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# Estimation of necrosis length, phenolic content, peroxidase and polyphenoloxidase activity in cocoa plants (*Theobroma cacao* L.) after *Phytophthora megakarya* inoculation

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## Abstract

Cocoa (*Theobroma cacao* L.) is the principal culture of income for many countries of the world. In Cameroon, black pod disease caused by *Phytophthora megakarya* is one of the most economically destructive diseases of cocoa. Hybridation is a reliable method of ameliorating cocoa production. The aim of this study was to select new genotypes with resistance to *P. megakarya*. Necrotic lesions, total polyphenols (TPP) and activities of antioxidant enzymes [peroxidase (POX) and polyphenoloxidase (PPO)] were conducted on leaves of three parental clones (T79/467, SNK413 and ICS40) and their hybrids (families F70, F30, F90 and F95) derived from reciprocal crossing after inoculation. 96% of the hybrid's genotype manifested a positive heterosis effect for the development of lesion size suggesting the existence of hybrid vigour. The F30.03, F30.07, F70.04, F70.07, F90.03, F95.01, F95.08 and F95.11 genotypes showed a significant increase ($P<0.05$) of biochemical components negatively correlated with the necrosis length and this increase was genotype-dependent. Those hybrids can be considered as elite clones. Furthermore, the pair of parental clones (T79/467-SNK413 and T79/467-ICS40) has showed good aptitudes for the combination of the characters studied and no maternal effect was detected in their transmission.

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## Introduction

Cocoa (*T. cacao* L.) is one of the main plants which impact positively the economy for many tropical countries in the word. Its broad beans are used in different domains such as pharmaceutical, cosmetic and food processing industries. However, the crop is attacked by several pathogens, causing serious losses in production. In Cameroon, black pod disease caused by *P. megakarya*, is one of the main hindrance to cocoa production (Nyasse *et al*., 2002). Yield lost per year can reach 40% of the world production and vary according to the geographic area as well as the *Phytophthora* species (Flood *et al.* 2004, Ndoumbe-Nkeng *et al*., 2004). In optimum conditions, losses can reach 80 % (Bowers *et al*., 2001). However, crop alternation can be applied to reduce the disease. There are also several fungicides effective against the disease. The pathogen is increasingly resistant against the fungicides used to control it, and using fungicides leads to the environmental pollution and concerns about human health that constitute a separate dimension of the problem (Walter *et al*., 2005). To avoid or limit the use of chemical control, which is extremely costly; research, was initiated towards genetic control, by breeding cocoa cultivars less susceptible to the disease and exploiting field resistance factors. Moreover, it has become very important to determine plant-pathogen interactions and discover which type of resistance is evolved from cultivating resistant species. In addition, it is necessary to develop new strategies to control diseases and increase resistance against disease in economically important plant species. Initially, resistance can be attributed to the accumulation of fungitoxic compounds after infection of cocoa leaves (Djocgoue *et al.,* 2011; Ondobo *et al*., 2014; Manga *et al*., 2016). Many authors showed the involvement of biochemical markers in the plant defense mechanism against *P. megakarya*. Omokolo and Boudjeko (2005), Effa *et al.* (2016), Manga *et al.* (2016) reported phenols as resistance factors in resistance of *T. cacao* to *P. megakarya*. Murthy *et al.* (2014), have shown that on mechanisms of biological control by PGPR reveals that several strains protect the plants from pathogen attack by strengthening the epidermal and cortical cell walls with deposition of newly formed barriers beyond infection sites including callose, lignin and phenolics and by activating defense genes encoding chitinase, peroxidase (POX), phenylalanine ammonialyase (PAL) and polyphenol oxidase (PPO).

This study investigates deals with the induction of defensetotal phenolics and activities of antioxidant such as, peroxidase (POX), polyphenol oxidase (PPO), following inoculation with *P. megakarya*.

## Materials and methods

*Cocoa plant material*

Three cocoa clones, with different sensibility to *P. megakarya* available in gene banks of the Cameroon Cocoa Development Corporation (SODECAO) at Mengang station, were used to create four progenies: one local Trinitario (SNK413, tolerant to *P. megakarya),* one Trinitario introduced from Trinidad (ICS40, high sensibility to *P. megakarya)* and one Forastero (T79/467 tolerant to *P. megakarya)*. Crossings were realized in May, June, July 2012 using hand pollination techniques (Cilas, 1991). The four progenies obtained were:

F30: (♀) SNK413 × (♂) T79/467
F70: (♀) T79/467× (♂) SNK 413
F90: (♀) T79/467 × (♂) ICS40
F95: (♀) ICS40× (♂) T79/467

*Production of seedlings and grafts*

Seeds from pods harvested in experimental field were sown in the nursery and 366 hybrids plants were obtained. Parental plantlets were obtained through grafting by using bud wood from the three clones listed above. This grafting was done on non-specific young cocoa plantlets.

