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Heterosis, heterobeltiosis, narrow-sense and broad-sense heritabilities for *Phytophthora megakarya* tolerance in two populations of *Theobroma cacao* L.

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A 2x2 diallel mating design was set up to estimate quantitative genetic parameters after leaf artificial inoculation tests in nursery and field for three consecutive years. F12 and F40 families of two reciprocal crosses viz., F12: ♀SCA12 × ♂ICS40 and F40: ♀ICS40 × ♂SCA12 were used to study heterosis (mid-parent), heterobeltiosis (better-parent), heritability (narrow-sense and broad-sense) and to quantify total polyphenols (TPP) and total flavonoids (TF) contents after inoculation of the leaves of parental and hybrid cocoa genotypes with *Phytophthora megakarya*. The results of heterosis and heterobeltiosis exhibited hybrid vigor of 86.66 and 85%, respectively. The highest heritability was identified [narrow-sense heritability (h^2: 0.668 for F12 and 0.682 for F40) and broad-sense heritability (H^2: 0.765 for F12 and 0.672 for F40)] for necrosis length in 2014. With regards to low narrow-sense heritability, it ranged from 0.019 to 0.162 while corresponding broad-sense heritability ranged from 0.476 to 0.562 for biochemical compounds. Among parent clones, ICS40 and SCA12 proved to have the general combining abilities (GCA). The use of these parental lines and understanding the gene flow of tolerance to black pod disease (Bpd) in cocoa will help researchers to develop a targeted breeding approach in generating new generation of tolerant genotypes cocoa.

Key words: Cocoa, heterosis, heterobeltiosis, heritability, black pod disease, tolerance.

INTRODUCTION

Cocoa tree (*Theobroma cacao* L.) is a woody plant belonging to the Magnoliopsideae class. Because of its ecological requirements, cocoa farming has been largely practiced in Africa (73% of world production, with 4.552 million tons), where four countries are among the five best producers in the world: Côte d'Ivoire (42%), Ghana

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(19%), Nigeria ranked behind Indonesia (7%), Ecuador (6.5%) and Cameroon (6%) (Anonymous, 2017). Although Cameroon is the fourth world cocoa producer, her cocoa crop is experiencing production setbacks due to black pod disease (Bpd) caused by an oomycete denominated *Phytophthora megakarya* (Nyasse, 1997).

Currently, disease control relies heavily on the use of chemical fungicides which has in turn increased the production costs and reduced growers' profits. In addition, there is a growing concern on the impact of chemical fungicides on the environment, which are costly and polluting for cocoa farmers. Genetic control based on hybridization allows the development and adoption of genotype tolerants to Bpd. This control strategy is the most preferred to improve production (Djocgoue et al., 2010; Ondobo et al., 2017).

Diallel cross analysis for a fixed set of populations provides a basis for preliminary determination of heterotic groups. Thus, knowledge on the nature and magnitude of genotypic and phenotypic variability present in the crop species plays a vital role in formulating a successful breeding program to evolve superior cultivars. Hence, heterosis per se is commercially useful.

However, heterosis (mid-parent) and heterobeltiosis (better-parent) are useful in shaping the direction that future hybrid breeding program should take. It also identifies the cross combinations which are promising in conventional breeding programs.

Studies on heterosis would help to generate breeding strategies of hybrid cocoa production. Obtaining genetic information from diallel cross progenies is a common practice of plant breeders working with allogamous crops.

However, the estimation of heritability serves as a useful guide to the breeder. By relying on heritability, the breeder is able to appreciate the production of the variation that is due to genotypic (broad-sense heritability) or additive (narrow-sense heritability) effects. If heritability of a character is very high, selection for the character should be fairly easy. Thus, estimates of heritability are useful in predicting the transmission of characters from the parents to their offspring (Parikh et al., 2016).

The present study was conducted firstly to estimate heterosis (mid-parent and better-parent) and heritability (broad-sense and narrow-sense) for necrosis length traits in a 2x2 diallel set of cocoa leaf and secondly to identify hybrids of *T. cacao* that are tolerant to Bpd and to observe good aptitudes for the combination of parent.

MATERIALS AND METHODS

Plant material

The experiment was conducted at the Cocoa Development Corporation (SODECAO) experimental field at Mengang Station. Two cocoa parental clones viz., SCA12 (moderately tolerant to Bpd and Forastero group) and ICS 40 (sensitive to Bpd and local Trinitario group) were used to produce two reciprocal offspring populations (F12: ♀SCA12 × ♂ICS40 and F40: ♀ICS40 × ♂SCA12) by hand-pollination techniques for three seasons in three years (2012/2013, 2013/2014 and 2014/2015). These parents were crossed using 2x2 full diallel mating design. The parents were grafted in the nursery using bud wood.

Plant inoculation

P. megakarya-lebdi isolate [obtained from the Central Laboratory of Phytopathology at IRAD (Research Institute for Agricultural Development)] was grown on V8 agar medium (20% V8 vegetable juice, 0.3% Ca_2CO_3, 1.5% agar and 1000 ml distilled water) and incubated in the dark at 25 ± 2°C.

