REPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie
****
UNIVERSITE DE YAOUNDE I
****
FACULTE DES SCIENCES
****
CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES DE LA VIE, SANTE ET ENVIRONNEMENT

REPUBLIC OF CAMEROON
Peace-Work-Fatherland
****
UNIVERSITY OF YAOUNDE I
****
FACULTY OF SCIENCE
****
POST GRADUATE SCHOOL FOR LIFE SCIENCES, HEALTH AND ENVIRONMENT

DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE VEGETALES

DEPARTMENT OF PLANT BIOLOGY

Mycorrhizal and environmental variations of *Prunus africana* (hook.f.) kalkman in relation with its bioactive contents for its domestication and sustainable management in Cameroon

Thesis submitted for the award of the Doctorate/Ph.D degree in Plant Biology

Option: Plant Biotechnology

By:

KAMKO Julie Doriane

Master in Plant Biotechnology

Registration Number: 12S0115

Advisors:

Pr NGONKEU MANGAPTCHE Eddy Leonard

Associate Professor

University of Yaoundé 1(Cameroon)

Pr. EIMERT Klaus

Associate Professor

Hochschule Geisenheim University (Germany)

Year: 2025

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PROTOCOL LIST

UNIVERSITÉ DE YAOUNDÉ I **Faculté des Sciences** Division de la Programmation et du Suivi des Activités Académiques		THE UNIVERSITY OF YAOUNDE I **Faculty of Science** Division of Programming and Follow-up of Academic Affairs
LISTE DES ENSEIGNANTS PERMANENTS		**LIST OF PERMANENT TEACHING STAFF**

ACADEMIC YEAR: 2025/2026

(By Department and Grade)

ACTUALISATION DATE: 24 Septembre 2025

ADMINISTRATION

DEAN: OWONO OWONO Luc, *Professor*

VICE-DEAN / DPSAA: NDJIGUI Paul-Désiré, *Professor*

VICE-DEAN / DSSE: NYEGUE Maximilienne Ascension, *Professor*

VICE-DOYEN / DRC : NOUNDJEU Pierre, *Associate Professor*

Head of administrative and financial division: NDOYE FOE Florentine Marie Chantal, *Associate Professor*

Head of academic affairs, research, and education division DAARS: AJEAGAH Gideon AGHAINDUM, *Professor*

1- DEPARTMENT OF BIOCHEMISTRY (BC) (43)			
N°	**NAMES**	**GRADE**	**OBSERVATIONS**
1.	BIGOGA DAIGA Jude	Professor	Present
2.	FEKAM BOYOM Fabrice	Professor	Present
3.	KANSCI Germain	Professor	Present
4.	MBACHAM FON Wilfred	Professor	Present
5.	MOUNDIPA FEWOU Paul	Professor	**Head of Department**
6.	OBEN Julius ENYONG	Professor	Present
7.	NGUEFACK Julienne	Professor	Present
8.	NJAYOU Frédéric Nico	Professor	Present
9.	ACHU Merci BIH	Associate Professor	Present
10.	ATOGHO Barbara MMA	Associate Professor	Present

11.	AZANTSA KINGUE GABIN BORIS	Associate Professor	Present
12.	BELINGA née NDOYE FOE F. M. C.	Associate Professor	*Head DAF / FS / UYI*
13.	DJUIDJE NGOUNOUE Marceline	Associate Professor	Present
14.	EFFA ONOMO Pierre	Associate Professor	Present
15.	EWANE Cécile Annie	Associate Professor	Present
16.	KOTUE TAPTUE Charles	Associate Professor	Present
17.	LUNGA Paul KEILAH	Associate Professor	Present
18.	MBONG ANGIE M. Mary Anne	Associate Professor	Present
19.	MOFOR née TEUGWA Clotilde	Associate Professor	*Dean FS / UDs*
20.	NANA Louise épouse WAKAM	Associate Professor	Present
21.	NGONDI Judith Laure	Associate Professor	Present
22.	TCHANA KOUATCHOUA Angèle	Associate Professor	Present
23.	AKINDEH MBUH NJI	Lecturer	Present
24.	BEBEE Fadimatou	Lecturer	Present
25.	BEBOY EDJENGUELE Sara Nathalie	Lecturer	Present
26.	DAKOLE DABOY Charles	Lecturer	Present
27.	DJUIKWO NKONGA Ruth Viviane	Lecturer	Present
28.	DONGMO LEKAGNE Joseph Blaise	Lecturer	Present
29.	FONKOUA Martin	Lecturer	Present
30.	KOUOH ELOMBO Ferdinand	Lecturer	Present
31.	MANANGA Marlyse Joséphine	Lecturer	Present
32.	OWONA AYISSI Vincent Brice	Lecturer	Present
33.	Palmer MASUMBE NETONGO	Lecturer	Present
34.	PECHANGOU NSANGOU Sylvain	Lecturer	Present
35.	WILFRED ANGIE Abia	Lecturer	Present
37.	BAKWO BASSOGOG Christian Bernard	Lecturer	Present
38.	ELLA Fils Armand	Lecturer	Present
39.	EYENGA Eliane Flore	Lecturer	Present
40.	MADIESSE KEMGNE Eugenie Aimée	Lecturer	Present
41.	MANJIA NJIKAM Jacqueline	Lecturer	Present
42.	MBOUCHE FANMOE Marceline Joëlle	Lecturer	Present
43.	WOGUIA Alice Louise	Lecturer	Present

2- DEPARTMENT ANIMAL BIOLOGY (BPA) (52)			
1.	AJEAGAH Gideon AGHAINDUM	Professor	*DAARS/FS*
3.	DIMO Théophile	Professor	Present
4.	DJIETO LORDON Champlain	Professor	Present
5.	DZEUFIET DJOMENI Paul Désiré	Professor	Present
6.	ESSOMBA née NTSAMA MBALA	Professor	*Vice Dean/FMSB/UYI*
7.	FOMENA Abraham	Professor	Present
8.	KEKEUNOU Sévilor	Professor	**Head of Department**
9.	NJAMEN Dieudonné	Professor	Present
10.	NJIOKOU Flobert	Professor	Present
11.	NOLA Moïse	Professor	Present
12.	TAN Paul VERNYUY	Professor	Present
13.	TCHUEM TCHUENTE Louis Albert	Professor	*Service Inspector* *Coord Prog/MINSANTE*
14.	ZEBAZE TOGOUET Serge Hubert	Professor	Present
15.	ALENE Désirée Chantal	Maître de Conférences	*Chief Service/MINESUP*
16.	BILANDA Danielle Claude	Maître de Conférences	Present
17.	DJIOGUE Séfirin	Associate Professor	Present
18.	JATSA B. Hermine épse MEGAPTCHE	Associate Professor	Present
19.	LEKEUFACK FOLEFACK Guy B.	Associate Professor	Present

20.	MBENOUN MASSE Paul Serge	Associate Professor	Present
21.	MEGNEKOU Rosette	Associate Professor	Present
22.	MONY Ruth épse NTONE	Associate Professor	Present
23.	NGUEGUIM TSOFACK Florence	Associate Professor	Present
24.	NGUEMBOCK	Associate Professor	Present
25.	TOMBI Jeannette	Associate Professor	Present
26.	GOUNOUE KAMKUMO Raceline	Associate Professor	Present
27.	MAHOB Raymond Joseph	Associate Professor	Present
28.	NOAH EWOTI Olive Vivien	Associate Professor	Present
29.	ATSAMO Albert Donatien	Lecturer	Present
30.	BASSOCK BAYIHA Etienne Didier	Lecturer	Present
31.	DONFACK Mireille	Lecturer	Present
32.	ESSAMA MBIDA Désirée Sandrine	Lecturer	Present
33.	ETEME ENAMA Serge	Lecturer	Present
34.	FEUGANG YOUMSSI François	Lecturer	Present
35.	GONWOUO NONO Legrand	Lecturer	Present
36.	KANDEDA KAVAYE Antoine	Lecturer	Present
37.	KOGA MANG DOBARA	Lecturer	Present
38.	LEME BANOCK Lucie	Lecturer	Present
39.	METCHI D. Mireille Flaure épse Ghoumo	Lecturer	Present
40.	MOUNGANG Luciane Marlyse	Lecturer	Present
41.	MVEYO NDANKEU Yves Patrick	Lecturer	Present
42.	NGOUATEU KENFACK Omer Bébé	Lecturer	Present
43.	NJUA Clarisse Yafi	Lecturer	*Chief Div./U. Bamenda*
44.	TADU Zephyrin	Lecturer	Present
45.	TAMSA ARFAO Antoine	Lecturer	Present
47.	YEDE	Lecturer	Present
48.	YOUNOUSSA LAME	Lecturer	Present
49.	AMBADA NDZENGUE GEORGIA E.	Lecturer	Present
50.	KODJOM WANCHE Jacguy Joyce	Lecturer	Present
51.	NDENGUE Jean De Matha	Lecturer	Present
52.	ZEMO GAMO Franklin	Lecturer	Present

3- DEPARTMENT OF PLANT BIOLOGY (BPV) (33)			
1.	AMBANG Zachée	Professor	**Head of Department**
2.	DJOCGOUE Pierre François	Professor	Present
3.	ZAPFACK Louis	Professor	Present
4.	BIYE Elvire Hortense	Professor	Present
5.	ANGONI Hyacinthe	Associate Professor	Present
6.	MAHBOU SOMO TOUKAM. Gabriel	Associate Professor	Present
10.	MALA Armand William	Associate Professor	Present
11.	MBARGA BINDZI Marie Alain	Associate Professor	*DAAC /UDla*
12.	NDONGO BEKOLO	Associate Professor	*CE / MINRESI*
13.	NGALLE Hermine BILLE	Associate Professor	Present
14.	NGODO MELINGUI Jean Baptiste	Associate Professor	Present
15.	NGONKEU MAGAPTCHE Eddy L.	Associate Professor	*CT / MINRESI*
16.	TONFACK Libert Brice	Associate Professor	Present
17.	TSOATA Esaïe	Associate Professor	Present
18.	ONANA Jean Michel	Associate Professor	Present
29.	DJEUANI Astride Carole	Associate Professor	Present
20.	GOMANDJE Christelle	Lecturer	Present
21.	MAFFO MAFFO Nicole Liliane	Lecturer	Present

22.	NNANGA MEBENGA Ruth Laure	Lecturer	Present
23.	NOUKEU KOUAKAM Armelle	Lecturer	Present
24.	NSOM ZAMBO Annie Claude épse PIAL	Lecturer	*On secondmentUNESCO MALI*
25.	Godswill NTSOMBOH NTSEFONG	Lecturer	Present
26.	KABELONG BANAHO Louis-Paul-R.	Lecturer	Present
27.	KONO Léon Dieudonné	Lecturer	Present
28.	LIBALAH Moses BAKONCK	Lecturer	Present
29.	LIKENG-LI-NGUE Benoit Constant	Lecturer	Present
30.	TAEDOUNG Evariste Hermann	Lecturer	Present
31.	TEMEGNE NONO Carine	Lecturer	Present
31.	MANGA NDJAGA JUDE	Lecturer	Present
32.	DIDA LONTSI Sylvere Landry	Lecturer	Present
33.	METSEBING Blondo-Pascal	Assistant	Present
33	BOLIE Hubert	Assistant	Present
33	MACHE NKOUANDEU Pasma	Assistant	Present
34	MAFFO FOKOU Adèle	Assistant	Present
34	METSEBING Blondo-Pascal	Assistant	Present
36	NTONMEN YANKEU Amandine Flore	Assistant	Present
37	ONANA EBODE Clotaire	Assistant	Present

4- DEPARTMENT OF INORGANIC CHEMISTRY (CI) (28)			
1.	GHOGOMU Paul MINGO	Professor	*Minister in Charge of Miss.PR*
2.	NANSEU Njiki Charles Péguy	Professor	Present
3.	NDIFON Peter TEKE	Professor	*CT/MINRESI*
4.	NENWA Justin	Professor	Present
5.	NGAMENI Emmanuel	Professor	*DEAN FS/U. Ngaoundere*
6.	NGOMO Horace MANGA	Professor	*Vice Chancelor/UB*
7.	ACAYANKA Elie	Associate Professor	Present
8.	EMADACK Alphonse	Associate Professor	Present
9.	KAMGANG YOUBI Georges	Associate Professor	Present
10.	KEMMEGNE MBOUGUEM Jean C.	Associate Professor	Present
11.	KENNE DEDZO GUSTAVE	Associate Professor	Present
12.	MBEY Jean Aime	Associate Professor	Present
13.	NDI NSAMI Julius	Associate Professor	**Head of Department**
14.	NEBAH Née NDOSIRI Bridget NDOYE	Associate Professor	*CT/ MINPROFF*
15.	NJIOMOU C. épse DJANGANG	Associate Professor	Present
16.	NJOYA Dayirou	Associate Professor	Present
17.	NYAMEN Linda Dyorisse	Associate Professor	Present
18.	PABOUDAM GBAMBIE AWAWOU	Associate Professor	Present
19.	TCHAKOUTE KOUAMO Hervé	Associate Professor	Present
20.	BELIBI BELIBI Placide Désiré	Lecturer	*Chief Service/ ENS Bertoua*
21.	CHEUMANI YONA Arnaud M.	Lecturer	Present
22.	KOUOTOU DAOUDA	Lecturer	Present
23.	MAKON Thomas Beauregard	Lecturer	Present
24.	NCHIMI NONO KATIA	Lecturer	Present
24.	NJANKWA NJABONG N. Eric	Lecturer	Present
24.	PATOUOSSA ISSOFA	Lecturer	Present
25.	SIEWE Jean Mermoz	Lecturer	Present
26.	BOYOM TATCHEMO Franck W	Assistant	Present

27.	DANTIO NGUELA Christian Brice	Assistant	Present
28.	LEKENE NGOUATEU Reine	Assistant	Present

5- DEPARTMENT OF ORGANIC CHEMISTRY (CO) (37)			
1.	Alex de Théodore ATCHADE	Professor	*Vice-Dean / DPSAA / UYI*
2	DONGO Etienne	Professor	*Vice-Dean / FSE / UYI*
3.	NGOUELA Silvère Augustin	Professor	*Head of Department / UDS*
4.	PEGNYEMB Dieudonné Emmanuel	Professor	**Head of Department/** *Recteur UBertoua*
5.	WANDJI Jean	Professor	Present
6.	MBAZOA née DJAMA Céline	Professor	Present
7.	AMBASSA Pantaléon	Associate Professor	Present
8.	EYONG Kenneth OBEN	Associate Professor	Present
9.	FOTSO WABO Ghislain	Associate Professor	Present
10.	KAMTO Eutrophe Le Doux	Associate Professor	Present
11.	KENMOGNE Marguerite	Associate Professor	Present
12.	KEUMEDJIO Félix	Associate Professor	Present
13.	KOUAM Jacques	Associate Professor	Present
14.	MKOUNGA Pierre	Associate Professor	Present
15.	MVOT AKAK CARINE	Associate Professor	Present
16.	NGO MBING Joséphine	Associate Professor	*Cell Cihef / MINRESI*
17.	NGONO BIKOBO Dominique Serge	Associate Professor	*C.E.A/ MINESUP*
18.	NOTE LOUGBOT Olivier Placide	Associate Professor	*DAAC/U. Bertoua*
19.	NOUNGOUE TCHAMO Diderot	Associate Professor	Present
20.	TABOPDA KUATE Turibio	Associate Professor	Present
21.	TAGATSING FOTSING Maurice	Associate Professor	Present
22.	TCHOUANKEU Jean-Claude	Associate Professor	*Dean / FS / UYI*
23.	YANKEP Emmanuel	Associate Professor	Present
24.	ZONDEGOUMBA Ernestine	Associate Professor	Present
25.	NGNINTEDO Dominique	Chargé de Cours	Present
26.	NGOMO Orléans	Lecturer	Present
27.	NONO NONO Éric Carly	Lecturer	Present
28.	OUAHOUO WACHE Blandine M.	Lecturer	Present
29.	OUETE NANTCHOUANG Judith Laure	Lecturer	Present
30.	SIELINOU TEDJON Valérie	Lecturer	Present
31.	MESSI Angélique Nicolas	Lecturer	Present
32.	TCHAMGOUE Joseph	Lecturer	Present
33.	TSAFFACK Maurice	Lecturer	Present
34.	TSAMO TONTSA Armelle	Lecturer	Present
35.	TSEMEUGNE Joseph	Lecturer	Present
36.	MUNVERA MFIFEN Aristide	Assistant	Present
37.	NDOGO ETEME Olivier	Assistant	Present

6- DEPARTMENT OF COMPUTER SCIENCE (IN) (22)			
1.	ATSA ETOUNDI Roger	Professor	*Head Div. / MINESUP*
2.	FOUDA NDJODO Marcel Laurent	Professor	*Head Dpt / ENS UYI* *Head IGA. / MINESUP*
3.	NDOUNDAM Réné	Lecturer	Present
4.	TSOPZE Norbert	Lecturer	Present
5.	ABESSOLO ALO'O Gislain	Lecturer	*Vice-Director / MINFOPRA*

6.	AMINOU Halidou	Lecturer	**Head of Department**
7.	DJAM Xaviera YOUH - KIMBI	Lecturer	Present
8.	DOMGA KOMGUEM Rodrigue	Lecturer	Present
9.	EBELE Serge Alain	Lecturer	Present
10.	HAMZA Adamou	Lecturer	Present
11.	JIOMEKONG AZANZI Fidel	Chargé de Cours	Present
12.	KOUOKAM KOUOKAM E. A.	Lecturer	Present
13.	MELATAGIA YONTA Paulin	Lecturer	Present
14.	MONTHE DJIADEU Valery M.	Lecturer	Present
15.	OLE OLE Daniel Claude Delort	Lecturer	*Vice director ENSET Ebolowa*
16.	TAPAMO Hyppolite	Lecturer	Present
17.	BAYEM Jacques Narcisse	Assistant	Present
18.	EKODECK Stéphane Gaël Raymond	Assistant	Present
19.	MAKEMBE S. Oswald	Assistant	Present
20.	MESSI NGUELE Thomas	Assistant	Present
21.	NKONDOCK. MI. BAHANACK.N.	Assistant	Present
22.	NZEKON NZEKO'O Armel Jacques	Assistant	Present

6- DEPARTEMENT DES ENERGIES RENOUVELABLES (ER) (1)			
1.	**BODO Bertrand**	**Professor**	***Head of Department***

7- DEPARTMENT OF MATHEMATICS (MA) (33)			
1.	AYISSI Raoult Domingo	Professor	**Head of Department**
2.	KIANPI Maurice	Associate Professor	Present
3.	MBANG Joseph	Associate Professor	Present
4.	MBEHOU Mohamed	Associate Professor	Present
5.	MBELE BIDIMA Martin Ledoux	Associate Professor	Present
6.	NOUNDJEU Pierre	Associate Professor	*Head of Programmes & Diplôma Services/FS/UYI*
7.	TAKAM SOH Patrice	Associate Professor	Present
8.	TCHAPNDA NJABO Sophonie B.	Associate Professor	*Director/AIMS Rwanda*
9.	TCHOUNDJA Edgar Landry	Associate Professor	Present
10.	AGHOUKENG JIOFACK Jean Gérard	Lecturer	*Cell Chief/ MINEPAT*
11.	BOGSO ANTOINE MARIE	Lecturer	Present
12.	CHENDJOU Gilbert	Lecturer	Present
13.	DJIADEU NGAHA Michel	Lecturer	Present
14.	DOUANLA YONTA Herman	Lecturer	Present
15.	KIKI Maxime Armand	Lecturer	Present
16.	LOUMNGAM KAMGA Victor		
17.	MBAKOP Guy Merlin	Lecturer	Present
18.	MBATAKOU Salomon Joseph		
19.	MENGUE MENGUE David Joe	Lecturer	*Head Dpt /ENS U. Maroua*
20.	MBIAKOP Hilaire George		
21.	NGUEFACK Bernard	Lecturer	Present
22.	NIMPA PEFOUKEU Romain	Lecturer	Present
23.	OGADOA AMASSAYOGA	Lecturer	Present

24.	POLA DOUNDOU Emmanuel	Lecturer	Present
25.	TCHEUTIA Daniel Duviol	Lecturer	Present
26.	TETSADJIO TCHILEPECK M. E.	Lecturer	Present
27.	BITYE MVONDO Esther Claudine	Assistante	Present
28.	FOKAM Jean Marcel	Assistant	Present
29.	GUIDZAVAI KOUCHERE Albert		
30.	MANN MANYOMBE Martin Luther		
31.	MEFENZA NOUNTU Thiery	Assistant	Present
32.	NYOUMBI DLEUNA Christelle		
33.	TENKEU JEUFACK Yannick Léa	Assistant	Present

8- DEPARTMENT OF MICROBIOLOGY (MIB) (24)			
1.	ESSIA NGANG Jean Justin	Professor	**Head of Department**
2.	NYEGUE Maximilienne Ascension	Professor	*Vice-Dean / DSSE/FS/UYI*
3.	ASSAM ASSAM Jean Paul	Associate Professor	Present
4.	BOUGNOM Blaise Pascal	Associate Professor	Present
5.	BOYOMO ONANA	Associate Professor	Present
6.	KOUITCHEU MABEKU Epse KOUAM Laure Brigitte	Associate Professor	Present
7.	RIWOM Sara Honorine	Associate Professor	Present
8.	NJIKI BIKOÏ Jacky	Associate Professor	Present
9.	SADO KAMDEM Sylvain Leroy	Associate Professor	Present
10.	ESSONO Damien Marie	Lecturer	Present
11.	LAMYE Glory MOH	Lecturer	Present
12.	MEYIN A EBONG Solange	Lecturer	Present
13.	NKOUDOU ZE Nardis	Lecturer	Present
14.	MONI NDEDI Esther Del Florence		
15.	TAMATCHO KWEYANG Blandine P.	Lecturer	Present
16.	TCHIKOUA Roger		
17.	TOBOLBAÏ Richard	Lecturer	Present
18.	NKOUE TONG ABRAHAM	Assistant	Present
19.	SAKE NGANE Carole Stéphanie	Assistant	Present
20.	EZO'O MENGO Fabrice Télésfor	Assistant	Present
21.	EHETH Jean Samuel	Assistant	Present
22.	MAYI Marie Paule Audrey	Assistant	Present
23.	NGOUENAM Romial Joël	Assistant	Present
24.	NJAPNDOUNKE Bilkissou	Assistant	Present

9. DEPARTMENT OF PYSICS (PHY) (43)			
1.	BEN- BOLIE Germain Hubert	Professor	Present
2.	DJUIDJE KENMOE épouse ALOYEM	Professor	Present
3.	EKOBENA FOUDA Henri Paul	Professor	*Vice-Rector / U. Ngaoundéré*
4.	ESSIMBI ZOBO Bernard	Professor	Present
5.	NANA ENGO Serge Guy	Professor	Present
6.	NANA NBENDJO Blaise	Professor	Present
7.	NDJAKA Jean Marie Bienvenu	Professor	**Head of Department**
8.	NJANDJOCK NOUCK Philippe	Professor	Present
9.	NOUAYOU Robert	Professor	Present
10.	PEMHA Elkana	Professor	Present

11.	SAIDOU	Professor	*Head of Center/IRGM/MINRESI*
12.	TABOD Charles TABOD	Professor	*Dean FSUniv/Bda*
13.	TCHAWOUA Clément	Professor	Present
14.	WOAFO Paul	Professor	Present
15.	ZEKENG Serge Sylvain	Professor	Present
16.	BIYA MOTTO Frédéric	Associate Professor	*DG/HYDRO Mekin*
17.	BODO Bertrand	Associate Professor	Present
18.	ENYEGUE A NYAM épse BELINGA	Associate Professor	Present
19.	EYEBE FOUDA Jean sire	Associate Professor	Present
20.	FEWO Serge Ibraïd	Associate Professor	Present
21.	HONA Jacques	Associate Professor	Present
22.	MBINACK Clément	Associate Professor	Present
23.	MBONO SAMBA Yves Christian U.	Associate Professor	Present
23	MELI'I Joelle Larissa		
24.	NDOP Joseph	Associate Professor	Present
25.	SIEWE SIEWE Martin	Associate Professor	Present
26.	SIMO Elie	Associate Professor	Present
27.	VONDOU DerbetiniAppolinaire	Associate Professor	Present
28.	WAKATA née BEYA Annie	Associate Professor	*Director/ENS/UYI*
29.	WOULACHE Rosalie Laure	Associate Professor	*On internship Since february 2023*
29.	ABDOURAHIMI	Lecturer	Present
30.	CHAMANI Roméo	Lecturer	Present
31.	EDONGUE HERVAIS	Lecturer	Present
32.	FOUEDJIO David	Lecturer	*Cell. Chief/ MINADER*
34.	MVOGO ALAIN	Lecturer	Present
35.	ABDOURAHIMI	Lecturer	*Present*
36.	AYISSI EYEBE Guy François Valérie	Lecturer	Present
37	CHAMANI Roméo	Lecturer	Present
38.	OTTOU ABE Martin Thierry	Lecturer	Present
39.	TEYOU NGOUPOU Ariel	Lecturer	Present
40.	KAMENI NEMATCHOUA Modeste	Assistant	Present
41.	LAMARA Maurice	Assistant	Present
42.	NGA ONGODO Dieudonné	Assistant	En poste
43.	WANDJI NYAMSI William	Assistant	En poste
10- DEPARTMENT OF EARTH SCIENCES (ST) (42)			
1.	BITOM Dieudonné-Lucien	Professor	*Dean / FASA / UDs*
2.	NDAM NGOUPAYOU Jules-Remy	Professor	Present
3.	NDJIGUI Paul Désiré	Professor	**Head of Department**
4.	NGOS III Simon	Professor	Present
5.	NKOUMBOU Charles	Professor	Present
6.	NZENTI Jean-Paul	Professor	Present
7.	ABOSSOLO née ANGUE Monique	Associate Professor	*Vice-Dean / DRC*
8.	BISSO Dieudonné	Associate Professor	*Director/Project 'Barrage Memve'ele'*
9.	EKOMANE Emile	Associate Professor	Present
10.	FUH Calistus Gentry	Associate Professor	*Sec. of State/MINMIDT*
11.	GANNO Sylvestre	Associate Professor	Present
12.	GHOGOMU Richard TANWI	Associate Professor	*Head od Deaprtment / U. Maroua*
13.	MOUNDI Amidou	Associate Professor	*CT/ MINIMDT*
14.	NGO BIDJECK Louise Marie	Associate Professor	Present
15.	NGUEUTCHOUA Gabriel	Associate Professor	*CEA / MINRESI*

16.	NJILAH Isaac KONFOR	Associate Professor	Present
17.	NYECK Bruno	Associate Professor	Present
18.	ONANA Vincent Laurent	Associate Professor	*Head of Maintenance & Materials service / UYII*
19.	TCHAKOUNTE J. épse NUMBEM	Associate Professor	*Cell. Chief / MINRESI*
20.	TCHOUANKOUE Jean-Pierre	Associate Professor	Present
21.	TEMGA Jean Pierre	Associate Professor	Present
22.	YENE ATANGANA Joseph Q.	Associate Professor	*Chef Div. / MINTP*
23.	ZO'O ZAME Philémon	Associate Professor	*DG / ART*
24.	ANABA ONANA Achille Basile	Lecturer	Present
25.	BEKOA Etienne	Lecturer	Present
26.	ELISE SABABA	Lecturer	Present
27.	ESSONO Jean	Lecturer	Present
28.	EYONG JOHN TAKEM	Lecturer	Present
29.	MAMDEM TAMTO Lionelle Estelle	Lecturer	Present
30.	MBESSE CECILE Olive	Lecturer	Present
31.	METANG Victor	Lecturer	Present
32.	MINYEM Dieudonné	Lecturer	*CD/ U. Maroua*
33.	NGO BELNOUN Rose Noël	Lecturer	Present
34.	NOMO NEGUE Emmanuel	Lecturer	Present
35.	NTSAMA ATANGANA Jacqueline	Lecturer	Present
36.	TCHAPTCHET TCHATO De P.	Lecturer	Present
37.	TEHNA Nathanaël	Lecturer	Present
38.	FEUMBA Roger	Lecturer	Present
39.	MBANGA NYOBE Jules	Lecturer	Present
40.	NGO'O ZE ARNAUD	Assistant	Present
41.	KOAH NA LEBOGO Serge Parfait	Assistant	Present
42.	TENE DJOUKAM Joëlle Flore, épouse KOUANKAP NONO	Assistant	Present

Numerical distribution of Teachers of the Faculty of Sciences of the l'University of Yaoundé I

	NOMBER OF TEACHERS				
DEPARTMENT	**Professors**	**Assistants Professors**	**Lecturers**	**Assistants**	**Total**
BCH	8 (00)	15 (10)	13 (05)	07 (05)	**43 (20)**
BPA	14 (01)	16 (07)	18 (07)	04 (02)	**52 (16)**
BPV	06 (01)	12(02)	13 (07)	03 (00)	**34 (10)**
CI	07(01)	15(04)	05 (01)	01 (00)	**28 (06)**
CO	06 (01)	18 (04)	11 (04)	02 (00)	**37(09)**
IN	02 (00)	02 (00)	14 (01)	04 (00)	**22 (01)**
MAT	01 (00)	08 (00)	17 (01)	07 (02)	**33 (03)**
MIB	02 (01)	07 (03)	8 (04)	07 (02)	**24 (10)**
PHY	15 (01)	15 (04)	11 (01)	02 (00)	**43 (06)**
ST	08 (00)	17 (03)	15 (04)	03 (01)	**43(08)**
Total	**69 (07)**	**125 (40)**	**125 (30)**	**40 (12)**	**359 (89)**

A total of **338 (92)** with :

- Professors **65 (11)**
- Associate Professors **120 (41)**
- Lectures **124 (32)**
- Assistants **29 (09)**

( ) = Nomber of Women **92**

DEDICATION

This Ph.D thesis is dedicated to:

My beloved Parents,My father, His Majesty Michel Ngako, Chief of Ngoté village and my beloved mother Toukam Therèse (in mémorium).

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LIST OF ABBREVIATIONS AND ACRONYMS

AMF	: Arbuscular Mycorrhizal Fungi
BPH	: Benign Prostatic Hypertrophy
EcM	: Ectomycorrhizae
EnM	: Endomycorrhizae
ErM	: Ericoid Mycorrhizae
F	: Frequency
FAO	: Food and Agriculture Organization
FRE	: Fine Root Endophyte
GC	: Gas Chromatography
GPS	: Global Positioning System
IBA	: Indole-3-Butyric Acid
ITS	: Internal Transcribed Spacer
IUCN	: International Union for Conservation of Nature
Masl	: Meter at the sea level
MCa	: Mount Cameroon
MO	: Mount Oku
MMa	: Mount Manengouba
Mt	: Mount
MS	: Mass Spectrophotometry
PCA	: Principal Component Analysis
PLS-DA	: Partial Least Squares- Discriminant Analysis
TIC	: Total Ion Chromatography
ZA	: South Africa
CITES	: Convention on International Trade in Endangered Species of wild Fauna
CIFOR	: Center for International Forestry Research

LIST OF APPENDICES

ABSTRACT

Prunus africana (*P. africana*) is an endangered medicinal plant species native to Africa, whose exploitation became abusive over the years. If nothing is done, extinction could occur in the coming decades. *Prunus africana* also faces multiple challenges: the recalcitrance of its seeds to germinate, irregular fruiting, and allogamic reproduction. The consequences are the threats of the species and the genetic resources of the country as well. Therefore, sexual and asexual regeneration studies have been set to propagate the species. However, the mycorrhizal and environmental diversities and their influence on the bioactive content of different populations of *P. africana* are still poorly elucidated in Cameroon. In fact, mycorrhizal and metabolite profile characterization are necessary to define the spatial scales at which conservation and domestication strategies could be developed. This has justified the aim of this study, which was to develop a *Prunus africana* strategy of domestication and sustainable management in relation with its environment, mycorrhizal status and metabolite profiling interactions in Cameroon. To make it possible, firstly, soil, root and bark samples were collected in three main *P. africana* mountainous areas in Cameroon (Mount Cameroon, Mount Oku and Mount Manengouba); then, isolation and characterization of Arbuscular Mycorrhizal Fungi (AMF) species were done, and the fungi trapping culture followed by a pre-germination, multiplication and evaluation of AMF growth efficiency. Secondly, to see their influence on *P. africana* root systems, each AMF species multiplied has been used as biofertilizer on *P. africana* cuttings in a frame, where indol butyric acid (AIB) was used as positive control and water was applied as a negative control. Thirdly, a glasshouse pot experiment was carried out to assess the effect of inoculation with indigenous AMF on the phytochemical content and growth of 8-month-old vegetatively propagated *P. africana*. Finally, the content and constitution of bioactive components of each observed tree in the three main locations has been examined via qualitative and quantitative analyses by Gas Chromatography coupled with Mass Spectrometry. The results revealed that there was a high diversity of AMF associated with *P. africana* found in the roots and rhizosphere soil, and variable community structure possibly shaped in part by local edaphic factors. They showed the presence of six *P. africana* associated species of AMF belonging to the genera *Gigaspora* (one species), *Glomus* (three species) and *Acaulospora* (two species). Each AMF species displayed a great capacity of growth improvement and self-multiplication. Inoculated AM fungi increased the root density of *Prunus africana* cuttings with an average root number of 35.00 ± 10.57 for the more efficient species (*Gigaspora margarita)*,

compared to the positive control which had an average root number of 10.00 ± 1.88 and the negative control 4.00 ± 1.80. The Inoculation with AMF inoculum also promoted leaf formation, with no significant effect on total seedling dry weight. AMF inoculum from *Gigaspora margarita* had a higher effect on tannin content, while no significant difference was observed on the total phenol and flavonoid contents. The analysis of the metabolomic results has shown that *P. africana* from these three locations exsudated almost the same composition of metabolites but with different concentration. Among the bioactive compounds detected, beta-sitosterol, epicatechin, 3-O-p-coumaroylquinic acid and chlorogenic acid turned out to be the main interesting coumpounds in *P. africana* in Cameroon due to their pharmaceutical hight value. The results have shown an ubiquitous presence of beta-sitosterol where trees from the Mount Manengouba area have displayed the highest average concentrations. *P. africana* phenolic's compounds are more highly concentrated in trees in the Mount Oku area, where beta-sitosterol is lowly concentrated. Mount Cameroon seems to be the area with the lowest content of bioactive compounds in *P. africana* in the three studied locations. There was a pronounced variation in the metabolite concentration among and within populations of *P. africana* studied. Our results showed a positive correlation between mycorrhizal variations of the soil and the bark quality of *P. africana* with beta-sitosterol positively correlated to the *Glomus* sp. and negatively correlated to *Gigaspora margarita* and *Acaulospora* sp. in the environment.

These results could help to improve the domestication and the sustainable exploitation of *P. africana*'s natural resources and a formulation of an efficient biostimulant for the population as the AMFs are good for the medicinal use by influencing phenolic components content which are good for the defense against pests. An appropriate characterization of plant materials is essential for the successful conservation of plant resources, and to ensure their sustainable use in a sense that, knowing the contents, we could better choose trees for domestication and by domestication, we could protect wild populations from overuse.

Key words: *Prunus africana,* mycorrhizal characterization, biofertilizer, metabolite profiling, bioactive content, biostimulant.

RESUME

Prunus africana (*P. africana*) est une espèce ligneuse d'Afrique dont l'écorce à vertu médicinale fait l'objet de sa principale exploitation. Si rien n'est fait, l'extinction de cette espèce pourrait survenir dans les décennies à venir. *Prunus africana* est également confronté à de multiples contraintes : la récalcitrance de ses graines, une fructification irrégulière et une reproduction allogamique. Les conséquences etant la ménace de la survie de l'espece ainsi que la disparition des ressources génétiques du pays. Par conséquent, des études de régénération sexuée et asexuée ont été mises en place pour propager l'espèce. Cependant, l'influence des diversités mycorhiziennes et environnementales sur les constituants bioactifs des différentes populations de *P. africana* demeure encore mal élucidée au Cameroun. En effet, la caractérisation des profils mycorhiziens et métaboliques est nécessaire pour définir les échelles spatiales auxquelles des stratégies de conservation et de domestication pourraient être développées. Ceci a justifié l'objectif de cette étude qui était de développer une strategie de domestication et de gestion durable de *Prunus africana* en fonction de ses varitions et interactions environnementales, mycorhiziennes et métaboliques au Cameroun. Pour ce faire, premièrement, des échantillons de sol, de racines et d'écorce ont été collectés dans trois principales zones de prédilection de *P. africana* au Cameroun (Mont Cameroun, Mont Oku et Mont Manengouba) ; ensuite, l'isolement et la caractérisation des espèces de champignons mycorhiziens à arbuscules (CMA) ont été réalisés, s'en est suivie leur pré-germination et multiplication pour la formulation des biofertilisants. Deuxièmement, afin d'apprecier leur influence réelle sur les systèmes racinaires des boutures de *P. africana*, chaque espèce de CMA multipliée a été utilisée comme biofertilisant dans un chassis de propagation, où l'acide indol butyrique (AIB) a été utilisé comme contrôle positif et de l'eau a été appliquée comme contrôle négatif. Troisièmement, une expérience sous serre a été réalisée pour évaluer l'effet de ces CMA natifs sur les constituants phytochimiques et la croissance des boutures de *P. africana* âgées de 8 mois. Enfin, la constitution et la teneur des composants bioactifs de chaque arbre de *P. africana* observé dans les trois principaux monts ont été examinés via des analyses qualitatives et quantitatives par Chromatographie en Phase Gazeuse couplée à la Spectrométrie de Masse. Les résultats ont révélé qu'il existe une grande diversité de CMA associés à *P. africana* trouvés dans les racines et le sol de sa rhizosphère, ainsi qu'une structure communautaire variable, éventuellement façonnée en partie par des facteurs édaphiques locaux. Ces résultats ont montré par ailleurs la présence de six espèces de CMA associées à *P. africana* appartenant aux genres *Gigaspora* (une espèce), *Glomus* (trois espèces) et *Acaulospora* (deux espèces). Chaque espèce de CMA a démontré une grande capacité d'amélioration de croissance et d'auto-multiplication. Les champignons mycorhiziens ainsi inoculés ont augmenté la densité racinaire des boutures de *Prunus africana* avec un nombre moyen de 35.00 ± 10.57 racines par bouture pour l'espèce la plus efficiente (*Gigaspora margarita*), par rapport au contrôle positif (ayant totalisé un nombre moyen de 10.00 ± 1.88 racines par bouture) et au contrôle négatif

(avec un nombre moyen de 4.00 ± 1.80 racines par bouture). L'inoculation des Champignons mycorhiziens a également favorisé la formation des feuilles, sans effet significatif sur le poids sec total des plantules. L'inoculum constitué du CMA *Gigaspora margarita* a eu l'effet le plus élevé sur la teneur en tanins, alors qu'aucune différence significative des espèces de CMA étudiées n'a été observée sur la teneur totale en phénols et en flavonoïdes des plantules de *P. africana*. L'analyse des résultats métabolomiques a montré que *P. africana* des trois sites étudiés, exsudait quasiment la même composition en métabolites, néanmoins avec des concentrations différentes. Parmi les composés bioactifs détectés, la beta-sitostérol, l'épicatéchine, l'acide 3-O-p-Coumaroylquinique et l'acide chlorogénique se sont révélés être les principaux composés les plus efficients de *P. africana* au Cameroun, en raison de leur haute valeur pharmaceutique. Les résultats de cette étude ont également montré une omniprésence de la beta-sitostérol (composé phare de *P. africana*) dans les trois zones étudiées avec les arbres de la région du Mont Manengouba présentant les concentrations moyennes les plus élevées. L'étude a révélé que les composés phénoliques exudés par *P. africana* étaient plus concentrés dans les arbres de la région du Mont Oku, où la beta-sitostérol y est faiblement concentrée toutefois. En outre, le Mont Cameroun semblerait être la zone avec la plus faible teneur en composés bioactifs de *P. africana* dans les trois zones étudiées. Les résultats ont montré une variation prononcée de la concentration en constituants bioactifs entre au sein des populations de *P. africana* étudiées. Enfin, une corrélation positive aété observée entre les variations mycorhiziennes du sol et la qualité de l'écorce de *P. africana* avec précisement une correlation positive entre la beta-sitosterol et *Glomus sp* pourtant négativement corrélée à *Gigaspora margarita* et *Acaulospora spp* dans l'environnement de vie.

Ces résultats pourraient contribuer à améliorer la domestication et l'exploitation durable des ressources naturelles de *P. africana*, ainsi que la formulation d'un biostimulant efficace pour la population, car les CMA, influençant la teneur en composés phénoliques, pourraient dorénavant servir à usage médical. Une caractérisation appropriée du matériel végétal est essentielle pour une conservation réussie des ressources végétales et ainsi garantir leur utilisation durable dans le sens où, connaissant leurs constitutions en substances bioactives, nous pourrions mieux choisir les arbres à domestiquer et, par la domestication, nous pourrions protéger les populations sauvages de *P. africana* d'une exploitation abusive.

Mots clés : *Prunus africana*, caractérisation mycorhizienne, biofertilisant, profil métabolique, constituants bioactifs, biostimulant.

INTRODUCTION

INTRODUCTION

1. Context and justification

Prunus africana (Hook K.) is a plant of the Rosaceae family (Kalkman, 1965). The species is abondant in Afro-montane forests and grows preferentially between 900 and 3000 m altitude (Dorthe, 2003). The bark extracts are used for the treatment of benign prostatic hyperplasia that affects 50 percent of men above 50 (Stewart, 2003). Global demand for these extracts is estimated at more than 4,000 tons per year for a value of $ 220 million (Cunningham et *al*., 2002). *Prunus africana* bark (and leaves) is basic constituents of at least nineteen drugs sold by twenty-three companies in Europe and in North and South America (Cunnningham et *al.*, 2000). Since 1972, 50 percent of these products come from Cameroon, 20 percent from Madagascar and 7 percent from Uganda (Jimu, 2011). The overexploitation of the species has led to its classification in the IUCN Red List of Threatened Species and it is also listed in CITES Appendix II in 1995, as a species which needs special requirement to be exported (Anonyme, 2008, Tchatat, 2006). The overexploitation of bark, the ageing population and the prevalence rate of prostate disorders, which is more than 60% besides the interest represented by the extracts of bark of *P. africana*, are more than conditioning factors of the place occupied by this species on the market (Bombardelli et Morazzoni, 1997).

