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Inheritance of phenolic contents and antioxidant capacity of dehulled seeds in cowpea (*Vigna unguiculata* L. Walp.)

Jean-Baptiste Tchiagam Noubissié^{1*}, Emmanuel Youmbi², Nicolas Y. Njintang¹, Madi Aladji Abatchoua¹, Richard M. Nguimbou³, Joseph M. Bell²

¹University of Ngaoundéré, Faculty of Science, Department of Biological Sciences, P.O. Box 454 Ngaoundéré, Cameroon

²University of Yaoundé I, Faculty of Science, Department of Plant Biology, Unit of Genetics and Biotechnology, P.O. Box 812 Yaoundé, Cameroon

³University of Ngaoundéré, Higher School of Agro-industrial Science (ENSAI), Department of Food Sciences, P.O. Box 455 Ngaoundéré, Cameroon

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Abstract

The objective of the present research was to estimate the magnitude of genetic variability for total phenol content and antioxidant activity of cowpea seed and investigate the genetics of these traits using generation's means analysis. Seven pure lines, F₁ and F₂ generations as well as backcross populations (BC₁) from three hybrid combinations, were grown in Randomized Complete Block Design (RCBD) in Ngaoundéré (Cameroon). For biochemical analysis, flour samples were produced from dehulled seeds. Analysis of variance indicated significant differences ($p < 0.01$) among genotypes for phenolic contents and antioxidant capacity. No significant transgressive segregation was observed among generations. High values of heritability in broad-sense (0.81-0.93 for phenolics and 0.63-0.71 for antioxidants) were recorded indicating major role of genetic variance in the expression of these polygenic traits. In most of the crosses, genetic analysis showed significance ($p < 0.05$) of the effects of additive, dominance, and epistatic genes for both traits. Approximately, at least 12 genes affected the phenolic contents while the antioxidant capacity was controlled by a minimum of nine factors. At 10% level of selection, an increase of 7.89 to 17.80% and 9.01 to 13.13% was predicted respectively for polyphenols content and antioxidant activity. Correlation between the phenolic contents and antioxidant activity was significantly positive ($r = 0.74$). These results suggested that breeding for increased antioxidant activity in decorticated cowpea seeds, to enhance the importance of this food stuff for the human diet, can be quite successful through recurrent selection in later generations.

*Corresponding Author: Jean-Baptiste Tchiagam Noubissié ✉ jbnoubissitch@yahoo.fr

Introduction

Cowpeas (*Vigna unguiculata* L. Walp), are an important part of the staple diet in many developing countries since the earliest practice of agriculture (Phillips and Mcwatters, 1991). Cowpea has found utilization in various ways in traditional and modern food processing in the world (Rivas-Vega *et al.*, 2006). Traditionally in Africa, cowpeas are consumed as boiled vegetables using fresh and rehydrated seeds or processed into flour to make other food products (Murdock *et al.*, 2003; Odedeji and Oyeleke, 2011). To improve the appearance, texture, aroma and taste, and reduce the cooking time, the seeds are dehulled before they are used (Cai *et al.*, 2003; Mokgope, 2007). After the seeds are decorticated, the hulls are normally thrown away as waste. The health-promoting effects of dehulled cowpea flours derived from phenolic compounds and other antioxidants make this legume a potential source of functional food ingredients. Phenolic compounds (tannins, flavonoids and phenolic acids) are secondary metabolites in plants and as such are present in some plant foods (Manach *et al.*, 2004; Wu *et al.*, 2006). Their functions in plants are not always known, but some are structural polymers, UV screens, antioxidants, attractants and others are involved in non specific defense mechanisms (Papoulias *et al.*, 2009). They are able to form complexes with food nutrients such as minerals and proteins, thus rendering them less susceptible to enzymatic degradation and less available for absorption (Shanab, 2007; Golam *et al.*, 2011). Despite the antinutritional activity of phenolic compounds, they have also a beneficial role in the seeds (Malencic *et al.*, 2007; Rodriguez *et al.*, 2007). They are concentrated in the seed coats (Pree and Punia, 2000; Nzaramba, 2004; Mokgope, 2007; Shao *et al.*, 2011a) and they protect the seeds against the oxidative damage and microbial infections (Troszynska *et al.*, 2002; Rodriguez *et al.*, 2007). Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods (Rodriguez *et al.*, 2007; Shanab, 2007; Rumbaoa *et al.*, 2009). Phenols decrease the risk of heart

diseases by inhibiting the oxidation of low-density lipoproteins (Moure *et al.*, 2001). They are also known to possess antibacterial, antiviral, antimutagenic and anticarcinogenic properties (Moure *et al.*, 2001; Manach *et al.*, 2004).