Artificial inoculation of leaves of 22 genotypes was carried out in 2013, 2014 and 2015 (three-season period) adapted from Djocgoue et al. (2006). They were then inner surface sterilized with ethanol 70%. Three treatments were performed on each parental genotype and hybrid: (i) healthy, (ii) scarified (iii) and infected leaves. Agar disks (6 mm diameter) cut from 5-day-old fungal and straminopilous isolates were laid on the midrib after creating wounding with a sterilized razor blade. The scars were then covered with cotton that had been immersed in sterilized water. The necrosis length was measured at two days interval with a graduated ruler after inoculation. The samples were wrapped with aluminum foil every six days after inoculation.

Estimation of heterosis and heterobeltiosis

The values of heterosis over mid and better parents from F_1 were calculated for length necrosis.

(i) Mid-parent heterosis (Zahour, 1992) was computed using the formula:

$$\mathrm{MPH}(\%) = \frac{\mathrm{F_1} - \mathrm{MP}}{\mathrm{MP}} \times 100$$

(ii) Better-parent heterosis (or heterobeltiosis) was calculated using the procedure of Tang and Xiao (2013), following the formula below:

$$\mathrm{BPH}(\%) = \frac{\mathrm{F_1} - \mathrm{BP}}{\mathrm{BP}} \times 100$$

In these formulas, MPH (%) represents the percentage of mid-parent heterosis, BPH (%) is the better-parent heterosis percentage, F_1 refers to the means of hybrid genotype, MP is the means of mid-parent and BP is the means of better-parent.

Estimation of heritabilities (h^2 and H^2)

For necrosis length, two types of expression of heritability were calculated from formulas suggested by Falconer and Mackay (1996):

(i) Narrow-sense heritability (h^2) measures the variance additive genetic.

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

(ii) Broad-sense heritability (H^2) measures any genetic variance.

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

In these formulas, V_G is the genetic variance, V_p is the phenotypic variance, V_A is the additive genetic variance and V_E is the environmental variance.

Extraction and total polyphenolic contents

The total polyphenolic (TPP) content was determined using the Folin−Ciocalteu method (Georgé et al., 2005) with some modifications. Leaves (0.5 g) were mixed with 2 ml of acetone/water (70:30, v/v) and incubated at room temperature. The mixture was centrifuged (Labofuge 400R) at 4500 rpm for 5 min. The supernatants were applied three successive washes with cyclohexane eliminate lipids, carotenoids and chlorophyll (Wieslaw et al., 1988). 2.5 ml of Folin-Ciocalteu (1:10) reagent was added to 0.5 ml of diluted crude extract (1:10) in a test tube. The mixture was incubated for 2 min in the dark and at room temperature. Then, 2 ml of Na_2CO_3 (75 g/L) were added and the whole was stirred on a vortex and then incubated for 15 min at 50°C in a water bath in the dark. Afterwards, the mixture was cooled in an ice cube tray, the absorbance at 760 nm was immediately measured (Jenway 6305 spectrophotometer) against a blank in which the extract was replaced by distilled water. Results were expressed as gallic acid equivalents (GAE).

Extraction of flavonoids

This was done following the extraction protocol described by Counet et al. (2004), with minor modifications. The plant material (cocoa leaves) lyophilized (50 mg) and crushed was extracted with 2 ml of the acetone/water/acetic acid (70:28:2 v/v/v). After upheaval at vortex, the mixture was incubated at room temperature for 1 h in the dark. The bottom was centrifuged (Labofuge 4000R) at 4500 rpm for 5 min and the supernatant was recovered and constituted the raw extracts. Raw extract undergone three successive washes with 1 x 3 ml of hexane in order to remove lipids, carotenoids and chlorophyll (Wieslaw et al., 1988).

Total flavonoids contents

The total flavonoids (TF) content was determined using the aluminum trichloride ($AlCl_3$) method (Ayoola et al., 2008). 2 ml of distilled water and 150 µl of a 5% (w/v) sodium nitrite solution ($NaNO_2$) were added to 500 µL of diluted extract (1/10). The mixture was then incubated for six min in the dark and at ambient temperature (25 ± 2°C). 150 µL of the $AlCl_3$ solution (10% in methanol; m/v) was added. After incubation for 6 min, at room temperature and in the dark, 2 ml of 4% (w/v) sodium hydroxide (NaOH) and 200 µL were rapidly added to each tube. The reaction medium (5 ml) was homogenized before being incubated in the dark for 15 min, and the absorbance as recorded at 510 nm using a UV spectrophotometer (Jenway 6305). Quercetin was used for the construction of a standard curve and the results were expressed as mg quercetin equivalent (QE) per grams dry weight (DW) of plant material. The analyses were performed in triplicate.

