Variability and Genetics Correlations between Some Nutritional Components of Cowpea Leaves

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Abstract
In the northern part of Cameroon, cereals are the staple food. The diversification of this diet necessitates the introduction of foods rich in proteins and minerals and justifies the qualitative improvement of cowpea leaves. The purpose of this study is to determine the variability of the calcium, copper, magnesium, phosphorus, zinc content and the total protein content of cowpea leaves and to verify the correlations between these different nutrients of the cowpea leaves studied. Plant material consists of fifteen entries from national and international research institutes. Biochemical analyzes were performed on leaves from field trials using a randomized complete block device. Analysis of variance for all quantitative parameters studied showed a significant genotype effect ($p < 0.001$). Factor analysis has shown that to improve these nutritional traits one has to choose the parents with significant and positive correlations for the total mineral and protein contents in selection. The integration of these nutritional traits into the breeding program could combat food insecurity and food insecurity in the high Guinean savannah zone of Cameroon.

Keywords: Vigna unguiculata, minerals, crudes proteins, leaves

1. INTRODUCTION

Cowpea *Vigna unguiculata* (L.) Walp. is a diploid legume (Faris, 1964), preferably self-pollinated (Tchuenguem *et al.,* 2009**)** grown mainly in the tropical and subtropical savannah regions of Africa, Asia and South America (Maréchal, 1970). Cowpea is consumed from the seedling stage to the harvest (seedling, young leaves, young pods, immature pods, dry pods) and is used in the confection of several African dishes (Akundabweni *et al.,* 1991; Abizari *et al*., 2013). Cowpea leaves are eaten as vegetables (Abizari *et al.,* 2013; Fofiri Nzossié *et al.*, 2008).

In Botswana and Zimbabwe, cowpea leaves cooked in water are kneaded and the pulp thus obtained is compacted into small pellets, which are then dried to preserve them (Pasquet and Baudoin., 1997; Langyintuo *et al.,* 2003). Cowpea leaves are also used in the poultice to treat swelling, skin infections and dental pain (Grubben and Denton, 2004. These leaves contain many proteins, vitamins and minerals essential for human nutrition (Hall *et al.*, 2003). In poor countries access to meat, a source of protein and minerals is limited. As a result, their diet is based on cereals (Nguefack *et al.,* 2015). For this reason, the search for inexpensive and reliable alternative sources of proteins and minerals of vegetable origin seems necessary (Bhat and Karim., 2009). The agronomic bio-fortification of locally consumed cowpea genotype leaves seems to be a plausible solution to this problem.

The total mineral and protein content of cowpea leaves varies with agro ecological zones, genotypes and stage of harvest, which justifies this research. The genetic improvement of the nutritional value of cowpea leaves and the selection of genotypes therefore represent major scientific and nutritional issues. Information on the genetic variability of cowpea leaf food composition is the first step towards the development of improved cultivars. To this end, it is necessary to determine the variability of some mineral elements and the crudes proteins content of cowpea leaves. It would also be wise to check the correlations between these different nutrients of the cowpea leaves studied.

2. Material and methods

2.1. Experimental site

Research was conducted at the University of Ngaoundere experimental farm in Dang (latitude 7°28' N, longitude 13°34' E, altitude 1.100m).This region belongs to the high Guinean savannah agro ecological zone (Adamawa, Cameroon). The soil is of ferruginous type, developed on basalt and it has a brown reddish clay texture, with a relatively high fertility.

Soil surface (0–15cm) analysis revealed a pH value of 5.3; with 9.6mg.kg^{-1} organic matter, total N content of 0.09% and Bray P content of 34mg.kg^{-1}.

Climate is characterized by two seasons with an average annual rainfall of 1480mm that is fairly distributed over the rainy growing period (April to September). Average annual temperature is 22°C, while annual hygrometry is about 70% (Westphal *et al.*, 1985).

2.2. Plant material

Experimental material was composed of 15 pure lines of cowpea including two improved varieties from the National Institute of Agricultural Research for Development (IRAD, Cameroon): 24-130 and VYA; six registered genotypes originated from the International Institute for Tropical Agriculture (IITA, Nigeria): IT81D98, IT97K-573-1-1, 24-125B, IT98K-205-8, IT93K-693-2 and IT97K-819-118; four registered genotype originated from Burkina and IITA in a vulgarization program in Chad: KVX414-22-2 (Burkina), KVX61-1 (Burkina), Vita5 (IITA) and Gorom (Burkina); one variety selected in Botswana (B301); one registered genotype from the Institute of Environment and Agricultural Research of Burkina (INERA): (TVX32-36) and one improved variety from the National Institute of Agronomic Research of Niger (INRAN): (TN5-78).

