Biochemical diversity in the genus *Coffea* L.: chlorogenic acids, caffeine and mozambioside contents

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Summary

The extent and nature of biochemical diversity in green coffee beans was established by a large sampling of genus Coffea, representing nine species from the Guineo-Congolian region, nine species from east Africa and seven species from Madagascar. HPLC analyses were used to determine the contents of caffeine, chlorogenic acids and mozambioside . Data processed by principal component analyses showed the existence of two metabolic pathways. One leads to the synthesis of small quantities of chlorogenic acids ($\lt 2.5\%$ dmb) and caffeine ($< 0.3\%$ dmb) while the other leads to large concentrations of chlorogenic acids ($> 4.5\%$ dmb) and caffeine $(>0.4\%$ dmb). Their distribution in the genus *Coffea* is discussed in relation to the biogeographic origin of the plant material.

Introduction

The genus Coffea L. embraces about a hundred species which present great genetic diversity structured in three biogeographic areas: Madagascan floristic region (Chevalier, 1947), east Africa (Bridson & Verdcourt, 1988) and the Guineo-Congolian region (Lebrun, 1941; Chevalier, 1947). Some species, such as C. canephora Pierre and C. liberica Hiern, are widely distributed, from Guinea to east Zaire. Other species are confined and have specific ecological adaptations : for example, C. humilis A. Chev. to ombrophilous forest in western Côte-d'Ivoire and C. congensis Froehner to the banks of the Congo (Zaire) river which are liable to flooding and its tributaries in central Africa. All species are diploid $(2n = 22)$, except the allotetraploid C. arabica L. which occupies the southern Abyssinian plateau. This species and a diploid taxon, C. sp. Moloundou recently discovered in central Africa (Anthony et al., 1985; de Namur et al., 1987), are distinguishable by their self-fertility.

Genetic results show that the limits of species or groups of species defined by taxonomists do not always coincide with the barriers encountered in interspecific hybridizations (Berthaud & Charrier, 1988). Several traits, other than macro-morphological descriptors, have been used to analyse the diversity of this multispecific complex: e.g. morpho-phenology (Charrier, 1978; Berthaud, 1986; Anthony, 1992), pollen (Lobreau-Callen & Leroy, 1980) and isozymes (Berthou et al., 1980; Berthaud, 1986; Anthony, 1992). The caffeine content (Charrier & Berthaud, 1975) and some physico-chemical characteristics of coffee beans (Santa Ram et al., 1982) have also been studied on a restricted number of coffee species. The taxonomic interest of biochemical components, such as chlorogenic acids and caffeine, has already been established (Clifford et al., 1989).

The presence of chlorogenic acids and caffeine in coffee beans has been known since the beginning of the XIXth century. The chlorogenic acids are the most widely distributed conjugates of the hydroxycinnamic aids (Chassevent, 1969). They accumulate in the seeds with fruit maturity (Clifford & Kazi, 1987) and they contribute to fruit resistance against biological and mechanical stress (Macheix et al., 1990) and to the beverage quality (Clifford, 1985) . Caffeine is a methylxanthine synthesized in small quantity in coffee leaves and transported to fruits and seeds where its accumulation increases with the maturation (el Hamidi & Wanner, 1964; Baumann et al., 1976). It produces various physiological effects on the human body, in particular the stimulation of the central nervous system. This component also has an influence on the beverage quality (Macrae, 1985).

Our biochemical study of diversity is based on a large sampling of the genus Coffea. The analysed characteristics are the major individual chlorogenic acids, caffeine and mozambioside, a new diterpene glycoside recently detected in C. pseudozanguebariae Bridson beans by Prewo et al. (1990). Its structure is similar to mascaroside found in C . $viannevi$ J. F. Leroy, a Madagascan species (Ducruix et al., 1977). The organization of the diversity is discussed in relation to the biogeographic origin of species .