*Leaf inoculation and analysis*

The leaf test is an artificial inoculation method that can be used to assess the resistance of genotypes. Briefly, leaves from one or 3-month-old were washed thoroughly with distilled water and sterilized with ethanol (70%). Three treatments were realized one each parental genotype and hybrid: (i) healthy leaves,

(ii) wounded leaves, (iii) wounded and infected leaves. The inner surface of leaves were scarified by a sterilized razor along the midrib and inoculated by deposition of a mycelium disc (6mm) of *P. megakarya* obtained from a 7-day-old potato dextrose agar (PDA) culture medium and incubated at 25-26°C in the totally dark and humid chamber. The scars were then covered with cotton that had been immersed in sterilized water. The isolate uses of *P. megakarya* belong to "phy-" strain and provided from the Institute of Agriculture Research for Development (IRAD), Nkolbisson research station. Necrotic lesions appeared two days after inoculation and the size of these lesions was measured every two days until day 6.

In order to emphasize the effects of sex of parents on transmission of resistance, two genetic parameters were estimated: the heterosis (Zahour, 1992) and the heritability ($h^2$)(Falconer, 1974).

*Total phenolic compound contents*

Total phenolic content was determined following the method of Singleton and Rossi (1965). A sample (50mg) was extracted with 1ml of 70% aqueous ethanol at room temperature. The mixture was centrifuged at 1000g for 15min. The supernatant (200µl) was mixed with 1.5ml of Folin-Ciocalteu reagent, and allowed to stand at room temperature for 5min; then 1.5ml of sodium bicarbonate solution (0.566M) was added to the mixture. After 60min, absorbance was read at 725nm. Results were expressed as gallic acid equivalents. The concentration used was in a range between 0.02 and 0.1mg/ml.

*Enzyme assays*

*POX activity assay*

The POX activity was determinated spectrophotometrically according to a modification of the method described by Rodriguez and Sanchez (1982).The assay mixture contained 1ml of 0.05M phosphate-citrate buffer PH 4.6, 1ml of 40mMguaiacol and 0.5ml of 26mM $H_2O_2$. The mixture was incubated for 15min at 25°C and finally 20µl of the enzyme extract were added to the cuvette. Changes in the absorbance at 420 nm were measured for 5min using a SCHIMADZU UV-120-01 spectrophotometer. POX activity was expressed as "ΔA420nm/min/g fresh weight".

*PPO activity assay*

PPO activity was also assayed spectrophotometrically as described by Van Kammenn and Broumer (1964), using catechol as a substrate. The reaction mixture, containing 2.5ml of 0.1 M acetate buffer PH 6.0 and 0.5 ml of 10 m Mcatechol was incubated at 25°C for 3min and finally 20µl of the enzyme extract was added to the cuvette. Changes in the absorbance at 330 nm were measured for 1min using SCHIMADZU UV-120-01 spectrophotometer. Enzyme activity was expressed as "△A330nm/min/g fresh weight".

*Statistical analyses*

Data presented are the means ± SE of at least three independent experiments. ANOVA and Tukey test permitted to compare the susceptibility level of better progenies were performed with the assistance of SPSS 17.0 for windows.Principal component analyses (PCA) were realized to describe the variability of length necrosis data and biochemical components respectively. This analysis was performed with SPAD 5.5 software package.

**Results**

*Hand pollination*

Hand pollination test was less successful in F95, F90 and F70 family with 12.76, 15.38 and 16% respectively. These results were better in F30 (39.13%) family (Table 1).

**Table 1.** Rate of success of hand-pollination.

| Families | Crossing | Number of tests | Number of success | Success (%) |
|---|---|---|---|---|
| F30 | (♀) SNK413 × (♂) T 79/467 | 92 | 36 | 39.13% |
| F70 | (♀) T 79/467 × (♂) SNK 413 | 110 | 18 | 16% |
| F90 | (♀) T 79/467 × (♂) ICS40 | 104 | 16 | 15.38% |
| F95 | (♀) ICS40 x (♂) T 79/467 | 94 | 12 | 12.76% |

*Evolution of necrotic lesions*

On leaves wounded and inoculated with agar disc containing *P. megakarya* mycelium, the development of the necrosis appears two days after inoculation in both parent clones and their hybrids excepted F70.04 and F95.03 genotypes.