According to Pree and Punia (2000), Warington *et al.* (2002), Cai *et al.* (2003) and Nzaramba (2004) a large genetic variability for the phenolic compounds content and antioxidant capacity exists in cowpea, with pigmented varieties as preferred parental material. The genetic analysis of the phenolic compounds and antioxidants of *V. unguiculata* seeds is important for nutraceutical and functional applications (Siddhuraju and Becker, 2007). Manach *et al.* (2004) noted that environmental and genetic factors have a major effect on polyphenols content. So far, a comprehensive assessment of the inheritance of polyphenols and antioxidants of dehulled cowpea seeds has not been reported (Phillips and Mcwatters, 1991; Siddhuraju and Becker, 2007). Knowledge of the genetic basis and heritability of these health beneficiary phytochemical profiles is essential for efficient development of new cultivars for food processing industries and breeders. The choice of an efficient breeding procedure depends to a large extent on knowledge of the genetic system controlling the characters to be selected (Allard, 1960). Thus, the current study was designed to assess the variability in seven genotypes, determine the types of gene action controlling the total phenolic compounds content and the antioxidant capacity of dehulled seeds flour, identify the genetic and environmental components of variance, evaluate heritability and gain from selection, estimate the minimum number of effective factor controlling these traits under the conditions of the Guinea savannah zone of Cameroon.

Material and methods

Experimental site

The research was conducted from 2008 to 2010 at the University of Ngaoundéré experimental farm, at Dang, Adamawa region (northern Cameroon),

which is intersected by 13°34' East longitude and 7°28' North latitude and has an elevation of 1115.0 m above the mean sea level. This region belongs to the Guinea savannah agroecological zone. The soil is ferruginous type, developed on basalt and has a brown reddish clay texture. The 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). The average annual temperature is 22°C, while the annual hygrometry is about 70% (Aladji Abatchoua, 2010).

Plant material

The study was carried out on seven cowpea genotypes (Table 1) including two local varieties, four breeding lines developed by the Institute of Agricultural Research for Development (IRAD, Maroua station, Cameroon) and a improved variety of the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria). The patterns of inheritance, including genetic effects, were studied in three hybrid combinations obtained from the following crosses: Bafia x Niébé Hosséré (NH); Bafia x IT97K-573.1.1 (IT573) and CRSP x Niébé Hosséré (NH). All crosses were made with emasculation on plants growing in pots. The F₁ seeds were produced for each hybrid combination in rainy season 2009. From July to November 2009, natural self-fertilisation of F₁ hybrids produced F₂ generations, while backcrossing of F₁ to P₁ (BC_{P1}) and F₁ to P₂ (BC_{P2}) produced the backcross generations. The process was replicated during the rainy season 2010, so that the seeds of the F₁, F₁ reciprocal, F₂, and backcross generations could be evaluated under the same cultivation conditions.

Field trials

During the growing season 2010, all seven pure lines, the three F₁ and three F₂ hybrids, and the six backcrosses were arranged in a triplicated randomized complete block design (RCBD). Each plot unit consisted of one row of 1.5m length x 0.5m broad for parents and F₁, 5m length x 0.5m broad for F₂ and backcrosses generations. Plants were

spaced 30cm apart and watered as necessary. NPK mineral fertilizer (7% N, 14% P₂O₅, 7% K₂O) was broadcasted 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 first at three weeks after plant emergence and subsequently one during the vegetative stage and close the crop maturity. A total of 12 plants for the parents and the F₁ generations, 15 plants for backcross generations and 24 for F₂ generations were selected. The pods for each selected plant were harvested at maturity; the seeds removed manually and were dried until the average moisture content of 15%.

Production of flours and extraction of samples

For the biochemical analyses, a random sample 20 seeds per selected plant was used for the production of flour according to the method described by Phillips and Mcwatters (1991). The seeds were soaked in a ratio (1/3) (w/v) during 2 h and dried at 60°C for 12 h in a hot-air fan drier. They were decorticated and crushed in a hammer mill through a 1500 µm sieve. Sample homogenate (0.5 g) was extracted as outlined by Pree and Punia (2000) with 10mL of ethanol 70°, centrifuged at 5000G for 10 min and filtered through Whatman N°3 paper. The supernatant was adjusted with ethanol at 10 mL.