2. Problem Statement

Overexploitation of the species, the recalcitrance of seeds to germinate, its irregular fruiting (every 2 to 3 years) limits the regeneration process and affect the availability of seedlings in Cameroon (Cunningham et *al.*, 2002). Another major contraint is the allogamy mode of reproduction, which constitutes a barrier to *in-situ* conservation (Munjuga et *al.*, 2000); this was confirmed by the discovery of a self-incompatibility system in *Prunus africana* (Faha, 2016). Therefore, the relatively long juvenile period (15-20 years) and a short duration of reception of the stigmas of each flower, added to the uneven distribution, often of low density. The low percentage of *Prunus africana* in the forest show that there is reason to be concerned about the actual population size in the forest, the variability of their long-term reproduction genetic, molecular and environmental variability among populations for in situ conservation (Munjuga et *al.*, 2000).

To avoid extinction of *Prunus africana*, several conservation measures have been set both nationally and internationally. But there are often problems of technical capacity to better ensure the control and monitoring of the species. In addition, since 2007, sustainable

management of *P. africana* in Cameroon and the DRC has been a priority for FAO, SNV, CIFOR and ICRAF (Anonymous, 2008). Few works have been done to improve on the conservation techniques.

Preliminary tests of micropropagation show that up to 100 percent of germination is obtained after 6-month storage at 4 °C with the use of growth regulators; for micropropagation, microcutting was successful with the use of cytokinines TDZ, which gave multiple shoots (Nzweundji et *al*., 2020). Also, vegetative propagation by cuttings showed a rooting rate of 80% (Tchoundjeu et *al.,* 2002, Avana, 2006). However, the low field recovery rates remain a challenge. But these studies were confronted to the enhancing plant growth, the inaccessibility of the seeds and embryonic state of *P. africana* domestication. Therefore, studies on genetic and environmental diversity relating to the bioactive constituents have been established.

Forest destruction and disturbance can have long-term consequences for species' diversity and ecosystem processes such as seed dispersal. Understanding these consequences is a crucial component of conserving vulnerable ecosystems. Avana et *al*. (2004) studied the genetic diversity of *Prunus africana* using AFLP and microsatellite markers compared to RAPD markers to provide larger indices of diversity and genetic distances between populations. Many studies are already done concerning genetic diversity and molecular characterization using different species of *Prunus* (Sharma and Sharma 2010; Ozyurt et *al.,* 2013; Bouhadija et *al*., 2015; Tamazizt et *al.,* 2015; Zeinalabetini et *al.,* 2016). However, the limits were the use of very few primers and, therefore, a very small genetic variability possible detected in *Prunus africana* especially. Also, Kadu et *al*., (2005) showed the existence of close genetic links between the *Prunus africana*'s populations of western Kenya and those of Cameroon despite the geographical distance. Then, Farwig et *al* (2006) prove that forest destruction and disturbance may have long-term consequences for species diversity. They also prove that human activities such as fragmentation and selective logging of forest can threaten population viability by modifying ecological and genetic processes of *Prunus africana* (Farwig et *al*., 2008). Guajardo et *al* (2008) show that information from genetic maps has demonstrated that all *Prunus* species share a similar genomic structure. Kadu et *al*. (2011), in the study of the phylogeny of *Prunus africana* in Africa, showed that haplotype similarities have revealed genetic proximities between western Uganda and East Africa, testifying the existence of an old corridor between East and West Africa. They also showed that there was unexpected diversity between *P. africana* accessions of West Africa,

whereas *P. africana* accessions of East and South were very close, with 13 common dominant haplotypes of 22 analyzed. These studies were limited since markers reveal low polymorphism. Also, the large-scale study of *P. africana* in many countries at the same time might not precisely reveal their phylogeny, due to a restricted sampling of individuals.

Concerning bioactive components, Kadu et *al*. (2013) showed that the increasing order of average concentrations of bioactive elements of *P. africana* in Africa is: lauric acid (18 mol/l), myristic acid (22), n-docosanol (25), ferulic acid (49), beta sistosterone (198), beta sitosterol (490) and ursolic acid (743). There is a very large variation in concentration between populations for ursolic acid in the order of 66%, and a rather low variation for beta sistosterol (20%). The environmental parameters (altitude, rainfull …) do not influence the concentrations of these substances. Ursolic acid concentration is negatively correlated with the size of the tree. The beta sitosterol concentrations are dependent on the haplolytic diversity (certain levels of concentrations are correlated with specific haplotypes). There is also an independent evolution of the metabolism of the bark within different phytogeographic lineages, certainly due to the environmental parameters shown by RADP molecular markers that are not polymorphic enough. Non specific microsatellites in this study could constitute one of its limits, associated with the small number of individuals sampled. Tchouakionie et *al.,* (2014) showed that the beta sitosterol concentrations of the *P. africana* bark in Cameroon vary from 0 to 38.65 ug / ml, and that there is no significant correlation between the concentration of beta sitosterol, the altitude and the chemical constituents of the soil. The *P. africana* populations of Cameroon are clustered into three groups, according to environmental criteria. This work aimed at assessing the effects of AMF on plant growth and propagation efficiencies in *P. africana* trees from different areas in Cameroon, as well as their potential impact on the composition and content of medicinally important bioactive compounds in their bark. This knowledge could be used to further specific conservation efforts for *P. africana* in Cameroon.

3. Research questions

- What contribution can the knowledge of metabolites profiling, environmental and mycorrhizal status of *Prunus africana* have on its sustainable management and conservation strategy in Cameroon?
- What is the link between the different Mycorrhizae association and the bioactive compounds of *Prunus africana* in Cameroon?

- Could the environment and mycorrhizae difference between individuals of *P. africana* influence its growth and development in vegetative propagation?
- Could the phylogenetic difference between individuals of the same population of *P. africana* influence its growth and development?
- It could exist a correlation between the mycrrhizal status and environmental parameters of *P. africana* and its bioactive contents.

4. Hypotheses

- There may be mycorrhizal diversity between *P. africana* populations in Cameroon.
- The environmental and Mycorrhizal diversity of *P. africana* could affect the composition and content of its phytochemical compounds.
- The environmental and Mycorrhizal interaction could affect the growth and development of *P. africana* cuttings in vegetative propagation.
- The content of the phytochemical constituents could vary according to the different genotype that could exist between *P. africana's* different groups.
- There may exist a correlation between environmentals parameters, mycorrhiral status and bioactives constituents of *P. africana*.

5. Objectives

General objective

To develop a *Prunus africana* strategy of domestication and sustainable management in relation with its environment, mycorrhizal status and metabolite profiling interactions in Cameroon.

Specific objectives:

- investigate the AMF diversity in the roots and rhizospheric soil of *P. africana* in Afromontane forests in Cameroon;
- evaluate the AMF effect on the growth of vegetatively propagated *P. africana* in the nursery;
- evaluate the effect of AMF on the phytochemical profile of vegetatively propagated *P. africana*;
- establish the metabolite profile of *Prunus africana* in Cameroon;
- Determine the correlation between the mycorrhizal and environmental variation of *P. africana* in relation with its bioactive constituents and contents.

CHAPTER I. LITERATURE REVIEW

CHAPTER I. LITERATURE REVIEW

I.1. Overview on *Prunus africana*

I.1.1. Description, taxonomy, and distribution of *Prunus africana*

Prunus africana (Hook. f.) Kalkman. syn. *Pygeum africanum* (Hook. f.) (Kalkman et *al*., 1965), is an evergreen canopy tree which grows up to 30–45 meters in height, with approximatively a 1.5-meter diameter for the adult trees (Fig. 1). The bark of young *P. africana* trees is glabrous, smooth, and green to green-reddish, whereas the mature one is grey to black and occasionally with brown dots or patches equivalent to crack or lenticels appear dilated bark. Mature bark is also slightly fissured and sometimes tessellated with rectangular adherent scales (Muñoz *et al.*, 2006; Kotina *et al.*, 2016). The leaves are simple, alternately arranged, usually stipulate with the stipules often adnate to the petiole. The flowers are creamy-white, small, androgynous with 10 to 20 stamens, insect-pollinated, fragrant, and distributed in 70 mm axillary racemes (Ingram & Nsawir, 2007). *P. africana* flowers are hermaphrodite pollinated by insects and possibly birds, fruits are transversely ellipsoid, indehiscent bilobed drupe, deep red to purple black, weighing approximatively 0.5 g, with an intensely bitter taste (Betti, 2008; Hall *et al.*, 2000). The seeds are recalcitrant and germinate after between 50 and 120 days. Seeds lose their viability quickly if they are not stored in a moist atmosphere, such that after six months, just 20% of seeds are viable (Muñoz *et al.*, 2006). The tree is generally single stemmed, developing multi-stems when saplings are browsed or cut, with large trees having weak resprouting capability (Betti et *al*., 2008).

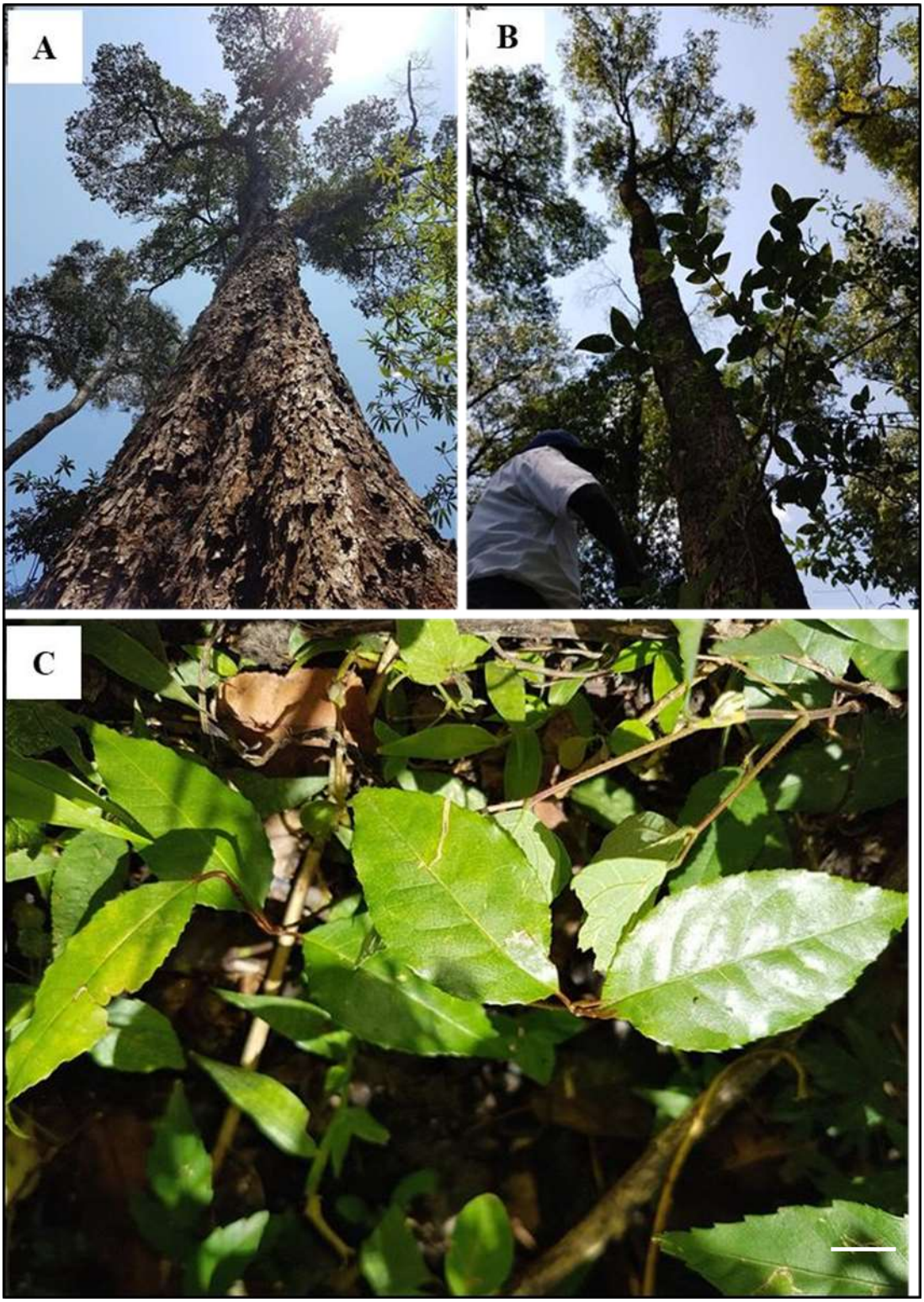


Fig. 1. *Prunus africana* in its natural habitat. A) Mature tree in Kakamega forest; B) Mature tree in Chuka forest; C) *P. africana* leaves of young plants. (Tchiechoua, 2020)

P. africana belongs to the genus *Prunus*, which comes from the classical Latin name of the plum tree and the Greek '*prunos*' (plum). The specific name means 'of Africa'. Thus, it is the only member of the genus *Prunus*, which comprises of more than 200 species, endemic to

Africa (Kalkman et *al*., 1965). The genus *Prunus* is a part of the Rosaceae family that have about 3200 species (Dharani et *al*., 2002).

P. africana is a broadly distributed tree in sub-Saharan Africa and circumscribed in Afromontane Forest habitats of Central, Western, Eastern and Southern Africa (Kadu *et al.*, 2011; Stewart et *al*., 2003; Vinceti *et al.*, 2013). In western tropical Africa, *P. africana* is found in Nigeria (Mambila plateau, Gashaka Gumti National Park and southeast of the country); in Central Africa, the species is found in Cameroon (Bamenda Highlands, Mt. Kilum, Mt. Manengouba, Ademawa plateau and Mount. Cameroon) and Equatorial Guinea (Pico Basile and Grand Caldera de Luba on the island of Bioko); in Eastern tropical Africa, *P. africana* occurs in Burundi (Albertine Rift, Mount Heha/Ijenda, Mount. Bururi and Teza Forest), Democratic Republic of Congo (Kivu Region, Rwenzori and Virunga Mountains and Kahuzi-Biega National Park), Ethiopia (northwest Highlands to Lake Tana and southeast Highlands to Horar, Harerge, Illubador, Kefa, Arsi and Wolega), Kenya (Kakamega Forest, Mount. Kenya, Mount. Elgon, Mau Forest), Rwanda (Virunga Mountains, Mukura and Nyungwe Forests), Sudan (Imatong Mountains), Tanzania (northeast of the country including Mount Kilimanjaro) and Uganda (Kalinzu, Bwindi, Mgahinga, Mount. Elgon and Imatong Mountains) (Betti *et al.*, 2016; Farwig *et al.*, 2008). In Southern Africa, the species is found in Angola (Bailundu Highlands and Mount Moco), Lesotho, Malawi (Mount. Mulanje, Zomba and Vipya plateaus), Mozambique (Mount Chiperone, Chimanimani Mountains and Mount Gorongosa), Sout Africa (Mpumalanga through KwaZulu/Natal to the Knysna Forest), Swaziland (Forest patches near Malolotja and Mbabane), Zambia and Zimbabwe (Chimanimani, Nyanga and Chirinda Forest Reserve) (Betti et *al*., 2008). *P. africana* also occurs in island countries, namely Madagascar (Zahamena Strict Nature Reserve, Montadia, Antsevable and Manakumbahiny-Est, Tsaratanana Mountains, Ambatondrazaka and Moramanga, Tampoketsan Ankazobe and Ankaratra Mountains), Sao Tome-Principe (central Principe, near the volcanic plugs of Joao Dias Pai e Filho and montane Sao Tome) and Grand Comoros (Betti et *al*., 2008; Dawson & Powell, 1999) (Fig.2).

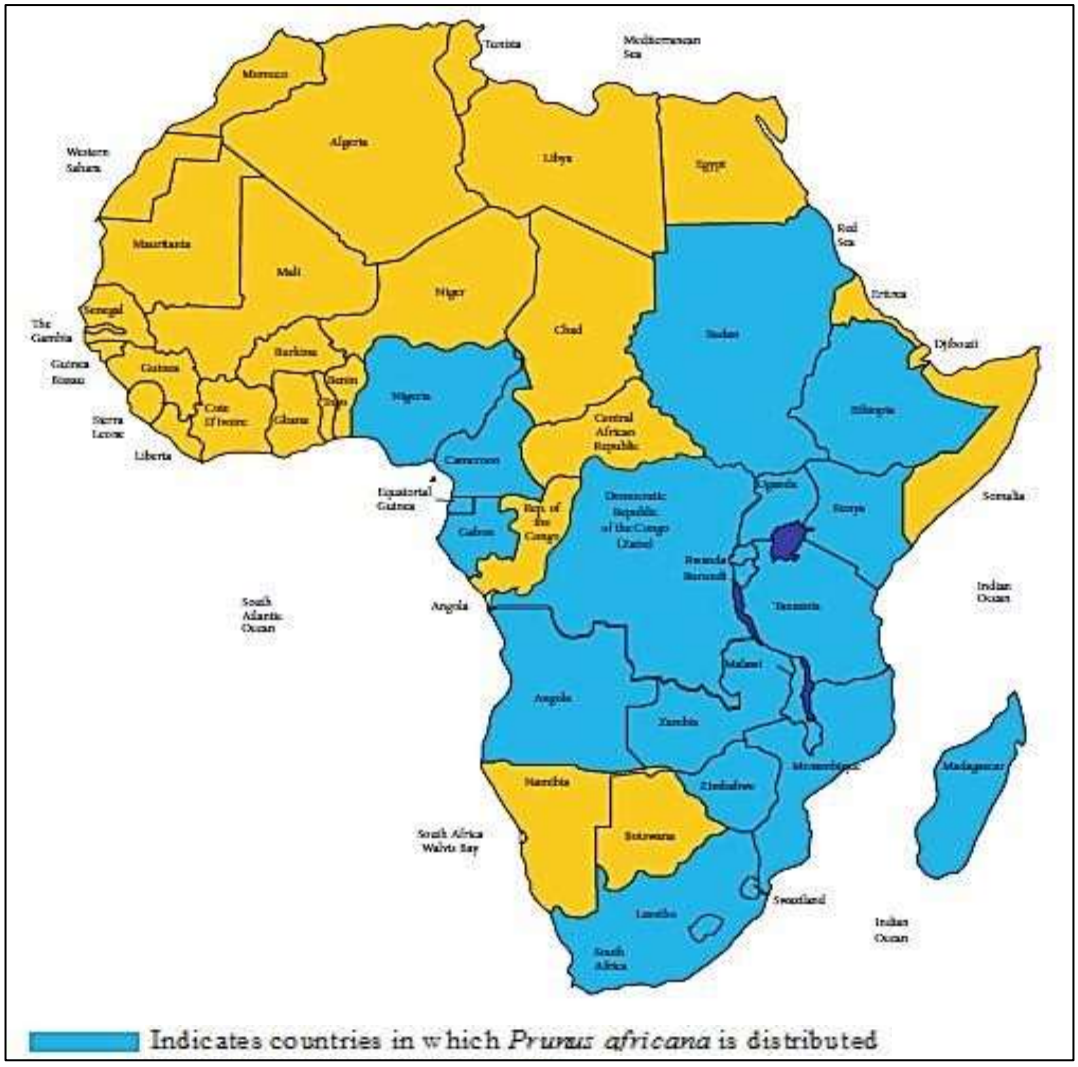


Fig.2. Distribution of *Prunus africana* in Africa (Komakech et *al*., 2017)

I.1.2. Importance of *Prunus africana*

Prunus africana is an important multipurpose medicinal tree, which has been used traditionally for many centuries in Africa. The local populations in Africa use its bark, stem, roots, and the leaves for different purposes (Koros *et al.*, 2016). *P. africana* is also used as firewood, charcoal production, and timber for furniture, lorry bodies, bridges, flooring, tool handles, poles and house building (Betti, 2008; Koros *et al.*, 2016). The bark of the mature tree is used by herbalists as a remedy for stomach-ache, an infusion for the treatment of lack of appetite, urinary and bladder infections, chest pain, malaria, microbial infections, and kidney disease (Betti, et *al*., 2008; Bii *et al.*, 2010; Koros *et al.*, 2016; Mwitari *et al.*, 2013; Otieno & Analo, 2012). Since the 1700s, the plant has attracted the interest of European explorers, who were inspired by the people of Kwa-Zulu Natal who used the bark for the treatment and healing of difficulties in passing urine (Cunningham *et al.*, 2016; Ingram *et al.*, 2009). The

refined extracts from *P. africana* bark have been used since the mid-1960s for treatment of benign prostatic hyperplasia (BPH) (Cunningham *et al.*, 2002; Fashing, 2004; Ingram *et al.*, 2009; Jimu, 2011; Komakech *et al.*, 2017; Koros *et al.*, 2016). Recent publications report that extracts from *P. africana* bark could be a suitable alternative solution for chemoprevention and chemotherapy of prostate cancer (Cunningham *et al.*, 2002; Fashing et *al*., 2004; Jimu et *al*., 2011; Komakech *et al.*, 2017; Koros *et al.*, 2016).

I.1.2.1. Genetic variation

P. africana has a wide but disjunct distribution in highland forest 'islands' across Africa (Kalkman et *al*., 1965), and genetic variation can be expected to have diverged accordingly. Analysis by Dawson and Powell (1999) using molecular markers (random amplified polymorphic DNA, RAPD) indicated this to indeed be the case. Analysing 10 populations sampled from Cameroon, Ethiopia, Kenya, Madagascar and Uganda, data revealed most genetic variation among countries (66%, $P < 0.001$), indicating the importance of regional approaches for conservation. Variation among individuals within populations, and among populations within Cameroon and Madagascar, was also highly significant, confirming the importance of developing genetic management strategies that also take account of genetic variation at a country level. Despite the geographic distance between Uganda and Cameroon, Ugandan material was more similar to West African populations than that from Kenya and Ethiopia. This is consistent with theories by White et *al.* (1983) relating to historical climate differences and migration corridors during glacial periods. Data indicated that Malagash populations were most distinct and therefore of particular conservation concern.

I.1.2.2. Reproductive biology

Few studies on the reproductive biology of *P. africana* have been conducted. Seed of *P. africana* is recalcitrant in nature, which limits *ex situ* seed storage (Jaenicke *et al*., 2000; Sunderland and Nkefor 1997). The best conditions for seed storage were achieved when seeds from mature (purple) fruit were harvested directly from trees and depulped immediately after collection, followed by storage, without drying, at 5° Celsius. However, even under these conditions, germination was only 35% after 12 months of storage (Jaenicke *et al*. 2000). Long-term seed storage of *P. africana* as a means of *ex situ* conservation, is therefore not advisable, although short-term storage across planting seasons is possible. According to studies by Munjuga *et al*. (2000), the species is predominantly outcrossing. Flowering and fruiting in each population may be spread over a relatively long period of time, with a short stigma

receptivity of individual flowers (Munjuga *et al*., 2000). Combined with the frequent low density, patchy and variable size class distribution of *P. africana* in forest (Ewusi *et al*., 1992; Ewusi *et al*., 1997), these observations raise concerns regarding effective population sizes of exploited natural stands and their long-term reproductive viability for *in situ* conservation.

I.1.2.3. Ecosystem function of *P. africana*

The Afromontane Forest 'islands' or other highland forests, which *P. africana* inhabits, have been classified as being important conservation targets (Davis *et al*., 1994). According to Thomas and Cheek (1992), 42 plant species are strictly endemic to Mount Cameroon. Bwindi Impenetrable Forest in Uganda is one of the most diverse forests in East Africa and contains half the world's endangered mountain gorilla (*Gorilla gorilla beringei*) population (Cunningham et *al*., 1996; Wild and Mutebi 1996). Kakamega Forest in Kenya is considered to be the eastern edge of the Guinea-Congolian Forest block and as such is considered of particularly high conservation value (Kokwaro et *al*., 1988). Although, the situation varies greatly with location, these forested areas are often under threat from agricultural clearance because they coincide with regions of high population density (Cunningham et *al*., 1996; Cunningham *et al*., 1997; Watts and Akago 1994; Wild and Mutebi 1996). Other activities may further contribute to the degradation of these highly diverse forests. The possible keystone function of *P. africana* within these forests in maintaining their integrity is therefore a key consideration. However, although the fruit of *P. africana* is eaten by a number of threatened bird and mammal species (Cunningham and Mbenkum 1993), none of these appear to be critically dependent on *P. africana but* appear to be generalist frugivores that may feed off a number of plants. Moreover, the density of mature *P. africana* trees in forest is generally low (Ewusi *et al*., 1992; Ewusi *et al*., 1997; Nzilani et *al*., 1999), suggesting that the quantity of fruit produced is limited and unlikely to compose a large part of the diet of bird and mammal species.

The most important impact of *P. africana* harvesting on the ecosystems in which it occurs may be indirect. According to the Bioko Primate Protection Programme (BPPP), in 1997, new access routes opened into the forest of Pico Basile on Bioko to harvest *P. africana*. These gave bush meat hunters easier access to the habitat of a seriously threatened endemic subspecies of the primate Preuss's guenon (*Cercopithecus pruessi insularis*), which has contributed to its endangered status (BPPP 1999). In Cameroon, the Fon of Banso considered that commercial harvesting of *P. africana* bark had aggravated forest clearance by changing local perceptions

of forest use, from being a community resource to an asset to be exploited for personal gain (Cunningham and Mbenkum 1993)

I.1.2.4. Policy and regulation

I.1.2.4.1.International

Internationally, the main regulation that influences the development of conservation strategies for *P. africana* is CITES. Listing of *P. africana* on Appendix II of the Convention indicates that trade in both wild and cultivated material must be licensed at export and import. However, a difficulty in implementation has been the identification of *P. africana* products in international trade, which has lead to unreported export and, particularly, import of *P. africana* (Cunningham *et al*. 1997). An additional difficulty is since some countries have not to date nominated *National CITES Authorities*.

I.1.2.4.2. National and local

In the countries where exploitation is highest (Cameroon and Madagascar), a number of national regulations address the harvesting of *P. africana* bark (reviewed by Ndibi and Kay 1997 for Cameroon; Walter and Rakotonirina 1995 for Madagascar). Regulations of the two countries differ widely, however, with the Malagash framework in particular being considered inadequate from a sustainable use perspective. Conservation activities in Madagascar could therefore benefit if harvesting regulations were adapted which were more in line with those applied in Cameroon. However, also in Cameroon, it seems as if the regulations for sustainable harvesting are not always well understood. For example, harvesting licenses specify that bark should only be stripped from one quarter of the standing trees, but the level of tree mortality and rate of bark recovery following present practices remain a concern (Sunderland and Tako 1999; Cunningham and Mbenkum 1993; Ewusi *et al.*, 1992). Inadequate inventory data on the size of populations is an additional constraint to the possibilities to determine sustainable harvesting levels (Ewusi *et al*., 1997). Apart from the development of regulations and quotas for harvesting to ensure sustainability, their enforcement is often difficult. In areas where *P. africana* is currently exploited, harvesting regulations are not consistently adhered to, due to lack of awareness and resources, and institutional weaknesses (Cunningham *et al*., 1997; Ndibi & Kay 1997; Mbenkum and Fisiy 1992). In Cameroon, for example, at least 900 tons of bark are reported to have been harvested illegally around Mount Cameroon between 1994 and 1996, when export licenses

were awarded to three Cameroonian entrepreneurs to fulfil a large order for bark export to Italy (Cunningham *et al.*, 1997). In Madagascar, almost all bark is reportedly harvested illegally (Walter and Rakotonirina 1995), and includes harvest in protected areas (Ian Dawson, personal observations). In Cameroon, where recent moves have been taken to issue a much-reduced quota for bark collection from the Mount Cameroon area, it will be important to ensure, that resulting sustainable harvesting will not be counteracted by increased illegal exploitation (James Acworth, Mount Cameroon Project, personal communication).

Thus, even when regulations exist to promote the sustainable management of *P. africana* from wild populations, such formal regulations alone are unlikely to be effective in ensuring conservation of the resources. As a result of verified problems, there have been recent moves, promoted by European countries, to place *P. africana* on Appendix I of CITES, which prohibits all commercial trade (Nouhou Ndam, Mount Cameroon Project, personal communication).

I.1.2.5. Community management issues

Local human communities are an important determinant in the success of conservation efforts, as they are often involved in harvesting *P. africana* bark and can derive long-term benefits from sustainable utilization strategies. One of the strongest efforts to encourage community participation in the sustainable management of *P. africana* has been made in Mount Cameroon, where the company handling and exporting bark signed special agreements with two villages in 1997 for the sustainable management and production of *P. africana* (Laird and Lisinge 1998). Elements of the agreement included payment of relatively high prices per unit weight of bark to villagers, and the training of collectors in harvesting techniques. Prescribed harvesting involves no cutting of trees and is monitored by a committee.

Community management of forests can help overcome difficulties in enforcement of regulations (Ndibi and Kay 1997). However, since approaches to forest resource use differ widely among communities, even within a region (Watts and Akogo 1994), the involvement of communities in conservation efforts must be location-specific rather than prescriptive. This makes the development of management plans a lengthy process and, often, longer than the window available for effective conservation action.

I.1.2.6. Markets and economics

As the world population ages, the demand for treatments for benign prostatic hyperplasia (BPH) is likely to increase. Coupled with trends toward the use of herbal products, the future world's demand for *P. africana* bark may increase considerably (Simons *et al*. 1998). In this context, unless alternative sources of bark can be developed through cultivation, or alternative remedies for BPH are found, natural stands of *P. africana* are in danger of depletion. In fact, several other herbal remedies are used to treat BPH, and these may become more popular if the available stocks of *P. africana* continue to decrease (Cunningham *et al*., 1997). The collection of bark provides a relatively small return to harvesters compared to profits of the companies marketing the product (Simons *et al.* 1998). However, the poverty of collectors and lack of alternative sources of income mean that they will make considerable efforts to harvest trees. In Madagascar, for example, villagers are willing to walk long distances (for several days) into the forest to harvest bark of *P. africana* (Walter and Rakotonirina 1995). Only extremely remote populations of the species are likely to be not viable economically for harvesting purposes.

I.1.3. Management of trade on Prunus africana bark

The international trade of the bark of *P. africana* is one of the main factors, which have led to its overexploitation during decades in Africa. Unsustainably harvested bark of wild P. *africana* is more internationally traded than any other medicinal plants in Africa (Cunningham *et al.*, 2016). The global demand for the bark is approximately 4000 tonnes per year, and it is supplied by a few African countries, led by Cameroon exporting 62%, Madagascar, 20%, and Uganda and Equatorial Guinea 7% each (Jimu et *al*., 2011). A set of frameworks and regulatory laws have been initiated at local and international levels by the Convention on International Trade in Endangered Species of wild fauna and flora (CITES) to regulate the trade of *P. africana* bark in order to limit the genetic erosion and to achieve sustainable conservation of *P. africana*.

For instance, Cameroon went through several regulations and laws promulgating trade in *P. africana* bark trading since the 1980s (Ingram et *al*., 2014). From 1983 to 2014, around seven bans were declared by the administrative and traditional leaders on the harvest and trade of *P. africana* bark (Cunningham *et al.*, 2016). The CITES committee in 2007 recommended to the Cameroonian government the reduction of trade of the annual quota of *P. africana* bark from 2000 tonnes/year to a reasonable quota based on the scientific data and reports

(Amougou *et al.*, 2010). The persisting unsustainable wild harvest of the bark at the international level led CITES to list *P. africana* in Appendix II of CITES in 1995. Therefore, the trade on *P. africana* bark needs to be controlled to avoid utilization that was incompatible with the species' survival (Cunningham *et al.*, 2016). To emphasize the international action, the International Union for Conservation of Nature (IUCN) classified *P. africana* as a vulnerable species in 1998 (World Conservation Monitoring Centre, 1998). In 2007, the European Union enacted a ban on the importation of the bark of *P. africana* in Europe (Cunningham *et al.*, 2016; Cunningham *et al.*, 2014; Jimu et *al*., 2011).

I.1.3.1. Options for conservation

I.1.3.1.1. Forest

In the context of the presently high levels of illegal harvesting without management, *in situ* conservation of *P. africana* in countries where the species is widely exploited will be possible only in very limited cases, in which strict monitoring of harvesting can be assured, strong community involvement in sustainable harvesting can be sustained, or trees are too inaccessible to exploit. Although traditional community beliefs connected with forest conservation are being eroded (Cunningham and Mbenkum 1993), in specific cases they may form a basis for *in situ* conservation efforts. For example, in Embu District of Kenya, over 250 sacred groves of forest have been identified in land otherwise cleared for agriculture. Many of these groves contain *P. africana* (Meru Traditional Healers Group, personal communication). The utility of these groves for conservation purposes depends on their size and isolation from each other, but remnant *P. africana* trees on farmland are expected to provide a degree of gene flow between small natural groves, which may allow population viability to be maintained (Ard Lengkeek, personal observations). In most areas, *P. africana* does not appear to be a keystone species within the ecosystems in which it occurs. Therefore, *in situ* management strategies should focus on conservation of representative forest blocks rather than on the management of *P. africana*. However, where specific interventions can be undertaken to promote the recovery of *P. africana* populations in harvested areas, these should include opening the canopy around, and clearing the undergrowth beneath, seed bearing trees (Ndam et *al*., 1998).

I.1.3.1.2. Enrichment planting and plantations

To date, successful plantations and enrichment plantings have been primarily limited to Kenya, where *P. africana* has been planted by the Forest Department for timber production. Although these stands provide a useful resource also for bark harvesting, they are of limited utility for conservation as they are often of unknown origin and as they may have a narrow genetic base. However, their success indicates that planting efforts could be successful also in Cameroon and Madagascar. Proper attention should be given to site choice and tree management practices, in addition to the origin and genetic variation of the reproductive materials used. Well-managed plantations could serve as *ex situ* conservation stands, and as sources of planting material for future reforestation and on-farm cultivation. In areas in Madagascar where natural stands are particularly threatened and a forest management culture is not welldeveloped (Walter and Rakotonirina 1995; Dawson et *al*., 1997), establishing *ex situ* conservation stands should be a priority. In cases where harvesting has removed all mature trees from natural tree populations, vegetative propagation techniques, such as cuttings and grafting, may be used to safeguard genetic resources of *Prunus africana* (Jaenicke *et al*., 2000).

I.1.3.1.3. Small-scale farmer cultivation

According to Cunningham (1994; 1996), the conservation of Afromontane forest can be improved through providing opportunities for small-scale farmers to cultivate useful forest products outside of protected areas. Trees planted on-farm can be an important genetic resource if attention is paid to the origin and genetic variation of the cultivated material. As natural forests contract as a result of agricultural expansion, the management of farmland also for the conservation of biodiversity becomes increasingly important. Research indicates that, in some areas, the number of trees planted on small-holder farms has increased together with human population density. While natural forest has been cleared, however, more trees have been planted on-farm to compensate this loss (Arnold and Dewees, 1995; 1998). Agroforestry may be a particularly appropriate method for conservation in highland areas of Africa where population density and the pressure on natural forests are especially high. In areas of exploitation, this system of “conservation through cultivation” is likely to be more effective than attempting to sustainably manage *P. africana* within natural forest.

In Cameroon, where considerable planting of *P. africana* by small-scale farmers has already taken place (Cunningham *et al.,* 1997), measures are underway to assess the current genetic base of material planted by farmers, using RAPD analysis (Ian Dawson, unpublished data).

Despite the merits of on-farm tree planting, there are serious constraints for its expansion. First, the intermediate nature of seed (Jaenicke *et al.,* 2000) limits seed availability. Second, although a large tree can yield large quantities of seed, seed yields fluctuate widely between years. Seed shortage is likely to be exacerbated in future years as the size of natural populations of trees diminish. Since the approximate time to the first flowering and fruiting in *P. africana* is 15-20 years, the establishment of seed stands is an urgent priority, as is the further development and adoption of vegetative propagation practices (Jaenicke *et al.,* 2000). Other difficulties with cultivation include the relatively long-time scale until bark harvesting is possible (approximately 15 years after planting), access to markets for bark from cultivated trees, and policy issues concerning tree tenure (Simons *et al*., 1998).

I.1.3.1.4. Regeneration strategies of *Prunus africana*

Regeneration is the main pillar that stakeholders should consider for a sustainable strategy for *P. africana* conservation. The regeneration of *P. africana* can be done *in-situ* (natural regeneration) or *ex-situ* by farmers (artificial regeneration through seed and cuttings). *In-situ* regeneration is a natural propagation of the tree through seedlings or by digging out wildlings from the forest (Macharia, 2000). Due to the light demand statute of *P. africana*, *in-situ* regeneration in an open forest is suitable to maintain its population (Ingram, 2014; Koros *et al.*, 2016; Solefack *et al.,* 2016). The *in-situ* regeneration of *P. africana* could be relatively easy to establish in Kenyan Afromontane (Orwa *et al.,* 2010), whereas a poor natural regeneration of *P. africana* has been reported from Nyanga National Park in Zimbabwe (Jimu *et al.,* 2012). However, the availability of viable seeds is the main limitation of *P. africana ex-situ* regeneration. Therefore, vegetative regeneration can be a better alternative for the propagation of *P. africana* out of its natural habitat. Prior investigations on vegetative propagation of *P. africana* were reported in Cameroon (Tchoundjeu *et al.,* 2002) and in Ethiopia (Kebede *et al.,* 2013). They recommended the use of juvenile leafy stem cutting of *P. africana* for effective regeneration. Nursery experiments undertaken by these authors showed that rooting media such as sand and/or sawdust were suitable and could ensure the development of roots.

I.1.4. Phytochemical profile of *Prunus africana*

In response to environmental variations such as light, temperature, soil microorganisms, soil water, nutrients, soil heavy metal, and salinity, plants can produce different kinds of phytochemicals to find their balance in the environment (Guerriero *et al.*, 2018; Molyneux *et al.*, 2007; Yang *et al.*, 2018). Phytochemicals are chemical components produced by plants through their secondary metabolism. These secondary metabolites have gained interest in industries for various applications, mainly in pharmaceutical industries (Guerriero *et al.*, 2018).

The pharmacological value of *P. africana* bark in the treatment of benign prostatic hyperplasia (BPH), a noncancerous enlargement of the prostate gland that occurs especially in some men over 50 years (Kadu *et al.*, 2012), has motivated the unsustainable overexploitation of the tree in the last few decades in Africa. Indeed, *P. africana* bark (Fig.3), contains known and unknown groups of phytochemical components (Kadu *et al.*, 2012). The known compounds are mainly made up of ursolic acid, oleanolic acid, atraric acid, ferulic acid, N-butylbenzene-sulfonamide, β-sitosterol and lauric acid (Kadu *et al.*, 2012; Komakech *et al.*, 2017; Nyamai *et al.*, 2016; Nyamai *et al.*, 2015).

Fig.3. *Prunus's* bark from Mt Cameroon (credit Cifor report, 2008)

I.1.5. Factors influencing the phytochemical profile of *P. africana*

I.1.5.1. Environmental factors

Environmental conditions are among the critical factors that influence the biosynthesis of phytochemical components. The environmental factors may influence the types, the contents, and the proportions of phytochemical compounds. The tannin content in *Potentilla fruticose* (Rosaceae) appeared to be significantly and negatively correlated to altitude (Liu *et al.*, 2016). Also, the annual sunshine duration and altitude were significantly and positively correlated to the flavonoids content, rutin content, and antioxidant activity (Liu *et al.*, 2016). The same authors showed that annual mean temperature was significantly and negatively correlated to the content of total phenolics, while altitude was significantly and positively correlated to the content of total phenolics. Some research showed how light, temperature, soil water, soil fertility, and salinity were able to change the phytochemical contents in diverse plant species (Yang *et al.*, 2018). The studies related to *P. africana* revealed that the concentration of most of the phytochemical compounds were not correlated to the environmental parameters (temperature, precipitation, altitude) (Kadu *et al.*, 2012; Tchouakionie *et al.*, 2014). However, the concentration of ferulic acid in the bark of *P. africana* is significantly and positively correlated to annual precipitation (Kadu *et al.*, 2012).

I.1.5.2. the effect of soil microorganisms on the phytochemical profile

Several studies have demonstrated the influence of soil microorganisms on the quantity and quality of phytochemical compounds in different parts of other plant species (Banchio *et al.*, 2008; Copetta *et al.*, 2006; Egamberdieva *et al.*, 2015; Wu *et al.*, 2007). The application of AMF increased the concentration of kaurenoic acid (terpenoids), and also P, K, Cu, Zn and B in the leaves of *Mikania laevigata* (Asteraceae) (Lazzari *et al.*, 2018). On the other hand, the concentration of dicaffeoylquinic acid in the leaves of mycorrhized *Mikania glomerate* (Asteraceae) was lower, compared to the non-mycorrhized plants (Lazzari *et al.*, 2018). *Glomus bagyaraii* has been detected to be the best symbiont to *Coleus forskohlii* (Lamiaceae), for the production of forskolin, a diterpene compound used in pharmaceutical industries (Sailo & Bagyaraj, 2005). The application of AMF increased the concentration of phenolic compounds in the fruits of Strawberry and the leaves of *Amburana cearensis* (Fabaceae), respectively (Cecatto *et al.*, 2016; de Oliveira *et al.*, 2015). Application of AMF on *Ocimum gratissimum* (Lamiaceae) induced a lower concentration of phenolic compounds with a higher concentration of essential oil, compared to the control without AMF (Hazzoumi *et al.*, 2015).

At 90 days, seedlings of *Salvia miltiorrhiza* (Lamiaceae) inoculated with different species of AMF showed higher concentration of tanshinones and the accumulation of salvianolic acid. As far as AMF are concerned, no study has been conducted on their effect on the phytochemical compounds in *P. africana*. However, previous studies have confirmed the presence of AMF in the rhizosphere and roots of *P. africana* in Ethiopia (Wubet *et al.*, 2004) and Cameroon (Tchiechoua, 2012, Kamko, 2018).

