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Change in amino acids content during germination and seedling growth of *Cola* sp.

Pierre Effa Onomo[1, 2], Nicolas Niemenak[2]*, Denis Omokolo Ndoumou[2] and Reinhard Lieberei[3]

[1]Department of Biochemistry, Faculty of Sciences, University of Yaounde I, P.O Box 812, Yaounde, Cameroon.
[2]Laboratory of Plant Physiology, Department of Biological Sciences, Higher Teachers' Training College, University of Yaounde I, PO Box 47, Yaounde, Cameroon.
[3]Biozentrum KleinFlottbek, Abteilung Nutzpflanzenbiologie, University of Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany.

Accessions of *Cola acuminata* and *Cola anomala* were analysed based on the variation of free amino acids during germination and seedling growth. These compounds were determined by high performance liquid chromatography (HPLC) methods. In the non-germinated seeds of *C. acuminata*, arginine (Arg) was the major amino acid found in all six accessions and valine (Val) was the least present, while in *C. anomala*, gamma (γ)-aminobutyric acid (GABA) was predominant and isoleucine (Ile) was less abundant. During germination, the seeds of the two species accumulated large amount of GABA and very little tryptophan. Principal component and cluster analyses of *Cola sp.* categorized the accessions into two groups distinguishing the two *Cola* species. The differences between the groups were related not only to the absence/presence of histidine (His) and phenylalanine (Phe), but also to amide amino acids asparagine (Asn), glutamine (Gln) and Arg contents. In *Cola sp.*, free amino acids varied significantly during these two processes indicating their high utilization. These metabolites can be considered as crucial in the control of germination.

Key words: *Cola* sp., amino acids, germination, seedling growth.

INTRODUCTION

Cola is a perennial genus which belongs to the Sterculiaceae family. Known as non-timber forest products, *Cola* is cultivated by subsistence farmers in association with cocoa and/or coffee as a shade plant. *Cola* nuts are consumed at fresh or fermented state for their excitant properties. The popularity of this crop resulted from its reputation as a stimulant, increasing energy and strength, dispelling drowsiness and staying off hunger (Morton,

1992). These properties could be attributed to the richness of the seeds in purine alkaloids, polyphenols and sugars (Niemenak et al., 2008). The seeds are used in the production of several pharmaceutical drugs, wines and liquors (Oladokun, 1982; Leung and Foster, 1996; Blancke, 2001).

In spite of this importance, the cultivation of *Cola* sp. has decreased due to difficulty encountered in the germination of the seeds as well as the slow development of the seedlings. The poor germination of *Cola* seeds is attributable to their high content in polyphenols. It was described by Obeng and Brown (1997) that *Cola* seeds contain more starch and glucose, but the status of amino acids in *Cola* is not yet described. The recalcitrance of *Cola* seeds for germination could be investigated by the mobilization of their reserves during metabolic process.

Mobilization of reserves has been described as a crucial process that controls germination (Eastmond et al., 2000; Finkelstein and Lynch, 2000; Eastmond and

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*Corresponding author. E-mail: niemenak@yahoo.com. Tel: 237 77 66 13 98.

Abbreviations: HPLC, High performance liquid chromatography; **GABA,** γ-amino-butyric acid; **BSA,** bovine serum albumin; **PVP,** polyvinylpyrrolidone; **PVPP,** polyvinyl-polypyrrolidon; **OPA,** O-phthalaldehyde; **NEAA,** non essential amino acids; **EAA,** essential amino acids; **PC,** principal component.

Table 1. Geographic distribution of *Cola* sp. accessions studied.

Species	Number	Origin	Longitude	Latitude
C. acuminata	01	Zoaetele	11°53'	3°15'2"
	02	Ombessa	11°15'3"	4°36'
	03	Okola	11°23'3"	4°1'1"
	04	Mbangassina	11°24'	4°33'3"
	05	Bokito	6°4"	4°34'2"
	06	Sangmelima	11°48'	2°38'
C. anomala	01	Bafang	10°11'	5°29'
	02	Bangoua	10°29'	5°12'
	03	Bamenda	10°10'	5°47'
	04	Foumbot	10°38'	5°30'
	05	Dschang	10°3'	5°27'
	06	Mamfe	9°19'	5°45'2"

Graham, 2001; Pritchard et al., 2002). In particular, mobilization of nitrogen reserves as a source of energy and nutrients to supply expanding new tissues is proposed to take part in the control of germination (Garciarrubio et al., 1997).

Several analytical works based on ^{14}C labelling showed that amino acids in germinating seeds contribute a large amount of carbon substrate to the respiratory system and sugar synthesis, as shown in neoglucogenic oilseeds such as castor bean (Lea and Joy, 1983). Considerable inter-conversion of amino acids occur following storage-protein hydrolysis in germinating seeds (Lea and Joy, 1983). This process contributes to the production of a complete spectrum of amino acids that can be used either as protein building blocks or precursors for key metabolites, and that can play determinant roles in the control of germination and seedling development (Gallardo et al., 2002).

Reportedly, GABA (γ-amino-butyric acid) levels in plants are enhanced during germination and high stress conditions, such as mechanical stimulation, hypoxia, cytosolic acidification, water, darkness, and drought (Serraj et al., 1998; Snedden and Fromm., 1998; Shelp et al., 1999). GABA may have a role in pH regulation (Bown and Shelp, 1997), nitrogen (N) storage (Selman and Cooper, 1978), plant development (Baum et al., 1996), and in the plant's defense against phytophagous insects (Ramputh and Bown, 1996). Recently, there has been an increased interest in the utilization of GABA as a bio-active plant component.

Cola seeds are distinguished from other Sterculiaceae by their especially high level of polyphenols, alkaloids and sugars content and are therefore an important source of vegetable alkaloid in both food and industry. The content of total amino acids including protein amino acids and non-protein amino acids (especially GABA) in *Cola* sp. is not known.

The aim of this study is therefore to determine changes in amino acids content during germination and seedling growth in two *Cola* species in order to know if these metabolites can be crucial in the control of germination. High performance liquid chromatography (HPLC) method was used to obtain the most accurate evaluation of the amino acids in seeds and seedlings. Chemotypic variation between *Cola acuminata* and *Cola anomala* based on free amino acids were also undertaken.