Statistical analysis

Data obtained from the observation were analyzed. SPSS (version 24.0 for windows) was used to perform analysis of variance (ANOVA). The significance of differences was determined by Tukey's multiple comparison technique. The Rho Spearman correlation was used to determine the correlation between all the parameters evaluated in the different treatments. Hierarchical classification and principal component analysis (PCA) of necrotic surface area data was performed with SPAD 5.5 software package.

RESULTS AND DISCUSSION

Length of necrosis

The development of necrosis for each of the three years: 2013, 2014 and 2015 was observed in parents (SCA12 and ICS40) and in hybrid populations F12 and F40 (Figure 1). However, individuals F12-36 [2.53±0.4 cm (2013), 2.26±0.42 cm (2014) and 5.93±0.31 cm (2015)], F12-37 [1.98±0.01 cm (2013), 1.93±0.35 cm (2014) and 3.13±0.25 cm (2015)] and F12-39 [2.70±0.21 cm (2013), 2.60±0.35 cm (2014) and 4.67±0.25 cm (2015)] showed a decrease in the development of necrosis at the level of 2014 (Figure 1A). For this purpose, the same slides were observed in individuals F40-31 [4.03±0.21 cm (2013), 3.77±0.23 cm (2014) and 7.20±0.36 cm (2015) and F40-34 [2.18±0.35 cm (2013), 2.17±0.32 cm (2014) and 5.40±0.26 cm (2015)] (Figure 1B). However, F12-38 and F40-36 are considered highly susceptible to ICS 40 (moderately sensitive).

Heterosis and heterobeltiosis

Six days after infection of the leaves, the hybrid F12 and F40 populations showed a positive heterosis effect of 86.66% (Table 1) on a continuous way (during the years 2013, 2014 and 2015). Likewise, genotypes F12-33 (+11.95) and F12-35 (+0.63), which showed a negative heterosis effect in the year 2013, on the other hand, they were again effective (positive heterosis) in the years 2014 and 2015.

Positive heterobeltiosis was manifested by a performance of hybrid genotypes superior to that of the better parent (SCA12). Only 85% of hybrid genotypes displayed negative values for the parameter studied after three years of evaluation (Table 1). Individuals F12-35 (-7.55) and F40-39 (-0.20) in 2015 showed that their positive heterobeltiosis is very small because their value was nearby to 0. Moreover, in hybrids F12-38 and F40-36 a continuously negative heterobeltiotis (periods 2013, 2014 and 2015) (Table 1).

Broad-sense and narrow-sense heritabilities

Narrow-sense and broad-sense heritabilities estimates for necrosis character and biochemical parameters were obtained for the two populations from reciprocal crosses. Values of heritability in 2014 period were h^2 = 0.686 and

