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*Full Length Research Paper*

# Breeding of parental and tolerant hybrids of *Theobroma cacao* L. to *Phytophthora megakarya* Bras. and Griff.

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**The development of resistant varieties can be done according to a genetic approach. Estimation of genetic parameters for the quantitative traits of cocoa genotypes (*Theobroma cacao* L.) was made from a 5 × 5 diallel mating design. The objective of this study was to identify tolerant genotypes to black pod disease (BPD) through genetic analyzes. The inoculation of the leaves of parental cocoa genotypes and their offsprings with *Phytophthora megakarya* was performed in the nursery for two seasons (dry and rainy). Percentage success for crosses made using hand pollination was low (31.22%) and fair for grafting (60.68%). Observations made on necrosis length on the 2nd, 4th and 6th days after inoculation showed increasing sensibility of the clones to BPD in the order SNK 413<T 79/467<T 79/501<SNK 16<SCA 12. 84.37 and 76.04% of hybrid genotypes exhibited positive heterosis (hybrid vigor) in dry and rainy seasons respectively. Narrow sense ($h^2$) and broad-sense ($H^2$) heritabilities was high in two reciprocal crosses [F30 ($h^2$= 0.699 and $H^2$=0.624) and F70 ($h^2$= 0.601 and $H^2$=0.643)].**

**Key words:** *Theobroma cacao*, *Phytophthora megakarya*, tolerant, heterosis, heritability.

## INTRODUCTION

Cocoa is native to tropical America and is a cash crop in many tropical countries. It is an important source of foreign exchange, but several factors account for its low productivity. Some of these factors include aging plantations (Tijani, 2005), poor farming practices, presence of insects, presence of rodents and disease infestations especially black pod disease (BPD) (Ndoumbe-Nkeng, 2002). In Cameroon, approximately 80% of its production losses caused by BPD in areas where climatic conditions are favorable (Despréaux et al., 1988).

Presently, chemical control can be used to manage the disease, but the costs of pesticides is usually too expensive for African farmers. To ensure sustainable production, improvement programs are increasingly focused on developing hybrids that are tolerant to BPD but information on its genetic control has not been fully elucidated.

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The selection of cocoa genotypes with reduced susceptibility to black pod disease therefore remains a priority. Despite, many researches on cocoa (Tarjot, 1969; Blaha and Lotodé, 1976), selection for BPD resistant genotypes still remains futile. Many authors suggest the reaction of different *Phytophthora* spp. stem from partial resistance, probably polygenic (Partiot, 1975; Blaha and Lotodé, 1977). Different plant material evaluation methods have been proven (Blaha, 1974). This hypothesis was eventually disproved when experimental evidences showed that the interaction between the host followed the principles of gene-for-gene concept (Brading et al., 2002; Kema et al., 2002). Given that different types of gene actions are important in different crosses, the breeding strategy for developing a desirable genotype should be based on the gene action involved in that particular cross (Manga et al., 2016).

The hybridization technique for cocoa based on vigor, precocity, productivity and disease resistance, has helped to obtain improved genotypes (Eskes and Lanaud, 1997; Djocgoue et al., 2006). This hybridization is usually accompanied a heterotic effect. Heterosis is characterized by faster growth, better vegetative and generative development, better performance and improved tolerance to adverse environmental conditions. Hybrids are characterized by high productivity and environmental adaptability is mainly due to the non-additive gene effect of the parental genotypes (Tahi et al., 2000; Djocgoue et al., 2006; Manga et al., 2016). For this reason, the use of heritability is an effective genetic parameter for selection of quantitative traits in a determined reproductive system. Nyasse et al. (1995) and Djocgoue et al. (2011) reported that additive gene effects were important for transmission of character length of necrosis (tolerance gene at *P. megakarya*). Effa et al. (2015) and Manga et al. (2016) showed that selection based on family performance or progeny test should be more effective. Heritability tends to be only moderate (Simon et al., 1998), but progress in breeding for resistance may still be possible.

Studies on the analysis of data collected from an experiment involving diallel mating design showed that resistance to BPD is an heritable trait, and it is controlled by polygenes and additive gene effect (Djocgoue et al., 2006). Therefore it would be possible to develop cocoa genotypes that would possess genes that are tolerant to BPD. The objective of the present work was to identify hybrids of *T. cacao* that are tolerant to BPD.

## MATERIALS AND METHODS

### Cocoa plant and *P. megakarya* Oomycete material

This study was carried out on the experimental farms of SODECAO (Cameroon Cocoa Development Corporation) station in Mengang. Five parental clones of *T. cacao* and their progenies used in this study are listed in Table 1. These parents were crossed using 5×5 full diallel mating design. The use of the five parents produced twenty different families. But in this study, only eight families made up of 8 to 10 individuals each according to their reaction to BPD have been selected.

A strain «*Lebdi*» of *P. megakarya* obtained from the Central laboratory of Phytopathology at the IRAD (Research Institute for Agricultural Development) Nkolbisson (central region, Cameroon). It was isolated in from a cocoa plantation of Leboudi (central region, Cameroon). The fungus maintained its aggressiveness by culturing on V8 agar medium, and the isolate is inoculated onto cocoa pods.

### Hand-pollination and grafting

Crossing using hand-pollination techniques (Cilas, 1991) was conducted at the field in March and May, 2012. Seeds from the pods harvested from the experimental farms were sown in the nursery and 1086 hybrids plants were obtained. The parents were grafted in the nursery using bud wood. This vegetative-propagated (grafting) was done on non-specific young cocoa plantlets. This two techniques (hand-pollination and grafting) study was carried out at the SODECAO station at Mengang.

### Leaf inoculation

The pathogenicity test was carried out in the nursery on leaves scarified along the midrib as described by Djocgoue et al. (2006) and Ondobo et al. (2014). The leaf test is an artificial inoculation method that can be used to assess the level of resistance in the genotypes. The inner surface of leaves was sterilized with ethanol 70%. Agar discs (6 mm diameter) cut from 5-day-old fungal and straminopilous isolates (oomycete) were laid on the midrib after creating wounds with a sterilized razor blade. The scars were then covered with cotton that had been immersed in sterilized water. The necrosis length was measured using a graduated ruler. The necrosis length was measured by a graduated ruler every two days after inoculation. The experiment was repeated thrice.

### Estimation of the heterosis and heritability

The estimate of mid-parent heterosis was computed using the formula from Zahour (1992):

$$\mathrm{HF}(\%) = \frac{\mathrm{F1} - \frac{\mathrm{P1} + \mathrm{P2}}{2}}{\frac{\mathrm{P1} + \mathrm{P2}}{2}} \times 100$$

Where: HF (%) = Mid-parent heterosis in percent; F1 = Means of hybrid genotype; P1 and P2 = Means of the two parents.

For necrosis length, heritabilities were estimated according to Falconer and Mackay (1996) following the formulas:

1. Broad-sense heritability

$$H^2 = \frac{V_G}{V_P} = \frac{V_G}{V_G + V_E}$$

2. Narrow sense heritability:

$$h^2 = \frac{V_A}{V_P} = \frac{V_A}{V_G + V_E}$$

Where: $V_G$ = Genetic variance; $V_P$ = Phenotypic variance; $V_A$ = Additive genetic variance; $V_E$ = Environmental variance.

**Table 1.** Origin and sensibilities of cocoa parental clones and their progenies of cocoa.

| S/N | Parental clones/families | Origin | Collection | Group |
|---|---|---|---|---|
| 1 | SNK 16 | Cameroon | Nkoemvone | Trinitario |
| 2 | SNK 413 | Cameroon | Nkoemvone | Trinitario |
| 3 | T 79/467 | Ghana | Tafo | Forastero |
| 4 | T 79/501 | Ghana | Tafo | Forastero |
| 5 | SCA 12 | Equateur | - | Forastero |
| 6 | F10: SCA 12 × T 79/467 | Cameroon | Mengang | Forastero x Forastero |
| | F80: T 79/467 × SCA 12 | Cameroon | Mengang | Forastero x Forastero |
| 7 | F15: T 79/501 × SCA 12 | Cameroon | Mengang | Forastero x Forastero |
| | F61: SCA 12 × T 79/501 | Cameroon | Mengang | Forastero x Forastero |
| 8 | F16: SNK 16 × T 79/501 | Cameroon | Mengang | Trinitario x Forastero |
| | F79: T 79/501 × SNK 16 | Cameroon | Mengang | Forastero x Trinitario |
| 9 | F30: SNK 413 × T 79/467 | Cameroon | Mengang | Trinitario x Forastero |
| | F70: T 79/467 × SNK 413 | Cameroon | Mengang | Forastero x Trinitario |

**Total phenolic compound contents**

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

**Total soluble amino acid and total soluble sugar contents**

Amino acid contents were determined by using ninhydrine method of Yemm and Cocking (1955) with slight modifications. The incubation mixture containing 100 mL of the ethanol extract, 1 mL of 80% ethanol, 1 mL of 0.2 M citrate buffer (pH 5) and 2 mL of acetonic ninhydrin solution (1% ninhydrin and 0.006% KCN in acetone) was incubated for 15 min at 100°C. The mixture was cooled for 5 min under tap water before adding 8 mL of distilled water. The absorbance of the purple product was recorded at 570 nm (Hitachi spectrometer U-200). Glycine equivalents were calculated from a standard curve obtained with pure analytical grade glycine.