MATERIALS AND METHODS

Plant materials

Mature fresh seeds of six accessions of *C. acuminata* ((Pal. De Beauvois) Schott and Endlicher), and six of *C. anomala* (Schott and Endlicher) were harvested randomly from trees which were at least 10 years old from different sites in Cameroon (Table 1). These accessions belong to those which were found more recalcitrant for germination in Cameroonian *Cola* germplasm in a previous study (Effa, 2007). 20 seeds for each accession were then collected and morphometric parameters (size, weight, cotyledon number) and colour were evaluated. Thereafter, red seeds of 5 cotyledons for both *C. acuminata* and *C. anomala* with weight between 15 to 20 g were used for germination. The seeds were kept in moist condition in a cold room for 8 days to activate the process of germination. Germination is considered by the emergence of radicle. The seedlings were harvested after 15 days of germination. In this study, cotyledons and seedlings axes were separated. Non-germinated seeds, germinated seeds and seedlings were analysed at Biozentrum KleinFlottbek, University of Hamburg, Germany where upon arrival, they were lyophilised and stored at -20°C.

Chemicals

All solvents used were of analytical grade purchased from Merck (Darmstadt, Germany). Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Tetrahydrofuran was from Lichrosolv ®, gradient grade. The solution degasser consisted of the Degassex DG-4400 from Phenomenex. Other common reagents used were of HPLC grade otherwise stated.

Determination of sugars and amino acids content

Sugars and amino acids were extracted from boiling ethanol 80°C

Table 2. Proteins, amino acids and sugars in mature seeds (I), germinated seeds (II) and seedlings (III) of six accessions of *Cola acuminata* (mg/g of dry wt ± S.D.).

Parameters	Zoaetele	Okola	Ombessa	Sangmelima	Bokito	Mbangassina
Proteins						
I	1.58±0.18[a]	1.85±0.21[a]	1.22±0.23[a]	1.29±0.14[a]	1.55±0.22[a]	1.44±0.19[a]
II	1.2 5±0.12[a]	1.63±0.17[a]	1.03±0.14[a]	1.05±0.16[a]	1.20±0.11[a]	1.22±0.18[a]
III	1.12±0.16[a]	1.46±0.21[a]	1.01±0.19[a]	1.88±0.16[a]	1.05±0.14[a]	1.12±0.15[a]
Amino acids						
I	2.51±0.23[a]	2.19±0.20[a]	2.30±0.24[a]	2.45±0.25[a]	2.71±0.19[a]	2.48±0.31[a]
II	3.91±0.32[b]	3.21±0.34[b]	3.30±0.38[b]	3.15±0.28[b]	3.49±0.33[b]	3.62±0.31[b]
III	4.85±0.39[c]	4.10±0.37[c]	3.70±0.34[b]	4.07±0.41[c]	4.85±0.38[c]	4.92±0.36[c]
Sugars						
I	3.51±0.25[a]	4.17±0.21[a]	5.37±0.32[a]	4.92±0.38[a]	5.13±0.36[a]	4.98±0.29[a]
II	3.42±0.31[a]	4.09±0.28[a]	5.38±0.35[a]	4.75±0.37[a]	5.05±0.34[a]	4.65±0.36[a]
III	3.25±0.18[a]	3.95±0.21[a]	5.37±0.34[a]	4.66±0.31[a]	4.94±0.35[a]	4.49±0.28[a]

and later heating under reflux for 30 min (Singh et al., 1990). Alcoholic extract was collected by filtration using Whatman No 3 filter paper. The residue was recovered and recycled. This solution constitutes the crude extract which served for analysis of soluble sugars and amino acids. Soluble sugars were determined following the Anthron method (Ashwell, 1957) and amino acids were determined with ninhydrin using the Yemm and Cocking (1955) method.

Determination of proteins content

Protein extracts were prepared by mixing 250 mg of *Cola* nut with 1.2 ml of 50 mM Tris-HCL buffer pH 7.3, containing 0.5 M $CaCl_2$ and 2% (w/v) polyvinylpyrrolidone (PVP), at 4°C for 1 h. Homogenate was centrifuged (6000 g, 4°C, 45 min) and the resulting supernatant used as crude extract of protein. Total protein was quantified by the linearized method of Bradford (1976) using the ratio of A_{650}/A_{450} versus protein concentration. Bovine serum albumin (BSA) was used as standard.

Analysis of free amino acids

Free amino acid contents were analysed according to the method described by Rhosius et al. (2006). 50 - 100 mg lyophilised *Cola* seeds were powdered and stirred at 4°C for 1 h with 100 - 200 mg polyvinyl-polypyrrolidon (PVPP) and 5 - 10 ml distilled water. Immediately after adding water, the pH was adjusted to 2.5 with 10% aqueous trifluoroacetic acid. The homogenate was centrifuged for 10 min at 5,000 rpm. The clear supernatant solution was filtered through a 0.45 µm filter (Multoclear, CS-Chromatography). About 30 µl of each sample were lyophilised (1 h at -20°C, 0.05 mbar°C) directly into the vial and kept at -20°C until analysis.

Free amino acids were derivatised with O-phthalaldehyde (OPA) prior to the HPLC analysis. Chromatographic separation was performed with a LiChroCART 250-4 (Merck) provided with precolumn Lichrospher 100 RP-18 (5 µm) (Merck, VWR international). Chromatographic analyses were made with a reverse phase binary gradient [A: 1.6 L sodium acetate solution/glacial acetic acid (50 mmol l^{-1}; pH 6.2), 50 ml MeOH (Lichrosolv ®, gradient grade); 20 ml tetrahydrofuran (Lichrosolv ®, gradient grade); B: 200 ml sodium acetate solution/glacial acetic acid (50 mmol l^{-1}; pH 6.2), 800 ml MeOH (Lichrosolv ®, gradient grade)] at a flow rate of 1.3 ml min^{-1}. The OPA derivatization procedure was performed according to Rhosius et al. (2006). 20 µl of the derivative samples were injected

into a column for separation and were subsequently detected with Hitachi F-1050 fluorescence spectrophotometer (λ_{ex} = 334 nm, λ_{em} = 425 nm). The column temperature was 30°C. The autosampler was from Merck-Hitachi AS- 4000. The solution degasser consisted of the Degassex DG-4400 from Phenomenex. Quantification was calculated via peak area of chromatograms from standard mixture containing 1 - 10 pmol μl^{-1} of each amino acid. Total free amino acid concentrations were obtained by summation of the individual amino acid concentration.

