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Amino acids, carbohydrates and heritability of resistance in the *Theobroma cacao/Phythophthora megakarya* interaction

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Summary. The black pod disease of cocoa (*Theobroma cacao*), caused by *Phythophthora megakarya*, is responsible for 80% loss of cocoa production in Cameroon. The principal method of ameliorating crop losses is through the use of black pod resistant and high productivity hybrid cocoa clones. In order to assess the possible role of amino acids and carbohydrates in the defence of *T. cacao* against *P. megakarya*, comparative analyses (quantitative and qualitative) of sugars and amino acids were carried out on leaves of parental genotypes, ICS95, ICS84 and hybrids (families F25:♀ICS84 X ♂ICS95 and F30: ♀ICS95 X ♂ICS84). A reduction in soluble sugar contents of parental genotypes ICS84, ICS95 and 30% of hybrid genotypes was noted under conditions of infection. Qualitative analyses of sugars showed that most cases of infection were characterized by the disappearance of sucrose and the persistence of glucose. Amino acids content increased in 70% of genotypes after injury or infection. In parental tolerant clone ICS84 and hybrid genotypes F3011, F2551 and F2552, proline appeared solely during conditions of infection, suggesting its implication in the defence mechanism of *T. cacao* against *P. megakarya*. A significant positive relationship was observed between amino acid contents and the severity of necrosis. There was a very weak relationship between sugar and amino acid contents in parental genotype, and those of the progeny. PCA of the length of necrosis, sugar level, amino acids and phenolics showed that under infection, the increase in content of phenolic compound was concomitant with reduction in amino acid content.

Key words: cocoa, black pod, metabolites, progenies.

Introduction

Cocoa (*Theobroma cacao* L.) is a commodity produced in the developing countries of the tropics. It is mainly consumed in the middle and high income countries of the world's temperate zone. Currently, over 50 countries are engaged in cocoa production and heavily rely only on cocoa exportation for their economic development, as this commodity contributes significantly to foreign exchange earnings. However, *Phytophthora* pod rot (Ppr), caused by *Phytophthora palmivora*, *P. megakarya*, *P. capsici* and *P. citrophthora*, is one of the prevalent and destructive diseases of cocoa (Iwaro *et al.,* 1997). Global losses from Ppr are very great, and have been estimated by Wood and Lass (1985) at about 30% of annual production. The selection criteria used for development of new cocao varieties in the 1960s were based on the yield earliness and bean weight. Resistance to disease, based on field observations, was taken into account only as a secondary selection criterion. Consequently, the develop-

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ment of high-yielding resistant crops has generally been considered to be the most effective and economical method for control of the disease (Iwaro *et al.,* 2000). Progress in this direction has been very slow, however, probably due to the narrow genetic base of most cocoa breeding programmes, low levels of resistance in base parents and poor screening methods (Bennett, 2003; Iwaro *et al.,* 2006).

In Cameroon cocoa production has not increased over several decades. This stagnation is partly due to high disease and pest incidence, and the lack of improved resistant and high-yielding varieties. Field performance of the planting material used by the farmers appears to be unsatisfactory. In most fields, only about 21% of *T. cacao* cultivated today is of selected varieties. The remaining 79% of trees are traditional populations. The selected varieties were obtained from the Nkoemvone (SNK) accessions (research station, southern Cameroon) with characteristics similar to those of the Criollo group (with high susceptibility to *Phytophthora* disease) (Laurent *et al.,* 1994). Traditional varieties as well as hybrids proposed by local research stations have often given low productivity, low vigour and high susceptibility to Ppr. Progress obtained until the 1970s in breeding for Ppr resistance has been relatively unadvanced (Efombagn *et al.,* 2006). However, Boudjeko *et al.* (2007) after crossing cocoa clones, obtained hybrid populations that had higher hybrid vigour than that of the best parent of the necrosis character. In addition, the value of heritability obtained was increased. Increased susceptibility of hybrids to pod rot is, according to farmers, probably due to inappropriate choice of parents.

Genetic improvement remains an alternative promising avenue to chemical control of Ppr, which is laborious and usually not cost-effective. Selecting cocoa trees that display lower susceptibility to the disease rot has therefore become a priority objective for numerous producing countries. This selection can be achieved using biochemical markers such as phenols, carbohydrates and amino acids. Disease development is likely to induce substantial changes in the carbohydrate and amino acid contents of host plants, and metabolic alterations that may favour or inhibit fungal development (Ayres *et al.,* 1996).

There is little published information testifying the participation of sugar in plant defence responses by either preventing or limiting invasion of pathogens (Graham *et al.,* 1990; Omokolo *et al.,* 1996). The nature of the sugar-based plant resistance to fungal pathogens is not clear. There is some direct and indirect evidence suggesting that sucrose, which is an easily accessible source of carbon in host cells and takes part in most physiological reactions, participates in plant resistance to fungal pathogens (Bugbee, 1973). Furthermore, carbohydrates, especially mannitol, are osmoprotectants (Tattini *et al.,* 1996), and because fungal pathogens usually require high values of water potential for optimum development, these substances may affect pathogen development. A decrease in water potential of host cells may limit fungal growth (Farrar, 1989; Jeun and Hwang, 1991). Furthermore, hexoses (sucrose) induce the expression of many genes, including plant resistance genes that determine the production of peroxidase and other pathogenesis-related (PR) proteins (Herbers *et al.,* 1996).

