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Biochemical Mechanisms of Resistance to Black Pod Disease in Cocoa (*Theobroma cacao* L.)

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ABSTRACT

Black pod disease caused by *Phytophthora palmivora* and *Phytophthora megakarya* is an important disease of cocoa and host plant resistance is one of the important components of management of this disease. Therefore, a diverse array of 12 cocoa genotypes was evaluated to identify biochemical characteristics conferring resistance to black pod disease. Resistance to black pod disease measured as leaf disc scores, pod lesion numbers, pod lesion sizes and natural field infection was associated with amounts of nitrogen, protein, soluble sugars, insoluble sugars, total polyphenols, flavonoids, tannins and lignin in leaf and pod of cocoa. The levels of these biochemical compounds in leaf and pod increased after inoculation with *P. megakarya*. Principal component analysis shows that 90% of the total variability in the eight original biochemical variables is captured in the first two principal components and 95% in the first three principal components. The first two principal components were defined by healthy pod lignin and healthy pod insoluble sugar and principal component three was mainly defined by healthy leaf protein. Phytochemical characterization of the 12 cocoa genotypes by cluster analysis revealed two major clusters. Cluster one consisted of Imc67, Na33, T79/501, T63/971, Imc53 and Sca9 which were susceptible to black pod and cluster two was made up of Pa150, T60/887, Sca6, Imc76, Pa7/808 and T85/799 which were resistant. Correlation coefficients, multiple and step-wise regressions indicated that insoluble sugar, flavonoid, tannins and lignin were the most reliable biochemical factors and these could be used as marker traits to screen and select for resistance to black pod disease of cocoa.

Key words: Black pod disease, *Phytophthora* species, *Theobroma cacao* L., resistance mechanisms, polyphenol, proteins, carbohydrates

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an important crop of industrial and nutritional value. Diseases, however, reduces yield and quality of product and increase the cost of production. Black pod is a serious cocoa disease in many production regions, particularly West Africa which produces 70.7% of the world's cocoa (ICCO, 2010). Variable and only partially effective control has been achieved

by protection through fungicides. The combined practice of farm sanitation and adequate fungicide application is being used to control the disease. However, such practices raise the production costs, pollute the environment and are therefore frequently economically unviable. The ideal solution for the control of the disease has been the replacement of susceptible cultivars by others with durable resistance to the *Phytophthora* species as it involves very little cost input by the farmers.

Genetic studies have shown that resistance to *Phytophthora* pod rot infection is polygenic and could be improved by recurrent selection (Iwaro *et al.*, 1997a; Ndoumbe *et al.*, 2001; Nyassé *et al.*, 2007). Different ways of assessing resistance were tested, mainly by observing infection levels in the field and by carrying out artificial inoculation tests on attached or detached pods (Iwaro *et al.*, 2005). One of the major drawbacks of these methods appears to be the long time lapse between development of new crosses and evaluation of resistance. Assessing resistance in an early stage, using organs other than pods has been considered by using inoculations of leaf discs (Nyassé *et al.*, 1995; Despreaux, 2004; Iwaro *et al.*, 2003; Nyassé *et al.*, 2002; Tahi *et al.*, 2000, 2006). The results of the leaf disc and detached pod inoculation tests, when carried out under standardized conditions, have been shown to be significantly correlated among themselves and with field level of infection (Iwaro *et al.*, 2005; Tahi *et al.*, 2000, 2006; Nyadanu *et al.*, 2009a).

Cocoa plant resistance to black pod disease appears to be a complex character and depends on the interplay of a number of componential factors which finally sum up in the expression of resistance to black pod disease (Van der Vossen, 1997). Biochemical activities in host tissues play a significant role in the resistance of plants against fungi when present in sufficient amounts either prior to or following infection (Agrios, 2005). The evidence of this could be seen from the fact that a particular pathogen will not infect certain plant varieties even though no structural barriers of any kind seem to be present or to form in these varieties. Many authors showed the involvement of chemical compounds in the plant defence mechanism against pests and diseases. Omokolo and Boudjeko (2005) reported phenols as resistance factors in resistance of cocoa to *Phytophthora* species. Omokolo *et al.* (2002) analyzed the amino acid and carbohydrate contents in nine clones of *T. cacao* with different degrees of susceptibility to the disease and reported a negative relationship between the lesion size and the amino acid and carbohydrate contents in the cortex. Lignin has been repeatedly reported to have a role in plant disease resistance (Vance *et al.*, 1980; Bell, 1981; Wally and Punja, 2010). There is little information about the role of specific polyphenols; flavonoids, tannins and lignins in cocoa susceptibility to black pod disease. This study was carried out to determine alterations in biochemical factors in cocoa genotypes after inoculation with *P. megakarya* and their possible relationship to black pod disease resistance.

MATERIALS AND METHODS

The experimental material consisted of a diverse array of 12 cocoa genotypes (Pa7/808, Na33, T60/887, T63/971, Imc76, Pa150, Sca9, Imc67, Imc53, Sca6, T85/799 and T79/501) maintained at the germplasm plot of Cocoa Research Institute of Ghana (CRIG). The experiments were conducted at CRIG during 2009/2010 and 2010/2011 seasons.

Inoculum preparation: An 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 disc test: Leaf disc preparation and inoculation as described by Nyassé *et al.* (1995) and Tahi *et al.* (2006) was carried out. The flushes of leaves were tagged for the cocoa genotypes. The ages of the leaves for each treatment were established by following the growth of young flushes from bud break in the field. Leaves of good physiological condition (young lignified leaves) without insect attacks and of similar age and exposure to sunlight were collected. Sixteen leaf discs of 1.5 cm diameter from each genotype were made with a cork borer and replicated four times. Leaf discs were placed with their abaxial surface upwards on wetted plastic foam in five trays of 70 cm long, 60 cm wide and 15 cm high. Discs of the cocoa genotypes were randomly arranged in groups of 12 within each tray, giving 16×12 = 192 discs per tray. The discs were inoculated and incubated at room temperature (25°C) in plastic trays and covered with another plastic tray in the laboratory to prevent direct sunlight until observations were made. Symptoms were scored 6 days after inoculation using a 0-5 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 Nyassé *et al.* (1995). The experiment was carried out twice.

Detached pod test: Detached pod test at penetration and post-penetration stages of infection as described by Iwaro *et al.* (1997b) was carried out. The flowers of the cocoa genotypes were selfed (using hand pollination) so that their exact ages could be determined at the time of harvest.

Inoculated pods were, arranged in a randomized complete block design and incubated at room temperature in moist plastic chamber boxes. Three replicates consisting of five pods each were assayed. After incubation for four days, the number of infection sites per inoculum site was counted. On the 7th day of incubation, sizes of the established lesions were traced on a transparent paper. The lesion sizes were determined from brown paper cutouts trimmed to the size of each lesion and were measured with a leaf area meter.

Field observations: Natural pod infections in the field were evaluated in 2009 and 2010 on individual crosses of the 6×4 factorial and 6×6 diallel mating designs. Pods infected by *Phytophthora* (Bp), rodent damaged pods (R) and healthy ripe pods (H) and Other Damages (OD) were counted each month after each harvesting round. The percentage (%) of rotten pods (Bp) was estimated in relation to the total number of pods produced by the cross:

$$\text{Percentage black pod (\%BP)} = \frac{\text{Bp}}{\text{Bp+H+R+OD}} \times 100$$

Biochemical composition of cocoa genotypes: The extraction and analysis of nitrogen involved the digestion of healthy and *P. megakarya* infected leaves and pods of cocoa types, using the micro-Kjeldahl method as described by Jackson (1964) with minor modification. Protein was estimated by multiplying N content with 6.25. For the determination of total polyphenols, the Folin-Ciocalteu reagent method (Marigo, 1973) was used. The soluble and insoluble sugars in cocoa pod husk and leaf tissue were quantified using method of DuBois *et al.* (1956). Lignin was quantified in cocoa leaf and pod tissues using the lignin thioglycolic acid (LTGA) procedure (Doster and Bostock, 1988). The Condensed Tannins (CT) were assayed colorimetrically by the method of Price *et al.* (1978). Flavonoids were determined by the aluminum chloride colorimetric method from the procedure reported by Woisky and Salatino (1998).

