some areas and the latent infection in cocoa and in certain forest trees and the high cost of control by cutting out diseased trees (Legg, 1979). It was generally agreed that breeding for resistance to the virus would be the long term solution to the problem. There were an indication that resistance from different cocoa populations could be accumulated to give progenies of higher resistance since resistance to CSSV was found to be additive and polygenically inherited (Lockwood, 1981).

Black pod disease caused by *Phytophthora palmivora* and *Phytophthora megakarya* in Ghana (Dakwa, 1987; Luterbacher and Akrofi, 1993; Opoku *et al.*, 1999) is one of the most prevalent and destructive diseases of cocoa (*Theobroma cacao* L.). *P. megakarya* is gradually spreading to all cocoa growing areas in Ghana. The development of high-yielding, resistant material is generally agreed to be the most effective and economic control method (Adomako, 2007, 2006; Nyadanu *et al.*, 2009; Cilas and Despreaux, 2004; Iwaro *et al.*, 2000).

The incidence of canker in Ghana dates back to the 1920's (Dade, 1928). *Phytophthora* canker is characterized by discolorations of bark tissues on the trunk and branches (Firman and Vernon, 1970). Canker reduces the yield potential of the tree by destroying flower cushions (Firman, 1974) and also serves as an important source of inoculum for pod infection (Griffin *et al.*, 1981). Despite its economic importance, cocoa canker has received little attention probably due to difficulties in field identification. Severe canker infections resulting in the death of many cocoa trees, particularly in the *P. megakarya* infected cocoa growing areas of Western, Ashanti and Brong Ahafo Regions of Ghana was reported. At the early stage of development and under dry conditions, scraping the bark to expose the canker lesions and or painting the scraped lesion with fungicides generally halts the advancement of the canker. However, under high rainfall and humidity, the canker may quickly girdle the stem and kill the tree (Akrofi, 2003). Breeding for resistance has been considered as the most effective to control the disease.

Efforts at developing resistant materials of major cocoa diseases in Ghana have been targeted at developing cocoa materials having resistance to only one of the pathogens at a time. Information on whether varieties resistant to one disease would necessarily be resistant to other diseases is not available. However, since the pathogens of CSSVD, black pod and canker continuous to spread to cocoa growing areas, real farm situations require cultivars having multiple disease resistance to CSSVD, black pod and canker. This is because it is only rare that only one pathogen is present in a particular field. Economic advantages of such cultivars with multiple disease resistance to the major diseases of cocoa are obvious; they are less expensive and less labour-intensive for the grower, reduce yield loss and minimize the need for pesticide applications, resulting in less pollution of the environment.

Multiple Disease Resistance (MDR) has been a major objective of research of many pathologists and breeders working on different crops (Jansky and Rouse, 2003). In Ghana however, MDR of

cocoa varieties to the three major cocoa diseases, that is, black pod, CSSVD and trunk canker have not been reported. The objective of this research was to develop cocoa hybrids from parents that have been known to be tolerant to black pod, CSSVD and trunk canker and evaluate them for multiple disease resistance to these diseases.

MATERIALS AND METHODS

Plant material: Six cocoa genotypes, Alpha B36, Pa7/808, Pound 7, T17/524, T65/238 and T65/326 were selected for this study among the accessions held at the Cocoa Research Institute of Ghana. These cocoa genotypes were used as parents in a 6x6 full diallel mating design. The progenies from the diallel mating design were grown under shade in a polybag. The crosses were arranged in a randomized complete block design with 20 seedlings per cross. The origins of the parents are listed in Table 1.

Evaluation of black pod disease resistance

Inoculum preparation: *Phytophthora palmivora* and *Phytophthora megakarya* were grown on carrot agar medium and from a ten-day-old culture, a zoospore suspension was obtained by inundating each culture plate (9 cm diameter) with 10 mL sterile distilled water (chilled to 10°C), refrigerated for 25 min (5°C) and incubated in the dark at 25°C for 30 min. The zoospore concentration of the suspension was determined using a haemocytometer and adjusted to 200,000 mL^{-1}.