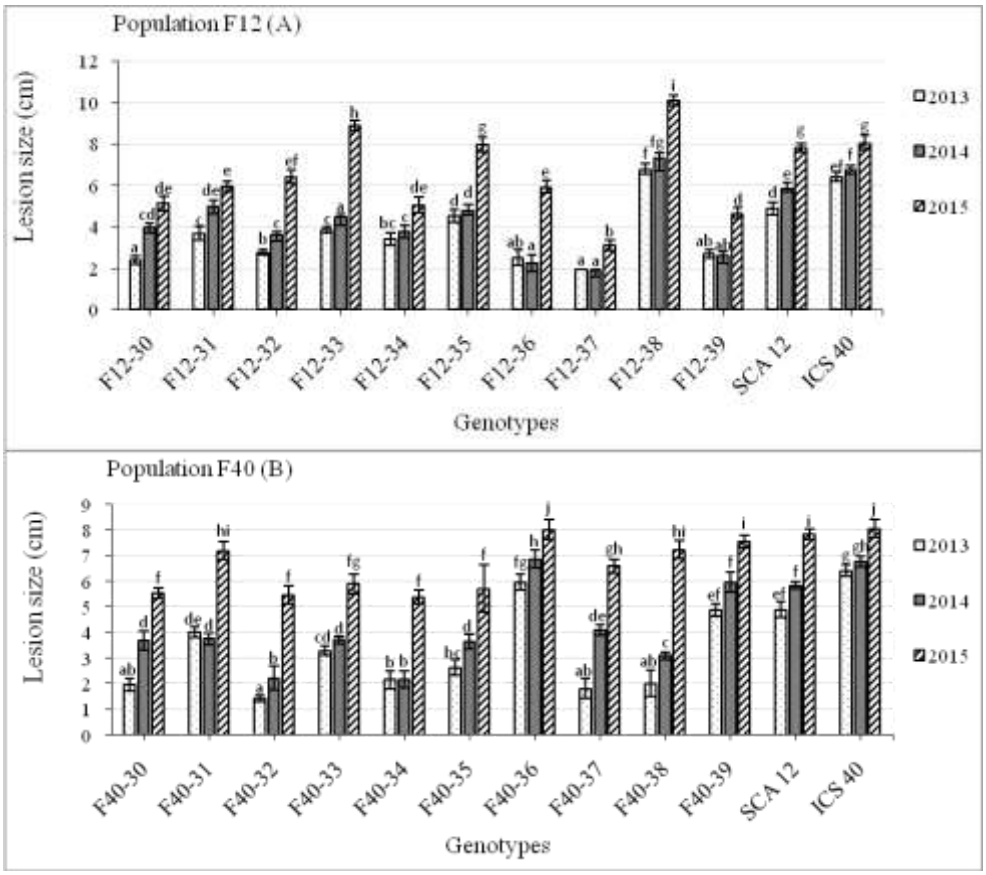

Figure 1. Average lesion size (cm) of the midrib in two clones (SCA 12 and ICS 40) and their progenies F12 (A) and F40 (B) of *T. cacao* L., 6 days after inoculation with mycelium of *P. megakarya* during 2012/2013, 2013/2014 and 2014/2015 cocoa seasons. Values with the same letter for same genotype and in the same family are not significantly different ($P < 0.05$). Values are means of 3 replicates.

H^2 = 0.765 for F12 and h^2 = 0.682 and H^2 = 0.672 for F40. These values were the highest of the other two years (2013 and 2015) (Table 2). Concerning (additive gene effects) and broad-sense (additive + dominance + epistasis effects), the values obtained in the (♀) SCA12 x (♂) ICS40 family (h^2 = 0.431 and H^2= 0.515 for 2015) was not significant to that obtained in the (♀) ICS40 x (♂) SCA12 (h^2 = 0.301 and H^2= 0.576 for 2015). The observation was the same for the 2013 and 2014 periods (Table 2).

Principal component analysis (PCA) and hierarchical cluster

In the F12 family, the first two principal components (PC) generated from all data represented 98.15% of the total variability of necrosis during three years of study. The year 2015 was the dominant feature of the first axis [PC1 (91.45% of total variability)], while necrosis in the years 2013 and 2014 was characterized as the highest of the second axis [PC2 (6.70% of total variability)] (Figure 2A).

The F40 family was dominated by necrosis in 2014 with 91.06% total variability, while necrosis progressed rapidly in the years 2013 and 2015 with a PC2 characterized by a total variability of 6.16% (Figure 2B). However, genotypes F40-36, F40-39, SCA12 and ICS40 which formed a group had higher necrosis sizes in all years 2013, 2014 and 2015.

Cluster analysis (5% dissimilarity) also classified genotypes by their affinities with the necrosis length character studied. The first cluster composed of genotypes that shared only 37% similarity among them. The second cluster consisted of two parents (SCA12 and ICS40) and eight hybrids correlated with 37.5% similarities. On the other hand, F12-38 (cluster 3) and F12-37 (cluster 4), differentiated themselves from other genotypes by their behavior (performance for F12-38 and vulnerable for F12-37) (Figure 3).

The ranking of parents and their offspring for necrosis length allowed classify more efficient genotypes other than the best parent “SCA12”. F12, F40, families

Table 1. Heterosis and heterobeltiosis values (%) for length of necrosis of hybrid populations F12 and F40 over three years.

Genotype	MPH			BPH		
	2013	2014	2015	2013	2014	2015
F12						
F12-30	-35.43	-37.08	-57.63	-34.47	-32.14	-51.02
F12-31	-24.95	-20.76	-34.69	-23.83	-14.53	-24.49
F12-32	-19.08	-42.16	-50.75	-17.87	-37.61	-43.06
F12-33	+11.95	-28.84	-31.51	+13.62	-23.25	-20.82
F12-34	-36.27	-40.25	-39.45	-35.32	-35.56	-30.00
F12-35	+0.63	-23.93	-20.04	+2.13	-17.95	-7.55
F12-36	-25.37	-64.18	-55.34	-24.26	-61.37	-48.37
F12-37	-60.59	-69.41	-65.05	-60.00	-67.01	-59.59
F12-38	+27.04	+16.16	+20.04	+28.94	+25.30	+38.78
F12-39	-41.30	-58.80	-52.34	-40.43	-55.56	-44.90
F40						
F40-30	-30.40	-41.36	-65.23	-29.36	-36.75	-59.80
F40-31	-9.43	-40.25	-28.86	-8.09	-35.56	-17.76
F40-32	-31.24	-64.66	-74.76	-30.21	-61.88	-70.82
F40-33	-25.79	-40.89	-41.75	-24.68	-36.24	-32.65
F40-34	-32.08	-65.45	-61.69	-31.06	-62.74	-55.71
F40-35	-27.88	-42.16	-53.57	-26.81	-37.61	-46.33
F40-36	+1.05	+8.72	+5.38	+2.55	+17.26	+21.84
F40-37	-16.98	-34.71	-67.70	-15.74	-29.57	-62.65
F40-38	-9.01	-50.71	-64.17	-7.66	-46.84	-58.57
F40-39	-4.82	-5.23	-13.68	-3.40	+2.22	-0.20
PHe (%)	**80**	**90**	**90**	**80**	**85**	**90**

MPH: Mid-parent heterosis, BPH: better-parent heterosis, PHe: positive heterosis.