Materials and methods

Materials

The material analysed consisted of dried seeds supplied from the living collections of coffee resources centres in Côte-d'Ivoire, Madagascar, Tanzania and Brazil . Some samples have been placed in the herbarium of the Royal Botanic Gardens (Kew, United Kingdom). In total, 68 samples belonging to 25 Coffea species have been analysed :

- -22 samples representing 9 species originated in central and west Africa,
- -39 samples representing 9 species originated in east Africa,

-7 samples representing 7 species originated in Madagascar.

All samples were from spontaneous forms, except C. arabica which is represented by cultivars and mutants. Psilanthopsis kapakata A. Chev. was the only species of the genus Psilanthopsis described by Chevalier (1939). Sound arguments based on morphological (Bridson, 1982; Leroy, 1982), cytogenetical (Carvalho & Monaco, 1959; Louarn, 1982) and biochemical studies (Santa Ram et al., 1982) suggest its assignment to genus Coffea subgenus Coffea and explain its inclusion in this study.

Analytical methods

The methods and the equipment for $HPLC¹$ analyses have been described by Clifford et al. (1989) . Contents are recorded on percentage of dry mass basis (% dmb). When the sample analysed consisted of only one or two seeds from herbarium specimens a 10% moisture content was assumed because of their higher dehydration. The lower limits of integration are 0.04% for 5-caffeoylquinic acid and 0.02% for caffeine, and these values have been recorded for each component visually detectable on the chromatograms at concentrations less than the lower limit of integration.

Data coding and analysis

For the known chlorogenic acids, the contents of 3-CQA, 4-CQA and 5-CQA are cumulated in the total content of caffeoylquinic acids (CQA) . In the same way, the sum of 3,4-diCQA, 3,5-diCQA and 4,5-diCQA contents gives the total content of dicaffeoylquinic acids (diCQA). In contrast, the content of feruloylquinic acids (FQA) is based only on 5-FQA. Among the 276 nm-absorbing components, only two were observed at concentrations sufficient for the data to be used in the statistical analysis : caffeine (CAF) and mozambioside (MOZ).

The data table comprises 68 samples and five quantitative variables representing the contents of CQA, diCQA, FQA, CAF and MOZ (Appendix 1). The total content of chlorogenic acids is reported in a calculated variable, CGA. The global

^{&#}x27;High Performance Liquid Chromatography

Fig. 1. Representation of the 68 samples on the plane 1-2 of the P.C.A. The first axis corresponds to a decrease in the contents of caffeine, dicaffeoylquinic and feruloylquinic acids . The second axis constitutes a ranking on the basis of mozambioside content .

diversity is described from a principal component analysis (P.C.A.) with standardized variables (Hottelling, 1933). This analysis permits the data to be summarized as a reduced number of independant factors. The variability of species represented by at least four samples, i.e. C. arabica, C. canephora, C. eugenioides S. Moore and C. sessiliflora Bridson, is compared by Bartlett's test (Bartlett, 1937) . Although in total six samples of C. stenophylla G. Don have been analysed, these have differed markedly in composition and been assigned by P.C.A. to two different subgroups within group A (see results). Accordingly it is inappropriate to analyse the within species variability by Bartlett's test.

Results

General diversity of the genus Coffea

The principal component analysis using the contents of 68 samples explains, in a statistical sense, 80% of the variability by two factors $(Fig. 1)$. The first factor separates the samples according to their contents of caffeine, dicaffeoylquinic and feruloylquinic acids. The second factor constitutes a ranking on the basis of mozambioside content.

These two independant factors provide evidence for the existence of three groups A, B and C, for which biochemical characteristics are summarized in Table 1. Group A embraces 15 species² with large contents of caffeoylquinic and dilarge contents of caffeoylquinic and di-
caffeoylquinic acids $(CGA > 4.5\%$ dmb) and caffeoylquinic acids $(CGA > 4.5\%$ dmb) caffeine $(>0.4\%$ dmb). The mean contents are at

