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Effects of Cocoa Swollen Shoot Virus Infection on Foliar Resistance to *P. palmivora* and *P. megakarya* and its Implications in Selection and Breeding Against Black Pod Disease

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ABSTRACT

Cocoa Swollen Shoot Virus (CSSV) occurs in all cocoa growing regions of Ghana and other parts of the West African sub-region. Cocoa swollen shoot virus disease has a long latent phase and so infected leaves might inadvertently be used in leaf disc tests. However, the effect of cocoa swollen shoot virus infection of leaves on the results of leaf disc test is not known. The objective of this study was to understand the effect of CSSV on leaf disc test to help select cocoa genotypes resistant to *Phytophthora* species. Leaf disc test with virus infected and healthy cocoa leaf tissues were therefore conducted to determine the effect of the virus infection on the results of leaf disc test. Significant differences were observed among the cocoa genotypes in resistance to *Phytophthora* species. Interaction between cocoa genotypes and *Phytophthora* species was not significant. However, cocoa genotypes × *Phytophthora* species × virus strains interaction was significant. Leaf discs from genotypes infected by CSSV were more resistant to *Phytophthora* species than those from healthy genotypes. The findings suggest that resistance of cocoa genotypes to *Phytophthora* species is significantly affected by CSSV and depend on the type of CSSV strain. Selecting and breeding for resistance to black pod disease using leaf disc test on cocoa genotypes with unknown CSSV status could be waste of time and resources. Care must therefore be taken in the collection of leaf samples to undertake leaf disc test especially in West Africa where the CSSV is prevalent. It is suggested that cocoa swollen shoot virus status of leaf samples be detected using CSSV diagnostic tools before using them for leaf disc test.

Key words: *Theobroma cacao* L., leaf disc test, black pod disease, *Phytophthora* species, cocoa swollen shoot virus, resistance

INTRODUCTION

Cacao is an important crop in Ghana. The crop is the backbone of the Ghanaian economy and a source of income for farmers. However, Black pod disease caused by *Phytophthora* species causes substantial yield losses in cocoa (*Theobroma cacao* L.). Losses can reach 100% of annual production

if no control measures are taken (Dakwa, 1987; Opoku *et al.*, 2007). Beans from affected pods are unfit for consumption. Several species of this pathogen have been identified in different production zones. The most widespread species is *P. palmivora*, which exists in virtually all cocoa producing countries. In Ghana, studies have revealed the existence of *P. megakarya* (Dakwa, 1987) which is considered to be the most aggressive species (Brasier and Griffin, 1979).

Therefore, selecting cocoa genotypes that are less susceptible to black pod disease is important. Many authors have suggested that the differences in reaction to *Phytophthora* spp. were due to partial, probably polygenic resistance (Tan and Tan, 1990; Cilas and Despreaux, 2004). Different ways of testing planting materials have been tried. Adult plant reactions in field tests have been the basis for evaluation of cocoa to pod rots caused by *Phytophthora* species. Non-uniform field inoculum distribution and micro-environmental effects often affect classification of genotypes in field tests (Nyasse *et al.*, 2002). The low efficiency of field screening for disease reaction in breeding for resistance has emphasized the need for improved evaluation methods (Nyasse *et al.*, 2002).

Different artificial inoculation tests were developed in order to enable an early prediction of cocoa resistance to *Phytophthora* species. Some tests used roots (Tahi *et al.*, 2000) or stems (Blaha, 1974) but they were destructive to the tree. Iwaro *et al.* (2005) reported usefulness of the detached pod test for assessment of cocoa resistant to *Phytophthora* pod rot. The use of detached pods necessitates waiting until the cocoa trees are bearing before the resistance levels of the cocoa genotypes can be determined. Leaf disc test was developed on leaves by Nyasse *et al.* (1995). Leaf disc tests has been reported to correlate with the rot rate observed in the field (Tahi *et al.*, 2006; Nyadanu *et al.*, 2009), making it possible to use this early test in selection processes and thereby speed up selection cycles whose length is often the main obstacle to genetic progress in perennial species.