I.2. Mycorrhizal Fungi

I.2.1. Mycorrhizal association and types

The association between plant roots and soil fungi is known as mycorrhiza (Brundrett &Tedersoo, 2018; Smith & Read, 2008). The term Mycorrhiza comes from the Greek 'mycos' for fungus and 'rhiza' for root (Frank et *al*., 1885). In this bidirectional association, plants provide sugar and lipids for the development of the fungal structures (Bago *et al.*, 2000; Keymer *et al.*, 2017; Smith & Read, 2008). In exchange, the fungi supply mineral nutrients (mainly inorganic phosphate (Pi) and nitrates) and promote water uptake (Hodge & Storer, 2015; Smith & Read, 2008). Moreover, fungi ensure the resistance to biotic and abiotic stresses and can, therefore, facilitate the adaptation of plants in a given ecosystem (Guo, 2018; Willis *et al.*, 2013). About 85 % of terrestrial plants form mycorrhiza structures, which can be clustered in four main groups: the endomycorrhizas (EnM) group is the most widespread with 71 % of colonized plants, followed by orchid mycorrhizas (OrM) group with 10 % of the colonized plants, then ectomycorrhizas (EcM) group representing 2.0 % of colonized plants and the ericoid mycorrhiza (ErM) group with only 1.5 % of the colonized plants (Fig. 4) (Brundrett & Tedersoo, 2018).

Each group forms a specific symbiotic structure with a particular morphological character in the roots' tissues of the host plant (Fig.4; Table I). Ectomycorrhizas, OrM and ErM are associations formed of Ascomycota and Basidiomycota, whereas EnM are formed of Mucoromycota phylum with two subphyla (Mucoromycotina and the Glomeromycotina) (Feijen *et al.*, 2018; van der Heijden *et al.*, 2015; Spatafora *et al.*, 2016; Strullu-Derrien *et al.*, 2018). Previous studies have found that fungi from the subphylum Mucoromycotina were able to form symbiosis associations with the earliest groups of land plants (Bidartondo *et al.*, 2011; Field *et al.*, 2012, 2016; Spatafora *et al.*, 2016). Recent studies revealed that the fungus known as fine root endophyte (FRE), which was named as *Glomus tenue*, initially *rhizophagus tenuis*

and placed into the phylum Glomeromycota, has now been grouped with *Endogone* spp. (Walker *et al.*, 2018). Therefore, the species was renamed *Planticonsortium tenue* and placed into the subphylum Mucoromycotina (Walker *et al.*, 2018). The concept of fungi of phylum Glomeromycota has been the oldest to form symbiotic relationships with the first plant species on earth about 460 to 480 million years ago (Pirozynski & Malloch, 1975), and is opposite to the recent works (Field *et al.*, 2015). Indeed, these new findings could allow the endomycorrhizal network community to develop advanced molecular tools to understand the evolution that occurred from the earliest to the contemporary plant-endomycorrhizas association. The present study focuses on Glomeromycotina subphylum, broadly known as arbuscular mycorrhizal fungi (AMF), the most prevalent type of mycorrhizal-plant symbioses.

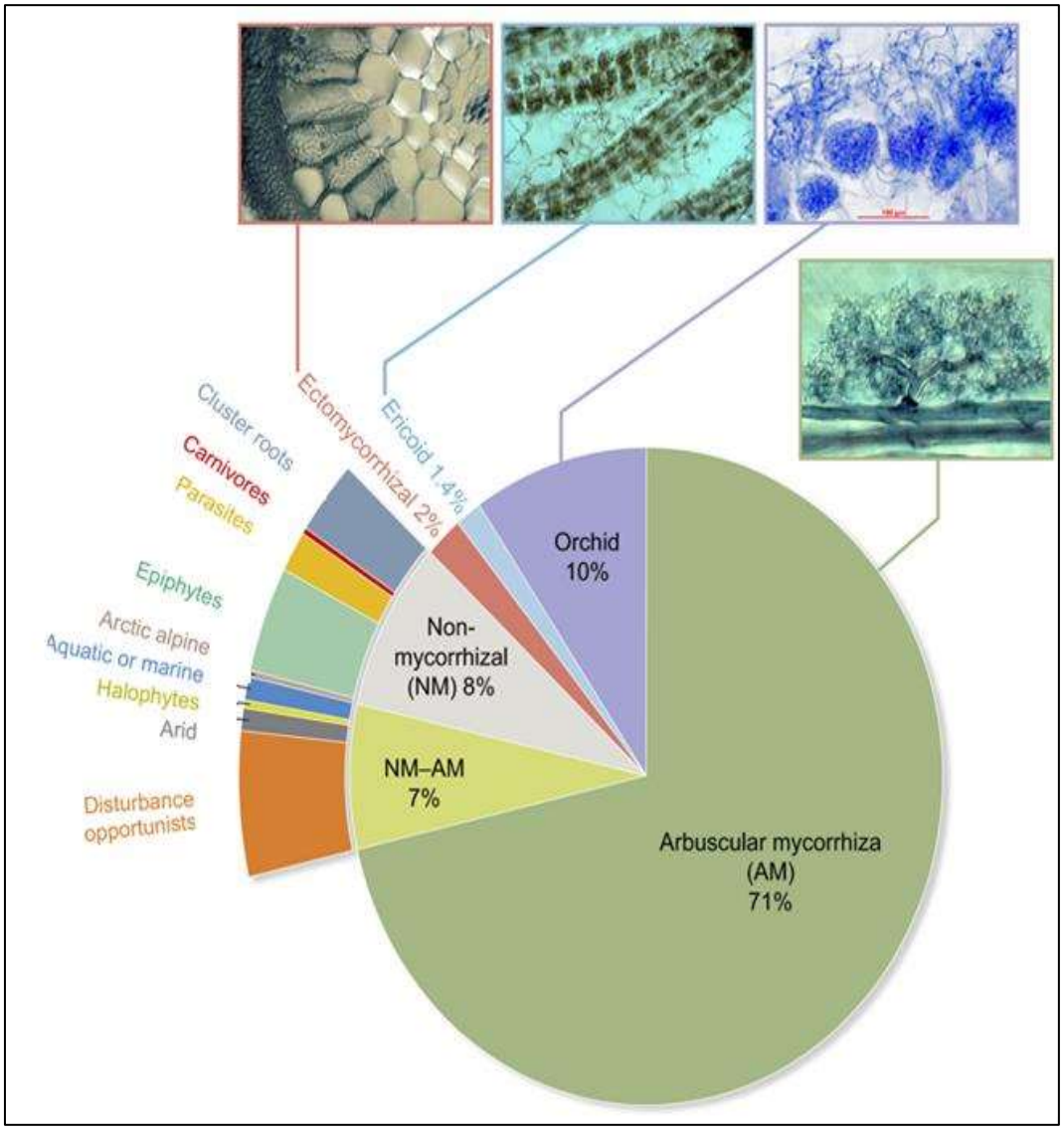


Fig.4. the proportion of mycorrhizal fungi and their host plants (Brundrett & Tedersoo, 2018)

Table I**.** Types of mycorrhizal fungi and their characteristics (Brundrett & Tedersoo, 2018)

Parameters	Endomycorrhizal	Ectomycorrhizal	Ericoid	Orchid
Morphological Characters	Arbuscules present; Vesicles present/absent; colonization from root surface mycelia or neighboring cells	Hartig net present; differentiated hyphal mantle present; no intracellular colonization	Hyphal coils in cells present; each cell is separately colonized from root surface; no (or patchy, undifferentiated) mantle	Hyphal pelotons within root cells present; old pelotons digested by the plant; colonization from root surface mycelia or from neighboring cells
Plant dependency	Mostly obligatory (survival with reduced competition)	Mostly obligatory (survival with reduced competition)	Obligatory	Obligatory for seedling development and adult nutrition
Benefits supplied to plants	Nutrition (mineralized nutrients), limited protection	Nutrition (mineralized, simple organic nutrients), protection	Nutrition (mineralized, simple organic nutrients), limited protection?	Nutrition (mineralized, organic nutrients, carbon energy), limited protection?
Benefits to fungi	Carbon energy, habitat, deep water from trees	Carbon energy, deep water from trees	Carbon energy? Habitat in roots and soil via allelopathy	None (probably cannot support fungi)
Presence of cheating associations	In plants (multiple groups)	In plants (Monotropoideae, Pyroleae),fungi conditionally	Not known	All germinating seedlings, and many adult plants (at least partially)
Fungal associations: phylogenetic groups	Glomeromycota, (or Mucoromycota)	Four Endogonomycetes lineages, 33–34 Pezizomycetes lineages, 45– 48 Agaricomycetes lineages (Tedersoo & Smith, 2017)	Groups within Helotiales, Chaetothyriales, Serendipitaceae, Pekloderma	EcM and saprotrophic Agaricomycetes and Pezizomycetes, especially Tulasnellaceae, Ceratobasidiacea and Serendipitacea e (Sebacinales)

I.2.2. Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi are microscopic organisms, found in the rhizosphere and the roots of 71 % of terrestrial plants (Brundrett & Tedersoo, 2018). They are the most prevalent of all the different types of mycorrhizae. AMF are present in almost all ecosystems, from desert to arable lands, from tropical to temperate forests (Brundrett et *al*., 2009; van der Heijden *et al.*, 2015). AMF belong to the monophyletic family Glomeromycota (Schussler *et al.*, 2001). The main characteristic of AMF is the formation of tree-like subcellular structures inside the cells of the roots known as arbuscules. These arbuscules play a key role in the nutrient exchange between the two symbionts (Armstrong & Peterson, 2002; Choi *et al.*, 2018). Indeed, 70-80 % of the overall plant's Pi (inorganic Phosphate), are provided by the fungus (Hoeksema *et al.*, 2010), through the mycelium network called hyphae. In return, *c* 3 to 20 % of the carbon assimilated by the plant may be allocated to the fungus (Treseder *et al.*, 2018). The establishment of the symbiotic association between the arbuscular mycorrhizal fungus and the roots of the host plant occurs after a series of chronological synchronized events. All these events are carried out through a dialogue between the molecular compounds from the two symbionts (Choi *et al.*, 2018; Kamel *et al.*, 2017) (Fig.5). Based on their morphology in cell roots, AMF can be grouped into two classes: *Arum*-type and *Paris*-type. The former is common to cultivated plants and is characterised by intercellular hyphae and intracellular arbuscules, and the latter is found in most of the plants in natural ecosystems,

with intracellular hyphal coils and arbusculate coils (Muthukumar & Prakash, 2009).

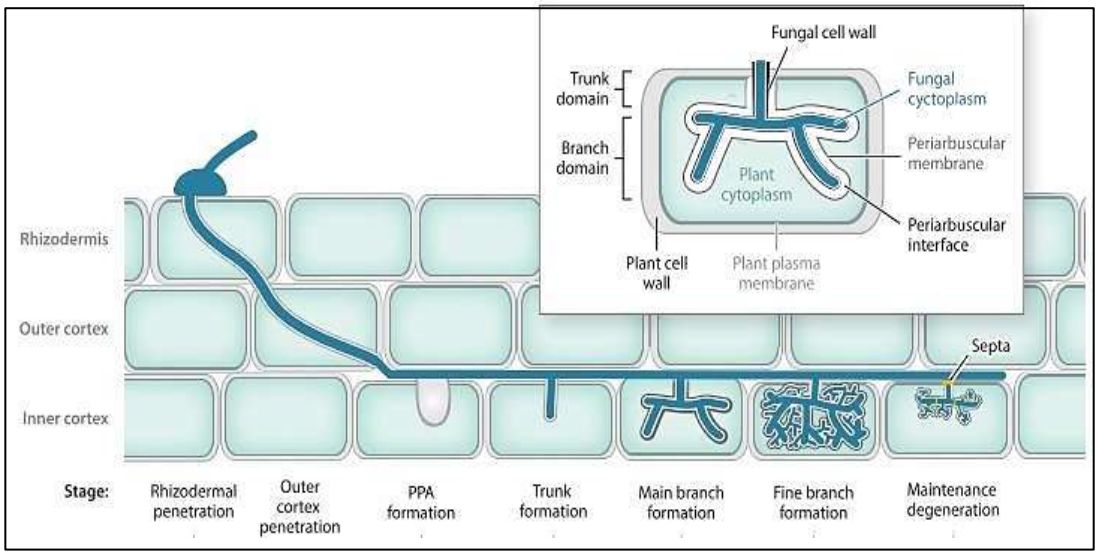


Fig.5. Mycorrhizal structures development in the root (Choi *et al.*, 2018)

I.2.3. Taxonomy and phylogenetic classification of AMF

Arbuscular mycorrhizal fungi taxonomy and phylogenetic classification have undergone many variations over the past decades, due to the use of different tools for identification (small subunit (SSU), internal transcribed spacer (ITS), and large subunit (LSU) of rRNA gene, β-tubulin genes, bioinformatics tools, morphological characteristics). Because of the morphological resemblance of their spores and those of *Endogone* spp., AMF were initially classified in the order of Murocales, the genus *Endogone* of the Endogonaceae family (Link, 1809). Morton's (1990) research has concluded that AMF's spores were asexual whereas *Endogone* spp. were sexual. Therefore, AMF could not be classified as a member of the Endogonaceae family. They were then grouped in the order of Glomerales in the phylum Zygomycota (Morton & Benny 1990).

However, recent genomic analyses reported the presence of genes involved in the sexual life cycle in the genome of AMF; but so far, no physical or functional evidence of AMF sexuality has been observed (Ropars *et al.*, 2016). In the early 2000s, new taxa of AMF were proposed based on the molecular analyses of ribosomal genes (rRNA). AMF species were relocated from the phylum Zygomycota to a new monophyletic phylum Glomeromycota (Schüßler *et al.*, 2001). Then, an *in silico* phylogenetic analysis suggested a new classification whereby, AMF were grouped into the clade of Zygomycetes, within a new subphylum Glomeromycotina in the phylum Mucoromycota (Spatafora *et al.*, 2016). The increasing advance and use of molecular biology and bioinformatics tools have promoted various complementary and at times, contradictory information on AMF taxonomy for the past decades. For instance, Schüßler & Walker (2010) published a species list of Glomeromycota with new families and new genera in comparison with the one previously published by Schüßler *et al.* (2001).

I.2.4. Arbuscular mycorrhizal fungi diversity

Recent reports indicated that the diversity of AMF in the roots and rhizosphere of various medicinal plant species could be varied based on the growing season, soil properties, local climate conditions, and environmental factors (Pandey *et al.*, 2018; Solaiman & Anawar, 2015). Due to their microscopic and clonal development, AMF are difficult to measure or identify in their natural habitat (soil and roots) (Gorzelak *et al.*, 2012). Previously, the investigation of the diversity of AMF was only based on spore morphology, whereby families and genera could be identified mainly by describing the modes of spore formation, and species

on the spore colour, size, and subcellular structures, in particular phenotypic and histochemical characteristics of spore wall components (Brundrett *et al.*, 1996). However, because of the genetic variability of their spores, it might be confusing to base the identification of AMF on spore structures (Gorzelak *et al.*, 2012; Morton & Redecker, 2001). Nowadays, the advent of molecular tools, based on DNA identification has allowed refining the taxonomy and phylogeny of AMF (Redecker *et al.*, 2013).

I.2.5. Medicinal plants associated with arbuscular mycorrhizal fungi

Some of the above tools, from the microscopic to the molecular methods, have been used to investigate the AMF community in the roots and/or rhizosphere soil of medicinal plants. Many studies have previously used the morphological methods. For example, a high density of AMF in the rhizosphere soil and roots of three medicinal plants was reported, namely *Leptadenia reticulata* (Apocynaceae), *Mitragyna parvifolia* (Rubiaceae), and *Withania coagulans* (Solanaceae) from seven districts in Western Rajasthan, India (Panwar & Tarafdar 2006). They identified five genera of AMF with species from *Glomus* genera as predominant. They isolated 10 species from *Glomus* genera namely *G. aggregatum, G. ambisporum, G. constrictum, G. convolutum, G. fasciculatum, G. geosporum, G. intraradices, G. mosseae, G. rubiforme* and *G. sinuosum*; three species from *Acaulospora* genera namely *A. elegans, A. sporocarpia* and *thomii*; three species from *Scutellospora* genera namely *S. heterogama, S. minuta* and *S. pellucida*; one species from *Paraglomus* genera *namely P. occultum* and one species from the genera *Gigaspora namely Gi. albida*. From 31 medicinal plant species collected in the Garden of Medicinal Plants of the Faculty of Pharmacy in Krakow, it was found that 30 were colonized by AMF (Zubek & Błaszkowski, 2009).

Based on the type of arbuscules, 23 were *Arum*-type while only five were *Paris*-type and two were identified as intermediate. *Glomus* species were the most predominant in the rhizosphere soil and root of the three medicinal species of the genera *Cassia* (Fabaceae) viz, *C. alata*, *C. occidentalis* and *C. sophera* (Chatterjee *et al.*, 2010). The higher the *Glomus* colonization rate is, the better is the antimicrobial activity of the plant (Chatterjee *et al.*, 2010). In Kanyakumari, a district in Southern India, species from *Glomus* genera were also found to be the dominant among all AMF found in the roots and rhizosphere of three medicinal plants *(Eclipta prostrata* (Asteraceae), *Indigofera aspalathoides* (Fabaceae), and *I. tinctorial* (Fabaceae)). A total of 21 AMF species were identified namely *Acaulospora delicata, A. morrowae, A. scrobiculata, A. bireticulata, Gigaspora margarita, Gi. decipiens, Glomus aggregatum, G. fasciculatum, G. fulvum, G. geosporum, G. hoi, G. Intraradices, G. macrocarpum, G. microcarpum, G.*

mosseae, *G. pakistanica*, *G. rubiforme*, *G. versiforme*, *Scutellospora calospora*, *S. heterogama*, *S. pellucida*. The two *Indigofera* species had a higher Shannon index, colonization rate and AMF species richness (Sundar *et al.*, 2011). In the rhizosphere soil of 14 cultivars of *Paeonia suffruticosa* (Paeoniaceae) collected in three geographical locations in China, 31 AMF species have been found with 21 species belonging to genera *Glomus* (*G. aggregatum*, *G. austral*, *G. caledonium*, *G. cerebriforme*, *G. claroideum*, *G. clarum*, *G. constrictum*, *G. convolutum*, *G. coronatum*, *G. etunicatum*, *G. fragile*, *G. fulvum*, *G. globiferum*, *G. hoi*, *G. microcarpum*, *G. mosseae*, *G. sinuosum*, *G. tenue*, *G. tortuosum*, *G. trimurales*, *G. vesiculiferum*); seven species from Acaulospora genera (*A. bireticulata*, *A. denticulate*, *A. elegans*, *A. excavate*, *A. foveata*, *A. rehmii*, *A. spinose*) and three species form *Scutellospora* genera *S. cerradensis*, *S. minuta*, *S. nigra*. The most commonly distributed species was *Glomus aggregatum* (Shi *et al.*, 2013). Sixty-six AMF species were found in the rhizosphere soils of 20 medicinal plant species in Zhangzhou, China, as follow, 38 species of *Glomus* genus, 12 of *Acaulospora* genus, nine of *Scutellospora* genus, two of *Gigaspora* and *Funneliformis* genus each and one of *Septoglomus*, *Rhizophagus* and *Archaeospora* genus each (Wang & Jiang, 2015). *Glomus* was the most dominant genus found in this study. Besides microscopic investigation, some researchers have identified AMF associated with medicinal using molecular methods.

By targeting the ITS region (5.8S/) of the rDNA, in the roots of *P. africana* collected in Ethiopia, 109 sequences belonging to the phylum Glomeromycota (currently known as subphylum Glomeromycotina) were found (Wubet *et al.*, 2004). The family Glomeraceae had 20 sequences and the genus *Glomus* was the most abundant. The community of AMF in the roots and rhizosphere soil of *Phellodendron amurense* (Rutaceae) was explored by targeting the 18S region of rDNA using nested PCR, denaturing gradient gel electrophoresis (DGGE) and sequencing. 20 sequences belonging to two groups of AMF, namely *Glomus* spp. and *Scutellospora* spp. were identified (Cai *et al.*, 2009). The AMF community was associated with four medicinal herbs in China, namely *Mentha haplocalyx* (Lamiaceae), *Perilla frutescens* (Lamiaceae), *Glycyrrhiza uralensis* (Fabaceae) and *Astragalus membranaceus* (Fabaceae) (Shi *et al.*, 2018). They indicated that the diversity of AMF and bacteria were different among the species, suggesting preferential association between medicinal plants and rhizosphere soil microorganisms.

I.2.6. Effect of arbuscular mycorrhizal fungi on plant growth and nutrient uptake

Several beneficial effects of AMF on the host plant have been reported from the experimental pot-studies in the greenhouse to the field (Gibert *et al.*, 2018). Indeed, mycorrhization increases the absorption area around the root by increasing the area of the surface in contact with the soil, increasing the absorption of mineral nutrients, such as phosphorus (P), zinc (Zn), copper (Cu), nitrogen (N), magnesium (Mg) and potassium (K). This higher nutrient acquisition influences plant growth and tolerance to environmental stresses directly (de Oliveira *et al.*, 2015; Smith & Read, 2008). Pots containing *Amorpha crenulate* (Fabaceae) and *Jacquemontia reclinate* (Convolvulaceae) seedlings were inoculated with mixture of indigenous AMF from rhizosphere soil and roots of these two endangered plant species, and the results showed a significant increase of the dry weight and total P of the seedlings (Fisher & Jayachandran, 2002). It has been shown that the inoculation of *Piper nigrum* (Piperaceae) with *Funniliformis mosseae* in the nursery enhanced growth and produced healthy cuttings (Wimalarathne *et al.,* 2014). The inoculation of *Viola tricolor* (Violaceae) with *Funniliformis mosseae* and *Rhizophagus irregularis* in pot culture, showed a higher concentration of Cu and Mg in leaves compared to the control without AMF inoculum (Zubek *et al.,* 2015). P and K content of *Lycium barbarum* (Solanaceae) reported to be higher when applied AMF (*Rhizophagus irregularis*) as an inoculum (Zhang *et al.,* 2017). More recently, it was observed that AMF inoculum enhanced the concentration of N, Cu, Mn, and Fe in leaves and was able to transfer more P from roots to leaves (Attarzadeh *et al.,* 2019). Medicinal plant species inoculated with AMF have been reported to have higher mineral uptake and growth compared to the non- inoculated plants (Solaiman & Anawar, 2015). Vegetatively propagated *Mikania glomerata* (Asteraceae) and *M. laevigata* (Asteraceae), two medicinal plants, have been reported with an increase of P, K, Cu Zn, and B concentration by inoculating a mixture of *Rhizophagus irregularis* in plastic pots (Almeida *et al.*, 2018).

I.2.7. Effect of arbuscular mycorrhizal fungi on phytochemical compounds in plants

The first reports on the effect of AMF on medicinal plant species were done on *Datura stramonium* (Solanaceae) (Wei & Wang, 1989) and *Schizonepeta tenuifolia* (Lamiaceae) (Wei & Wang, 1991). These authors showed that the different AMF species enhanced

phytochemical compounds in the respective plant species. Since then, several studies have been carried out confirming the modulating effect of AMF species on secondary metabolites content in different parts (leaves, roots and barks) of several medicinal plant species.

Glomus fasciculatum was used to enhance the oil content and oil yield of three cultivars of *Mentha arvensis* (Lamiaceae) in field conditions (Gupta *et al.*, 2002). Eleven AMF species were screened for their effect on the forskolin content in *Coleus forskohlii* (Lamiaceae), and it was found that the forskolin content varied with AMF species (Sailo & Bagyaraj, 2005). Plants inoculated with the AMF had higher forskolin contents than non-inoculated control plants. It was found that the concentration of alpha-terpineol increased when *Ocimum basilicum* were inoculated with *Glomus rosea* compared to other mycorrhizal fungi species and control (Copetta *et al.,* 2006). The total phenolic and vinblastine compounds in the leaves of *Catharanthus roseus* (Apocynaceae), a medicinal plant was found to be enhanced by the application of AMF (De la Rosa-Mera *et al.,* 2011). *Amburana cearensis* (Fabaceae) in pots were inoculated with three different AMF, namely *Gigaspora albida*, *Claroideoglomus etunicatum*, *Acaulospora longula* and after 160 days of growth, the total phenols, flavonoids and tannins were significantly higher in plants inoculated with *Claroideoglomus etunicatum* compared to non-inoculated control plants and other species (De Oliveira *et al.,* 2015). In the same vein, the concentration of diterpene kaurenoic acid in mycorrhizal (*Glomus spp* species) Mikania *laevigata* was found four times higher than the control plants (Almeida *et al.*, 2018).

However, other studies have also reported that AMF does not always have a positive effect on the phytochemical in medicinal plant species. For example, no effect has been observed on the concentration of phenolic and rosmarinic acid of *Salvia officinalis* (Lamiaceae) inoculated with AMF (Nell *et al.,* 2009). In another study of the same species, it was shown that AMF (acaulospora spp) inoculation decreased the leaf concentration of phenolic and flavonoid compounds (Geneva *et al.,* 2010). Similar results were also observed in the concentration of tricaffeoylquinic acid in Mikania *laevigata* (Almeida *et al.* (2018).

I.3. State of Metabolomics

I.3.1. Primary and secondary metabolism

Primary metabolism is biological reactions essential to maintain life in living organisms. Plant converts sunlight energy to chemical energy, such as ATP, NADPH, by the mediation of chlorophyll in chloroplasts and synthesize sugars and starch from CO_2 by using ATP and

NADPH+. These carbohydrates are stored and used for differentiation and formation of plant tissues (Alu'datt et *al*., 2017).

Secondary Metabolites are formed only in specific organisms, or groups of organisms, they are species specific. Secondary metabolites are not necessarily produced under all conditions, and in most cases the function of these compounds and their benefit to the organism is not yet known. It is this area of secondary metabolism that provides most of the pharmacologically active natural products (Yang et *al*., 2022).

Plant secondary metabolites are widely distributed in various higher plant organs such as, spices, grains, legumes, and nuts. They play important roles in diverse physiological processes such as plant quality, colour, flavor, and stress resistance (Bodoira and Maestri, 2020)

The natural antioxidant, antimicrobial, anticarcinogenic, and antiinflammatory activities of secondary compounds have become a hot spot in terms of research and utilization at present. Secondary compounds possess a common chemical structure, usually comprising an aromatic ring with one or more hydroxyl substituents that can be divided into several classes, and the main group is the phenolic compounds, including flavonoids, phenolic acids, tannins, stilbenes, and lignans (Alu'datt et *al*., 2017). In recent years, with the increasing recognition for their bioactivity values, secondary compounds have been found to exert various effects such as antioxidant (Martins and *al*., 2017), antimicrobial, anticarcinogenic, antiinflammatory, and prevention of cardiovascular diseases, cancers, diabetes, and diseases associated with oxidative stress (Yasir et *al*.,2016),

For example, four marker compounds from *Dendrobii herba* exhibit antiinflammatory activity by targeting different inflammation-related cytokines (Mo et *al*., 2017). The synergistic action of catechin, vanillic, and protocatechuic acids can inhibit the adhesion of uropathogenic *Escherichia coli* on silicone surfaces. The chemical composition of a plant product is determined by qualitative chemical analysis using various solvents for extraction. Primarily, extraction methods should be selected and optimized along with the corresponding analytical techniques, including the used solvents, the sources, and the properties of the compound itself (Tanase et *al*., 2019). In the research or development of metabolites compounds, exploring qualitative or quantitative approaches to analyzing these bioactive compounds should be prioritized in abundant different natural sources, which contribute to developing rapid, sensitive, and reliable methods. Many different methods have been explored

or improved in the past few years. General approaches allow the quantitation of a global estimation of phenolic compounds' content, which is mainly achieved by spectrophotometry methods. However, more specific analyses are based on the identification of individual metabolite classes, typically by high-performance liquid chromatography (HPLC) or gas chromatography (GC), and their detection by sensitive detectors, such as mass spectrometry.

I.3.2. Secondary compounds identified from plants

Over 8000 molecules have been reported in the secondary compounds family and the list continues expanding. Secondary compounds are mainly classified according to their chemical structures into phenolic acids, flavonoids, tannins, phenolic lignans, and phenolic stilbenes It is found that secondary compounds have two basic structures, one is the C6–C3–C6 ring structure, including flavonoids, partial phenolic acids, and condensed tannins, and the other is the C6–C1 structure, mainly including partial phenolic acids and hydrolyzed tannins (Gowd et *al*., 2017).

I.3.2.1. Flavonoids

Flavonoids are a series of compounds with diphenylpropanes (C6–C3–C6) as the basic skeleton and two aromatic rings connected to each other through the central 3-carbon bridge. Flavonoids are currently the most diverse phenolic compounds which are closely related to flower color formation of plants and play an important role in plant–environment interaction (such as preventing ultraviolet [UV] damage, resisting disease, affecting root nodules formation of legumes, etc). The most common flavonoids found in nature are anthocyanins, flavones, flavonols, flavanones, isoflavones, flavanonols, and other subclasses.

For example, epicatechin, a flavonoid isolated from the Mexican medicinal plant *Geranium mexicanum* HBK could affect the virulence properties of a human pathogen (Bolanos et *al*., 2014). Another major flavonoid, kaempferol, which is obtained from *Kalancho blossfeldiana* Poelln, has antiherpes potential (Urmenyi et *al*., 2016). Flavanones are the major flavonoids of citrus; they are found as hesperitin in oranges, eriodictyol in lemons, and aglycones naringenin in grapefruit. In a glass of orange juice, between 40 and 140 mg flavanone glycosides can be found (Kaur et *al*., 2014). Isoflavones are a subclass of flavonoids, hold structural similarities to estrogens. Isoflavones are sometimes referred to as "phytoestrogens," which are especially abundant in soybeans. Studies have shown that they can be used to prevent some important diseases, such as hypertension (Maaliki, 2019) and

cancer (George and Dellaire, 2017), and ameliorate intestinal health (Landete et *al*., 2016). Anthocyanins are the largest type of water-soluble vacuolar pigments, appearing as red, blue, or purple, and occur in all plant tissues, including flowers, stems, leaves, roots, and fruits. Anthocyanins are abundant in cereals, red wine, and some root vegetables such as onions, and others. However, they are generally seen in certain fruits such as cherries, red berries, and pomegranates. Anthocyanins exert protective effects in preserving cardiovascular health by reducing the risk of myocardial infarction and cardiovascular disease-related mortality (Kriga et *al*., 2019). Furthermore, anthocyanins have shown antiobesity (Xie et *al*., 2018), and anti-diabetes effects through multiple mechanisms.

I.3.2.2. Phenolic acids

Phenolic acids belong to a major class of secondary compounds in plants and are present in free and bound forms. Phenolic acids can be divided into two subgroups: hydroxybenzoic acid (HBA) and hydroxycinnamic acid (HCA). HBAs are based on a C6–C1 structure and include p-HBA, protocatechuic, vanillic etc. However, HCAs are aromatic compounds with a 3-carbon side-chain (C6–C3), including coumaric, caffeic, ferulic, and sinapic acids (Zhang et *al*., 2016). HBAs are found abundantly in oilseeds, cereals, coffee, cowpea, black currant, raspberry, squash shells and seeds, and blackberry. HCAs are majorly sourced from coffee, cherries, cereals, peaches, spinach, citrus juices and fruits, plums, tomatoes, potatoes, and almonds. Studies have shown that phenolic acids have various biological functions. For instance, caffeic acid (CAA) and chlorogenic acid (CHA) are important members of HCA with natural antioxidant and cardio-protective properties. CAA and CHA exhibit blood pressure-lowering properties and reduce activities of key enzymes linked to the pathogenesis of hypertension in cyclosporine-induced rats (Agunloye et *al*., 2019). Gallic acid has been shown to specifically target the adipose tissue to suppress lipogenesis, improve insulin signaling, and concomitantly combat raised proinflammatory response and oxidative stress, as well as reduce excessive lipid storage in obese subjects (Dludla et *al*., 2019). Vanillic acid is an effective inhibitor of Hypoxia-inducible factor 1 (HIF-1) and can cause significant inhibition of tumor growth in a xenografted tumor model, providing new perspectives into the mechanism of its antitumor activity (Gong et *al*., 2019).

I.3.2.3.Tannins

Tannins are a group of secondary compounds that are widely present in cereals, leguminous seeds, and, predominantly in many fruits and vegetables, where they possess

various biological activities including antimicrobial, antiparasitic, antiviral, antioxidant, antiinflammatory, immunomodulation, etc (Smeriglio et *al*., 2017). Tannins are classified according to their chemical structures into hydrolyzable tannins (tannic acid) and condensed tannins (proanthocyanidins). Hydrolyzable tannins consist of gallic acid and its dimeric condensation product, hexahydroxydiphenolic acid esterified to a polyol, which is mainly glucose. Condensed tannins have been identified in longan bark, the content of extractable condensed tannins in longan bark is 198.3 ± 8.7 mg/g (Chai et *al*., 2018). In brown seaweeds, HBAs, rosmarinic acid, and quinic acid derivatives have been characterized in *Ascophyllum nodosum*, *Bifurcaria bifurcata*, and *Fucus vesiculosus* (Agregan et *al*., 2017). Phlorotannins are oligomers of phloroglucinol, which is restricted to brown seaweeds, where they exert functions as primary and secondary metabolites. Phlorotannins have a wide range of biological activities, such as antioxidant, antibacterial, antiviral activity, antitumor, anticardiovascular disease, antidiabetic syndrome, liver protection, and hyaluronidase lysinase inhibition, which is of great significance for the development of marine drugs (Cotas et *al*., 2020).

I.3.3. Extraction methods of secondary compounds

The chemical composition of a plant product is determined by qualitative chemical analysis using various solvents for extraction. To extract bioactive phenolic compounds from a wide variety of plant materials, including fruits, leaves and roots, researchers use multiple techniques and methods (Chai et *al*., 2018). The choice of method and solvent used for extraction is a particularly important step to obtain an optimal concentration of natural compounds in the extract. It is important to select an efficient extraction method and proper work phases to assure high performance and increased stability of the extracted compounds. Many conventional and original means can be used to extract phenolic compounds from plant samples, such as solid–liquid extraction (SLE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), or enzyme-assisted extraction (EAE) (Agregan et *al*., 2017).

I.3.4. Secondary compounds analysis methods

Secondary compound quantification depends on different parameters, such as the chemical nature of compounds, extraction method used, particle size, standard selection, and interfering substances and impurities. With the advancement of analytical science, numerous

methods have been used for quantifying phenolic compounds from plant materials, such as spectrophotometry, HPLC, GC, and their combinations (Smeriglio et *al*., 2017).

I.3.4.1. Spectrophotometry

Spectrophotometry is a simple and fast technique which can among many things be used for quantifying phenolic compounds from plant materials and is mainly based on the principle for measuring the various structures present in the phenolic compounds. The Folin–Ciocalteu assay has been widely used to detect phenolic compounds in plants for many years. This assay is based on a chemical reduction involving reagents containing tungsten and molybdenum (Gogia et *al*., 2014). The Folin–Ciocalteu method is a modified method of the Folin–Denis assay, which slightly changes the composition of the reagent used. The general principle of this analysis is to prepare an extract of phenolic compounds from the material and to add the Folin-Ciocalteu reagent, sodium carbonate (35% or 0.1 N), and distilled water. The solution prepared in this way is allowed to react for 15 to 120 min. In general, the flavonoid content is often measured with spectrophotometry. In addition, total phenolic quantification and the condensed tannin content can also be estimated by spectrophotometry. Spectroscopy is the common technique used for quantifying different classes of phenolic compounds because of its simplicity and low cost (Gong et *al*., 2019).

I.3.4.2. Gas chromatography

GC is a useful technique utilized for the separation, identification, and quantification of some phenolic compounds in plants, such as tannins, flavonoids, and anthocyanins. It employs the evaporation temperature specific to each compound to separate it from the solution by passing the sample through a heated column where it is divided between an inert gas under pressure and a thin layer of nonvolatile liquid covered with an inert substrate inside the column.The derivatization and volatility of phenolic compounds are the main components detected by GC.
Vaiciulyte et *al*. (2019) have applied GC–FID (flame ionization detector) to detect carvacrol obtained from *Thymus pulegioides* L. In recent years, GC coupled with a MS detector has become
widespread in measuring complex compounds because of its high selectivity and sensitivity in quantitation. For example, the low-molar-mass fraction of hydrophilic extracts in Norway spruce knotwood, which are mainly lignans, has been characterized by GC–MS (Smeds et *al*., 2016). The columns most used in the GC technique to analyze phenolic compounds include

30 m long capillary columns, with an outer diameter of 0.25 to 0.30 mm, and an inner diameter of 0.25 μm. Helium is usually used as the carrier gas.

GC: Stationary Phase: Column.

GC: Mobile Phase: Gas (N_2, He, H_2 etc.);

Gradient: Temperature (low to high)

I.3.4.3. High-Pressure liquid chromatography

HPLC is the most used technique for the separation and detection of phenolic compounds. It is a versatile and adaptable instrument with various advantages, such as high selectivity, sensitivity, resolution, precision, and sample behavior (Naczk et *al*., 2006). The method's principle lies in the separation of compounds from complex mixtures on the basis of their solubility and/or interaction between a less polar stationary phase and a more polar mobile phase. Thus, some factors affect HPLC analysis of phenolic compounds, such as column types, applied detectors mobile phase, and the properties of the tested compounds. To obtain information about a specific phenolic compound, it is necessary to compare its retention time with the standard. But some classes of polyphenols, mainly flavonoid glycosides and proanthocyanidins lack standards, so this is a major disadvantage when using the HPLC technique.

I.3.4.4. HPLC–Mass spectrometry

Phenolic compounds can be analyzed by HPLC combined with tandem MS. HPLC assisted by MS detection is an advanced analytical technique that exhibits high sensitivity and selectivity. This approach can measure structural information about unknown compounds from crude or partially purified samples of natural sources (Mocan et *al*., 2014). Recently, numerous studies on phenolic compounds analyses have been focused on the assessment of methods that involve different couplings between HPLC and MS. Chen et *al.* (2014) have analyzed the phenolic compounds in Jerusalem artichoke (*Helianthus tuberosus* L.) responding to salt stress by HPLC/tandem MS. In recent years. In addition, it could provide structural information about unknown compounds. Overall, this technique is currently the best analytical approach to studying phenolic compounds of various biological resources and the most effective tool in analyzing their structure. However, its main disadvantage is the high cost of the device.

I.3.5. Biological activities of metabolite compounds

Natural plants, especially those with biological activities, represent an important source of chemical compounds that are useful in a wide range of applications. Secondary compounds play an essential role in natural antioxidant, antimicrobial, and antiinflammatory effects as well as the treatment of diseases such as obesity, cancer, and diabetes. Some important biological activities of phenolic compounds are illustrated below (Li et *al*., 2016).

I.3.5.1. Antioxidant activity

Metabolite compounds have shown promising antioxidant properties, with their potential being directly related to the type of solvent used in the extraction, but also with plant origin, growing conditions, harvesting time, and storage conditions. The study of the antioxidant potential of secondary metabolites derived from plant species is one of the hot topics among the scientific community (Avello et *al*., 2013). Biological activity tests indicated that the secondary extracts of tartary buckwheat bran may contain functional compounds that exert antioxidant activities. In vitro (oxygen radical antioxidant capacity) and ex-vivo (cellular antioxidant activities, CAA), as well as antiproliferative activity against human liver cancer cells (HepG2) *in vitro* (Li et *al*., 2016). The secondary compounds extracted from three species of the medicinal plants (*Buchenavia tetraphylla*, *Buchenavia tomentosa*, and *Lippia sidoides*) provide the main components to the antioxidant potential (Teixeira et *al*., 2017). The stem of *Dendrophthoe falcata* (Loranthaceae) plant shows a high content of phenolic and flavonoid compounds and very high antioxidant activities.

In addition to the concentration of phenols, the antioxidant activity of phenolic compounds is in direct relation with their chemical structures such as number as well as the position of the hydroxyl groups (Stanković et *al*., 2015) Because of seasonal variations, the trends of different phenolic compounds are also different. Contents of both total phenolics and total flavonoids were strongly correlated with antioxidant activity (Shi et *al*., 2018). The total phenolics, total flavonoid, and antioxidant capacity of all blueberry's cultivars increased nonlinearly with ripening.

I.3.5.2. Antiinflammatory activity

It has been demonstrated that, besides the essential antioxidant effect, phenolic compounds reduce lipid peroxidation and DNA damage (Velmurugan et *al*., 2018). The extracted phenolic compounds from *Ficus hirta* Vahl. exhibit pronounced inhibitory effects

on the lipopolysaccharides (LPS) induced nitric oxide (NO) production in murine macrophage RAW 264.7 compared to indomethacin, which suggested that hairy fig could be served as an antiinflammatory agent for health products (Cheng et *al*., 2017). Most of the phenolic compounds isolated from durian shells display pronounced inhibitory activities on LPS-induced NO production in RAW 264.7 cells, indicating the potential to serve as antiinflammatory agents (Feng et *al*., 2017). A variety of phenolic compounds and stilbene derivatives in different parts of germinated peanut suggests that the peanut sprout exerts high anti-inflammatory effects and may be related to the polyphenolic content and antioxidant properties.

I.3.5.3. Anticoronavirus properties

The highly contagious novel disease COVID-19 caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has resulted in a major international concern all over the globe. However, antiviral therapeutic options that specifically target this new coronavirus remain limited. Natural compounds with a great diversity of chemical structures may provide an alternative approach for the discovery of new antivirals. Studies have shown that natural phenolic compounds have anticoronavirus properties in supportive and prophylaxis treatments (Dejani et *al*. 2021). A recent review presented that resveratrol stably binds to the viral protein/angiotensin-converting enzyme 2 (ACE2) receptor complex of SARS-CoV-2, indicating it to be a promising agent in the therapeutics of COVID-19 by disrupting the virus S protein (Wahedi et *al*., 2021). Curcumin has been suggested as a potential treatment option for patients with COVID-19 because it inhibits ACE2 and suppresses the entry of SARS-CoV-2 into the cells (Zahedipour et *al*., 2020). In the prophylaxis and treatment of COVID-19, the antiviral activity of green tea and black tea has also been proven and emphasized. Theaflavin, the compound responsiblen for the orange/black color of black tea, is a potent inhibitor of the RNA polymerase of SARS-CoV-2 (Lung et *al*., 2020). Catechin gallate and gallocatechin gallate also showed high inhibitory activity against SARS-CoV-2 N protein in a concentration-dependent manner and affected virus replication (Ghosh et *al*., 2021).