2.3. Field Experiments

During the growing season 2015, all 15 pure lines were arranged in a triplicated randomized complete block design (RCBD). Each plot unit was consisted of one row of 4m length x 0.5m broad, spaced 30cm apart. Cowpea seeds were sown at an intra-row spacing of 20cm.Three seeds were sown per hole and after germination one healthy seedling was retained at each hole after thinning.

NPK mineral fertilizer (7% N, 14% P2O5, 7% K2O) was used at rate of 60kg per ha on the experimental plots prior to planting. All standard agronomic practices i.e., hoeing, weeding and irrigation etc. were adopted uniformly. Mechanical weed controls was regularly done three weeks after plant emergence and subsequently during the vegetative stage and maturing stage. Cowpea plants were fully protected against insects through regular spray of insecticides (Optimal® 20 SP and Cypercal®).

2.4. Biochemical analysis

Crudes proteins content was determined by the Kjeldahl'smethod (AOAC, 2002.). Mineral contents were determined by Atomic Absorption Spectrometry (IUPAC, 1997).

2.5. Statistical analysis

The analysis of variance (ANOVA) and Duncan's nutrient test were performed using the STATGRAPHICS PLUS version 5.0 software (Statgraphics, 1997). Estimates of genetic correlation coefficients were provided by the XLSTAT Version 2007.8.04 program.

3. RESULT

3.1. Variability

The calcium, copper, magnesium, phosphorus, zinc and crudes proteins contents for the 15 pure lines tested are shown in Table I. The analysis of variance showed a significant genotype effect ($p < 0.001$).

The genotype IT97K-573-1-1 (8.40mg.kg^{-1}) contains more calcium. In contrast, TN5-78 genotype (4.60mg.kg^{-1}) contains less calcium. The average production of calcium is 6.40mg.kg^{-1} with a coefficient of variation of 16.8%. For copper content, the richest copper genotypes are 24-130 (13.7mg.kg^{-1}) and 24-125B (13.5mg.kg^{-1}) while TN5-78 genotype (9.67mg.kg^{-1}) is the poorest copper. The average copper content is 11.86mg.kg^{-1} with a coefficient of variation of 10.4%. For phosphorus content, genotypes with high phosphorus levels are: TVX32-36 (0.42mg.kg^{-1}) and B301 (0.44mg.kg^{-1}). In contrast, the low phosphorus genotype is VYA (0.19mg.kg^{-1}). The average phosphorus content is 0.31mg.kg^{-1} with a coefficient of variation of 25.5%. The richest zinc genotype is IT98K-205-8 (44.4mg.kg^{-1}). On the other hand, the genotype TVX32-36 (35.4mg.kg^{-1}) has low zinc content. The average zinc content is 39.5mg.kg^{-1} with a coefficient of variation of 6.66%. For crudes proteins content, IT97K-573-1-1 genotype (26.5%) contains more crudes proteins. On the other hand, the genotype TVX32-36 (18.6%) is low in crudes proteins. The average crudes proteins production is 23.0% with a coefficient of variation of 9.30%.

3.2. Factor analysis

Figure 1 shows the mapping of genotypes on the axis plane F1 and F2 and explains 73.14% of the results. On this map, we see that the IT97K-573-1-1, B301 and IT81D-98 genotypes are positively correlated with the F1 axis, which explains 43.78% of the results. The 24-125B and 24-130 genotypes are strongly correlated with the F2 axis (29.36%).