Data analysis

Multi-way analysis of variance (ANOVA) was conducted using the statistical package for the social sciences (SPSS) 7.5 software. Principal component analysis (PCA) was performed to establish associations among accessions by using the SPAD 4.1 statistical software package. Cluster analyses with free amino acids, using the unweighted pairwise group methods with arithmetical average (UPGMA) on the basis of Nei (1978) genetic distance, were performed with the assistance of SPAD 4.1. A dendrogram was built to represent the structure of the genetic diversity within our collection.

RESULTS

**Soluble proteins, sugars and amino acids content**

Proteins, amino acids and sugars in mature seeds, germinated seeds and seedlings of six accessions of both *C. acuminata* and *C. anomala* are presented in Tables 2 and 3, respectively. Whatever the process (germination and seedling growth), the level of proteins and sugars are not significantly different in all accessions of the two *Cola* species studied (Tables 2 and 3).

However, the amounts of amino acids varied significantly during the two processes. In *C. acuminata*, from non-germinated to germinated seeds, among the six accessions, Zoaetele displays high increase (55.8%) while Sangmelima showed the lowest (28.6%) (Figure 1).

During seedling growth and according to the evolution of

Table 3. Proteins, amino acids and sugars in mature seeds (I), germinated seeds (II) and seedlings (III) of six accessions of *C. anomala* (mg/g of dry wt ± S.D.).

Parameters	Bafang	Bamenda	Bangoua	Mamfe	Dschang	Foumbot
Proteins						
I	0.99±0.11[a]	1.15±0.10[a]	1.31±0.12[a]	1.54±0.14[a]	1.35±0.13[a]	1.64±0.13[a]
II	1.24±0.09[a]	1.03±0.07[a]	1.13±0.12[a]	1.38±0.16[a]	1.24±0.11[a]	1.29±0.13[a]
III	1.05±0.10[a]	0.96±0.06[a]	1.02±0.09[a]	1.29±0.05[a]	1.15±0.08[a]	1.17±0.07[a]
Amino acids						
I	3.80±0.25[a]	3.17±0.27[a]	3.27±0.29[a]	3.09±0.21[a]	3.15±0.24[a]	3.36±0.22[a]
II	4.83±0.33[b]	4.20±0.37[b]	4.42±0.31[b]	4.13±0.32[b]	4.18±0.35[b]	4.51±0.39[b]
III	4.63±0.37[b]	4.99±0.41[c]	5.18±0.43[c]	4.92±0.39[c]	5.12±0.42[c]	5.42±0.44[c]
Sugars						
I	7.06±0.56[b]	7.17±0.59[a]	6.37±0.48[a]	6.92±0.51[a]	6.13±0.42[a]	6.98±0.46[a]
II	5.43±0.35[a]	7.09±0.48[a]	6.38±0.41[a]	6.75±0.45[a]	6.05±0.43[a]	6.65±0.41[a]
III	5.80±0.39[a]	6.95±0.43[a]	6.44±0.35[a]	6.66±0.41[a]	5.94±0.32[a]	6.49±0.38[a]
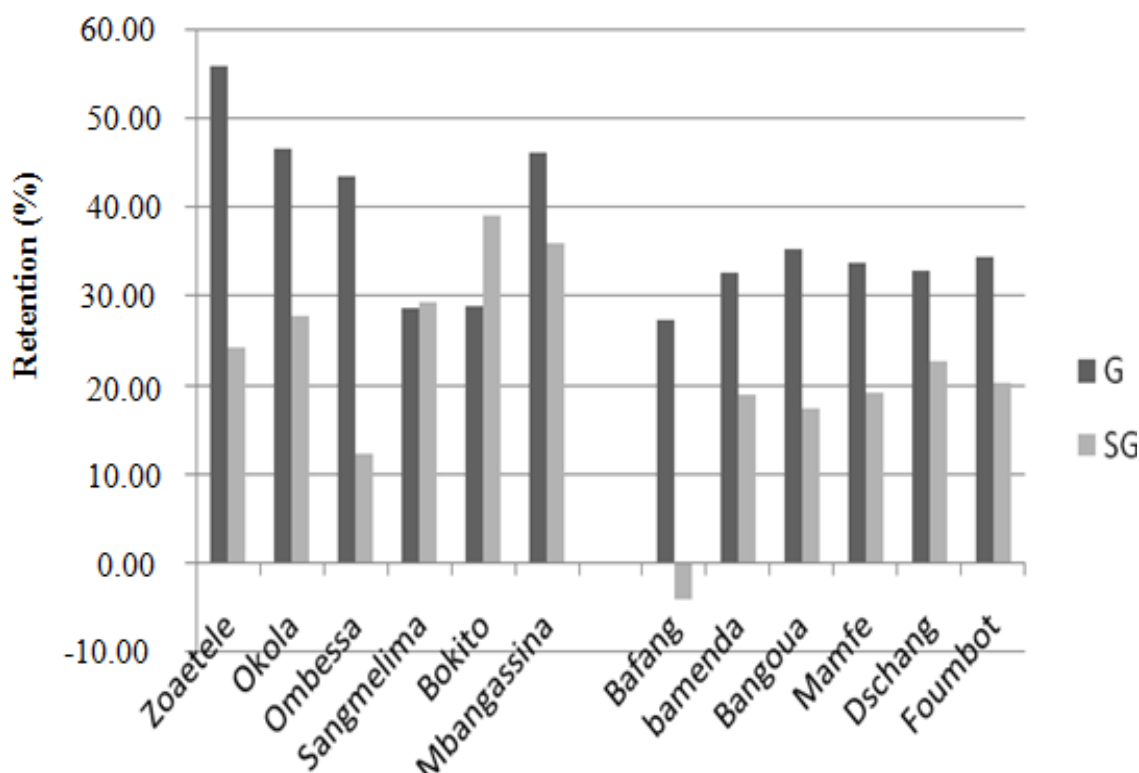
Figure 1. Effect of germination and seedling growth on the soluble amino acids content. G: increase of amino acids during germination, SG: increase of amino acids during seedling growth.

amino acids content, Bokito presents the better increase (39%) and Ombessa the lowest (12.1%).

In *C. anomala*, the variation of amino acids comprises between 27.1% (Bafang) and 35.2% (Bangoua) from mature to germinated seeds (Figure 1). From germinated seeds to seedling, this variation was around -4.1% (Bafang) and 22.5% (Dschang). Free amino acids content results are given in µg/g dry wt.