CHAPTER II. MATERIALS AND METHODS

CHAPTER II. MATERIALS AND METHODS

II.1. MATERIALS

II.1.1. Study sites

The zones of study were targeted in two administrative areas named the South-West and the North-West Regions of Cameroon, a country in Central Africa. The choice of these two areas is justified by the presence of natural regeneration and plantations of *P. africana* (fig.6 and 7)

Fig.6.1. Sampled Sites for bark and AMF of *Prunus africana* in the mono modal rain forest and western highlands ecological zones of Cameroon. Source Ciford, 2007

●: *Prunus africana* Population

Fig.6. Distribution of *Prunus africana* in Cameroun and in Africa (Jimu et *al*., 2011).

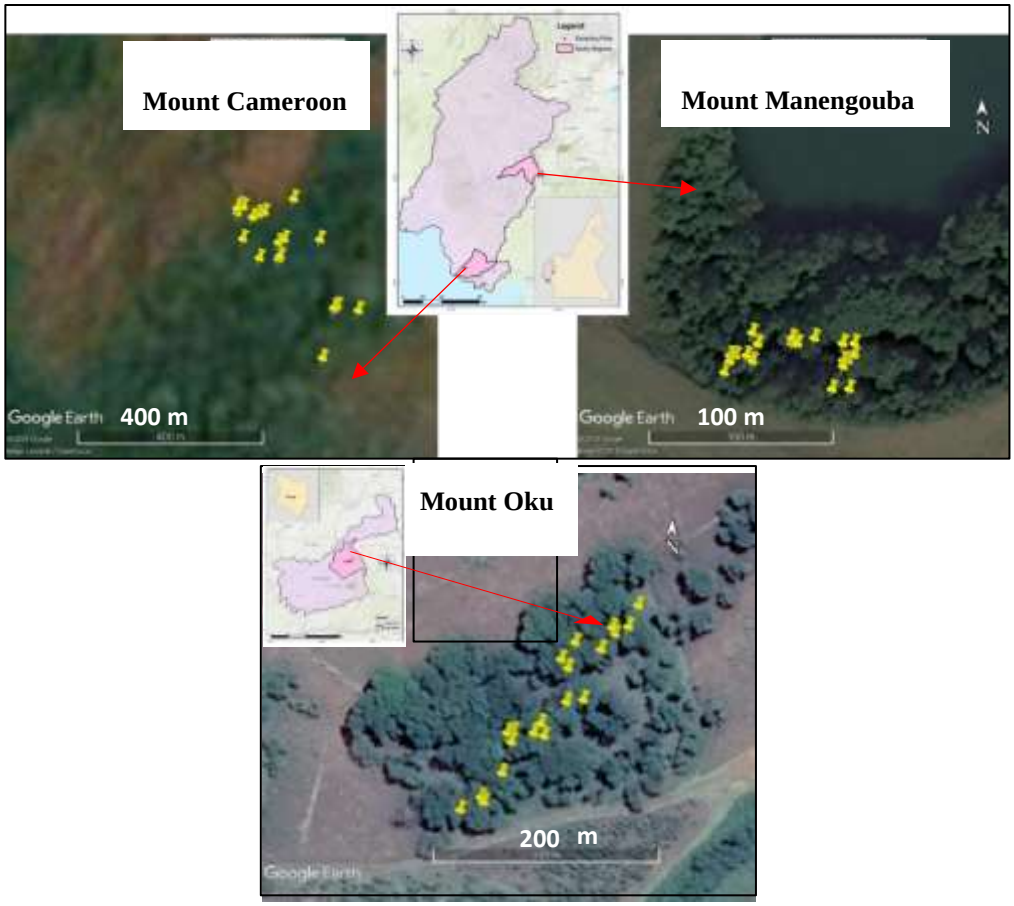


Fig.7. Sampling sites where fine roots, rhizosphere soils and barks of *Prunus africana* were collected In Cameroon. The yellow pins show the GPS location of all *P. africana* trees sampled (Tchiechoua et *al.*, 2022 modified).

II.1.2. Description of sites

Three sites were chosen for this study, Mount Cameroon, Mount Oku, and Mount Manengouba.

II.1.2.1. Mount Cameroon

II.1.2.1.1. Location (agroecological zone: IV)

The Mount Cameroon site is at the coast of the Gulf of Guinea (Biafra) in the Southwest Province of Cameroon (4°44 'N, 9°11'42.01"E). Mount Cameroon, which includes the research site, rises steeply in as much as 18 km from sea level to 4095 m at the summit. The selected site for fieldwork was Mapanja (4° 07' N and 9° 05' E). Mapanja is located on the eastern slope of Mount Cameroon, at about 950 m of altitude. It is about 25 km from Limbe, a coastal town in the Southwest Province. Other villages around this site include Likombe,

Bojongo, Saxenhof and Etinde. The population of the village can be estimated at 250-300 inhabitants. The Bakweri forms the only ethnic group in Mapanja (Environment 91, 1991).

II.1.2.1.2. Climate

There is no meteorological station in Mapanja, the climate here does not differ greatly from that of Tole which is at an altitude 700 m above sea level. The climate is a modified equatorial type with one wet season (March–September) and dry season (length: 3-4 months). The mean maximum and minimum rainfall are respectively 4978 mm and 1503 mm. The mean annual temperature is 22 °C (Fraser et *al*., 1998). Soil temperature is even lower.

II.1.2.1.3. Geology

Mount Cameroon and the Bamenda highlands are formed largely of igneous materials, both volcanic and plutonic (Anon et *al*., 1998). Three main periods of volcanic activity and one of plutonic uplift have been reviewed by Conrade et *al*. (1974). The second period of volcanic activity, "the middle series", gave rise to much of the Bamenda Highlands, but appears to have contributed little to the Mount Cameroon area. The "lower black series" of volcanic activity gave rise to basalts that formed the heart of Mount Cameroon. The geology of Mount Cameroon shows recent volcanic rocks (basalts, tufts and volcanic ash). Its geomorphology shows numerous features derived from recent episodes of volcanic activities. The absence of settlement over most of the main massif is due to the free draining volcanic lava that drains rapidly and eliminates permanent surface water supplies but instead feeds the numerous springs that well up from the foot of the mountain. The elevation of mountain increases steadily up to the summit with very steep slopes in some parts.

II.1.2.1.4. Soils

Mount Cameroon is constituted of soils on volcanic ash, basalt and tufts as parent material and is dominated in places by solidified lava. Soil type is mainly Humic Andosols following the FAO classification. The soils are deep (84-110 cm) but stony and rocky in some places, dark yellowish brown when moist. The Ah horizon is dark from 20-50 cm thick when moist, loam, and moderately medium granular and subangular blocky structure. The soil is generally acidic. Clay minerals include allophanes and, less commonly, imogolite, and/or humus complexes of aluminium and iron. These soils are notorious for highly variable

exchange properties. The organic matter content is high. The Cation Exchange Capacity (C.E.C) is high particularly in humid areas (Fraser et *al*., 1998).

II.1.2.1.5. Vegetation

There is a clear gradation of the vegetation from the lower slopes to the summit of Mount Cameroon. Fallow and farms are dominated by *Vernonia spp Polyscias fulva, Maaranga spp, Arundinaria madagascariensis* and *Xanthosoma saghitifolium.* Mountain forest depicts species like *Nuxia congesta, Prunus africana, Syzygium staudii,* and *Entandrophragma angolense.* The forests are often covered with clouds and mist making trees rich in orchids, mosses and epiphytes. The vegetation is classified as humid montane forest as they all surpass 800 m of altitude (Macleod et *al.*, 1986).

II.1.2.1.6. Land uses

The research site is populated by the Bakwerians who live in small houses made of zinc and wood. They carry out agricultural activities but also hunting, gathering and harvesting of Non-Timber Forest Products (NTFP). Crop cultivation is not very intense here as farming is mostly subsistence. Most areas that are not very stony are cultivated. They follow the normal cropping calendar. The soil is used for mixed agriculture on a permanent basis, practically without the use of chemical fertilizers. Macabo, taro, plantains, and maize are the major food crops planted here, while oil palm, tea, and rubber are the major cash crops. For NTFPs, the exploitation of *Prunus africana* and hunting are the major activities. Workers of *Plantecam* trained villagers on how to carry out sustainable bark harvesting (Anon et *al*., 1998).

II.1.2.2. Mount Oku (Kilum mountain forest)

II.1.2.2.1. Location (agroecological zone : IV)

The Kilum mountain forest (6° 12’ N and 10° 28’ E) is found in the Oku sub-division, which is about 40 km from Kumbo, a town in the Northwest region of Cameroon. Mount Oku, which includes this site, is about 3011 m above sea level. The Kilum mountain forest vegetation was chosen for the fieldwork. Entry points to the forest were usually between 2050 and 2100 m. The Oku tribe is the only ethnic group in the locality (Fig.2.2) (Environment 91, 1991).

II.1.2.2.2. Climate

Climatic data collected in the area show that rainfall is between July and September. Rainfall varies from 1780-2290 mm per year but the summit probably receives rainfall in excess of 3050 mm per year. In general, January and February have the lowest relative humidity (average 45-50 %). The monthly average exceeds 80 % in July and August. During the rainy season, mist and low clouds occur frequently. Mean maximum temperature is 20-22 °C and mean minimum is 13-14 °C. Between 2000-2500 m the temperature reads 14.5-17 °C and above 2500 m the temperature reads 14.5°C (Macleod et *al*., 1986).

II.1.2.2.3. Geology

Mount Oku, which includes the Kilum mountain forest in the Bamenda Highlands, is the second highest peak (3011 m) along the mountain chain (Macleod et *al*., 1986). Mount Oku is formed on volcanic rocks (tertiary basalt and trachyte lava) (Macleod et *al*., 1986). The geomorphology of Mount Oku shows a ridge structure as it rises up to the summit with very steep slopes.

II.1.2.2.4. Soils

Soils of Mount Oku are formed on basalt and trachyte, but the greater part of the mountain is on the massive trachyte (Kips et al., 1987). On the FAO soil classification system, the dominant soil types are Umbric alisols (basalt), Umbric cambisols and Haplic acrisols (trachyte). They are deep (100-150 cm) to very deep (150-200 cm). The AP horizon of alisols and cambisols is similar, very dark greyish brown or very dark brown silt loam or silty clay loam, moderately structured, very strongly or strongly acid. Clay minerals are kaolinite and gibbsite. Soil organic matter is very high and mineralization rate of organic nitrogen is high. Levels of available phosphorus are high in alisols and medium to low in cambisols. Total base exchangeable capacity is medium to low. For acrisols, the AP horizon is brown to dark brown silt loam or silty clay loam, moderately structured, strongly to very strongly acidic. Cambisols contain minerals such as biotite, olivine, pyroxenes, and amphiboles. Other ferric oxides and hydroxides include goethite and hematite (Kips et *al*., 1987).

II.1.2.2.5. Vegetation

The upper montane forests of the natural sites has similar vegetation. The Kilum or Oku mountain forest is rich in *Podocarpus milanjianus, Arundinaria alpina* and *Prunus africana*; the forests most resemble those of the highlands of East Africa (Environment 91, 1991)

II.1.2.2.6. Land uses

The people of Oku living below the mountain at an altitude less than 2000 m carry out intensive agricultural activities during humid periods of the year. They cultivate maize, beans, potato, groundnuts and many other food crops. Cash crops produced here include cola nut. Tree nurseries perform a crucial role in improved resource management. Tree nurseries supply materials for soil conservation, agroforestry, firewood plantation, woodcarving, live fencing and demarcation of the Kilum forest reserve. Only *Prunus africana* is used in the last case while *Eucalyptus saligna, Cupressus benthamii, Casuarina equisetifolia* may be used in the other cases. Over 100,000 people depend on the forest for a wide variety of forest products: firewood, building materials, medicinal plants and bush meat. There are about 1000 beekeepers; their hives are kept almost exclusively in the forest where the bees find rich foraging. The honey is marketed through the Oku Honey Cooperative Society (Kips et *al*., 1987).

II.1.2.3. Mount Manengouba

II.1.2.3.1. Location (Agroecological zone: IV)

Manengouba is located in the Littoral Province of Cameroon (05° 06’ N and 10° 07’ E), approximately 18 km from Nkogsamba center town. Two ethnic groups constitute the population of the site: The Mboh and the Bamileke. The site is at altitude 800 m in the plain and 800-1000 m up the hill (Fig.2.3) (Yerima et *al*., 1998).

II.1.2.3.2. Climate

The climate of the area is humid tropical with two seasons. Manengouba shows an increase in temperature (27°c), as it is some 1000 m below Loum. Annual rainfall in the region is 1600-1900 mm.

II.1.2.3.3. Soils

Soil types are andosols, which have gradually lost their initial characteristics and are evolving towards Ferralsols (Yerima et *al*., 1998). Low parts of Manengouba are partially inundated during the wet seasons (March–September).

II.1.2.3.4. Vegetation

The vegetation is dominated by *P. africana* (Natural and artificially regenerated by Forestry administration in the early 1970s), *Voacanga africana, Ricinodendron heudelotii, Canarium schweinfurthii* and many other species of the Burseraceae. Cash crops include some coffee and cocoa. Food crops in the area include cocoyams, cassava, maize, sugarcane, plantains, *Arachis* and many other food crops cultivated in the Littoral and West Province (Kips et *al*., 1987).

II.1.2.3.5. Land uses

With increase in population, most of the land is used for agricultural activities. Coffee is the main cash crop in the area and is planted at altitudes above 800 m and also in the vicinity of settlements. Extensive rearing of goats, pigs and fowls is also done in this area.

Table II. Characteristics of experimental sites where soil and root samples were obtained for culturing and characterization of Arbuscular Mycorrhizal Fungi

Sample places	Climatic zone	Coordinates	Altitude (m)	Predominant vegetation	Soils characteristics		Environmental data		
					pH	%Org C	T°	Disturbance	Annual Precipitation
Mount Cameroon	Subequatorial humid	4º44'N, 9º 32'E	2231	Sterculiaceae, Ulmaceae,	6.87	8.95	17 – 22°c	0.13	2500 – 4000 mm/an
Mount Oku	Tropical humid and warn	7°15'N, 11°15'E	2335	Caesalpiniaceae; *Araliaceae*	4.50	7.61	14.5 °C	0.10	1500 – 2000mm/an
Mount Manenguba	pseudo-tropical humid	5°6'N, 10° 7'E	1935	*Ericaceae, Tilbaceae, Pittosporaceae*	6.11	7.95	18-23°c	0.14	2500 – 4000 mm/an

P: available P, Org C: Organic Carbon. Source: WorldClim (http://www.worldclim.org). Disturbance data from Farwig et *al*.,2006

Fig.8. Sampled Sites for bark and arbuscular mycorrhizal fungi isolates of Prunus africana in the mono modal rain forest and western highlands ecological zones of Cameroon. Source Cifor, 2007

II.2. METHODS

II.2.1. Investigate the AMF diversity in the roots of *P. africana* in Afromontane forests in Cameroon

II.2.1.1. Field surveys and selection of trees

The tools used for data collection on the field consisted of a GPS receiver of Garmin T5 brand to record altitudes and coordinates, a digital camera of HP Photosmart E 427 brand, a digital meter of 7.5 m for appreciating the circumference of the stem, plastic bags for pocketing in a separate way the various samples and a cutlass to carve out the selected samples of bark and dig out soils.

For the mycorrhizal screening of *P africana*, different numbers of trees were selected in three sites. Identification of *P. africana* in the field was done with the help of local assistants. In each site, trees were selected at various altitudes: in Oku mountain forest, between 1950–2670 m; in Manengouba, between 750 and 1760 m; and in Mt Cameroon, at 700 and 800 m. Altitude was recorded with a GPS reveiver. Chosen trees were spatially separated and selected at random. Phenology of *P. africana* varied during the sampling period (May to July). In Oku mountain forest, some trees bore fruits, others had seedlings under their canopy. In Manengouba and Mt Cameroon, all trees sampled did not have fruits. Soil temperature also varied with site (though it was not measured, one could note differences upon sensation on sampling). Seedlings, saplings, juvenile and mature *P. africana* trees were identified, and their girth at breast height (gbh) were determined. Different size classes were determined to describe seedlings, saplings, juvenile and mature *P. africana* trees. Seedlings were plants of small size, < 10 cm gbh with 3-5 true leaves and were less than or more than 1.5 m in height. Saplings were trees of intermediate size between seedlings and juvenile having gbh between 10 and 49 cm. Juveniles were identified as trees between 50 and 99 cm gbh, whereas mature trees were those having gbh greater than 99 cm (Onguene and Kuyper, 2001). A rolling meter tape was used to measure the girth at breast height for each selected tree.

II.2.2. Collection of root samples

Experimental field works

Fine root samples were traced from the stem base of each selected *Prunus africana* tree. Samples were collected at four different cardinal points around each selected tree and pooled to form one sample per tree. For seedlings, the whole plant was uprooted with the surrounding soil. A secator was used to cut bigger roots of 1 cm in diameter and the remainder portion underground was carefully dug with a trowel in order to get the fine roots. Roots were rinsed in water to remove dirt and remaining soil particles. In total, one composite sample was collected per tree, 20 samples were collected per location. The roots were then cut into smaller fragments of 3-5 cm in length, placed in small plastic bags and then preserved in 50 % alcohol (Brundrett et *al*., 1996; Melville and Peterson, 1994). The samples were transported to regional biological control and applied microbiology laboratory of the Institute of Agricultural Research for Development (IRAD), Yaoundé, Cameroon.

II.2.3. Estimation of AMF colonization in *Prunus africana* roots

In the laboratory, fine roots from the root samples were selected and cut into 1-2 cm length. Then, they were rinsed three times in plastic vials with tap water to remove alcohol. Using a modified procedure for staining mycorrhizal roots with Fuchsin acid stain and Trypan blue (Onguene and Kuyper, 2001), root fragments were cleared with 10 % potasium hydroxyde (KOH) for 24 hours. *P. africana* roots are pigmented, so they were bleached after immersion for six hours in alkaline peroxyde at room temperature. Alkaline peroxyde was prepared by adding 3 ml of ammonium hydroxyde (NH_4OH) and 30 ml of 10 % peroxyde to 567 ml of tap water. Roots were thoroughly rinsed two to three times with deionized water to remove the alkaline peroxide. Root fragments were thereafter acidified with 1 % hydrochloric acid for three minutes before staining in a solution of Fuchsin acid for 2-3 days. The Fuchsin acid staining solution was prepared from 1750 ml of lactic acid, 126 ml of glycerin, 126 ml of filtered tap water, and 0.2 g of Fuchsin acid. The stained roots were destained for 2-3 days in a destaining solution. The destaining solution was prepared as the staining solution in the same proportion except for the absence of Fuchsin acid. Each root sample was placed and arranged in a petri dish before observation under a dissecting microscope (Nikon SMZ 645, type 102, Nikon Inc.) at magnification 40x. Percentage mycorrhizal colonization was measured using the gridline-intersect method (Giovannetti and Mosse, 1980). The gridline-intersect method has the advantage of providing both the proportions of colonized root and total root length. The roots of

P. africana were dark even after bleaching. Thus, internal hyphae often could be recognized only after squashing a root portion. It was assumed that when hyphae were firmly attached to the outside of the root or when the entry point of the hyphae could be seen, internal colonization was present. AM fungi do not cause obvious morphological changes of roots; however, they produce arbuscules and, in many cases, vesicles in roots (Sylvia et *al*., 1994). The absence or presence of arbuscules and vesicles were examined using the compound microscope, auxiliary bodies were additionally noted. Root samples of *P. africana* were considered arbuscularly mycorrhized if the above internal and external features were found. The frequency and intensity of AMF structures in root fragments were evaluated as described by Trouvelot *et al.* (1986) using the MYCOCALC software (www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

II.2.4. Investigate the AMF diversity in the rhizosphere soil of *P. africana* in Cameroon

II.2.4.1. Collection of soil samples

The method used for soil sampling was that of Sieverding (1991). For each site, an area of one hectare was delimited and subdivided into 20 quadrants measuring 25 × 20 m_2. In each of these quadrants, one plant of *P. africana* was randomly selected; the roots were collected from 0 to 45 cm together with a soil sample around the rhizosphere of the tree using an auger. At each quadrant, a total of 500 g of soil sample was collected and soil samples from the same site were mixed to make a composite sample of 10 kg of soil per site. Since the propagules of arbuscular mycorrhizal fungi are generally concentrated in the uppermost few centimetres of soil and reach their maximum concentration in the rhizosphere (Cuenca et *al*., 1998), soil samples were collected at 0–20 cm depth using a sterilised auger and placed into plastic bags. Soil and root samples were collected in May (Mt Cameroon), June (Oku Mountain forest) and July (Manengouba), 2022. Twenty mature *P. africana* trees (≥ 30 cm dbh (diameter at breast hight) were randomly selected (see section 3.3.1) and rhizosphere soil samples were collected from each tree at four cardinal points, and at about 20 cm distance from the trunk. Each sample consisted of approximately 50 g of soil aseptically collected (soil collection was done with an auger sterilised with 70 % ethanol after each sample being collected) at a depth of 30 cm after removing the surface soil litter. The four samples from each tree were then pooled to form a composite sample of 200 g per tree. Subsequently, the composite sample per tree was further pooled to form a composite sample of 4 kg from all sampled trees per site. A subsample of 500 g from each composite sample was air-dried at room temperature for soil chemical analyses.

II.2.4.2. Soil chemical analyses

Soil samples were analysed to determine the pH, electrical conductivity (EC), percentage of carbon (% C), total nitrogen (% N), available phosphorus (P), potassium (K), sodium (Na), and calcium (Ca), present in the soil samples at the Soil, Plant and Water analysis Laboratory of IRAD Cameroon as described by Odee *et al*. (2002)

II.2.4.3. Trapping and isolation of AMF's spores in soil rhizosphere of *Prunus africana*

Trapping culture was established to trap the AMF species in the collected soil samples (Ngonkeu et *al*., 2009) using *Sorghum bicolor* (Poaceae) and *Vigna unguiculata* (Fabaceae) as host plants (Figure 2.4). A layer of about 100 g of the collected soil samples was placed between two layers of sterilized sand in a pot of 2 L. The sand collected and used for raising seedlings was autoclaved twice at 121 ºC for one hour. The *S. bicolor* and *V. unguiculata* seeds were sterilized using 70% ethanol for 2 min, followed by 1% sodium hypochlorite solution for 3 min. The seeds were soaked in distilled water for 24 h and then sown in the first layer of the trap pot and placed in the glasshouse (Figure 9). After germination, the seedlings were watered regularly with tap water. After 3 months of growth, watering was withheld to create a draught stress for a period of one month to induce sporulation.

II.2.4.3. 1. Spore extraction

A modified wet and decanting method of spore extraction followed by sugar gradient centrifugation was used (Onguene et *al*., 2000). 100 g portions of collected soil samples were taken and air-dried on greenhouse benches for a minimum of five days. Air-dried soil samples were then sieved through a 2 mm sieve to remove gravel and debris. 25 g portions of the soil from each sample containing some fine roots and organic matter (dry weight basis) were weighted and soaked in 100 ml of tap water for at least 30 minutes. The soil sample was re-suspended by stirring briskly for 1-2 minutes breaking larger soil particles. The sample was left to stand for 30 seconds for the sedimentation of coarse sand. The suspension was decanted through a series of three sieves (850 μm over a 500 μm and this over a 250 μm) to a recipient. The pellet was re-suspended in 25 ml of tap water and this suspension was decanted through the sieves. This process was repeated three times. Centrifugation was done in the Soil Chemistry Laboratory of the International Institute of Tropical Agriculture (IITA) using a Torvall T6000B centrifuge at a maximum speed of 3600 rpm. Sieving of each sample was transferred into four centrifuge tubes and with a steady stream of water from a washed bottle; they were equally

weighed on an electronic balance before centrifuging. The samples were centrifuged at 2000 revolutions per minute (rpm) for 5 minutes. The supernatant was discarded carefully to avoid disturbing the pellet. The pellet was re-suspended in 20 ml of sugar solution by gently stirring with a glass rod. The sugar solution was prepared by dissolving 1000 g of saccharose in 1 liter of filtered water. They were centrifuged again at 2000 rpm for 2 minutes. The sugar suspension was gently poured on to a 38 µm sieve and rapidly rinsed with abundant water to remove sugar. Spores were washed from the sieve and then trapped on a filter paper placed in a funnel. Each filter paper was later placed in a petri dish for spore enumeration and morphotyping.

II.2.4.3.2. Spore enumeration and morphotyping

Spore count was carried out under a dissecting microscope at magnification 40x. A square mesh of 0.5 cm X 0.5 cm was printed on a tracing paper. The filter paper, which was slightly wetted, was placed on the tracing paper. Spores were counted by scanning horizontally leaving from one square to another, using a manual counter. Describing characteristics such as spore colour, shape and ornamentation were used for morphotyping. Spore types were assigned to AMF types based on their shape, colour, size, hyphal attachment, and spore content as described by the International Culture Collection of Arbuscular and Vesicular Mycorrhizal Fungi (INVAM) (http://fungi.invam.wvu.edu/the-fcastroungi/species-descriptions.html) and Brundrett *et al.* (1996). Spores abundance was estimated in 100 g of dry soil.

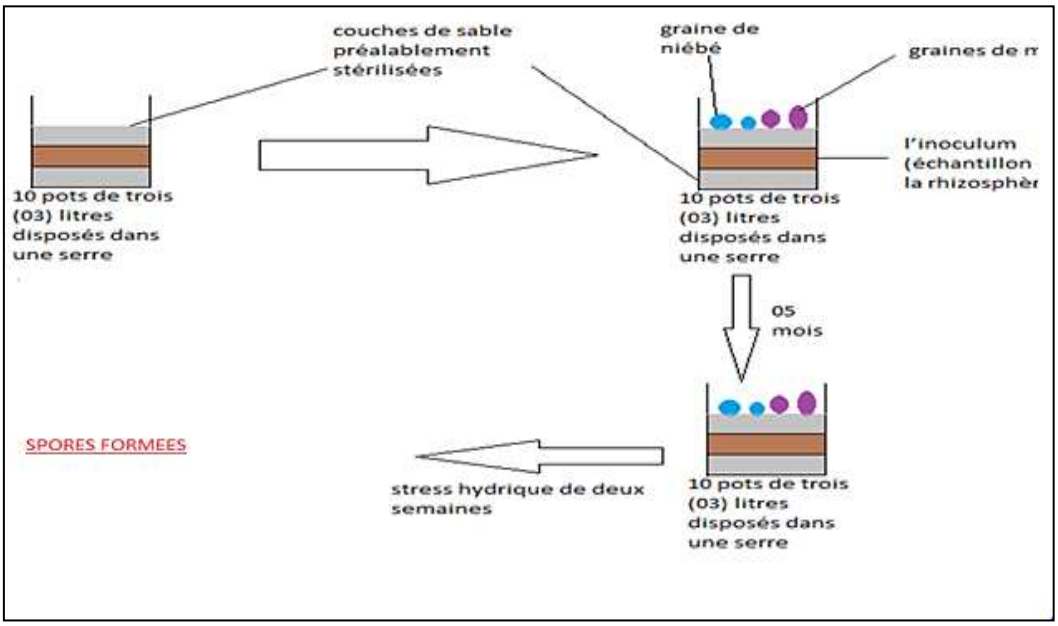


Fig.9. Trapping system with the collected soil placed between sterilized sand

II. 2.4.3.3. Bioassays for measuring colonization potential of *P. africana* soils

Soil samples from the Oku, Cameroon and Manengouba mountain forest was collected in the vicinity of the different size classes of *P. africana*: seedlings, saplings, juvenile and mature trees. The soils were wrapped in black plastic bags. To prevent drying out, they were kept on greenhouse benches under cool and shaded conditions. After six weeks, soils from the different developmental stage seedlings, saplings, juvenile and mature trees were mixed (all classes mixed) and used as treatments for a provenance. 700 g portions of mixed soil samples were transferred into small plastic bags. Pre-germinated seeds of cowpea (*Vigna unguiculata*), and *Sorghum vulgare* var. Zouave were sown in the filled bags. The seeds were sterilized in 70 % alcohol for 30 seconds, rinsed three times before placing them in petri dishes for pre-germination. The seeds were pre-germinated on wetted filter paper placed in petri dishes for 48 hours in the dark under obscurity and sowing was done using forceps (Kamko, 2018). The bags were arranged on greenhouse benches in a randomized complete block design with five replicates per treatment. There were four treatments resulting from two provenances. Watering was done with a cup to prevent splashes. Plants were grown for one month under greenhouse conditions in IRAD-Nkolbisson. Assessment of mycorrhizal colonization potential was done by examining the presence or absence of roots colonized by arbuscular mycorrhizal fungi after carrying out the staining procedure as indicated above (Brundrett *et al.*, 1996).

II.2.5. Assessment of AMF effect on the morphological growth of vegetatively propagated *Prunus africana* cutting in nursery

II.2.5.1. Production of AMF Inoculum

To optimize and intensify the activity of the identified AM fungi, purification of the strains was performed using the method described by Ngonkeu et al. (2009). The pre-germinated spores were inoculated on the roots of a mycotrophic plant (*Sorghum bicolor*) and then gently introduced into 3L pots filled with sterilized sand. In order to test if the AM fungi efficacy of promoting growth dependent on the number of spores initially present in the medium, an experiment was conducted with six treatments and one negative control without inoculation. One treatment consisted of 6 *Sorghum* plants (Bafia's variety) inoculated with one AMF specie with two variables (1 spore and 10 spores) and three replicates. The *S. bicolor* seeds were surface sterilized using 70% ethanol for two min, followed by 1% sodium hypochlorite solution for three min, then rinsed with sterile distilled water 10 times. The sterilized seeds were then

transferred to Petri dishes containing 0.8 % water agar and kept in the oven at 30 °C for seeds to germination. After three days, two seedlings were put in a folded filter paper and a single healthy spore of AMF was placed on the root. The plants of a treatment (same AMF species) were grouped in a tray of 8 m_2. The negative treatment consisted of plants not inoculated with AM fungi. The plants were regularly watered by capillary action for three months and growth parameters such as stem length, number of leaves and leaf surface area were recorded to verify the effectiveness of the mycorrhizal symbiosis. Then the plants were subjected to drought stress to stimulate sporulation. Finally, extractions were carried out to confirm the multiplication of spores and the reliability of obtaining a biofertilizer the efficiency of which could be evaluated on *Prunus* cuttings.

II.2.5.2. Production of *Prunus africana* seedling

II.2.5.2.1. Construction of low-technology non-mist Propagators

Low-technology non-mist propagators were constructed following Leakey *et al.* (1990) and were used to produce vegetatively propagated *P. africana* cuttings. The propagators consisted of an aluminium frame enclosed in wooden side and bottom panels, with a removable clear glass cover (Fig.10). The propagator was designed to allow manual watering to a depth of 15 cm below the surface of the rooting medium, and maintenance of appropriate humidity using a hand sprayer. A layer of gravel was placed to a depth 10 cm at the base of the propagator, followed by a layer of small stones (5 cm) and gravel with 1 cm thickness. The rooting medium for *P. africana* cuttings consisted of a layer of sand placed at the top of the gravel to a depth of 20 cm.

II.2.5.2.2. Preparation and planting of leafy stem cuttings of *Prunus africana*

The efficacy of the identified AM fungi strains was evaluated on the vegetative propagation of *Prunus africana* cuttings in a greenhouse following the method described by Leakay et *al*., (1994). A treatment consisted of *Prunus africana* cuttings placed in substrates (sand and sawdust) inoculated with a biofertilizer (inoculum produced, containing infected root fragments, hyphae and spores). Once in the glasshouse, leafy stem cuttings with one node and a trimmed leaf were dipped in 200 mg/L indole-3-butyric acid (IBA) which is a root growth inducing hormone and was used as a positive control (Tchoundjeu *et al.*, 2002). The surface area of leaves ranged from 20 to 40 cm^2 and the stem length from 2.5-4 cm. After dipping the leafy stem cuttings in IBA for 1 min, cuttings were planted in the rooting media. The negative control included cuttings grown on a substrate with no AM fungi applied (no biofertilizer). The

experimental unit consisted of 15 cuttings. After 25 days of propagation, the cuttings of the different treatments were evaluated based on their root density in comparison with that of the controls and the experiment was repeated three times.

Fig.10. Prunus africana cuttings planted into the sand and sawdust substrate in a propagator.

All rooted cuttings were transplanted to plastic bags containing sterilized substrate. The substrate used was a mixture of soil mixed with sand at a ratio of (1:1 v/v). The substrate was sterilized twice at 121 °C for 1 h.

II.2.5.3. Experiment design and layout

The experimental design was a 4-factor completely randomized 3-block design containing three inoculation treatments. The inoculation treatments were as follows: AH+E, SH+E, AH+SE, SH+SE, non-sterilized soil (NS) collected at Irad Knolbisson soil, and autoclaved (121 ºC, 1 h) sand as a non-inoculated control treatment. For mycorrhizal inoculation, 500 g of inoculum produced, containing infected root fragments, hyphae and spores from the inoculated substrate were introduced in pots containing the vegetatively propagated *P. africana*. The experiment was carried out in the greenhouse for a period of three months from October 2018 to January 2019, with a photoperiod of 12 h, and average day and night temperatures of 25 ºC and 14 ºC, respectively. The watering was done manually on a daily basis (Avana et *al*., 2006)

II.2.5.4. Measurement of arbuscular mycorrhizal infection in roots

At the end of three months, cuttings were harvested by carefully uprooting from the substrate. Approximately 2 g of fresh roots were subsampled from each seedling and stored in 50 % ethanol to evaluate the mycorrhizal colonization (Tchiechoua et *al*., 2022). After washing the fine roots with distilled water, they were cleared using 10 % KOH at room temperature for 24 h, treated with 30 % H_2O_2 for 30 min, washed, acidified with 10 % HCl at room temperature for 15 min and stained with 0.05 % Trypan blue in lactoglycerol (5m/l°) at 121 ºC for 5 min (Phillips & Hayman, 1970). About 30 root fragments of approximately 1 cm were analysed under the microscope. The following mycorrhizal parameters were assessed: frequency (F), the intensity in roots system (AIRS), the intensity in the root fragment (AIRF), abundance in the root system (AARS), and abundance in the root fragment (AARF). These parameters were evaluated as described by Trouvelot *et al.* (1986) using the MYCOCALC software (www.dijon.inra.fr/mychintec/Mycocalcprg/download.html).

II.2.5.5. Measurement of growth parameters of vegetatively propagated *Prunus africana* cuttings

To quantify cuttings growth, the number of leaves appearing after the inoculation date were counted; the average leaf surface area was measured using a fast and accurate method (Chaudhary *et al.*, 2012). Cuttings heights were also measured and the shoot weight and the total weight biomass were determined after drying at 50 ºC to constant weight for 48h in a drying oven.

II.2.6. Assessment of AMF effect on the phytochemical content of vegetatively propagated *Prunus africana* cuttings in nursery

II.2.6.1. Qualitative analysis

Preliminary phytochemicals screening was carried out on vegetatively propagated *P. africana* to check the presence of saponins, anthraquinones, terpenoids and alkaloids. Phytochemical screeming highlights the presence of families of actives molecules; It is a qualitative study used to know the overall chemical composition of extracts (Dohou et *al*., 2003; Senhaji et *al*., 2005; Kumar et *al*., 2010).

II.2.6.1.1. Test for saponins

A foam test was used to screen for saponins in the samples, by adding 1 ml of ethanolic extracts and 5 ml of distilled water in a test-tube. The mixture was then vigorously shaken and then allowed to stand for 10 min and the formation of foam suggested the presence of saponins. Foam formation was scored as the saponins follows: (i) no foam, (ii) < 3 mm high formation of foam was classified as poor, (iii) > 3, 6 mm high was moderate, and (iv) > 6 mm was recorded as abundant (Kumar et *al*., 2010).

II.2.6.1.2. Test for anthraquinones

Prunus africana dried powder (0.5 g) was boiled for five min in 10 % hydrochloric acid and filtered while hot. Then 2 ml of chloroform and 2 ml of a 10 % ammonia solution were added to the filtrate. The formation of pink colour in the aqueous layer indicated the presence of anthraquinones (Harborne et *al*., 1998, Price *et al.*, 1978).

II.2.6.1.3. Test for terpenoids

A volume of 5 ml of plant water extract was mixed with 2 ml chloroform and drops of concentrated sulphuric acid added to the mixture. The formation of a reddish-brown precipitate indicated the presence of terpenoids (Dohou et *al*., 2003).

II.2.6.1.4. Test for alkaloids

A volume of 10 µl of plant ethanolic extract was spotted at equidistance from each other on silica gel-coated plates and then eluted with a methanol-sulfuric acid solution. The plates were dried, sprayed with Dragendorff reagent and the formation of redbrown colour was an indicator of the presence of alkaloids in the extract (Senhaji et *al*., 2005).

II.2.6.2. Quantitative analyses

Tannin, flavonoids, and total phenols were quantified in the vegetatively propagated *P. africana*.

II.2.6.2.1. Determination of tannins content

Condensed tannins were assayed using the vanillin-hydrochloric acid method as described by Price *et al.* (1978). Extraction was done using 4 % HCl in methanol using a shaker (Labortechnik KS 250b, Germany). After separation using a refrigerated centrifuge (Kokusan, Type H-2000C, Japan) at 4,500 rpm for 10 min at 25°C, extraction was repeated on the residue from the first extraction using 1 % HCl in methanol and standards were prepared using catechin

hydrate. Absorbance for all prepared solutions was read at 500 nm and tannin content calculated as percent catechin equivalent (CE) using a standard calibration curve.

II. 2.6.2.2. Determination of flavonoids content

Flavonoids were determined using the aluminium chloride colorimetric method (Lamaison & Carnet, 1990) with 0.3 mL of 5 % in H20 sodium nitrite solution added to a mixture of 1 mL of plant extract and 4 mL of distilled water. Then 10 % aluminium chloride in H20 was added to a mixture and after 5 min, 2 mL of 1 M sodium hydroxide was added and the volume made up to 10 mL with distilled water. Absorbance was measured at 415 nm using a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). The quantity of total flavonoids was calculated from the calibration curve of a standard prepared from quercetin

II.2.6.2.3. Determination of total phenol content

Total phenol contents in samples were determined using a modified FolinCiocalteu method (Ragazzi & Veronese, 1973). Fifty mL of methanol were added to 5 g of ground sample and shaken for three hours. Then kept for 72 hours and filtered. After being centrifuged for 10 min at 150 rpm at 25 ºC, 1 ml of the supernatant was filtered, then mixed with 2 mL of Folin-Ciocalteu and vortexed. After two hours. The absorbance of the mixture was determined at 765 nm against a blank.

II.2.6.3. Statistical analyses

Statistical analyses were performed using the SPSS package (SPSS Inc., 1993). Data of spore count and percent root colonization were tested for normality and homogeneity of variances using the Levene test. Spore count numbers were log-transformed and percent root colonization were arcsine square-root transformed before a two-way analysis of variance (ANOVA) with site, size class distribution and altitude.

II.2.7. Assess the Metabolite profile of *Prunus africana* in Cameroon

II.2.7.1. Samples collection

The collection of barks of *P. africana* individuals was carried out following the described method of Kadu et *al*., 2014. It took place in three main natural location of *P. africana* in

Cameroon and these barks were ground in fine powder, labeled and dried for a total of 15 samples.

Disk-bark samples were collected from trees that located at four cardinal points and the centre of each site. The bark samples were collected using a standardized (same settings for all samples) cordless drill. Barks were taken from trees in the North, South, West and East orientation avoiding any area of bark damage. So, barks were collected by moving up or down at each orientation. The four barks disks collected from each tree were immediately pooled into a calico bag.

Twenty disk-bark samples were collected from five trees of each site (three sites), making a total of 60 disk-barks for 15 total samples. Upon arrival at the station, the samples were immediately taken to the Molecular Parasitology laboratory. They were ground in fine powder, with a mortar and pestle under liquid nitrogen. Afterwards, they were labeled and completely dried in a SpeedVac (Model SC117 and ST3182, Savant 2018,) for four hours. A final dry bark of each sample was labeled, put into a 50ml Eppendorf tube and stored at room temperature and transported to the Plant stress laboratory at the Molecular and Cell Biology Department in South Africa.

II.2.7.2. Metabolite profiling of Prunus africana in three locations in Cameroon

Test of different extraction methods of metabolite compounds

In the goal to set up the most appropriate and efficient extraction method which would allow us to have the best qualitative results (polar and non-polar compounds), we have decided to test three different methods: Ethyl Acetate (Acetone) + Ribitol modified from Kadu et *al*. (2014); Ethyl acetate + Methanol + Ribitol modified from Kadu et *al*. (2014) and MTBE + Methanol + Ribitol (Mohamed et *al*., 2020). The study involved the collection of samples from three different locations (Mount Cameroon: C, Mount Manengouba: M, and Mount Oku: O), which were then analyzed using gas chromatography/mass spectrometry (GC/MS). A certain weight of the five trees ground bark from locations (depending on the method) were prepared for the extractions. After the extracted samples were dried and derivatized, 1 microlitre of sample was injected into an Agilent GCMS (Model 7890A). The chromatograms from three locations and one blank were analysed with Agilent MassHunter Quantitative Analysis software.

II.2.7.2.1. First experimentation

For this first trial on GC-MS, we tried to familiarize ourselves with the whole extraction and analysis process, to have an overview on the metabolite exuded by *P. africana* from the three different locations in Cameroon and finaly to set up the appropriate GC conditions for the final experimentation.