Table I: Biochemical composition of genotypes in cowpea leaves

Genotypes	Calcium ($mg.kg^{-1}$)	Cuivre ($mg.kg^{-1}$)	Iron ($mg.kg^{-1}$)	Magnesium ($mg.kg^{-1}$)	Phosphorus ($mg.kg^{-1}$)	Zinc ($mg.kg^{-1}$)	Crudes Proteins (%)
24-125B	$5.45±0.21^{f}$	$13.48±0.04^{gh}$	$370.52±28.55^{i}$	$0.330±0.03^{h}$	$0.34±0.02^{bcde}$	$37.74 ± 0.34^{gh}$	$24.25±2.02^{bcd}$
24-130	$7.25±0.44^{b}$	$13.68±0.25^{h}$	$425.63±35.33^{h}$	$0.370±0.03^{h}$	$0.36±0.07^{abcd}$	$36.67 ± 0.25^{ghi}$	$25.52±1.31^{ab}$
B301	$8.40±0.91^{a}$	$12.29 ±0.30^{e}$	$625.12±35.44^{c}$	$0.835±0.02^{c}$	$0.45±0.01^{ab}$	$36.31 ± 0.27^{i}$	$25.44±1.01^{abc}$
Gorom	$5.60±0.61^{e}$	$11.64±0.19^{d}$	$440.29±14,55^{fgh}$	$0.455±0.01^{g}$	$0.25±0.08^{def}$	$40.63 ± 0.18^{de}$	$21.96±1.23^{ef}$
IT81D-98	$7.35±0.22^{b}$	$11.74±0.34^{d}$	$755.08±7.44^{a}$	$0.960±0.02^{b}$	$0.36±0.05^{abcd}$	$39.60 ± 0.14^{e}$	$22.48±0.77^{ef}$
IT93K-693-2	$6.45±0.31^{cd}$	$13.26±0.37^{g}$	$470.33±28.11^{f}$	$0.545±0.01^{f}$	$0.33±0.04^{cde}$	$38.25 ± 0.35^{fg}$	$23.20±0.33^{de}$
IT97K-573-1-1	$8.25±0.44^{a}$	$12.66±0.27^{f}$	$770.45±28.74^{a}$	$1.030±0.03^{a}$	$0.46±0.03^{a}$	$40.30 ± 0.28^{de}$	$26.50±0.51^{a}$
IT97K-819-118	$5.25±0.34^{f}$	$10.69±0.28^{c}$	$525.05±35.98^{e}$	$0.525±0.04^{f}$	$0.26±0.01^{def}$	$41.30 ± 0.28^{cd}$	$22.28±1.03^{ef}$
IT98K-205-8	$6.70±0.63^{c}$	$10.26±0.35^{b}$	$435.17±21.00^{gh}$	$0.460±0.06^{g}$	$0.24±0.04^{ef}$	$44.40 ± 0.14^{a}$	$21.11±0.92^{fg}$
KVX414-	$6.35±0.82^{d}$	$12.38±0.18^{ef}$	$545.68±7.77^{de}$	$0.425±0.04^{g}$	$0.25±0.01^{def}$	39.52 ±	$24.51±1.41^{cd}$

22-2						0.03^{ef}	
KVX61-1	5.60±0.31^{e}	12.28±0.32^{e}	465.11±21.88^{fg}	0.335±0.02^{h}	0.34±0.08^{bcde}	36.64 ± 0.19^{hi}	19.96±0.91^{gh}
TN5-78	4.65±0.72^{g}	9.68±0.25^{a}	660.05±14.50^{b}	0.640±0.02^{e}	0.27±0.02^{def}	42.34 ± 0.23^{bc}	21.71±1.13^{f}
TVX32-36	5.60±0.51^{e}	10.53±0.04^{bc}	637.38±17.66^{bc}	0.545±0.01^{f}	0.43±0.10^{abc}	35.35 ± 0.21^{i}	18.66±2.21^{h}
VITA5	6.70±0.93^{c}	12.63±0.18^{f}	625.95±35.43^{c}	0.855±0.01^{c}	0.27±0.02^{def}	39.74 ± 0.33^{e}	25.04±0.09^{bc}
VYA	6.40±0.01^{e}	10.74±0.34^{c}	565.67±21.62^{h}	0.730±0.03^{d}	0.20±0.01^{f}	43.29 ± 0.23^{ab}	23.20±2.13^{de}
Means	6.40±0.11	11.9±1.23	554.67±12.14	0.60±0.23	0.316±0.08	39.47±2.63	22.9±2.13
LSD	0.18	0.29	32.55	0.041	0.11	1.28	1.38
CV (%)	16.83	10.4	21.9	37.4	25.5	6.66	9.30

Means with the same subscript within the same column do not differ ($p > 0.05$); LSD (0.05): least significant difference at 5% level