Table 2. Narrow-sense (h^2) and broad-sense (H^2) heritabilities values for the length of necrosis after three consecutive years of study.

Reciprocal crossings	2013		2014		2015	
	h^2	H^2	h^2	H^2	h^2	H^2
F12: (♀) SCA 12 x (♂) ICS 40	0.419	0.466	0.668	0.765	0.431	0.515
F40: (♀) ICS 40 x (♂) SCA 12	0.412	0.514	0.682	0.672	0.301	0.576

respectively presented 7 (F12-37> F12-39 >F12-36 > F12-30 > F12-32 >F12-34 > F12-31) and (F40-32 > F40-34 >F40-30 > F40-35 > F40-33 > F40-38 > F40-37) genotypes with the susceptibility level less than the best parent (Table 3). Intermediate genotypes with the susceptibility level less than the sensitive moderately parent “ICS40” presented 2 (F12-33 > F12-35) and 2 (F40-31 > F40-39) (Table 3).

Phenols and flavonoids

Biochemical component in the leaves of hybrids and parents inoculated with *P. megakarya* (Figure 4) showed significant variations among the healthy, scarified, scarified and inoculated, non-inoculated and parents for total polyphenols and flavonoids content between tolerant and susceptible hybrids. Tolerant hybrids, F12-37 and F40-32, had accumulated relatively higher TPP (5.44 and 11.91 mg EAG/g DW) and TF content (2.63 and 7.53 mg EAG/g DW), respectively. This was contrary to susceptible hybrids recorded low content (Figure 4). However, Table 4 shows the heritability values in the narrow-sense [h^2 was low (varying between 0.019 to0.063)] and in the broad-sense [H^2 was high (ranging from 0.476 to 0.562)] of the accumulation of biochemical