Determination of total phenols content (TPC)

The cowpea seeds flours were analyzed for total phenols content (TPC) using the Folin-Ciocalteu reaction as described by Gao *et al.* (2000) and using gallic acid as standard. Diluted extracts, 0.2 mL, was mixed with 1.0 mL of 1:10 diluted Folin-Ciocalteu phenol reagent followed by adding 0.8 mL of 7.5% (w/v) mL sodium carbonate. After 1 h, absorbance was measured at 765 nm using UV-visible spectrophotometer. Quantification of TPC was based on gallic acid standard curve generated by preparing 0-10 µg of gallic acid. The TPC were expressed as milligrams of gallic acid equivalents/100 g extract.

Antioxidant activity (AOA) determination

The antioxidant activity of the flour was evaluated by 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) free radical scavenging assay as described by Brand-Williams *et al.* (1995). Extract (200 µL) was added to 1000 µL of 100 µM methanolic DPPH, vortexed and kept in the dark at room temperature. The decrease in absorbance of the resulting solution was monitored at 517 nm for 30 min. DPPH solution (5 mg/100 mL) was used as standard and antioxidant activities (AOA) were expressed as percentage of inhibition using the following equation:

$$\text{AOA (\%)} = 100 (\text{DRc} - \text{DRs}) / \text{DR}$$

where, DRc was the degradation rate of the control and DRs the degradation rate of the sample.

Statistical analysis

The means of generations for each combination or those of the pure lines were subjected to analysis of variance (ANOVA) using STATGRAPHICS PLUS statistical package program. Differences in means performance were tested using the Least Significant Difference (LSD) or by the Student's t-test at 5% level of probability. Pearson's linear correlation coefficients were used to assess the relationships between TPC and AOA.

Genetic analysis

Estimates of the genetic parameters were obtained with the variance of parents P_1 (V_{P1}) and P_2 (V_{P2}), F_1 (V_{F1}), F_2 (V_{F2}), RC_{P1} (V_{BCP1}) and RC_{P2} (V_{BCP2}) generations for each hybrid combination. Variance components were estimated as described by Kearsy and Pooni (1996) and Eschghi and Akhundora (2010) using the following equations:

$$\text{Additive variance: } V_A = 2V_{F2} - (V_{BCP1} + V_{BCP2}),$$

$$\text{Phenotypic variance: } V_P = V_{F2},$$

$$\text{Environmental variance in } F_2: V_E = 1/4 (2V_{F1} + V_{P1} + V_{P2}), \text{ and genetic variance: } V_g = V_P - V_E.$$

In addition, broad-sense (h^2) and narrow-sense (h_n^2) heritabilities were estimated using the variance component method (Mather and Jinks, 1982) and the variance of F_2 and backcross generations (Warner, 1952, respectively as:

$$h^2 = V_g / V_P = [V_{F2} - (V_{P1} + V_{P2} + 2V_{F1}) / 4] / V_{F2}$$

$$h_n^2 = V_A / V_P = [V_{F2} - (V_{BCP1} + V_{BCP2}) / 2] / V_{F2}$$

Response to selection was estimated (Allard, 1960) with 10% selection intensity (selection differential $K = 1.75$) as: Genetic advance $GA = K \times (V_{F2})^{1/2} \times h_n^2$.

The genetic advance expressed as percentage of mean (GA%) was measured by the following formula: $GA\% = (GA \times 100) / X_o$; where $X_o =$ average of original F_2 plants.

Heterosis (H%) and heterobeltiosis (HB%) were quantified as deviation of F_1 value from the mid-parent [$MP = (P_1 - P_2) / 2$] and from the better parent (BP) as outlined by Fonseca and Patterson (1968): $H\% = [(F_1 - MP) / MP] \times 100$ and $HB\% = [(F_1 - BP) / BP] \times 100$