Using leaves to develop a non-destructive early test that could be repeated on the same plants is particularly an attractive idea, however, the effect of Cocoa Swollen Shoot Virus (CSSV) infection of leaves on the results of leaf disc test is not known. CSSV is of great economic importance in West Africa. CSSV is endemic, in the Eastern Region of Ghana, where it was first discovered (Brunt, 1975; Brunt and Kenten, 1971). The disease which is caused by the CSSV, can now be found in all cocoa growing regions of Ghana and other parts of the West African sub-region. Leaf chlorosis, stem swellings, stunted growth, dieback of shoots and reduced growth of lateral roots are the main symptoms of cocoa swollen shoot virus. The infection of swollen shoot virus passes through lengthy latent phases where symptoms could be absent or inconspicuous. The infected leaves might inadvertently be used in leaf disc tests. The effect of viruses on resistance of plants to fungal infection is not clear. While some authors reported that viral infection increased susceptibility of host plants to fungal infection (Bovey, 1963; Russel, 1966; Campbell, 1969), others reported that viral infection increased resistance of plants to fungi (Wilson, 1958; King *et al.*, 1964).

The recent reports of outbreak of cocoa swollen shoot virus in Mabang in the Brong Ahafo Region of Ghana where leaf samples are being collected from clonal materials for leaf disc test informed this study. The objective was to understand the effect of CSSV on leaf disc test to help select cocoa genotypes that are resistant to *Phytophthora* species.

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. Alpha B36, Pa7/808, Pound 7, T17/524, T65/238 and T65/326 were used as parents in a

Table 1: Origin of progenies used

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)

6×6 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. The origins of the progenies are listed in Table 1.

Leaf disc test of healthy crosses

Inoculum preparation: The isolates of *P. palmivora* and *P. 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 sampling: Seedlings of 17 months old were used for the screening test. 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. Five leaves were harvested from each genotype. 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 tap water, blotted dry with Whatman number 3 papers 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, 12 discs of 1.5 cm in diameter were made with cork borer per leaf next day after harvesting the leaves. All the discs from the same plant were mixed. Leaf discs were placed with their abaxial surface upwards on wetted plastic foam of 1 cm thick and imbibed with 2.5 L of distilled water in four trays of 70 cm×60 cm×10 cm. The discs from the same plant were aligned in completely randomized rows of 15 discs per tray. Inoculation was carried out the same day, after preparation of all leaf discs. Leaf discs from each tree, 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 medium-sized, 4 = large uniform brown lesions and 5 = very large brown lesions, often expanding necrotic spots, 2 = larger number and size of necrotic spots, 3 = coalescence of brown spots into

outside the area covered by the inoculum droplet) as described by Nyasse *et al.* (1995). The experiment was carried out twice.

Leaf disc test of cocoa swollen shoot virus infected crosses: After evaluating the resistance levels of the healthy seedlings, the leaves were stripped off and patch grafting method of CSSV inoculation (Posnette, 1940) was used to inoculate 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. Leaves of uniform age were collected from the infected seedlings and were inoculated with *P. palmivora* and *P. megakarya* using leaf disc test as described above.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) in a Randomized Complete Block Design (RCBD) using GenStat® 11th version (GenStat, 2008). The residual plots were inspected to confirm data conformed to normality. The significance of mean differences among genotypes was evaluated at $p<0.05$.

RESULTS

Significant differences ($p<0.001$) were observed among the healthy leaves (uninoculated with CSSV) of the crosses for resistance to *P. palmivora* and *P. megakarya* (Table 2). The scores of *P. palmivora* inoculated crosses varies from 1.44 for Pound 7 × Pa7/808 to 3.87 for T17/524 × Pa7/808 with percentage coefficient of variation of 30.1 (Table 3). The scores of *P. megakarya* inoculated seedlings varies from 1.57 for Alpha B36 × Pound 7 to 3.67 for T65/238 × T65/238 and T17/524 × Pound 7 (Table 3). In general the scores of *P. megakarya* inoculated crosses were higher than scores of *P. palmivora* inoculated crosses (Table 3). There were no significant interaction ($p>0.05$) between the *Phytophthora* species and the crosses. Similarly, there was no interaction between the *Phytophthora* species and the strand numbers (Table 2).

There were no significant interaction between the viral strains and the crosses (Table 2). Crosses × *Phytophthora* species x viral strains and strand number × *Phytophthora* species × viral strains interaction was significant (Table 2).