This study involved the collection of samples from three different locations (Mount Cameroon: C, Mount Manengouba: M, and Mount Oku: O), which were also analyzed using Gas Chromatography/Mass Spectrometry (GC/MS). Samples of the same weight of ground bark from each of five trees from the same location were mixed for extraction (MTBE-MeOH method: Mohamed et *al*., 2020). After the extracted samples were dried and derivatized, one microlitre of sample was injected into Agilent GCMS. The chromatograms from three locations and one blank were analysed with Agilent MassHunter Quantitative Analysis software 3.0.

Method

MTBE + Methanol + Ribitol (Internal standart**)**

30 mg of the bark powder of each location were extracted with 3:1 MTBE: MeOH +ribitol (18.75 ml MTBE , 6.2 ml of methanol, 50 µl Ribitol (4µg/ml) in methanol) in 1.5 Eppendorf tubes using an orbital shaker (1000t/min for 30 min). The extracts were incubated it 15 min in a sonicator ice water bath (Model UE06SF-D, Wiggens 2020, Cape Town ZA), then centrifuged (Model 1-14k, Sigma Laborzentrifugen 2018, Cape Town ZA) at 14000 rpm at room temperature for 5 minutes. 700 µl of 3:1 H2O: MeOH was added to the upper fraction and the mix was centrifuged at 14000 rpm at room temperature for 5minutes. Finally, 400 µl of each lower phase were transfered to a new 1.5 ml Eppendorf tube and then dried in a Speed Vac (Model SC110 and RT4104, Savant 2022, Cape Town ZA) at 15000 rpm at -20°c and store at -80°C freezer the same day.

Samples preparation:

- Take all samples (from the three locations)
- Label Eppendorf tubes for the three location: 1C for Mount Cameroon; 2O for Mount Oku; 3M for Mount Manengouba **(**1C; 2O; 3M)
- Take 5 mg bark from each tree per location
- Mix all trees for each location

- Weight 30 mg of samples from each location (three locations in total)
- Take the 30 mg of samples for each location for the extraction

MTBE: Methyl Tert-Butyl Ether

Buffer:

- Add pre-cooled 1ml (-20°C, keep on ice) 3:1 MTBE: MeOH +ribitol (final concentration 4 µg/ml) to 30 mg dry material of each location

25 ml MTBE mix: (Ribitol final concentration: 4 µg/ml): keep buffer in a -20°C freezer, during the extraction keep it on ice.

- 50 µl Ribitol (4µg/ml) in methanol
- 18.75 ml MTBE
- 6.2 ml Methanol

Extraction method:

- Suspend the ground tissue with a vortex mixer
- Incubate the samples at 4°C orbital shaker 1000 t/min (Model 88881045 Thermo Fisher Scientific 2020, Cape Town ZA) for 30 minutes.
- Then incubate it 15 min in a sonicator ice water bath (Model UE06SF-D, Wiggens 2020, Cape Town ZA)
- Centrifuge (Model 1-14k, Sigma Laborzentrifugen 2018, Cape Town ZA) at 14000 rpm at room temperature for 5 minutes
- Transfer 700 µl of fraction 1 to 1.5 Eppendorf; keep the pellet for protein extraction
- Add 700 µl of 3:1 H2O: MeOH to fraction 1 and mix well
- Centrifuge at 14000 rpm at room temperature for 5minutes
- Carefully remove all the upper phase with micro-pipettes using sterile tips
- Using new sterile tips, carefully transfer 400 µl of the lower phase to a new 1.5 ml Eppendorf.
- Dry all the fractions and pellets in a Speed Vac (Model SC110 and RT4104, Savant 2022, Cape Town ZA) at 15000 rpm at -20°c and store at -80°C freezer the same day at 15000 rpm.

II.2.7.2.2. Second experimentation: Test of different extraction methods for metabolite compounds

The second experimentation aimed to set up the most appropriate and efficient extraction method resulting in the best qualitative results (polar and non-polar compounds). We decided to test three differents methods: Ethyl Acetate (Acetone) + Ribitol, Ethyl acetate + Methanol + Ribitol and MTBE + Methanol + Ribitol (already run in the first experiment). The study involved the collection of samples from three different locations (Mount Cameroon: 1C, Mount Manengouba: 3M, and Mount Oku: 2O), which were then analyzed using gas chromatography/mass spectrometry (GC/MS). A certain weight of ground bark samples from the five trees from the correspondent locations (depending on the method) were prepared for the extractions. After the extracted samples were dried and derivatized, one microlitre of sample was injected into the Agilent GCMS. The chromatograms from three locations and one blank were analysed with Agilent MassHunter Quantitative Analysis software 3.0.

Method

a) Ethyl Acetate (Acetone) + Ribitol

30 mg of the bark powder of a pooled sample of one location was extracted with 10 ml ethyl acetate + 20 µl ribitol (2 mg/ml stock) in 1.5 Eppendorf tubes using an orbital shaker (1000t/min for 30 min) using a sonicator ice water bath (Model UE06SF-D, Wiggens 2020, Cape Town ZA) for 15 minutes. The extracts were centrifuged (Model 1-14k, Sigma Laborzentrifugen 2018, Cape Town ZA) at 14000 rpm room temperature for two minutes. 800 µl of supernatants were filtered through S&S 595½ folded filters (0.22 µm pore size filter, Schleicher & Schüll, Dassel, Germany) into 24 ml vials and dry in a Speed Vac (SC110 Savant 2022, Cape Town ZA), then 200 µl were finally stored at -80 C for derivatization.

Samples preparation:

- Randomly choose one location
- Choosen location: 3M
- Weight 15 mg of bark from each tree (five trees in total) as a pooled sample
- Take 30 mg of the pool for the extraction

Buffer:

- 10 ml total: (ribitol final concentration: 4 µg/ml)

- 10 ml ethyl acetate + 20 µl ribitol (2 mg/ml stock).

Extraction method:

- Add 10 ml ethyl acetate to 30 mg dry material; Sonicate (Model UE06SF-D, Wiggens 2020, Cape Town ZA) in an ice water bath for 15 minutes.
- Centrifuge (Model 1-14k, Sigma Laborzentrifugen 2018, Cape Town ZA) at 14000 rpm for two minutes
- Take 800 µl supernatant. Filter with 0.22 µm pore size filter.
- Dry in a Speed Vac (Model SC110 and RT4104, Savant 2022, Cape Town ZA), 200 µl for derivatization.

b) Ethyl acetate + Methanol + Ribitol (Internal standart)

30 mg of the bark powder of a pooled sample of one location was extracted with 8 ml ethyl acetate + 2 ml methanol + 20 µl ribitol (2 mg/ml stock) in 1.5 Eppendorf tubes using a thermos shaker (Model 88881045 Thermo Fisher Scientific 2020, Cape Town ZA) at 1000/min (16.7 Hz) at 4°C for 70 minutes. The extracts were centrifuged (Model 1-14k, Sigma Laborzentrifugen 2018, Cape Town ZA) at 14000 rpm room temperature for two minutes. 800 µl of supernatants were filtered through S&S 595½ folded filters (0.22 µm pore size filter, Schleicher & Schüll, Dassel, Germany) into 24 ml vials and dry in a Speed Vac (SC110 Savant 2022, Cape Town ZA), then 200 µl were finally stored at -80 C for derivatization.

Samples preparation:

- Take all the five trees for the three location 1C, 2O, 3M
- Choosen location: 3M
- Weight 15mg of bark from each tree (5 trees in total)
- Take 30 mg of the pool for the extraction

Buffer:

- 10 ml total: (ribitol final concentration: 4 µg/ml)
- 8 ml ethyl acetate + 2 ml methanol + 20 µl ribitol (2 mg/ml stock).

Extraction method:

- Add 1 ml buffer to 30 mg dry material;

- Shake in a thermos shaker (Model 88881045 Thermo Fisher Scientific 2020, Cape Town ZA) at 1000/min (16.7 Hz) at 4°C for 70 minutes.
- Centrifuge (Model 1-14k, Sigma Laborzentrifugen 2018, Cape Town ZA) at 14000 rpm for two minutes.
- Take 800 µl supernatant. Filter with 0.22 µm pore size filter.
- Dry in a Speed Vac (Model SC110 and RT4104, Savant 2022, Cape Town ZA), 200 µl for derivatization.

II.2.7.2.3. Final experimentation

The final experimentation has the goal to use the best extraction method to have the metabolites profiling of all the samples (Genotypes) from the total observed *P. africana* population.

The study involved the collection of samples from the same previous locations (Mount Cameroon: C, Mount Manengouba: M, and Mount Oku: O), which were then analyzed using gas chromatography/mass spectrometry (GC/MS). Same weight of three trees' ground bark from the total three P. *africana* populations were prepared for the extraction (Ethyl acetate + Methanol + Ribitol method). After the extracted samples were dried and derivatized, 1 microliter of sample was then injected into Agilent GCMS. The chromatograms from plants of three locations with an elaborated QC (quality control) and one blank were analyzed with Agilent MassHunter Quantitative Analysis software and the normalized data were analyzed using MetaboAnalyst version 5.0 with an open-source JavaServer Faces Technology using the PrimeFaces library (v12.0) and the back-end computations based on the open-source MetaboAnalyst_R package.

Method (Ethyl acetate + Methanol + Ribitol)

30 mg of the bark powder of a pooled sample of one location was extracted with 8 ml ethyl acetate + 2 ml methanol + 20 µl ribitol (2 mg/ml stock) in 1.5 Eppendorf tubes using a thermos shaker (Model 88881045 Thermo Fisher Scientific 2020, Cape Town ZA) at 1000/min (16.7 Hz) at 4°C for 70 minutes. The extracts were centrifuged (Model 1-14k, Sigma Laborzentrifugen 2018, Cape Town ZA) at 14000 rpm room temperature for three minutes. 800 µl of supernatants were filtered through S&S 595½ folded filters (0.22 µm pore size filter, Schleicher & Schüll, Dassel, Germany) into 24 ml vials and dry in a Speed Vac (SC110 Savant 2022, Cape Town ZA), then 200 µl were finally stored at -80 C for derivatization.

Samples preparation:

- Take bark samples from the five trees for the three location 1C, 2O, 3M
- Weight 30 mg of bark from each tree (15 trees in total)-record actual weight (can be 30 mg +/- 2 mg)
- Take 30 mg for the extraction

Buffer:

- 25 ml extraction buffer:
- 20 ml ethyl acetate
- 4.9 ml methanol
- 2 mg/ml ribitol

Ribitol will be 8 µg/ml in the buffer, 1.6 µg in 200 µl extraction buffer used for GC Extraction:

- Add 1 ml cold buffer to 30 mg dry material, vortex and mix well.
- Shake in a thermo shaker (Model S2030, New Brunswick Scientific 2022, Cape Town ZA) 1000/min at 4°C for 70 minutes (or incubate on a shaker in the cold room)
- Centrifuge (Model 1-14k, Sigma Laborzentrifugen 2018, Cape Town ZA) at 12000 rpm for three minutes.
- Take 800 µl supernatant. Filter with 0.22 µm filter.
- Dry 200 µl for derivatization.
- After filtering the samples, transfer 20 ul from each sample to an Eppendorf.
- Mix well, quick spin.
- Transfer 200 µl of mixed sample into a new Eppendorf as QC sample, dry it in Speed Vac (-20°),

Blank: Transfer 200 µl of extraction buffer to a new Eppendorf as blank, dry it in Speed Vac (-20°),

<u>GC derivitization:</u>

1. Take the samples out of -80°C freezer, dry the tubes in a Speed Vac (Model SC110 and RT4104, Savant 2022, Cape Town ZA), for 20-30 minutes.
2. Add freshly prepared 40 µl MeOX (MethylOxolane) 20 mg/ml in pure Pyridin)) in a fume hood.
3. Prepare one blank sample (it's the sample went through same extraction method, it should contain the same amount of ribitol), follow the same procedure.

4. Shake 284 /min=4.73Hz at 37°C for 2 hours.
5. Quick spin.
6. Add 70 µl MSTFA (Trimethylsilyl-Methyl-Trifluoroacetamide)-prewarm MSTFA at room temperature.
7. Shake at 37°C for 30 minutes.
8. Transfer to the GC vial. Inject 1 µl, try different split ratio.
9. Randomize the sample order (QC Samples and +blank)

II.2.7.3. Metabolite compounds analysis methods

II.2.7.3.1. GC-MS condition

The analyses were performed using an Agilent Model 7890A equipped with 7000C mass quadruple mass spectrometer. Samples of 1 ul were injected by the autosampler at temperatures of 250 °C and passed through a J&W 122-5532G DB-5ms+DG column with a carrier gas helium at flow rate of 1ml/min. The column temperature was 80°C for 2 mins, followed by increase to 320°C at a rate of 5 °C/min and maintained at 320 for seven minutes. The GC included an Electron impact (EI) of ionization energy 70ev with ion source of 230oc with a mass scan range of 70-500.

II.2.7.3.2. Peak identification

The separated constituents were identified by comparing their mass spectra with those in International standard library and the new compounds with the NIST08 MS library (National Institute of Standards and Technology, Gaithersburg, MD, USA) by comparing their retention indices (RIs) and Mz with literature values (Dool et *al*, 2014). The RIs were calculated relative to a C7-C30 Ribitol standard (Sigma Aldrich, St Louis, MO, USA) separated on the MS capillary column under the same GC-MS analysis conditions (Obistioiu et *al*., 2014). Each constituent was quantified based on the comparison of its peak area with that of the internal standard (Ribitol).

II.2.7.3.3. Metabolomic Statistical Analysis

Statistical analysis was performed on obtained data after normalizing Pareto Scaling (normal distribution curve) and log10-transformed in MetaboAnalyst5.0 (https://www.metaboanalyst.ca accessed on 8 February 2023). Differences in the metabolomics profiles of samples were analyzed with unsupervised principal component analysis (PCA) and

supervised partial least squares-discriminant analysis (PLS-DA). Significant variables were defined based on cross-validated *p*-values derived from one-way analysis of variance (ANOVA) with Bonferroni correction for false discovery rates (FDR). Multiple comparisons and post hoc analyses were done using Tukey's Honestly Significant Difference (Tukey's HSD). Fisher LSD test were used to determine which compounds varied significantly between groups at $p < 0.05$. Results were visualized in the form of hierarchical cluster analyses incorporating heat maps. All significant metabolites were mapped into metabolomic pathways based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

CHAPTER III. RESULTS AND DISCUSSION

CHAPTER III. RESULTS AND DISCUSSION

III.1 RESULTS

The soil characteristics of the sampling sites are shown in table XI, Data revealed that all soils were acidic with a pH < 7. Significant differences (P < 0.05) were observed for almost all parameters regardless of the sample soil.

Table XI. Chemical parameters of Prunus africana rhizosphere soil samples

Sample sources	pH	EC (mS/cm)	%C	%N	P (ppm)	K (ppm)	Na (ppm)	Ca (ppm)
Mount Cameroon (MC)	6.8a	0.26a	14.92a	0.57b	37.33a	255.31b	81.49a	4085.16d
Mount Manengouba (MM)	6.12b	0.22a	12.49b	0.86c	35.14b	565.09a	76.68b	4338.70c
Mount Oku (MO)	4.9c	0.12b	8.3c	0.43a	6.04c	555.18a	11.66d	4469.78b

Values followed by the same letter do not differ significantly according to the ANOVA test, $P < 0.05$, n = 3; EC: Electromagnetic Conductivity

III.1.1. Isolated and identified Arbuscular Mycorrhizal Fungi structures from roots and zhizospheric soils of *P. Africana*

Roots and soils samples collected showed different mycorrhyzal characteristics depending on their sampling site. Thus, we obtained more morphotypes in Mount Cameroon site (9) compared to Mount Oku (6) and Mount Manengouba (4). Root staining revealed the presence of mycorrhizal fungus propagules such as vesicles and hyphae inside the roots, characteristic of endomycorrhizal symbiosis (Fig.11)

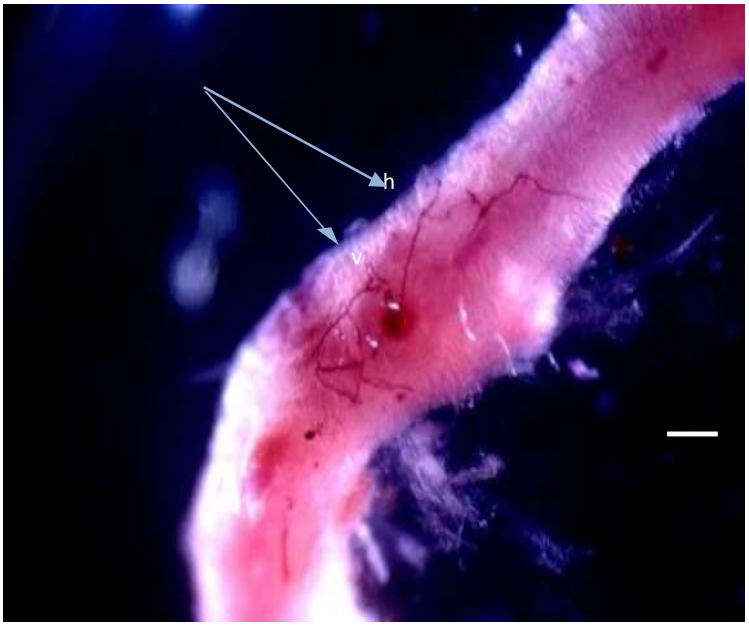


Fig. 11. Mycorrhizal fungi structures (h: Hyphea and v: Vesicles) in roots fragments of *P. africana*

III.1.2. Isolated and identified Arbuscular Mycorrhizal Fungi spores from zhizospheric soils of *P. africana*

Several number of morphotypes (15) were observed and labelled from T1 to T15, with T1 identified to be a ubiquitous morphotype. Table 1 shows the number of morphotypes that were isolated per site, followed by the number of morphotypes that were retained after separation of morphotypes into specific and non-specific strains to *P. africana* (Fig.12). The characterization of the morphotypes specific to *P. africana* allowed the identification of six species of AMF belonging to three genera, namely *Gigaspora*, *Acaulospora* and *Glomus* (Table III and IV).

Table III. *P. africana*-specific Morphotypes per site

Sites morphotype	Mt Ca	Mt Oku	Mt Ma
Morphotypes isolated	9	6	4
Morphotypes retained	2(T1,T7)	3(T1,T10,T11)	3(T1,T14,T15)

T: Morphotypes

The characterization of these morphotypes specific to *P. africana* from Mount Cameroun, Mount Manengouba and Mount Oku, allowed the identification of six species of AMF belonging to three genera, namely *Gigaspora (Gigaspora margarita), Acaulospora* (Acaulospora sp) and *Glomus* (Glomus aggregatum) (Table III).

Table IV. Identification of characterized morphotypes

Morphotypes(T)	**Color**	**Form of spore**	**Diameter of spore**	**Genera /species**
T1	chestnut	Globular	125- 200 µm	*Glomus aggregatum*
T7	white	Globular	45-125 µm	*Gigaspora margarita*
T10	chestnut	Sub-globular	125-250 µm	*Acaulospora* sp.
T11	Yellow-bright	Sub-globular	100-250 µm	*Acaulospora* sp.
T14	Yellow-dusky	Globular	125-250 µm	*Glomus* sp.
T15	Yellow-bright	Globular	45-125 µm	*Glomus* sp.

A total of six species of AMF belonging to three genera, namely *Gigaspora (Gigaspora margarita), Acaulospora* (Acaulospora sp) and *Glomus* (Glomus aggregatum) observed from the three different mounts are presented in Figure 12.

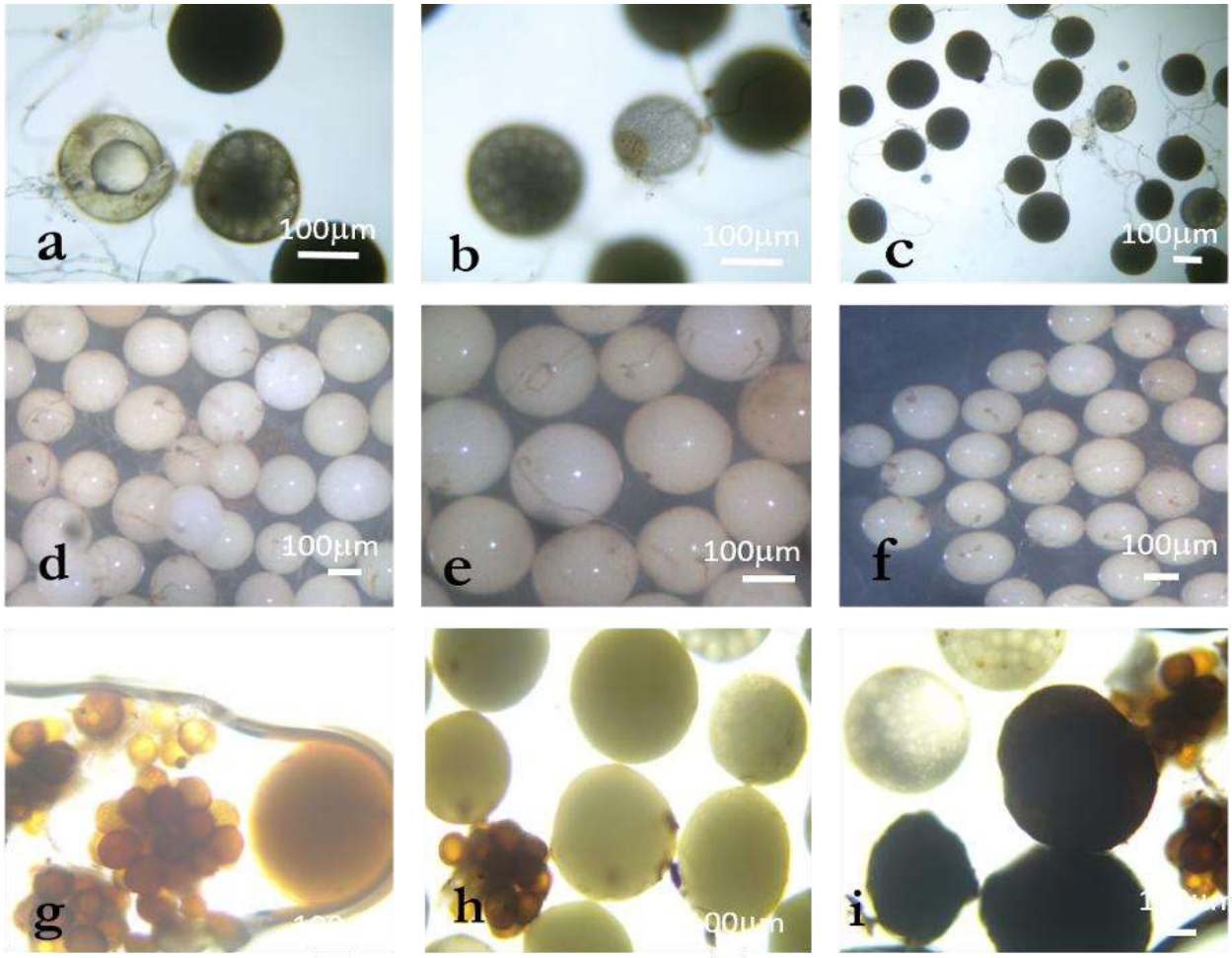
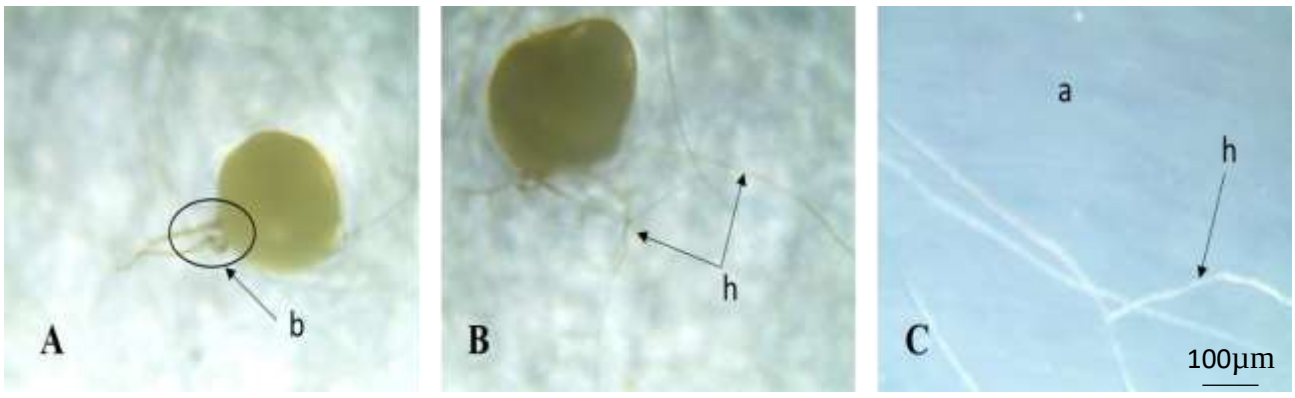


Fig.12. Spores of the identified species of AMF observed; **a, b** and **c**: *Acaulospora*; **d, e** and **f**: *Gigaspora*; **g, h** and **i**: *Glomus*

The pre-germination of AMF spores on agar media carried out after 5 days in the dark allowed us to visualize the effective initiation of spore germination of AM fungi species



identified with visible colonization of the culture medium by the mycelial hyphae

Fig.13. Germination and growth of spores on agar medium. A: b = germination loop of the spore on filter paper; B: h = mycelial filaments from germination of the spore on the filter paper; C: a = agar medium; E=100µm

III.1.2. *Efficiency* of AMF Evaluated on the vegetative development of *Prunus africana*

The extraction of the spores after their pregermination (Fig.13) carried out in 100 g of substrate after purification has demonstrated the effectiveness of their multiplication from one or ten spores, thus making it possible to obtain an inoculum of biofertilizer that does not depend on the number of spores seeded (Table V).

Table V. Number of AMF spores obtained after 6 weeks of multiplication

Morphotypes	1 spore	10 spores
T1	75	145
T7	56	93
T10	18	45
T11	43	82
T14	108	206
T15	33	101

T: morphotypes

The staining of the roots after purification of AMF species allowed us to assess the presence of mycorrhizal fungus propagules inside these roots, testifying to the effectiveness of symbiosis (Fig.14). Growth parameter data were recorded and analyzed to assess the efficacy of the mycorrhizal strains used as biofertilizer. The inoculated plants showed a significant growth difference compared to control treatments (Table VI). Regarding the length of the stem, inoculated plants recorded a higher growth than non-inoculated plants both after one and six weeks. For instance, plants inoculated with morphotype T7 had an average stem length of 19 and 40 cm after one and six weeks, respectively, while control plants recorded an average length of 12 and 24 cm during the same period. For the number of leaves, plants inoculated with morphotype T7 registered an average number of 5 and 9 leaves after one and six weeks respectively, while the control plants registered an average of 3 and 6 during the same period. For the length of leaves, plants inoculated with morphotype T7 had an average length of 14 and 30 cm after one and six weeks respectively, while the control plants registered an average of 11 and 28cm during the same period. However, the growth promotion did not depend on the number of spores inoculated.

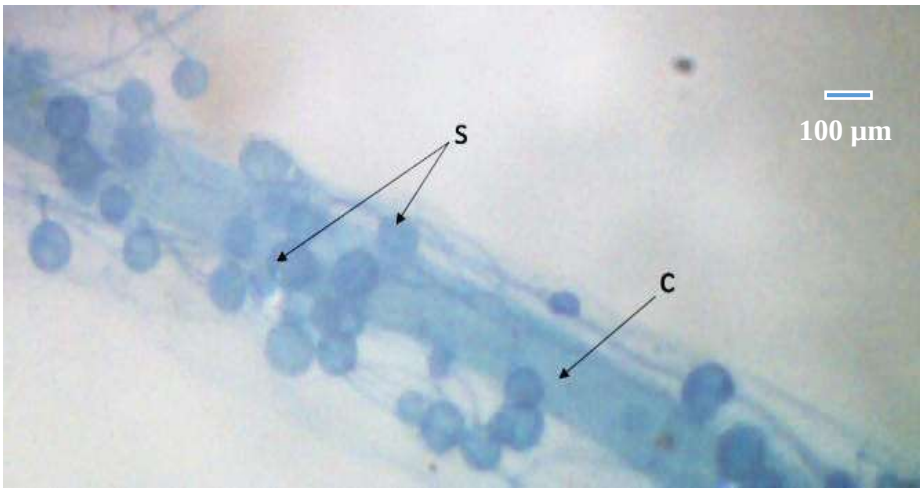


Fig. 14. Colored roots of *Sorghum bicolor* plants after trapping (s = Intra-root spores, c= root cortex)

Table VI. Summary of the influence of treatments (AMF species) on the growth parameters of P. africana cuttings

	LS		NL		LF	
Morphotype	S1	S6	S1	S6	S1	S6
Control	12.15±1.56d	23.83±23.83c	3.00±00c	5.50±0.84d	11.38±1.90c	27.72±2.77a
T10	15.75±3.3b	20.7±2.44e	3.50±0.84c	5.67±0.82d	9.92±2.40d	28.67±5.87a
T1	14.18±1.4c	19.50±3.02d	3.17±0.41c	4.33±0.52c	8.28±1.97d	23.28±3.44b
T14	15.38±2.2b	38.67±5.57a	3.17±0.41c	5.17±0.75d	11.42±1.36c	24.50±2.53b
T7	18.95±3.1b	40.19±9.85a	4.50±0.55b	8.67±6.67a	13.68±1.42b	29.58±3.97a
T11	17.02±3.24a	32.50±8.02b	3.17±0.41c	6.00±0.63b	11.20±2.80c	28.15±1.63a
T15	17.28±1.37a	26.50±3.89b	3.33±0.52c	6.33±0.52b	12.17±2.71c	27.15±3.36a

Caption: LS = length of the stem in cm, NL = number of leaves, LF = length of the leaf in cm, S1 = week1 and S6 = week6.

About 70 % of cuttings were rooted with 10 % having shoots. For the root density evaluation, there was a significant difference in the root promotion effects (Table VII). On the 25th post-inoculation day, the most effective mycorrhizal strain was T7 corresponding to the species of *Gigaspora margarita* with an average root number of 35 per cutting (Fig.15). The low performing strain was T10 corresponding to the species of *Acaulospora sp*. with an average of 10.78 roots per cutting. During the experimentation, the negative control (no inoculation) had the smallest number of roots (4) and the positive control (AIB) had an average root number of 10 (Table VII).

Table VII. Avearge number of roots produced by cuttings per treatment groups

Treatment	Number of roots
Control (negative)	4.00 ± 1.80^{a}
AIB (positive control)	10.00 ± 1.88^{de}
T1 (*Glomus aggregatum*)	24.71 ± 6.88^{b}
T7 (*Gigaspora margarita*)	35.00 ± 10.57^{f}
T10 (*Acaulospa* sp.)	10.78 ± 3.72^{de}
T11 (*Acaulospora* sp.)	17.43 ± 4.29^{c}
T14 (*Glomus* sp.)	20.36 ± 4.52^{bc}
T15 (*Glomus* sp.)	15.00 ± 3.60^{cd}

Values followed by the same letter do not differ significantly according to the ANOVA test, $P < 0.05$

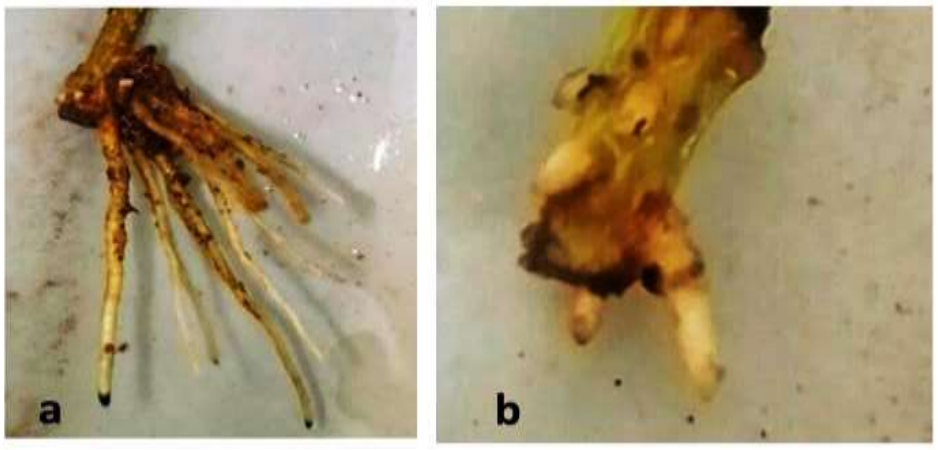


Fig.15. *P. africana* cuttings, a: root system of the inoculated cuttings; b: root system of un-inoculated cuttings after 21 days

III.1.3. Effect of AMF inoculum on growth parameters

III.1.3.1. Mycorrhizal colonization parameters

AMF colonized all samples examined, except the non-inoculated control plants. In the colonized roots, vesicles, internal and external hyphae were observed. Significant differences were only observed in AMF frequency with 39 % and 64 % in arbuscules abundance in a root

fragment (AARF), with 10.5 % and 29.6 % respectively in Innoculated and Non Innoculate substrate.

In General, AMF colonization frequency was significantly higher in inoculated treatments with 72 %, 70 %, 68 %, and 51 % respectively for E1, E2, E3 (E1: *Gigaspora margarita*), E2 (Glomus sp.), E3 (*Acaulospoa* sp.) compared to non-sterilized soil (NS) at 38 % and the non-inoculated control treatments had no AMF infections

III.1.3.2. Growth parameters of *Prunus africana* cuttings

It was observed that shoots from leafy stem cuttings from inoculated soil had higher growth performance compared to those from non-inoculated except for the leaf surface area parameter irrespective of treatment when considering the number of leaves, shoot height (cm), leaf surface area (cm^2), shoot weight (g) and total weight (g). ANOVA and Tukey's test showed a significant difference at $p \leq 0.05$ (Table VIII).

Table VIII. Mean value of growth parameters of vegetatively propagated *Prunus africana*.

Growth parameters	Mean ± Std. Error	
	Inoculated Soil	Non-inoculated soil
Number of leaves	3.782a ± 0.075	1.873b ± 0.075
Height (cm)	5.384a ± 0.260	3.745b ± 0.260
Leaf surface area (cm^2)	4.403a ± 0.558	4.831b ± 0.558
Shoot weight (g)	0.263b ± 0.025	0.132b ± 0.025
Total weight (g)	0.451b ± 0.037	0.237b ± 0.037

Mean values followed by the same letter are not significantly different at $p \leq 0.05$.

The different treatments did not have any significant effect on shoot weight ($p = 0.64$; $p \geq 0.05$) and total weight ($p = 0.77$; $p \geq 0.05$). However, a variable growth response to inoculation was also observed. Cuttings inoculated with E1 (*Gigaspora margarita*), E2 (*Glomus sp*.), E3 (*Acaulospoa* sp.) had significantly higher growth (number of leaves and height) respectively than the non-inoculated control treatment. Also, cuttings inoculated with AMF had lower leaf surface area than non-inoculated treatment. There was no significant difference in shoot and total dry weight among the treatments.

III.1.4. Effect of AMF inoculum on phytochemical content

III.1.4.1. Phytochemical screening

Table IX shows that saponin was only present in cuttings grown on soil inoculated with AMF inoculum from E1 and E2 and absent in cuttings on non-inoculated soil, excepted in the treatment NS. Similar observations were also made for anthraquinones in the cuttings on both substrates, excepted for the treatment NS where this phytochemical was present in cuttings from inoculated and non-inoculated soil. On the other hand, terpenoids and alkaloid were present in all cuttings from both soils irrespective of the treatment (Table IX).

Table IX. Phytochemical screening in vegetatively propagated *Prunus africana* seedlings inoculated with AMF spores.

	Saponins		Anthraquinones		Terpenoids		Alkaloids	
Treatment	Inoculated soil	Non-inoculated soil	Inoculated soil	Non-inoculated soil	Inoculated soil	Non-inoculated soil	Inoculated soil	Non-inoculated soil
E1	+	-	+	-	++	++	++	++
E2	+	-	+	-	+	+	++	++
E3	-	-	-	-	++	+	++	++
NS	-	+	++	++	++	+	++	++
Con	-	-	+	+	+	++	++	++

Quantities are scored as follows: absent (-), poor (+), moderate (++), and high (+++). Con: control -CMA

In contrast to the growth performance, vegetatively propagated *P. africana* on non-inoculated soil showed higher amounts of phytochemical contents (tannins, flavonoids, and phenols) than cuttings from inoculated soil (Table X).

Table X. Mean value of phytochemicals of vegetatively propagated *Prunus africana* per source.

	Mean ± Std. Error	
Phytochemical parameters	Non-Inoculated soil	Inoculated soil
Tannin (mg/100g)	191.892d ± 5.789	392.905a ± 5.789
Flavonoids (mg/100g)	173.341c ± 2.248	226.385b ± 2.548
Total phenol (mg/100g)	60.229e ± 0.834	126.606a ± 0.834

Mean values followed by the same letter are not significantly different at $p \leq 0.05$.

On the other hand, cuttings inoculated with inoculum E1 (Gigaspora *margarita*), produced significantly higher tannin content, while total phenol and flavonoid content were significantly high with inoculum E3 (*Acaulospora* sp.), compared to other inoculum treatments. However, there was no significant difference ($P > 0.05$) in flavonoids content, among E1, E2, E3 and non-inoculated control. A similar observation was made on total phenol content, with no significant differences between E3 and E1 inoculum.

III.1.5. Metabolite Analysis of *Prunus africana* from Three Locations in Cameroon

III.1.5.1. Soil chemical properties of samples

The soil characteristics of the sampling sites are shown in table XI, Data revealed that all soils were acidic with a pH < 7. Significant differences (P < 0.05) were observed for almost all parameters regardless of the sample soil.

Table XI. Chemical parameters of *Prunus africana* rhizosphere soil samples

Sample sources	pH	EC (mS/cm)	%C	%N	P (ppm)	K (ppm)	Na (ppm)	Ca (ppm)
Mount Cameroon (MC)	6.8a	0.26a	14.92a	0.57b	37.33a	255.31b	81.49a	4085.16d
Mount Manengouba (MM)	6.12b	0.22a	12.49b	0.86c	35.14b	565.09a	76.68b	4338.70c
Mount Oku (MO)	4.9c	0.12b	8.3c	0.43a	6.04c	555.18a	11.66d	4469.78b

Values followed by the same letter do not differ significantly according to the ANOVA test, $P < 0.05$,

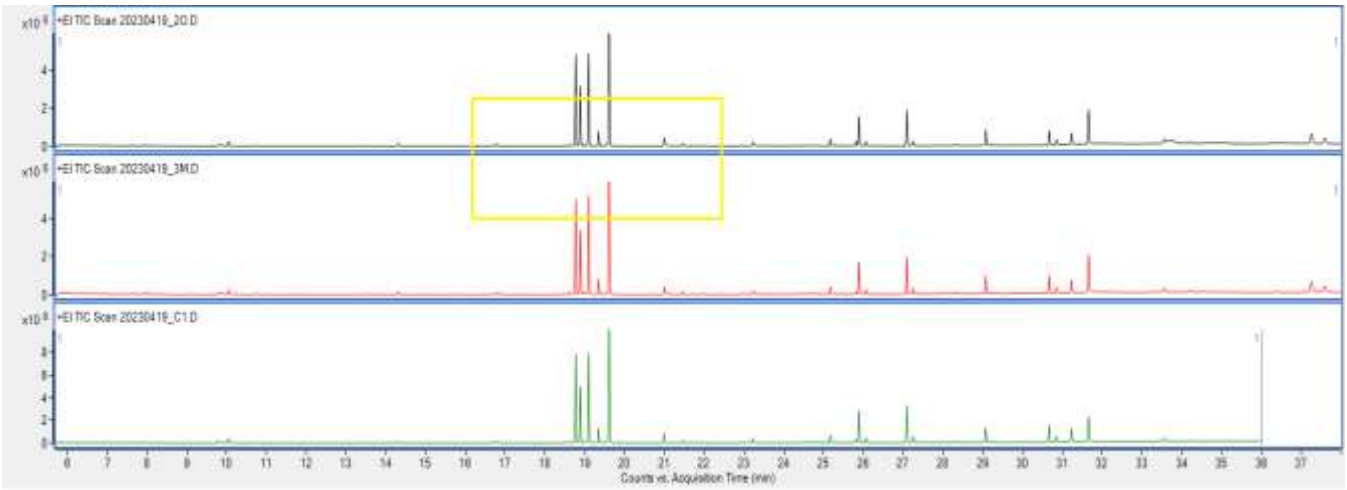
n = 3; EC: Electromagnetic Conductivity

III.1.5.2. First experimentation: Chromatogram and spectrum analysis

Several compounds were identified from *P. africana* metabolites based on location from the three different extraction methods. The ratio of the compounds was different within each method.

For the different locations studied independently from the extraction method used, the ratio between the components within each method is also quite different.

The main compound used against prostate cancer and hyperplasia, **beta-sitosterol**, was found in all three locations in deferent concentration and from the three different extraction methods.



Peack = mz/rt, x-axis= rt Area= volt/min= relative respons

Fig.17. Total Ion Chromatography (TIC) of *P. africana* metabolite profiling from three locations in Cameroon (O, M and C)

III.1.5.2.1. *Prunus africana* Metabolites profile based on three locations in Cameroon

The chromatogram analysis of sugars and few primary metabolites (Yellow rectangle in Fig.16 and Fig.17) from the three locations showed that the Mount Cameroon population exhibited higher concentrations of metabolites.

Triterpenes such as Phytosterol (beta-sitosterol) (Fig.18), which have significant pharmaceutical roles, were generally detected in all samples" from all extraction methods (for example, see red rectangle in Fig.19).