Statistical analysis: Data were subjected to analysis of variance (ANOVA) using GenStat® 11th version (GenStat, 2008). Significance of differences among the genotypes for each trait was tested by F-test. When the ANOVA showed significant genotypic differences, the significance of differences between the genotypic means was judged by Least Significant Difference (LSD) at $p \leq 0.05$. Simple correlation, multiple and step-wise regression analysis was performed to understand the relationship between the biochemical traits and resistance to black pod disease in leaf and pod. Principal components and cluster analysis was also done to identify the most important biochemical factors and diversity of cocoa genotypes in biochemical contents.

RESULTS

Relative susceptibility of cocoa genotypes to *Phytophthora* species: The reaction of the 12 cocoa genotypes used in this study to *P. palmivora* and *P. megakarya* is presented in (Table 1). Significant differences ($p<0.001$) in mean severity values were detected among the genotypes in the inoculation tests (Pod Lesion Number (PLN), Pod Lesion Size (PLS) and Leaf Disc Score (LDS)). The PLN on pods among the genotypes varied from 2.93 for Sca6 to 15.23 for Imc67. Significantly smaller PLS were recorded on Sca6, T60/887 and Pa150, respectively, than for the other genotypes. PLS on Imc76 and Sca9 were moderate; but significantly larger sizes were produced on the rest of the genotypes. Disease severity scores of LDS varied from 1.57 for Sca6 to 4.24 for T63/971. The lesion number, lesion sizes and leaf disc scores increased when *P. megakarya* was used as the inoculum.

Biochemical characteristics: The total polyphenol content in the leaf and pod of cocoa genotypes varied significantly ($p<0.001$). The polyphenol content in the leaf and pod of Sca6, T60/887 and Imc53 was significantly higher than the other genotypes (Table 2, 3). Polyphenols in leaf of cocoa were significantly higher than in pod. Comparison of amount of Polyphenols in healthy and infected leaf and pod showed that total polyphenol content increased or accumulated after infection of leaf and pod (Table 2, 3). The accumulation was more in Sca6, T60/887, Imc53 and T85/799 than the other genotypes.

Table 1: The parental mean values for pod lesion number, pod lesion size and leaf disc scores of the 12 cocoa varieties after inoculation with *P. palmivora* and *P. megakarya*.

Parents	PLN (Pp)	PLN (Pm)	PLS (Pp)	PLS (Pm)	LDS (Pp)	LDS (Pm)
Pa7/808	4.23±0.30^{ef}	7.60±0.84^{fg}	31.30±2.59^{d}	49.60±2.85^{de}	1.44±0.04^{c}	2.1±0.050^{c}
Na33	9.83±0.89^{ab}	13.70±0.70^{ab}	62.30±3.07^{b}	76.40±4.45^{b}	2.96±0.05^{ab}	3.10±0.05^{abc}
T60/887	3.00±0.10^{f}	6.00±0.87^{g}	25.80±1.55^{d}	33.30±3.14^{f}	1.58±0.06^{c}	2.00±0.05^{c}
T63/971	9.67±0.97^{abc}	13.20±0.67^{bc}	78.30±3.81^{a}	103.30±8.46^{a}	2.89±0.05^{ab}	4.24±0.05^{a}
Imc76	4.97±0.24^{e}	8.67±0.43^{ef}	40.80±2.33^{c}	58.10±3.67^{cd}	2.05±0.05^{abc}	2.57±0.04^{bc}
Pa150	3.53±0.18^{ef}	7.23±0.45^{fg}	28.70±1.68^{d}	39.50±1.47^{ef}	1.62±0.05^{c}	2.09±0.04^{c}
Sca9	7.37±0.29^{d}	10.53±1.05^{de}	42.30±2.89^{c}	51.90±3.14^{d}	1.95±0.04^{bc}	2.51±0.04^{c}
Imc67	11.23±0.93^{a}	15.23±0.87^{a}	61.18±1.04^{b}	67.20±1.05^{bc}	3.25±0.05^{a}	3.81±0.06^{ab}
Imc53	8.03±1.05^{cd}	11.73±0.68^{cd}	61.14±1.48^{b}	67.30±1.32^{bc}	2.08±0.05^{abc}	2.91±0.05^{bc}
Sca6	2.93±0.29^{f}	5.97±1.41^{g}	26.53±0.67^{d}	30.00±0.22^{f}	1.57±0.06^{c}	1.91±0.05^{c}
T85/799	4.70±0.06^{ef}	8.40±0.31^{f}	23.97±2.26^{d}	34.60±2.03^{f}	1.72±0.05^{bc}	2.42±0.04^{c}
T79/501	8.90±0.50^{bcd}	12.60±0.70^{bc}	65.24±6.41^{b}	76.9±6.32^{b}	2.27±0.05^{abc}	2.94±0.04^{bc}
LSD	1.78	1.86	8.761	1.81	0.14	0.15
SE	1.06	1.20	5.17	6.98	0.90	0.91

PLN: Pod lesion number, PLS: Pod lesion size, LDS: leaf disc score, Pp: *Phytophthora palmivora*, Pm: *Phytophthora megakarya*, Different letters indicate significant differences between means at the level of $p<0.05$, LSD: Least significant difference

Table 2: Polyphenolic, nitrogen, protein and carbohydrate composition of 12 cocoa genotypes evaluated in healthy and *P. megakarya* infected leaf

	Healthy leaf					*P. megakarya* infected leaf				
Genotypes	Polyphenol	N	Protein	Sol.s	Ins.s.	Polyphenol	N	Protein	Sol.s	Ins.s.
Pa7/808	7.42±0.21^{bc}	2.97±0.04^{ab}	38.96±0.20^{cd}	10.65±0.19^{cdef}	40.39±0.68^{d}	11.87±0.33^{bc}	3.49±0.15^{abc}	34.26±0.20^{cd}	15.98±0.29^{cd}	60.59±1.03^{c}
Na33	2.10±0.27^{f}	1.88±0.04^{c}	25.50±0.44^{f}	9.76±0.28^{cdefg}	27.93±1.67^{fg}	3.36±0.43^{f}	2.25±0.04^{e}	31.80±1.12^{ef}	14.63±0.42^{cde}	41.90±2.49^{de}
T60/887	9.49±0.08^{a}	3.03±0.01^{ab}	40.75±0.25^{bc}	17.00±0.19^{a}	57.62±0.93^{a}	15.18±0.13^{a}	3.54±0.01^{ab}	35.92±0.15^{bc}	25.49±0.28^{a}	86.44±1.39^{a}
T63/971	5.39±0.48^{de}	2.89±0.03^{b}	38.41±0.90^{d}	5.52±2.48^{h}	20.44±0.67^{h}	8.62±0.77^{de}	3.26±0.15^{d}	33.54±0.58^{de}	8.28±3.71^{f}	30.65±0.99^{f}
Imc76	6.25±0.45^{cd}	3.08±0.04^{a}	42.03±0.64^{ab}	11.66±0.78^{bcde}	37.14±1.09^{d}	9.99±0.71^{cd}	3.58±0.03^{a}	37.43±0.69^{ab}	17.50±1.17^{cd}	55.71±1.63^{c}
Pa 150	6.17±0.42^{cd}	2.99±0.02^{ab}	38.48±0.05^{d}	13.04±1.02^{bcd}	47.28±1.71^{c}	9.87±0.66^{cd}	3.49±0.02^{abc}	33.74±0.05^{de}	19.57±1.53^{bc}	70.92±2.57^{b}
Sca9	4.18±0.21^{e}	2.96±0.08^{ab}	40.92±0.79^{bc}	9.46±0.44^{defg}	31.12±0.66^{ef}	6.68±0.34^{e}	3.47±0.08^{abc}	37.20±1.77^{ab}	14.18±0.67^{de}	46.69±0.99^{d}
Imc 67	4.20±0.49^{e}	1.80±0.02^{c}	24.21±0.07^{f}	7.11±0.37^{fgh}	20.89±0.68^{h}	6.72±0.79^{e}	2.30±0.02^{e}	30.43±0.06^{f}	10.66±0.56^{ef}	31.34±1.03^{f}
Imc 53	9.21±0.23^{a}	2.99±0.01^{ab}	41.54±0.31^{ab}	6.66±0.74^{gh}	25.82±1.40^{g}	14.74±0.36^{a}	3.35±0.02^{cd}	36.87±0.48^{ab}	10.00±1.12^{ef}	38.73±2.10^{e}
Sca 6	8.46±0.07^{ab}	3.14±0.02^{a}	43.35±0.55^{a}	15.30±0.05^{ab}	53.82±1.06^{b}	13.54±0.11^{ab}	3.64±0.15^{a}	38.65±0.69^{a}	22.95±0.07^{ab}	80.74±1.58^{a}
T85/799	7.54±0.86^{bc}	3.05±0.06^{ab}	40.80±0.77^{bc}	13.20±0.14^{bc}	48.68±1.30^{c}	12.07±1.38^{bc}	3.54±0.06^{ab}	36.05±0.65^{bc}	19.80±0.22^{bc}	73.02±1.95^{b}
T79/501	6.94±0.55^{c}	2.87±0.03^{b}	35.73±0.13^{e}	8.80±0.38^{efgh}	31.82±1.36^{e}	11.01±0.88^{c}	3.37±0.04^{bcd}	32.48±0.08^{def}	13.20±0.57^{def}	47.73±2.04^{d}
Lsd (0.05)	1.26	0.11	1.82	2.49	3.31	2.02	0.23	1.49	3.74	4.97
S.e	0.88	0.07	1.07	1.73	2.30	1.41	0.14	0.88	2.60	3.45