For carbohydrate determination, proteins were removed from the ethanolic extract after treatment with basic lead acetate. The carbohydrate extracts were then determined by using anthron method: 1mL of the extract was incubated in 5 mL of anthron solution (0.12 g anthron in 100 mL 6.5 M $H_2SO_4$) at 90°C for 10 min. The absorbance of the green product was measured at 630 nm. Results were expressed in µg eq. glucose by reference to the standard.

**Proline contents**

Proline contents were determined spectrophotometrically (Bates et al., 1973). Acid-ninhydrin was prepared by heating 0.7 g ninhydrin in 15 mL glacial acetic acid and 10 mL of 6 M phosphoric acid, with agitation till dissolution and stored at 4°C. Approximately 0.3 mg of plant material was homogenized in 8 mL of 3% aqueous sulfosalicylic acid and the homogenate filtered through Whatman No2 filter paper. 2 mL of filtrate was reacted with 2 mL acid-ninhydrin and 1.5 mL of glacial acetic acid in a test tube for 1 h at 100°C, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene, mixed vigorously with a test tube stirrer for 20 s. The chromophore containing toluene dissolved in the aqueous phase, warmed to room temperature and the absorbance read at 520 nm (Hitachi spectrometer U-200) using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows: [(µg proline/mL × mL toluene) / 115.5 µg/µmole]/[(g sample)/5] = µmoles proline/g of fresh weight material.

**GABA contents**

The GABA content was determined by following the procedure of Baum et al. (1996) with a minor modification. Ground cocoa leaves (200 mg) were added to a solution (800 mL) of methanol: chloroform: water mixture (12:5:3, volume basis) in a centrifuge tube. The tube was vortexed and then centrifuged at 12,000 g at 4°C for 15 min. The supernatant was collected in a flask and the residue was extracted again with a chloroform: water (3:5, v/v) solution (800 mL). The second supernatant was combined with the first supernatant. The collected sample was dried and then re-dissolved in water. The sample was then filtered through a 0.45 mm filter and the GABA content was measured by an amino acid analysis system (Waters, Milford, MA) after 6-aminoquioly-N-hydroxysuccinimidyl carbonate (AQC) derivatization.

**Statistical analysis**

Data presented are the means ± SE of three independent experiments. Analysis of variance (ANOVA) and the Tukey's test were performed using SPSS version 20.0. Principal component analysis (PCA) was used to describe the variability of necrosis length data. This analysis was performed with SPAD version 5.5.

## RESULTS

### Hand-pollination and grafting

Table 2 presents the results obtained from hand-

**Table 2.** Percentage of success of hand pollination of different hybridizations.

| S/N | Famillies | Reciprocal crossing | Tests | Successful | % of successful | Observation |
|---|---|---|---|---|---|---|
| 1 | F10 | (♀) SCA 12 × (♂) T 79/467 | 105 | 22 | 20.95 | Viable |
| | F80 | (♀) T 79/467 × (♂) SCA 12 | 108 | 25 | 23.14 | Viable |
| 2 | F15 | (♀) T 79/501 × (♂) SCA 12 | 105 | 43 | 40.95 | Viable |
| | F61 | (♀) SCA 12 × (♂) T 79/501 | 90 | 33 | 36.66 | Viable |
| 3 | F16 | (♀) SNK 16 × (♂) T 79/501 | 102 | 42 | 41.17 | Viable |
| | F79 | (♀) T 79/501 × (♂) SNK 16 | 100 | 31 | 31 | Viable |
| 4 | F30 | (♀) SNK 413 × (♂) T 79/467 | 92 | 36 | 39.13 | Viable |
| | F70 | (♀) T 79/467 × (♂) SNK 413 | 110 | 18 | 16 | Viable |
| | Total | | **812** | **250** | **31.12** | |

**Table 3.** Percentage of successful transplants parental clones.

| Parents | Test graft | Successfull graft | Percent of successful |
|---|---|---|---|
| T 79/501 | 60 | 48 | 80 |
| T 79/467 | 60 | 25 | 41.66 |
| SNK 16 | 60 | 32 | 53.33 |
| SNK 413 | 60 | 44 | 73.33 |
| SCA 12 | 60 | 33 | 55 |
| **Total** | **240** | **148** | **60.68** |

pollination. The overall rate of success was 31.12%, with F10, F80 and F70 families having a lesser success of 16, 20.95 and 23.14% respectively. On the other hand, F15 (40.95%) and F16 (41.17%) families had the best percentage. The viability of all the seeds from reciprocal crosses showed the compatibilities of clones. However, after obtaining the hybrid plantlets four parental clones were obtained by grafting. The success rates varied between 41.66 and 80% (Table 2).

## Development of the lesion

The rate of development of infection was genotype dependent. In all trials, there was a highly significant variation between the reactions of parental clones and their progenies to BPD (Table 3). On 2$^{nd}$ day after infection, necrotic lesions were observed in all parental and almost all hybrid genotypes, except for F15.02, F61.07 and F70.04 in the dry season (ds) and F15.08 and F70.08 genotypes in the rainy season (rs) (Table 4). From the fourth day, the necrosis evolved regularly in all the inoculated genotypes till the sixth day (Table 4). On the 4$^{th}$ day after infection, lowest necrotic lesions was recorded in 100 and 100% individuals of F10, 100 and 87.5% for F15, 100 and 62.5% for F30 in ds and rs respectively (Table 4). On the 6$^{th}$ day after infection by mycelium, the highest necrosis length was recorded for genotypes F79.01 (7.36±0.63 cm), F79.03 (5.35±0.77 cm), F61.04 (5.27±1.67 cm), and F79.02 (5.03±0.50 cm) in the ds, and subsequently in the rs, F79.05 (6.30±0.17 cm), F79.07 (6.42±0.22 cm), F30.07 (6.63±0.38 cm) F79.08 (6.15±0.12 cm) and F30.02 (5.77±0.39 cm) genotypes also had the highest necrosis length (Table 4).

## Heterosis and heritability

The percentage of genotypes with positive heterosis was higher in the ds (84.37%) than in rs (76.04%). Reciprocal families F10/F80 (best pair) had a hybrid vigor of 100 and 83.33% in dry and rainy seasons, respectively (Table 5). On the other hand, F16/F79 families had the lowest heterosis in ds (52.08%) and rs (68.75%) (Table 5).

The heritabilities (narrow-sense and broad-sense) for necrosis length were obtained for the eight families from reciprocal crosses. Concerning narrow sense heritability, the value obtained in the F10 family ($h^2$ = 0.229) was not significant to that obtained in F80 ($h^2$ = 0.306) (Table 6). However, such observations were made in reciprocal families F15-F61, F16-F79 and F30-F70. These values are coupled, respectively 0.212-0.203, 0.278-0.132 and 0.699-0.601 (Table 6). On the other hand, the broad-sense heritability showed that reciprocal families F10 ($H^2$ = 0.329) and F80 ($H^2$ = 0.396), F15 ($H^2$ = 0.156) and F61 ($H^2$ = 0.232), F16 ($H^2$ = 0.375) and F79 ($H^2$ = 0.313) had