In the F70 family, six days after inoculation, the mean lesion length varied between 0.67 and 2.77 cm in the F70.04 and the F70.10 hybrids respectively. As far as, the genotype F30.09 has displayed the low development of necrotic lesions in sixth day (0.73 cm) (Table 2).

**Table 2.** Average lesion size (cm) on the midrib of *T. cacao* leaves from hybrids derived from F30 and F70 families.

| | Average necrosis length (cm) | | |
|---|---|---|---|
| Génotypes | Day 2 | Day 4 | Day 6 |
| **Parents** | | | |
| T79/467 | 1.04±0.02 c | 2.20±0.10 c | 3.07±0.36 e |
| SNK413 | 0.98±0.01 a | 2.37±0.02 c | 2.76±0.01 d |
| **F70** | | | |
| F70.01 | 0.23±0.12 a | 1.10±0.17 ab | 2.03±0.61 c |
| F70.02 | 0.33±0.15 a | 1.40±0.85 ab | 2.17±0.32 c |
| F70.03 | 0.27±0.06 a | 1.20±0.55 ab | 1.43±0.35 b |
| F70.04 | 0a | 0.33±0.53 a | 0.67±0.12 a |
| F70.05 | 0.30±0.17 a | 0.53±0.35 a | 0.73±0.25 a |
| F70.06 | 0.33±0.06 a | 1.00±1.68 ab | 1.60±0.72 b |
| F70.07 | 0.23±0.06 a | 0.63±0.26 a | 1.57±0.90 b |
| F70.08 | 0.13±0.15 a | 0.47±0.25 a | 1.27±0.49 ab |
| F70.09 | 0.30±0.20 a | 1.50±0.06 ab | 2.00±2.23 c |
| F70.10 | 0.27±0.12 a | 2.37±1.25 c | 2.77±1.66 cd |
| F30 | | | |
| F30.01 | 0.23±0.21 a | 1.33±0.64 ab | 1.93±1.10 b |
| F30.02 | 0.33±0.31 a | 1.00±0.80 ab | 1.83±1.01 b |
| F30.03 | 0.43±0.42 b | 1.77±1.14 b | 2.33±1.36 c |
| F30.04 | 0.20±0.17 a | 0.53±0.40 a | 1.33±0.57 ab |
| F30.05 | 0.43±0.25 b | 2.20±1.47 c | 2.63±1.58 d |
| F30.06 | 0.17±0.06 a | 1.60±0.44 b | 2.10±0.10 c |
| F30.07 | 0.30±1.73 a | 0.37±0.21 a | 1.00±0.70 ab |
| F30.08 | 0.23±0.12 a | 0.60±0.52 a | 1.07±0.40 ab |
| F30.09 | 0.13±0.15 a | 0.50±0.26 a | 0.73±0.25 a |
| F30.10 | 0.20±0.03 a | 0.53±0.35 a | 1.13±0.76 ab |

Values with the same letter in the same column and in the same family are not significant ($P < 0.05$) different. Values are means of 3 replicates.

In the F90 family, the hybrid F90.02 presented the largest lesion (7.43 cm) at day 6. This value remained greater compared to the value to its parents. The individual F95.13 also displayed the largest lesion in its family (5.30 cm); but this value was comprised between the values of its parents (Table 3).

*Heterosis*

At day 2, all hybrids of F70 family studied displayed a positive heterosis. This hybrid vigour disappeared at day 4only for F70.10 hybrid (+3.71). At day 6, all individuals displayed this vigour.

In the F30 family, all hybrids manifested a positive heterosis at day 2, 4 and 6, excepted F30.05 hybrid which lost this vigour at day 4 (table 4).

In F90 and F95 families, 2, 4 and 6 days after infection, all hybrids presented a positive heterosis excepted the individuals F90.02 and F95.13 which lose their vigour 4 and 6 days following infection respectively (Table 4).

*Variation of phenolic contents*

In the two families (F70 and F30), an increase of phenol compounds, of 72 (SNK413) to 216% (F70.07) was observed in all the genotypes studied after inoculation. After infection all hybrids displayed more phenolic compounds than the tolerant parent SNK413 (Fig. 1).Accumulation of phenolic compounds is less important in F90.01, F95.02 and F95.13 hybrids in biotic stress.

Meanwhile F90.03, F90.02, F95.01, F95.08 and F95.11 hybrids displayed more phenolic contents following infection by mycelium of *P. megakarya* (Fig. 1).

*POX and PPO activities*

In our study, infection induced an increase of the POX and PPO activities in all individuals of families (F70 and F30) excepted in F70.04 and F30.07 hybrids for POX activity, and in F70.09 and F70.10 hybrids for PPO activity.