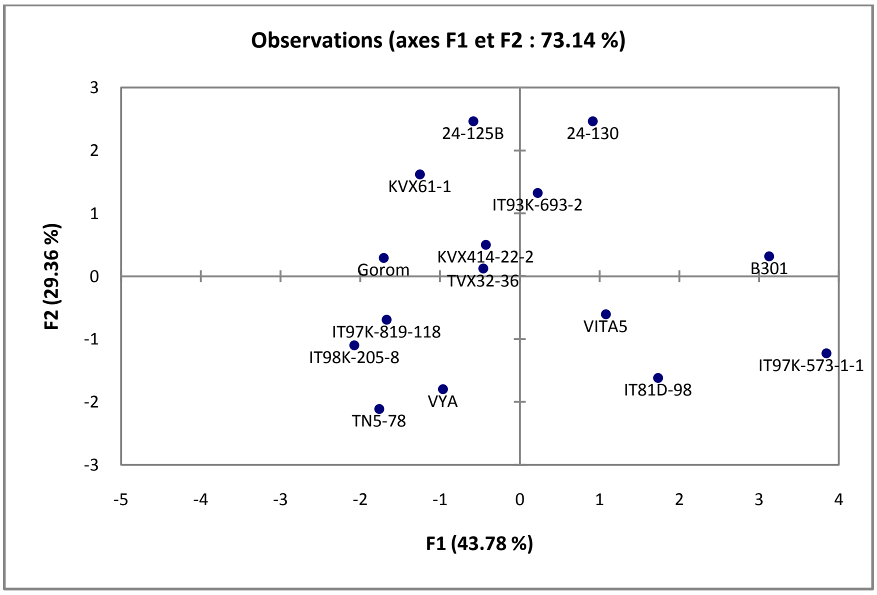

Figure 1: Mapping representation of genotypes on the axis plane F1 x F2

These observations seem unclear and inconclusive for the other individuals because of the weak contribution of these two significant axes in this study. It is therefore important to highlight the contributions of each on the different axes (Table II). Only genotypes with at least 5% contribution on each axis are considered significant.

The genotypes IT97K-573-1-1 (32.147%), B301 (21.263%), IT98K-205-8 (9.384%), TN5-78 (6.763%), IT81D-98 (6.523%), Gorom (6.344%) and IT97K-819-118 are correlated to F1 axis. Genotypes 24-125B (19.755%), 24-130 (19.771%), TN5-78 (14.389%), VYA (10.448%), KVX61-1 (8.531%), IT93K-693-2 (5.713%) and IT81D-98 (8.456%) are correlated to F2 axis. IT81D-98 and TN5-78 genotypes are highly correlated to both axes. This could be explained by the fact that these axes are very insignificant.

Figure 2 illustrates the correlation circle of variables studied (biochemical traits of cowpea leaves). This circle explains 73.14% of the results expressed in this study. Traits such as calcium content, magnesium content, phosphorus content and crude protein content are correlated with F1 axis which accounts for 43.78% of the results. Copper content, iron content, and zinc content are correlated with F2 axis (29.36%).

Table II: Contribution of cowpea genotypes on the main axes

Genotypes	F1	F2	F3	F4	F5	F6	F7
24-125B	0.75	19.76	1.72	5.73	12.01	7.67	3.34
24-130	1.82	19.77	2.92	2.11	0.44	6.27	3.53
B301	21.26	0.33	0.78	7.45	2.14	22.53	18.00
Gorom	6.34	0.28	1.12	0.01	0.43	0.98	3.82
IT81D98	6.52	8.46	2.81	0.02	18.56	5.26	2.10
IT93K-693-2	0.11	5.71	0.58	0.19	3.65	11.96	1.43
IT97K-573-1-1	32.15	4.87	0.30	0.02	14.22	14.40	8.73
IT97K-819-118	6.08	1.53	0.02	1.43	5.68	0.59	1.73
IT98K-205-8	9.38	3.88	3.46	53.06	0.62	2.80	0.706
KVX414-22-2	0.40	0.82	4.30	2.41	2.03	19.34	40.52
KVX61-1	3.42	8.53	9.14	0.17	6.61	2.88	2.36
TN5-78	6.76	14.39	1.83	13.28	16.66	1.07	0.89
TVX32-36	0.46	0.05	57.91	0.24	0.03	1.62	0.18
VITA5	2.52	1.18	5.16	13.71	13.02	0.14	7.60
VYA	2.02	10.45	7.98	0.20	3.93	2.50	5.07