Significant differences were also observed among the New Juaben CSSV strain 1A infected crosses for resistance to *P. palmivora* and *P. megakarya* (Table 4). Nsaba CSSV strain infected crosses also varies significantly ($p<0.05$) in leaf discs scores for resistance to *P. palmivora* and *P. megakarya* (Table 4). The leaf disc scores of New Juaben CSSV strain 1A infected crosses inoculated with *P. palmivora* varies from 0.74 for Pound 7 × Alpha B36 to 2.18 for Pound 7 × T17/524 with percentage coefficient of variation of 44.0. The New Juaben CSSV strain 1A infected

Table 2: ANOVA parameters for main effects and interactions of crosses, strand numbers, Phytophthora species and virus strains

Sources of variation	df	F. ratio	p-value
Crosses	35	159.13	<0.001
Strand number	19	4.34	<0.001
Phytophthora species	1	54.26	<0.001
Virus strain	1	499.91	<0.001
Crosses × *Phytophthora* species	35	13.49	0.081
Crosses × virus strain	35	1.28	1.00
Phytophthora species × strand number	19	6.73	1.00
Virus strain × strand number	19	0.12	1.00
Crosses × virus strain × *Phytophthora* species	35	3.21	0.003

Table 3: Genotypic differences in leaf disc scores of healthy cocoa genotypes after inoculation with *P. palmivora* and *P. megakarya*

	Lead disc scores caused by:	
Crosses and their reciprocals	*P. palmivora*	*P. megakarya*
Alpha B36 × Pa7/808	1.99±0.12	1.94±0.11
Alpha B36 × Pound7	1.55±0.09	1.57±0.07
Alpha B36 × T17/524	2.63±0.08	3.26±0.10
Alpha B36 × T65/238	2.54±0.09	2.43±0.09
Alpha B 36 × T65/326	1.88± 0.07	1.82±0.08
Alpha B36 × Alpha B36	1.99±0.09	2.27±0.07
Pa7/808 × Alpha B36	1.73±0.09	1.93±0.11
Pa7/808 × Pa7/808	2.27±1.00	2.35±0.10
Pa7/808 × Pound7	1.93±0.11	1.93±0.12
Pa7/808 × T17/524	2.69±0.13	3.26±0.11
Pa7/808 × T65/238	1.98±0.09	2.32±0.08
Pa7/808 × T65/326	2.13±1.09	1.83±0.10
Pound 7 × Pa7/808	1.44±0.08	1.58±0.08
Pound 7 × Alpha B36	1.48±0.09	1.76±0.08
Pound 7 × Pound7	2.34±0.08	2.24±1.00
Pound 7 × T17/524	3.08±0.09	3.51±0.09
Pound 7 × T65/238	2.31±0.09	2.26±0.09
Pound 7 × T65/326	2.15±0.08	2.38±0.07
T17/524 × Pa7/808	3.87±0.09	3.59±0.08
T17/524 × Pound7	2.47±0.07	3.67±0.06
T17/524 × Alpha B36	2.56±0.06	2.63±0.08
T17/524 × T17/524	2.64±0.07	2.45±0.07
T17/524 × T65/238	2.45±0.08	2.33±0.07
T17/524 × T65/326	2.46±0.07	3.64±0.06
T65/238 × Alpha B36	1.94±0.09	2.15±0.08
T65/238 × Pa7/808	2.45±0.06	2.43±0.09
T65/238 × Pound 7	1.92±0.11	1.91±0.10
T65/238 × T17/524	2.57±0.07	3.27±0.11
T65/238 × T65/238	3.59±0.08	3.67±0.06
T65/238 × T65/326	2.86±0.09	2.81±0.09
T65/326 × Alpha B36	2.27±0.05	1.86±0.08
T65/326 × Pa7/808	2.13±0.07	1.94±0.09
T65/326 × Pound7	1.96±0.09	2.09±0.08
T65/326 × T17/524	3.74±0.07	2.85±0.06
T65/326 × T65/238	2.65±0.08	2.78±0.09
T65/326 × T65/326	1.98±0.09	1.95±0.09
LSD	0.24	0.25
SE	0.68	0.69
Mean	2.27	2.36

SE: Strandard error, Higher leaf disc score means susceptibility to *Phytophthora* species

crosses inoculated with *P. megakarya* varies from 0.72 for Pound 7 × Pa7/808 to 2.77 for T65/238 × T17/524 with percentage coefficient of variation of 43.7.