Involvement of amino acids in the relationship between host and parasite has been established (Hwang, 1983; Jeun and Hwang, 1991; Nemec, 1995; Omokolo *et al.*, 2002; Omokolo and Boudjeko, 2005), indicating an accumulation during the development of infections. Amino acids might act directly to inhibit fungal development, or indirectly by their implication in the metabolic ways associated with resistance to diseases (Graham *et al.,* 1990). Phenylalanine and tyrosin, which are substrates of secondary metabolite synthesis pathways, might be possible sources of nitrogen for the synthesis of preformed antimicrobial compounds which are stored as glycosyl precursors (glycosides, cyanogenic compounds, amygdalin, cysasin) in vacuoles (Van Etten *et al.,* 1994). After infection of resistant genotypes of some host species, accumulation of certain specific amino acids such as glutamine, histidine, glycine and arginine were observed in tomatoes (Hassan *et al.,* 1994; Starrat and Lazarovits, 1996), tyrosine and alanine in wheat (Tyuterev and Tarlakovskii, 1994) and asparagin, glutamic acid, proline, glycine and arginine in citrus (Nemec, 1995).

Aromatic amino acids are substrates of phenylpropanoid pathways which lead to the biosynthesis of phenols, salicylic acid (Sticher *et al.,* 1997), accumulation of the phytoalexines (Dixon *et al.,* 1995), and synthesis of various substrates involve

in lignification processes.

De novo synthesis of defence proteins implies the same for amino acids. This is the case of the glycoprotein rich in hydroxyproline or proline which is involved in the hardening of the cell walls (Cassab, 1998) and the polygalacturonases rich in leucine (Stotz *et al.,* 2000).

Our previous studies demonstrated that following cocoa pod infection, an accumulation of amino acids and carbohydrates was observed in host tissue. Accumulation of these components explains, at least in part, the differences observed during disease development among cocoa clones (Omokolo *et al.,* 2002).

The present study investigated the resistance of cocoa progenies (F25 and F30) from ICS95×ICS84 reciprocal crosses for resistance to *P. megakarya*, by characterising alterations in composition and amounts of amino acids and sugars in two hybrid populations of *T. cacao*. Our findings contribute to the evaluation of the role of these metabolites in cocoa tolerance to this disease, and provide useful information for the development of new strategies for management of Ppr of cocao.

Materials and methods

Plant material

Two *T. cacao* clones, both Trinitario types, introduced from Trinidad and of different susceptibilities to Ppr (Bartley 2000), (ICS84: less susceptible and ICS95 moderately susceptible) were used to produce two reciprocal hybrid families. For this experiment, parents and hybrid genotypes were chosen based on severity (length) of necrosis developed and phenolic content accumulated 6 d after inoculation (Djocgoue *et al.,* 2007) (Table 1). This choice permitted genotypes to be considered which have or have not manifested hybrid vigour for necrosis development and phenolic content.

Table 1. Mean lesion size (cm) on the midribs of *Theobroma cacao* leaves and total soluble phenolic content (μg mg^{-1} fresh weight) for parental clones ICS95 and ICS84, and F30 and F25 hybrid families, 6 d after inoculation with *Phytophthora megakarya*.

Genotype	Lesion size (cm) [a]	Soluble phenolic content (μg mg^{-1} Fw) [a]
Parents		
ICS84	8.42±0.29cd	2.17±0.06def
ICS95	9.20±0.54d	1.80±0.05c
F30		
F30111	6.76±0.017a	2.03±0.12cde
F30152	6.67±0.68a	2.97±0.12g
F3018	7.87±0.25bc	2.27±0.06ef
F3019	8.87±0.25d	1.97±0.08cd
F3022	7.00±0.21ab	2.83±0.05g
F3032	6.57±0.80a	3.44±0.19h
F3048	7.72±0.62bc	0.916±0.04a
F3094	6.55±0.41a	2.34±0.09f
F25		
F2548	6.70±0.35b	2.06±0.06ab
F2551	7.75±0.48c	1.93±0.26ab
F2552	5.65±0.12a	3.01±0.05d
F2563	7.80±0.4c	1.92±0.14ab
F2591	8.20±0.68cd	1.89±0.09cd

[a]Means with same letter in the same column are not significantly different at $P \leq 0.05$ (Duncan's test).