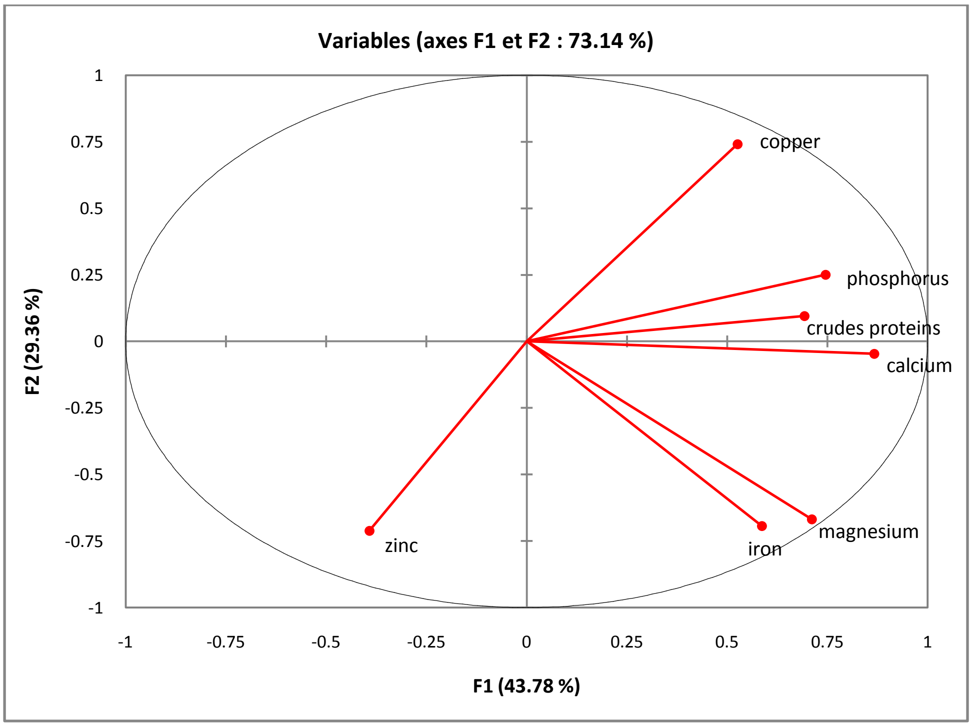

Figure 2: Circle of correlation of the traits on the axis plane F1x F2

The contribution of each trait in the representation of axes (Table III) makes it possible to better appreciate their significance. Thus, calcium content (24.50%), phosphorus content (18.15%), magnesium content (16.47%), protein content (15.66%), iron content (11.22%), copper content (9.01%) and zinc content (5.03%) have a strong contribution to the construction of F1 axis. Copper content (26.72%), zinc content (24.62%), iron content (23.35%) and magnesium content (21.72%) are significant on F2 axis.

Table III: Contribution of traits on the main axes

Traits	F1	F2	F3	F4	F5	F6	F7
Calcium	24.50	0.10	3.89	55.02	9.40	5.15	2.01
Copper	9.01	26.72	7.93	8.06	12.84	29.01	6.44
Iron	11.22	23.35	8.42	14.46	1.39	2.09	39.07
Magnesium	16.47	21.72	0.00	3.15	6.01	7.94	44.71
Phosphorus	18.12	3.05	23.49	4.56	37.56	13.21	0.01
Zinc	5.03	24.62	22.49	5.15	7.52	27.96	7.23
CrudesProteins	15.66	0.44	33.86	9.59	25.27	14.64	0.53

Pearson's correlation matrix (Table IV) shows existing links between the different traits studied. Values close to 1 and shown in bold are significant ($p <0.05$). Thus, a positive and significant correlation exists between calcium and magnesium ($r = 0.605$), calcium and phosphorus ($r = 0.529$), calcium and crudes proteins ($r = 0.647$), copper and crudes proteins ($r = 0.622$) and finally between iron and magnesium ($r = 0.883$). On the other hand, a negative and significant correlation exists between copper and zinc ($r = - 0.574$) and between phosphorus and zinc ($r = -0.701$).

4. Discussion

The results showed that calcium content is a variable trait. The average calcium content is higher than the results of Gerrano *et al.*, 2015 and Tchiégang and Kitikil, 2004 on cowpea leaves. However these results are inferior to those of Yoka *et al.*, 2014. These variations in the results would result from the fact that calcium richness depends on age of the harvested leaf (Megueni *et al.*, 2011). These different levels may also be due to variations in agro ecological conditions and determination methods (Yoka *et al.*, 2014).

Table IV: Pearson's correlation matrix for selected traits

Variables	Calcium	Cuivre	Iron	Magnesium	Phosphorus	Zinc	Crudes proteins
Calcium	**1**						
Cuivre	0.431	**1**					
Iron	0.390	-0.265	**1**				
Magnesium	**0.605**	-0.088	**0.883**	**1**			
Phosphorus	**0.529**	0.367	0.399	0.337	**1**		
Zinc	-0.158	**-0.574**	0.058	0.174	**-0.701**	**1**	
Crudes proteins	**0.647**	**0.622**	0.159	0.417	0.200	-0.011	**1**

Values in bold are significantly different from 0 at a level of significance $p < 0.05$

The average variability of calcium content seems insufficient and needs improvement (Hallberg *et al.,* 1992; Heaney, 1996). Genotypes with a good basis for this program are 24-130, B301, IT81D-98 and IT97K-573-1-1. On copper content of cowpea leaves, the results are lower than those reported by Yoka *et al.*, 2014 but higher than those of Gerrano *et al.*, 2015.

These results seem to show that copper content varies according to the agro ecological zones. Genotypes 24-125B and 24-130 appear to be better offspring for breeding this trait in the high Guinean savannah zone of Cameroon. Iron content results are higher than those of Okonya and Maass, 2014. The absorption of dietary iron is poor (Roberts and Heyman, 2000). For this reason, the improvement of cowpea leaves for a good source of iron is necessary and could help to recover a nutritional problem such as anemia in poor populations (Nguefack *et al.*, 2015). The best breeding stock seems to be IT81D-98 and IT97K-573-1-1.