 2 Group A: C. arabica, C. brevipes, C. canephora, C. congensis, C. eugenioides, C. humilis, C. liberica, C. mufindiensis, C. racemosa, C. sessiliflora, C. stenophylla, C. sp. F, C. sp. Moloundou, C. sp. Nkoumbala, Psilanthopsis kapakata

acids (CGA), caffeoylquinic (CQA), dicaffeoylquinic (diCQA), feruloylquinic (FQA) acids, caffeine (CAF) and mozambioside (MOZ)								
Components	CGA	COA	diCOA	FOA	CAF	MOZ		
Group A	$4.53 - 9.90$	$2.84 - 9.76$	$0.04 - 1.68$	$0.04 - 1.03$	$0.46 - 3.19$	$0.00 - 0.07$		
$(N = 56)$	6.79	5.61	0.71	0.34	1.47	0.02		
Group B	$0.93 - 2.41$	$0.80 - 1.95$	$0.00 - 0.18$	$0.06 - 0.16$	$0.02 - 0.28$	$2.28 - 2.71$		
$(N = 4)$	1.72	1.43	0.11	0.10	0.10	2.48		
Group C	$0.14 - 1.40$	$0.14 - 1.19$	$0.00 - 01.20$	$0.00 - 0.04$	$0.00 - 0.09$	$0.00*$		
$(N = 8)$	0.88	0.85	0.03	0.01	0.04	0.00		

Table 1. Biochemical characterisation of the three diversity groups: minimum, maximum and mean (% dmb) of contents of chlorogenic

*Mozambioside content in C. vianneyi is not reported because it corresponds to mascaroside (see footnote to Appendix 1)

least four times the corresponding mean values for the two other groups. The feruloylquinic acid content appears less discriminant. In contrast, the species of groups B and C present small contents of total chlorogenic acids $(<2.5\% \text{ dm b})$ and caffeine ($< 0.3\%$ dmb). Group B is formed by two species: $C.$ pseudozanguebariae and $C.$ salvatrix Swynn. & Phil., characterized by a very large mozambioside content ($> 2.2\%$ dmb). The species³ of group C have no mozambioside . The species $C.$ vianneyi is peculiar: it contains mascaroside which unexpectedly has the same retention time as mozambioside in our experimental conditions (Rakotomalala, personal communication). In addition, the species of this group C have less than half the total chlorogenic acids observed in group B. The biochemical composition of green beans from group C is very poorly defined at present, and it is far from clear what replaces the chlorogenic acids and caffeine.

Diversity of species with large contents of chlorogenic acids and caffeine (group A)

The diversity within group A has been studied thoroughly with a new principal component analysis (Fig. 2). The sense of the first factor is not modified. Most of the species from central and west Africa have large contents of caffeine, dicaffeoylquinic and feruloylquinic acids, in contrast to species from east Africa. The species C. *arabica* takes an intermediate position. The second factor represents a gradient of represents caffeoylquinic acids content. For the east African

³Group C: C. farafanganenis, C. homollei, C. perrieri, C. rhamnifolia, C. tetragona, C. vatovavyensis, C. vianneyi, C. sp. A315

species, this gradient separates C . mufindiensis Bridson, C . sp. F Bridson and two samples of C . sessiliflora which are very rich in caffeoylquinic acids.

Two Guineo-Congolian species cannot be placed with other species from the same geographic group: P. kapakata originated in Angola and C. stenophylla is endemic to western Africa. The C. stenophylla samples are separated according to
their source: the samples supplied from supplied Côte-d'Ivoire contain twice as much chlorogenic acids and caffeine as those taken from herbaria or supplied from Brazil and Tanzania.

The within species variability of caffeoylquinic acids content differs in a highly significant manner between species (Table 2): e.g. C. sessiliflora $(\sigma = 2.15)$ is four times as variable as C. arabica $(\sigma = 0.58)$. Highly significant differences also exist for caffeine content but these variations are linked to between-species differences of the mean . The smaller variations of C . arabica could have been induced by the very restricted genetic basis of the sampling.