Leaf inoculation

An artificial inoculation leaf test was used to assess the resistance of genotypes. Briefly, whole detached leaves from 1 or 2-month-old plants were washed thoroughly with tap water and sterilized with 70% ethanol for 30 s. The experimental design consisted of three replicates with six leaves in each of the three conditions: healthy (without treatment) (H), wounded (W)/injury and infection (I). The inner surface of the leaves were scarified along the midribs and each inoculated by placing a mycelium disc (4 mm diam.) of *P. megakarya*, obtained from a 7-day-old PDA culture previously incubated at 25–26°C in a dark and humid chamber. Control leaves were inoculated with sterile agar discs using the same methods. The isolate of *P. megakarya* used was collected from a naturally infected pod from the Nkolbisson Research Station in Cameroon. To determine the involvement of amino acid and sugar contents in the defence mechanism in the *T. cacao*/*P. megakarya* relationship, principal components analyses (PCA) were carried out for necrosis, and contents of soluble amino acids, sugars and phenols, with these parameters assessed at 6 days after inoculation.

Extraction from leaf tissues

Soluble carbohydrates and soluble amino acids were extracted from leaf tissues in 80% ethanol using the method of Singh *et al.,* (1990). Two grams of leaves were crushed in 6 mL of 80% ethanol and boiled under reflux for 30 min. The ethanol-soluble extracts were filtered through filter paper (Whatmann No. 1) and concentrated under vacuum at 50°C prior to analysis. The operation was repeated twice and the three extracts from each sample were mixed.

Determination of total and individual amino acids and carbohydrates

Total amino acid content was determined by the 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 in tap water before adding 8 mL of distilled water. The absorbance of 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.

Identification of individual amino acids in the extract was carried out by thin layer chromatography using butanol, acetic acid and water at 4:1:1 ratio. For each sample, a volume of extract containing 4 μg of amino acid was deposited on silica gel at 1 cm from the bottom of the plate. The chromatograms were air-dried and sprayed with a 1% ethanolic ninhydrin solution. Spots were developed after drying the chromatograph plates at 110°C for 10 min. in an oven. Amino acids were identified by comparing the Rf and the colour of samples with that of the pure standard amino acid from Sigma-Aldrich, (Saint-Quentin Fallavier, France).

For carbohydrate determination, proteins were removed from the ethanolic extract after treatment with basic lead acetate. The carbohydrate extracts were then determined by the anthron method of Yemm and Wills (1954): one mL of the extract was incubated with 5 mL 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.

Identification of individual sugars was also performed by thin layer chromatography using methanol:ethyl acetate:acetic acid:water (1:6:5:1). Sugars were identified by spraying the chromatograph plates with a solution containing 2 mL of 50% diphenylamine in aniline, 100 mL acetone and 15 mL phosphoric acid. Spots were developed after drying plates at 110°C for 10 min. Pure analytical grades of sucrose, glucose and fructose were used as standards for this assay

Estimation of the heritability

For the different parameters measured, heritability was estimated according to Falconer (1974). This estimation considers the regression slope between means of parameters for parents and progeny.

Statistical analyses

Data presented are the means ± SE of three independent experiments. Analysis of variance

and the Duncan's test were used to compare the susceptibility levels of progenies resulting from different crosses in order to assess hybrid vigour and to compare amino acid and sugar contents. The hierarchical classification of parents and their progenies was obtained using PCA. Statistical analyses were performed using the SAS-system (Anonymous 1997).

Results

Variation of the sugar content

In healthy conditions, the content of total sugars was much greater in the leaves of clones ICS95 and lessfor ICS84 and hybrids (F2551, F2548 and F2552 with respective values of 11.80, 6.43 and 5.43 mg g^{-1} FW). Concentrations remained very low in the F2563 hybrid. Injury resulted in a reduction in total soluble sugar content for parents and 60% of hybrid genotypes, but gave significant increases in sugar contents for hybrids F3019, F3022 and F2548. Infection was characterized by decreases in sugar contents in parental genotypes and 60% of hybrid genotypes, but increases (up to 10 mg g^{-1} FW), with 700, 400, 240, 120 and 85% increases for hybrid genotypes F30152, F3048, F3022, F3019 and F2548 respectively (Table 2).

The different parental and hybrid genotype regroupings based on the total soluble sugar contents obtained in the different conditions of treatment were achieved from hierarchical classifications at 95% homogeneity.

In the F25 family, five groups were distinguished when all conditions of treatment were taken into account. The first group consisted of hybrids F2552 and F2591, the second of hybrid F2563, the third of hybrid F2551and the parent ICS84, while the fourth and fifth groups consisted

Table 2. Mean total soluble sugars content (mg g^{-1} fresh weight) in healthy, wounded or *Phytophthora megakarya*-inoculated leaves of F30 and F25 hybrid families of *Theobroma cacao*.