The results on the magnesium content corroborate those obtained by Tchiégang and Kitikil, 2004. This rate is well above the required average (Rayssiguier *et al.*, 2001), which could explain the deficiencies in this micronutrient in the high Guinean savannah of Cameroon. To improve this trait, genotypes B301, IT81D-98, IT97K-573-1-1 and VITA5 are better indicated for this program. The results on phosphorus content are lower than those of Megueni *et al.*, 2011 in the high Guinean savannah of Cameroon.

Also on cowpea leaves and in the same area, Noubissié *et al.*, 2011 reported the range of 282 to 468mg.kg^{-1}. In the Democratic Republic of Congo, Yoka *et al.*, 2014 obtained a phosphorus content of cowpea leaves of 40.7mgkg^{-1}. This disparity in results can be explained by the fact that phosphorus accumulation in genotypes depends on aboveground biomass and phosphorus bioavailability in soil (Raposo *et al.*, 2004; Nwoke *et al.*, 2005; Ojo *et al.*, 2006; Ojo *et al.*, 2007). It is subject to very important variations; it depends mainly on the nature of the species, the age and the organ analyzed (Megueni *et al.*, 2011); it also depends, but to a lesser extent, on the richness of the soil (Nordeide *et al.*, 1996); it is very weakly dependent on the presence of other elements giving rise to antagonisms with phosphoric acid (Agbo *et al.*, 2009). In view of the fact that phosphorus is limiting in most soils in the Sudano-Sahelian zone, and that farmers rarely use fertilizer for cowpea cultivation, the selection of genotypes such as B301, IT97K-573-1-1 and TVX32-36 is necessary for the creation of varieties adapted to the agro ecological zone of the high Guinean savannah.

The overall mean value for zinc was similar to that reported by Mamiro *et al.*, 2011 in Tanzania. Similar results were also reported by Yoka *et al.*, 2014 on cowpea leaves

(441mg.kg^{-1}). However, Gerrano *et al.*, 2015 reported low zinc content on cowpea leaves (26.23mg.kg^{-1}). This could mean that zinc accumulation is a function of agro ecological zones. Mirand, 2003 had demonstrated nutrient and functional interactions between nutrients, which justifies the selection of higher zinc content varieties whose uptake could help its poor populations to develop their immune defense system.

The best genotypes for this program are Gorom, IT97K-573-1-1, IT97K-819-118, IT98K-205-8, TN5-78 and VYA. For the crudes proteins content, the results are lower than those of Adu - Daapah, 1999. Relatively higher values have been reported in Tanzania (Mamiro *et al.*, 2011), Nigeria (Nielsen *et al.*, 1997), Uganda (Okonya and Maass, 2014), Brazil, (Santos *et al.*, 2012) and Ghana (Ahenkora *et al.*, 1998). This slight difference in levels is due to the age at which the leaves are harvested (Adeyanyu, 2009).

The high crudes proteins content is a nutritional quality sought for the fight against protein malnutrition in the northern part of Cameroon where despite the pastoral potential, vulnerable populations have limited access to meat products (Nguefack *et al.*, 2015). The material currently distributed has a low crudes proteins level (Tchiégang and Kitikil, 2004). It would be wise to select or create genotypes with high protein levels. In this respect, genotypes IT97K-573-1-1, B301 and 24-130 constitute the basic spawners that can be integrated in the varietal creation programs.

The study showed that there is a significant and positive correlation between a few traits in selection. Positive and significant correlations suggest a close genetic association between these traits. Thus, a positive and significant correlation exists between calcium and magnesium, calcium and phosphorus, calcium and crudes proteins, copper and crudes proteins and finally between iron and magnesium. These associated traits can be improved simultaneously.

5. CONCLUSION

A wide genetic variability was noted for the biochemical traits of cowpea leaves in the high Guinean savannah zone. Factor analysis showed that there are positive genetic correlations for minerals and crudes proteins. The biochemical traits of leaves are essential criteria for the acceptability of cowpea varieties but are not found in sufficient quantity in all the leaves of the genotypes studied. It is therefore imperative to make a creative improvement in order to combine them adequately in a genotype.

REFERENCES

Abizari A., Pilime N., Armar-Klemesu M. and Brouwer I.D., 2013. « Cowpeas in Northern Ghana and the factors that predict care givers' intention to give them to school children ». *PLoS One*, 8:8

Adeyanyu A.O., 2009. Genetics of harvest index and leaf-yield indices in cowpea. *J. Crop Improv*, 23, 3:266-274.