Leaf disc test

Field leaf sampling: The new flushes from bud break of the genotypes were tagged to obtain average ages of the leaves for each experiment. For each of the inoculation series, leaves were collected from all the 36 crosses. Leaves were harvested from the progenies of each cross. The average ages of the leaves for each treatment were established by following the growth of young flushes from bud break. After collecting the mature leaves, they were placed in labelled polyethylene bags into which a few drops of distilled water were sprayed before hand. The bags were then kept in the dark till the next morning to minimize effect of leaf sampling time that may occur with large time lapses between harvesting of leaves (Tahi, 2003). The leaves were washed thoroughly with tapwater, blotted dry with Whatman number 3 paper and then surface sterilised with 70% ethanol.

Preparation of leaf discs and inoculation method: Leaf disc inoculation as described by Nyasse *et al.* (1995) was carried out. In total, 15 discs of 1.5 cm in diameter were taken per leaf from the seedling progenies with a cork borer the next day after harvesting the leaves. All the discs from the same cross were mixed. Leaf discs were placed with their abaxial surface upwards on

Table 1: Origin of parents of progenies

Alpha B36	The Tafo alphabetical code B36
Pa7/808	Selections made by Pound at Parinari
Pound7	Material collected in the headwaters of the Amazon by Pound
T17/524	Seedling progenies collected from Trinidad by Posnette in 1944
T65/238	Seedling progenies collected from Trinidad by Posnette in 1944
T65/326	Seedling progenies collected from Trinidad by Posnette in 1944

wetted plastic foam of 1 cm thick and imbibed with 2.5 L of distilled water in four trays of 70×60×10 cm. The discs from the same plant were aligned in totally randomized rows of ten discs per tray. Inoculation was carried out the same day, after preparation of all leaf discs. After the concentration of zoospores were determined with a hemacyatometer and adjusted to 200,000 mL^{-1}, droplets of 10 µL were placed on each disc. Leaf discs from each cross, placed in rows, were inoculated across the rows so as to inoculate a disc from each tree in succession in order to randomize any effect of the spore batch equally over the different genotypes. The discs were incubated at room temperature of 25°C in plastic trays lined with moist plastic foam and covered with another plastic tray in the laboratory avoiding direct sunlight until observations were carried out.

Observation of symptoms on leaf discs: Symptoms were scored 6 days after inoculation using a 0 to 5 point scale depending on the size of necrosis (0 = absence of symptoms, 1 = very small necrotic spots, 2 = larger number and size of necrotic spots, 3 = coalescence of brown spots into medium-sized, 4 = large uniform brown lesions and 5 = very large brown lesions, often expanding outside the area covered by the inoculum droplet) as described by Nyasse *et al.* (1995). The experiment was repeated twice.

Evaluation of swollen shoot resistance: Patch grafting method of inoculation (Posnette, 1940) was used to evaluate the resistance levels of the progenies. A patch from the bark of a source plant with the phloem tissues attached was carefully put into a slit made in the recipient rootstock and held firmly with budding tape. Two strains of the virus were used; severe New Juaben CSSV strain 1A and Nsaba CSSV strain. The number of plants showing symptoms of CSSV disease in the first, second and third flush leaves after inoculation was recorded over a period of 5 months under a controlled environment. The severity of symptoms was rated on a 0-6 scale as follows:

0: Healthy (no symptoms)
1: Red vein banding of leaves
2: Chlorotic flecking of leaves
3: Chlorotic vein clearing and green vein banding of leaves
4: Diffused flecking of leaves
5: Fern pattern and swollen shoot
6: Dead plant

Evaluation of canker resistance: The 36 crosses were screened for resistance to *Phytophthora* canker. The stems of 10 month old test seedlings were inoculated with agar discs of *P. palmivora* and *P. megakarya*. About 0.5 m from the ground, the bark of each seedling was sterilized with 70th ethanol and inoculated with 1 mm^{-2} agar plug taken from the margins of actively growing colonies of 10-day old cultures of *P. palmivora* and *P. megakarya*. Plastic film was then wrapped over the inoculated site and tightly secured with adhesive tape. 12 months later, the tape was removed and the observed lesions (the length and width) measured with a measuring tape. The canker lesions on outer bark of the seedlings were measured and the canker lesions inside was also measured after scraping the bark.