C. acuminata

Large amounts of amino acids were found in non-germinated seeds except for glycine (Gly), tyrosine (Tyr), tryptophan (Trp), valine (Val), isoleucine (Ile) and leucine (Leu) (Table 4). The major amino acid found in all six accessions was arginine (Arg) while Val was the lowest. Contrary to *C. anomala*, *C. acuminata* lacks phenyalanine

Table 4. Free amino acids in mature seeds (I), germinated seeds (II) and seedlings (III) of six accessions of *Cola acuminata* (µg/g of dry wt).

Parameters	Zoaetele			Okola			Ombessa		
	I	II	III	I	II	III	I	II	III
NEEA									
Asp	1666.3[a]	2857.8[b]	4309.5[c]	1658.9[a]	2845.1[b]	4302.3[c]	1658.9[a]	2845.1[b]	4302.3[c]
Glu	1028.0[a]	2354.5[b]	3934.9[c]	1034.1[a]	2362.4[b]	3939.2[c]	1025.1[a]	2359.7[b]	3980.6[c]
Ser	186.3[a]	459.7[b]	578.7[c]	192.5[a]	484.7[b]	605.8[c]	175.4[a]	498.6[b]	597.6[c]
Gly	70.2[a]	127.8[b]	153.5[c]	93.4[a]	186.7[b]	245.1[c]	102.8[a]	214.5[b]	301.2[c]
Arg	1984.6[a]	3056.8[b]	3724.2[c]	1854.3[a]	2894.3[b]	3896.7[c]	1785.3[a]	2853.4[b]	3924.0[c]
Ala	246.4[a]	1148.9[b]	1454.9[c]	352.8[a]	1453.8[b]	2015.9[c]	301.9[a]	1884.6[b]	2534.2[c]
Gln	208.3[a]	689.6[b]	925.9[c]	288.2[a]	715.8[b]	985.3[c]	302.5[a]	755.1[b]	1054.3[c]
Asn	1563.8[a]	1867.3[b]	2004.9[c]	1684.1[a]	1927.0[b]	2562.3[c]	1725.5[a]	1986.4[b]	2245.7[c]
EEA									
Thr	146.8[a]	386.7[b]	451.0[c]	185.2[a]	412.8[b]	505.4[c]	211.7[a]	400.7[b]	545.3[c]
His	123.7[a]	185.8[b]	247.5[c]	178.5[a]	254.3[b]	318.4[c]	194.1[a]	277.8[b]	385.6[c]
Val	48.2[a]	98.6[b]	124.1[c]	54.2[a]	105.6[b]	203.1[c]	58.2[a]	87.1[b]	112.7[c]
Ile	59.7[a]	75.1[b]	88.9[b]	62.3[a]	88.9[b]	101.8[c]	69.2[a]	85.6[b]	98.0[c]
Leu	58.3[a]	73.3[b]	84.3[b]	63.0[a]	85.2[b]	112.2[c]	66.3[a]	80.5[b]	99.0[c]
Phe	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tyr	73.8[a]	108.1[b]	129.6[c]	95.3[a]	163.0[b]	196.5[c]	77.5[a]	152.4[b]	190.5[c]
Trp	50.3[a]	64.8[b]	73.5[c]	55.3[a]	70.6[b]	89.3[c]	63.0[a]	78.9[b]	94.5[c]
Lys	1349.7[a]	1872.6[b]	2059.6[c]	1420.3[a]	1985.6[b]	2425.5[c]	1652.2[a]	2145.3[b]	2325.0[c]
NPAA									
GABA	3128.8[b]	4097.8[c]	1241.3[a]	1371.8[a]	2854.8[b]	3546.8[c]	1054.3[a]	2788.0[b]	3850.4[c]

	Sangmelima			Bokito			Mbangassina		
	I	II	III	I	II	III	I	II	III
NEEA									
Asp	1650.2[a]	2849.4[b]	4300.1[c]	1649.5[a]	2836.8[b]	4294.2[c]	1648.9[a]	2855.2[b]	4296.3[c]
Glu	1082.1[a]	2381.6[b]	3959.8[c]	1054.3[a]	2380.5[b]	3905.1[c]	1080.9[a]	2375.4[b]	3995.8[c]
Ser	200.9[a]	518.9[b]	615.8[c]	197.6[a]	578.4[b]	625.4[c]	154.6[a]	475.9[b]	599.7[c]
Gly	85.2[a]	175.7[b]	233.9[c]	140.3[a]	264.7[b]	351.9[c]	117.6[a]	267.5[b]	365.4[c]
Arg	1625.8[a]	2875.3[b]	3985.2[c]	1715.3[a]	3003.8[b]	4120.3[c]	1657.4[a]	2775.1[b]	3125.3[c]
Ala	422.3[a]	2004.7[b]	3215.3[c]	321.5[a]	1895.3[b]	2351.9[c]	198.3[a]	982.3[b]	1652.0[c]
Gln	321.4[a]	784.6[b]	970.3[c]	308.5[a]	784.6[b]	1147.2[c]	287.5[a]	765.1[b]	991.7[c]
Asn	1457.3[a]	1684.6[b]	2542.0[c]	1465.7[a]	1925.7[b]	2473.5[c]	1671.3[a]	1985.4[b]	2547.1[c]
EEA									
Thr	224.3[a]	450.3[b]	487.0[b]	199.2[a]	444.7[b]	541.3[c]	202.3[a]	456.8[b]	622.7[c]
His	202.3[a]	298.0[b]	385.4[c]	155.4[a]	312.5[b]	403.4[c]	200.4[a]	335.7[b]	413.8[c]
Val	58.9[a]	88.4[b]	110.8[c]	62.4[a]	89.3[b]	140.3[c]	52.8[a]	94.6[b]	133.8[c]
Ile	65.0[a]	84.2[b]	98.5[c]	60.3[a]	79.2[b]	96.4[c]	62.4[a]	88.4[b]	102.5[c]
Leu	65.1[a]	80.6[b]	98.0[c]	60.3[a]	85.2[b]	98.4[c]	62.4[a]	78.3[b]	95.4[c]
Phe	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tyr	89.1[a]	140.6[b]	175.3[c]	70.2[a]	99.4[b]	118.6[c]	85.2[a]	132.4[b]	178.1[c]
Trp	65.2[a]	75.4[b]	102.3[c]	56.2[a]	69.1[b]	77.5[b]	60.3[a]	86.2[b]	100.7[c]
Lys	1250.2[a]	1658.3[b]	2002.5[c]	1236.5[a]	1987.2[b]	2332.1[c]	1432.1[a]	1984.2[b]	2225.1[c]
NPAA									
GABA	1125.3[a]	3028.6[b]	3876.4[c]	1452.6[a]	3102.4[b]	4004.3[c]	1145.3[a]	2845.7[b]	3864.2[c]

NEAA: Non essential amino acids; EAA: Essential amino acids; NPAA: Non proteic amino acids; ND: not detected

(Phe) and contains histidine (His). In germinated seeds, GABA is much present and tryptophan less present. In this stage, none of the amino acids disappear and there is a significant increase of all amino acids.