Adu-Daapah H.K., 1999. Les feuilles de niébé se mangent aussi. *Spore* CTA, Wageningen, France n° 78, 8p.

Agbo E., Kouamé C., Mahyao A., N'Zi J.C. and Fondio L., 2009. Nutritional importance of indigenous leafy vegetables in Côte d'Ivoire. *Acta Hort*. 806, ISHS, 1:361-366.

Ahenkora K., Adu Dapaah H.K. and Agyemang A., 1998. Selected nutritional components and sensory attributes of cowpea (*Vigna unguiculata* [L.] Walp) leaves. *Plant Foods Hum. Nutr*., 52:221-229.

Akundabweni L.S., Paul C.P. and Singh B.B., 1991. Evaluation de lignées d'élite de niébé pour un usage double (feuilles/fourrage et graines). *In :* la recherche à IITA, 3:6-7

AOAC, 2002. *Official methods of analysis of AOAC International.*17e édition current through 1re révision. Association of Analytical Communities, Gaithersburg, MD, Etats-Unis.

Bhat R. and Karim A.A., 2009. Exploring the nutritional potential of wild and underutilized legumes.*Compr. Rev. Food Sci. Food Saf.*, 8:305-33.

Faris D.G., 1964. The chromosomes of *Vigna sinensis* (L.) *Savi. Canad. J. Genet. Cytol.*, 6:255-258

Fofiri Nzossié E.J., Ndamé J-P., Temple L., Ndjouenkeu R. and Simeu Kamdem M., 2008. Croissance urbaine et innovations dans les filières vivrières: Cas du maïs et du niébé dans les savanes du Cameroun. 2èmes journées de recherches en sciences sociales. INRA SFER CIRAD, 11-12 décembre 2008, Lille, France, 24p

Gerrano A.S., Jansen van Rensburg W., Laurie S. and Adebola P., 2015. Genetic variability in cowpea [*Vigna unguiculata* (L.) Walp.] genotypes. *S. Afr. J. Soil and Plant*, 32:165–174.

Grubben G.J.H. and Denton O.A., 2004. *Ressources Végétales de l'Afrique Tropicale: Légumes* (Vol. 2). Fondation PROTA Backhuys Publishers: Wageningen – Leyden, 737p

Hall E., Cissé N., Thiaws S., Elaward H.O.A., Eblers J.D., Ismail A.M., Fery R.L., Robert P.A., Kitch L.W., Murdock L., Boukar O., Phillips R.D. and Mc Walters K.H., 2003. Development of cowpea cultivars and germoplasm by bean/cowpea CRSP. *Field Crops Research*, 82:103-134

Hallberg L., Rosander-Hulten L., Brune M. and Gleerup A., 1992. Calcium and iron absorption: mechanism of action and nutritional importance. *Eur. J. Clin. Nutr.*, 46:317-327.

Heaney R.P., 1996. Calcium. *In :*Belizekian JP, Rais LG, Rodan GD eds. Principles of bone biology. New York :Academic Press, pp. 1007-1018.

IUPAC, 1997. Compendium of Chemical Terminology, 2è éd.

Langyintuo A.S., Lowenberg-DeBoer J., Faye M., Lambert D., Ibro G., Moussa B., Kergna A., Kushwaha S., Musa S. and Ntoukam G., 2003. Cowpea supply and demand in West and Central Africa. *Field Crops Research*, 82:215-231

Mamiro P.S., Mbwaga A.MA, Mamiro D.P., Mwanri A.W. and Kinabo J.L., 2011. Nutritional quality and utilization of local and improved cowpea varieties in some regions in Tanzania. *Afr. J. Food Agr. Nutr. Dev*., 11:4490–4506.

Maréchal R., 1970. Données cytologiques sur les espèces de la sous-tribu des Papilionaceae-Phaseoleae-Phaseolinae. Deuxième série. *Bull. Jard. Bot. Nat. Belg.*, 40:307-348.

Megueni C., Awono E.T. and Ndjouenkeu R., 2011. Effet simultané de la dilution et de la combinaison du Rhizobium et des mycorhizes sur la production foliaire et les propriétés physico-chimiques des jeunes feuilles de *Vigna unguiculata* (L.) Walp. *J. Appl. Biosci.*, 40:2668–2676.

Mirand P.P., 2003. Protéines et lipides végétaux : interactions nutritionnelles et fonctionnelles. *Oléagineux, Corps Gras, Lipides*, 10, 1:61-65.