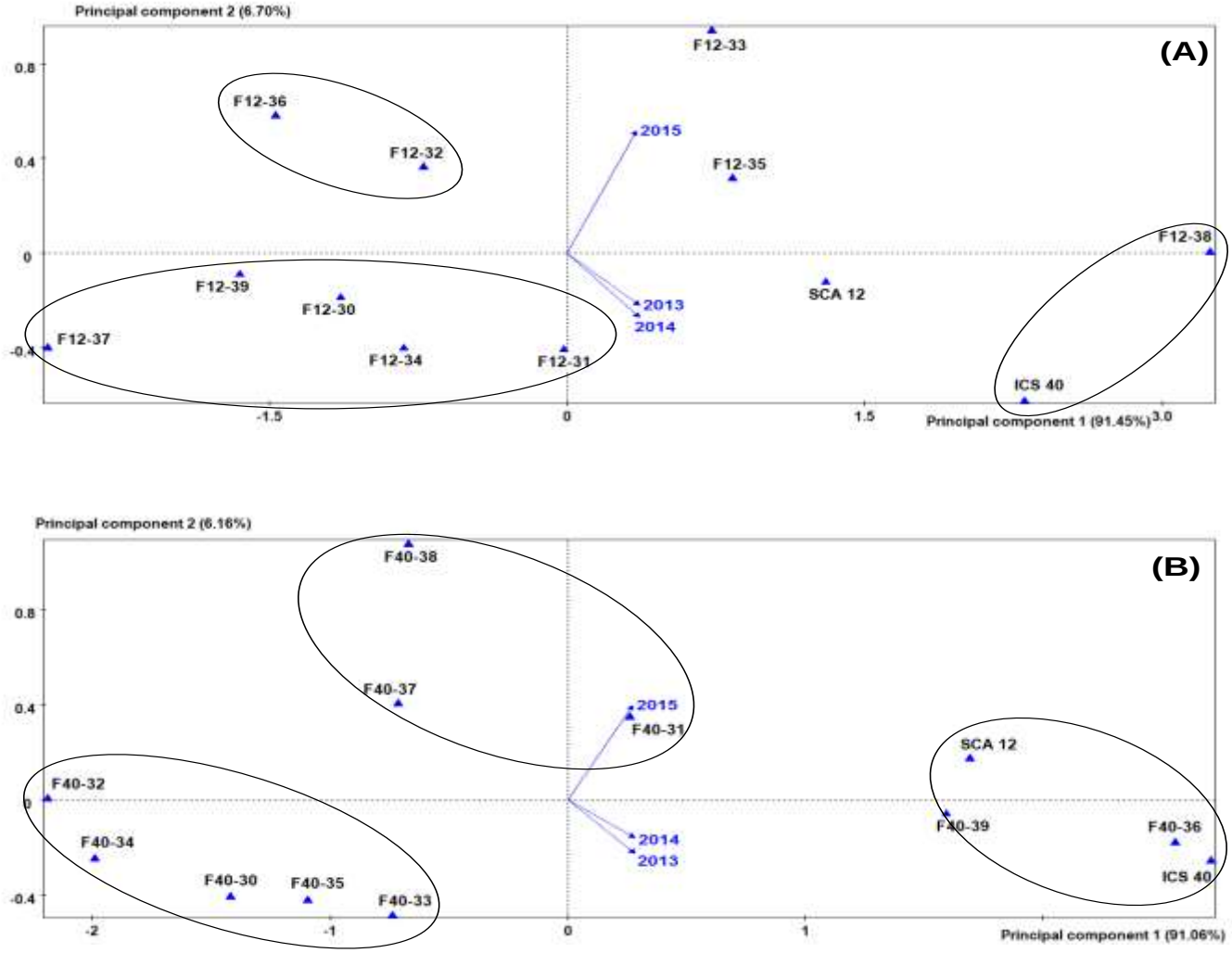

Figure 2. Principal component analysis based on the development of cocoa leaf necrosis between 2013 and 2015 of parental and hybrid genotypes of F12 (A) and F40 (B) reciprocal populations.

compounds of two reciprocal populations.

Correlation coefficients

The trials that were carried out revealed significant correlation in the two families studied. The significant negative correlation was noticed between the necrosis length and biochemical component [F12: TPP *(r* = -0.959, *P<0.05*) and TF (*r* = -0.944) and F40: TPP *(r* = -0.886, *P<0.01*) and TF (*r* = -0.771, *P<0.05*)] (Table 5). Besides, a significant positive correlation between TPP and TF (F12 and F40 populations, *P*<0.05) was recorded (Table 5).

DISCUSSION

In this study, we attempted to estimate heritability (narrow-sense and broad-sense), heterosis (mid-parent and better-parent) and understand the role of metabolites in the interaction *T. cacao* L./*P. megakarya*. The length of necrosis and the biochemical compounds (TPP and TF) were determined in the leaves of the parental and hybrid genotypes of *T. cocoa* L. In order to achieve this objective, the production of the plant material (grafting and hand-pollination) was the fundamental element of this work.

The experiment and field work took three consecutive years of assessment of the tolerance level of F12 and F40 populations in nurseries and fields. Necrotic lesion size was observed 48 h after inoculation of the fungal mycelia. This confirmed that this duration is necessary for the interaction between the two entities and the beginning of resistance of cocoa tissues. The length of necrosis was favored by the vascularization of midrib which facilitates the progression of the disease. These results have been already obtained by Effa et al. (2016)

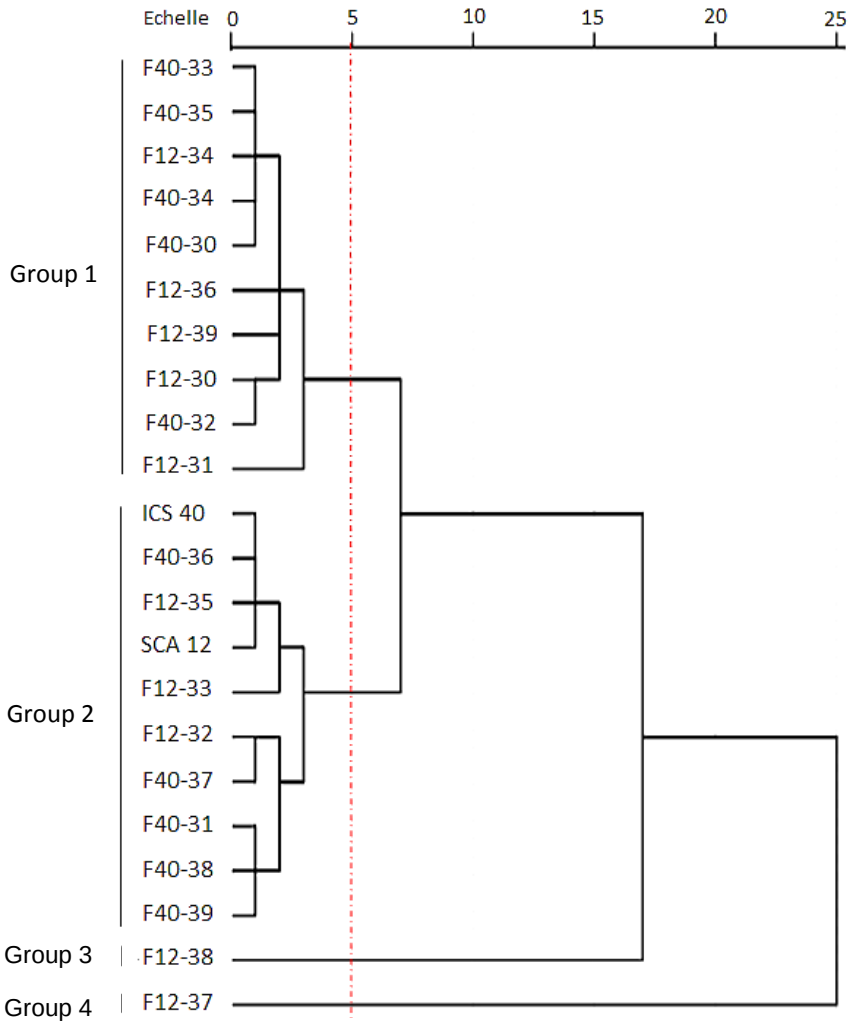

Figure 3. Dendrogram of the F12-F40 populations and their parents, SCA 12 and ICS 40 based on the length of necrosis of the years 2013, 2014 and 2015.