Statistical analysis: All the data obtained were analysed using the Genstart statistical software (Version 10.0) to perform analysis of variance. The residual plots were inspected to confirm data

conformed to normality. The relationships among resistance of cocoa genotypes to black pod, swollen shoot virus and *Phytophthora* canker diseases were tested by Spearman's correlation analysis.

RESULTS

Resistance to black pod disease: The study showed significant differences ($p<0.001$) in leaf disc score rating of resistance levels among the crosses to *P. palmivora* and *P. megakarya* (Table 2). The

Table 2: Mean leaf disc and CSSV severity scores of the cocoa crosses

	Mean leaf disc score (LDS)		Mean CSSV score*	
Crosses	LDS Pp	LDS Pm	1A	Nsaba
Alpha B36×Pa7/808	1.99±0.12	1.94±0.11	2.28±0.29	2.32±0.21
Alpha B36×Pound7	1.55±0.09	1.77±0.07	4.08±0.24	2.05±0.28
Alpha B36×T17/524	2.63±0.08	3.26±0.10	1.82±0.24	2.85±0.23
Alpha B36×T65/238	2.54±0.09	2.43±0.09	3.00±0.25	3.13±0.15
Alpha B 36×T65/326	1.88± 0.07	1.82±0.08	3.20±0.22	2.38±0.14
Alpha B36×Alpha B36	1.99±0.09	2.27±0.07	2.72±0.31	1.97±0.23
Pa7/808×Alpha B36	1.73±0.09	1.93±0.11	3.38±0.17	2.52±0.25
Pa7/808×Pa7/808	2.27±1.00	2.35±0.10	2.70±0.19	3.07±0.22
Pa7/808×Pound7	1.93±0.11	1.93±0.12	2.55±0.19	2.00±0.19
Pa7/808×T17/524	2.69±0.13	3.26±0.11	2.68±0.19	2.30±0.19
Pa7/808×T65/238	1.98±0.09	2.32±0.08	2.97±0.31	2.25±0.23
Pa7/808×T65/326	2.13±1.09	1.83±0.10	1.52±0.22	2.73±0.12
Pound 7×Pa7/808	1.44±0.08	1.55±0.08	3.95±0.18	2.48±0.31
Pound 7×Alpha B36	1.48±0.09	1.76±0.08	3.20±0.30	2.70±0.24
Pound 7×Pound7	2.34±0.08	2.24±1.00	2.47±0.25	2.62±0.23
Pound 7×T17/524	3.08±0.09	3.51±0.09	2.58±0.20	2.28±0.32
Pound 7×T65/238	2.31±0.09	2.26±0.09	3.23±0.25	2.65±0.21
Pound 7×T65/326	2.15±0.08	2.38±0.07	3.82±0.29	2.90±0.25
T17/524×Pa7/808	2.87±0.09	3.19±0.08	2.83±0.32	1.88±0.24
T17/524×Pound7	2.47±0.07	2.27±0.06	3.20±0.25	3.32±0.14
T17/524× Alpha B36	2.56±0.06	2.63±0.08	2.78±0.26	2.82±0.19
T17/524×T17/524	2.64±0.07	2.45±0.07	3.17±0.15	2.73±0.17
T17/524×T65/238	2.45±0.08	2.33±0.07	2.22±0.22	2.60±0.23
T17/524×T65/326	2.46±0.07	2.64±0.06	3.03±0.23	2.17±0.18
T65/238×Alpha B36	1.94±0.09	2.15±0.08	3.23±0.21	2.53±0.17
T65/238×Pa7/808	2.45±0.06	2.43±0.09	3.12±0.21	3.01±0.19
T65/238×Pound 7	1.92±0.11	1.91±0.10	3.11±0.28	3.33±0.27
T65/238×T17/524	2.57±0.07	3.27±0.11	2.62±0.26	2.65±0.19
T65/238×T65/238	2.59±0.08	2.67±0.06	3.17±0.20	2.23±0.19
T65/238×T65/326	2.86±0.09	2.81±0.09	3.40±0.27	2.40±0.24
T65/326×Alpha B36	2.27±0.05	1.86±0.08	4.08±0.22	3.88±0.21
T65/326×Pa7/808	2.13±0.07	1.94±0.09	3.88±0.24	2.96±0.27
T65/326×Pound 7	1.96±0.09	2.09±0.08	4.00±0.25	2.50±0.19
T65/326×T17/524	2.74±0.07	2.85±0.06	4.00±0.25	2.50±0.19
T65/326×T65/238	2.65±0.08	2.78±0.09	2.28±0.18	2.13±0.19
T65/326×T65/326	1.98±0.09	1.95±0.09	3.20±0.23	2.40±0.21
LSD	0.24	0.25	0.66	0.61
SE	0.68	0.69	1.86	1.70
Mean	2.27	2.36	3.05	2.59