Seedlings are characterised by high level of aspartic acid (Asp) and a low level of Trp. This specie lacks Phe. At germination, seedling growth is also accompanied by a drop of all amino acids detected.

C. anomala

In mature non-germinated seeds, GABA is predominant and Ile was the lowest (Table 5). In germinated seeds and seedlings, GABA remains the highest and Trp the lowest. His was not detected.

The germination process has a positive effect on the amino acid contents in the two *Cola* species studied. Germination brought about significant increases (P ≤ 0.05) in non essential amino acids (NEAA), such as alanine (Ala), serine (Ser) and glutamic acid (Glu) (for *C. acuminata*), asparagine (Asn) and Ala (for *C. anomala*) (Figure 2). Among essential amino acids (EAA), the germination caused an increase in threonine (Thr) and Val (*C. acuminata*) and Val and His (*C. anomala*) (Figure 2).

Among NEAA, the effect of germination is more prominent in *C. acuminata* than *C. anomala* for Asp, Glu, Ser, Gly, Arg, Ala and glutamine (Gln). For EAA, germination process had the same effect on Ile and Leu for the two *Cola* species; this physiological process brought about a great increase on Thr, Tyr, Trp and GABA in *C. acuminata* than *C. anomala* (Figure 2).

Principal component (PC) analysis was used to visualise the variations in the samples (Figure 3). The two principal components generated from all the data represented 93.44% of the total variability. All free amino acids detected (except Tyr) were the dominating features in the first principal component (88.3% of the total variability), while Tyr was the feature with the highest weight in the second principal component (5.14% of the total variability) (detailed analysis not shown).

Examining a two-dimensional scores plot in the space defined by PC1 and PC2 showed that the distribution of samples follows a specific pattern. The differences between the groups are related not only to the absence/presence of His and Phe, but also to Asn, Gln and Arg contents.

Dendrogram of *Cola* sp. generated by unweighted pair group method (UPGAM) based on hierarchical cluster analysis using data of free amino acids is presented in Figure 4. This dendrogram permits a better visualisation of the distribution and it displays two main groups. The first group A consisted of *C. anomala* accessions while group B is characterised by *C. acuminata* accessions.

DISCUSSION

Soluble proteins, sugars and amino acids content

The germination of seeds is accompanied by metabolism

of the reserve proteins stored in the cotyledons. The hydrolysis of these molecules produces amino acids and peptides that may either remain in the storage tissue or be translocated in the developing plant. These amino acids and their derivatives are used for the synthesis of new proteins and to supply metabolic intermediates and energy needed by the seedling prior to the onset of photosynthesis (Ashton, 1976; Derbyshire et al, 1976). The low variation of proteins observed during germination and seedling growth of *Cola* sp. May be due to equilibrium between hydrolysed proteins and the new synthesized proteins. Different authors have shown that the germination process increases the protein content in lupin and peas, although others have observed no changes or lower protein content in sprouts, and results seem to depend, not only on the seed cultivar, but also on the germination conditions (Dagnia et al., 1992; Urbano et al., 2005b).

Sugars did not vary significantly during germination and seedling growth of *Cola*. In contrast, Blöchl et al. (2007) found that pea germination is accompanied by an increase of soluble sugars. Amounts of amino acids varied significantly during the two processes. Seeds would be a site of intense synthetic activities of amino acids. Sutclife and Bryant (1977) on pea and Rozan et al. (2001) on lens also noticed the positive influence of germination on the amino acids content. In fact, the main stages of proteolytic breakdown take place within the protein bodies by the combined action of endopeptidases and exopeptidases. The first step occurs at the onset of germination, and involves the *de novo* synthesis of an endopeptidase (proteinase A) which catalyses limited proteolysis of the insoluble storage proteins, converting them to soluble peptides (Ferreira et al., 1995). In this way, the modified proteins become susceptible to the action of proteinase B, carboxypeptidases, aminopeptidases and dipeptidases, which are unable to attack the native proteins of non-germinated seeds but readily convert the soluble peptides into amino acids (Műntz et al., 1985; Shutov and Vaintraub, 1987).

Free amino acids content

Large amounts of GABA are found in non germinated seeds, germinated seeds and *Cola* seedlings. In *Castanea sativa* L., GABA also represents a major amino compound during germination and early seedling growth (Desmaison and Tixier, 1986). After germination, the content of free amino acids increased dramatically. The seedlings were the sites of high amino acids biosynthetic activity, resulting in the high contents of free protein amino acids (Morot-Gaudry et al., 2001) which support the synthesis of proteins and the development of the plant. On the other hand, during germination, storage proteins can undergo proteolysis and results in the increase of free amino acids (Miflin and Lea, 1980).

Considerable level of Glu, GABA, Asp and Arg was

Table 5. Free amino acids in mature seeds (I), germinated seeds (II) and seedlings (III) of six accessions of *C. anomala* (µg/g of dry wt).