Table 3. Classification of the different parents and their progenies according to their susceptibility to black pod disease.

	F12: (♀) SCA12 x (♂) ICS40	**F40: (♀) ICS40 x (♂) SCA12**		
- Sensitive	F12-37^{d}	F40-32^{d}		
	F12-39^{cd}	F40-34^{d}		
	F12-36^{cd}	F40-30^{cd}		
	F12-30^{cd}	F40-35^{cd}	1	
	F12-32^{cd}	F40-33^{cd}		
	F12-34^{c}	F40-38^{cd}		
	F12-31^{c}	F40-37^{c}		
	SCA 12^{bc}			2
	F12-33^{b}	F40-31^{b}		
	F12-35^{b}	F40-39^{b}		
	ICS 40^{a}			
+ Sensitive	F12-38a	F40-36^{a}		

1, Fourteen tolerant hybrids; 2, four intermediate tolerant hybrids. *Values with the same letter in the same column and in the same family are not significantly different (Tukey's test, $P < 0.05$).

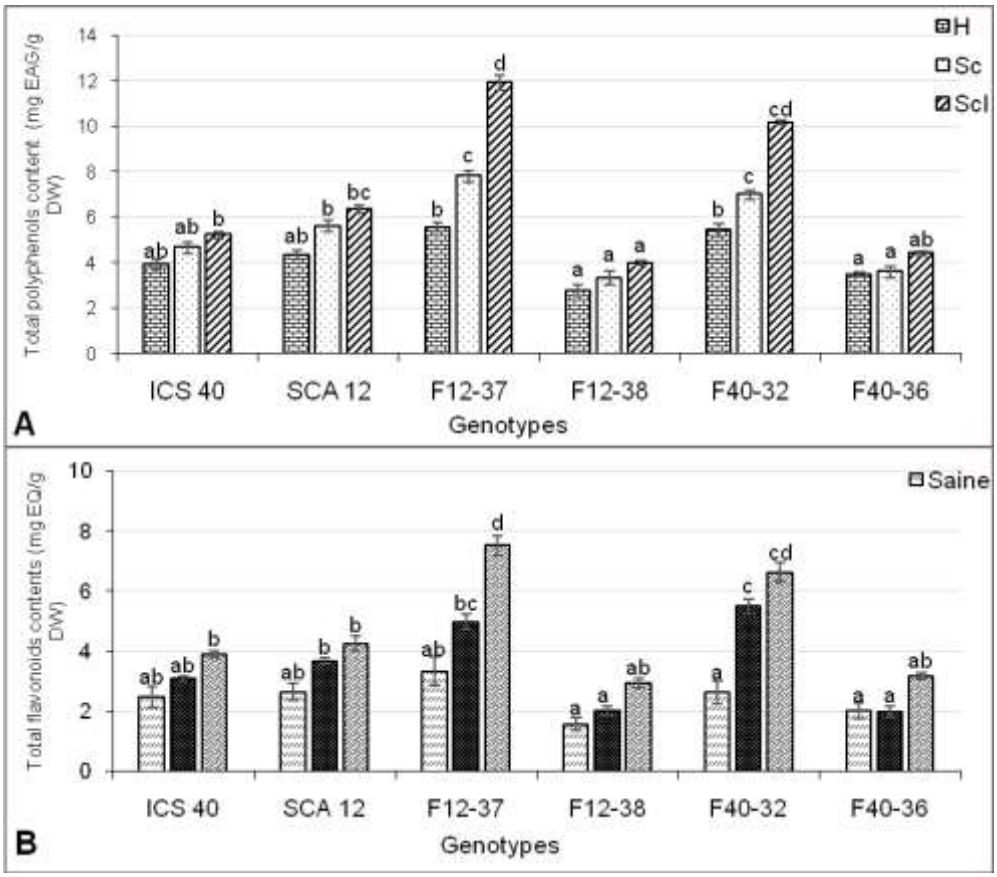

Figure 4. Variations of total polyphenol (A) and flavonoids (B) content in three conditions of treatment (H=Healthy, Sc=scarified and Scl= scarified and inoculated). Values with the same letter in the same family are not significantly (P < 0.05) different. Values are means of 3 replicates.

Table 4. Narrow -sense (h^2) and broad-sense (H^2) heritabilities values of the TPP and TF content of the four reciprocal crosses.

Reciprocal crossings		h^2		H^2	
		TPP	TF	TPP	TF
1	F12 : (♀) SCA 12 × (♂) ICS 40	0.155	0.063	0.562	0.531
2	F40 : (♀) ICS 40 × (♂) SCA 12	0.162	0.019	0.477	0.476

Table 5. Coefficients of correlation between tolerance tests and biochemical compounds of populations F12 and F40.