N: Nitrogen, Sol.s.: soluble sugar, Ins.s.: Insoluble sugar. S.e.: Standard error, Different letters indicate significant differences between means at the level of $p<0.05$ (Least significant difference)

Table 3: Polyphenolic, nitrogen, protein and carbohydrate composition of 12 cocoa genotypes evaluated in healthy and *P. megakarya* infected pod

	Healthy pod					*P. megakarya* infected pod				
Genotypes	Polyphenol	N	Protein	Sol.s.	Ins.s.	Polyphenol	N	Protein	Sol.s.	Ins.s.
Pa7/808	1.84±0.02^{bc}	0.68±0.23^{abc}	21.25±0.23^{cd}	12.51±0.09^{e}	51.14±3.65^{c}	3.12±0.04^{bc}	1.18±0.08^{ab}	24.66±0.64^{cd}	17.52±0.12^{c}	71.59±5.11^{c}
Na33	0.75±0.03^{f}	0.57±0.44^{bcd}	18.80±0.44^{ef}	9.24±0.47^{ef}	34.42±1.15^{fg}	1.28±0.06^{gf}	0.92±0.09^{c}	22.20±0.46^{ef}	12.94±0.66^{ef}	48.19±1.61^{fg}
T60/887	2.14±0.09^{a}	0.73±0.15^{abc}	23.04±0.15^{bc}	17.51±0.13^{a}	62.33±0.75^{b}	3.63±0.17^{a}	1.14±0.03^{ab}	26.46±0.15^{bc}	24.52±0.19^{a}	87.27±1.05^{b}
T63/971	1.20±0.04^{d}	0.56±0.89^{cd}	20.57±0.89^{de}	9.07±0.55^{f}	30.32±0.24^{gh}	2.04±0.06^{e}	0.69±0.08^{d}	23.97±0.89^{de}	12.70±0.78^{f}	42.45±1.73^{gh}
Imc76	1.62±0.09^{bc}	0.78±0.69^{a}	24.31±0.69^{ab}	12.77±0.65^{c}	46.02±1.66^{d}	2.76±0.15^{cd}	1.27±0.04^{a}	27.73±0.65^{ab}	17.88±0.91^{c}	64.43±2.32^{d}
Pa150	1.88±0.02^{ab}	0.68±0.05^{abc}	20.75±0.05^{de}	15.82±0.51^{b}	59.43±2.87^{b}	3.20±0.04^{abc}	1.19±0.01^{a}	24.13±0.05^{de}	22.15±0.72^{b}	83.20±4.02^{b}
Sca9	0.86±0.05^{ef}	0.66±0.12^{abcd}	24.89±0.12^{ab}	10.80±0.76^{d}	38.81±0.42^{ef}	1.46±0.08^{gf}	1.00±0.10^{bc}	28.28±0.12^{ab}	15.13±1.07^{d}	54.34±0.58^{ef}
Imc67	1.03±0.03^{def}	0.49±0.05^{d}	17.17±0.05^{f}	8.36±0.58^{f}	26.24±5.83^{h}	1.75±0.05^{efg}	0.89±0.12^{c}	20.55±0.07^{f}	11.70±0.81^{f}	36.73±8.16^{h}
Imc53	1.55±0.02^{c}	0.69±0.46^{abc}	23.84±0.46^{ab}	9.59±0.59^{def}	40.67±0.99^{e}	2.63±0.03^{d}	1.13±0.09^{ab}	26.59±0.45^{bc}	13.42±0.83^{def}	56.94±1.39^{e}
Sca6	1.90±0.02^{ab}	0.82±0.55^{a}	25.62±0.55^{a}	16.56±0.53^{ab}	69.02±2.09^{a}	3.24±0.04^{ab}	1.23±0.26^{a}	29.04±0.54^{a}	23.18±0.75^{ab}	96.63±2.92^{a}
T85/799	1.76±0.19^{bc}	0.74±0.79^{ab}	23.10±0.79^{bc}	13.86±0.19^{c}	53.50±2.06^{c}	2.98±0.33^{bcd}	1.25±0.16^{a}	26.49±0.28^{bc}	19.40±0.27^{c}	74.90±2.88^{c}
T79/501	1.13±0.12^{de}	0.57±0.08^{bcd}	19.36±0.08^{ef}	10.48±0.60^{de}	36.20±1.73^{f}	1.92±0.19^{ef}	0.94±0.01^{c}	22.08±0.07^{ef}	14.67±0.85^{de}	50.68±2.42^{f}
LSD (0.05)	0.24	0.11	1.35	1.48	6.02	0.40	0.31	1.34	2.07	8.42
SE	0.17	0.06	0.79	1.03	4.18	0.28	0.18	0.79	1.44	5.86

N: Nitrogen, Sol.s.: Soluble sugar, Ins.s.: Insoluble sugar. SE: Standard error, Different letters indicate significant differences between means at the level of $p < 0.05$, LSD: Least significant difference

There were significant differences in protein and nitrogen in the leaf and pod of the cocoa genotypes tested. Nitrogen content in cocoa genotypes remained almost unchanged after infection of leaf and pod with *P. megakarya* (Table 2, 3). The protein content increased after infection of cocoa leaf and pod with *P. megakarya*. In general, the cocoa genotypes; T60/887, Pa150, Pa7/808, Sca6, had more protein than the other genotypes (Table 2, 3).

Soluble and insoluble sugar (carbohydrates) content varied significantly ($p<0.001$) among the cocoa genotypes. Constitutively, carbohydrate content was higher in the clones Sca6, Pa150 and T60/887 than the other genotypes (Table 2, 3). Comparison between healthy and infected leaf and pods showed that carbohydrate content increased in infected pods and leaves than the healthy ones. The increment was more in T60/887, Sca6, T85/799 and Pa150 than the other genotypes (Table 2, 3).