**Table 4.** Length of necrosis of the parents and their offsprings in the nursery.

| Genotypes | | Length necrosis in cm (dry season) | | | Length necrosis in cm (rainy season) | | |
|---|---|---|---|---|---|---|---|
| | | Day 2 | Day 4 | Day 6 | Day 2 | Day 4 | Day 6 |
| **Parents** | **SCA 12** | **1.42±0.47$^{b}$** | **3.45±1.02$^{d}$** | **4.90±0.78$^{d}$** | **1.20±0.15$^{ab}$** | **2.33±0.29$^{b}$** | **5.85±1.05e** |
| | **T 79/467** | **1.04±0.02$^{b}$** | **2.20±0.19$^{c}$** | **3.07±0.11$^{c}$** | **1.47±0.22$^{b}$** | **2.31±0.23$^{b}$** | **4.36±0.42$^{d}$** |
| **F10** | F10.01 | 0.17±0.05$^{a}$ | 0.37±0.05$^{a}$ | 2.67±0.46$^{bc}$ | 0.69±0.15$^{a}$ | 2.33±0.17$^{b}$ | 3.81±1.21$^{c}$ |
| | F10.02 | 0.23±0.05$^{a}$ | 0.54±0.05$^{a}$ | 2.10±0.36$^{b}$ | 0.23±0.15$^{a}$ | 0.47±0.05$^{a}$ | 1.43±0.05$^{a}$ |
| | F10.03 | 0.27±0.25$^{a}$ | 0.70±0.20$^{a}$ | 1.73±0.58$^{a}$ | 0.62±0.12$^{a}$ | 1.27±0.20$^{a}$ | 2.07±0.61$^{b}$ |
| | F10.04 | 0.10±0.17$^{a}$ | 0.60±0.26$^{a}$ | 2.13±0.89$^{b}$ | 0.60±0.17$^{a}$ | 1.36±0.14$^{a}$ | 4.63±1.16$^{d}$ |
| | F10.05 | 0.30±0.17$^{a}$ | 0.75±0.32$^{a}$ | 1.36±0.40$^{a}$ | 1.13±0.27$^{ab}$ | 1.63±0.40$^{a}$ | 3.90±0.17$^{c}$ |
| | F10.06 | 0.23±0.05$^{a}$ | 0.70±0.10$^{a}$ | 1.40±0.10$^{a}$ | 0.30±0.10$^{a}$ | 0.63±0.15$^{a}$ | 1.23±0.20$^{a}$ |
| | F10.07 | 0.17±0.05$^{a}$ | 0.53±0.35$^{a}$ | 1.07±0.11$^{a}$ | 0.57±0.11$^{a}$ | 1.47±0.21$^{a}$ | 2.88±0.44$^{b}$ |
| | F10.08 | 0.10±0.03$^{a}$ | 0.93±0.23$^{a}$ | 2.27±0.92$^{b}$ | 0.80±0.23$^{a}$ | 1.63±0.28$^{a}$ | 2.97±0.28$^{b}$ |
| **F80** | F80.01 | 0.17±0.05$^{a}$ | 0.70±0.26$^{a}$ | 1.57±0.55$^{a}$ | 0.97±0.28$^{a}$ | 3.83±0.67$^{c}$ | 4.73±1.33$^{d}$ |
| | F80.02 | 0.17±0.07$^{a}$ | 0.53±0.23$^{a}$ | 0.90±0.10$^{a}$ | 1.26±0.22$^{ab}$ | 4.00±1.20$^{cd}$ | 4.97±1.66$^{d}$ |
| | F80.03 | 0.23±0.15$^{a}$ | 0.53±0.20$^{a}$ | 1.73±0.55$^{a}$ | 0.53±0.05$^{a}$ | 1.20±0.16$^{a}$ | 2.36±0.29$^{b}$ |
| | F80.04 | 0.20±0.11$^{a}$ | 1.60±0.36$^{b}$ | 2.30±0.60$^{b}$ | 2.05±0.81$^{c}$ | 3.16±1.23$^{c}$ | 4.40±0.37$^{d}$ |
| | F80.05 | 0.23±0.05$^{a}$ | 0.90±0.45$^{a}$ | 1.93±0.60$^{ab}$ | 2.12±0.23$^{c}$ | 2.63±0.35$^{b}$ | 3.91±1.03$^{c}$ |
| | F80.06 | 0.27±0.05$^{a}$ | 0.43±0.11$^{a}$ | 1.00±0.30$^{a}$ | 0.83±0.47$^{a}$ | 1.69±0.36$^{a}$ | 2.95±0.66$^{b}$ |
| | F80.07 | 0.13±0.05$^{a}$ | 0.63±0.15$^{a}$ | 1.03±0.15$^{a}$ | 0.67±0.27$^{a}$ | 1.47±0.81$^{a}$ | 2.67±0.58$^{b}$ |
| | F80.08 | 0.67±0.27$^{a}$ | 1.47±0.81$^{b}$ | 2.67±0.58$^{bc}$ | 1.37±0.09$^{b}$ | 1.73±0.23$^{a}$ | 2.53±0.44$^{b}$ |
| **Parents** | **SCA 12** | 1.42±0.47$^{b}$ | 3.45±1.02$^{bc}$ | 4.90±0.78$^{cd}$ | 1.20±0.15$^{b}$ | 2.33±0.29$^{b}$ | 5.85±1.05e |
| | **T 79/501** | 0.72±0.06$^{a}$ | 2.10±0.17$^{ab}$ | 3.67±0.48$^{bc}$ | 1.56±0.31$^{b}$ | 2.13±0.20$^{b}$ | 4.13±0.38$^{c}$ |
| **F15** | F15.01 | 0.60±0.17$^{a}$ | 2.20±0.79$^{ab}$ | 2.80±0.92$^{ab}$ | 0.63±0.11$^{a}$ | 1.20±0.17$^{a}$ | 3.83±0.28$^{c}$ |
| | F15.02 | 0$^{a}$ | 2.53±0.46$^{ab}$ | 3.00±0.08$^{b}$ | 0.60±0.17$^{a}$ | 1.14±0.25$^{a}$ | 2.77±0.20$^{b}$ |
| | F15.03 | 0.50±0.10$^{a}$ | 1.93±0.12$^{a}$ | 2.13±0.23$^{a}$ | 0.67±0.05$^{a}$ | 1.33±0.11$^{a}$ | 2.93±0.15$^{b}$ |
| | F15.04 | 0.47±0.21$^{a}$ | 2.83±0.15$^{b}$ | 3.33±0.49$^{b}$ | 0.70±0.15$^{a}$ | 1.00±0.05$^{a}$ | 3.25±0.25$^{c}$ |
| | F15.05 | 0.60±0.10$^{a}$ | 1.10±0.26$^{a}$ | 1.34±0.35$^{a}$ | 1.03±0.20$^{a}$ | 2.95±0.15$^{b}$ | 4.64±0.25$^{d}$ |
| | F15.06 | 0.40±0.16$^{a}$ | 1.30±0.07$^{a}$ | 1.87±0.12$^{a}$ | 0.56±0.16$^{a}$ | 0.77±0.21$^{a}$ | 1.97±0.35$^{a}$ |
| | F15.07 | 0.20±0.05$^{a}$ | 1.50±0.52$^{a}$ | 2.46±0.06$^{ab}$ | 0.43±0.23$^{a}$ | 0.63±0.36$^{a}$ | 1.58±0.05$^{a}$ |
| | F15.08 | 0.53±0.12$^{a}$ | 2.40±0.35$^{ab}$ | 4.23±0.58$^{c}$ | 0$^{a}$ | 1.47±0.34$^{a}$ | 2.63±0.23$^{b}$ |
| **F61** | F61.01 | 0.67±0.15$^{a}$ | 1.37±0.47$^{a}$ | 1.70±0.35$^{a}$ | 0.73±0.15$^{a}$ | 2.10±0.40$^{b}$ | 3.40±0.28$^{c}$ |
| | F61.02 | 0.50±0.17$^{a}$ | 1.97±0.25$^{a}$ | 2.83±0.42$^{ab}$ | 0.67±0.20$^{a}$ | 2.46±0.51$^{b}$ | 4.87±0.17$^{d}$ |
| | F61.03 | 0.53±0.06$^{a}$ | 1.57±0.15$^{a}$ | 2.06±0.21$^{a}$ | 0.63±0.11$^{a}$ | 1.30±0.16$^{a}$ | 2.57±0.51$^{b}$ |
| | F61.04 | 0.72±0.12$^{a}$ | 4.80±0.69$^{cd}$ | 5.27±1.67$^{d}$ | 0.66±0.20$^{a}$ | 1.78±0.31$^{ab}$ | 2.73±0.30$^{b}$ |
| | F61.05 | 0.75±0.15$^{a}$ | 3.00±0.87$^{b}$ | 3.33±0.99$^{b}$ | 0.70±0.12$^{a}$ | 2.50±0.29$^{b}$ | 3.90±0.30$^{c}$ |
| | F61.06 | 0.67±0.15$^{a}$ | 1.17±0.57$^{a}$ | 1.50±0.44$^{a}$ | 0.90±0.28$^{a}$ | 2.84±0.17$^{b}$ | 5.34±0.23e |
| | F61.07 | 0$^{a}$ | 2.53±0.46$^{ab}$ | 3.03±0.31$^{b}$ | 1.03±0.21$^{a}$ | 2.93±0.23$^{b}$ | 4.00±0.25$^{c}$ |
| | F61.08 | 0.73±0.12$^{a}$ | 2.20±0.61$^{ab}$ | 3.80±0.20$^{bc}$ | 0.77±0.17$^{a}$ | 2.50±0.23$^{b}$ | 3.70±0.11$^{c}$ |
| **Parents** | **SNK 16** | 2.24±0.22$^{c}$ | 3.09±0.12$^{bc}$ | 4.09±0.44$^{c}$ | 1.06±0.21$^{a}$ | 2.97±0.06$^{b}$ | 5.27±0.44$^{d}$ |
| | **T 79/501** | 0.72±0.06$^{a}$ | 2.10±0.17$^{a}$ | 3.67±0.48$^{bc}$ | 1.56±0.31$^{b}$ | 2.13±0.20$^{b}$ | 4.13±0.38$^{c}$ |
| **F16** | F16.01 | 1.76±0.32$^{b}$ | 3.33±0.04$^{bc}$ | 4.16±0.52$^{c}$ | 2.07±0.21$^{c}$ | 3.17±0.35$^{bc}$ | 4.23±0.37$^{c}$ |
| | F16.02 | 1.33±0.40$^{ab}$ | 2.46±0.41$^{b}$ | 3.32±0.73$^{b}$ | 0.60±0.10$^{a}$ | 1.40±0.17$^{a}$ | 3.31±0.25$^{b}$ |
| | F16.03 | 1.93±0.28$^{bc}$ | 4.80±0.69$^{d}$ | 5.18±0.04$^{cd}$ | 0.67±0.06$^{a}$ | 3.20±0.26$^{bc}$ | 4.13±0.21$^{c}$ |
| | F16.04 | 1.66±0.45$^{b}$ | 3.50±1.00$^{c}$ | 4.46±0.27$^{c}$ | 0.60±0.13$^{a}$ | 1.53±0.15$^{a}$ | 2.63±0.12$^{a}$ |
| | F16.05 | 1.63±0.65$^{b}$ | 3.20±0.72$^{bc}$ | 3.52±0.86$^{bc}$ | 0.83±0.12$^{a}$ | 1.83±0.14$^{a}$ | 3.44±0.20$^{b}$ |
| | F16.06 | 1.20±0.52$^{ab}$ | 2.13±0.11$^{a}$ | 2.90±0.52$^{b}$ | 1.36±0.23$^{ab}$ | 4.73±0.25$^{cd}$ | 6.20±0.26e |
| | F16.07 | 1.56±0.11$^{b}$ | 2.86±0.23$^{b}$ | 3.63±0.40$^{bc}$ | 0.83±0.23$^{a}$ | 2.53±0.21$^{b}$ | 4.70±0.20$^{c}$ |
| | F16.08 | 0.73±0.05$^{a}$ | 2.00±0.51$^{a}$ | 2.46±0.25$^{a}$ | 1.22±0.68$^{ab}$ | 1.43±0.46$^{a}$ | 2.48±0.84$^{a}$ |
| **F79** | F79.01 | 1.23±0.25$^{ab}$ | 5.83±0.76e | 7.36±0.63e | 0.70±0.14$^{a}$ | 1.53±0.25$^{a}$ | 2.90±0.17$^{a}$ |