**Table 3.** Average lesion size (cm) on the midrib of *T. cacao* leaves from hybrids derived from F90 and F95 families.

| | Average necrosis length (cm) | | |
|---|---|---|---|
| Génotypes | Day 2 | Day 4 | Day 6 |
| Parents | | | |
| T79/467 | 1.04 ±0.02 b | 2.20±0.10 cd | 3.07±0.32 d |
| ICS40 | 1.91 ±0.09 b | 3.41±0.01 e | 6.43±0.03 g |
| F90 | | | |
| F90.01 | 0.17±0.11 a | 0.80±0.30 a | 1.20±0.26 a |
| F90.02 | 0.20±0.10 a | 4.37±1.25 e | 7.43±2.18 e |
| F90.03 | 0.20±0.10 a | 2.00±0.50 c | 2.53±0.55 cd |
| F90.04 | 0.13±0.05 a | 2.80±0.91 d | 3.57±1.25 d |
| F90.05 | 0.13±0.08 a | 1.33±0.75 b | 3.20±1.15 d |
| F90.06 | 0.13±0.02 a | 0.43±0.23 a | 0.73±0.05 a |
| F90.07 | 0.20±0.10 a | 0.60±0.17 a | 1.13±0.15 a |
| F90.08 | 0.13±0.08 a | 0.50±0.20 a | 1.00±0.45 a |
| F90.09 | 0.13±0.05 a | 1.36±0.63 b | 1.86±0.90 b |
| F92.10 | 0.23±0.05 a | 0.57±0.38 a | 1.73±0.58 b |
| F90.11 | 0.27±0.15 a | 0.67±0.30 a | 2.50±0.87 cd |
| F90.12 | 0.20±0.10 a | 0.63±0.40 a | 1.37±0.32 ab |
| F95 | | | |
| F95.01 | 0.10±0.30 a | 1.27±0.61 de | 2.70±0.60 cd |
| F95.02 | 0.10±0.10 a | 0.40±0.17 a | 1.20±0.30 a |
| F95.03 | 0a | 0 a | 0.83±0.20 a |
| F95.04 | 0.10±0.03 a | 0.57±0.38 a | 1.47±0.50 ab |
| F95.05 | 0.13±0.05 a | 0.37±0.11 a | 1.13±0.32 a |
| F95.06 | 0.17±0.11 a | 0.53±0.32 a | 1.60±0.60 b |
| F95.07 | 0.13±0.11 a | 0.63±0.35 a | 4.50±0.50 e |
| F95.08 | 0.20±0.10 a | 1.47±0.45 b | 2.67±0.90 cd |
| F95.09 | 0.13±0.15 a | 0.70±0.40 a | 1.33±0.50 a |
| F95.10 | 0.13±0.05 a | 0.67±0.29 a | 1.97±0.40 b |
| F95.11 | 0.13±0.02 a | 0.97±0.30 ab | 1.80±0.95 b |
| F95.12 | 0.33±0.05 a | 0.73±0.50 a | 1.43±0.23 ab |
| F95.13 | 0.23±0.05 a | 2.43±0.67 cd | 5.30±1.38 f |
| F95.14 | 0.13±0.05 a | 0.80±0.27 a | 2.20±0.80 c |
| F95.15 | 0.17±0.15 a | 0.20±0.17 a | 0.60±0.10 a |

Values with the same letter in the same column and in the same family are not significant ($P < 0.05$) different. Values are means of 3 replicates.

The tolerant parentSNK413 presented the best POX activity (Fig. 2). The two hybrids F70.07 and F30.07 and the parent T79/467 displayed a great PPO activity in biotic stress (Fig. 3).

In F90 and F95 families, POX and PPO activities are also induced in infected leaves. Two hybrids distinguished themselves by the highest increase observed in infected condition: F95.01 hybrid for POX activity and F90.03 for PPO activity (Fig.2-3).

*Principal component analysis (PCA) and Correlation coefficients*

The two-dimensional plane formed by PC1 (74.17%) and PC2 (11.90%) components retained in total, 86.07% of the original variance. Principal components (PC1 and CP2), Characterized by necrosis, TPP, POX and PPO variables, showed that distribution of samples followed a pattern of four groups (Fig. 4).

In our trials, a significant negative correlation is noticed between the necrosis length and biochemical parameters ($P<0.01$) (Table 5). Besides, a significant positive correlation between all the studied biochemical parameters ($P<0.05$ and $P<0.01$) were registered.