There were significant differences in specific polyphenols (flavonoids, tannins and lignin) content in the leaf and pod of cocoa genotypes evaluated. The specific polyphenols were significantly higher in T60/887, Pa150, Sca6 and T85/799 than in the other genotypes. The contents of these specific polyphenols increased in leaf and pod of the cocoa genotypes after infection with *Phytophthora megakarya* for 5 days. Table 4 and 5 shows the contents of flavonoids, tannins and lignin in leaf and pod, respectively for the cocoa genotypes.

Relationship between biochemical characters and resistance to *Phytophthora* species: The association between resistance to black pod disease caused by *P. palmivora* and *P. megakarya* (PLN, PLS, LDS and F.I.) and biochemical traits estimated from healthy and *P. megakarya* infected leaf and pod are presented in Table 6a and b, respectively.

Most of the biochemical characters investigated in cocoa leaf and pod under healthy and *P. megakarya* infected conditions showed significant strong negative correlation with resistance to *P. palmivora* and *P. megakarya* resistance measured as pod lesion number, pod lesion size and leaf disc score. There was significant and negative correlation between total phenols, sugars and proteins and resistance to black pod disease resistance. However, nitrogen, protein and total

Table 4: Flavonoids, tannins and lignin composition of 12 cocoa genotypes evaluated in healthy and *P. megakarya* infected leaf

	Healthy leaf			*P. megakarya* infected leaf		
Genotypes	Flavonoids	Tannins	lignin	Flavonoids	Tannins	Lignin
Pa7/808	6.59±0.17^{c}	42.25±0.21^{d}	32.64±0.86^{c}	9.89±0.26^{d}	54.92±0.27^{d}	43.94±0.86^{c}
Na33	4.13±0.19^{e}	33.26±0.41^{f}	20.46±0.94^{e}	5.53±0.18^{f}	43.24±0.53^{e}	24.09±0.99^{f}
T60/887	8.59±0.10^{ab}	43.97±0.34^{c}	42.50±0.51^{ab}	12.88±0.15^{b}	57.16±0.44^{bc}	53.80±0.51^{a}
T63/971	5.53±0.17^{d}	29.82±0.35^{g}	27.39±0.84^{d}	6.97±0.09^{e}	38.76±0.46^{f}	32.02±1.25^{e}
Imc76	6.85±0.17^{c}	42.09±0.34^{d}	33.92±0.82^{c}	10.28±0.25^{d}	54.72±0.44^{d}	45.22±0.82^{c}
Pa150	8.00±0.37^{b}	45.23±0.29^{b}	39.62±1.82^{b}	12.01±0.55^{c}	58.79±0.38^{a}	50.92±1.82^{ab}
Sca9	6.80±0.21^{c}	42.46±0.43^{d}	33.68±0.43^{c}	10.21±0.48^{d}	55.20±0.23^{d}	44.98±0.35^{c}
Imc67	5.74±0.31^{d}	34.03±1.21^{f}	28.43±0.72^{d}	6.62±0.71^{e}	44.23±0.48^{e}	34.40±0.25^{e}
Imc53	6.53±0.18^{c}	38.92±0.58^{e}	34.61±1.23^{c}	4.82±0.53^{f}	56.41±0.38^{c}	38.62±0.83^{d}
Sca6	8.67±0.15^{a}	45.83±0.63^{ab}	44.81±0.52^{a}	14.38±0.39^{a}	58.23±0.71^{ab}	51.42±0.26^{ab}
T85/799	6.83±0.24^{c}	46.47±0.15^{a}	35.63±1.17^{c}	11.21±0.36^{c}	58.20±0.19^{ab}	49.92±1.17^{b}
T79/501	5.74±0.07^{d}	34.03±0.28^{f}	28.43±0.33^{d}	6.62±0.09^{e}	44.23±0.37^{e}	34.40±0.74^{e}
LSD (0.05)	0.63	0.82	3.1	0.86	1.07	3.41
SE	0.34	0.47	1.77	0.49	0.61	1.94

Different letters indicate significant differences between means at the level of p<0.05, LSD: Least significant difference

Table 5: Flavonoids, tannins and lignin composition of 12 cocoa genotypes evaluated in healthy and *P. megakarya* infected pod

	Healthy pod			*P. megakarya* infected pod		
Genotypes	Flavonoids	Tannins	Lignin	Flavonoids	Tannins	Lignin
Pa7/808	13.85±0.36^{d}	67.59±0.32^{d}	68.54±1.80^{b}	20.77±0.55^{c}	87.87±0.32^{cd}	73.84±1.80^{c}
Na33	8.68±0.40^{f}	53.22±0.65^{f}	42.97±1.98^{de}	13.02±0.60^{e}	69.19±0.65^{e}	44.60±1.58^{f}
T60/887	18.03±0.22^{ab}	70.35±0.54^{c}	89.26±1.07^{a}	27.05±0.32^{ab}	91.45±0.54^{b}	94.56±1.07^{ab}
T63/971	11.62±0.36^{e}	47.71±0.56^{g}	57.52±1.77^{c}	17.43±0.54^{d}	62.02±0.56^{g}	61.49±1.65^{de}
Imc76	14.39±0.35^{cd}	67.34±0.54^{d}	71.24±1.72^{b}	21.59±0.52^{c}	87.55±0.54^{d}	76.54±1.72^{c}
Pa150	16.81±0.77^{b}	72.36±0.47^{b}	83.19±3.81^{a}	25.21±1.16^{b}	94.07±0.47^{a}	88.49±3.81^{b}
Sca9	4.39±0.22^{g}	67.94±1.40^{d}	70.72±2.51^{b}	21.43±0.43^{c}	88.32±1.40^{cd}	76.02±2.07^{c}
Imc67	16.81±0.46^{b}	54.44±0.65^{f}	59.71±1.32^{c}	18.09±0.56^{d}	70.78±0.65^{e}	64.00±1.63^{d}
Imc53	15.36±0.72^{c}	56.33±0.82^{e}	48.22±2.89^{d}	20.67±2.60^{c}	64.82±0.82^{f}	57.66±2.53^{e}
Sca6	18.72±0.48^{a}	74.21±1.74^{a}	38.19±0.78^{e}	28.43±0.28^{a}	89.32±1.74^{c}	97.43±0.87^{a}
T85/799	14.29±0.49^{cd}	67.94±0.24^{d}	70.72±2.46^{b}	21.43±0.75^{c}	88.32±0.24	76.02±2.46^{cd}
T79/501	12.06±0.14^{e}	54.44±0.45^{f}	59.71±0.69^{c}	18.09±0.21^{d}	70.78±0.45^{e}	64.00±0.51^{d}
LSD (0.05)	1.32	1.32	6.52	1.97	1.72	6.07
SE	0.75	0.75	3.72	1.13	0.98	3.47

SE: Standard error, Different letters indicate significant differences between means at the level of p< 0.05, LSD: Least significant difference

polyphenol in healthy leaf was not significantly (p>0.05) correlated with pod lesion number, pod lesion size and leaf disc scores caused by *P. palmivora* and *P. megakarya* (Table 6a). Specific phenols; flavonoids, tannins and lignins was significantly and negatively correlated with resistance to *Phytophthora* species resistance. However, the negative correlation between lignin in healthy pod husk and PLN, PLS and LDS caused by *P. palmivora* and *P. megakarya* was not significant (p>0.05) (Table 6b). Under *P. megakarya* infected infection, however, biochemical traits in leaf and pod were significantly correlated with PLN, PLS and LDS. Correlation coefficients of nitrogen, protein, total polyphenol, soluble sugar, insoluble sugar, flavonoids, tannins and lignin in healthy and infected leaf and pod were significant and negative with mean field infection in 6×4 factorial