**Table 4.**Contd.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | F79.02 | 1.10±0.10$^{a}$ | 4.23±0.25$^{d}$ | 5.03±0.50$^{cd}$ | 0.90±0.17$^{a}$ | 3.26±0.25$^{c}$ | 5.03±0.15$^{d}$ |
| | F79.03 | 2.60±0.40$^{c}$ | 3.63±0.70$^{c}$ | 5.35±0.77$^{cd}$ | 0.78±0.06$^{a}$ | 1.33±0.15$^{a}$ | 3.30±0.30$^{b}$ |
| | F79.04 | 0.63±0.11$^{a}$ | 1.00±0.06$^{a}$ | 2.00±0.17$^{a}$ | 0.80±0.20$^{a}$ | 2.94±0.20$^{b}$ | 4.57±0.31$^{c}$ |
| | F79.05 | 1.83±0.23$^{b}$ | 3.33±0.66$^{bc}$ | 4.12±0.06$^{c}$ | 1.20±0.26$^{ab}$ | 4.37±0.23$^{cd}$ | 6.30±0.17e |
| | F79.06 | 0.70±0.04$^{a}$ | 1.76±0.49$^{a}$ | 2.46±0.30$^{a}$ | 0.87±0.15$^{a}$ | 3.15±0.32$^{bc}$ | 5.00±0.50$^{d}$ |
| | F79.07 | 1.46±0.72$^{b}$ | 2.45±0.89$^{b}$ | 3.83±0.56$^{bc}$ | 1.26±0.21$^{ab}$ | 4.70±0.26$^{cd}$ | 6.42±0.22e |
| | F79.08 | 0.70±0.09$^{a}$ | 1.76±0.49$^{a}$ | 1.46±0.30$^{a}$ | 0.80±0.10$^{a}$ | 3.03±0.45$^{bc}$ | 6.15±0.12e |
| **Parents** | **T 79/467** | 1.04±0.02$^{ab}$ | 2.20±0.19$^{c}$ | 3.07±0.11$^{c}$ | 1.47±0.33$^{b}$ | 2.31±0.05$^{b}$ | 4.36±0.12$^{d}$ |
| | **SNK 413** | 0.98±0.01$^{a}$ | 2.37±0.02$^{c}$ | 2.76±0.01$^{c}$ | 1.16±0.31$^{b}$ | 2.06±0.16$^{b}$ | 3.70±0.46$^{c}$ |
| | F30.01 | 0.23±0.08$^{a}$ | 1.33±0.64$^{b}$ | 1.93±0.10$^{b}$ | 0.55±0.15$^{a}$ | 1.23±0.25$^{ab}$ | 2.21±0.25$^{b}$ |
| | F30.02 | 0.33±0.01$^{a}$ | 1.00±0.80$^{a}$ | 1.80±0.01$^{b}$ | 0.50±0.12$^{a}$ | 2.88±0.50$^{b}$ | 5.77±0.39e |
| | F30.03 | 0.43±0.12$^{a}$ | 1.77±0.14$^{b}$ | 2.34±0.36$^{b}$ | 0.23±0.11$^{a}$ | 1.33±0.64$^{ab}$ | 1.92±0.13$^{a}$ |
| **F30** | F30.04 | 0.45±0.15$^{a}$ | 2.20±0.47$^{c}$ | 2.60±0.58$^{b}$ | 0.80±0.17$^{a}$ | 2.70±0.20$^{b}$ | 4.15±0.42$^{d}$ |
| | F30.05 | 0.17±0.06$^{a}$ | 1.60±0.44$^{b}$ | 2.10±0.10$^{b}$ | 1.33±0.25$^{b}$ | 2.26±0.35$^{b}$ | 3.33±0.31$^{c}$ |
| | F30.06 | 0.26±0.12$^{a}$ | 0.62±0.12$^{a}$ | 1.07±0.40$^{a}$ | 0.23±0.02$^{a}$ | 0.60±0.12$^{a}$ | 1.07±0.40$^{a}$ |
| | F30.07 | 0.13±0.05$^{a}$ | 0.50±0.06$^{a}$ | 0.72±0.25$^{a}$ | 0.63±0.15$^{a}$ | 2.43±0.30$^{b}$ | 6.63±0.38f |
| | F30.08 | 0.20±0.09$^{a}$ | 0.53±0.15$^{a}$ | 1.13±0.76$^{a}$ | 1.16±0.31$^{b}$ | 2.06±0.16$^{b}$ | 3.70±0.46$^{c}$ |
| | F70.01 | 0.23±0.06$^{a}$ | 1.10±0.17$^{a}$ | 2.03±0.61$^{b}$ | 0.50±0.13$^{a}$ | 0.86±0.25$^{a}$ | 2.26±0.19$^{b}$ |
| | F70.02 | 0.33±0.15$^{a}$ | 1.41±0.85$^{b}$ | 2.17±0.32$^{b}$ | 0.50±0.20$^{a}$ | 2.73±0.26$^{b}$ | 3.70±0.12$^{c}$ |
| | F70.03 | 0.27±0.03$^{a}$ | 1.20±0.55$^{b}$ | 1.43±0.35$^{a}$ | 0.33±0.10$^{a}$ | 2.80±0.21$^{b}$ | 3.57±0.24$^{c}$ |
| **F70** | F70.04 | 0$^{a}$ | 0.32±0.03$^{a}$ | 0.68±0.12$^{a}$ | 0.67±0.06$^{a}$ | 2.53±0.72$^{b}$ | 4.55±0.64$^{d}$ |
| | F70.05 | 0.30±0.07$^{a}$ | 0.54±0.05$^{a}$ | 0.73±0.25$^{a}$ | 0.75±0.17$^{a}$ | 2.93±0.52$^{b}$ | 4.73±0.46$^{d}$ |
| | F70.06 | 0.34±0.06$^{a}$ | 1.00±0.68$^{a}$ | 1.60±0.72$^{b}$ | 0.89±0.31$^{a}$ | 1.73±0.27$^{ab}$ | 3.98±0.38$^{c}$ |
| | F70.07 | 0.13±0.04$^{a}$ | 0.47±0.25$^{a}$ | 1.27±0.49$^{a}$ | 0.13±0.05$^{a}$ | 0.47±0.25$^{a}$ | 1.27±0.49$^{a}$ |
| | F70.08 | 0.27±0.12$^{a}$ | 2.37±0.25$^{c}$ | 2.76±0.66$^{c}$ | 0$^{a}$ | 0.33±0.03$^{a}$ | 0.67±0.12$^{a}$ |

*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.

low values (Table 6). F30 ($H^2$ = 0.624) and F70 ($H^2$ = 0.643) families had relatively high values (Table 6).