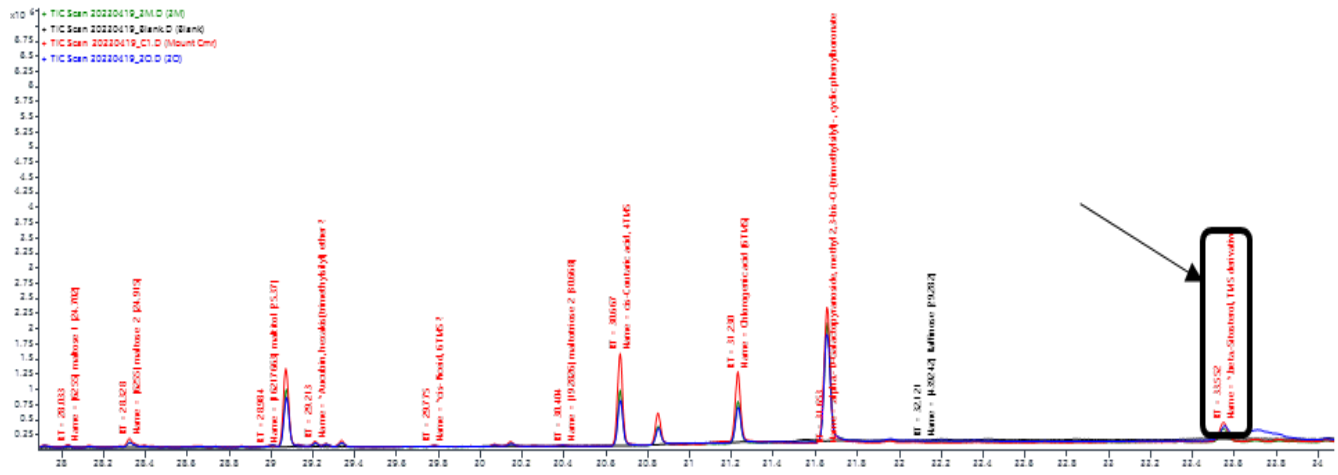


Fig. 18 Total Ion Chromatogram of metabolites exudates of P. africana from the Mount Oku region after MTBE extraction

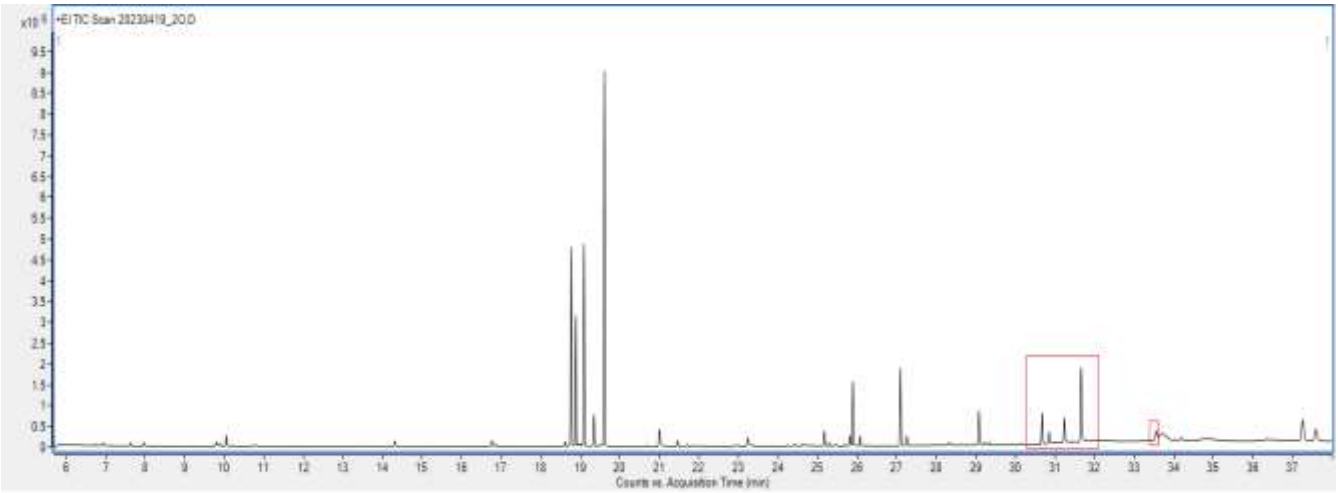


Fig. 19. Total Ion Chromatography (TIC) of P. africana metabolite profiling with beta-sitosterol targeted (outlined in the rectangle) from three locations in Cameroon after MTBE extraction.

We also find in all samples, significant amounts of bioactive constituents (phenolic compounds) like **Chlorogenic acid and quinic acid** (Fig.20 and 21), which are, according to the *South african National Cancer Institute (NCI drug dictionary)*, polyphenols with potential antioxidant and chemopreventive activities. Chlorogenic acid scavenges free radicals, which inhibits DNA damage and may protect against the induction of carcinogenesis. In addition, this agent may upregulate the expression of genes involved in the activation of the immune system and enhance activation and proliferation of cytotoxic T-lymphocytes, macrophages, and natural killer cells. Chlorogenic acid also inhibits the activity of matrix metalloproteinases.

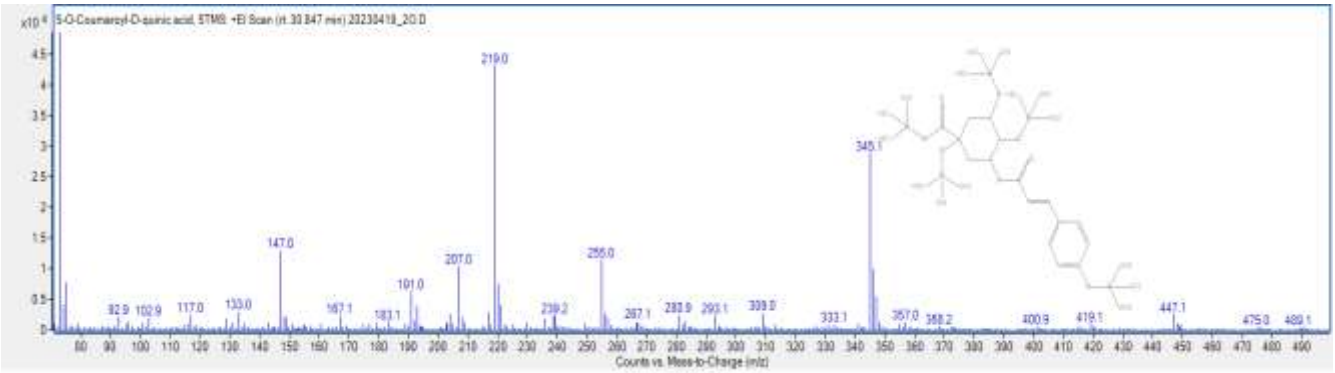


Fig.20. 5-O-Coumaroyl-D-quinic acid spectrum from *P. africana* population 2O

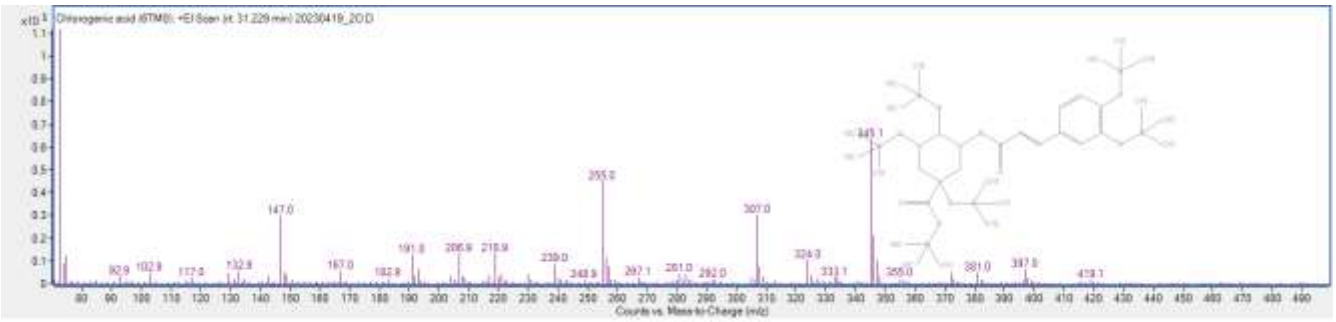


Fig.21. Chlorogenic acid spectrum from *P. africana* population 2O

III.1.5.3. Second experimentation: Different Extraction Methods Comparison

Ethyl acetate +methanol helps us to have more significant bioactive constituents than the other methods (Table XII).

From the three location, the comparison of the concentration of the six most efficient antioxidants found in *Prunus* in Cameroon shows that beta-sitosterol, the actual most significant metabolite used against prostate cancer and hyperplasia/hypertrophia, is most concentrated in the trees of Mount Manengouba forest, whereas the new found antioxydant (chologenic acid, epicatechin, and quinic acid) are most concentrated in trees of Mount Cameroon forest (Table XII).

	1C MTBE	2O MTBE	3M MTBE
epicatechin	114945.01a	106635.33b	120733.96a
5-O-Coumaroyl-D-quinic acid	2022.54c	1515.32c	1535.30c

Table XII. Concentration comparison of the six most significant P. afrinana bioactive constituent from the different locations with the same extraction method

4-O-Coumaroyl-D-quinic acid	129939.40a	93517.11c	109053.82a
3-O-Coumaroyl-D-quinic acid	34830.93d	25959.56d	28850.52c
Chlorogenic acid	50385.04c	37140.00d	41810.01b
beta-sitosterol	13280.69b	14085.42c	14215.66c

MTBE: Methyl Tert-Butyl Ether; **EM**: Ethyl acetate + Methanol; **E**: Ethyl acetate

The concentration comparison of the six most efficient bioactive metabolites in *P. africana* with different extraction methods shows that, among the three tested methods, the Ethyl acetate+ Methanol method is best for the above-mentioned compounds (it has the most constant ratio (most consistent measurement in the repeats) compared to the others) (Table XIII).

Table XIII. Concentration comparison of the six most significant bioactive compounds in P. africana from one location (ME) with three different extraction methods

	3M MTBE	3M EM	3M E
epicatechin	120733.96a	52848.18b	19260.62d
5-O-Coumaroyl-D-quinic acid	1535.30b	1377.89c	0.00
4-O-Coumaroyl-D-quinic acid	109053.82a	76960.18b	2769.46d
3-O-Coumaroyl-D-quinic acid	28850.52b	19553.30d	508.70c
Chlorogenic acid	41810.01b	26882.66c	950.78d
beta-sitosterol	14215.66a	7262.43c	84678.52b

III.1.5.4. Final experimenttion

III.1.5.4.1. Metabolite Compounds Analysis Methods

The average content from three repeats and standard deviations of the most efficient bioactive compounds of our study show that there is a slight variation of concentrations between locations.

The difference of concentration between locations for the beta-sitosterol it is not high but significant (Fig.22). We can observe that *P. africana* plants from Mount Cameroon have the lowest concentration of almost total components. There is a variation of concentration of the main sugars, fructose and sucrose, between the three locations added to appreciate the results. The general view has shown that trees from the Mount Oku area may have the highest concentrations of bioactive contents of *P. africana* among the three targeted locations in Cameroon (Fig.22).

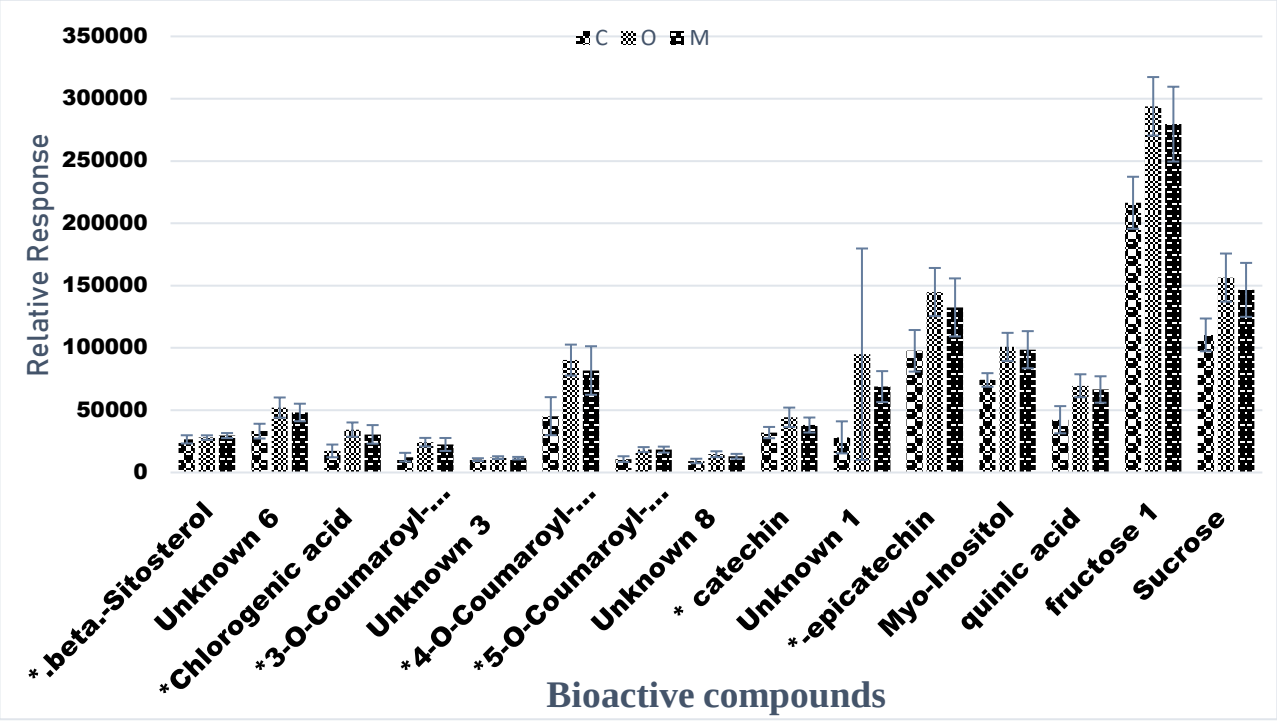


Fig.22. Average and standard deviation concentration of the most efficients bioactive contents of Prunus africana in three locations in Cameroon.

beta-sitosterol, the most efficient bioactive content known in *Prunus africana* has been found in all the locations with diferent concentration. Fig.23 is showing result from trees from Mount Oku).

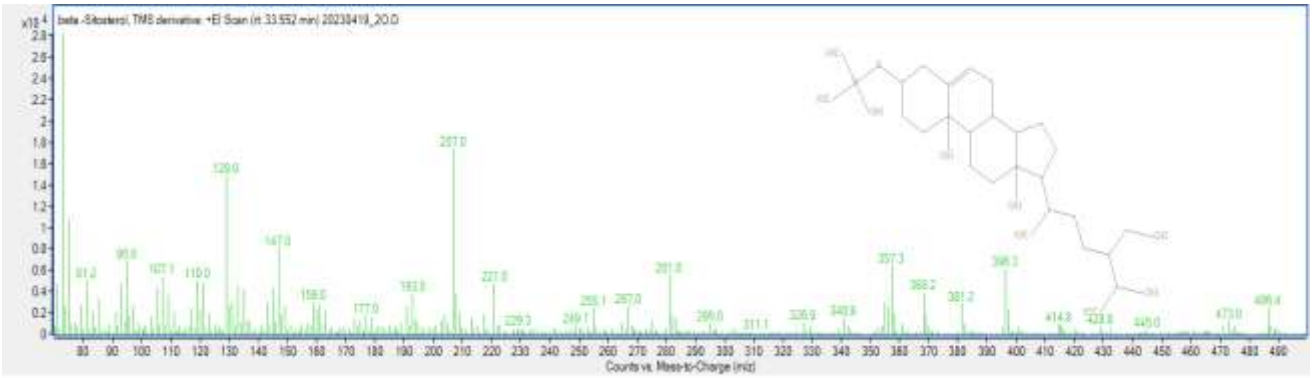


Fig.23. beta-sitosterol spectrum obtained from the Total Ion Chromatography in the three *Prunus* populations in the Mount Oku forest.

Trees from Mount Manengouba forest has the higher concentration of beta-sitosterol among the three locations (Fig.24).

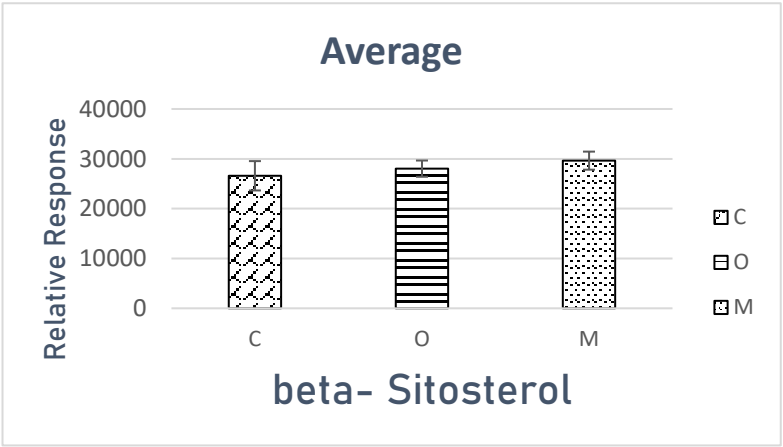


Fig.24. Average and standart deviation concentration of beta-sitosterone from Prunus africana in three locations in Cameroon. C: Mt Cameroon; O: Mt Oku; M: Mt Manengouba.

III.1.5.4.2. Statistical Analysis of the Metabolite Profiling Data

Metabolite profiling is a powerful tool that has contributed to the understanding of plant physiology, including phenotypic differences, gene annotations, metabolite regulation, and characterization of stress responses. Metabolic profiling of *Prunus africana* has been accomplished using GC-MS techniques. In the present research one study statistical analysis was used to investigate modifications to metabolite pathways. However, metabolic profiling of *P. africana* was never carried out. Data normalization results, performed with MetaboAnalyst assistance prior statistical analysis, are shown on Figure 25. PCA, PCA-DA an PLS-DA were applied to normalized GC-MS data from the three sample groups in order to identify possible variations in the metabolite composition and concentration between and within samples. Out of the 18 principal components, the first three (PC1, PC2 and PC3) were responsible for major

variation (84.8%) in study groups (Fig. 25). Score plots clearly demonstrated that all three groups of samples, MG, OG, and MG cluster into three very distinct groups (Fig. 25). Further PCA-Discriminant Analysis resulted in three components, which contributed equally to 100% of variation (Fig. 31). In the present study we assumed that each tree corresponds to a particular genotype (G).

From PCA results, there is clear separation between the three populations (with MG anOG are completely overlapping each other) few of the genotypes sharing bioactive compounds within different populations (Fig.25).

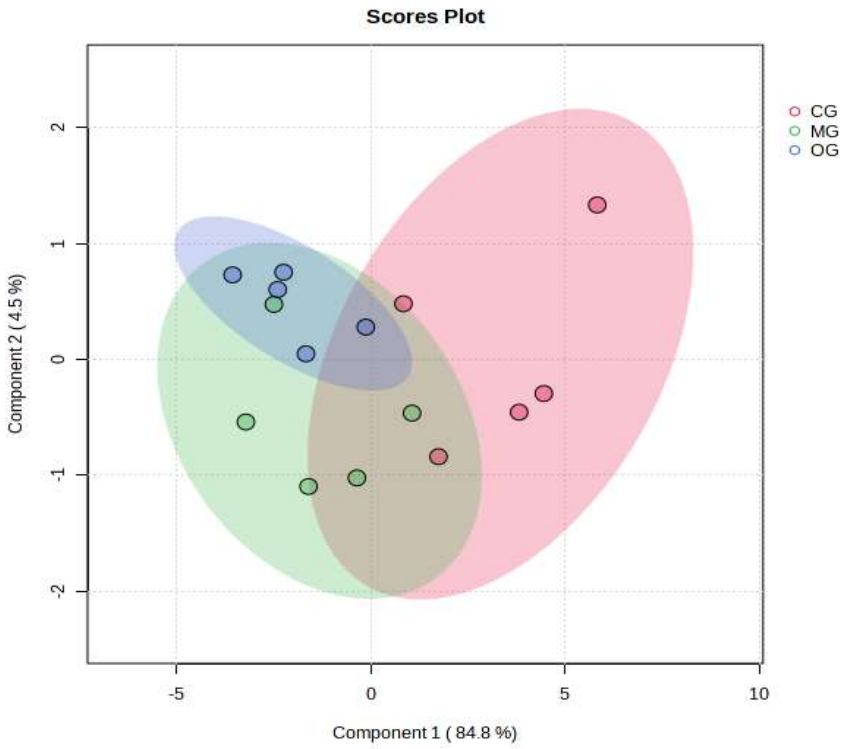


Fig.25. PCA scores plot of bioactive compound from the total genotypes of the three locations in Cameroon.

The Normalized concentration box plot of the top three bioactive compounds in *Prunus* in terms of their pharmaceutical effect has shown that the sterols are more highly concentrated in the plants of the Manengouba forest. (Fig.26,27,28). From figures 26,27,28, MG (Mount Manengouba samples) shows a high accumulation of sterols followed by OG and lower levels were observed in CG population as indicated in the Box Plot.

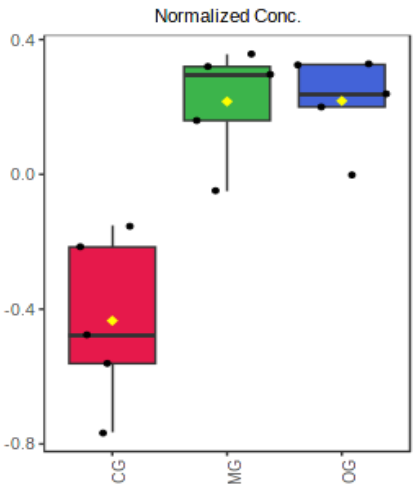


Fig.26. 5-O-Coumaroyl-D-quinicacid 5TMS derivative, normalized concentration Box Plot

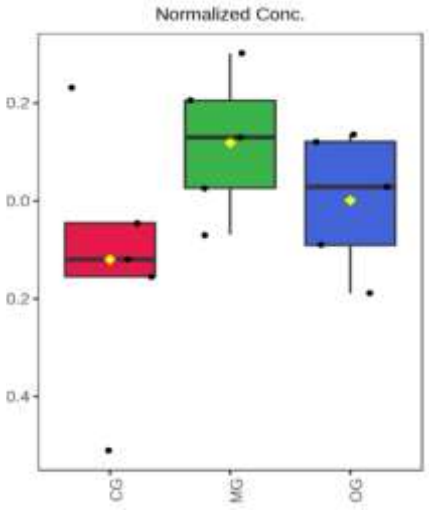


Fig.27. beta-Sitosterol TMS derivative, normalized concentration Box Plot

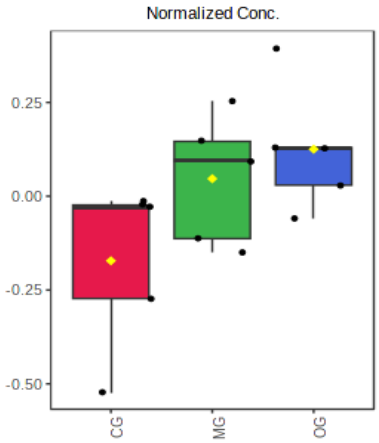


Fig.28. Unknown3: beta-Sitosterone? 441 147 159 derivative Normalized Concentration Box Plot

From the heat map of location, most of the bioactive compounds are accumulated in class OG with beta- sisterol highest accumulation in MG and less abundance of compounds in CG. (Fig.29**).**

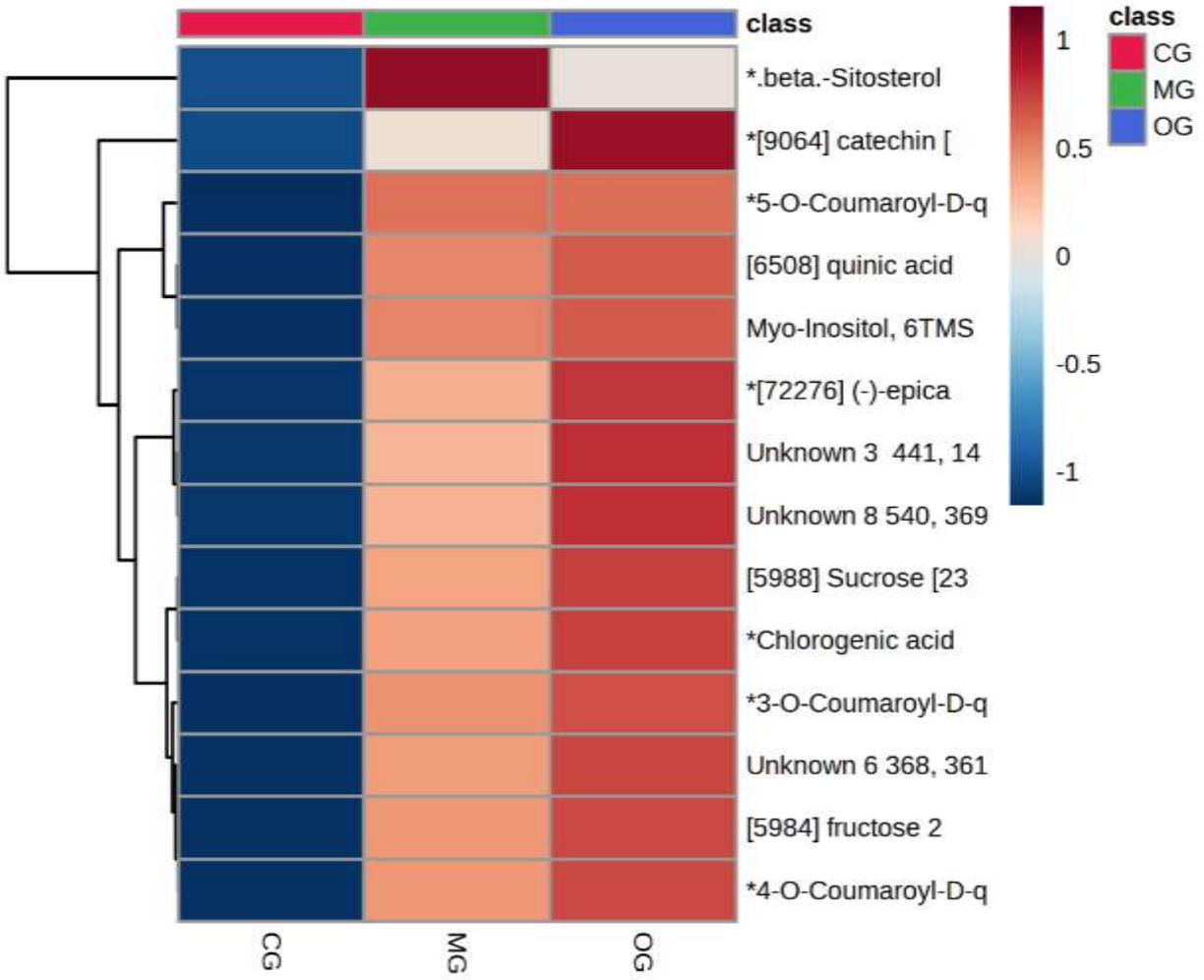


Fig.29. Heat Map of the accumulation of bioactive compounds within trees from three locations in Cameroon. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under this study.

The Heat Map of the accumulation of bioactive compounds within different Genotypes in the three locations (Fig. 30) clearly illustrated the differences of sterols and other bioactive compound concentration within different genotypes across the three populations with genotype M2 showing highest accumulatiom of sterols followed by C4 and least accumulation in C1 (Fig.30)

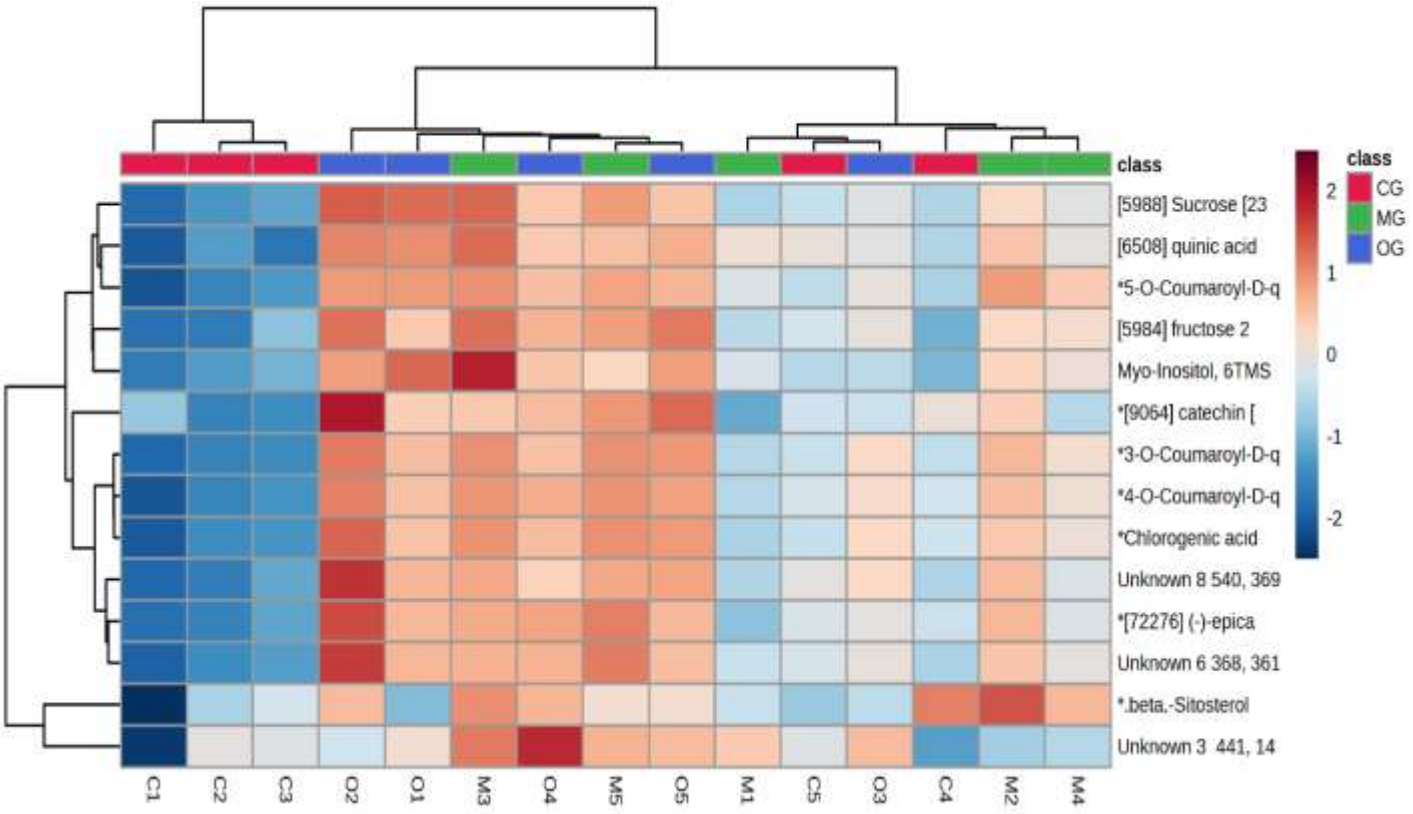


Fig.30. Heat Map of the accumulation of bioactive compounds within different Genotypes in three locations (class) in Cameroon. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under this study.

The list of 15 the most important features is shown on Figure 32 alone with the indication of relative metabolite concentrations in each group. When groups compared side by side, there are metabolites that having larger score in the particular pare (Fig. 31)

There was a clear separation in the production of beta sitosterol from specific populations from other compounds as showned in the PCA loading results (Fig.31) and this was clealy showned by the VIP scores figure shown below where MG showed a high accumulation of the compound compared to other populations (Fig.32).

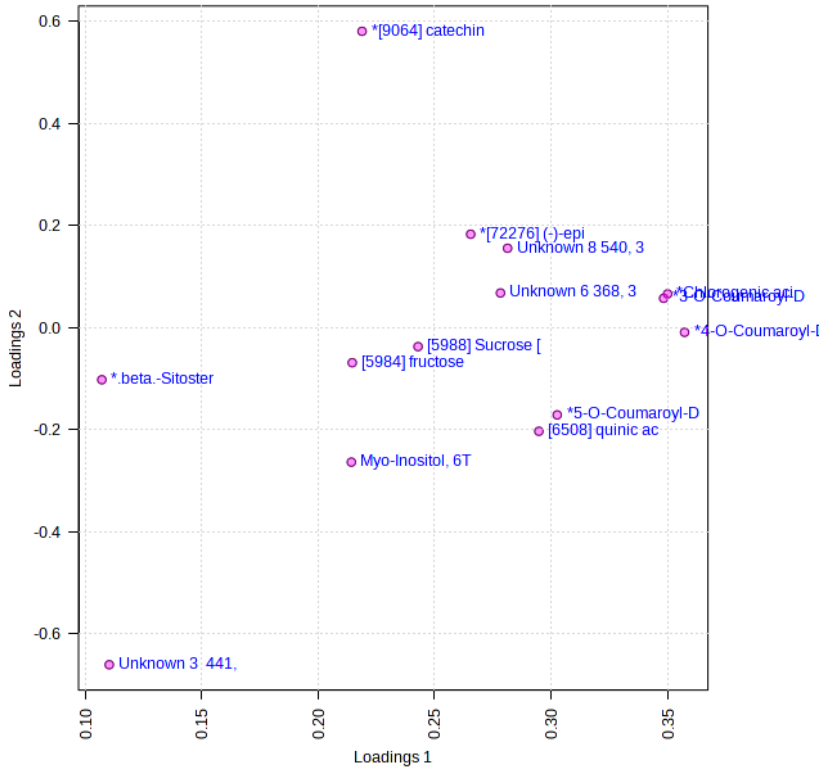


Fig.31. PCA loadings of Important Features (bioactive contents) identified by PLS-DA (MetaboAnalyst).

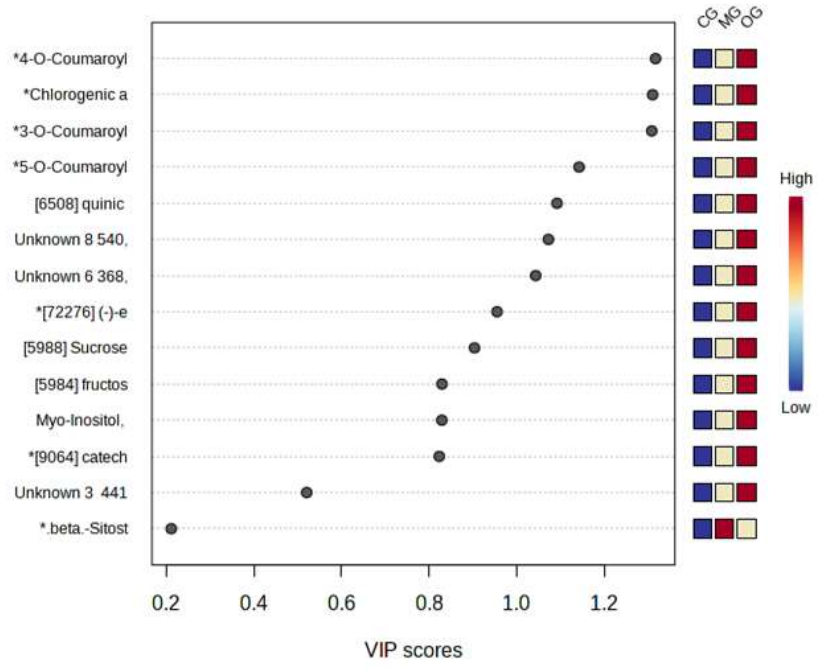


Fig.32. Important features (candidates to metabolic markers) identified by PLS-DA (MetaboAnalyst). The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under current study

A one-way anova is showing that, among the bioactive compounds analysed, there are twelve significant features for the total group under current study (Fig.33 and Table XIV)

Table XIV. Significant variables defined in Prunus africana in Cameroon based on cross-validated p-values derived from one-way analysis of variance (ANOVA) with Bonferroni correction for false

	f.value	p.value	Log10(P)	FDR
5-O-Coumaroyl-D-quinic acid, 5TMS	19.379	0.000175	3.7579	0.002445
3-O-Coumaroyl-D-quinic acid, 5TMS	13.808	0.000772	3.1122	0.003245
fructose 2	12.907	0.001021	2.9908	0.003245
beta-sitosterol	11.725	0.001505	2.8226	0.003245
Chlorogenic acid	11.719	0.001507	2.8218	0.003245
quinic acid	11.587	0.001577	2.8023	0.003245
4-O-Coumaroyl-D-quinic acid, 5TMS	11.503	0.001623	2.7898	0.003245
Myo-Inositol	10.814	0.002065	2.6852	0.003473
8 540, 369, 271, beta-sitosterone	10.596	0.002233	2.6511	0.003473
Sucrose	9.928	0.002857	2.5441	0.004
epicatechin	8.108	0.005917	2.2279	0.007531
catechin	4.9669	0.026817	1.5716	0.031286

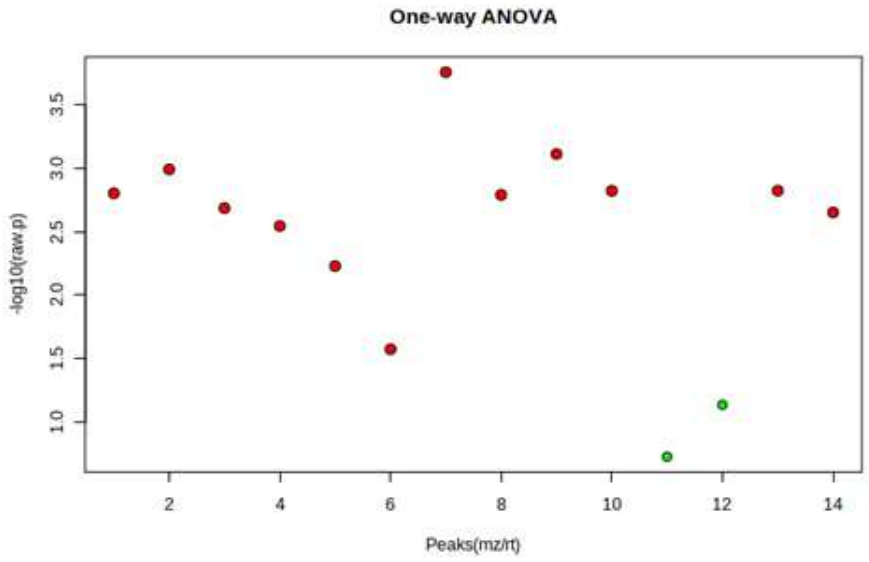


Fig. 33**.** Significant variables defined in *Prunus africana* in Cameroon based on cross-validated p-values derived from one-way analysis of variance

III.1.5.4. Metabolic pathway analysis

Pathway analysis performed with MetaboAnalysis assistance revealed a number of impacted metabolic pathways. The overall pathway impact picture is illustrated in figure 34. This data presented in greater details in Table XV where number of hits and p values are depicted. The most impacted metabolic appears to be Stilbenoid, diarylheptanoid and gingerol metabolism; Flavonoid, Phenylpropanoid and Galactose metabolism. However, impact consequences are not straightforward and metabolic flux is involved. Changes in flux can be illustrated with Figure 34 where some metabolites do not follow the pattern of key metabolite. This indicates flux redistribution upon potential influence of mycorrhizae and environment. This confirms a high complexity and sensitivity of metabolic networks exploited by plant in order to survive and adjust to environmental challenges.

Table XV. Metabolic Pathway Impact table generated by MetaboAnalyst from *Prunus africana* in Cameroon

Pathway names	**Total**	**Expected**	**Hits**	**Raw p**	**Holm p**	**Holm adjust**	**FDR**	**Impact**
Stilbenoid, and gingerol biosynthesis	8	0.0323	2	0.0003	3.422	0.036296	0.0268	0.264
Flavonoid biosynthesis	47	0.1901	3	0.0005	3.252	0.05308	0.0268	0.049
Galactose metabolism	27	0.1092	2	0.0045	2.339	0.43046	0.1465	0.042
Phenylpropanoid biosynthesis	46	0.1861	2	0.0130	1.884	1	0.3130	0.0582
Ascorbate and aldarate metabolism	18	0.0728	1	0.0707	1.150	1	1	0
Starch and sucrose metabolism	22	0.0890	1	0.0859	1.065	1	1	0.0889
Phosphatidylinositol signaling system	26	0.1051	1	0.1008	0.996	1	1	0.0328
Inositol phosphate metabolism	28	0.1132	1	0.1086	0.965	1	1	0.1025
Steroid biosynthesis	45	0.1820	1	0.1690	0.771	1	1	0.0072

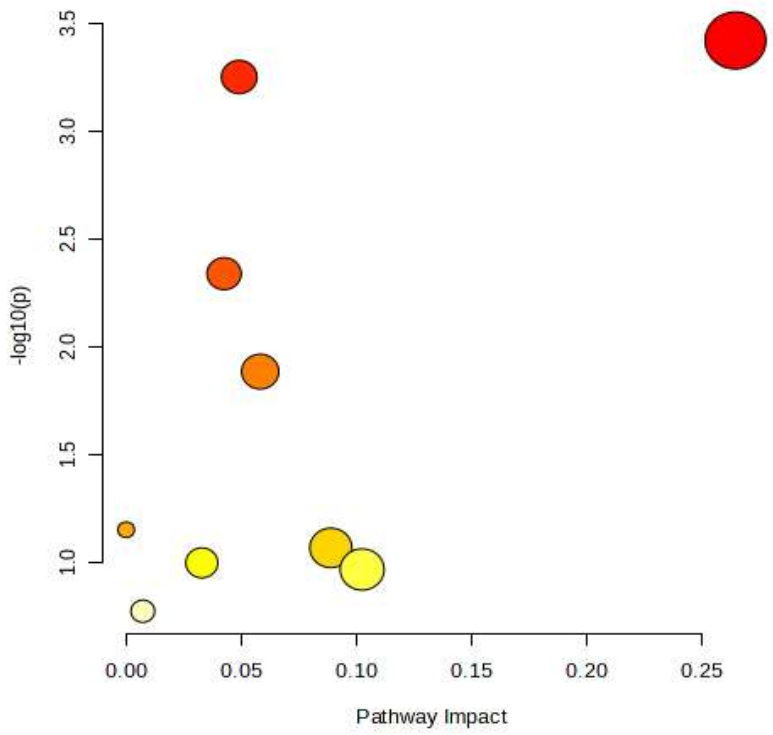


Fig. 34. Metabolic Pathway Impact enrichment analysis of metabolites varying in the metabolome of *Prunus africana* in Cameroon.