Gene effects based on a six parameters were estimated using the nonweighted generation means analysis described by Gamble (1962) and are defined as follows: mean [m] = F_2 ; additive [a] = $BC_{P1} - BC_{P2}$; dominance [d] = $-0.5P_1 - 0.5P_2 + F_1 - 4F_2 + 2BC_{P1} + 2BC_{P2}$; additive x additive [aa] = $-4F_2 + 2BC_{P1} + 2BC_{P2}$; additive x dominance [ad] = $-0.5P_1 + 0.5P_2 + BC_{P1} - BC_{P2}$ and dominance x dominance [dd] = $P_1 + P_2 + 2F_1 + 4F_2 - 4BC_{P1} - 4BC_{P2}$. The minimum number of genes (N) controlling the traits was estimated following Lande (1981) as:

$$N = (P_2 - P_1)^2 / 8 [2V_{F2} - (V_{BCP1} + V_{BCP2})].$$

These effective factor formulas assume that the segregating genes for each trait are all located in one parent, not linked, have equal effects, with no genotype x environment effects, no epistatic and dominance effects (Mather & Jinks, 1982).

Results

Genetic variability

Analysis of the variance (Table 2) for the phenolic compounds contents (TPC) and antioxidant activity (AOA) of seeds flours from the seven lines tested, showed the presence of significant genotypic differences ($p < 0.05$). The TPC varied from 85.15 to 294.73 mg GAE/100 g (mean = 142.72 mg GAE/100 g) while the AOA ranged between 21.22% (VYA) and 97.48% (Bafia) (mean = 48.04%).

Table 1. Characteristics of the seven studied cowpea pure lines.

Genotypes	Origin	Growth habit	Seed color	Seed index (g)
Bafia	Local Mbam	Erect	Brown	17.22
BR1 (IT81D985)	IRAD Maroua	Erect	White	21.33
CRSP	IRAD Maroua	Semi-erect	White	16.44
IT97K-573.1.1	IITA Ibadan	Semi-erect	White	23.33
Lori Niébé	IRAD Maroua	Semi-erect	White	20.55
Niébé Hosséré (NH)	Local Maroua	Semi-erect	White	19.67
VYA	IRAD Maroua	Erect	White	21.00

IRAD: Institute of Agricultural Research for Development (Maroua station, Cameroon); IITA: International Institute of Tropical Agriculture (Ibadan, Nigeria).

Table 2. Genetic variability of phenolic content and antioxidant activity of dehulled seeds in seven cowpea pure lines.

Genotypes	Phenolic content (mg GAE/100g)	Antioxidant capacity (%)
BR-1	102.16±1.95 ^d	46.56±3.22 ^b
CRSP	122.60±5.12 ^c	33.93±3.66 ^c
Bafia	294.73±4.00 ^a	97.48±3.50 ^a
Lori Niébé	118.00±3.33 ^c	34.86±2.25 ^c
IT97K-573-1-1	189.11±7.15 ^b	50.96±3.31 ^b
Niébé Hosséré	122.33±3.83 ^c	51.31±3.39 ^b
VYA	85.15±5.97 ^e	21.22±4.18 ^d
Mean	147.72±4.47	48.04±3.35
LSD (0.05)	10.85	6.12

LSD: Least significant difference; For each parameter, means followed by the same letter are not significantly different at 0.05 probability.

Table 3. Phenolic content of cowpea seeds in parents and in F₁, F₂ and backcross generations of three hybrid combinations.

Parents and generations	Phenolic content of cowpea seed flour (mg GAE/100g)		
	Bafia (P ₁) x NH (P ₂)	Bafia (P ₁) x IT573 (P ₂)	CRSP (P ₁) x NH (P ₂)
P ₁ (n = 12)	294.73 ± 4.00 ^a	294.73 ± 4.00 ^a	122.60 ± 5.12 ^a
P ₂ (n = 12)	122.33 ± 3.83 ^c	189.11 ± 7.15 ^b	122.33 ± 3.83 ^a
F ₁ (n = 12)	199.48 ± 8.45 ^b	215.68 ± 8.18 ^c	137.71 ± 3.76 ^a
F ₂ (n = 24)	138.34 ± 21.23 ^c	199.89 ± 16.41 ^c	125.60 ± 16.23 ^a
BC _{P₁} (n = 15)	186.72 ± 19.15 ^b	218.66 ± 16.22 ^b	127.00 ± 15.46 ^a
BC _{P₂} (n = 15)	131.78 ± 15.68 ^c	205.75 ± 11.22 ^{bc}	125.16 ± 10.90 ^a

n = number of plants sampled in each generation; within a cross, means followed by the same letter are not significantly different at 0.05 probability.