Genotype	Healthy[a]	Wounded[a]	Inoculated[a]
Parents			
ICS84	9.82 ± 0.32c	5.59 ± 0.24c	5.94 ± 0.36d
ICS95	14.66 ± 1.04d	3.36 ± 0.64b	2.41 ± 0.34a
F30			
F30111	17.83 ± 0.17f	6.49 ± 0.74c	6.09 ± 0.35c
F30152	1.70 ± 0.53a	3.75 ± 0.64b	15.60 ± 1.21f
F3018	16.37 ± 1.88e	2.80 ± 0.18b	2.25 ± 0.28a
F3019	5.33 ± 0.98b	8.23 ± 0.39d	10.69 ± 0.21 e
F3022	4.71 ± 0.12b	11.13 ± 1.20 e	17.24 ± 0.51g
F3032	4.00 ± 0.37b	1.23 ± 0.19a	1.56 ± 0.20a
F3048	1.87 ± 0.40a	3.72 ± 0.52b	10.19 ± 0.70e
F3094	2.28 ± 0.20a	2.67 ± 0.21b	4.04 ± 0.76b
F25			
F2548	6.43 ± 0.69b	8.38 ± 0.77[e]	11.85 ± 0.35[e]
F2551	11.80 ± 0.97d	4.58 ± 0.46c	5.61 ± 0.56c
F2552	5.44 ± 0.38b	4.51 ± 0.58c	4.21 ± 0.23b
F2563	1.54 ± 0.15a	1.69 ± 0.13a	5.60 ± 0.24b
F2591	5.48 ± 0.73b	3.73 ± 0.29bc	3.74 ± 0.36b

[a]See Table 1.

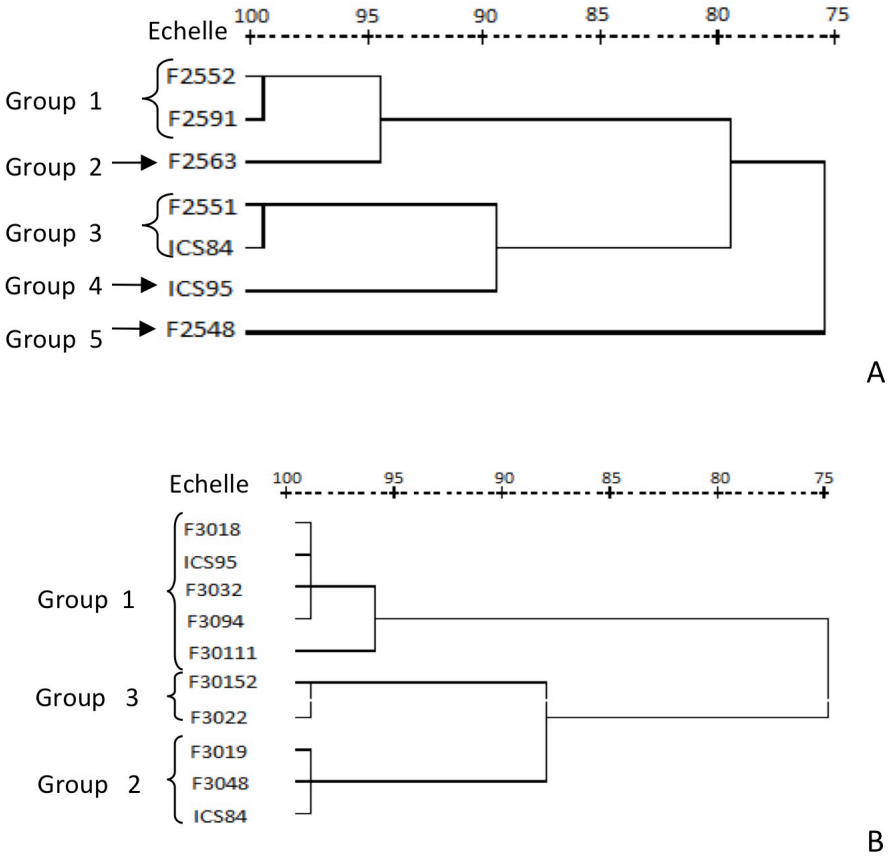

Figure 1. Hierarchical classification obtained with total sugar contents of ICS 84 and ICS 95 clones and F25 hybrid(A) and F 30 hybrid (B) families of *Theobroma cacao*.

of the parent ICS95 and the hybrid F2548 respectively (Figure 1 A).

In the F30 family, only three groups were distinguished considering all conditions of treatment. The first group comprise hybrids F3032, F3018, F30111 and F3094 as well as the parent ICS95. The second group composed hybrids F30152 and F3022, while the third contained hybrids F3048 and F3019 and the parent ICS84 (Figure 1B).

Variation of the amino acid contents

In healthy conditions, clone ICS84 (less susceptible) had significantly more amino acid (0.37±0.035 mg g^{-1} FW) than the susceptible clone ICS95 (0.18±0.03 mg g^{-1} FW). Injury and infection resulted to increases in amino acid contents of ICS84 and ICS95 clones by 26% and 179%, respectively in the case of infection (Table 3).

Among the hybrids of the F30 family, increased amino acid contents were significant in the genotypes F30152 (0.24±0.01 mg g^{-1} FW), F3019 (0.18 ± 0.06 mg g^{-1} FW) and F3022 (0.24±0.07 mg g^{-1} FW) in the healthy condition. Injury and infection gave increased amino acid contents of 67% in the genotypes. This increase was greatest in the genotypes F3019 (47%), F3022 (52%) and F3094 (133%) (Table 3).

Within the hybrids of the F25 family, amino acid contents were significantly greater in genotypes F2563 (0.6±0.05 mg g^{-1} FW), F2591 (0.39±0.08 mg g^{-1} FW) and F2548 (0.31±0.01 mg g^{-1} FW). In this family, infection was characterized

Table 3. Mean total amino acid content (mg g^{-1} fresh weight) in healthy, wounded and *Phytophthora megakarya*-inoculated leaves of F30 and F25 hybrid families of *Theobroma cacao*.