Parameters	Bafang			Bamenda			Bangoua		
	I	II	III	I	II	III	I	II	III
NEAA									
Asp	1050.1[a]	1237.5[b]	1508.6[c]	1070.2[a]	1252.5[a]	1528.9[b]	1059.1[a]	1245.3[b]	1513.4[c]
Glu	1165.3[a]	1225.4[a]	2139.8[b]	1169.1[a]	1222.7[a]	2121.6[b]	1154.6[a]	1227.4[b]	2125.1[c]
Ser	126.2[a]	145.3[a]	248.5[b]	115.2[a]	149.1[b]	229.8[c]	111.6[a]	148.1[b]	221.5[c]
Gly	47.1[a]	51.0[a]	97.2[b]	41.2[a]	63.2[b]	94.6[c]	42.3[a]	54.6[b]	92.3[c]
Arg	69.6[a]	76.3[a]	191.9[b]	64.2[a]	76.9[a]	194.6[b]	67.8[a]	74.3[b]	194.8[c]
Ala	176.2[a]	257.0[b]	419.0[c]	175.4[a]	256.2[b]	417.1[c]	172.4[a]	258.4[b]	417.6[c]
Gln	150.0[a]	170.0[a]	193.2[b]	141.0[a]	165.0[b]	187.4[c]	145.3[a]	164.1[b]	185.6[c]
Asn	398.2[a]	721.1[b]	1807.0[c]	388.1[a]	732.1[b]	1801.7[c]	380.2[a]	739.4[b]	1807.8[c]
EAA									
Thr	69.6[a]	76.3[a]	191.9[b]	72.1[a]	78.3[a]	189.4[b]	65.4[a]	77.7[a]	194.6[b]
His	ND	ND	ND	ND	ND	ND	ND	ND	ND
Val	32.0[a]	73.9[b]	106.2[c]	36.2[a]	75.6[b]	102.1[c]	35.0[a]	74.2[b]	101.7[c]
Ile	16.7[a]	48.2[b]	89.6[c]	18.7[a]	51.6[b]	93.7[c]	16.4[a]	54.1[b]	90.9[c]
Leu	42.5[a]	57.1[b]	142.3[c]	44.2[a]	58.4[b]	149.7[c]	45.7[a]	57.2[b]	145.9[c]
Phe	32.6[a]	45.5[b]	47.2[b]	33.8[a]	47.1[b]	49.6[b]	34.5[a]	44.8[b]	48.9[b]
Tyr	75.8[a]	100.3[b]	112.6[b]	70.2[a]	105.2[b]	118.2[b]	78.5[a]	104.2[b]	118.1[c]
Trp	30.6[a]	36.8[a]	45.6[b]	36.2[a]	39.5[b]	48.2[b]	32.4[a]	39.8[a]	48.4[b]
Lys	69.6[a]	76.3[a]	191.9[b]	72.1[a]	82.7[b]	196.2[c]	72.0[a]	81.2[b]	196.1[c]
NPAA									
GABA	1663.2[a]	2309.6[b]	2502.7[b]	1652.1[a]	2302.1[b]	2486.3[b]	1650.6[a]	2300.0[b]	2485.3[c]

Parameters	Mamfe			Dschang			Foumbot		
	I	II	III	I	II	III	I	II	III
NEAA									
Asp	1061.6[a]	1244.2[b]	1506.8[c]	1059.3[a]	1240.2[b]	1500.6[c]	1053.4[a]	1230.6[b]	1515.6[c]
Glu	1146.0[a]	1218.5[a]	2129.4[b]	1129.4[a]	1225.5[a]	2131.4	1120.2[a]	1221.3[a]	2137.5[b]
Ser	115.2[a]	149.1[b]	231.5[c]			[b]	111.6[a]	147.3[b]	227.1[c]
Gly	44.3[a]	52.6[a]	98.4[b]	111.2[a]	147.4[b]	233.6[c]	42.5[a]	58.4[b]	96.1[c]
Arg	60.1[a]	74.9[b]	197.5[c]	44.1[a]	55.2[b]	91.7[c]	72.3[a]	78.4[a]	201.5[c]
Ala	185.4[a]	262.8[b]	424.8[c]	67.2[a]	72.6[a]	198.8[b]	178.4[a]	250.3[b]	430.2[c]
Gln	149.6[a]	156.3[a]	192.7[c]	180.1[a]	265.4[b]	429.2[c]	142.5[a]	167.2[b]	189.0[b]
Asn	391.5[a]	723.1[b]	1840.7[c]	145.6[a]	161.0[a]	189.5[b]	396.4[a]	755.2[b]	1824.9[c]
				382.3[a]	734.6[b]	1808.5[c]			
EAA									
Thr	67.2[a]	79.2[b]	194.3[c]	68.2[a]	77.3[a]	194.2[b]	70.6[a]	73.4[a]	199.5[b]
His	ND	ND	ND	ND	ND	ND	ND	ND	ND
Val	35.8[a]	75.8[b]	112.2[c]	38.2[a]	77.0[b]	104.9[c]	35.3[a]	77.4[b]	112.8[c]
Ile	16.5[a]	54.5[b]	92.8[c]	17.1[a]	48.9[b]	92.1[c]	17.4[a]	54.9[b]	95.7[c]
Leu	41.8[a]	57.4[b]	147.5[c]	48.4[a]	59.0[a]	154.6[b]	47.0[a]	60.2[b]	156.2[c]
Phe	31.5[a]	44.9[b]	46.1[c]	35.4[a]	47.2[b]	52.6[b]	34.8[a]	47.2[b]	49.6[b]
Tyr	78.1[a]	98.2[b]	110.5[c]	78.0[a]	104.6[b]	125.2[c]	70.2[a]	101.9[b]	117.2[b]
Trp	32.1[a]	38.7[a]	48.1[b]	32.0[a]	38.9[a]	47.4[b]	34.0[a]	38.4[a]	46.7[b]
Lys	69.9[a]	74.3[a]	195.5[b]	69.8[a]	73.1[a]	194.8[b]	69.6[a]	75.1[a]	198.0[b]
NPAA									
GABA	1653.7[a]	2301.2[b]	2475.9[c]	1654.1[a]	2305.4[b]	2495.3[b]	1656.1[a]	2301.6[b]	2488.5[b]