## Principal component analysis (PCA)

Principal component analysis (PCA) was carried out on the data from all the clones and hybrids studied during the development of necrosis. For the dry season, the first two principal component (PC) generated from all data represented 97.90% of the total variability of necrosis. In the first principal component, necrosis at 4$^{th}$ and 6$^{th}$ days after inoculation contributed to 86.64% of the total variability while in the second principal component, necrosis at the 2$^{nd}$ day after inoculation contributed 11.27% of the total variability (Figure 1). Examining a two-dimensional scores plot in the space defined by PC1 and PC2 shows that distribution of genotypes followed a specific pattern. Genotype F79.01 (highly susceptible to BPD) represented Group 1; group 2 constituted of three genotypes (F61.04, F79.02 and F16.03) that were susceptible to BPD; group 3 consisted of two parental clones (SCA 12 and SNK 16) and four hybrids (F16.04, F16.01, F79.06 and F79.03) that were moderately susceptible to BPD. Group 4 was very homogeneous and also distinguished the best parents in terms of increasing tolerance (T 79/501, T 79/467 and SNK 413) and 25 hybrids (list them)that were tolerance to BPD The genotypes in group revealed high tolerance (Figure 1).

For the rs, the first two principal components represented 95.30% of the total variability of necrosis length. Days 4, and 6 were the dominant features in the first PC1 (76.39% of the total variability) while necrosis at day 2 was the highest feature in the second PC2 (18.91% of the total variability) (Figure 2). Only F79.01 represented the first group, marked by a higher necrosis length. The second group included the susceptible parents (SCA 12 and SNK 16) and 23 hybrids that presented significant necrosis length; however, it was less important in the third group consisting of the best parents T 79/501, T 79/467, SNK 413 and hybrids that formed the group. The fourth group embodied 30 hybrids, characterized by lower necrosis length (Figure 2).

The ranking of parents for necrosis length, allowed classifying more efficient genotypes other than the best parent “SNK 413”. F10, F15, F30, F61, F70, F80 families respectively presented 6 (F10.02, F10.03, F10.05, F10.06, F10.07 and F10.08), 3 (F15.03, F15.06 and F15.07), 4 (F30.03, F30.05, F30.07 and F30.08), 1 (F61.03), 2 (F70.04, F70.05), 3 (F70.01, F70.07 and

**Table 5.** Estimation of mid-parent heterosis (%) of necrosis length from the parents and their offsprings in nursery.

| Genotypes | | Dry season | | | Rainy season | | | Genotypes | | Dry season | | | Rainy season | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 2 | Day 4 | Day 6 | Day 2 | Day 4 | Day 6 | | | Day 2 | Day 4 | Day 6 | Day 2 | Day 4 | Day 6 |
| F10 | F10.01 | -86.18 | -86.90 | -60.60 | -48.31 | +0.43 | -25.29 | F16 | F16.01 | +18.92 | +28.32 | +7.22 | +58.02 | +24.31 | -10 |
| | F10.02 | -81.30 | -80.88 | -77.42 | -82.77 | -79.74 | -71.96 | | F16.02 | -10.14 | -5.20 | -14.43 | -54.20 | -45.10 | -29.57 |
| | F10.03 | -78.05 | -75.22 | -56.59 | -53.56 | -45.26 | -59.41 | | F16.03 | +30.41 | +84.97 | +33.51 | -48.85 | +25.49 | -12.13 |
| | F10.04 | -91.87 | -78.76 | -42.28 | -55.06 | -41.38 | -9.22 | | F16.04 | +12.16 | +34.87 | +14.95 | -54.20 | -40 | -44.04 |
| | F10.05 | -75.61 | -73.45 | -51.57 | -15.36 | -29.74 | -23.53 | | F16.05 | +10.14 | +23.31 | -9.28 | -36.64 | -28.24 | -26.81 |
| | F10.06 | -81.30 | -75.22 | -74.91 | -77.53 | -72.84 | -75.88 | | F16.06 | -18.92 | -17.92 | -25.26 | +3.82 | +85.49 | +31.91 |
| | F10.07 | -86.18 | -81.24 | -74.15 | -57.30 | -36.64 | -43.53 | | F16.07 | +5.41 | +10.21 | -6.44 | -36.64 | -0.78 | 0 |
| | F10.08 | -91.87 | -67.08 | -33 | -40.07 | -29.74 | -41.76 | | F16.08 | -50.68 | -22.93 | -36.60 | -6.87 | -43.92 | -47.23 |
| F80 | F80.01 | -86.18 | -75.22 | -60.60 | -27.34 | +65.09 | -7.25 | F79 | F79.01 | -16.89 | +124.66 | +89.69 | -46.56 | -40 | -38.30 |
| | F80.02 | -86.18 | -81.24 | -77.42 | -5.62 | +72.41 | -2.55 | | F79.02 | -25.68 | +63.01 | +29.64 | -31.30 | +27.84 | +7.02 |
| | F80.03 | -81.30 | -81.24 | -56.59 | -60.30 | -48.28 | -53.73 | | F79.03 | +75.68 | +39.88 | +37.89 | -40.46 | -47.84 | -29.79 |
| | F80.04 | -83.74 | -43.36 | -42.28 | +53.56 | +36.21 | -13.73 | | F79.04 | -57.43 | -61.46 | -48.45 | -38.93 | +15.29 | -2.77 |
| | F80.05 | -81.30 | -68.14 | -51.57 | +58.80 | +13.36 | -23.33 | | F79.05 | +23.65 | +28.32 | +6.19 | -8.40 | +71.37 | +34.04 |
| | F80.06 | -78.05 | -84.78 | -74.91 | -37.83 | -27.16 | -42.16 | | F79.06 | -52.70 | -32.18 | -36.60 | -33.59 | +23.53 | +6.38 |
| | F80.07 | -89.43 | -77.70 | -74.15 | -49.81 | -36.64 | -47.65 | | F79.07 | -1.35 | -5.59 | -1.29 | -3.82 | +84.31 | +36.60 |
| | F80.08 | -45.53 | -47.96 | -33 | +2.62 | -25.43 | -50.39 | | F79.08 | -52.70 | -32.18 | -62.37 | -38.93 | +18.82 | +30.85 |
| F15 | F15.01 | -43.93 | -20.72 | -34.66 | -54.35 | -46.19 | -23.25 | F30 | F30.01 | -77.23 | -41.79 | -33.79 | -58.17 | -43.71 | -45.16 |
| | F15.02 | -100 | -8.83 | -29.99 | -56.52 | -48.88 | -44.49 | | F30.02 | -67.33 | -56.24 | -38.25 | -61.98 | +31.81 | +43.18 |
| | F15.03 | -53.27 | -30.45 | -50.29 | -51.45 | -40.36 | -41.28 | | F30.03 | -57.43 | -22.54 | -19.73 | -82.51 | -39.13 | -52.36 |
| | F15.04 | -56.07 | +1.98 | -22.29 | -49.28 | -55.16 | -34.87 | | F30.04 | -55.45 | -3.72 | -10.81 | -39.16 | +23.57 | +2.98 |
| | F15.05 | -43.93 | -60.36 | -68.73 | -25.36 | +32.29 | -7.01 | | F30.05 | -83.17 | -29.98 | -27.96 | +1.14 | +3.43 | -17.37 |
| | F15.06 | -62.62 | -53.15 | -56.36 | -59.42 | -65.47 | -60.52 | | F30.06 | -74.26 | -72.87 | -63.29 | -82.51 | -72.54 | -73.45 |
| | F15.07 | -81.31 | -45.95 | -42.59 | -68.84 | -71.75 | -68.34 | | F30.07 | -87.13 | -78.12 | -75.30 | -52.09 | +11.21 | +64.52 |
| | F15.08 | -50.47 | -13.51 | -1.28 | -100 | -34.08 | -47.29 | | F30.08 | -80.20 | -76.81 | -61.23 | -11.79 | -5.72 | -8.19 |
| F61 | F61.01 | -37.38 | -50.63 | -60.33 | -47.10 | -5.83 | -31.86 | F70 | F70.01 | -77.23 | -51.86 | -30.36 | -61.98 | -60.64 | -43.92 |
| | F61.02 | -53.27 | -29.01 | -33.96 | -51.45 | +10.31 | -2.40 | | F70.02 | -67.33 | -38.29 | -25.56 | -61.98 | +24.94 | -8.19 |
| | F61.03 | -50.47 | -43.42 | -51.93 | -54.35 | -41.70 | -48.50 | | F70.03 | -73.27 | -47.48 | -50.94 | -74.90 | +28.15 | -11.41 |
| | F61.04 | -32.71 | +72.97 | +22.99 | -52.17 | -20.18 | -45.29 | | F70.04 | -100 | -86 | -76.67 | -49.05 | +15.79 | +12.90 |
| | F61.05 | -29.91 | +8.11 | -22.29 | -49.28 | +12.11 | -21.84 | | F70.05 | -70.30 | -76.37 | -74.96 | -42.97 | +34.10 | +17.37 |
| | F61.06 | -37.38 | -57.84 | -64.99 | -34.78 | +27.35 | +7.01 | | F70.06 | -66.34 | -56.24 | -45.11 | -32.32 | -20.82 | -1.24 |
| | F61.07 | -100 | -8.83 | -29.29 | -25.36 | +31.39 | -19.84 | | F70.07 | -87.13 | -79.43 | -56.43 | -90.11 | -78.49 | -68.49 |
| | F61.08 | -31.78 | -20.72 | -11.32 | -44.20 | +12.11 | -25.85 | | F70.08 | -73.27 | +3.72 | -5.32 | -100 | -84.90 | -83.37 |