III.1.5.5. Metabolism History: Steroid Biosynthesis

Changes in flux can be illustrated with Figure 3.25 where some metabolites do not follow the pattern of key metabolite and we could clearly follow the steroid biosynthesis, especially of phytosterols (beta-sitosterole and beta-sitosterone).

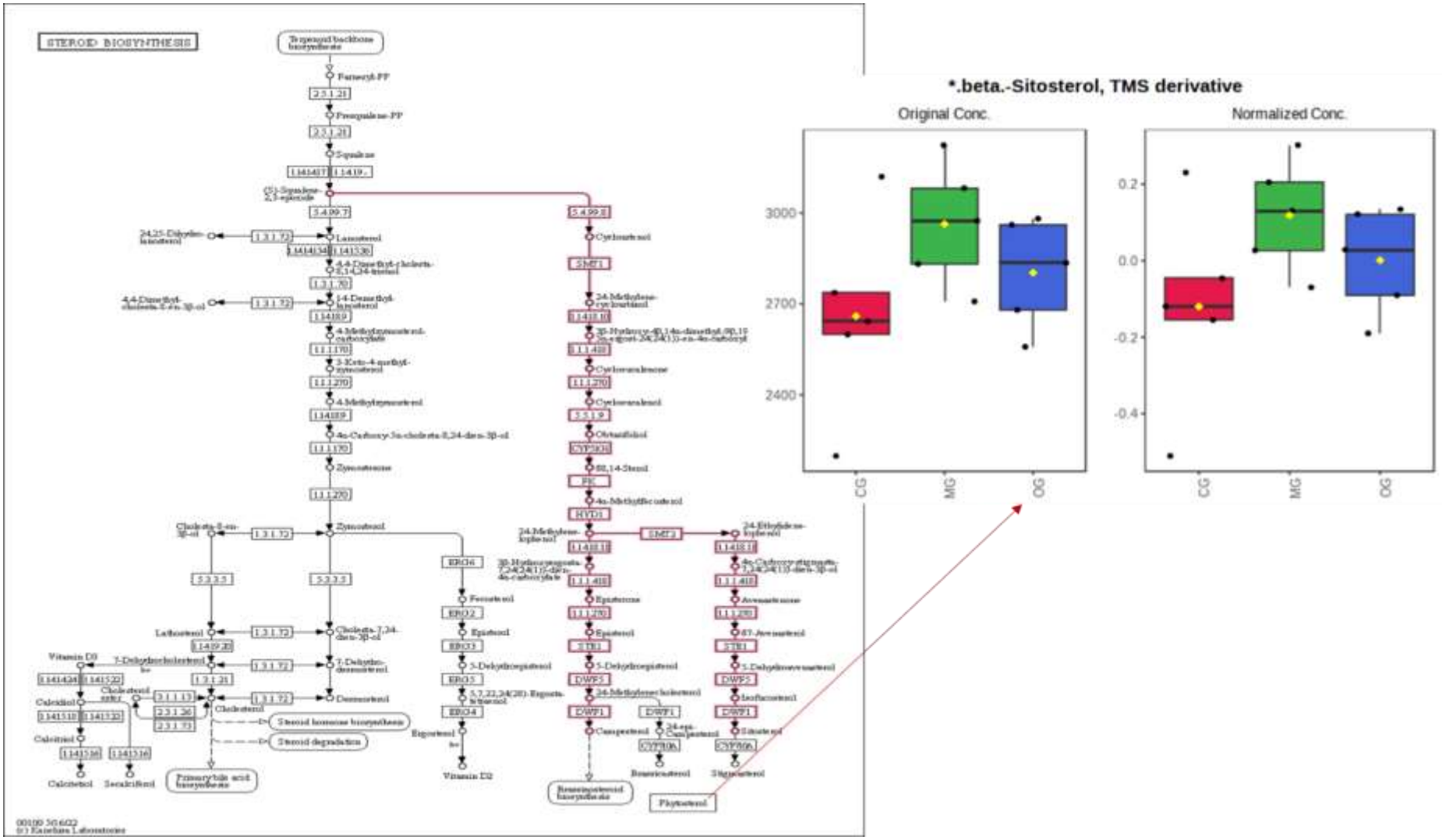


Fig.35: Plant cholesterol biosynthetic pathway overlaps with phytosterol metabolism: Steroid biosynthesis

III.1.6. Correlation between parameters

Based on the results from GC MS, we analysed possible correlations using the isolated AMF that were characterized. As the table XVI shows, we observed a positive correlation between the *Glomus sp* and sterols, as well as their negative correlation with *Gigaspora* and *Acaulospora spss.* This confirmes the findings from the GC results showing a high accumulation of beta-sitosterol in the population from Mount Manengouba compared to the rest of the populations.

Table XVI. Correlation table between beta-sitosterol and the native AMF species of *Prunus africana* in.

	Gigaspora spp	*Acaulospora Spp*	*Glomus spp*	*.beta.-Sitosterol*
Gigaspora spp	1			
Acaulospora Spp	-0.5	1		
Glomus spp	-0.5	-0.5	1	
beta-Sitosterol	-0.849347825	-0.032443364	0.881791189	1

III.2. DISCUSSION

III.2.1. Characterization and identification of Arbuscular Mycorrhizal Fungi

The number of morphotypes recorded in the Mount Cameroon (9), Mount Oku (6) and Mount Manengouba (4) sites reflects the diversity of these sites. These results did not match with those obtained by Nzweundji and *al*. (2015) in the same sites, which at that time had fewer morphotypes (6). This difference could come from the fact that the fungi present in these sites have adapted and evolved. Over time, the environment would have become favorable to accommodate new species according to the variations in soil composition influenced by the growth of different plant species (Ngonkeu et *al*., 2009).

The morphological identification carried out in this study made it possible to determine the different families of mycorrhizal fungi colonizing the roots of *P. africana* which were found to be: *Gigaspora margarita (*Gigasporaceae), *Acaulospora* sp (Acaulosporaceae) and *Glomus aggregatum* (Glomeracea). These species were characterized using morphological characters: the shape, size, color and external appearance of the spore. The morphological identification has many limitations (Schüßle et *al.*, 2001; Zeramdini et *al*., 2009) but the results obtained were in line with those of Tchiechoua et *al.* (2012). Growth of plants inoculated with one spore and that with several spores is in accordance with the work by Jansa et *al.* (2008) and Olfat and Jalil (2012) on root infections and multiplication of spores. This could be explained by the fact that the establishment of a mycorrhizal symbiosis depends on the quality of the information transmitted by the partners present, the information being transmitted by strigolactone for the plant to induce a mycfoctor response in the Fungi. This exchange is made independently of the amount of AM fungi in the medium or substrate (Jalil and Olfat 2012).

To ensure their symbiotic efficiency, the Arbuscular Mycorrhizal fungi spores identified were previously pre-germinated on an agar medium. This technique made it possible to have almost all spores germinated with young mycelial filaments active in the formation of mycorrhizal associations. This result is similar to that obtained by Ngonkeu et *al*., (2009), in the same culture substrate.

In this study, no non-mycorrhized roots were observed in trees of *P. africana* from native montane volcanic habitats. Implying that *P. africana* is mycotrophic. All samples were of the arbuscular mycorrhizal type, confirming previous data on the mycorrhizal status of species of

Prunus (Haug et *al*., 1994; Moyersoen et *al*., 2001; Pons et *al*., 1983). The presence of arbuscules in mycorrhizal roots is generally used to designate plants with functional AM. In this investigation, arbuscules were rarely observed in most root samples. Absence of hyphal coils was certainly overlooked. Internal hyphae and vesicles were equally common. Our observations of the lack or scarcity of arbuscules are consistent with those in tropical wet forests (Béreau et *al*., 1997; Onguene and Kuyper, 2001). The amount of arbuscules, hyphal coils and the amount of internal hyphae might depend on differences in root morphology. Smith and Smith (1997) reviewed structural diversity of AM and recognized two type, viz: *Arum*-type and *Paris*-type. In the *Arum*-type, extensive intercellular hyphae and arbuscules develop while in the *Paris*-type these structures are absent and hyphal coils commonly occur. *P. africana* trees typically exhibit the *Arum*-type AM colonization with variously shaped vesicles, suggestive that these mycobionts could belong to the Glomaceae. No significant attempt was made to identify spores from its rhizosphere for diversity study. Implying that a particular genus or species may show different colours and shapes at different ages.

Fractional mycorrhizal colonization of roots of *P. africana* in Mt Manengouba was different from that in the Oku Mountain and the Mount Cameroon site. In Mt Manengouba, *P. africana* trees were artificially re-introduced 39 years ago, while they grow naturally in later sites. Higher fractional mycorrhizal colonization of *P. africana* in native montane sites contrasted with low mycorrhizal colonization in artificially regeneration lowland possibly due to several causes such as: anthropic disturbances, having a toll, and insect and disease attack. Trees in Mt. Manengouba were seriously debarked to death though still standing. Root samples were collected from trees having foliage, which even did not give a picture of the evergreen canopy nature (shadowed by a higher canopy) of *P. africana* as defined by Dawson and Powell, (1999). Thus, lack of enough photosynthetic materials by these trees in Mt Manengouba is likely to reduce the extent of mycorrhizal root colonization when basing on the interaction between mycorrhizal fungi and host plant; that is, a mutually beneficial symbiosis (Onguene et *al*., 2000). That notwithstanding, the cash and food crop cultivation associated with the use of chemicals such as fungicides and inorganic fertilizers affect mycorrhizal colonization considerably. Fungicides are generally far less damaging to mycorrhizal population than fumigants but typically delay or reduce AM colonization (Mengue, 1982). Besides these differences, on the reason of the decline, the site affected

percentage root colonization. Further comparisons with the natural sites were not possible since some size classes were lacking in Mt Manengouba.

Characteristics of plant roots usually highly correlate with mycorrhizal formation and abundance. Coarse roots with no or few root hairs are often highly mycotrophic while highly branched, fine, long roots with numerous root hairs have often been observed to derive little nutritional benefit from mycorrhizae in controlled experiments. In this study, we observed that roots of *P. africana* were rather coarse without root hairs. They did show abundant AM colonization at all growth stages, implying that under its montainous native habitats, *P. africana* is highly mycotrophic. In view of the low available phosphorus of Mont Oku, Cameroon and Manengouba soils (Table XI), it is not surprising that all root samples of *P. africana* were mycorrhized. From this work, six species of AM fungi belonging to *Gigaspora*, *Glomus* and *Acaulospora* genera were identified to be dominant and active in the rhizosphere of *P. africana* in Cameroon. According to the five classes of mycorrhizal root colonization *P. africana* can be categorized as a highly mycotrophic mycorrhizal plant in its native Cameroon habitats. So, what can be the implication of its high mycotrophic characteristic on its vegetative propagation?

III.2.2. Efficiency of Arbuscular Mycorrhizal fungi on the vegetative growth of *Prunus africana* cuttings

Spores are organs for the perpetuation of species. Under favorable conditions, these spores establish symbiotic (mycorrhizal) relationships with plant roots for mutual growth. When these symbioses break up, arbuscular mycorrhizal species remain due to sporulation. The results of the multiplication of mycorrhizal fungi from a single spore and from several spores have shown that these beings multiply and colonize their living environment regardless of their starting number.

Functional diversity can be determined through an assessment of the variability in plant response to mycorrhizal inoculation. This was significantly recorded after inoculation of six AM fungi strains on *Sorghum* plants in a greenhouse. Morphologically identical spores introduced into culture gave an identical response on the growth and development of sorghum plants, thus demonstrate good identification. This result showed a slight difference from Ngonkeu's work (2009), which was probably due to a difference in precision during the morphological

characterization. Analysis of our data showed that there was no significant difference in growth of plants inoculated with one spore and that with several spores, in accordance with the work by Jansa et *al*., (2008) and Olfat and Jalil (2012) on root infections and multiplication of spores. This could be explained by the fact that the establishment of a mycorrhizal symbiosis depends on the quality of the information transmitted by the partners present, the information being transmitted by strigolactone for the plant to induce a mycfoctor response in the fungi. This exchange is made independently of the amount of AM fungi in the medium or substrate (Jalil and Olfat 2012).

The rooting capacity of *P. africana* cuttings can be improved by growth hormone (Avana et *al*., 2006). The results of this study showed that the contribution of indole butyric acid (AIB) treatment stimulated the rooting of *P. africana* cuttings, but mycorrhizal fungi induced a more pronounced development of the root system. This can be explained by the fact that mycorrhizal fungi, by intensifying the nutrient absorption surface of the plant partner, protect them against root pathogens, stimulate the production of their natural phytohormones (essential for rooting) and contribute to the increase of their active substances (Augé et *al*., 2004). In return, they receive their carbon nutrients from the plants (Ludwig-Müller and Güther, 2007). Hence, the contribution of the latter becomes incomparable to any other exogenous contribution, in line with the work by Veresoglou et *al*., (2012) on the impact of AM fungi on root development, nutrition and protection of plant cuttings.

Across size class distribution, extent of AM colonization of *P. africana* roots was significantly higher in young plants and moderate in mature trees. Similar data have been observed with Okoume (*Aucoumea klaineana* Pierre), an endemic tree species of forests of Gabon, and in the artificial regeneration in the Kienke reserve, South Cameroon (Onguene et *al*., 2000), implying that mycorrhiza is also essential in the early growth stages of *P. africana.* The negative linear correlation between size class distribution of *P. africana* and fractional colonization suggest that over the growing period, mature trees could exert a control over mycorrhizal colonization. Janos (1996) proposed a positive linear correlation between early seral status and mycorrhizal dependency: early successional plant species tend to be facultatively mycotrophic, conversely to late successional plants. Furthermore, the fact that seedlings have relatively smaller proportion of storage tissue to provide enough photosynthate, may explain why they are still highly colonized (St John and Coleman, 1983). The available information and our observations lead us to the conclusion that *P. africana* is obligatly mycotrophic. The hyphal mat model can explain this as thus: the model

suggests that hyphal bridges are abundant under closed canopy and may transport photosynthate from overstored plants, thereby allowing shaded seedlings to be partially heterotrophic for carbon. That notwithstanding, these runner hyphae can produce new colonization, which may lead to the overall increase in the seedling stage, as it is a beneficial interaction. Francis and Read (1984) stated in concordance that colonization of plant roots by AM fungi could provide channels for the transfer of carbon between associated plants through mycelium, which interconnects individuals.

Altitude significantly affected mycorrhizal colonization of *P. africana* roots and spore count of its rhizosphere. Highest values of both parameters were recorded above 2000 m altitude, respectively. This high altitude appears to be the ecological niche of *P. africana*. Usually, a forest dependence of mycorrhizal types is recognizable: in the lower tropical regions, AM associations dominate, whereas in the mixed subtropical forests most of the species show AM, but there are also several species with ectomycorrhizae. In the higher mountains, ectomycorrhizae predominates. The results of this report contradict this view of mycorrhizal occurrence. Among the six founded AMF species, *Gigaspora margarita* was found to be the best arbuscular mycorrhizal in the improvement of *Prunus africana* cuttings development. Their effects on root system development were more pronounced compared to those of plant growth hormone AIB. The use of these particular species in the vegetative regeneration of *Prunus africana,* can improve their root system density and enable cuttings to effectively resist field transplantation. This mycorrhizal symbiosis could represent an innovative approach to solve the problem of *Prunus africana* regeneration. So, as mycorrhizae appears to be appropriate for the vegetative propagation of *P. africana* what can be their reel implication on the growth of cuttings during transplantation as well as their impact on the bioactivity of the species?

III.2.3. Effect of AMF inoculum on the phytochemical compounds of vegetatively propagated *Prunus africana*

The bark tissue of *P. africana* contains different types of phytochemical compounds. The present study targeted three groups of phytochemical compounds, namely tannins, flavonoids and phenols. No significant difference was observed between treatments on the tannins content, except the treatment with *Gigaspora margarita* (Gm) inoculum that led to a higher concentration of tannins. Flavonoids, like many other polyphenol compounds, are essentially secondary metabolite products of plants and fungi. Also known to have antioxidant activities, flavonoids play a key role

in the management of prostate conditions (Katz et *al*., 2002; Madivoli *et al.*, 2018; Reale *et al.*, 2018; Sak, 2017). Previous studies have reported the presence of this group of phytochemical compounds in *P. africana* extracts (Jena et *al*., 2016; Komakech & Kang, 2019). The flavonoid and total phenol contents were significantly higher in non- inoculated cuttings compared to the inoculated cuttings. Other studies have reported a decrease in total phenol and flavonoids content in *Salvia officinalis* (Lamiaceae), a medicinal plant (Geneva *et al.*, 2010). In the present study, we also observed a similar response in *P. africana*.

However, other studies have shown an increase in total phenol and flavonoid in *Amburana cearensis* (Fabaceae) (de Oliveira *et al.*, 2015) and *Viola tricolor* (Violaceae) (Zubek *et al.*, 2015) when inoculated with AMF. Studies have shown variation of phytochemical content during the different growing stages of the plant growth. Indeed, Oszmiański *et al.* (2018) showed that the concentration of polyphenolic compounds in immature cranberry (*Vaccinium macrocarpon*, Ericaceae) fruits was lower, compared to the concentration of semi-mature fruits. In contrast to the results observed in this study (different plant species and different plant organs however), it has been reported an alteration of phenolic and glycoalkaloid content in *Solanum tuberosum* (Solanaceae) from initiation to the maturation (Kirui *et al.*, 2018).

This study showed that AMF inoculum produced with indigenous AMF spores from *P. africana* rhizosphere soil from the three Afromontane forests significantly enhanced the growth parameters including height, leaf surface area, and the number of leaves of *P. africana* cuttings in nursery. However, the response in phytochemical content of *P. africana* seedlings was variable. This study could significantly contribute to the *ex-situ* regeneration or cultivation of *P. africana* through the production of indigenous AMF inoculum. So, as it is shown that an appropriate AMF inoculum can improve the growth and adaptation of cuttings ex sittu, what can be its impact on the phytochemical content of *P. africana*?

III.2.4 Soil characteristics

In all native montane habitats, fractional mycorrhizal colonization significantly differed as well as extent of colonization with size class distribution. Mycorrhizal colonization was generally higher in Oku Mountain than in Cameroon. Both sites occur on rich organic, volcanic soils, low in total nitrogen compared to Manengouba Mountain (Table XI). Soil pH differs in all sites because Mt Cameroon is located in an active volcano conversely to Oku and Manengouba Mountain. It is

possible that the number of mycorrhizal propagules is limited by volcanic activity, since spore count was 1.33 time higher in Oku Mountain (400 spores per 25 g dry soil) than in Mt Cameroon (300 spores per 25 g dry soil).

These results clearly demonstrate that *P. africana* is a highly mycotrophic plant in its native Cameroon montane habitats with rich spore populations. We have determined that different native species of mycorrhizal inocula may elicit different growth responses. However, as the selection of mycorrhizal propagules in the rhizosphere of *P. africana* appears to be the first step for the successful harnessing of the mycorrhizal biotechnology to alleviate nursery problems related to poor cuttings growth, *Gigaspora margarita* was found to be the most appropriate arbuscular mycorrhizal in the improvement of *Prunus africana* cuttings development therefore with a negative correlation with its phytochemical contents. So, now Can we discover a link between the mycorrhizal variation and the bioactivity of *P. africana* in the environment?

III.2.6 Metabolite profile of *Prunus africana* form three locations in Cameroon

III.2.6.1. Extraction methods comparison

Extraction with method E is confusing because we probably made a mistake when preparing the buffer, especially in the concentration of the standard (ribitol). This is the main reason why some all concentrations with this method look high (beta-sitosterol).

The difference between the results of the final experimentation and the previous one (using the same extraction method) may belong to the shaker. We used, for the first experimentation, a very high-speed shaker (1000t/m) allowing for a higher extraction of secondary metabolites with less fatty acids, when for the previous one, we used an old and low speed shaker (280t/min). This still can't explain why our results are far different from those of Kadu et *al*. (2012) using an even slower shaker (180 t/min).

This may explain why, finally, Ethylacetate+ Methanol seems to be the best extraction method for *Prunus africana* metabolite profiling. It is the best in terms of quality (more compounds and best ratio than the others). This result is slightly different from what Kadu et *al*., found in 2012.

Contrary to Kadu et *al*. (2012), we did not have high amounts of non-polar compounds. This could be because the GC Column we used for experimentation was a polar Column (based on the

Principle of the GC, the first step of compounds separation is by interaction with the GC Column and the second is the separation by the Gas).

It is also possible that we did not have nonpolar compounds because of the sample collection, storage condition or travel conditions (some compounds may have been degraded and lost during sampling and travel).

In summary, our results show that almost the same composition of metabolites could be extracted from *Prunus africana* trees from three different locations in Cameroon.

Beta-sitosterol, the *P. africana* most important component, was generally detected in samples from all locations, with trees from the Mount Manengouba area showing the highest average concentrations (fig.24). The presence of triterpenes, such as phytosterol, in all samples has significant implications for the pharmaceutical industry. Also, some metabolites were only found in specific locations, possibly indicating the influence of environmental and mycorrhizal factors on metabolite composition. The occurance of certain significant compounds like chlorogenic acid and quinic acid, with high pharmaceutical value, were never studied in *P. africana* before. This constitutes one of the major findings of these first results. Interestingly, these phenolic compounds are more highly concentrated in trees from the Mount Cameroon area, where beta-sitosterol is low-concentrated.

III.2.6.2. Bioactive compounds constitution and concentrations within and among locations

Metabolite profiling is a powerful tool that has contributed to the understanding of plant physiology, including phenotypic differences, gene annotations, metabolite regulation, and characterization of stress responses. Metabolic profiling of *Prunus africana* has been accomplished using GC-MS techniques. In the present research study statistical analysis were used to investigate the metabolic pathways of *P. africana* trees in Cameroon. However, metabolic profiling of *P. africana* in Cameroon, as well as its metabolic pathways had never been studied before us. Data normalization results, performed with MetaboAnalyst assistance prior statistical analysis with R. PCA-DA and PLS-DA were applied to the normalized GC-MS data from Mount Cameroon (MC): Cameroon Genotypes (CG), Mount Oku (MO): Oku genotypes (OG), and Mount Manengouba (MM): Manengouba genotypes (MG) sample groups in order to identify possible variations in the metabolite composition between the three locations or populations sampled.

Out of the 25 main components, the first three (PC1, PC2 and PC3) were responsible for major variation (87.6%) in study groups. Score plots clearly demonstrated that all the three groups of samples, MC, MO, MM cluster into three very distinct groups (Fig. 25). Further PCA-Discriminant Analysis resulted in three components, which contributed equally to 100% of variation. The list of 15 most important features is shown on Fig. 32, with the indication of relative metabolite concentrations in each group. When groups compared side by side, there are metabolites that having larger score in the particular paire (Fig. 31). These results are the same with those obtained by Zuno-Floriano et *al*. (2012).

The concentrations of the *P. africana* bark constituents varied considerably among populations (locations) in Cameroon but the constitutions are mostly the same. For instance, for beta-sitosterol and catechin, the content in samples among locations varied erratically. β -sitosterol was previously reported in *P. africana* in Cameroon, but concentrations among locations were not significantly different (Tchouakionie *et al* 2014), even if the locations were not the same compared to our results. Low concentrations of catechin were also reported. However, for other species it has been described that the concentrations of both constituents decreased rapidly (in leaves) with time and eventually only traces were found in developing seeds (Munshi and Sukhija 1984). Thus, the question about whether genetic, environmental or mycorrhizal variations factors are causing the observed pattern in *P. africana* remains open as of yet.

The variation in concentration among different populations of the key compounds studied is shown in Fig.29. Quinic acid concentration, for instance, was found to be highest in plants from Mount. Oku and lowest in those from Mt Cameroon. In general, trees from Mount Oku Forest seems to be the one with the highest concentration of key bioactive compounds of *Prunus africana* in Cameroon, except of the most efficient compound beta sitosterol which *P. africana* trees from Mount Manengouba has the highest concentration when trees from Mount Cameroon have the lowest concentration in total bioactive constituent recorded (Fig.32). This can be explained by the vegetation, climate and geological characteristics of the Oku Mountain, which differ strongly from those of the two other mounts. The concentrations of sucrose in plants from Mount Oku shows that *Prunus africana* trees in this mountain are facing drought stress which can correlate with the concentrations of their bioactive contents. These results are shown by the Box plot (Fig. 26; 27; 28), with MG (Manengouba Genotypes) showing a high accumulation of sterols followed by OG

(Oku Genotypes) and lower levels were observed in CG (Cameroon Genotypes) population as indicated PLS-DA VIP scores.

The variation in concentration among genotypes (trees) of the key compounds studied showed clearly the differences of sterols and other bioactive compounds production within different genotypes across the three populations, with trees from the M2 area showing highest accumulatiom of sitosterol followed by C4 and least accumulation in C1, clearly illustrated by heat Map of the accumulation of bioactive compounds within different trees in three locations or populations (Fig.30). In location CG (population of mount Cameroon) for instance tree from the C5 area showed the highest accumulation of quinic acid followed by genotype C4 when C1, C2 and C3 showed just traces. This variation can be explained by the fact that there were probably anthropogenic factors such as severe deforestation and changes in land use starting approx. 1000–2000 years and these have had a dramatic impact on Afromontane ecosystems (Gade and *al*., 1996; Finch et *al.*, 2009).

We can see in the PCA analysis (Fig.25) that there is clear separation between the three populations with few of the genotypes (trees) sharing bioactive compounds within different populations. This can be explained by the existence of cooridors between locations and a possible presence of different genotypes: the observed differences may be due to genetic difference or differences in gene expression

. These conclusions are the same found by Kadu et *al*. in 2014, in the study on *P. africana* in Kenya, who reported the existence of a potential corridor between *P. africana* accessions belonging to the two countries.

We observed that, among the bioactive compounds analysed (Fig.22), there are twelve significant features for the total group showing a clear separation in the production of sterols from a specific population from other compounds as shown in the PCA loading results (Fig.31). This was clearly shown by the VIP scores figure, where MG displayed a higher accumulation of compounds as compared to other populations (Fig.32). In contrast to Kadu et *al*., (2014), we did not observe large amounts of non-polar compounds. This could be due to different methodical approaches using a polar GC Column (based on the GC Principle, the first step of compounds separation is by interaction with the GC Column, and the second is separation by Gas). It could also be possible that this contradiction could be an artifact introduced by the sample collection

process, storage or travel conditions (some compounds may have been degraded and lost during sampling and travel).

III.2.6.3. Metabolic pathway analysis

Pathway analysis performed with MetaboAnalysis assistance revealed a number impacted metabolic pathways. The overall pathway impact picture is illustrated with Figure 3.24. These data presented in greater details in Table XV where number of hits and p values are depicted. The most impacted appears to be stilbenoid, diarylheptanoid and gingerol metabolism; flavonoid, phenylpropanoid and galactose metabolism appears to be less present in *P. africana* trees in the studied locations. However, impact consequences are not straightforward and metabolic flux is involved. Changes in flux can be illustrated with Figure 33 where some metabolites do not follow the pattern of key metabolite. This indicates that *P. africana* flux redistribution is influenced by either gene flow, environment or mychorrhizae and its metabolites. This confirms a high complexity and sensitivity of metabolic networks used by the plant in order to survive and adjust to environmental challenges (Fig.33) as demonstrated by Cattupalli et *al.,* 2021.

III.2.7. Correlation of bioactive contents with mycorhyzae variations within and among locations

As the table XVI shows, we observed a positive correlation with the *Glomus spss* and sterols, as well as their negative correlation with *Gigaspora* and *Acaulospora spp***.** The general view shown a negative correlation between some mycorrhizal fungi species and *P. africana* phytochemical content when *Glomus spss* inversely showed a positive correlation with its pytochemichal content. These results were quite suprising because they were different from the results obtained on *P. africana* cuttings done in controlled environment. The negative linear correlation between size class distribution of *P. africana* and fractional colonization suggest that over growing period, mature trees could exert a control over mycorrhizal colonization as Janos et *al*., (1996) observed. We can deduce that there is a positive linear correlation between early seral status and mycorrhizal dependency on *P. africana* growth and a negative correlation with its phytochemical content observed in this study. At mature age, the correlation seems to be positive between *P. africana* phytochemical content and *Glomus spp* colonization. However, other studies have shown an increase in total phenol and flavonoid in *Amburana cearensis* (Fabaceae) (Oliveira *et al.*, 2015) and *Viola tricolor* (Violaceae) (Zubek *et al.,* 2015) when inoculated with AMF. Studies have shown

variation of phytochemical content during the different growing stages of the plant growth as we found. Indeed, Oszmiański *et al.* (2018) showed that the concentration of polyphenolic compounds in immature cranberry (*Vaccinium macrocarpon*, Ericaceae) fruits was lower, compared to the concentration of semi-mature fruits. Like the results we observed in this study, an alteration of phenolic and glycoalkaloid content has been reported in *Solanum tuberosum* (Solanaceae) from initiation to the maturation (Kirui *et al.,* 2018). Therefore, tannins, flavonoids and total phenols in *P. africana* could be effectively increased during the different growing phases and it has been correlated to the *Glomus spp* in the environment**.** Studies conducted on mycorrhizae (Beat et *al*. 1993) have previously shown that the combination of *Glomus spp* contributed to the over accumulation of terpenoids and this clearly indicates that the *Glomus spp* is likely to contribute and enhance production of most secondary metabolites in plants, though this hypothesis remains to be proven.

III.2.8. Synthesis

The findings of this study demonstrate the richness of *Prunus africana* in sugars, terpens, triterpenes and flavonoids, which are of potential pharmaceutical interest.

A pronounced variation in the concentration of selected bark constituents was found among and whithin populations. This suggests that spatial genetic effects are very likely to be present, especially for catechin. The concentration of beta sitosterol was not correlated with the concentration of the other constituents and thus canot be used as a good predictor of the concentration of the other constituents studied. Some environmental parameters (variation of mycorrhizal statute of the soil) did significantly correlate with constituent concentrations. For instance, concentration of beta-sitosterol was negatively correlated with some soil constituents when other was positively correlated with them. According to the litterature, Informal reports from pharmaceutical companies and bark traders indicate that trees older than 15 years produce a type of bark that is more highly appreciated, which suggests that those bark constituents which decrease with the age may not be so therapeutically important. However, it is also possible that traders may simply prefer a thicker bark for handling reasons. Further investigations are needed to cast more light on the environmental factors affecting bark quality. To fully understand opportunities for genetic gains through selection of populations and individuals, genetic field tests are necessary. The concentration of bark constituents originating from different Cameroonian cherry populations or locations present a very distinct geographical pattern. Therefore, it is concluded that the

molecular phylogeographic pattern is reflected in the spatial patterns of certain bark constituents, notably in catechin. This may suggest a dependent evolution of bark metabolism within different phylogeographic lineages (assuming there is genetic difference at this point). The data indicate that *P. africana* populations with very high concentrations of the studied constituents are located in Mount Oku, trees in a region that is also genetically distinct from the Cameroonian mainland and Mycorrhizal statute. The *P. africana* colonization of this Mountain would probably have been the result of a single dispersal. Our data also indicate that populations with very high concentrations of beta-sitosterol are located in Mount Manengouba a region composed of *P. africana* natural and artificially regenerated by Forestry administration in the early 1970s. This study also shows that data on spatio-chemical diversity are expedient for a conservation strategy for the African cherry. If a conservation strategy focuses exclusively on molecular/ metabolomic diversity, populations in Mount Cameroon and Manengouba will be prime regions since Mount Oku populations are not quite as diverse as the volcanic chain populations from other populations. However, *P. africana* populations from Mount Oku feature a relatively high concentration of all bark constituents (cf. Fig. 30) and, although it is unknown which role bark constituents may play for adaptation and inversely, it is highly recommended to include Oku populations into a Global conservation strategy. These Mountain populations appear also to be highly vulnerable due to bark overexploitation. Intraspecific variation of chemical compounds is common in many plant species, and often shows defined geographical patterns, that may reflect environmental differences within the range of a species.

Our Data indicate that mycorrhizal variations of the soil affect and influence the bark quality of *P. africana*. Tannins, flavonoids and total phenols in *P. africana* increase during the different growing phases and it been positively correlated to the *Glomus spp* and negatively correlated to *Gigaspora margarita* and *Acaulospora spp* in the environment. Studies conducted on mycorrhizae) by other authors have previously shown that the combination of *Glomus spp* contributed to the over accumulation of terpenoid and this clearly indicates that the *Glomus spp* is likely to contribute to and enhance production of most secondary metabolites in plants. These results can allow us to say that, the quality and concentration of bioactive compounds of *P. africana* seems to be closely linked to its growth and development environment (agroecological and edaphic conditions).

CONCLUSION, RECOMMENDATIONS AND PERSPECTIVES

CONCLUSION

The general objective of this work was to develop a *Prunus africana* strategy of domestication and sustainable management in relation with its environment, mycorrhizal statute and metabolite profiling interactions in Cameroon. The results clearly demonstrate that *P. africana* is a highly mycotrophic plant in its native montane habitats in Cameroon owing to lavish spore populations. From this work, six species of AM fungi belonging to *Gigaspora*, *Glomus* and *Acaulospora* genera were identified to be dominant and active in the rhizosphere of *P. africana* in Cameroon. Among the species, *Gigaspora margarita* was found to be the best arbuscular mycorrhizal for the improvement of *Prunus africana* cuttings development and growth. Their effects on root system development were more pronounced compared to those of plant growth hormone AIB. Thus, the use of these particular species as biofertilizer in the vegetative propagation of *Prunus africana*, can improve their root system density and enable cuttings to effectively resist field transplantation. Thus, the artificial regeneration of *P. africana* is greatly improved by inoculation with appropriate mycorrhizal fungi. As the study shows *Gigaspora margarita* is the most appropriate AMF for positive influence on vegetatively propagated cuttings and first step growth (transplantation). From this study, the physicochemical properties of the soils did not appear as a key factor shaping the AMF community composition and diversity in the rhizosphere soils of *P. africana*. The results showed that AMF inoculum produced with indigenous AMF spores from *P. africana* rhizosphere soil from the various Afromontane forests significantly enhanced the growth parameters including height, leaf surface area, and the number of leaves of *P. africana* seedlings in nursery. However, the response in phytochemical content of *P. africana* seedlings was variable. This mycorrhizal symbiosis could represent an innovative approach to mitigate the problem of *Prunus africana* regeneration. Our results also show that almost the same composition of metabolites could be extracted from *Prunus africana* trees from three different locations in Cameroon. Beta-sitosterol, was generally detected in samples from all locations, with trees from the Mount Manengouba area showing the highest average concentrations. The presence of triterpenes such as phytosterol in all samples and some high value polyphenols such as epicatechin, 3-O-p-coumaroylquinic acid, and chlorogenic acid has significant implications for the pharmaceutical industry. Also, some metabolites were only found in specific locations, possibly indicating the influence of environmental and mycorrhizal factors on metabolite composition. Interestingly, these phenolic

compounds are highest concentrated in trees from the Mount Oku area, where beta-sitosterol is less concentrated. There was a pronounced variation among and within populations of concentrations of selected bark constituents, suggesting a possible genetic effect influencing the constitution and content of phytochemicals in *P. africana* trees in these locations.

Finally, data and pathway analysis indicate that mycorrhizal variations of the soil affect and influence the bark quality of *P. africana*. Tannins, flavonoids and total phenols in *P. africana* increase during the different growing phases and it been positively correlated to the *Glomus* spp. and negatively correlated to *Gigaspora margarita* and *Acaulospora* spp. in the environment. This clearly indicates that the *Glomus* spp. is likely to contribute to and enhance the production of most secondary metabolites in *P. africana,* when *Gigaspora margarita* contributes to improve its vegetative propagation. These mycorrhizal symbioses could represent an innovative approach to solve the problem of *Prunus africana* regeneration by the formulation of an appropriated bioferlizer and its sustainable management in Cameroon.

RECOMMENDATIONS

The role of soil microorganisms in shaping plant communities and enhancing crop productivity has gained interest over the recent decades. Nevertheless, their application in the domestication and biotechnological production of forest species and non-timber forest products are particularly lagging behind. This study has demonstrated the potential of AMF as biofertilizers to improve *P. africana* production in nursery system, including vegetatively produced cuttings and even the quality of its bioactive content. The methods and protocols in this study could also be extended to other important medicinal plant or crop species. This study can be used as a tool by all stakeholders involved in the management and regeneration of *P. africana* to promote an efficient regeneration of the plant, for a permanent availability of the raw material necessary for drug production and ecosystem conservation. Based on this study, the following recommendations are proposed;

1- Farmers/foresters interested in *P. africana* production, should consider the source of the material to be used. Indeed, transferring the plant material from one location to another with different environmental conditions could be detrimental for the production of *P. africana* cuttings due to the lack of compatible microsymbionts such AMF in a given geographical area;

2- Cuttings should be produced in the same geographical area, to avoid any stress due to the change of environment conditions.

3- Native rhizosphere soil should be used if translocation of material is necessary, to ensure the regeneration and the establishment of *P. africana* in new location.

4- The production of AMF inoculum indigenous to each region where *P. africana* is endemic in Cameroon should be initiated and promoted, with prefence given to *Gigaspora margarita* for its vegetative growth.

5- To use inoculum made from indigenous AMF species (*Gigaspora margarita)* collected in the rhizosphere soil of *P. africana*.

6- Farmers/foresters interested in *P. africana* production should prefere environment rich in *Glomus spp* for a better constitution of bark of *P. africana* in quality and quantity.

7- Aditionnal studies should be done on biofertiser to insure their efficiency and avoid competivity with natives AMF species after transplantation in nature.

8- Pharmaceutical firms exploiting *P. africana*, should focus on the *P. africana* populations from Mount Oku area for their good quality bark in term of Phenolic compounds and to *P. africana* populations from Mount Manengoube area for the main and most important bioactive compound of the tree: the sterols (beta-sitosterol and beta-sitosterone)

PERSPECTIVES

For future studies, collection of samples from all the countries in Africa where *P. africana* is endemic for a holistic profiling of its microbiome could offer new perspective on the management of this important medicinal plant. Also, future studies will be required to assess the genetic diversity between *P. africana* populations, as well as the effect of single AMF species and different combinations and mixtures of AMF species inocula on the growth and bioactive content of *P. africana* cuttings at various stages of its development. This study is one of the first to report on the role of AMF on *P. africana* cuttings as well as on its bioactivity, and the early results obtained here will guide the design of new studies, especially using single spores with experimentation in the greenhouse as well as in the field with a longer period of observation to have an insight into the functional role of each AMF species found in the rhizosphere of *P. africana*.