The leaf disc scores of Nsaba CSSV strain infected crosses inoculated with *P. palmivora* varies from 0.43 for Alpha B36 × Pa7/808 to 2.55 for Pound 7 × T17/524 with percentage coefficient of variation of 44.1. The leaf disc scores of Nsaba CSSV strain infected crosses inoculated with

Table 4: Genotypic differences in leaf disc scores of New Juaben CSSV strain 1A and Nsaba CSSV strain infected cocoa genotypes inoculated with *P. palmivora* and *P. megakarya*

	1A infected leaf disc scores		Nsaba infected leaf disc scores	
Crosses and their reciprocals	*P. palmivora*	*P. megakarya*	*P. palmivora*	*P. megakarya*
Alpha B36 × Pa7/808	1.22±0.11	1.14±0.10	0.43±0.12	1.34±0.12
Alpha B36 × Pound7	0.79±0.08	0.96±0.06	0.93±0.09	1.13±0.08
Alpha B36 × T17/524	1.73±0.08	2.36±0.10	2.02±0.09	2.76±0.12
Alpha B36 × T65/238	1.64±0.09	1.53±0.08	1.92±0.09	1.79±0.10
Alpha B 36 × T65/326	0.98±0.07	0.92±0.07	1.15±0.09	1.07±0.09
Alpha B36 × Alpha B36	1.19±0.08	1.37±0.08	1.39±0.09	1.61±0.08
Pa7/808 × Alpha B36	1.05±0.07	1.15±0.09	1.29±0.09	1.30±0.14
Pa7/808 × Pa7/808	1.39±0.09	1.45±0.10	1.62±0.09	1.57±0.10
Pa7/808 × Pound7	1.16±0.08	1.24±0.09	1.35±0.10	1.45±0.11
Pa7/808 × T17/524	1.85±0.11	2.36±0.11	2.16±0.13	2.76±0.12
Pa7/808 × T65/238	1.16±0.09	1.42±0.07	1.35±0.10	1.67±0.09
Pa7/808 × T65/326	1.32±0.07	1.11±0.09	1.43±0.08	1.49±0.09
Pound 7 × Pa7/808	0.79±0.06	0.72±0.06	0.87±0.87	0.87±0.07
Pound 7 × Alpha B36	0.74±0.06	0.89±0.08	0.93±0.93	1.04±0.09
Pound 7 × Pound7	1.44±0.08	1.34±0.09	1.69±0.09	1.56±0.11
Pound 7 × T17/524	2.18±0.09	2.61±0.09	2.55±0.11	3.03±0.12
Pound 7 × T65/238	1.53±0.07	1.39±0.08	1.79±0.08	1.63±0.09
Pound 7 × T65/326	1.25±0.08	1.48±0.07	1.46±0.09	1.73±0.09
T17/524 × Pa7/808	1.97±0.09	2.30±0.08	2.30±0.10	2.69±0.09
T17/524 × Pound7	1.57±0.07	1.37±0.06	1.84±0.09	1.60±0.07
T17/524 × Alpha B36	1.66±0.06	1.73±0.08	1.94±0.08	2.02±0.09
T17/524 × T17/524	1.74±0.07	1.55±0.07	2.03±0.09	1.82±0.08
T17/524 × T65/238	1.55±0.08	1.43±0.07	1.82±0.09	1.67±0.08
T17/524 × T65/326	1.59±0.07	1.77±0.06	1.87±0.08	2.07±0.07
T65/238 × Alpha B36	1.07±0.08	1.27±0.07	1.26±0.11	1.49±0.09
T65/238 × Pa7/808	1.55±0.07	1.53±0.09	1.81±0.08	1.79±0.10
T65/238 × Pound 7	1.21±0.09	1.13±0.08	1.42±0.12	1.32±0.10
T65/238 × T17/524	1.67±0.07	2.77±0.11	1.95±0.09	2.77±0.13
T65/238 × T65/238	1.69±0.08	1.77±0.06	1.99±0.08	2.07±0.08
T65/238 × T65/326	1.96±0.09	1.91±0.09	2.29±0.10	2.24±0.10
T65/326 × Alpha B36	1.37±0.05	1.02±0.07	1.60±0.06	1.19±0.07
T65/326 × Pa7/808	1.29±0.07	1.13±0.08	1.52±0.08	1.32±0.09
T65/326 × Pound7	1.20±0.08	1.23±0.08	1.41±0.09	1.44±0.09
T65/326 × T17/524	1.84±0.07	1.95±0.06	2.15±0.08	2.28±0.08
T65/326 × T65/238	1.75±0.08	1.88±0.09	2.05±0.09	2.21±0.11
T65/326 × T65/326	1.15±0.07	1.16±0.08	1.35±0.09	1.36±0.09
LSD	0.22	0.23	0.32	0.34
SE	0.62	0.65	0.73	0.77
Mean	1.42	1.49	1.67	1.75