**Table 4.** Heterosis values (%) by comparison of necrotic lesion size between parents and their progenies derived from four families.

| Genotypes | | Heterosis values (%) of lesion necrosis size | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 2 | Day 4 | Day 6 | | | Day 2 | Day 4 | Day 6 |
| F30 | F30.01 | -77.23 | -41.79 | -33.45 | F70 | F70.01 | -51.86 | -51.86 | -30 |
| | F30.02 | -67.33 | -56.24 | -36.9 | | F70.02 | -38.73 | -38.73 | -25.17 |
| | F30.03 | -57.43 | -22.54 | -19.66 | | F70.03 | -47.48 | -47.48 | -50.69 |
| | F30.04 | -80.2 | -76.81 | -54.14 | | F70.04 | -85.56 | -85.56 | -76.9 |
| | F30.05 | -57.43 | +7.22 | -9.62 | | F70.05 | -76.81 | -76.81 | -74.83 |
| | F30.06 | -83.17 | -29.98 | -27.59 | | F70.06 | -56.24 | -56.24 | -44.83 |
| | F30.07 | -0.99 | -83.81 | -65.52 | | F70.07 | -72.43 | -72.43 | -45.86 |
| | F30.08 | -77.23 | -73.74 | -63.45 | | F70.08 | -79.43 | -79.43 | -56.21 |
| | F30.09 | -87.13 | -78.12 | -74.83 | | F70.09 | -34.35 | -34.35 | -21.72 |
| | F30.10 | -80.2 | -76.81 | -61.03 | | F70.10 | -3.72 | +3.71 | -4.48 |
| F90 | F90.01 | -88.47 | -71.48 | -74.74 | F95 | F95.01 | -93.22 | -54.72 | -43.16 |
| | F90.02 | -8644 | +55.79 | +56.42 | | F95.02 | -93.22 | -85.74 | -74.74 |
| | F90.03 | -86.44 | -28.7 | -46.74 | | F95.03 | -100 | -100 | -82.53 |
| | F90.04 | -91.19 | -0.18 | -24.84 | | F95.04 | -93.22 | -79.68 | -69.05 |
| | F90.05 | -91.19 | -52.58 | -32.63 | | F95.05 | -91.19 | -86.81 | -76.21 |
| | F90.06 | -91.19 | -84.67 | -84.63 | | F95.06 | -88.47 | -81.11 | -66.32 |
| | F90.07 | -86.44 | -78.61 | -76.21 | | F95.07 | -91.19 | -77.54 | -5.26 |
| | F90.08 | -91.19 | -82.17 | -78.95 | | F95.08 | -86.44 | -47.59 | -43.79 |
| | F90.09 | -91.19 | -51.52 | -60.84 | | F95.09 | -91.19 | -75.04 | -72 |
| | F90.10 | -84.41 | -79.68 | -63.58 | | F95.10 | -91.19 | -76.11 | -58.53 |
| | F90.11 | -81.69 | -76.11 | -47.37 | | F95.11 | -91.19 | -65.42 | -62.11 |
| | F90.12 | -86.44 | -77.54 | -71.16 | | F95.12 | -77.63 | -73.98 | -69.89 |
| | | | | | | F95.13 | -84.41 | -13.37 | +11.58 |
| | | | | | | F95.14 | -91.19 | -71.48 | -53.68 |
| | | | | | | F95.15 | -88.47 | -92.87 | -87.37 |

*Estimation of heritability*

The values of heritability ($h^2$) have been determined according to the size of lesions, TPP, POX and PPO (Table 6). Concerning these character values obtained various between 0.14 and 0.74.

**Discussion**

The objective of this study was to analyse the heritability of the resistance to *P. megakarya* in cocoa by the evaluation of the necrotic size in healthy, wounded and inoculated leaves. The variation of phenolic compounds and antioxidant enzyme activities was also performed in these conditions.

**Table 5.**Correlation coefficients between length of necrosis and biochemical compounds (F70, F30, F90 and F95 populations).

| | Necrosis | PPT | POX | PPO |
|---|---|---|---|---|
| Necrosis | 1 | -0.731** | -0.573** | -0.619* |
| PPT | | 1 | 0.602* | 0.722** |
| POX | | | 1 | 0.682** |
| PPO | | | | 1 |