Table 6a: Correlations of soluble sugar, insoluble sugar, protein, polyphenol, flavonoid, tannins and lignin estimated from healthy and infected leaf with lesion number, pod lesion size, leaf disc scores and natural field infection caused by *P. palmivora* and *P. megakarya*

	Biochemical traits													
	Healthy leaf							*P. megakarya* infected leaf						
Character	X1	X2	X3	X4	X5	X6	X7	X1	X2	X3	X4	X5	X6	X7
PLNPp	-0.72	-0.64	-0.87**	-0.93***	0.86**	-0.91***	-0.84**	-0.67	-0.64	-0.87**	-0.93***	-0.88**	-0.85**	-0.90***
PLNPm	-0.73	-0.64	-0.87**	-0.93***	-0.87**	-0.93***	-0.87**	-0.68	-0.65	-0.87**	-0.93***	-0.89**	0.84**	-0.91***
PLSPp	-0.51	-0.46	-0.88**	-0.91***	-0.79**	-0.94***	-0.76*	-0.55	-0.46	-0.88**	-0.90***	-0.87**	-0.86**	-0.87**
PLSPm	-0.44	-0.49	-0.87**	-0.88**	-0.81**	-0.94***	-0.79*	-0.54	-0.49	-0.86**	-0.88**	-0.82**	-0.88**	-0.86**
LDSPp	-0.80**	-0.70	-0.71	-0.83**	-0.79*	-0.89**	-0.79*	--0.69	-0.70	-0.72	--0.82**	-0.74*	-0.88**	-0.86**
LDSPm	-0.56	-0.52	-0.84**	-0.86**	-0.74	-0.93***	-0.73	-0.59	-0.52-	-0.84**	-0.87**	-0.76**	-0.89**	-0.80**
MFI+	-0.76*	-0.73	-0.87**	-0.79*	-0.82**	-0.70	-0.82**	-0.75*	-0.45	-0.63	-0.75*	-0.84**	-0.83**	-0.86**
MFI*	-0.7	-0.78*	-0.85**	-0.63	-0.87**	-0.79*	-0.86**	-0.71	-0.58	-0.79*	-0.74-	-0.87**	-0.78*	-0.80**

X1: %Protein, X2: Polyphenols, X3: Soluble sugars, X4: Insoluble sugars, X5: Flavonoid, X6: Tannins, X7: Lignin. MFI+: Mean field infection of factorial crosses, MFI*: Mean field infection of diallel crosses. Multiple linear regression equation; lesion numbers on pod caused by *P. palmivora* PLNPp: $22.2^{*}-0.05X_1+0.004X_2^{**}+0.14X_3-0.16X_4-0.73X_5^{**}-0.20X_6^{***}+0.17X_7^{**}$ (R^2: 95.9%, R^2(adj.) = 84.9%). Stepwise regression equation PLNPp: $25.49^{**}-2.8X_5^{**}-0.35X^{*}+0.42X^{***}$ (R^2: 86.19, R^2(adj.): 81.01%). Multiple linear regression equation; lesion numbers on pod caused by *P. megakarya* PLNPm: $26.4^{**}-0.17X_1+0.05X_2^{*}+0.52X_3-0.19X_4^{**}-1.05X_5^{*}-0.16X_6^{**}+0.20X_7^{**}$ (R^2= 96.3%, R^2(adj.) = 86.3%). Stepwise regression equation; PLNPm: $30.05^{***}-3.0X_5^{**}-0.35X_6^{*}+0.42X_7^{**}$ (R^2: 86.51, R^2(adj.): 81.45). Multiple linear regression equation; lesion sizes on pod caused by *P. palmivora* PLSPp: $131^{**}-2.97X_1-0.27X_2^{**}+5.58X_3^{*}-2.33X_4^{**}-29.5X_5^{**}-2.61X_6^{**}+6.37X_7^{***}$ (R^2= 99.3%, R^2(adj.) = 97.5%). Stepwise regression equation, PLSPp: $181.5^{**}-24.31X_5^{*}-3.49X_6^{**}+4.94X_7^{**}$ (R^2: 92.49, R^2 (adj.): 89.67). Multiple linear regression equation; lesion sizes on pod caused by *P. megakarya* PLSPm: $155^{**}-1.83X_1-1.00X_2+4.19X_3-1.86X_4^{*}-11.8X5^{**}-3.23X_6^{***}+2.38X_7^{***}$ (R^2: 97.5%, R^2 (adj.): 90.8%). Stepwise regression equation, PLSPm: $209.5^{**}-9.00X_5^{**}-3.78X_6^{**}+1.70X_7^{**}$ (R^2 = 88.17, R^2(adj.) = 83.73). Multiple linear regression equation; leaf disc scores caused by *P. palmivora* LDSPp: $6.20^{**}+0.04X_1^{*}-0.04X_2^{**}+0.08X_3-0.03X_4^{**}+0.14X_5^{*}-0.09X_6^{**}+0.007X_7^{*}$ (R^2: 97.5%, R^2(adj.): 90.7%). Stepwise regression equation, LDSPp: $6.09^{**}-0.06X_5^{*}-0.09X_6^{**}+0.003X_7^{*}$ (R^2: 79.81, R^2(adj.): 72.23). Multiple linear regression equation; leaf disc scores caused by *P. megakarya* LDSPm: $7.99^{**}+0.16X_1^{**}-0.03X_2-0.19X_3^{*}+0.03X_4^{**}+1.24X_5^{***}-0.15X_6^{**}-0.19X_7^{**}$ (R^2: 96.8%, R^2(adj.): 88.3%). Stepwise regression equation, LDSPm: $7.90^{**}+0.05X_5^{*}-0.15X_6^{**}+0.014X_7^{*}$ (R^2: 87.23, R^2(adj.): 82.44). *,**,***: Correlation and regression coefficients significant at p = 0.05, p<0.05 and p<0.001, respectively. Multiple and step-wise regressions were carried out with biochemical contents in healthy leaf samples

mating design (MFI+) in 2009 and 2010 (data not shown) and mean field infection in 6×6 diallel mating design (MFI*) in 2009 and 2010 (data not shown). However, correlation coefficients of nitrogen in healthy leaf and total polyphenols in infected leaf was not significant (p>0.05) with natural field infection (Table 6a, b).

Principal Component Analysis (PCA) showed that the first two components contributed to explain 0.904 (PC1 = 0.847 and PC2 = 0.058) of the total variation of biochemical traits in cocoa. Pod lignin, pod insoluble sugar and leaf insoluble sugar were the major contributors of total variation of biochemical traits in PC1. In PC2, pod lignin, leaf lignin, pod tannins and pod insoluble sugar were the major contributors to total variation (Table 7). Pod lignin and pod insoluble sugar dominated both PC1 and PC2. It is apparent that pod lignin and pod insoluble sugar defined PC1 and PC2 and leaf insoluble sugar the PC3. Graphic representation of the principal components analysis (Fig. 1) shows the 12 cocoa genotypes separated according to their biochemical characteristics. Genotypes T63/971 and Imc67 and Na33 and Imc53 are clearly classified as different from the rest. The PC1 which discriminates genotypes according to lignin and by amount of insoluble sugars, groups T63/971 and Imc67 as different because they have the least

Table 6b: Correlations of soluble sugar, insoluble sugar, protein, polyphenol, flavonoid, tannins and lignin estimated from healthy and infected pod husk with lesion number, pod lesion size, leaf disc scores and natural field infection caused by *P. palmivora* and *P. megakarya*