SE: Strandard error. Higher leaf disc score means susceptibility to *Phytophthora* species

P. megakarya varies from 0.87 for Pound 7 × Pa7/808 to 3.03 for Pound 7 × T17/524 with percentage coefficient of variation of 44.0.

Figure 1 shows the distribution of resistance levels of healthy crosses inoculated with *P. palmivora* and *P. megakarya*. Fig. 2 shows New Juaben CSSV strain 1A and Nsaba CSSV strain infected crosses inoculated with *P. palmivora* and *P. megakarya*. Comparative analysis of Fig. 1

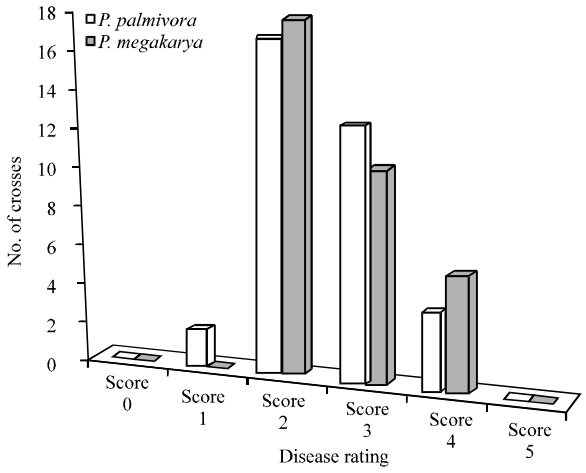

Fig. 1: Distribution of scores for resistance to *P. palmivora* and *P. megakarya* among the healthy crosses assessed by the leaf disc test. Higher leaf disc score means susceptibility to *Phytophthora* species

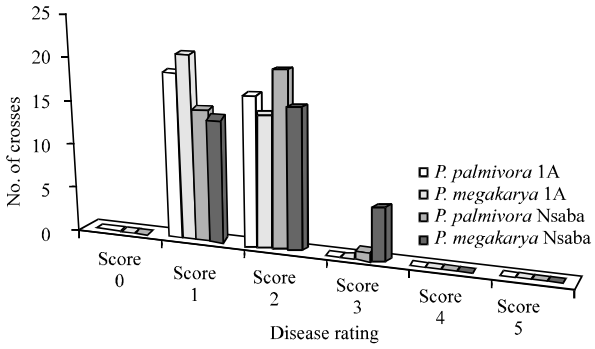

Fig. 2: Distribution of scores for resistance to *P. palmivora* and *P. megakarya* among 1A and Nsaba infected crosses assessed by the leaf disc test. Higher leaf disc score means susceptibility to *Phytophthora* species

and 2 shows that the disease rating of New Juaben CSSV strain 1A and Nsaba CSSV strain infected genotypes fall under leaf disc scores 1 and 2 while the healthy crosses fall under leaf disc scores 2,3 and 4. This suggests that the cocoa swollen shoot virus infection increased the resistance of the crosses to *P. palmivora* and *P. megakarya*.

DISCUSSION

The significant differences among the crosses of cocoa in their reactions to *P. palmivora* and *P. megakarya* indicate that leaf disc test effectively differentiated the various levels of resistance in the 36 cocoa crosses assessed in this study. Nyasse *et al.* (1995), Tondje *et al.* (1988) and Tahi *et al.* (2006) who studied the reaction of some cocoa genotypes to *P. palmivora* infection made similar observation. With all the genotypes planted under the same environmental conditions, the observed variations could probably be genetic. Tan and Tan (1990) and Simmonds (1994) observed similar variations in their studies on the resistance of cocoa progenies to *P. palmivora*.