*Mean CSSV tolerance score was based on the cumulative score (severity) of all symptoms of each plant, Higher score indicates higher level of susceptibility. Pp: *P. palmivora*, Pm: *P. megakarya*

mean disease severity was 2.27 and 2.36 for *P. palmivora* and *P. megakarya*, respectively. Interaction between crosses x Phytophthora species was not significant ($p>0.05$). Of the 36 crosses, Alpha B36×Pa7/808, Alpha B36×Pound7, Alpha B36×T65/326, Alpha B36×Alpha B36, Pa7/808×AlphaB36, Pa7/808×Pound7, Pa7/808×T65/238, Pound 7×Pa7/808, T65/238×Alpha B36, T65/238×Pound7, T65/326×Pound7 and T65/326×T65/326 were the most resistant (Table 2). The most susceptible crosses were T17/524×Pa7/808, T65/238×T65/238 and T65/326×T17/524 (Table 2).

Resistance to swollen shoot virus disease: Significant differences ($p<0.001$) were observed among the crosses in terms of severity of symptoms (tolerance) caused by New Juaben 1A virus strain and Nsaba virus strain (Table 2). The mean disease severity was 3.05 and 2.59 for 1A and Nsaba, respectively. Interaction between Crosses x virus strains was not significant ($p>0.05$). The most tolerant crosses were Alpha B36×T17/524, Pa7/808×T65/326, Alpha B36×Pa7/808, Pa7/808×Pound 7, T17/524×T65/238 and T65/326×T65/238 (Table 2). The most susceptible crosses were Alpha B36×Pound 7, T65/326×AlphaB36, T65/326×Pa7/808, T65/326×Pound7 and T65/326×T17/524 (Table 2).

Resistance to canker: The 36 hybrids varied significantly ($p<0.001$) in canker lesion sizes both before and after scraping (Table 3). The mean of canker lesion sizes before scraping was 12.43 and