	Biochemical traits													
	Healthy pod husk							*P. megakarya* infected pod husk						
Character	X1	X2	X3	X4	X5	X6	X7	X1	X2	X3	X4	X5	X6	X7
PLN*Pp*	-0.64	-0.88**	-0.94***	-0.96***	-0.47	-0.92***	-0.46	-0.67	-0.88**	-0.94****	-0.96***	-0.86**	-0.87**	-0.86**
PLN*Pm*	-0.67	-0.87**	-0.94***	-0.96***	-0.48	-0.91***	-0.45	-0.69	-0.87**	-0.95***	-0.95***	-0.86**	-0.87**	-0.88**
PLS*Pp*	-0.54	-0.75*	-0.88**	-0.88**	-0.47	-0.96***	-0.51	-0.58	-0.75*	-0.88**	-0.88**	-0.78**	-0.95***	-0.84**
PLS*Pm*	-0.53	-0.75*	-0.85**	-0.88**	-0.59	-0.94***	-0.41	-0.56	-0.69	-0.84**	-0.88**	-0.81**	-0.89**	-0.83**
LDS*Pp*	-0.68	-0.79*	-0.81**	-0.87**	-0.48	-0.86**	-0.43	-0.69	-0.79*	-0.80**	-0.87**	-0.79**	-0.80**	-0.76*
LDS*Pm*	-0.59	-0.69	-0.83**	-0.88**	-0.46	-0.92***	-0.36	-0.60	-0.69	-0.84**	-0.88**	-0.74	-0.87***	-0.73
MFI+	-0.46	-0.80**	-0.77*	-0.80**	-0.82**	-0.82**	-0.84**	-0.85**	-0.70	-0.56	-0.89**	-0.82**	-0.80**	-0.83**
MFI*	-0.76*	-0.85**	-0.46	-0.73	-0.86**	-0.77**	-0.81**	-0.82**	-0.78**	-0.74	-0.81**	-0.79**	-0.75*	-0.88**

X1: %Protein, X2: Polyphenols, X3: Soluble sugars, X4: Insoluble sugars, X5: Flavonoid, X6: Tannins, X7: Lignin. MFI+: Mean field infection of factorial crosses, MFI*: Mean field infection of diallel crosses. Multiple linear regression equation ; lesion numbers on pod caused by *P. palmivora* PLNPp: $21.0^{**}-0.013X_1-1.49X_2^{*}+0.016X_3-0.102X_4^{**}+0.124X_5^{*}-0.085X_6^{**}-0.023X_7^{**}$ (R^2 = 98.9%, R^2(adj.) = 95.8%). Stepwise regression equation, PLNPp: $25.72^{*}+0.02X_5^{**}-0.30X_6^{**}-0.01X_7^{***}$ (R^2 = 84.45, R^2(adj.) = 78.62). Multiple linear regression equation; lesion numbers on pod caused by *P. megakarya*, PLNPm: $26.3^{*}-0.26X_1^{*}-2.18X_2^{**}-0.21X_3-0.07X_4^{**}+0.17X_5^{*}-0.11X_6-0.007X_7^{*}$. ($R^2$ = 98.4, R^2(adj.) = 94.0). Stepwise regression equation; PLNPm: $30.63^{**}+0.013X_5^{**}-0.32X_6^{***}-0.007X_7^{*}$ (R^2 = 84.09, R^2(adj.) =78.12). Multiple linear regression equation; lesion size on pod caused by *P. palmivora* PLSPp: $151^{**}-1.59X_1-2.2X_2^{**}+1.73X_3-0.53X_4^{**}+0.52X_5^{**}-1.81X_6^{*}-0.15X_7^{**}$ (R^2 = 96.1%, R^2 (adj.) = 85.7%). Stepwise regression equation PLSPp: $173.6^{***}+0.46X_5^{*}-2.05X_6-0.10X_7^{**}$ (R^2 = 93.60, R^2(adj.) = 91.20). Multiple linear regression equation; lesion size on pod caused by *P. megakarya* PLS*Pm*: $179^{**}+2.47X_1^{**}+14.5X_2^{*}+5.00X_3-1.67X_4^{**}-1.74X_5-1.54X_6^{**}-0.28X_7^{*}$ (R^2 = 91.7%, R^2(adj.) = 69.6%). Stepwise regression equation PLSPm: $206.5^{**}-1.0X_5^{**}-2.19X_6^{**}+0.05X_7^{*}$ (R^2 = 89.75, R^2(adj.) = 85.91). Multiple linear regression equation; leaf disc scores caused by *P. palmivora* LDSPp: $4.80^{**}-0.005X_1^{**}+0.79X_2^{*}+0.49X_3-0.16X_4-0.06X_5+0.035X_6^{*}-0.037X_7^{**}$ (R^2 = 94.8%, R^2(adj.) = 81.1%). Stepwise regression equation, LDSPp: $5.87^{**}-0.005X_5^{*}-0.057X_6-0.002X_7^{**}$ (R^2 = 74.75%, R^2(adj.) = 65.28%). Multiple linear regression equation; leaf disc scores caused by *P. megakarya* LDSPm: $5.62^{***}+0.149X_1^{*}+1.70X_2+0.48X_3-0.17X_4^{**}-0.08X_5^{**}+0.02X_6^{**}-0.04X_7^{*}$ (R^2 = 94.2%, R^2(adj.) = 78.9%). Stepwise regression equation, LDSPm: $7.33^{**}+0.01X_1^{*}-0.08X_2^{**}+0.004X_3^{***}$ (R^2 = 85.16, R^2(adj.) = 79.60)

lignin and insoluble sugar content. Likewise, PC2 allows separation of genotypes T60/887 and Pa150 and Sca6 and T85/799 from the others because these genotypes have high contents of lignin and insoluble sugar. The angle between lignin and insoluble sugars in leaf and pod are very acute (Fig. 1). A close relationship was found between biochemical traits and pod lignin and pod insoluble sugar, on one hand and components of resistance to *P. palmivora* and *P. megakarya* in cocoa, on the other hand, thus suggesting the importance of lignin and insoluble sugar values of cocoa genotypes as a resistance mechanism against black pod disease.

Similarity and dissimilarities of the 12 cocoa genotypes in terms of biochemical traits were examined by means of hierarchical cluster analysis. The cluster analyses were based on similarity matrices representing absolute values of biochemical factors (Fig. 2) and the values of the principal components (Fig. 3). The cluster analyses show a wide variability among the genotypes and they are wide with a similarity distance of 0-150 (Fig. 2) and 0-70 (Fig. 3). Two major clusters were evident among the 12 cocoa genotypes. Cluster one consisted of Imc67, Na33, T79/501, T63/971, Imc53 and Sca9. Cluster two is made up of Pa150, T60/887, Sca6, Imc76, Pa7/808 and T85/799.

Multiple linear regression analysis indicated that biochemical contents explained 96.3 and 98.4% of the total variation in PLNPm, 97.5 and 91.7% of the variation in PLSPm, 96.8 and 94.2%

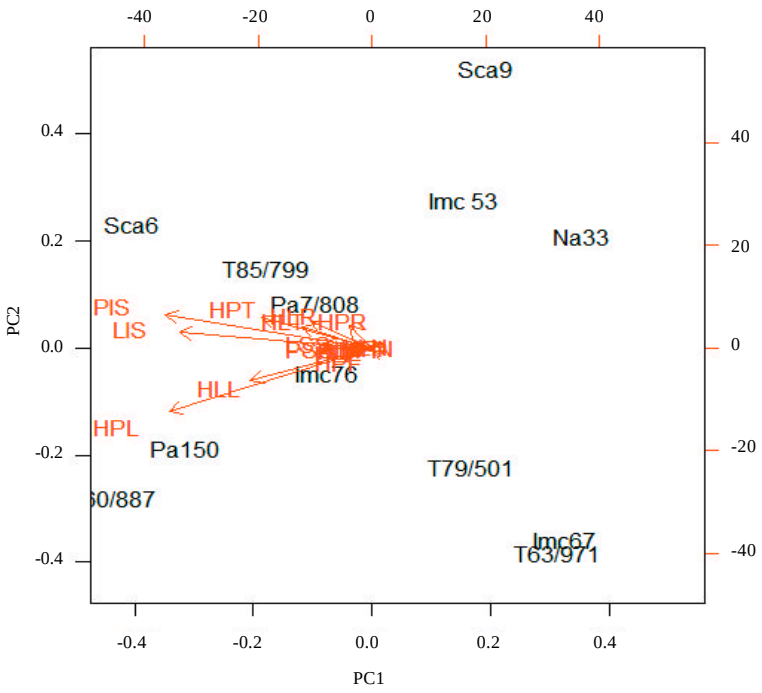

Fig. 1: A two-dimensional biplot of the principal component analysis on 12 cocoa genotypes and eight biochemical factors in leaf and pod of cocoa as variables