TPP : Total polyphenols ; POX : Peroxidase ; PPO; Polyphenoloxidase,

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

**Table 6.** Estimation of the narrow sense heritability ($h^2$) of resistance to *P. megakarya* in the F30, F70 F90 and F95 cocoa families

| | Reciprocal crossing | Value of heritability ($h^2$) | | | |
|---|---|---|---|---|---|
| | | Necrosis | PPT | POX | PPO |
| **1** | F30: (♀) SNK413 × (♂) T79/467 | 0.67 | 0.73 | 0.24 | 0.69 |
| | F70: (♀) T79/467 × (♂) SNK413 | 0.69 | 0.74 | 0.34 | 0.59 |
| **2** | F90: (♀) T79/467 × (♂) ICS40 | 0.65 | 0.56 | 0.14 | 0.41 |
| | F95: (♀) ICS40 x (♂) T79/467 | 0.58 | 0.60 | 0.21 | 0.52 |

Results of hand-pollination test were different according to the crosses used. The first cross displayed a loss and an average rate (16% and 39% for F70 and F30respectively) and the second cross shows15 and 12% success results for F90 and F95respectively. These results were similar to those found by Mossu (1990) in *T. cacao* L. and seem to be clone dependant and could be explained by genetic compatibility of the clones and the period of pollination.

The study shows considerable genetic variability among the individuals of cocoa for resistance to black pod disease.There was no significant difference between genotypes coming from reciprocal crosses when the lesion size on the main vein was evaluated.

This is in agreement with the investigations of Nyasse *et al.* (1995) after infection of discs of cocoa leaves with UPA134 x SNK64 and SNK64 x UPA164 by zoospores of *P. megakarya*.

The heterosis effect of each F70, F30, F90 and F95 families when comparing the development of necrosis, revealed high variability within both family. In fact, 100% of F70 hybrids, 100% of F30 individuals, 92% of F90 hybrids and 93% of F95 individuals presented a positive heterosis 6 days after inoculation, a testament to the hybrid vigour manifestation within these four families. Earlier studies of Ondobo *et al.* (2014) and Manga *et al.* (2016) have shownthat the differential response of cocoa hybrids further suggested that these was implied the presence of the additive and dominant gene effect in the transmission.

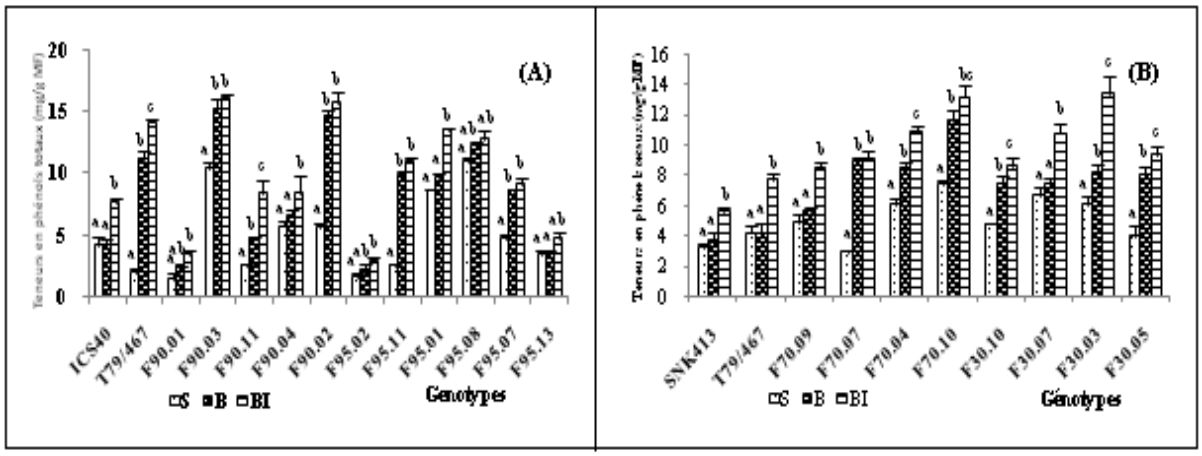


**Fig. 1.** Phenolic contents of cocoa clones (ICS40, T79/467 and SNK413) and their progenies (A: F90 and F95 families; B: F30 and F70 families) in different treatments (S:healthy; B: wounded; BI: inoculated). Values with the same letter in the same family are not significantly ($P < 0.05$) different. Values are means of 3 replicates.

In response to the infection, the host induces a cascade of pathogen inducible enzymes, which are implemented in defense against phytopathogens. In this study, enzyme activities of POX and PPO as well as account of TPP were found to differ significantly in resistant and susceptible genotypes.

However, it is suggested that accumulation of phenolic polymers and lignin in the infection region to inhibit the invasion of the pathogen might be a result of increased synthesis of phenolics (Djocgoue *et al*., 2007; Ondobo *et al*., 2013).