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APPENDICES

APPENDICES

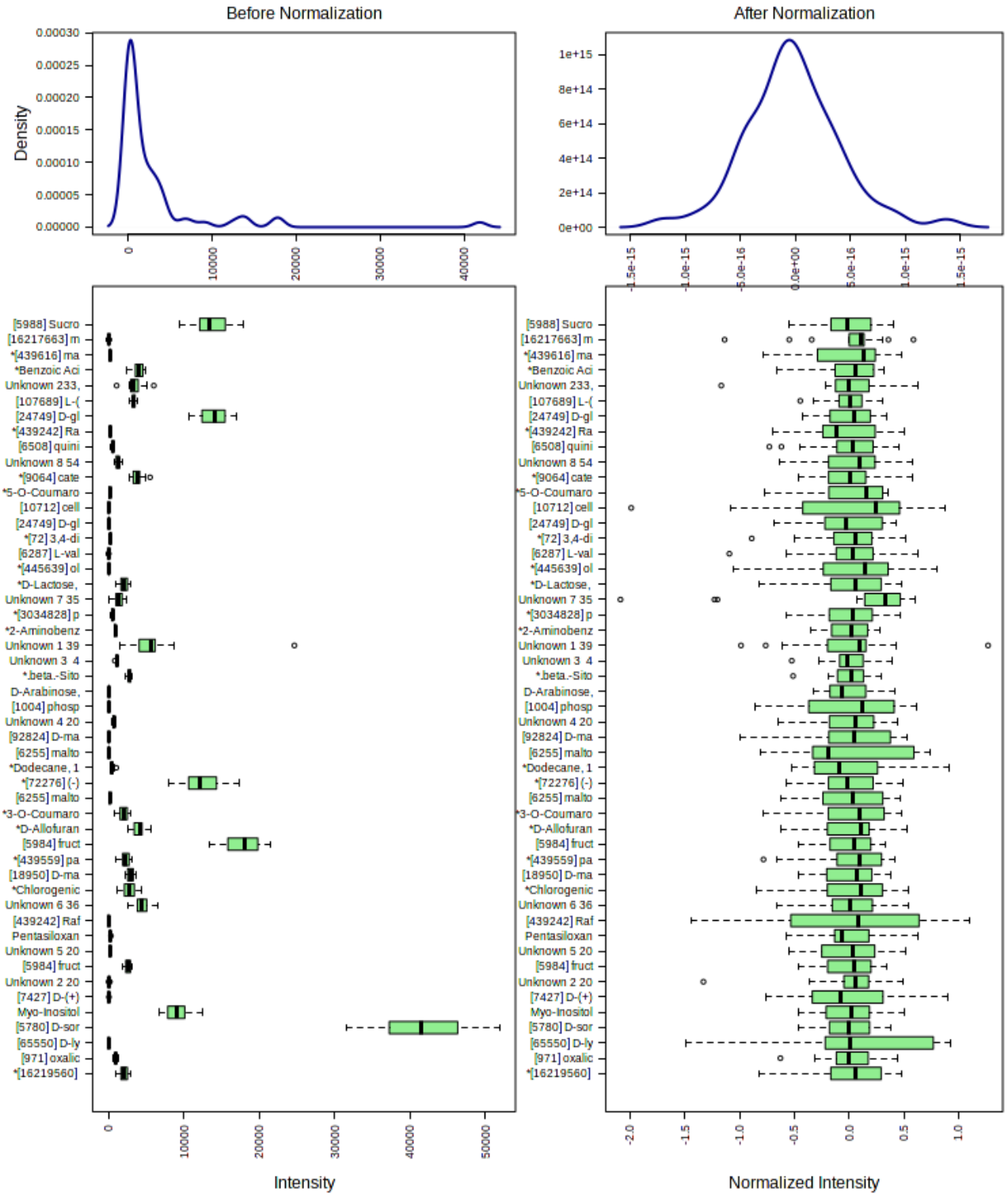
Appendice 1. Partial Least Squares-Discriminant analysis of the bioactive content of *P. africana* in three populations in Cameroon

	coef.mean	CG	MG	OG
*3-O-Coumaroyl-D-quinic acid, 5TMS	63.546	100	31.078	59.559
*4-O-Coumaroyl-D-quinic acid, 5TMS	63.334	99.683	30.961	59.359
*Chlorogenic acid (6TMS)	62.05	97.756	30.248	58.145
*5-O-Coumaroyl-D-quinic acid, 5TMS	57.523	90.966	27.737	53.865
[6508] quinic acid [17.076]	52.489	83.415	24.945	49.106
Unknown 6 368, 361, 169	47.923	76.566	22.412	44.79
Unknown 8 540, 369, 271, 149	47.466	75.881	22.159	44.359
*[72276] (-)-epicatechin [25.472]	41.678	67.198	18.948	38.887
[5988] Sucrose [23.988]	39.788	64.364	17.9	37.1
Myo-Inositol, 6TMS derivative [19.354]	37.73	61.277	16.759	35.155
[5984] fructose 2 [17.288]	36.41	59.297	16.027	33.907
*[9064] catechin [25.643]	30.524	50.467	12.761	28.343
Unknown 3 441, 147, 159	18.177	31.947	5.913	16.671
*.beta.-Sitosterol, TMS derivative	7.517	15.957	0	6.5939

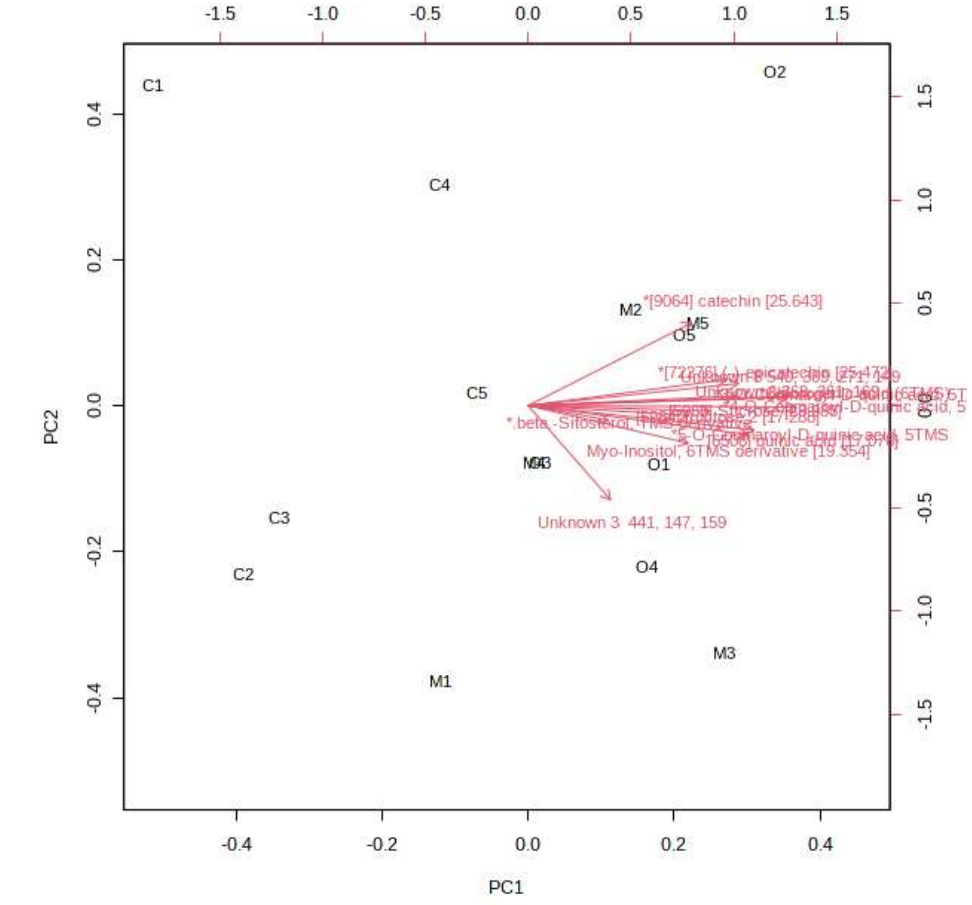
Appendice 2. Partial Least Squares-Discriminant analysis Coefficient between genotypes

	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7	Comp 8
C1	-2.3258	0.1083	0.15786	-0.17058	0.12413	-0.013	-0.08728	-0.0305
C2	-1.7657	0.020716	-0.11452	0.007971	-0.07112	0.057255	0.0782	-0.00301
C3	-1.5504	-0.00929	-0.05045	0.035593	-0.07396	0.071849	-0.07311	0.018051
C4	-0.57818	-0.39242	0.025391	-0.05907	-0.04576	0.004819	0.034293	0.041551
C5	-0.31261	0.088804	-0.00327	-0.09959	-0.13096	-0.14767	0.016817	0.026909
M1	-0.53671	0.10203	-0.13393	0.12496	0.05457	-0.01133	0.11125	-0.03772
M2	0.61792	-0.30807	-0.00356	0.06996	0.11186	0.000593	0.03841	-0.02969
M3	1.2173	0.027811	-0.26527	-0.07166	-0.01365	-0.01988	-0.14548	0.040598
M4	0.030148	-0.14683	-0.02999	0.1404	0.099574	-0.07841	-0.07764	-0.02618
M5	1.0564	0.05758	0.14289	0.028822	-0.08962	-0.04386	-0.01968	-0.07846
O1	0.82743	0.21481	-0.11053	-0.11199	0.15804	0.013047	0.10268	0.00357
O2	1.5265	-0.07717	0.10001	-0.24151	-0.03395	0.049044	0.077969	0.002443
O3	0.076603	0.13055	0.15442	0.19627	-0.02441	-0.02442	0.082841	0.083707
O4	0.73982	0.08051	-0.01077	0.071966	-0.15051	0.072494	-0.03707	-0.06082
O5	0.97727	0.10265	0.1417	0.078454	0.085762	0.069474	-0.10221	0.049546

Appendice 3. Normalized data for the Metabolites analysis



Appendice 4. PCA Biplot of bioactive compound of *P. africana* in three locations in Cameroon



Appendice 5. *P. africana* seeding from vegetative propagation with biofertilizer at fundong, North West Cameroon

Appendice 6. Laboratory materials from plant stress lab, Department of Molecular and Cell biology, UCT, South Africa

(a: Machines room; b: Milipore filter; c: Ice Sonicator water bath; d: SpeedVac, freeze drier; f: Agilent LC-MS Machine; g: Agilent GC-MS Machine; h: -80 freezer; i: Fluorescent Microscope; j: Library).

Appendice 7. List of some metabolite's analytes together with retention times and I^T (experimental and reference NIST Chemistry WebBook, SRD 69 values).

Chemical Class	Compound Name	^1t_R (min)	^2t_R (sec)	Ref. I^T
Polyalcohols	Glycerol 3TMS	15.38	1.59	1278
	*2,3-Butanediol,	28.15	1.67	1684
	Arabinitol 5TMS	28.44	1.69	1702
	Ribitol 5TMS	28.88	1.67	1717
	Glucitol 6TMS	34.21	1.72	1927
	Mannitol 6TMS	34.27	1.73	1925
	Myo-Inositol 6TMS	38.06	1.91	2073
Sugars	Arabinose 4TMS	27.27	1.71	/
	Ribose 4TMS	27.68	1.71	1668
	Fructose 5TMS (anti)	33.01	1.76	1867
	Glucose 5TMS	33.46	1.82	1898
	Mannose 6-phosphate 4TMS	43.09	2.16	/
	Sucrose 8TMS	51.17	3.06	2730
	Maltose 8TMS	51.47	1.93	2732
Flavan-3-ols	Catechin 5TMS	53.33	2.41	2840
	Epicatechin 5TMS	53.75	2.27	/
	Gallocatechin 6TMS	54.33	2.17	/
	Epigallocatechin 6TMS	54.50	2.32	2903
Phenolic acids	Quinic acid TMS	32.50	1.85	1853
	Chlorogenic acid 6TMS	57.83	2.56	3082
Others	(E)-Erythrono-1,4-lactone 2TMS	18.76	2.55	1380
	Xylonic acid lactone TMS	26.42	2.50	1627
	Ribono-1,4-lactone 3TMS	27.93	2.57	1677
	N-Acetyl-D-glucosamine 4TMS	37.92	2.32	/
	Galactose oxime 6TMS	38.81	1.80	/
	4-O-Coumaroyl-D-quinic acid, 5TMS	56.58	2.89	3012

Appendice 8. *Prunus africana* Metabolites profile from different extraction methods based on three locations in Cameroon

Metabolites	1C MTBE	2O MTBE	3M MTBE	3M EM	3M E
*2,3-Butanediol,	2795.55	2651.29	2981.37	1096.47	143.54
2-hydroxypyridine	2790.45	4191.55	4255.39	1899.50	18633.23
L-(+) lactic acid	5781.49	5492.07	4522.48	7022.00	21751.79
glycolic acid	243.560	302.91	333.15	584.68	4473.24
L-alanine 1	564.92	420.63	489.01	243.01	176.22
Hydroxylamine	3743.88	5708.70	5600.90	2222.80	26263.88
oxalic acid	5.25	13.39	4.72	19.40	0.00
oxalic acid 2	2912.51	4401.56	4706.58	1361.36	12531.48
L-valine 2	205.98	132.03	152.03	79.23	0.00
*Benzoic Acid	29322.32	24567.89	26477.45	12976.22	5098.01
*Benzene, 1,3-bis(1,1-dimethylethyl)-	13056.40	18186.59	20148.44	43.45	260.30
glycerol	17232.23	17346.59	16986.08	8861.22	6041.56
phosphoric acid	802.46	1056.93	1050.66	4522.77	2894.62
*Undecane, 2,9-dimet	3023.42	4194.15	4592.10	921.96	7968.94
succinic acid	1498.78	1568.43	1030.56	341.98	1063.83
*] mandelic acid	2635.60	2306.20	2810.10	1523.88	1071.84
D-malic acid	1446.17	1288.56	1360.08	9868.85	396.23
*Dodecane, 1-iodo-	4188.64	3116.72	4778.24	2067.23	14421.94
*2-Aminobenzoxazole	28132.75	35258.75	36648.95	1586.58	6873.22
Tyramine	2505.73	1629.76	1540.00	986.04	2593.50
D-lyxose 1	6.27	19.53	29.84	2.21	25.13
D-lyxose 2	556.00	70.03	58.74	24.33	368.65
D-Arabinose,	1001.55	962.62	875.54	523.78	764.17
D-(+)-Arabitol	1090.45	1194.01	1350.94	611.16	519.50
citric acid	477.70	508.01	350.55	36044.20	552.37
*3,4-dihydroxybenzoic	1319.38	1492.91	1484.03	1005.91	1459.61
* tagatose 1	1574.97	1244.43	960.64	497.66	1003.61

quinic acid	9388.97	8618.57	8688.40	26702.32	953.13
fructose 1	315733.57	289508.04	299595.71	163739.34	89973.67
fructose 2	214065.65	197462.46	206135.53	116222.85	66269.23
D-glucose 1	1525.63	1074.57	946.33	708.00	406.93
D-glucose 2	172600.18	160490.26	167791.53	87435.56	23192.18
D-mannose 2	43106.68	38056.49	40992.99	22566.94	5518.87
*D-mannitol	3128.51	2916.91	2969.96	1598.82	186.38
*D-Glucitol	2088.35	2076.45	2267.70	1182.18	0.00
D-sorbitol	579767.73	544957.05	549352.35	287434.81	46355.39
tyrosine 2	1195.19	965.76	957.70	397.35	83.73
palmitic acid	30292.38	30276.55	26006.05	10789.05	90370.39
Myo-Inositol	10279.59	9742.87	9326.31	6956.48	798.80
] D-allose 1	589.92	593.08	645.26	318.28	0.00
* linoleic acid	795.13	332.51	353.93	333.77	2935.69
* oleic acid	711.81	637.49	430.44	345.07	2520.96
* stearic acid	15965.55	12434.06	11532.95	5870.29	58324.90
*Glyceryl-glycoside	352.92	512.50	567.65	293.14	91.28
*Methyl 12-hydroxyst	2.69	8.03	0.00	1.81	114.96
*DAllofuranose,penta	37328.17	28842.36	32139.16	18218.20	8560.08
*D-(-)-Ribofuranose,	24277.22	33754.66	17981.24	0.00	5870.04
*Methyl galactoside,	152825.42	125535.07	135332.43	76248.68	33387.29
* Lactobionic acid 2	31974.29	26261.38	28329.91	31661.70	970.78
*D-Lactose, (isomer 1)	31974.29	26261.38	28329.91	31661.70	970.78
Sucrose	194591.24	163551.83	170687.85	107078.39	3004.73
* palatinose	24490.63	14888.48	20188.35	13759.96	726.22
cellobiose 1	576.22	600.59	740.97	520.57	#VALUE!
cellobiose 2	1383.69	1318.21	1387.37	891.17	363.18
maltose 1	1603.58	305.38	1363.58	155.46	130.31
D-(+) trehalose	312.40	446.04	0.00	190.76	82.28
maltose 2	2048.12	2340.85	1686.63	1369.58	356.88
Unknown 394, 465	2.55	0.00	7.15	21684.47	101984.53
maltitol	513.82	371.45	428.15	312.10	185.53

*D-(+)-melezitose?	295.23	366.50	440.62	285.33	185.53
* epicatechin	114945.01	106635.33	120733.96	52848.18	19260.62
*Aucubin,hexakis (trimethylsilyl)	1539.06	1583.23	1291.84	1005.45	53.28
*5-O-C-D-quinic acid	2022.54	1515.32	1535.30	1377.89	0.00
*Benzenamine,4-octyl	5.39	0.00	0.00	70596.26	202029.46
*maltotriose 2	50.09	49.19	29.49	0.00	252.90
*4-o-Co-D-quinic acid	129939.40	93517.11	109053.82	76960.18	2769.46
*3-o-Co-D-quinic acid	34830.93	25959.56	28850.52	19553.30	508.70
*Chlorogenic acid	50385.04	37140.00	41810.01	26882.66	950.78
Unknown1 119, 13	109143.24	135925.80	142842.31	6477.67	73501.65
Raffinose	54.62	123.21	370.51	5.12	295.67
*beta.-Sitosterol	13280.69	14085.42	14215.66	7262.43	84678.52
*Raffinose	2033.49	2572.07	2101.95	787.42	321.81
Unknown sitosteron	8217.62	8014.84	7643.23	2932.61	3360.36
Unknown 3 368, 361,	0.00	46529.21	50644.20	20648.77	0.00
Unknown 4 356, 272,	0.00	733.28	1656.38	643.24	364.38
Unknown 540, 369,	0.00	13353.78	14942.11	5796.42	427.68

Asterisk (*) denote compounds found without standards, by comparing their spectra to the ones in online library. Compounds with known anti-carcinogenic and anti-atherogenic properties are marked in red. Compounds were identified with GC library search (NIST14 or Fiehn Library

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Effect of Arbuscular Mycorrhizal Fungi Used as Biofertilizer for the Vegetative Propagation of *Prunus africana* (Hook.f.) Kalkman

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Abstract *Prunus africana* is a medicinal plant species whose exploitation became abusive over the years due to the high properties of its bark. It faces multiple challenges: the recalcitrance of its seeds and the irregular fruiting which makes its sexual regeneration difficult. Therefore, asexual regeneration has been set as the appropriate corridor for its domestication. Nonetheless, this way has encountered difficulties, particularly in the continuous growth of cuttings due to the weak development of its root systems. The optimization of plant mycorrhizal symbiosis is a key factor to overcome this constraint. This justifies the aim of this work which was to develop a method of producing *P. africana* cuttings inoculated with its native Arbuscular mycorrhizal fungi (AMF) to improve its regeneration. To undergo this purpose, soils samples were collected in three Mounts (Cameroon, Oku and Manengouba), then, isolation and characterization of AMF species was done after the fungi trapping culture followed by a pre-germination, multiplication and evaluation of their growth efficiency. To see their real efficiency on *Prunus* root system, each AMF species multiplied has been used as biofertilizer on *Prunus* cuttings in a frame, where indol butyric acid (AIB) was used as positive control and water was applied as a negative control. The result revealed the presence of six species of AMF belonging to the genera *Gigaspora* (1), *Glomus* (3) and *Acaulospora* (2). Each AMF species showed a great capacity of growth improvement and self-multiplication. Inoculated AM fungi increased the root density of *Prunus africana* cuttings with an average root number of 35 for the more efficient species (*Gigaspora margarita)*, compared to the positive control which had an average root number of 10 and the negative control 4. These results can contribute to the improvement of *P. africana* regeneration.

Keywords Domestication, Cuttings, Biofertilizer, Symbiosis, Root density

1. Introduction

Prunus africana (Hook.f.) Kalkman commonly known as Pygeum, is a mountainous tree species from tropical Africa (Betti et *al.*, 2014). It grows on volcanic soil and in cool climates at high altitude between 900 and 3000 m (anonymous, 2015). This plant is much better known for the healing properties of its back extracts, used for the manufacture of more than nineteen drugs, sold by European and American companies for more than 30 years for the treatment of benign prostatic hyperplasia (Cunningham et *al.*, 2002), as well as many other related disorders such as cancer. However, the plant produces a low quantity of secondary metabolites in their natural environment and this has justified the large exploitation of its bark to obtain the active compounds essential for global therapeutic needs. Thus, a world demand of 4000 tons per year (Cunningham et *al.*, 2002; Jimu, 2011) has resulted to an increase in the species' mortality rate due to barking (Sunderland and Tako, 1999). Domestication, the creation of large-scale nurseries and plantations are among other techniques put in place in order to maintain the availability of *P. africana* bark (Cunningham and Mbenkum, 1993). Nonetheless, this domestication

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remains limited to the problem of seed availability and adaptation of cuttings to weaning during transplantation, due mainly to the weak development of their root system. This is when rhizospheric microorganisms such as arbuscular mycorrhizal fungi (AMF) come into play, whose plant growth promotion abilities help to boost the development of the plant root system (Barea et *al.*, 2002). They play a key role in the production of phytohormones such as AIA (natural auxin) and ZR (Zeatin Riboside) (Torelli et *al.*, 2001; Ludwig-Müller and Güther, 2007) and ensure root system development (Morrison et *al.*, 1993; Johansson et *al.*, 2004; Smith and Read, 2010).

Arbuscular Mycorrhizal fungi (AMF) are symbiotic associations between plant and fungi. AM fungi get almost all their carbon as glucose from the host plant (Bago et *al.*, 2000). AMFs are obligate biotrophs, they need a plant partner to support their nutrition and complete their development cycle. However, the only phase in their life cycle where the fungus does not depend on the host plant is that of spore germination (Bago et *al.*, 2000), as they use their accumulated carbon reserves during their development and can therefore be induced *in vitro* under appropriate conditions (appropriate substrate) before their transfer to a young root system of a host plant. In return, AMF bring tremendous benefits to plants. They provide better nutrition in minerals such as phosphorus (Harrison, 2005; Helgason and Fitter, 2005; Feddermann et *al.*, 2010), nitrogen and other essential cations such as zinc, copper, manganese and iron (Liu et *al.*, 2000).

The presence of Arbuscular Mycorrhizal fungi (AMF) in the rhizosphere of *Prunus* plants amplify their root system. By obtaining all their carbon substances from their host plant, AM fungi in return with the help of their hyphal network, draw minerals and water from the soil, thus increasing their hosts' nutritional resources, mainly for phosphorus which also protects them against saline stress and several types of pests (Bago et *al.*, 2000; Helgason and Fitter, 2005). The work of Njeudeng (2001) and Nzweundji (2015) on the mycorrhizal status of *P. africana* in Cameroon, revealed that the plant is mycotrophic. It would therefore be wise to scrupulously define the mycorrhizal fungi species active in the rhizosphere of the plant in order to make their production profitable to the domestication of this plant. Hence the general objective of this study was to develop a method of production of *P. africana* cuttings inoculated with native mycorrhizal fungi in order to improve its regeneration and domestication. Specifically, it was to morphologically describe the mycorrhizal fungi subservient to *P. africana* then multiply them for production of plant-specific fertilizers as well as the evaluation of their efficacy on plant growth promotion.

2. Materials and Methods

Description of the sampling sites

Samples (soils and fine roots of *Prunus*) were collected from three sites: Mount Cameroon, Mount Manengouba and Mount Oku. Mount Cameroon is located in a wet forest area of the agro-ecological zone IV at 4100 m above sea level, with monomodal rainfall and a black-colored soil, furnished with granitic crystals; the geographical coordinates of sampling site: 4°44 'N, 9°32' E. Mount Manengouba is located at an altitude of 3211 m, humid forest area of the agro-ecological zone IV, also with single-mode rainfall, with a very dark black soil; the geographical coordinates of sampling site: 5° 6 'N, 10° 7'E. Mount Oku is located at 2000 m altitude; in the western highland area of the agro-ecological zone III, with a slightly black (brown) soil; the geographical coordinates of sampling site: 7° 15 'N, 11° 15' E.

The method used for soil sampling was that of Sieverding (1991). For each site, an area of one hectare was delimited and subdivided into 20 quadrants measuring 25 × 20 m^2. In each of these quadrants, one plant of *P. africana* was randomly selected, the roots were collected between 0 to 45 cm together with a soil sample around the rhizosphere of the tree using an auger. At each quadrant, a total of 500 g of soil sample were collected and soil samples from the same site were mixed to make a composite sample of 10 kg of soil per site. The roots were sampled at the same points of soil sampling at a rate of 8g per tree. The collected roots from the same site were also mixed and stored in 50% of alcohol solution.

Isolation and characterization of AMF spores

To assess the association between *Prunus* and AM fungi present in their rhizosphere, root staining was done by a modified method described by Ngonkeu (2009). The roots were cut and introduced into test tubes, 10% KOH was added and the mixture was allowed to stand for 15min at 90℃ in a water bath. Then 30% of hydrogen peroxide (H_2O_2) was introduced into the mixture for root thinning. The roots were removed and rinsed three times with distilled sterile water and then soaked in 10% HCl solution for 15 min at 90℃ in a water bath to remove excess of KOH so as to prepare the roots for staining. The roots staining was done with 0.01% methyl blue heated at 90℃ in a water bath for 35 min and rinsed three times with distilled sterile water before observation using an optical microscope (WILD M2B, Germany).

To optimize the density of the AM fungi spores, present in the soil samples collected, fungi trapping culture was done following the method described by Ngonkeu (2009). A 3L jar was subsequently filled at 1/3 with sterilized sand, completed to 2/3 volume with 1kg inoculum (collected soil) and then filled with sterilized sand. The sterilization was done at 121℃ for 1h in an autoclave (model PTS-B100L). Soil samples from each site were used to fill 10 pots on which two highly mycotrophic plants: *Vignia onguiculata* and *Sorghum bicolor* were planted and disposed in a greenhouse, where they were allowed to grow for three months, during which the plants were regularly watered. After three months of development, these plants were

subjected to water stress in order to stimulate the sporulation of mycorrhizal fungi in association with them.

To isolate the AMF spores obtained after trapping, extraction was performed according to the method proposed by Brundrett and *al*., (1996). After homogenization of the substrate, 100 g were removed and introduced into a beaker of 1000 ml. 300ml of distilled water was added followed by stirring of the mixture then allowed to stand for 15 seconds. Four sieves were arranged in decreasing order of mesh size 710 µm, 250 µm, 125 µm and 45 µm in which the supernatant of each mixture was filtered. Then the content of the last three sieves was collected, washed and observed with stereo microscope (WILD M2B, Germany). The observed spores were characterized and identified according to the Morton (1988) method based on size, shape, hyphae, presence or absence of the suspensory bulb, spore-forming saccules, germination loops, and colors. Then they were grouped by species and named Tx (x being a number assigned in the order of discovery) then counted according to the method described by Ngonkeu (2003).

In order to optimize the symbiosis between the identified AM fungi and the tall plant (*Soghum bicolor*), a purification and pre-germination of the spores on agar media were conducted following the method described by Ngonkeu (2009). In brief, the identified spores were introduced into Eppendorf tubes for sterilization with 3ml of 2% Chloramine T followed by vortexing for 20min. After removal of chloramine T, the spores were rinsed three times with sterile distilled water. 3ml of Streptomycin 0.025% was then introduced into the tubes; the mixture was stirred for 20 minutes followed by 3 times rinsing with sterile distilled water for 15 min. The disinfected spores were sown base on morphotype in Petri dishes containing agar medium (0.7%) at the rate of 4 spores per dish with three replicates. The Petri dishes were then sealed and incubated in the dark for at least 4 days at 30℃.

Evaluation of the identified AMF efficiency on the vegetative development of Prunus africana

To optimize and intensify the activity of the identified AM fungi, purification of the strains was performed using the method described by Ngonkeu (2009). The pre-germinated spores were inoculated on the roots of a mycotrophic plant (*Sorghum bicolor*) and then gently introduced into 3L pots filled with sterilized sand. In order to test if the AM fungi efficacy of promoting growth dependent on the number of spores initially present in the medium, an experiment was conducted with six treatments and one negative control without inoculation. One treatment consisted of 6 Sorghum plants (Bafia's variety) inoculated with one AMF specie with two variables (1 spore and 10 spores) and three replicates. The plants of a treatment (same AMF species) were grouped in a tray of $8m^2$. The negative treatment consisted of plants not inoculated with AM fungi. The plants were regularly watered by capillary action for three months and growth parameters such as stem length, number and leaf surface area were recorded to verify the effectiveness of the mycorrhizal symbiosis. Then the plants were subjected to water stress to stimulate sporulation. Finally, extractions were carried out to confirm the multiplication of spores and the reliability of obtaining a biofertilizer whose efficiency could be evaluated on *Prunus* cuttings.

The efficacy of the identified AM fungi strains was evaluated on the vegetative propagation of *Prunus* cuttings in a greenhouse following the method described by Leakay et *al*., (1994). A treatment consisted of *Prunus* cuttings sown on substrates (sand) inoculated with a biofertilizer (spores of AM fungi). Indol Butyric Acid (AIB) which is a root growth inducing hormone, was used as a positive control at a dose of 75 µg per cutting. The negative control included cuttings grown on a substrate with no AM fungi applied (no biofertilizer). The experimental unit consisted of 15 cuttings. After 25 days of propagation, the cuttings of the different treatments were evaluated on the basis of their root density in comparison with that of the controls and the experiment was repeated three times.

3. Results

Isolation and characterization of Arbuscular Mycorrhizal fungi spores

The soil samples collected showed different characteristics depending on their sampling site. Thus, we obtained more morphotypes in Mount Cameroon site (9) compared to Mount Oku (6) and Mount Manengouba (4). Root staining revealed the presence of mycorrhizal fungus propagules such as vesicles and hyphae inside the roots, characteristic of endomycorrhizal symbiosis (fig. 1).

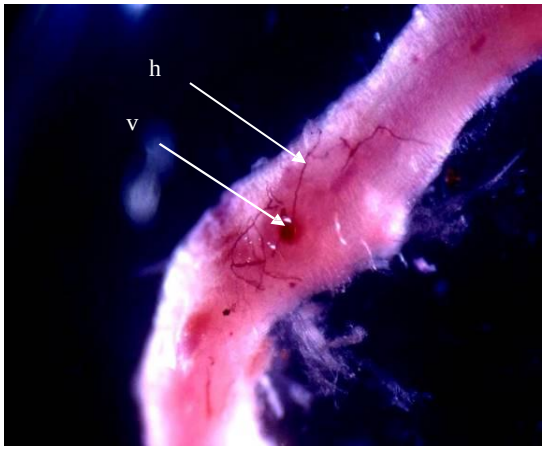

Figure 1. Arbuscular Mycorrhizal fungi structures (h: Hyphea and v: Vesicles) in roots fragments of *P. africana*

A set of 15 morphotypes were observed and labelled from T1 to T15 and T1 was identified to be the ubiquitous morphotype. Table 1 shows the number of morphotypes that were isolated per site, followed by the number of morphotypes that were retained after separation of morphotypes into specific and non-specific strains to *P. africana* (fig. 2). The characterization of the morphotypes specific to *P. africana* allowed the identification of 6 species

of AMF belonging to three genera, namely *Gigaspora*, *Acaulospora* and *Glomus* (Table 2).

Table 1. *Prunus*-specific Morphotypes per site

Sites morphotype	Mt Ca	Mt Oku	Mt Ma
Morphotypes isolated	9	6	4
Morphotypes retained	2(T1,T7)	3(T1,T10,T11)	3(T1,T14,T15)

The pre-germination of AMF spores on agar media carried out after 5 days under darkness allowed us to visualize the effective initiation of spore germination of AM fungi species identified with visible colonization of the culture medium by the mycelial hyphae (Fig 3).

Evaluation of the efficiency of AMF on the vegetative development of Prunus africana

The extraction of the spores carried out in 100 g of substrate after purification has demonstrated the effectiveness of their multiplication from one or ten spores, thus making it possible to obtain a biofertilizer that does not depend on the number of spores seeded (Table 3).

Table 2. Identification of characterized morphotypes

Morphotypes	Color	Form of spore	Diameter of spore	Genera /species
T1	chestnut	Globular	125- 200 µm	*Glomus aggregatum*
T7	white	Globular	45-125 µm	*Gigaspora margarita*
T10	chestnut	Sub-globular	125-250 µm	*Acaulospora sp*
T11	Yellow-bright	Sub-globular	100-250 µm	*Acaulospora sp*
T14	Yellow-dusky	Globular	125-250 µm	*Glomus sp*
T15	Yellow-bright	Globular	45-125 µm	*Glomus sp*

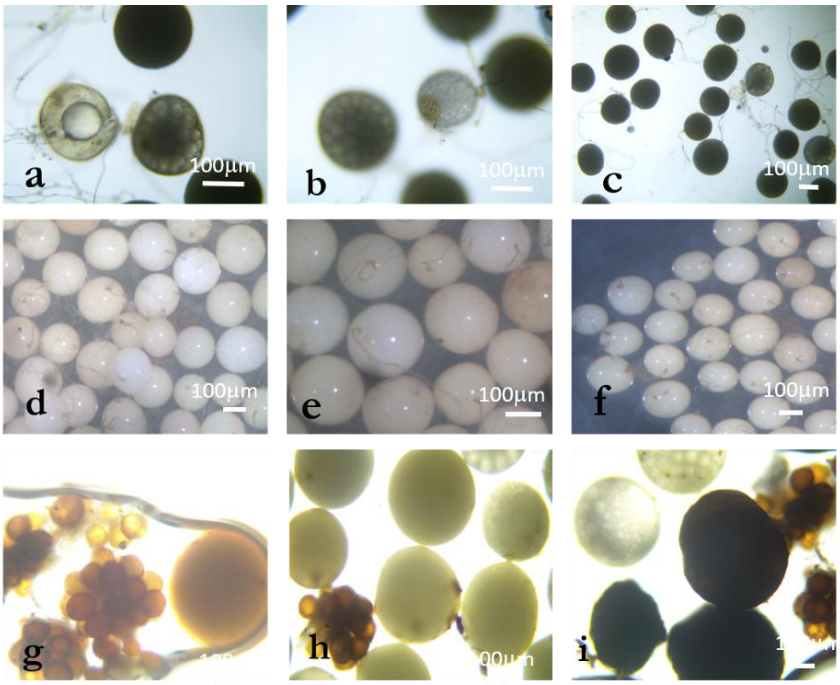

Figure 2. Spores of the characterized morphotypes; **a, b** and **c**: *Acaulospora*; **d, e** and **f**: *Gigaspora*; **g, h** and **i**: *Glomus*

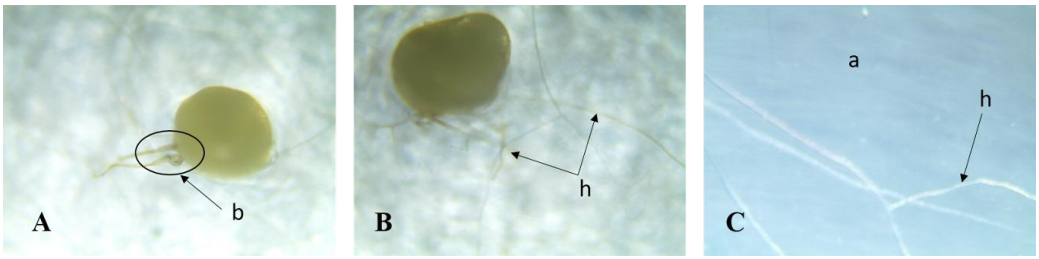

Figure 3. Germination and growth of spores on agar medium. A: b = germination loop of the spore on filter paper; B: h = mycelial filaments from germination of the spore on the filter paper; C: a = agar medium

Table 3. Multiplication of CMA spores

Morphotypes	1 spore	10 spores
T1	75	145
T7	56	93
T10	18	45
T11	43	82
T14	108	206
T15	33	101

The staining of the roots after purification of AMF species help us to assess the presence of mycorrhizal fungus propagules inside these roots, testifying to the effectiveness of symbiosis (Fig 4). Growth parameter data was recorded and analyzed to appreciate the efficacy of the mycorrhizal strains used as biofertilizer. The inoculated plants showed a significant growth difference compared to control treatments (Table 4). Regarding the length of the stem, inoculated plants recorded a higher growth than non-inoculated plants both after one and six weeks. For instance, plants inoculated with morphotype T7 had an average stem length of 19 and 40 cm after one and six weeks respectively, while control plants recorded an average length of 12 and 24 cm during the same period. For the number of leaves, morphotype T7 registered an average number of 5 and 9 leaves after one and six weeks respectively, while the control plants registered an average of 3 and 6 during the same period. For the length of leaves, morphotypes T7 had an average length of 14 and 30 cm after one and six weeks respectively, while the control plants registered an average of 11 and 28cm during the same period. However, the growth promotion did not depend on the number of spores inoculated.

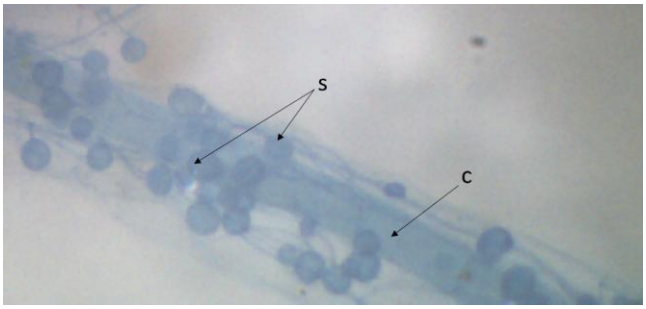

Figure 4. Colored roots of Sorghum bicolor plants after trapping (s = Intra-root spores, c= root cortex)

Table 4. Summary of the influence of the treatments according to the parameters

	LS		NL		LF	
Morphotype	S1	S6	S1	S6	S1	S6
Control	12.15±1.56	23.83±23.83	3.00±00	5.50±0.84	11.38±1.90	27.72±2.77
T10	15.75±3.3	20.7±2.44	3.50±0.84	5.67±0.82	9.92±2.40	28.67±5.87
T1	14.18±1.4	19.50±3.02	3.17±0.41	4.33±0.52	8.28±1.97	23.28±3.44
T14	15.38±2.2	38.67±5.57	3.17±0.41	5.17±0.75	11.42±1.36	24.50±2.53
T7	18.95±3.1	40.19±9.85	4.50±0.55	8.67±6.67	13.68±1.42	29.58±3.97
T11	17.02±3.24	32.50±8.02	3.17±0.41	6.00±0.63	11.20±2.80	28.15±1.63
T15	17.28±1.37	26.50±3.89	3.33±0.52	6.33±0.52	12.17±2.71	27.15±3.36

Caption: L.S = length of the stem in cm, N.L = number of leaves, LF = length of the leaf in cm, S1 = week1 and S6 = week6.

Table 5. Avearge number of roots produced by cuttings per treatment groups

Treatment	Number of roots
Control (negative)	4.00 ± 1.80^{a}
AIB (positive control)	10.00 ± 1.88^{de}
T1 (*Glomus aggregatum*)	24.71 ± 6.88^{b}
T7 (*Gigaspora margarita*)	35.00 ± 10.57^{f}
T10 (*Acaulospa sp*)	10.78 ± 3.72^{de}
T11 (*Acaulospora sp*)	17.43 ± 4.29^{c}
T14 (*Glomus sp*)	20.36 ± 4.52^{bc}
T15 (*Glomus sp*)	15.00 ± 3.60^{cd}

Same letters in column show no significant difference between treatment groups based on a Student-Newman-Keuls pairwise comparison test at 0.05 threshold.

For the root density evaluation, there was a significant difference in the root promotion effects (Table 5). 25 days post-inoculation, the most effective mycorrhizal strain was T7 corresponding to the species of *Gigaspora margarita* with an average root number of 35 per cuttings (fig. 5). The low performing strain was T10 corresponding to the species of *Acaulospora* sp. with an average of 10.78 roots per cuttings. During the experimentation, the negative control (no inoculation) had the smallest number of roots (4) and the positive control (AIB) had an average root number of 10 (Table 5).

Figure 5. *Prunus* cuttings, a: root system of the inoculated cuttings; b: root system of un-inoculated cuttings

4. Discussion

Isolation and characterization of Arbuscular Mycorrhizal Fungi

The number of morphotypes recorded in the Mount Cameroon (9), Mount Oku (6) and Mount Manengouba (4) sites has reflected the diversity of these sites. These results did not match with those obtained by Nzweundji and *al*. (2015) in the same sites, which at that time had fewer morphotypes (6). This difference could come from the fact that the fungi present in these sites have adapted and evolved. Over time, the environment would have become favorable to accommodate new species according to the variations in soil composition influenced by the growth of different plant species (Ngonkeu, 2009).

The morphological identification carried out in this study made it possible to determine the different families of mycorrhizal fungi colonizing the roots of *P. africana* which were found to be: *Gigaspora margarita (*Gigasporaceae), *Acaulospora* sp (Acaulosporaceae) and *Glomus aggregatum.* These species were characterized using morphological characters: the shape, size, color and external appearance of the spore. The morphological identification has many limitations (Schüßle et *al.*, 2001; Zeramdini, 2009) but the results obtained were in line with those of Tchiechoua et *al.,* (2015). As mycorrhizal fungi are obligatory symbionts, to ensure their symbiotic efficiency, the Arbuscular Mycorrhizal fungi spores identified were previously pre-germinated on an agar medium. This technique made it possible to have almost all spores germinated with young mycelial filaments active in the formation of mycorrhizal associations. This result is similar to that obtained by Ngonkeu (2009), in the same culture substrate.

Evaluation of the efficiency of Arbuscular Mycorrhizal fungi on the vegetative growth of Prunus africana cuttings

Spores are organs for the perpetuation of species. Under favorable conditions, these spores establish symbiotic (mycorrhizal) relationships with plant roots for mutual growth. When these symbioses break up, Arbuscular mycorrhizal species remain thanks to sporulation. This principle has been highlighted in this study. The results of the multiplication of mycorrhizal fungi from a single spore and from several spores have shown that these beings multiply and colonize their living environment regardless of their starting number.

Functional diversity can be determined through an assessment of the variability in plant response to mycorrhizal inoculation. This was significantly recorded after inoculation of 6 AM fungi strains on *Sorghum* plants in a greenhouse. Morphologically identical spores introduced into culture gave an identical response on the growth and development of *sorghum* plants, thus demonstrate good identification. This result showed a slight difference with Ngonkeu's work (2009), which was probably due to a difference in precision during the morphological characterization. Analysis of the data showed that there was no significant difference in growth of plants inoculated with one spore and that with several spores, in accordance with the work by Jansa et *al.*, (2008) and Olfat and Jalil (2012) on root infections and multiplication of spores. This could be explained by the fact that the establishment of a mycorrhizal symbiosis depends on the quality of the information transmitted by the partners present, the information being transmitted by Strigolactone for the plant to induce a Mycfoctor response in the Fungi. This exchange is made independently of the amount of AM fungi in the medium or substrate (Jalil and Olfat 2012).

The rooting capacity of *P. africana* cuttings can be improved by growth hormone (Avana, 2006). The results of this study showed that the contribution of indole butyric acid (AIB) treatment stimulated the rooting of *P. africana* cuttings, but mycorrhizal fungi induced a more pronounced development of root system. This can be explained by the fact that mycorrhizal fungi, by intensifying the nutrient absorption surface of the plant partner, protect them against root pathogens, stimulate the production of their natural phytohormones (essential for rooting) and contribute to the increase of their active substances (Augé, 2004). In return they receive their carbon nutrients from the plants (Ludwig-Müller and Güther, 2007). Hence, the contribution of the latter becomes incomparable to any other exogenous contribution, in line with the work by Veresoglou et *al.*, (2012) on the impact of AM fungi on root development, nutrition and protection of plant cuttings.

5. Conclusions

The general objective of this work was to assess the efficiency of native AMF species in the improvement of the growth of root cuttings to ameliorate the regeneration of *P. africana*. From this work, six species of AM fungi belonging to *Gigaspora*, *Glomus* and *Acaulospora* genera were identified to be dominant and active in the rhizosphere of *P. africana* in Cameroon. Among the species, *Gigaspora margarita* was found to be the best arbuscular mycorrhizal in the improvement of *Prunus africana* cuttings development. Their effects on root system development were more pronounced compare to those of plant growth hormone AIB. The use of these particular species in the vegetative regeneration of *Prunus Africana,* can improve their root system density and enable cuttings to effectively resist field transplantation. This mycorrhizal symbiosis could represent an innovative approach to solve the problem of *Prunus africana* regeneration.

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