Table 3: Mean canker lesions of the crosses before and after scraping

	Canker before scraping		Canker after scraping	
Crosses	Pp	Pm	Pp	Pm
Alpha B36×Pa7/808	11.04±0.81	27.45±1.09	27.45±1.09	24.83±1.27
Alpha B36×Pound7	13.78±0.79	28.96±1.34	28.96±1.34	29.11±1.19
Alpha B36×T17/524	12.85±0.97	18.76±0.79	18.76±0.79	27.52±1.46
Alpha B36×T65/238	5.68±0.82	14.87±1.23	14.87±1.23	15.85±1.32
Alpha B 36×T65/326	6.17±0.57	16.56±0.81	16.56±0.81	17.29±0.95
Alpha B36×Alpha B36	21.24±1.32	31.04±1.69	31.04±1.69	39.49±1.81
Pa7/808×Alpha B36	9.68±0.69	21.42±0.89	21.42±0.89	23.00±1.06
Pa7/808×Pa7/808	36.09±2.13	49.68±2.92	49.68±2.92	58.92±2.78
Pa7/808×Pound7	18.71±0.80	38.22±1.35	38.22±1.35	36.26±1.12
Pa7/808×T17/524	5.26±0.53	16.83±1.72	16.83±1.72	15.58±0.91
Pa7/808×T65/238	10.38±0.67	29.75±1.44	29.75±1.44	24.12±1.01
Pa7/808×T65/326	13.42±1.05	30.63±1.61	30.63±1.61	28.47±1.49
Pound 7×Pa7/808	3.93±0.39	14.39±0.63	14.39±0.63	12.78±0.79
Pound 7×Alpha B36	4.18±0.45	11.64±0.88	11.64±0.88	13.10±0.91
Pound 7×Pound7	4.43±0.37	11.77±0.71	11.77±0.71	14.30±0.69
Pound 7×T17/524	6.36±0.52	17.46±1.01	17.46±1.01	17.78±0.84
Pound 7×T65/238	5.07±0.60	13.21±1.05	13.21±1.05	15.18±0.99
Pound 7×T65/326	4.69±0.45	13.19±0.75	13.19±0.75	14.78±0.77
T17/524×Pa7/808	19.25±1.19	31.45±1.58	31.45±1.58	36.86±1.62
T17/524×Pound7	12.49±0.75	26.07±1.46	26.07±1.46	27.27±1.11
T17/524×Alpha B36	16.33±1.06	25.35±1.13	25.35±1.13	32.73±1.49
T17/524×T17/524	17.15±1.34	35.29±1.61	35.29±1.61	33.69±1.87
T17/524×T65/238	16.03±0.79	32.18±1.64	32.83±1.47	32.46±1.13

Table 3: Continue

	Canker before scraping		Canker after scraping	
Crosses	Pp	Pm	Pp	Pm
T17/524×T65/326	20.92±1.02	34.83±1.47	34.83±1.46	39.23±1.40
T65/238×Alpha B36	11.54±0.92	22.67±1.46	22.67±1.46	25.68±1.37
T65/238×Pa7/808	14.00±0.73	25.91±1.03	25.91±1.03	29.55±1.06
T65/238×Pound 7	14.21±1.28	26.03±1.23	26.03±1.23	29.36±1.83
T65/238×T17/524	11.69±1.15	27.01±1.19	27.01±1.19	25.62±1.69
T65/238×T65/238	24.27±1.57	31.41±2.00	31.41±2.00	43.47±2.13
T65/238×T65/326	13.80±0.89	16.99±0.84	16.99±0.84	29.11±1.29
T65/326×Alpha B36	8.72±0.41	21.81±0.75	21.81±0.75	21.72±0.65
T65/326×Pa7/808	14.79±0.91	27.37±1.03	27.37±1.03	30.59±1.30
T65/326×Pound7	5.48±0.49	15.18±0.84	15.18±0.84	16.21±0.82
T65/326×T17/524	13.55±1.14	29.11±1.87	29.11±1.87	28.53±1.65
T65/326×T65/238	10.32±0.70	22.67±1.11	22.67±1.11	23.92±1.10
T65/326×T65/326	9.84±0.49	26.19±0.99	26.19±0.99	23.52±0.76
LSD	2.56	2.53	3.69	3.66
SE	7.16	7.09	10.32	11.21
Mean	12.43	11.04	24.54	26.61

Larger lesion size indicates higher level of susceptibility. Pp: *P. palmivora*, Pm: *P. megakarya*