Table 7: Principal component analysis of biochemical traits in two plant parts (leaf and pod) of 12 cocoa genotypes

Biochemical traits	PC1	PC2	PC3	PC4
Healthy leaf nitrogen	-0.011	0.013	0.049	-0.037
Healthy pod nitrogen	-0.003	0.006	0.004	-0.002
Healthy leaf protein	-0.152	0.275	0.696	-0.456
Healthy pod protein	-0.056	0.234	0.205	-0.124
Healthy leaf polyphenol	-0.059	-0.014	0.184	-0.030
Healthy pod polyphenol	-0.016	-0.009	0.189	0.006
Healthy leaf soluble sugar	-0.124	0.043	-0.202	-0.034
Healthy pod soluble sugar	-0.120	-0.001	-0.053	-0.049
Healthy leaf insoluble sugar	-0.475	0.161	-0.429	-0.229
Healthy pod insoluble sugar	-0.511	0.347	-0.141	-0.177
Healthy leaf flavonoid	-0.067	-0.043	-0.035	-0.079
Healthy pod flavonoid	-0.066	-0.115	0.112	0.175
Healthy leaf tannins	-0.174	0.228	0.148	0.484
Healthy pod tannins	-0.273	0.325	0.105	0.624
Healthy leaf lignin	-0.299	-0.336	-0.167	-0.106
Healthy pod lignin	-0.500	-0.661	0.338	0.118
Proportion of variance	0.847	0.058	0.041	0.028
Cumulative proportion	0.847	0.904	0.946	0.974
Standard deviation	25.750	6.730	5.670	4.680

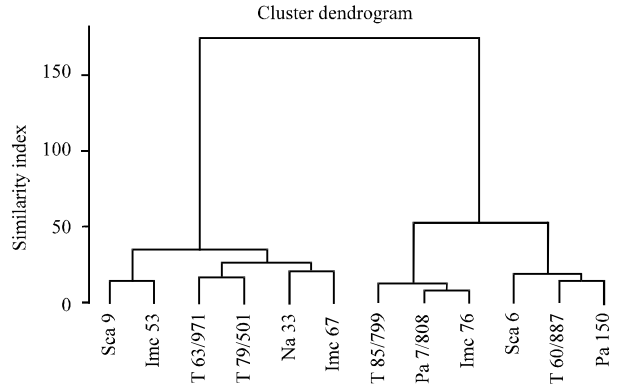

Fig. 2: Dendrogram of 12 cocoa genotypes based on absolute values of total phenols, soluble sugars, insoluble sugars, percentage nitrogen, percentage protein, flavonoid, tannins and lignin in leaf and pod of cocoa

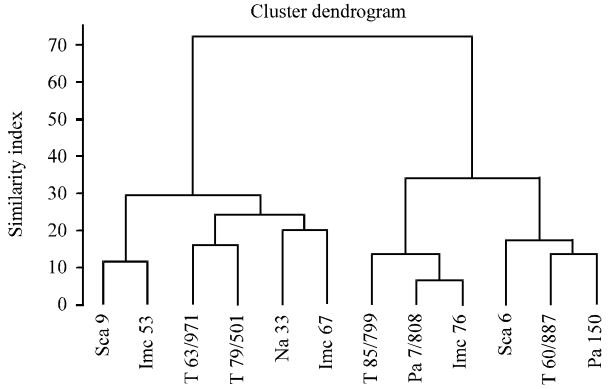

Fig. 3: Dendrogram of 12 cocoa genotypes based on principal components 1 and 2 of total phenols, soluble sugars, insoluble sugars, percentage nitrogen, percentage protein, flavonoid, tannins and lignin in leaf and pod of cocoa

of the variation in LDS*Pm* in healthy leaf and pod husk, respectively. Step-wise regression analysis indicated that flavonoid, tannin and lignin accounted for 86.51 and 84.09% of total variation in PLN*Pm*, 88.17 and 89.75% of the variation in PLS*Pm* and 87.23 and 85.16% of the variation in LDS*Pm* in healthy leaf and pod, respectively (Table 6a, b).

DISCUSSION

The study showed considerable genetic variability among cocoa genotypes for PLN, PLS and LDS caused by *P. palmivora* and *P. megakarya*. The differential response of cocoa genotypes further suggested that resistance to black pod disease was under genetic control and should therefore be liable to genetic improvement. Ample genetic variability for black pod resistance has also been reported in cocoa by Iwaro *et al.* (1997a), Nyadanu *et al.* (2009b), Nyassé *et al.* (2002) and Tahi *et al.* (2006).

Plant biochemical constituents have received considerable attention in relation to disease resistance. To develop cocoa cultivars with stable resistance to black pod disease, there is need to

use cocoa genotypes with different combinations of factors associated with black pod disease resistance. Therefore, there is a need to have a comprehensive understanding of the biochemical constituents that influence the expression of resistance to black pod in improved varieties and hybrids. Iwaro (1995) suggested that, post-penetration resistance which influences the extent of invasion of tissue by the pathogen and hence size of lesion was probably governed by biochemical factors. This study showed considerable genetic variability among cocoa genotypes for all biochemical traits investigated. The significant differences among cocoa genotypes in biochemical contents suggest that cocoa genotypes varied in biochemical contents. With the use of organs of uniform age and materials from the same environment, the observed variation could be genetic. This provides opportunity to improve biochemical contents of cocoa genotypes. This agrees with the results of Ndoumou *et al.* (1996) who also reported significant differences in carbohydrates, amino acids and phenol contents in cocoa pods from three clones after infection with *P. megakarya*.

In the present study, increased concentration of total polyphenol in leaf and pod were found after infection with *P. megakarya*. The accumulation was more in the less susceptible genotypes than the highly susceptible ones. This result indicates that the inhibitory effect of phenolics on fungal development depends on the level of these compounds in the plant tissue. Meifrein and Tanguy (1967), Ndoumou *et al.* (1995, 1996), Djocgoue (1998) and Djocgoue *et al.* (2007) reported that highly susceptible clones of cocoa contain less phenolics. The findings are also consistent with results reported by Musseti *et al.* (2000) and Siranidou *et al.* (2002) who, respectively, showed an increase in phenolic content potato wheat tissues when they were infected. Phenolics are known to be substrates in infected plants, for the synthesis of compounds involved in disease resistance, like pterocarpan phytoalexins and hydroxycinnamic acid esters (Dixon and Lamb, 1990) and for the production at or near the infection site of bioresistant phenylpropanoid polymers (lignins and suberins) which act as a barrier to the penetration or the propagation of the pathogen (Ebel and Grisebach, 1988; Davin and Lewis, 1992). The results also show that the less susceptible cocoa genotypes, T60/887, Sca6, T85/799 and Pa150 contain more carbohydrates (soluble and insoluble sugars) than the highly susceptible genotypes, Na33, Imc67 and Imc53. This suggests the role of soluble and insoluble sugars in resistance to black pod disease. The soluble sugars did not increase or accumulate after infection of cocoa leaf and pod with *P. megakarya*. However, the insoluble sugars increased in leaf and pod after infection with *P. megakarya*. The constant level of soluble sugars in leaf and pod after infection may be attributed to their utilization by the fungus for its growth. These results are in agreement with those reported on *Botryosphaeria* apple rot (Hwang, 1983), on charcoal rot of sorghum (Patil *et al.*, 1985), on *Phytophthora* blight of pepper (Jeun and Hwang, 1991) and on *Phytophthora* pod rot of cocoa (Ndoumou *et al.*, 1996; Omokolo *et al.*, 2002) who reported a decrease in soluble sugars after infection. Insoluble sugar variation during infection seems to be genotype-dependent and that the fungus does not utilize sugars of this group for its growth. The involvement of insoluble sugars in the resistance of black pod disease has not been reported in cocoa literature.