Table 4. Antioxidant capacity of cowpea seed flour in parents and in F₁, F₂ and backcross generations of three hybrid combinations.

Parents and generations	Antioxidant capacity of cowpea seeds (%)		
	Bafia (P ₁) x NH (P ₂)	Bafia (P ₁) x IT573 (P ₂)	NH (P ₁) x CRSP (P ₂)
P ₁ (n = 10)	97.48 ± 3.50 ^a	97.48 ± 3.50 ^a	51.31 ± 3.39 ^a
P ₂ (n = 10)	51.31 ± 3.39 ^c	50.96 ± 3.31 ^d	33.93 ± 3.66 ^c
F ₁ (n = 10)	83.55 ± 5.42 ^{bc}	59.46 ± 4.12 ^{bcd}	41.03 ± 3.76 ^b
F ₂ (n = 25)	74.84 ± 8.42 ^c	55.44 ± 7.06 ^{cd}	38.41 ± 6.00 ^{bc}
BC _{P₁} (n = 15)	92.39 ± 7.88 ^{ab}	65.67 ± 6.00 ^b	42.68 ± 3.15 ^b
BC _{P₂} (n = 15)	67.71 ± 8.9 ^d	59.33 ± 5.82 ^{bcd}	34.61 ± 3.88 ^c

n = number of plants sampled in each generation; means followed by the same letter are not significantly different at 0.05 probability.

Generation's means

Analysis of variance of the six generations means for the TPC and AOA in each of the three combinations are given in Tables 3 and 4. In all combinations, progeny density distribution of F₂ and BC₁ families were normal. No significant transgressive segregation was observed, and only in rare cases did F₂ individuals reach values of the best parent.

Variance components and heritability estimates

Variance components and heritability estimates for seed flour TPC and AOA are listed in Table 5. The genetic component of the variance (V_g) was larger than environmental variance (V_E) in all crosses for both traits and, the additive variance (V_A) appeared as the major part of the genetic variance. Estimates of variance components varied considerably between crosses. The broad-sense (h^2) and narrow-sense (h_n^2) heritability estimates ranged from 0.81 to 0.93 and 0.55 to 0.64 for TPC, while for AOA, h^2 and h_n^2 values varied between 0.63-0.71 and 0.33-0.59 respectively depending on crosses combinations. The differences observed between h^2 and h_n^2 reflected the presence of dominant genes.

Genetic advances

Expected genetic gain per cycle for selection at the 10% level (GA) ranged from 15.78 to 23.77 mg GAE/100g for TPC and of 3.46 to 7.95% for AOA (Table 5). Otherwise, in percentage of the initial F₂ mean, GA for three combinations varied from 7.89 to 14.46% for TPC and 9.01 to 13.13% for AOA (Table 5). Estimates of gain were larger for crosses

with a considerable difference between parents, i.e. Bafia x NH for TPC and Bafia x IT97K-573.1.1 for AOA.

Heterosis estimates

Mid-parent heterosis (H) and heterobeltiosis (HB) values for TPC were negative for all crosses except for combination CRSP x NH (Table 5). H and HB values for this characteristic ranged from -10.84 to 12.45% and from -32.31 to 12.32% respectively. Concerning the AOA, H values (Table 5) were negative for crosses Bafia x IT97K573-1-1 (-18.88%) and CRSP x NH (-3.73%) but positive for Bafia x NH cross (9.70%) while HB values were negative for all three combinations (-39.0 to -14.29%).

Gene effects

The means variation among generations for TPC and AOA was fitted to an additive-dominance model using six-parameter nonweighted generation means analysis described by Gamble (1962) (Table 6). Estimates of additive (a) and dominance (d) parameters deviated significantly from zero, and additive and dominance effects had always greatest magnitude for both traits. Additive and dominance effects, as well as additive x additive (aa), additive x dominance (ad) and dominance x dominance (dd) interactions were significant at $P = 0.05$ especially for combinations Bafia x NH and Bafia x IT97K 573-1-1. There were positive a and d effects as well as positive aa interactions for both traits and negative ad epistasis for TPC and AOA. Globally, dd epistasis showed positive effects for TPC and negative effects for AOA.

Table 5. Estimates of genetic parameters and genetic advance for total phenol content and antioxidant activity in cowpea seed flour of three hybrid combinations.