Genotype	Healthy	Wounded	Inoculated
Parents			
ICS84	0.37 ± 0.03c	0.50 ± 0.06f	0.49 ± 0.04e
ICS95	0.18 ± 0.03b	0.37 ± 0.04 e	0.51 ± 0.01e
F30			
F30111	0.10 ± 0.02a	0.13 ± 0.01b	0.14 ± 0.02ab
F30152	0.24 ± 0.02b	0.16 ± 0.02bc	0.054 ± 0.00a
F3018	0.06 ± 0.01a	0.04 ± 0.01cd	0.03 ± 0.01a
F3019	0.19 ± 0.01b	0.26 ± 0.01de	0.28 ± 0.02cd
F3022	0.24 ± 0.03b	0.32 ± 0.01b	0.35 ± 0.04d
F3094	0.09 ± 0.00a	0.20 ± 0.02a	0.21 ± 0.03bc
F25			
F2548	0.31 ± 0.01c	0.43 ± 0.03cd	0.42 ± 0.05d
F2551	0.08 ± 0.00a	0.14 ± 0.01a	0.17 ± 0.01b
F2552	0.21 ± 0.01b	0.10 ± 0.00a	0.09 ± 0.00a
F2563	0.60 ± 0.05d	0.56 ± 0.04 e	0.31 ± 0.03c
F2591	0.39 ± 0.05c	0.33 ± 0.04b	0.17 ± 0.03b

[a]See Table 1.

by decreased amino acid contents of 60% of the hybrid genotypes. This reduction was greater in F2563 and F2591.

Amino acid contents obtained from the different conditions of treatment were used to develop a hierarchical classification with groupings of the different progeny genotypes achieved at 95% of homogeneity. Four groups were distinguished from the F25 family with all conditions of treatments taken into account. The first group consisted of ICS95, ICS84 and hybrid F2548. This group was characterized by average amino acid content. The second group included on hybrid F2591. The third group, characterized by high amino acid content, included only hybrid F2563. The fourth group, characterized by low amino acid contents, include hybrids F2551 and F2552 (Figure 2A).

Hierarchical classification of individuals of the F30 family when all conditions of treatment were taken into account enabled the separation of three groups. The first consisted of parents (ICS95 and ICS84) with high amino acids contents. The second group included hybrids F3019 and F3022, average amino acid contents, and the third group, including hybrids F3052, F30111, F3094 and F3018, had low amino acid contents for all treatments (Figure 2B).

Thin layer chromatography (TLC)

The separation of crude extracts using thin layer chromatography for parental clones (ICS95 and ICS84) and families (F25 and F30), in the absence of all treatments, revealed the presence of three sugars, glucose, fructose and sucrose (Table 4). In conditions of injury and infection, sucrose did not occur in the less susceptible clone ICS84, but persisted in the fairly susceptible clone ICS95. For the hybrid genotypes in the absence of treatment, the separation of sugars also revealed the presence of the same three sugars in F30111, F3019