**Table 6.** Values of narrow-sense ($h^2$) and broad-sense ($H^2$) heritabilities for necrosis length for eight families (reciprocal crosses).

| S/N | Families | Reciprocal crosses | Narrow-sense heritability ($h^2$) | Broad-sense heritability ($H^2$) |
|---|---|---|---|---|
| 1 | F10 | (♀) SCA 12 × (♂) T 79/467 | 0.229 | 0.329 |
| | F80 | (♀) T 79/467 × (♂) SCA 12 | 0.306 | 0.396 |
| 2 | F15 | (♀) T 79/501 × (♂) SCA 12 | 0.212 | 0.156 |
| | F61 | (♀) SCA 12 × (♂) T 79/501 | 0.203 | 0.232 |
| 3 | F16 | (♀) SNK 16 × (♂) T 79/501 | 0.278 | 0.375 |
| | F79 | (♀) T 79/501 × (♂) SNK 16 | 0.132 | 0.313 |
| 4 | F30 | (♀) SNK 413 × (♂) T 79/467 | 0.699 | 0.624 |
| | F70 | (♀) T 79/467 × (♂) SNK 413 | 0.601 | 0.643 |

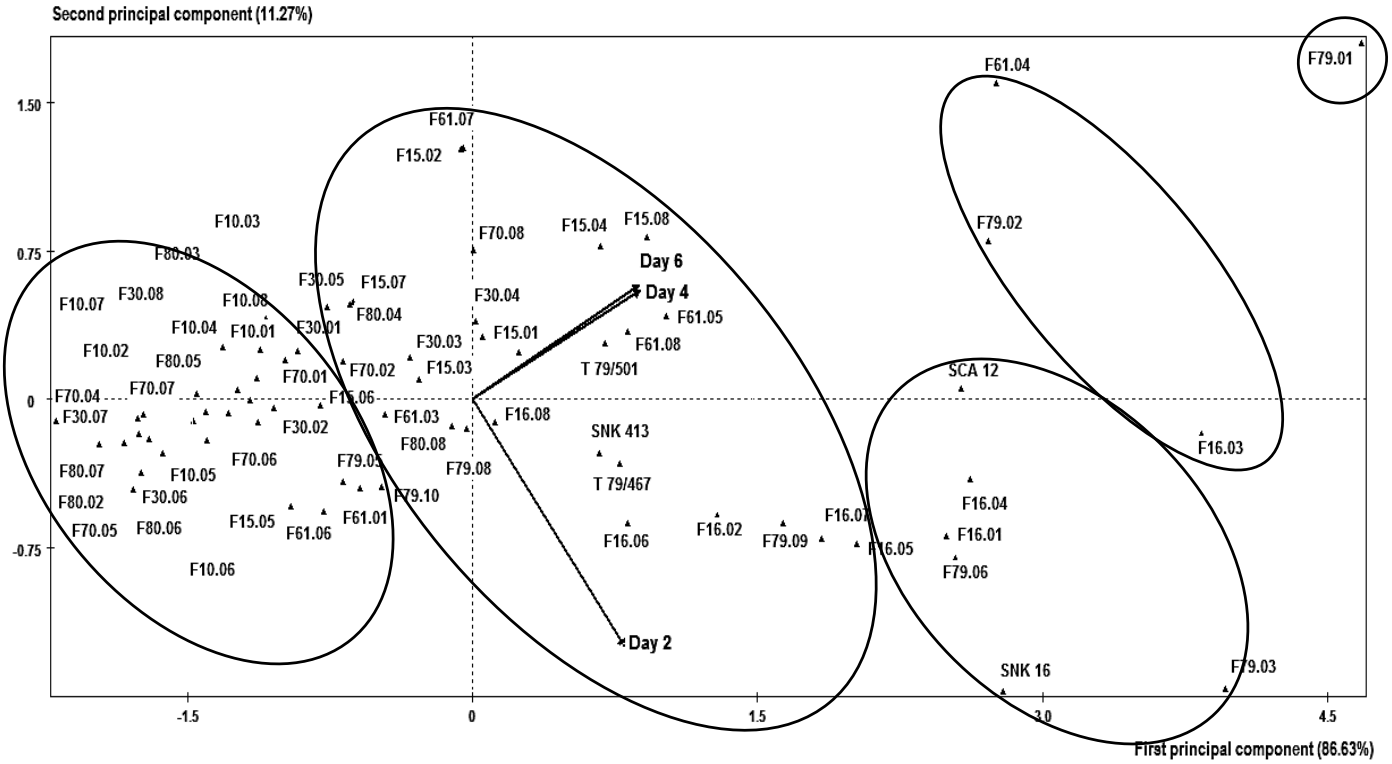


**Figure 1.** Principal component analysis based on the length of necrosis on *T. cacao* leaf 2nd, 4th and 6th days after inoculation for parental and hybrid cocoa genotypes in dry season.

F70.08) and 3 (F80.03, F80.07, F80.08) genotypes with the susceptibility level less than the best parent (Figure 3).

## Regression analysis between dry and rainy seasons for length of necrosis

There was a positive relationship between necrosis length in rainy and dry seasons after biotic stress (Figure 4), but as the time of infection increases, regression coefficients decreases (r = 0.25, 0.16 and 0.09).

## Variation of metabolite compounds between rainy and dry seasons

In order to study the variation of some metabolites after *P. megakarya* inoculation on cocoa leaves during rainy and dry seasons, the three parents and one hybrid from each family observed to be vigorous than the best parent SNK 413 were used. This study revealed that phenols, proline and GABA contents increased significantly during rainy season for about 80% of genotypes considered, but this was not observed for sugars where rainy season characterized by a decrease of this metabolite in all