NEAA: Non essential amino acids; EAA: essential amino acids; NPAA: non protein amino acids; ND: not detected.
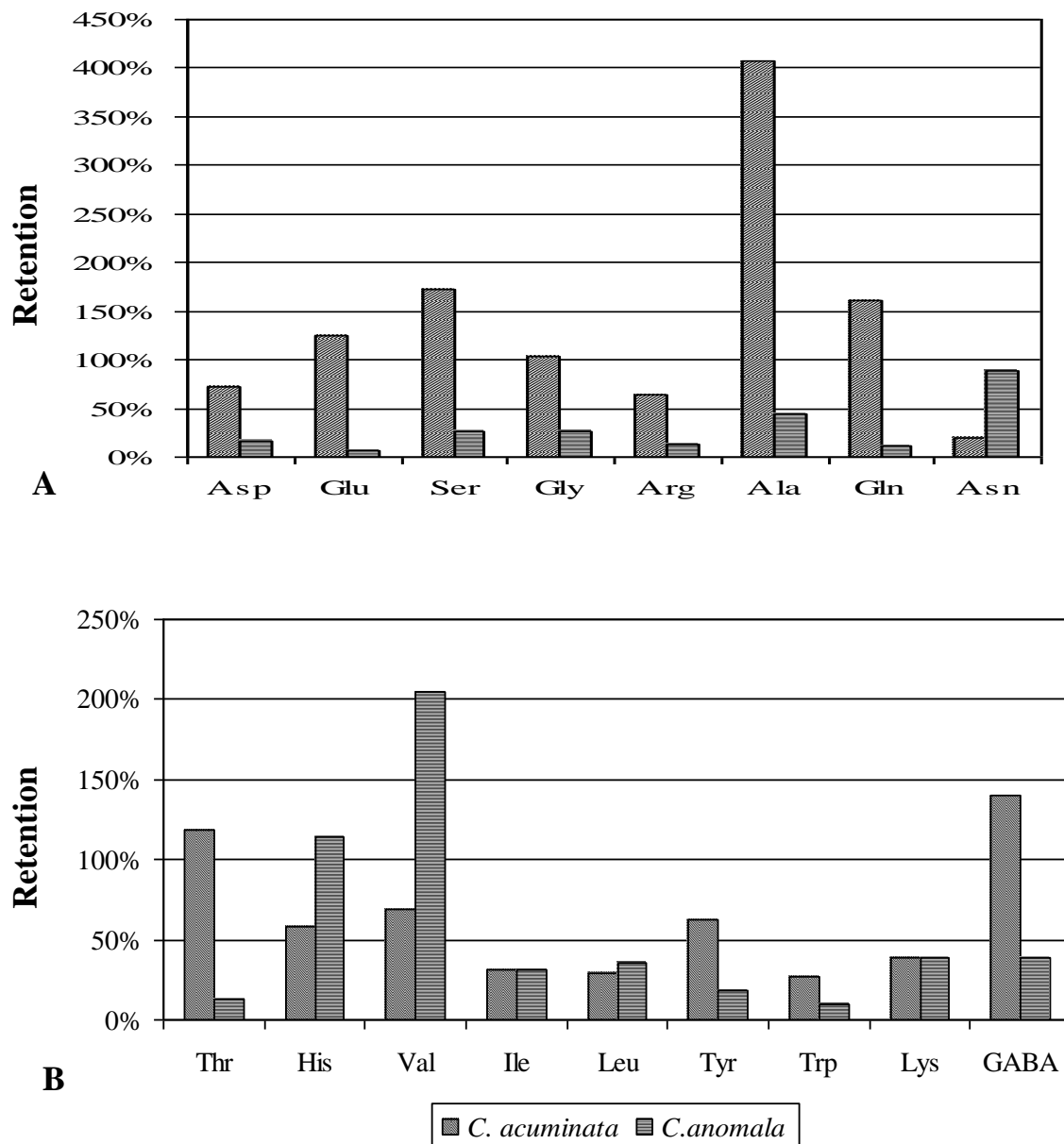
Figure 2. Effect of germination on the amino acid content of *C. acuminata* and *C. anomala* seeds. A: Non essential amino acids. B: Essential amino acids and GABA.

observed in all *Cola* sp. accessions studied. Glu is likely to play a pivotal role in the inter-conversion of amino acids in germinating seeds. Aminotransferases in plants preferentially use Glu and the corresponding keto-acid 2-oxoglutarate in transamination reactions. Furthermore, Glu is either the precursor or by-product of the degradation of specific amino acids such as Arg, Pro and His (Morot-Gaudry et al., 2001). Glutamate also leads to ammonium release in an oxidative deamination reaction catalyzed by glutamate dehydrogenase (GDH, EC 1.4.1.2) (Oh and Cha 2001). In a stress condition, decarboxylation of Glu produces GABA in the GABA shunt pathway.

GABA, a non protein amino acid has several physiological functions such as neurotransmission and induction of hypotensive, diuretic, and tranquilizing effects, as well as inhibition of cancer cell proliferation (Omori et al., 1987; Jakobs et al., 1993; Okada et al., 2000; Oh and Oh, 2004). Several lines of evidence suggest that plant extracts containing high levels of GABA are effective in blood pressure regulation (Omori et al., 1987; Nakagawa and Onota, 1996) and in the recovery of alcohol-related symptoms (Nakagawa and Onota, 1996; Oh and Cha, 2001).