11.04 cm^2 for *P. palmivora* and *P. megakarya*, respectively. The mean of canker lesion sizes after scraping was 24.54 and 26.61 cm^2 for *P. palmivora* and *P. megakarya*, respectively. The most tolerant crosses were Pound 7×Alpha B36, Pound 7×Pound 7, Pound 7×T65/326, Pound 7×T65/238, Pa7/808×T17/524, Alpha B36×T65/326 and Alpha B36×T65/238 (Table 3). The most susceptible crosses were Pa7/808×Pa7/808, T65/238×T65/238, T17/524×T65/326, T17/524×Pa7/808 and Pa7/808×Pound 7 (Table 3).

Multiple disease resistance: Combined resistance to black pod, cocoa swollen shoot virus disease and *Phytophthora* canker was not observed in any of the crosses tested. Two crosses, Alpha B36×Pa7/808 and Pa7/808×Pound 7 had combined resistance to both black pod and cocoa swollen shoot virus diseases (Table 2). Combined resistance to black pod disease and *Phytophthora* canker was observed in one cross, Alpha B36×T65/326 (Table 3). No cross was found to have combined resistance to *Phytophthora* canker and cocoa swollen shoot virus disease.

AlphaB36×Pound 7 and T65/326×Pound 7 which were observed to be resistant to black pod were observed to be susceptible to swollen shoot virus (Table 2). Pa7/808×Pound 7 which was observed to be resistant to both black pod disease and swollen shoot virus was found to be susceptible to *Phytophthora* canker (Table 2, 3).

Correlations among resistance to black pod, cocoa swollen shoot virus and *Phytophthora* canker diseases: Table 4 shows that the rank correlations between leaf disc score and severity scores of cocoa swollen shoot virus, leaf disc score and *Phytophthora* canker lesion sizes and severity scores of cocoa swollen shoot virus and *Phytophthora* canker were not significant ($p>0.05$). Figure 1-3 show graphical presentation of relationship between black pod disease and cocoa swollen shoot, black pod and canker and swollen shoot and canker, respectively. The regression coefficients (r^2) of Fig. 1-3 were 7.0, 1.5 and 4.5, respectively. The regression

Table 4: Spearman's rank correlation between black pod, swollen shoot virus disease and *Phytophthora* canker in the crosses of cocoa

Parameters	Correlation coefficients	Probability level
Leaf disc score Pp vs. CSSV 1A	-0.31	0.02
Leaf disc score Pp vs. CSSV Nsaba	-0.04	0.21
Leaf disc socre Pm vs. CSSV 1A	-0.28	0.02
Leaf disc score Pm vs. CSSV Nsaba	-0.26	0.15
Canker before scraping Pp vs. Leaf disc score Pp	0.29	0.02
Canker before scraping Pp vs. leaf disc score Pm	0.33	0.01
Canker after scraping Pm vs. leaf disc sscore Pp	0.28	0.03
Canker after scraping Pm vs. leaf disc score Pm	0.32	0.01
CSSV 1A vs. Canker before scraping Pp	-0.23	0.04
CSSV 1A vs. Canker before scraping Pm	-0.28	0.02
CSSV Nsaba vs. canker after scraping Pp	-0.26	0.03
CSSV Nsaba vs. canker after scraping Pm	-0.15	0.09

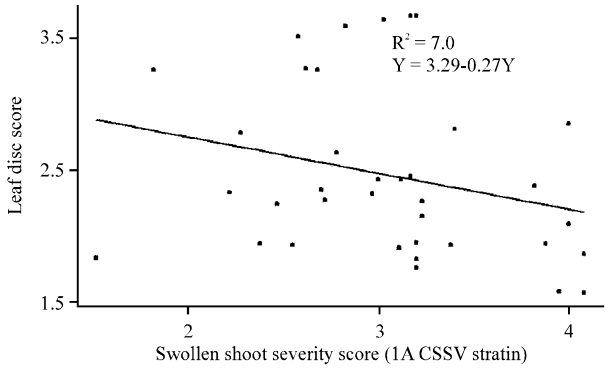

Fig. 1: Relationship between *P. megakarya* caused black pod and swollen shoot diseases resistance