F12	Necrosis	TPP	TF
Necrosis	1	-0.959*	-0.944
TPP		1	0.973**
TF			1
F40			
Necrosis	1	-0.886**	-0.771*
TPP		1	0.941
TF			1

*Correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level.

on the leaves attached to the plant (cocoa) in nursery and field. This result supports the findings of Nana et al. (2011), after the evaluation of necrotic lesions on cocoa pod cortex (SNK10 and SNK413).

Heterosis [mid-parent (86.66% for F12-40)] and heterobeltiosis [better-parent (85% for F12-40)] were evaluated for the development of necrosis character during three cocoa seasons (2013, 2014 and 2015), exhibited hybrid vigor, indicating good general aptitude for the combination of parents in reciprocal crosses (F12: ♀SCA12 × ICS40, F40: ♀ICS40 × ♂SCA12). From this, it is therefore suggested that these individuals demonstrate hybrid vigor, which would imply the presence of the additive and dominant gene effect in the transmission of character (Djocgoue et al., 2006). The results of the present investigation can be linked to those obtained previously by Manga et al. (2016). Such results show that tolerance to *P. megakarya* is controlled by the action of additive, dominant and epistatic genes. However, the role of dominant gene effect is more significant than that of additive gene effect. These observations corroborate those obtained by Ondobo et al. (2017) on the cocoa tree. Mohammadi et al. (2011) working on wheat, showed that the involvement of genes with additive and dominant effects in the estimation of heterosis (HPM and HMP) could be important in the selection of varieties.

These results matched with those of Djocgoue et al. (2010) and Manga et al. (2016), which showed a variation of strongly inheritable characters ($h^2 > 0.4$). The work of Ram and Jabeen (2016) have shown that high values in a narrow sense heritability for a particular trait indicate that genes are largely controlled in an additive model in *Abelmoschus esculentus* L. Moench and *Zea mays* L., respectively. In 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 be cytoplasmic but nuclear (Ondobo et al., 2017). In fact, nuclear transmission of characters involved genetic additive effects. Recent studies by Parikh et al. (2016) showed that additive genetic effects are more important than non-additive effects (dominance and epistasis) for powdery mildew resistance in wheat.

The high heritability values reported in this study suggest that most of the phenotypic variance in Bpd resistance observed in the two reciprocal crosses is attributable to genetic effects. Similar results were observed for quantitative traits in tobacco (Aleksoska and Aleksoski, 2012), where it was concluded that high broad-sense and narrow-sense heritability were indicative of traits being controlled by genetic factors as opposed to environmental effects. Estimates of heritabilities for broad-sense and narrow-sense are useful tools to predict the genetic gain resulting from a specific reciprocal crossing. However, general combining abilities (GCA) are predominant for intra- and inter-family selections. This means that the tolerance character is essentially transmitted additively (Ondobo et al., 2017).

Principal component analysis (PCA) and dendrogram based on necrosis length was used to categorize all the families; 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) towards parental gene combinations (Cilas et al., 2004).

Plant biochemical constituents have received considerable attention in relation to disease resistance. Most plants synthesize toxic compounds such as phenols and flavonoids during normal development, and their role in the resistance mechanism has been reported earlier by many authors (Nyadanu et al., 2013; Manga et al., 2016). These can be used as markers for selection of tolerance genotypes. Tolerant plants, when subjected to biotic stress, showed elevated levels of free phenolics and contained more lignin. Their role in resistance mechanisms was previously reported by Effa et al. (2016). These results are consistent with those of Ondobo et al. (2017), which emphasize that these compounds act as barriers against pathogen invasion and hence constitute part of host resistance mechanisms. This accumulation of flavonoids supported the assertion that these compounds are a major class of polyphenols involved in the resistance of cocoa against *P. megakarya*. Among these flavonoids, flavan-3-ol units are a major class with regards to the reaction of cocoa to stress (Effa et al., 2016). This is supported by the findings of Djocgoue et al. (2007) who reported that qualitative analysis of phenolics in leaves of cocoa showed a higher accumulation of the flavonoids (luteolin and apigenin derivatives) and some hydroxycinnamic acid derivatives.

The significant negative correlation between metabolites (TPP and TF) and length of necrosis trait reflects the importance of the biochemical compounds in tolerance expression. The findings indicate that TPP and TF play a role in disease resistance against *P. megakarya.* The results support the findings of many authors. Ngadze et al. (2012) disclosed that potato varieties with a high content of these compounds in tuber tissues can exhibit tolerance to pathogen attack. Nyadanu et al. (2013) suggested 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*.

Conclusion

The overall evaluation of 20 genotypes cocoa hybrids led to identification of the tolerant hybrids with about 85.83% of hybrid vigor. Narrow-sense heritability for Bpd tolerance observed in the current study indicates that

additive gene effects are more important than non-additive effects (dominance and epistasis) for Bpd tolerance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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