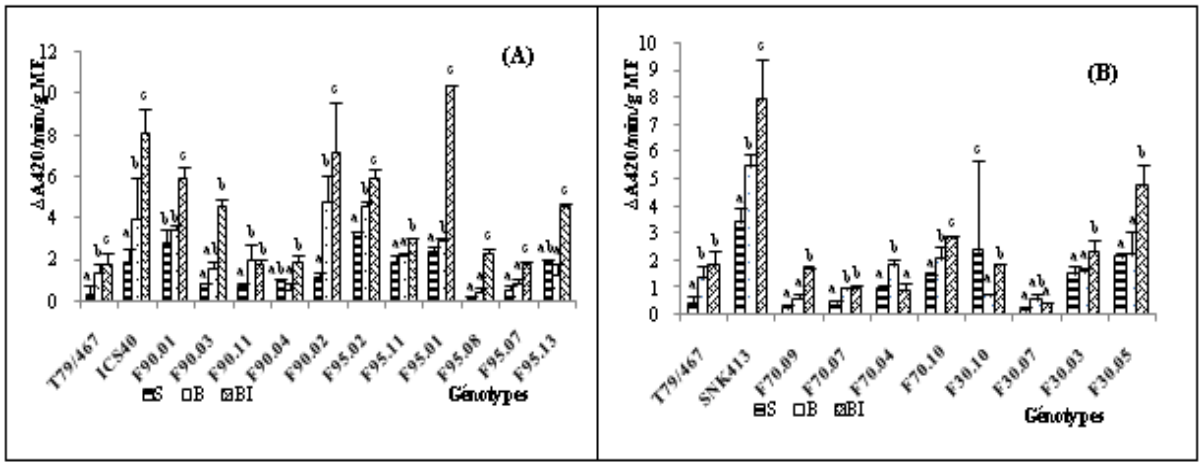


**Fig. 2.** Peroxidase activities in leaves of clones (ICS40, T79/467 and SNK413) and their progenies (A: F90 and F95 families; B: F30 and F70 families) in different treatments (S: healthy; B: wounded; BI: inoculated). Values with the same letter in the same family are not significantly ($P < 0.05$) different. Values are means of 3 replicates.

Likewise, activities of these two enzymes also increased significantly in wounded and inoculated leaves.PPO is also considered a general stress response to reactive oxygen species that are produced in excess amounts. However, the antioxidant potential of the PPO in the tissues of *Capsicum annuum* is not sufficient to block the oxidative damage in some cases (Koc and Ustum, 2012).

When PPO is inactivated under severe stress conditions, toxic properties of $H_2O_2$are inhibited by another antioxidant enzyme such as POX. The increase in PPO activity may contribute to the reduction of $H_2O_2$. These findings agree with of Ngadze *et al.* (2012), Effa *et al.* (2015) and Manga *et al.* (2016) observations who reported that tolerant genotype accumulates high amount of biochemical substances (TPP, TF, POX, PPO and PAL).

The importance of TPP, POX and PPO in defense mechanisms against *P. megakarya* cannot be underestimated. They also observed differences in responses among genotypes (genotype-dependence).

PCA of genotypes revealed a diagonal opposition between the necrosis length and biochemical parameters in the resistance and susceptibility genotypes. These experiments clearly show a positive correlation between amounts of TPP, POX and PPO ($P$<0.01).