Nguefack F., Adjahoung C.A, Keugoung B., Kamgaing N. and Dongmo R., 2015. Prise en charge hospitalière de la malnutrition aigüe sévère chez les enfants avec des préparations locales alternatives aux F-75 et F-100 : résultats et déficits. *Pan. Afr. Med. J.*, 21:329.

Nielsen S.S., Ohler T.A. and Mitchell T.A., 1997. Cowpea leaves for human consumption: Production, utilization, and nutrient composition. *In:* B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai (eds.). Advances in cowpea research. Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Center for Agricultural Sciences (JIRCAS), IITA, Ibadan, Nigeria.

Nordeide M.B., Hatloy A., Folling M., Lied E. and Oshaug A., 1996. Nutrient composition and nutritional importance of green leaves and wild food resources in an agricultural district, Koutiala, in southern Mali. *Int. J.F. Sce. Nut.*,47:455-478.

Noubissié T.J.B., Youmbi E., Njintang Y.N., Ndogonoudji A.A., Nguimbou, M.R. and Bell J.M., 2011. Genetic architecture of some leaf yield and quality attributes in dual-purpose cowpea (*Vigna unguiculata* L. Walp.). *American Journal of Experimental Agriculture*, 1, 4:400-413.

Nwoke O.C., Diels J., Abaidoo R. and Sanginga N., 2005. Low phosphorus availability in West African moist savanna soils: effect of sparing soluble P sources on growth of soybean, cowpea and maize. *Afr. Crop Sci. Proc.*, 7:1157-1161.

Ojo D.K., Bodunde J.G., Ogunbayo S.A. and Akinwale A.F., 2006. Genetics evaluation of phosphorus utilization in tropical cowpea (*Vigna unguiculata* (L.) Walp.). *African Journal of Biotechnology*, 5, 8:597-602.

Ojo D.K., Ogunbayo S.A., Idehen E.O., Akinwale A.F. and Odunwaye O.A., 2007. Genetics evaluation of phosphorus utilization in tropical cowpea (*Vigna unguiculata* (L.) Walp.). *J. Amer. Sci.*, 3, 1:77- 83.

Okonya J.S. and Maass B.L., 2014. Protein and iron composition of cowpea leaves: an evaluation of six cowpea varieties grown in eastern Africa. *Afr. J. Agr. Nutr. Dev.*, 14:9329–9340.

Pasquet R.S. and Baudoin J-P., 1997. Le niébé, *Vigna unguiculata*. *In:* Charrier A., Jacquot M., Hammon S. & Nicolas D. (eds.). L'amélioration des plantes tropicales. Montpellier, France: CIRAD-ORSTOM, pp. 483-505

Raposo R.W.C., Muraoka T., Basso L.C., Lavres J.J. and Franzini V.I., 2004. Acid phosphatase activity and leaf phosphorus content in soybean cultivars. *Sci. Agric*., 61, 4:439-445.

Rayssiguier Y., Boirie Y. and Durlach J., 2001. Magnésium. *In : Apports nutritionnels conseillés.* Paris : Tec et Doc. Lavoisier, pp. 146-149.

Roberts S. B. and Heyman M.B., 2000. Micronutrient shortfalls in young children's diets: Common and owing to inadequate intakes both at home and at child care centers », *Nutrition Reviews*, 58, 1:27-29.

Santos C.A.F., da Costa D.C.C., W.R., da Silva W.R. and Boiteux L.S., 2012. Genetic analysis of total seed protein content in two cowpea crosse. *Crop Science*, 52:2501-2506.

Statgraphics, 1997. Statgraphics plus for windows 3.0.Rockville, MD: Manugistics Inc.

Tchiégang C. and Kitikil A., 2004. Données ethno nutritionnelles et caractéristiques physico-chimiques des légumes-feuilles consommés dans la savane de l'Adamaoua (Cameroun). *Tropicultura*, 22, 1:11-18.

Tchuenguem F.F.N., Ngakou A. and Kengni B.S., 2009. Pollination and yield responses of cowpea (*Vigna unguiculata* L. Walp.) to the foraging activity of *Apis mellifera adansonii*

(Hymenoptera: Apidae) at Ngaoundere (Cameroon). *African Journal of Biotechnology*, 8:1988-1996

Westphal E., Embrechets L., Ferwerda J.D., Van Gils-Menus H.E. and Mustears M.J.W., 1985. *Cultures vivrières tropicales avec références spéciales sur le Cameroun*. Pudocwageningen, 514p.

Yoka J., Loumeto J.J., Djego J.G., Akouango P. and Epron D., 2014. Evolution des teneurs en éléments minéraux des feuilles de niébé (*Vigna unguiculata* (L.) Walp.) cultivées dans la zone de Boundji en République du Congo. *Journal of Applied Biosciences*, 79:6799–6807.