Discussion

The study of the biochemical composition in green beans of 25 species from the genus Coffea reveals the existence of two metabolic pathways . One leads to the synthesis of small quantities of chlorogenic acids $(<2.5\%$ dmb) and caffeine $(<0.3\%$ dmb) while the other leads to significant concentrations of chlorogenic acids $(>4.5\%$ dmb) and caffeine $(>0.4\%$ dmb). The link between caffeine and chlorogenic acids is well known in coffee trees (Navellier, 1959, Martin et al., 1987). In essentially

Fig. 2. Representation of the 15 species of group A on the plane 1-2 . The first axis corresponds to a decrease in the contents of caffeine, dicaffeoylquinic and feruloylquinic acids . The second axis constitutes a gradient of caffeoylquinic acids content .

caffeine-free samples, such as most Madagascan species, the chlorogenic acids are present at very low concentrations; the dicaffeoylquinic and feruloylquinic acids are generally absent. On the other hand, the large contents of dicaffeoylquinic and feruloylquinic acids are only found in species which contain at least 0.6% dmb of caffeine.

The first metabolic pathway belongs to all analysed species from Madagascar and to three species from east Africa (Fig. 3). In Madagascan species, the absence of caffeine has been known for a long time (Bertrand, 1901) and the total chlorogenic acids content is very small $\left($ < 1.5% dmb). However it must be noted that we have examined only 7 of the 50 species reported by botanists and it is now clear that they are not representative of the Madagascan diversity group (Clifford et al., 1991; Rakotomalala et al., 1992). In east Africa,

Table 2. Characteristics of species represented by at least four samples: mean (X in % dmb), standard deviation (σ in % dmb) and coefficient of variation (CV in %). For abbreviations see Table 1.

Species	COA			diCOA		FOA		CAF				
	X		CV		σ	$_{\rm CV}$	x	σ	CV			CV
C. canephora $(N = 6)$	5.62	1.11	19.8	1.29	0.30	23.3	0.89	0.10	11.2	2.53	0.48	19.0
C. arabica $(N = 21)$	5.26	0.58	11.0	0.64	0.15	23.4	0.31	0.06	19.4	1.42	0.30	21.1
C. sessiliflora $(N = 4)$	7.62	2.15	28.2	0.16	0.12	75.0	0.13	0.04	30.8	0.53	0.10	18.9
C. eugenioides $(N = 4)$	4.53	0.79	17.4	0.23	0.10	43.5	0.21	0.10	47.6	0.58	0.10	17.2

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Fig. 3. Distribution of the biochemical diversity in genus Coffea.

C. pseudozanguebariae has been the first caffeinefree species discovered on mainland Africa (Hamon et al., 1984). The biochemical peculiarity of C. salvatrix beans had been observed by Santa Ram et al. (1982). The profiles of these two species, obtained on chromatograms, suggest the existence of affinities, indeed a common origin, with the Madagascan species. Leroy (1982) had previously expressed the hypothesis that coffee trees from Kenya and Madagascar might be derived from a common origin centre. The mozambioside synthesis could be the result of a separated evolution on the African continent. According to Prewo et al. (1990), this diterpene glycoside is as bitter as caffeine and could play a similar role in secondary plant metabolism. The third species which presents affinities with Madagascan species is C. rhamnifolia (Chiov.) Bridson which is endemic to the coastal plains of Somalia . As a matter of fact, the analysed sample is almost chlorogenic acids-free (only 0.14% dmb of caffeoylquinic acids) and caffeinefree (0.06% dmb). It could be a relict form as proposed by Leroy (1982) for other coffee trees with sympodial development.

The second metabolic pathway producing large quantities of chlorogenic acids and caffeine is present in other east African species and in all Guineo-Congolian species. The contents of caffeine, dicaffeoylquinic and feruloylquinic acids form a gradient as the geographic location progresses through Africa, from east to west. However, two species collected from central Africa are classified with east African species because of their small contents: P . kapakata and C . sp. Moloundou. From the observation of taxonomic
criteria. Bridson (1982) and Leroy (1982) Bridson (1982) and Leroy (1982) concluded that P . kapakata coffee trees, growing in the south of Luanda (Angola), have many affinities with coffee trees from east Africa. Their history could be connected with the migratory track described by White (1981, 1990) for some mountain taxa during arid Pleistocene periods. This track follows the southern side of the Congo basin, at an altitude often above 1.000 m, then passes along the atlantic coast up to Mount Cameroun. The species C . sp. Moloundou has been discovered near the frontier between Cameroun and Congo (Anthony et al., 1985; de Namur et al., 1987). The morphological observations made in a collection in Cote-d'Ivoire confirm its resemblance to east African species. This second metabolic pathway also exists in Madagascar. Clifford et al. (1991) have recently detected the presence of chlorogenic acids (6 .6% dmb) and caffeine $(0.7\%$ dmb) in the Madagascan taxon C. kianjavatensis J. F. Ler.. According to Rakotomalala et al. (1992), two other Madagascan taxa, C. lancifolia A. Chev. and C. sp. $A801$, present similar characteristics .