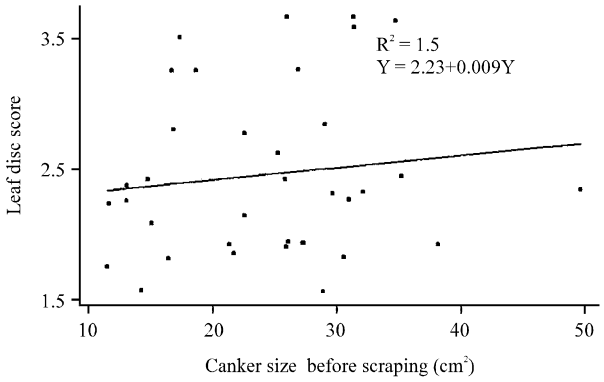

Fig. 2: Relationship between *P. megakarya* caused black pod and canker diseases resistance

coefficients shows that the associations among resistances to black pod, cocoa swollen shoot virus and stem canker diseases are not strong. This suggests that resistance to one disease could not be used to predict resistance to another disease.

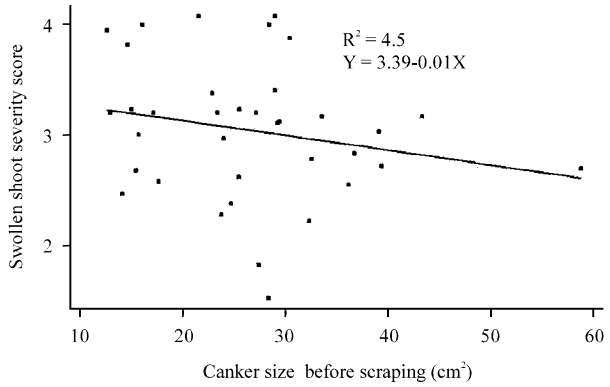

Fig. 3: Relationship between *P. megakarya* caused swollen shoot and canker diseases resistance

DISCUSSION

Due to limited availability of resources for present-day agricultural research, an extensive evaluation of an entire germplasm for a particular disease is difficult and also time consuming. Thus, the concept of multiple disease resistance that involves selection of varieties with combined resistance to a number of diseases has been put forward to save time and yield loss of crops (Steffenson and Smith, 2006; Fetch *et al*., 2003; Mmbaga and Sauve, 2004; Panella *et al*., 2008; Jansky and Rouse, 2003; Pande *et al*., 2006).

The study shows considerable genetic variability among the hybrids of cocoa for resistance to black pod, cocoa swollen shoot virus and *Phytophthora* canker. The differential response of cocoa hybrids further suggested that these characters are under genetic control and should therefore be liable to genetic improvement. This agrees with findings of Iwaro *et al*. (1997), Nyadanu *et al*. (2009), Nyasse *et al*. (2002) and Tahi *et al*. (2006). Adomako (2006) and Thresh *et al*. (1988) reported significant differences among genotypes of cocoa for resistance to swollen shoot virus. Resistance to CSSV has been found to be largely polygenic (additive) (Lockwood, 1981). The significant differences among cocoa genotypes for CSSV resistance indicates that it might be possible to increase resistance by accumulating the different resistant factors, since CSSV resistance genes are neither confined to one particular population nor to any type of progeny but scattered among them (Adomako, 2006). The significant differences in *Phytophthora* canker in this study agrees with reports of Firman and Sundaram (1970) and Okey *et al*. (1996) who also reported genetic variability among cocoa genotypes for resistance to *Phytophthora* canker in cocoa.