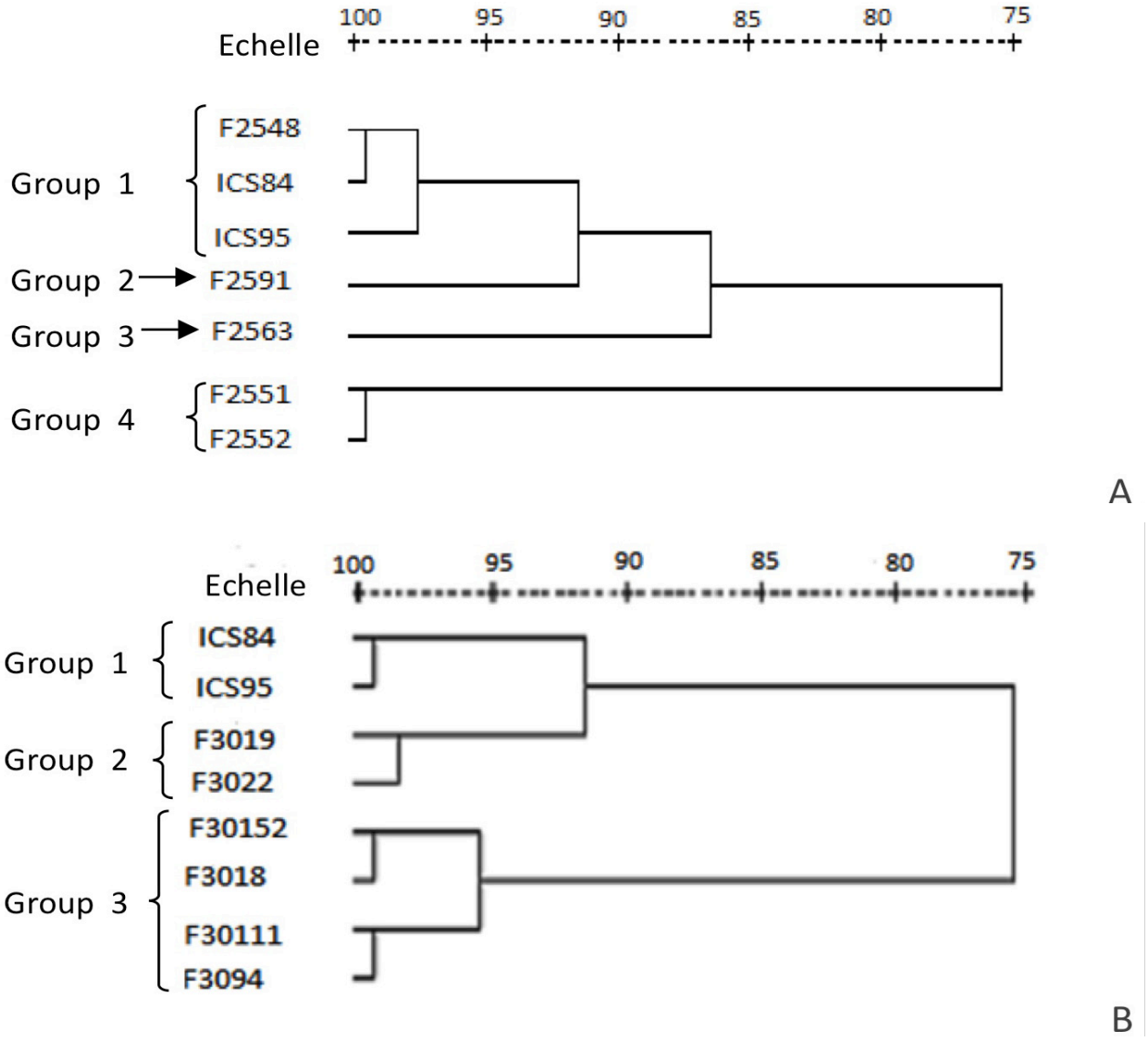

Figure 2. Hierarchical classification obtained with amino acid contents of ICS 84 and ICS 95 clones and F25 hybrid (A) and F 30 hybrid (B) families of *Theobroma cacao*.

and F2552 genotypes. Infection and injury lead to decreasesof sucrose in F30111 and F2551 genotypes, while sucrose persisted in genotypes F3019 and F2552 (Table 4).

The crude amino acid extracts in parental and hybrid genotypes under different conditions of treatment were separated on thin layer chromatography. Two amino acids (aspartate and cystein) were detected in the parental genotypes. In the less susceptible genotype ICS84 and the hybrids F3011, F2552 and F2551, proline was detected in conditions of injury and infection. The intensity of the spotlight obtained was greater in conditions of infection. In the mildly susceptible clone ICS95, conditions of infection and injury gave increases in the intensity of proline spotlight. In this clone, leucine appeared alongside, in the healthy condition and disappeared in conditions of injury and infection. Amino acids were also separated in four hybrid genotypes of these crosses. Aspartate was observed in

Table 4. Summary of thin layer chromatography analyses of sugars in healthy (H), wounded (W) and *Phytophthora megakarya*-inoculated (I) leaves of F30 and F25 hybrid families of *Theobroma cacao*.

Genotype	Treatment	Sugar[a]		
		Glucose	Fructose	Saccharose
ICS84	H	+	+	+
	W	++	-	-
	I	++	+	-
ICS95	H	+	+	+
	W	+	+	-
	I	+	+	+
F30111	H	+	+	+
	W	+	+	-
	I	+	+	-
F3019	H	+	+	+
	W	++	+	+
	I	++	+	+
F2552	H	++	+	+
	W	++	+	+
	I	++	+	+
F2551	H	+	-	+
	W	+	+	-
	I	+	+	-

[a] -, Absent; +, present; ++, increased intensity.

genotypes of F30111, F3019 and F2552 but absent in F2551 for all conditions of treatment. Cysteine and glycine were present in all hybrid genotypes studied in both healthy and injury conditions. In the infected condition, these two amino acids were deficient in the F3019 genotype. Proline is absent in all analyzed hybrid genotypes irrespective of the condition of treatment (Table 5).

Heritability and correlation

Heritability of total soluble sugar contents were estimated using the regression between the average contents in sugars of parents and those of the progeny. The calculated heritability (h^2) was 0.35 in the F25 family and 0.25 in the F30 family. There was a very weak relationship between sugar contents in the parental genotype and those of the progeny.

Free amino acid contents obtained in the different conditions of treatment in the parental and hybrid genotypes permitted estimation of the heritability within the limits of this character with respect to quantitative genetics. Heritability of the accumulated amino acids was weak for the different progeny genotypes ($h^2 = 0.14$ for F25 families and $h^2 = 0.27$ for F30 families). As was the case with sugars, there was a very weak relationship between amino acid contents of parents and those of progenies.