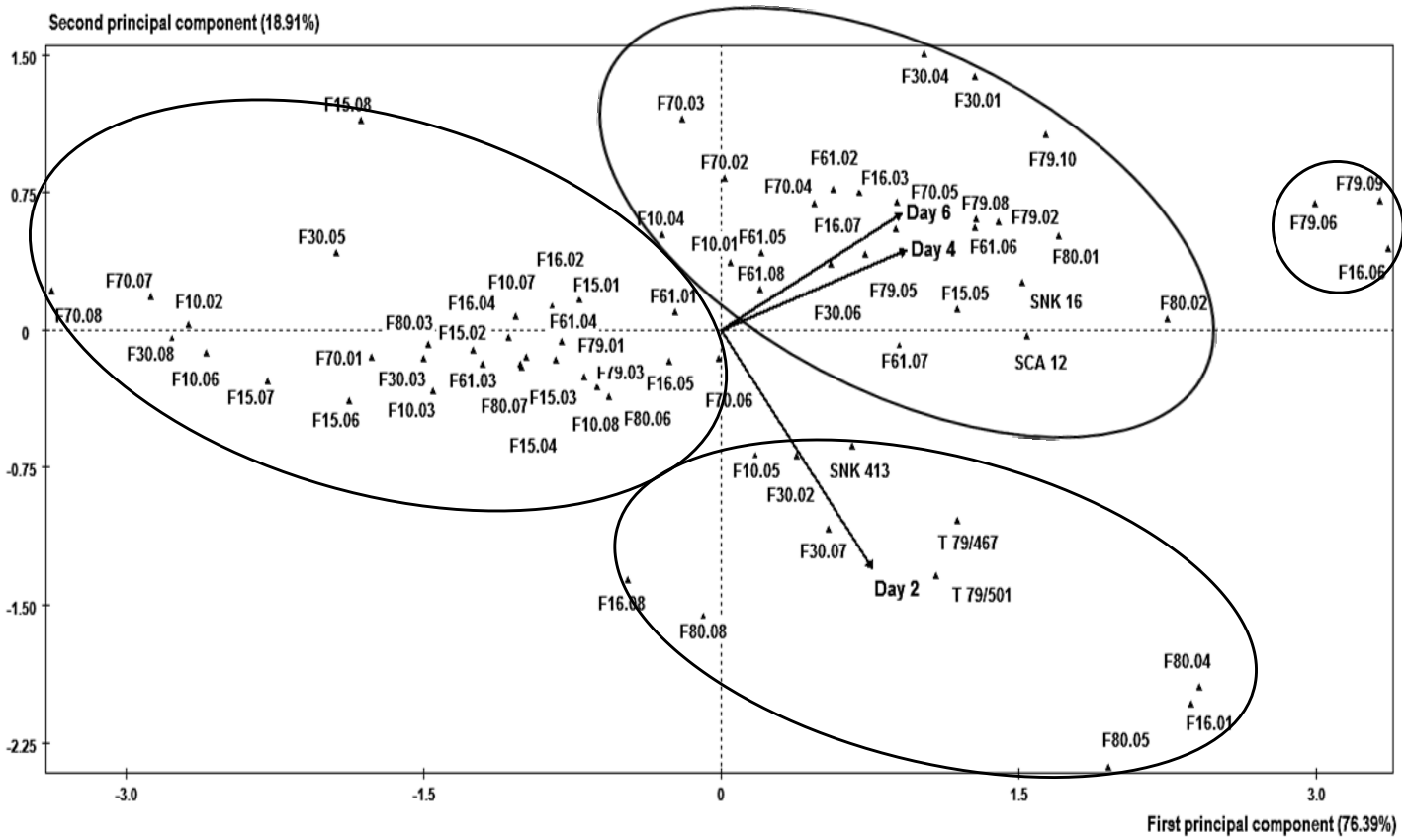


**Figure 2.** Principal component analysis based on the length of necrosis on *T. cacao* leaf 2nd, 4th and 6th days after inoculation for parental and hybrid cocoa genotypes in rainy season.

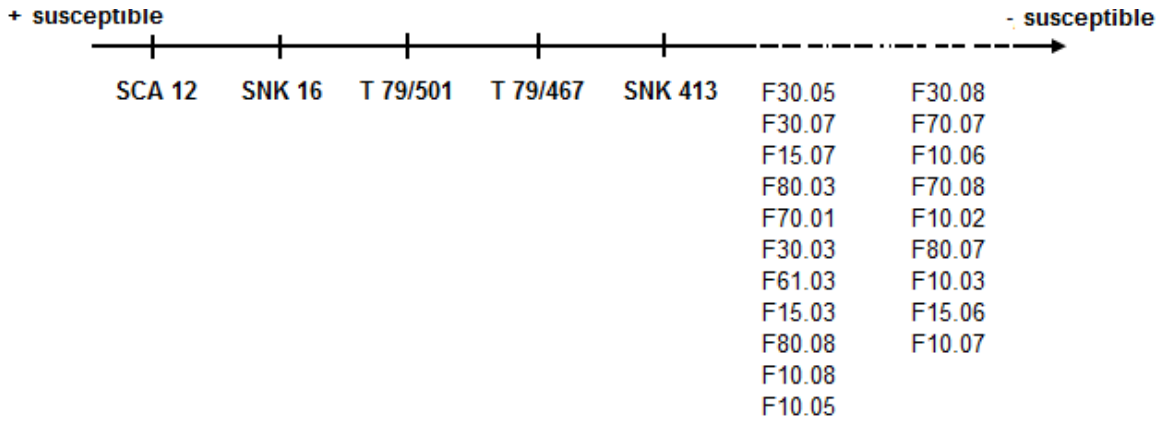


**Figure 3.** Classification of the different parents and their progenies according to their susceptibility to black pod.

individuals (Figure 5).

## DISCUSSION

The production of parental and hybrid genotypes of cocoa was achieved through hand pollination and grafting. The percentage of successful crosses r recorded for hand pollination (31.12%) was low. This could be due to genetic incompatibility between clones and also the period of pollination. This result is similar to those found by Mossu (1990) and Ondobo et al. (2014).

The percentage of success for the regeneration parents formed by grafting varied from 41.66 to 80% depending

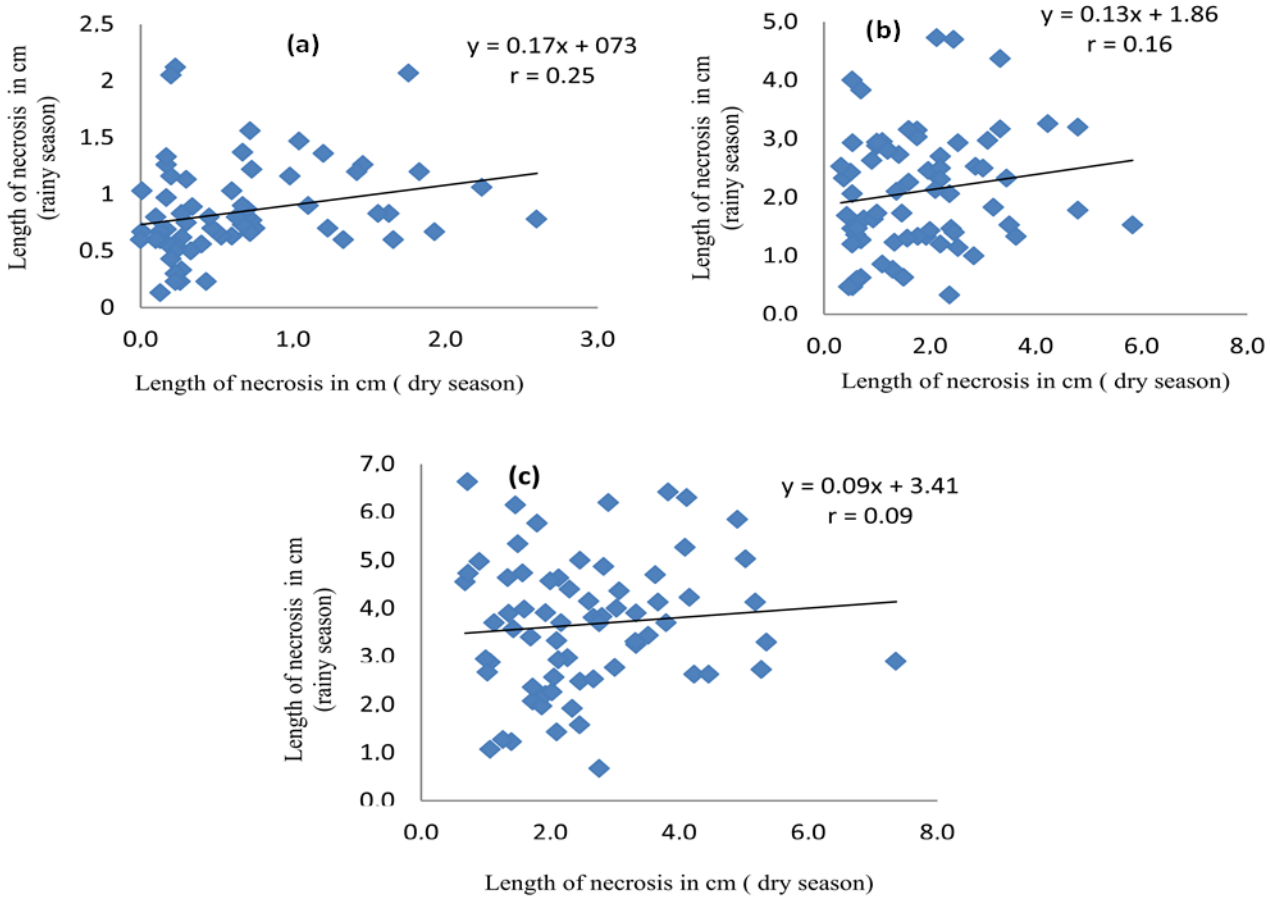


**Figure 4.** Relationship between length of necrosis in rainy and dry seasons on cocoa leaves after inoculation with mycelium of *P. megakarya* on (a) 2nd day (b) 4th day (c) 6th day.

on the clone used. According to Akinnifesi et al. (2008), they observed that the success of grafting for *Uapaca kirkiana* depends on the skill of the grafter, management practices used after grafting, and the technique used. In this study, the period of grafting and the non-compatibility of rootstock and graft could have caused the low success rate.

The appearance of necrosis on the midrib of the leaves infected in nursery, confirms the presence of *P. megakarya* mycelium. This result is in agreement with the findings of Nyasse (1997). from the leaf disc test done in the laboratory, Efombagn et al. (2011) on pods in field, Djocgoue et al. (2010) and Ondobo et al. (2014) on the leaves attached to the plant in nursery. There were significant ($P<0.05$) differences in the necrosis length of susceptible SCA 12 and SNK 16 as compared to T 79/501, T 79/467 and SNK 413.