Interaction of the hybrids with the *Phytophthora* species and the viral strains was not significant, indicating that the hybrids did not change their relative ranking for black pod, swollen shoot and *Phytophthora* canker resistance across the *Phytophthora* species and the viral strains. The non-significance of the host genotypes x *Phytophthora* species and host genotypes x viral strains has important implications in cocoa breeding for resistance to black pod, cocoa swollen shoot virus disease and *Phytophthora* trunk canker. The levels of resistance of cocoa genotypes could be identified using any of the *Phytophthora* species and viral strains since resistance has been shown to be *Phytophthora* species or viral strain non-specific in this study. However, the use of the most aggressive species of Phytophthora or viral strains could lead to the identification of useful levels of resistance against the pathogens. The results of the present study support the previous results of Van der Vossen (1997), Iwaro *et al*. (1997) and Surujdeo-Maharaj *et al*. (2001) who reported that interaction between *Phytophthora* species and cocoa genotypes was not significant.

Combined resistance to black pod, cocoa swollen shoot virus disease and *Phytophthora* canker was not observed in any of the crosses tested. The results show that Alpha B36×Pound 7 and T65/326×Pound7 which were observed to be resistant to black pod were susceptible to swollen shoot virus. Pa7/808×Pound 7 which was observed to be resistant to both black pod disease and swollen shoot virus was found to be susceptible to *Phytophthora* canker. These findings suggest that, a cocoa cultivar resistant to one disease may not necessarily be resistant to another disease of cocoa. These findings agrees with the results of Okey *et al.* (1996) who observed SCA 6, a well known black pod disease resistant cocoa variety, to be susceptible to *Phytophthora* trunk canker. The insignificant Spearman's rank correlations among results of resistance to black pod, cocoa swollen shoot virus and *Phytophthora* canker further suggest that cocoa genotypes resistant to one disease may not be resistant to another disease of cocoa. These findings suggest that selecting cocoa genotypes for multiple disease resistance to the major diseases of cocoa could be difficult. This agrees with works of other authors. Wang *et al.* (2007) reported that breeders frequently face complex choices in designing efficient crosses and selection strategies aimed at combining desired genes into a single target genotype. Mohler and Singrun (2004) also reported that is difficult to select plants with multiple resistance genes based on phenotype alone due to epistatics. Gene pyramiding using Marker Assisted Selection (MAS) is a practical approach to achieving multiple and durable resistance (Schafer and Roelfs, 1985; Mundt, 1990; Singh *et al.*, 2001; Castro *et al.*, 2003). Pyramiding of genes for all the three major diseases of cocoa could be accomplished through MAS using tightly linked gene-specific molecular markers. The establishment of related molecular studies would enhance selection especially regarding the accumulation of resistance genes in one genotype in order to increase resistance level and the durability of such new cacoa cultivars.

Alpha B36×Pa7/808 and Pa7/808×Pound7 with combined resistance to black pod and cocoa swollen shoot virus diseases and Alpha B36×T65/326 with combined resistance to black pod disease and *Phytophthora* canker are potential promising materials for multiple disease resistance breeding in cocoa.

CONCLUSIONS

Selection and breeding of cocoa genotypes for multiple disease resistance to black pod, cocoa swollen shoot and *Phytophthora* canker diseases of cocoa based on phenotypic data alone would be difficult. Future studies should consider pyramiding of genes for these diseases using tightly linked gene-specific molecular markers. The study identifies Alpha B36×Pa7/808 and Pa7/808×Pound 7 to have multiple disease resistance to black pod and cocoa swollen shoot virus and Alpha B36×T65/326 to have multiple disease resistance to black pod and *Phytophthora* canker. These crosses could therefore be valuable sources of resistance for cocoa breeding programs for multiple disease resistance.

ACKNOWLEDGMENTS

Financial support provided by Ghana Cocoa Growing Research Association (GCGRA), UK, is much appreciated. Technical support provided by Messrs Mawuli Adoblanui, Emmanuel Ewe and Ernest Akortia and Madam Mercy Ofori, all of CRIG is highly acknowledged.

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