Levels of relationship between severity (length) of the necrosis, the total soluble sugars and amino acids as well as their statistical significance, were estimated. The PCA of these data permitted assessment of the different relationships, and es-

Table 5. Summary of thin layer chromatography analyses of amino acids in healthy (H), wounded (W) and *Phytophthora megakarya*-inoculated (I) leaves of F30 and F25 hybrid families of *Theobroma cacao*.

Genotype	Treatment	Amino acid [a]					
		Aspartate	Cystein	Glycine	Proline	Leucine	Tyrosine
ICS84	H	+	+	-	-	-	-
	W	++	+	-	-	+	-
	I	++	+	-	++	+	-
ICS95	H	+	+	-	+	+	+
	W	++	+	-	++	-	-
	I	++	+	-	++	-	-
F30111	H	++	+	+	-	-	+
	W	++	+	+	-	-	+
	I	++	+	+	++	-	+
F3019	H	++	+	+	-	-	-
	W	++	+	+	-	-	-
	I	++	-	-	-	-	-
F2552	H	++	+	+	-	-	-
	W	++	+	+	-	-	-
	I	++	+	+	++	-	-
F2551	H	-	+	+	-	-	-
	W	-	+	+	-	-	+
	I	-	+	+	+	-	-

[a] See Table 4.

tablishment of different genotype distributions in relation to the valued parameters. A significant positive relationship was observed between amino acid contents and length of necrosis. This relationship was 0.857 and 0.418 respectively for F25 and F30 families. Genotypes with high amino acid contents in infected conditions developed severe necrosis. On the other hand, a significant negative relationship was observed between amino acids and phenolic compounds (-0.525 at $P<0.001$) (Figure 3). The relationships were not statistically significant between sugar content and severity of necrosis, and content of sugars and phenolic compounds.

Discussion

Total soluble sugars were analyzed in leaves of the parental and hybrid clones of *T. cocoa* in different conditions of treatment. The main results show a reduction in soluble sugar contents of parental genotypes ICS84, ICS95 and about 30% reduction in the hybrid genotypes under conditions of infection by *P. megkarya*. These results are in accordance with those of Kabsch (1982) who reported that under infection conditions, there was a reduction of glucose and fructose contents in cucumber leaves. Similarly, Abood and Lösel (2003) demonstrated reduction of hexoses in infected cu-