The non-significance of the host genotypes x Phytophthora species has important implications in cocoa breeding for *Phytophthora* resistance. The levels of resistance of cocoa genotypes could be identified using any of the *Phytophthora* species since resistance has been shown to be species non-specific in this study. The results found in the present study support the previous results of Van der Vossen (1997), Iwaro *et al.* (1998) and Surujdeo-Maharaj *et al.* (2001) who reported non-significance of genotype x *Phytophthora* species in cocoa. In each progeny studied, differences in the reactions of strand numbers (families) to *P. palmivora* and *P. megakarya* measured by the leaf disc test were highly significant. Such differences were also reported in previous studies with plants maintained in nursery conditions (Tahi *et al.*, 2007; Efombagn *et al.*, 2007) and in the field (Tahi *et al.*, 2006).

The significant interactions between cocoa genotypes and *Phytophthora* species and viral strains indicate that the resistance levels of cocoa genotypes to *Phytophthora* species were influenced by infection of cocoa swollen shoot virus. The findings suggest that resistance of cocoa genotypes infected by CSSV to *Phytophthora* species depend on the type of viral strain. This has serious implications in selection and breeding for black pod disease resistance. Resistance due to the influence of one strain of virus could not be used to predict resistance due to the influence of another virus strain since the results of resistance to *Phytophthora* species due to influence of the virus is viral strain specific.

The results show that inoculation with cocoa swollen shoot virus increased resistance of cocoa genotypes to *P. palmivora* and *P. megakarya*. This agrees with findings of Wilson (1958) and King *et al.* (1964) who reported that viral infection increased resistance of plants to fungus. The observed increased resistance of cocoa to *Phytophthora* species upon cocoa swollen shoot virus infection may have been due to effect of virus infection on the metabolism and transport of biochemical contents in affected plants. Adomako and Hutcheon (1974) reported that starch, sucrose and reducing sugars were found to accumulate in virus infected tissues of cocoa. They stated that among the factors possibly contributing to the stunted growth of virus infected plants may be inhibition of synthesis of host structural and protoplasmic material and the diversion of substantial amounts of photosynthates and high energy phosphate toward host defence reactions. These effects have been reported for some other host-virus systems (Goffeau and Bove, 1965; Hirai and Takahashi, 1967; Mackenzie and Haselkorn, 1972; Bedbrook and Matthews, 1972). Omokolo *et al.* (2002) reported importance of soluble sugars in the resistance of cocoa to black pod disease. Phenolic compounds, implicated in resistance of cocoa to *Phytophthora* species Djocgoue *et al.* (2007), Spence (1961), Tan *et al.* (2004), Omokolo and Boudjeko (2005), have been reported by Holden (1957) and Manu (2006) to accumulate in CSSV-infected cocoa leaves. These findings suggest that the increased resistance of cocoa genotypes as a result of CSSV infection in this work could be due to the defence systems of CSSV-infected cocoa genotypes switched on before inoculation with *Phytophthora* species and thus causing the genotypes to appear or behave resistant. A black pod susceptible variety which behaved as if resistant could therefore be selected in situations where latent virus infected plants are screened for resistance using leaf disc test. The implications of this are obvious as it would lead to waste of resources and time. It is therefore important to be careful that leaves are not infected with CSSV before using them in leaf disc test. This could be done by involving virus diagnosis tools to detect CSSV status of leaf samples before using them in screening cocoa genotypes for resistance to black pod disease in leaf disc test. Adequate testing of all breeding materials for resistance to black pod disease is obviously essential, to prevent the production of very susceptible varieties.

Alpha B36 × Pa7/808, Pound 7 × Alpha B36 and Pound 7 × Pa7/808 were the most resistant progenies and could therefore be very important in breeding for black pod disease resistance in cocoa.

CONCLUSION

The cocoa genotypes varied significantly in resistance to *P. palmivora* and *P. megakarya*. Interaction among cocoa genotypes, *Phytophthora* species and viral strains was significant. The results revealed that cocoa swollen shoot virus infection increased resistance of cocoa genotypes to *Phytophthora* species. Care must therefore be taken in the collection of leaf samples to undertake leaf disc test especially in West Africa where the cocoa swollen shoot virus is prevalent.

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