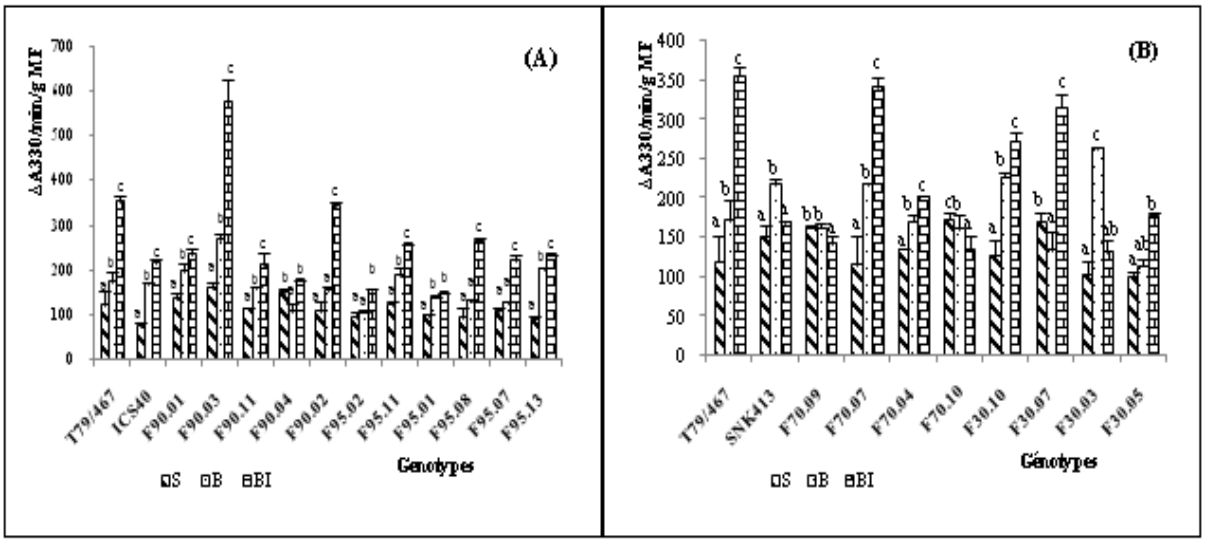


**Fig. 3.** Polyphenoloxidase activities in leaves of clones (ICS40, T79/467 and SNK413) and their progenies (A: F90 and F95 families; B: F30 and F70 families) in different treatments (S: healthy; B: wounded; BI: inoculated). Values with the same letter in the same family are not significantly (P < 0.05) different. Values are means of 3 replicates.

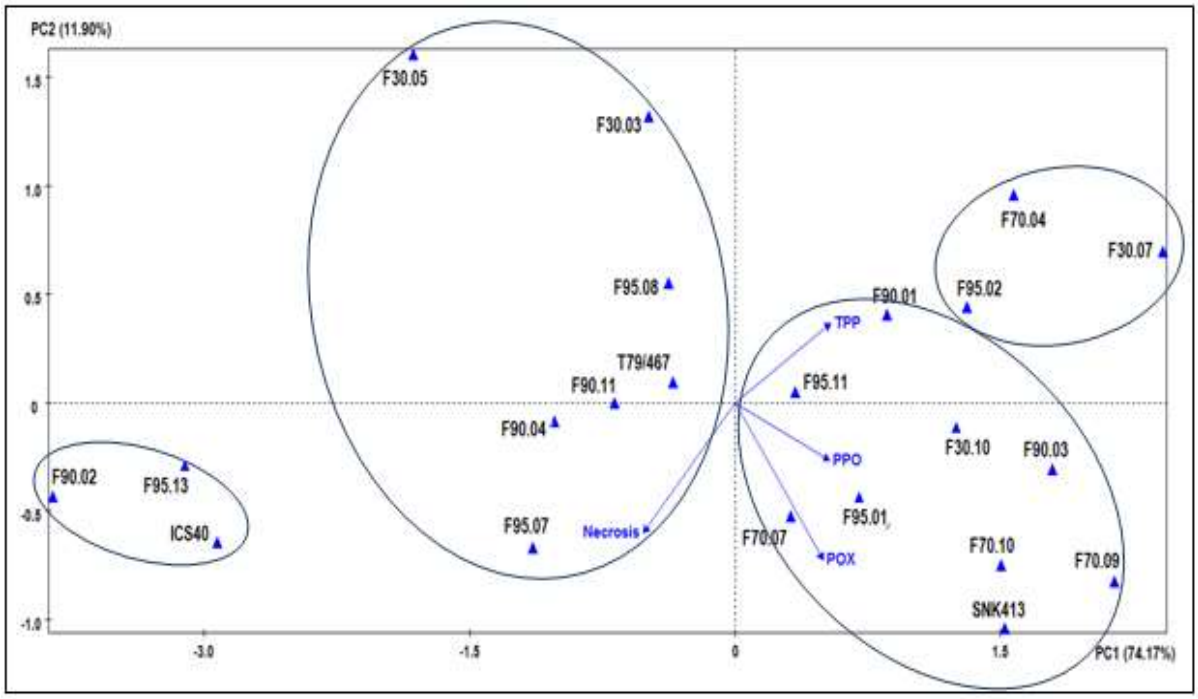


**Fig. 4.** Biplot graph with dispersion of 22 genotypes according to the principal components (PC1 and PC2) and vectors projection of the resistance parameter: length necrosis (necrosis), Total polyphenols (PPT); Peroxidase (POX); Polyphenoloxidase (PPO).

Values of their heritability in the four reciprocal crossings were not significant different.

Transmission of the character would thus seem to be governed by primarily additive effect suggesting a nuclear origin of the transmission of these characters. The same observations were described by Manga *et al.* (2016) in the same plant.

Of the varieties tested, F30.03, F30.07, F70.04, F70.07, F90.03, F95.01, F95.11 and F95.08 were identified as the most tolerance to pathogen attack like the best parent (SNK413). The parental clones used, showed a good suitability to the combinations.

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