The nitrogen content remains constant after infection of cocoa leaf and pod with *P. megakarya*. High percentage protein content was observed in less susceptible genotypes as compared to the highly susceptible genotypes indicating their role in disease resistance. It is worth to note that this variable increased significantly in pods after infection with *P. megakarya* and decreased significantly in the infected leaves of cocoa genotypes but such decrease was not observed in the diseased leaves of Na33 and Imc67. The result of percentage proteins show non-consistent pattern in leaf and pod. This pattern could be explained by differences in structure and the synthesis of

disease related molecules like phenols and hydroxyproline-rich glycoproteins which generally accumulate in fungus infected plants in leaf and pod (Esquerre-Tugaye, 1979).

There were more flavonoid, tannins and lignin content in leaf and pod of less susceptible cocoa genotypes, than the highly susceptible genotypes suggesting their involvement in resistance to *Phytophthora* pod rot in cocoa. An increased level of these specific phenolic compounds among cocoa genotypes with high resistance levels could be due to reaction of the genotypes against *Phytophthora* colonization. The role of flavonoid, tannins and lignin in resistance to black pod disease has not been reported in cocoa literature. However, the antimicrobial property of these specific phenolic compounds has been well reported (Smith and Banks, 1986; Hahlbrock and Scheel, 1989). Plant phenolics, particularly flavonoid and lignin are the most widespread classes of secondary metabolites known to be involved in the plant-microbe interactions (Morandi, 1996). Generally, the accumulation of phenolics following infection by a pathogen involves the neosynthesis of specific phenol compounds (Conceicao *et al*., 2006). Qualitative analysis of phenolics in leaves of cocoa showed a higher accumulation of some luteolin derivatives and apigenin derivatives (flavonoids) and some hydrocinnamic acid derivatives (Djocgoue *et al*., 2007).

The strong negative correlation between biochemical traits and pod lesion number, pod lesion size and leaf disc score of *Phytophthora palmivora* and *P. megakarya* suggests that as biochemical factors increase, infection caused by *Phytophthora* species in cocoa leaf and pod reduces. The findings from this study suggest that total polyphenols, soluble sugars, insoluble sugars, nitrogen, proteins, flavonoid, tannins and lignin are involved in resistance of cocoa to black pod disease caused by *P. palmivora* and *P. megakarya*. The results support the findings of many authors. Gabler *et al*. (2003) stated that contact with underlying plant tissues presents the invading pathogen with a different set of barriers, most notably preformed antibiotic compounds and phytoalexins induced by the plant. El-Hassni *et al*. (2004), Tan *et al*. (2004), Omokolo and Boudjeko (2005) showed the involvement of phenolics in the cocoa plant defence mechanism against pest and diseases. Djocgoue (1998) observed an accumulation of phenolics during the development of necrosis within the mesocarp of the cortex in cocoa's pod infected by *P. megakarya*. He noticed that the most tolerant cocoa clones have pods richer in phenolic content than the susceptible cocoa clones. Omokolo *et al*. (2002) reported amino acids and carbohydrates in the cortex of cocoa clones resistant to *P. megakarya*, inhibiting expansion of lesions. Resistance of apple (*Malus domestica*) to *Venturia inaequalis* has been shown to be related to the high content of catechin and the proanthocyanidin present in leaves (Treuter and Feucht, 1990). Islam *et al*. (2003) reported seed coat tannins of common bean (*Phaseolus vulgaris* L.) to be negatively correlated with susceptibility to angular leaf spot, bacterial blight, anthracnose and *Empoasca*. Increased lignin formation in the outer periderm tissues of carrot (*Daucus carota* L.) was reported by Wally and Punja (2010) to be involved in resistance to *Alternaria radicina* and necrotrophic pathogens in carrot. Peltier *et al*. (2009) also reported soybean stem lignin concentration to be related to resistance to Sclerotinia stem rot caused by *Sclerotinia sclerotiorum*, an economically important disease of soybean (*Glycine max*). This was further explained by Ride (1978) who suggested that lignification might hinder fungal growth through plant tissue by forming walls more resistant to mechanical penetration, dissolution by fungal enzymes and restricting diffusion of enzymes and toxins from the fungus to the host.

The insignificant correlation of proteins, nitrogen and total polyphenols with some of the components of resistance could be due to their low levels in the tissues of the cocoa genotypes. The

stronger association between biochemical traits and the components of resistance under *P. megakarya* infected conditions of leaf and pod than in the healthy leaf and pod suggests that the biochemical factors accumulate during or after infection.

There is a need to identify some biochemical traits that contribute mostly to the biochemical variation of the cocoa genotypes and strongly correlate with other biochemical factors. To do this, principal components analysis (PCA), was carried out to indicate the most reliable biochemical characters to be used as markers to screen and select for resistance to black pod disease. The PCA demonstrated how pod lignin and pod insoluble sugar are the main features of the 12 cocoa genotypes associated with resistance to black pod disease because they are the main variables contributing to the first PC1 of PCA. The lines that connect test entity (in this case the biochemical traits) to the biplot origin are vectors. The cosine of the angle between the vectors of two biochemical traits approximates the correlation between them (Yan and Tinker, 2006). The acute angle between lignin and insoluble sugar in leaf and pod indicates a positive correlation between them implying that cocoa genotypes that were high in lignin content were also high in pod insoluble sugar and vice versa. This also suggests that biochemical contents in leaf could be used to predict biochemical contents in pod. These results confirm the possibility to use cocoa leaf resistance to *Phytophthora* species to predict cocoa pod resistance and furthermore could offer a theoretical explanation about the results obtained by Nyassé *et al.* (1995), Nyassé *et al.* (2002), Iwaro *et al.* (1997b), Tahi *et al.* (2000), Blaha *et al.* (2000) and Nyadanu (2008) who reported that resistance of leaf and pod to *P. palmivora* and *P. megakarya* were positive and significantly correlated.

From the PCA, the relative importance lignin and insoluble sugar have in the conformation of PC1 may be deduced, allowing this discrimination of genotypes Imc67 and T63/971 (with low lignin and insoluble sugar) and T60/887, Pa150 and Sca6 (with high lignin and insoluble sugar) from other genotypes.

It is clear from the dendrogram that the clusters were not mere groupings of genotypes according to their biochemical characters but also as distinct genetic groups. Within the sub-clusters of these two clusters, susceptible cocoa genotypes; Imc67, Na33, T79/501, T63/971, Imc53 and Sca9 and resistant cocoa genotypes Pa150, T60/887, Sca6, Imc76, Pa7/808 and T85/799 were more similar and most closely related genotypes.

The principal components, correlation coefficients, multiple and step-wise regressions indicated that insoluble sugar, flavonoid, tannins and lignin are the most reliable biochemical factors and these could be used as marker traits to screen and select for resistance to black pod disease of cocoa.

CONCLUSION

There was considerable genetic diversity among the cocoa genotypes based on black pod disease resistance and biochemical composition. Resistance in cocoa to black pod disease is the result of combination of many biochemical factors and cannot be ascribed to a single biochemical factor.

The present studies based on a diverse array of cocoa genotypes with different levels of resistance to black pod disease provided a rational comparison of the contribution of different biochemical traits associated with black pod resistance and pinpoint those that can be used as markers to screen and breed for resistance to this disease. The use of biochemical factors such as flavonoids, tannins, lignin and insoluble sugars which have high association with black pod disease resistance could lead to sustainable improvement of cocoa genotypes against black pod disease.

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