Forty hybrid genotypes would have shown lower necrosis length to *P. megakarya* relative to the best parent (SNK 413). Earlier studies (Nyasse et al., 2002, Efombagn et al., 2011; Ondobo et al., 2014) showed that tolerance to black pod disease was under genetic control and can be improved genetically. Otherwise, the effects of global combinations aptitudes to parental genotypic was substantial for the rot rate of cocoa pods (Cilas et al., 2004; Ondobo 2014), suggesting a primary and additive transmission of resistant characters (Tan and Tan, 1990).

The manifestation of hybrid vigor was observed between dry season (84.37%) and rainy season (76.04%) in all the hybrid families. Cilas et al. (1998) and Djocgoue et al. (2006) showed that, individuals which exhibit hybrid vigor, would imply the presence of the additive and dominant gene effect in the transmission of character. However, negative heterosis observed in hybrid genotypes could be explained by the epistatic effect of some genes which tend to mask genes controlling tolerance to *P. megakarya*. Similar results were also obtained by Djocgoue et al. (2007) and Ondobo et al. (2014) when leaves of *T. cacao* were inoculated with *P. megakarya*. Early studies of Mohammadi et al. (2012) showed that, resistance to *Septoria tritici* blotch is controlled by additive, dominance and epistatic gene action, but the role of the dominant gene effect is greater than the others.

The values of narrow sense ($h^2$) and broad-sense ($H^2$) heritabilities of necrosis length (dry and rainy season) in

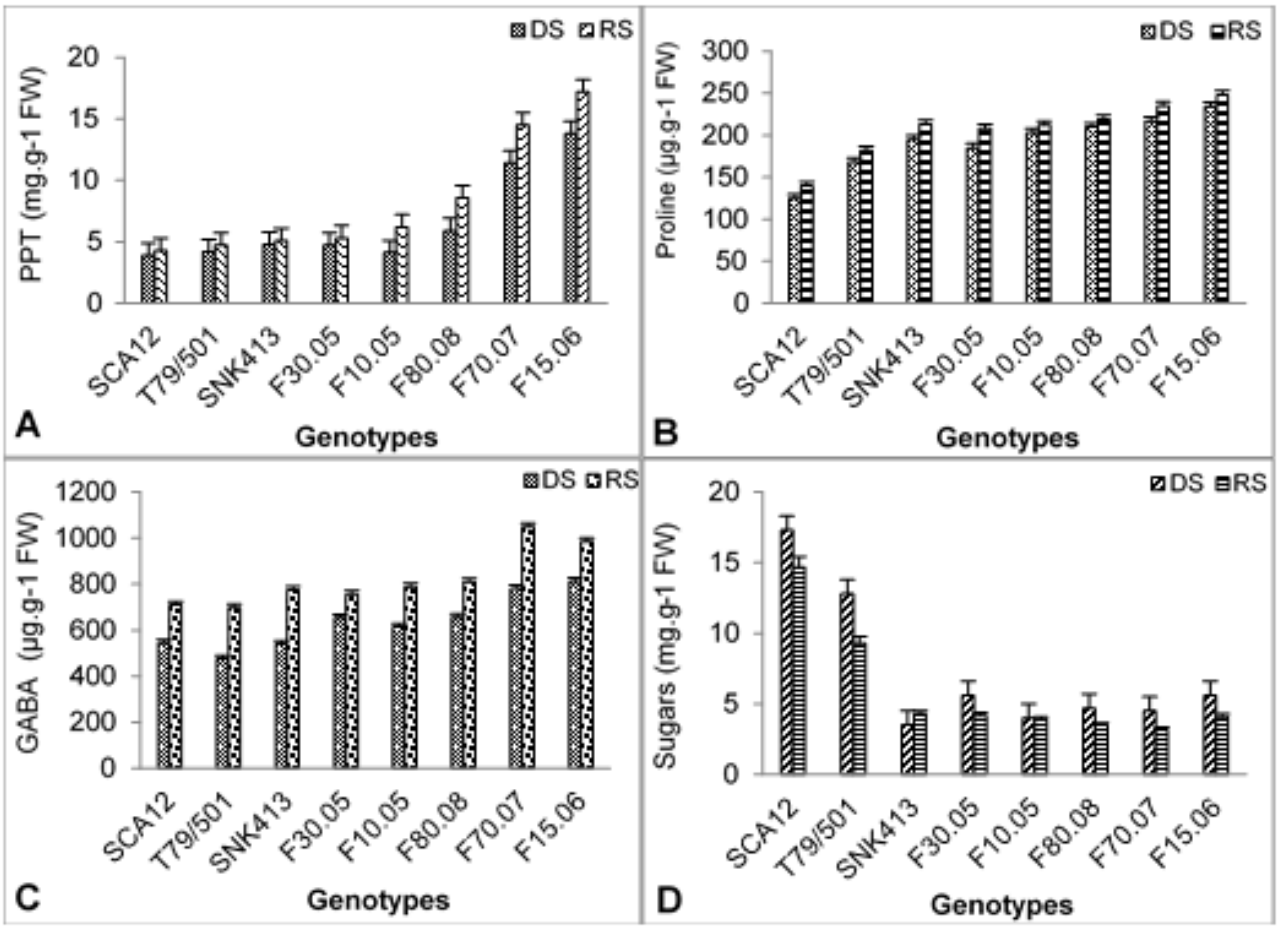


**Figure 5.** (a) Content of phenolic compounds, (b) proline, (c) GABA and (d) sugars during dry season (DS) and rainy season (RS) on cocoa leaves 6th day after inoculation with mycelium of *P. megakarya.*

the reciprocal crossings were not significant. The studied character, the absence of a significant difference between the heritability values from reciprocal crossing portrays the absence of maternal heritability. This finding suggested that the transmission of this character would not cytoplasmic but nuclear (Djocgoue et al., 2011). Alone values of heritability in the two reciprocal crossings [F30 ($h^2$ = 0.699 and $H^2$ = 0.624) and F70 ($h^2$ = 0.601 and $H^2$ = 0.643)] was showed a variation of character strongly inheritable ($h^2$ > 0.4). These results matched with those of Djocgoue et al. (2010) and Manga et al. (2016). In addition, in the experimental conditions of the present investigations, the parental and hybrid genotypes were planted in the same plot, and this had the effect of minimizing environment related effects, rendering the heritability estimations more trustworthy (Cilas, 1991). Therefore, selection should be conducted in the advanced generations of selfing when the breeding materials can be duplicated for extensive evaluation. Then, selection based on family performance or progeny test should be more effective (Phudenpa et al., 2004).

PCA based on necrosis length categorized all the families into five (dry season) and four (rainy season) groups. Each group consists of similar individuals characterized by low and high length of necrosis. The tolerant hybrids characterized by low and intermediate length of necrosis were considered as elites. This result confirms a good aptitude (cross) to parental gene combinations (Cilas et al., 2004; Ondobo et al., 2013).

Regression studies showed that *P. megakarya* is more virulent in the rainy season than in dry season and this virulence decreases with time of infection. During rainy season, oomycete develop their sporangia rapidly because of high humidity. The sporangia of many oomycetes may germinate directly to form an infection hypha, or else in the presence of abundant water they may differentiate, through specialized cleavage vesicles, into 10 to 30 zoospores that can individually disperse to initiate sites of infection (Birch and Cooke, 2004). In this study, necrosis appeared two days after young leaves of cocoa were inoculated.

Variation of metabolites in cocoa genotypes 6th day after inoculation with *P. megakarya* was seen in the increase of phenols, proline and GABA and a decrease in

sugar contents. This increase is more important in rainy season than dry season. Phenols and amino acids have been considered by several authors (Djocgoue et al., 2011; Omokolo and Boudjeko, 2005) as markers of resistance to fungal infections in plants. Del Rio et al. (2003) in the study of the enhancement of phenolic compounds in olive plants (*Olea europaea* L.) and their influence on resistance against *Phytophthora* sp. noticed that the HPLC-MS studies pointed to an increase in the phenol content of leaves 120 days after treatment with 0.3% Brotomax. These authors stated that oleuropein, catechin and tyrosol are some of the main phenolic compounds produced after *Phytophthora* sp. attack plants. Increase in proline content in the rainy season as compared to the dry season contrasted the result of Szabados and Savoure (2009). They noticed that during osmotic stress, proline biosynthesis is augmented in the chloroplasts, and this is controlled by the stress induced P5CS1 gene in Arabidopsis.

## Conclusion

Resistance tests on leaf were used to select clones and hybrid families less sensitive. The estimation of genetic parameters has achieved comparative hybrid tests of genotypes, for the most effective characters among those analyzed and to identify the parental genotypes that show good combining potentials. 84.37 and 76.04% of hybrid genotypes exhibited positive heterosis (hybrid vigor) in dry and rainy seasons respectively. However, inoculation periods (dry and rainy season) did not show a high distinction. Transmission of the character would thus seem to be governed by primarily additive gene effect suggesting a nuclear origin of the transmission of these characters.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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