On the African mainland, the distribution of biochemical diversity coincides with species distribution: there is a discontinuity in the rift region, in the east of Zaire. According to White (1979), similarities between the east African and the Guineo-Congolian flora are rare in total: 10 .1 % near Lake Victoria and only 2 .4% near the

coast. The evolution in these two environments separated since the rift formation, about 30 million years ago, has not modified qualitatively the biochemical composition of coffee beans, but it has generated quantitative variations in the contents of caffeine, dicaffeoylquinic and feruloylquinic acids . The synthesis of these compounds is probably limited by the rapidity of fruit development in east Africa: 1.5 to 4 months, except for C. arabica and C. eugenioides, contrast to 7 to 15 months in the Guineo-Congolian region.

Conclusions

This study of variations in biochemical composition of green coffee beans has been focused on the genus Coffea, and particularly the subgenus Coffea. It has shown that the diversity in composition can be marked for species endemic to east Africa and Madagascar, and has given new insights to the classification of species endemic to the African continent. In order to fully understand the interrelationships with this multispecific complex, it is necessary to extend the study to species of Coffea subgenus Paracoffea (Leroy) Leroy, which are specifically adapted to dry climates, and the closely related genus Psilanthus Hook. f. which is found in Africa, south east Asia and Oceania.

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Appendix 1

Data table

Appendix 1. Data table (for abbreviations see Table 1)

Continued overleaf

 $Appendix$ 1. Data table continued

Code	Identification	Source	CQA	diCQA	FOA	CAF	MOZ
SES ₃	C. sessiliflora Bridson	Côte-d'Ivoire	9.76	0.04	0.13	0.65	0.00
A315	C. sp. A315	Madagascar	1.12	0.00	0.00	0.02	0.00
MOLO	C. sp. Moloundou	Côte-d'Ivoire	2.84	1.68	0.13	0.62	0.00
NKOU	C. sp. Nkoumbala	Côte-d'Ivoire	6.70	1.62	0.41	2.00	0.00
CSPF	$C.$ sp. F Bridson	Côte-d'Ivoire	8.99	0.47	0.11	0.99	0.00
STEO	C. stenophylla G. Don	Côte-d'Ivoire	7.66	0.70	0.28	2.07	0.00
STEI	C. stenophylla G. Don	Côte-d'Ivoire	5.94	0.56	0.34	2.64	0.00
STE ₂	C. stenophylla G. Don	Brazil	6.04	0.46	0.16	1.53	0.04
STE3	C. stenophylla G. Don	Kew	5.41	0.53	0.10	1.50	0.04
STE4	C. stenophylla G. Don	Tanzania	4.62	0.24	0.19	0.62	0.04
STE ₅	C. stenophylla G. Don	Côte-d'Ivoire	7.59	1.63	0.28	2.07	0.00
TETR	C. tetragona Jumelle & Perrier	Madagascar	1.19	0.20	0.01	0.03	0.00
VATO	C. vatovavyensis J. F. Ler.	Madagascar	0.96	0.00	0.00	0.02	0.00
VIAN	C. vianneyi J. F. Ler.	Madagascar	0.78	0.00	0.00	0.05	$0.55*$
KAPA	<i>Psilanthopsis kapakata A. Chev.</i>	Tanzania	4.11	0.61	0.34	1.04	0.07

*Content in C . vianneyi reported as mozambioside corresponds to mascaroside which unexpectedly has the same retention time in our experimental conditions (Rakotomalala, personal communication)