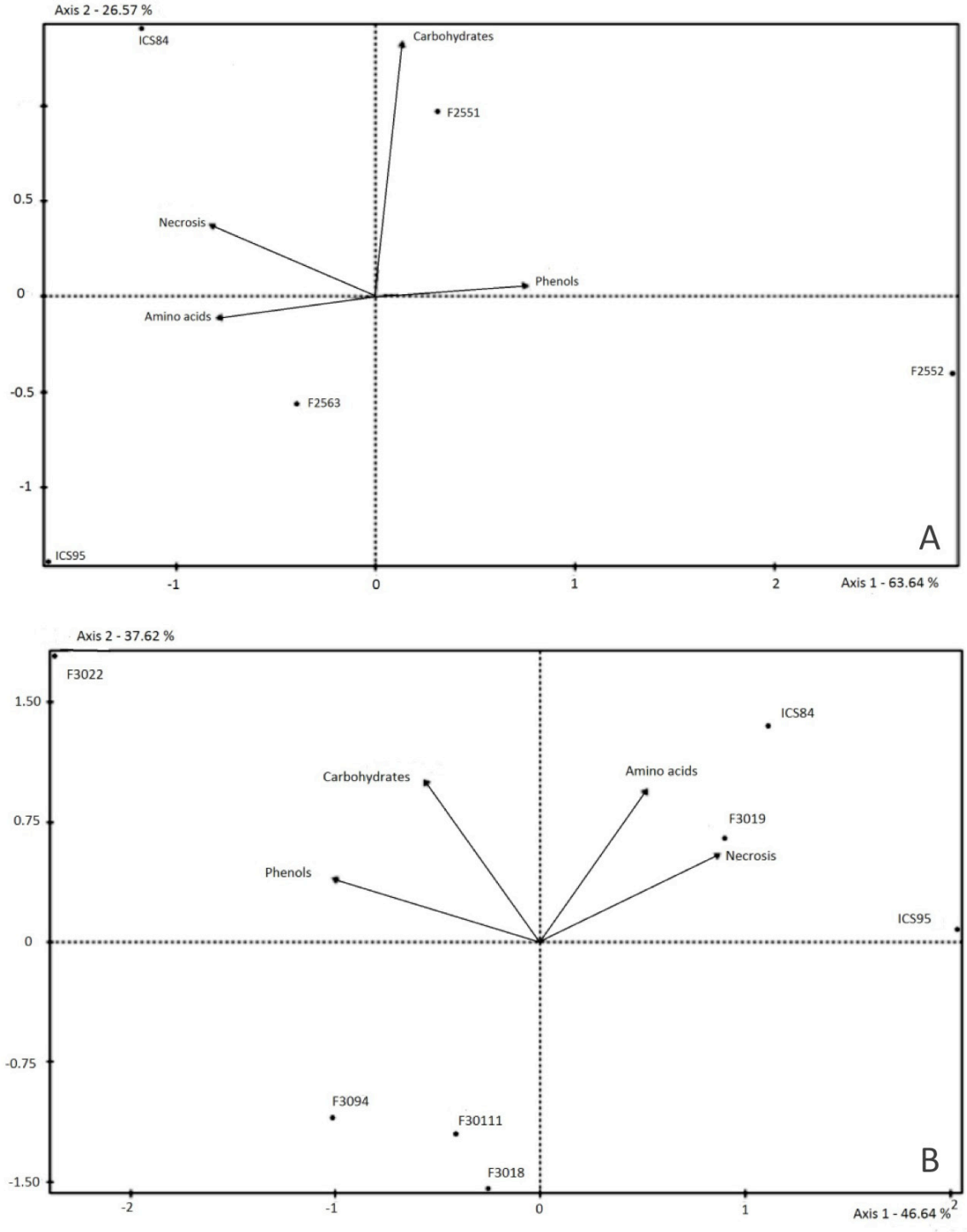

Figure 3. Principal component analyses obtained from severity of necrosis and metabolite contents from F25 (A) and F30 (B) families of *Theobroma cacao*.

cumber leaves 3 days after inoculation. However, the trend of variation of the total soluble sugar contents in plants after infection was quite variable. Andrew *et al.,* (2005) showed that the infection did not affect the sugar content of leaves of turnip and *Arabidopsis*. On the other hand, accumulation in total soluble sugar after infection has been reported in tobacco (Shalitin *et al*., 2002), papaya (Guthrie *et al*., 2001), cucumber (Técsi *et al*., 1994; Shalitin and Wolf, 2000) and potato (Sindelarova *et al*., 1999). Soluble carbohydrates may be involved in host metabolism associated with the synthesis of various defence chemicals such as phenolics, phytoalexins, phytoanticipin compound and lignin

Altering the sugar content of leaves has been shown to be a possible way to control diseases (Lukens, 1970). Specific carbohydrates like sucrose, glucose and galactose have been correlated with disease resistance in some plant-pathogen interactions. Qualitative analysis of the sugars has shown that most cases of infection are characterized by the disappearance of sucrose and the persistence of glucose. This result is in agreement with the works of Evers *et al*. (2003) and Omokolo and Boudjeko (2005) who documented a possible relation between carbohydrate content and host resistance. In the present study, sucrose persisted under infection conditions in the genotypes F3019, F2552 and ICS95. Thus, the role of the carbohydrate composition and its alteration during infection by *P. megakarya* still not fully resolved.

Values of the heritability within the limits of the accumulation of soluble sugars in the two reciprocal crossings were not significant. This suggests the absence of a maternal heritability. Based on this fact, the heritability of the character might be nuclear and non- cytoplasmic. Soluble sugars could be implicated in varied ways in the mechanisms of plant defence against pathogens such that their level of heritability becomes weak. This makes it difficult to exploit soluble sugar content as a criterion for effective genotype detection in relation to resistance against cocoa pod rot.

Free amino acids were analyzed in leaves of the parental and hybrid clones of *T. cocoa* under different conditions of treatment. Generally, the infection was characterized by increases in amino acids contents. These results are similar to those obtained by Lepka *et al*. (1999) showing that in infected tobacco leaves, amino acid contents were greater than those of the healthy leaves. Free amino acids are important indicators of the plant conditions, arising as a consequence of protein degradation in tissues under programmed cell death (Scarpari, *et al*. 2005). The increase in amino acids under infection conditions may also indicate pathogen effects on molecular transportation via phloem tissues. Indeed, inhibition of metabolite translocation due to the presence of pathogen in infected plants has been described by Guthrie *et al*. (2001) in papaya and by Maust *et al*. (2003) in coconut.

A significant negative relationship was observed between the severity of necrosis and content of phenolic compounds. Furthermore, it is well known that amino acids are precursors of synthesis pathways of phenolic compounds (Sticher *et al*., 1997; Djocgoue *et al*., 2007). Our results indicate indirect implication of amino acids in the defence mechanism of cacao. Besides, these amino acids could be used for the *de novo* synthesis of protein that might contribute to the inhibition of the development of fungi (Cui *et al*., 2000).

Qualitative analysis of amino acids by thin layer chromatography revealed that accumulation of some soluble amino acids is genotype dependent, or could be due to infection. Nemec (1995) showed an accumulation of amino acids in cirus after infection by *Fusarium solani*. This leads to the suggestion that, in the *T. cacao/P. megakarya* interaction, the induction of the defence mechanism is not characterised by a single metabolic signal, in spite of the modification of certain gene expression (Bailey *et al*., 2005). In the tolerant parental clone ICS84, proline appears solely in infected leaf tissue, suggesting this compound is implicated in the defence mechanism in *T. cacao/P. megakarya* interaction. Similar results were reported by Omokolo *et al*. (2002) after cocao pod infection. Otherwise, no significant difference was observed between values of the narrow sense heritability of amino acids contents in two reciprocal crossings. Under infection and injury conditions, very few genotypes had hybrid vigour for the amino acid content. Besides, the heritability values in the strict sense of this character were very low. A relationship was observed between content in amino acids of parents and progenies.

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