An analysis of all the samples irrespective of the species, displayed a dendrogram with two main groups. The
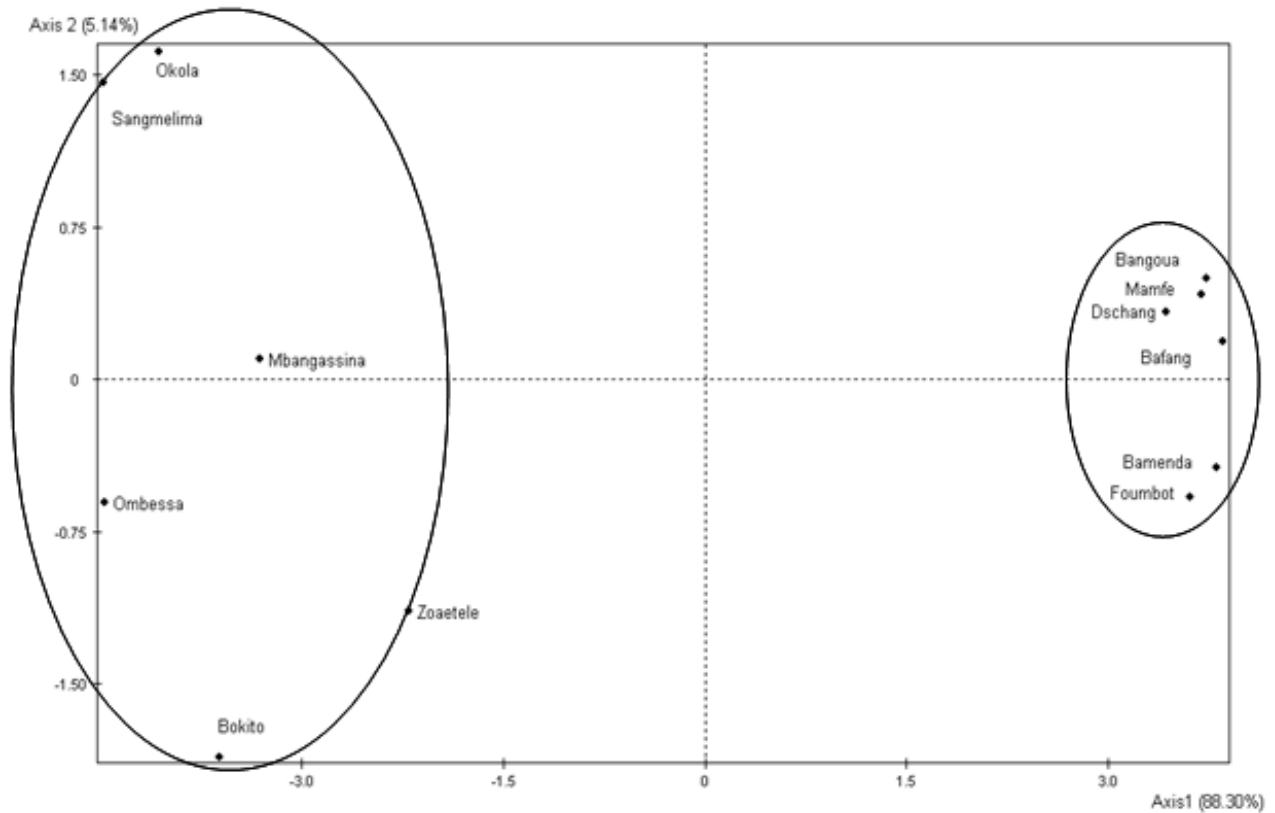
Figure 3. Loading plot of the first two components for free amino acids from all the Cola sp. Accessions analysed.
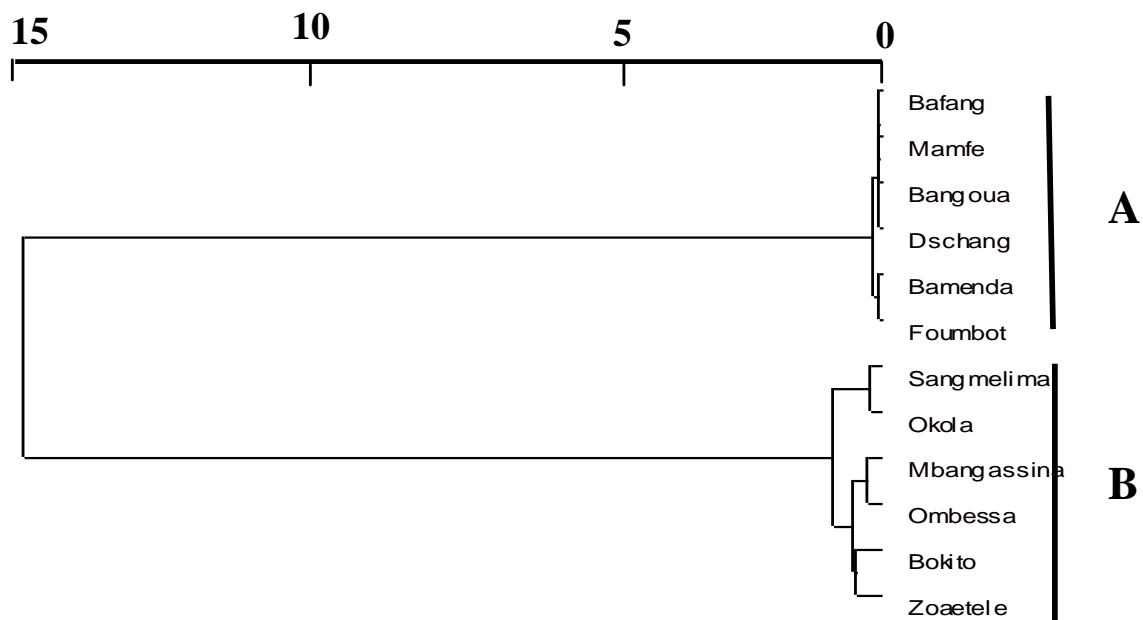

Figure 4. Dendrogram of Cola sp. Generated by unweighted pair group method (UPGMA) based on hierarchical cluster analysis using data of free amino acids.

first group (A) consisted of *C. anomala* accessions while group B is characterised by *C. acuminata* accessions.

This latter specie distinguished *C. anomala* by their high contents in amide amino acids (Asn and Gln), Asp, and

Arg. In the same way, Niemenak et al. (2008) found that *C. anomala* accessions exhibited high the obromine content while *C. acuminata* accessions displayed high caffeine and catechin contents. Differences between the 2 species were also observed in zymotypic and morpho-typic variations. In terms of isozymes, *C. anomala* displayed a greater level of diversity for peroxidase, poly-phenoloxidase and amylase than *C. acuminata* (Effa et al., 2006a). According to morphotypes, a collection of the Cameroonian *Cola* sp. germplasm exhibited 2 morp-hotypes for *C. acuminata* (Effa et al., 2006b) and 6 for *C. anomala* (Effa et al., 2009). In the same way, based on isozymes studies recently carried out in Côte d'Ivoire, *Cola nitida* germplasm displayed two distinct groups (Sié et al., 2005) characterised by some specific morp-hological and agronomical traits (Sié et al., 2009). The variability of free amino acids shows that for a clonal selection of *Cola* sp., these metabolites must be taken into account. This approach in variety identification could be efficient for the creation of "core" collections of *Cola* germplasm.

Conclusion

In *C.acuminata* and *C.anomala*, soluble amino acids varied significantly during germination and seedling growth while the variation of soluble sugars and proteins were not important. HPLC analysis of free amino acids confirmed the high increase of these metabolites during the two processes studied. Amino acids were then considered as crucial for the control of germination of *Cola* seeds. Glu, which played a pivotal role in interconversion of amino acids in germinating seeds and GABA, a non protein amino acid with some healthful functions was highly represented in all *Cola* sp. accessions. Principal component and cluster analyses based on free amino acids distin-guished the two *Cola* species.

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