Genetic Parameter	Bafia x NH		Bafia x IT97K-573		CRSP x NH	
	TPC	AOA	TPC	AOA	TPC	AOA
M	208.53	74.39	241.92	74.22	122.46	42.62
V_P	450.71	70.90	269.28	49.84	263.41	36.00
V_E	43.36	20.63	50.24	14.28	17.29	13.29
V_g	407.35	50.27	219.04	35.56	246.12	22.71
V_A	288.63	38.78	149.56	29.80	168.92	11.95
h^2	0.9038	0.7090	0.8134	0.7135	0.9343	0.6308
h_n^2	0.6401	0.5470	0.5552	0.5980	0.6413	0.3319
H%	-4.34	9.70	-10.84	-18.88	12.45	-3.73
HB %	-32.31	-14.29	-26.82	-39.00	12.32	-20.03
GA	23.77	7.95	15.78	7.28	18.17	3.46
GA%	17.18	10.62	7.89	13.13	14.46	9.01

M: Parents' means; V_P : Phenotypic variance; V_E : Environmental variance; V_g : Genetic variance; V_A : Additive variance; h^2 : Broad-sense heritability; h_n^2 : Narrow-sense heritability; H%: Mid-parent heterosis; HB%: Heterobeltiosis; GA: Genetic advance; GA%: Genetic advance in percentage of means

Table 6. Estimates of gene effects and the minimum number of effective factors for phenolic content and antioxidant activity in three crosses of cowpea.

Genetic componen	Bafia x NH		Bafia x IT97K-573		CRSP x NH	
	TPC	AOA	TPC	AOA	TPC	AOA
[m]	138.34	74.84	199.89	55.44	125.60	38.41
[a]	54.94**	24.68**	12.91**	6.34*	1.84	8.07**
[d]	74.48**	29.99**	23.02**	13.48**	17.17**	-0.65
[aa]	83.64**	20.84**	49.26**	28.24**	1.92	0.92
[ad]	-31.26**	1.60	-39.90**	-16.92**	1.70	-0.63
[dd]	95.38**	-25.15**	17.12**	-10.88**	14.11**	11.78**
N	12.86	/	9.32	9.07	/	0.81

* and ** : Estimates significantly different from zero at $p = 0.05$ and $p = 0.01$ respectively. TPC: Total phenol content; AOA: Antioxidant activity; [m]: Mean; [a]: Additive effects; [d]: dominance effects; [aa]: Additive x additive interaction; [ad]: Additive x dominance interaction; [dd]: dominance x dominance interaction; N: Minimum number of effective genes

Number of effective factors

Estimates of the minimum number of effective factors (genes) controlling TPC of cowpea seed flour ranged between 9 and 12 depending on crosses (Table 6). For the AOA of cowpea flour, it is likely that the number of genes controlling this trait was about 9 (Table 6).

Phenotypic correlation

The interrelationship between seed flour TPC and their AOA over all parents and crosses was moderately high ($r = 0.74$, $P = 0.001$).

Discussion

Cultivar significantly affected phenolic accumulation and antioxidant capacity of flours from dehulled cowpea seeds. The guinean variety 'Bafia', with a brown seed coat, possessed the higher AOA and TPC levels. According to Warington *et al.* (2002), Nzaramba (2004), and Siddhuraju and Becker (2007), pigmented cowpea varieties had favorable factors that enhance AOA of seeds. The present study showed that there are also differences in these traits among white cowpea genotypes. The levels of the total phenols found are lower than reported concentration in whole seeds which are in

order of 0.8 – 0.9% (Pree and Punia, 2000) and 0.03 – 0.4% (Cai *et al.*, 2003). Odedeji and Oyeleke (2011) noted that flour produced from whole seeds presents better functional properties compared to the dehulled seed flour which is common practice in processing of cowpea. Many authors outlined that AOA and TPC varied both among species (Adom and Liu, 2002; Manach *et al.*, 2004; Shanab, 2007) and among genotypes within a species (Anttonen and Karjalainen, 2005; Oomah *et al.*, 2005; Kravic *et al.*, 2009; Akond Massum *et al.*, 2010; Aladji Abatchoua, 2010). In common bean varieties, the phenolic compounds concentration of seeds ranged from 117 to 440 mg GAE/100 g (Heimler *et al.*, 2005); 223 and 1247 mg GAE/100 g (Wu *et al.*, 2006) and from 587 to 1414 mg GAE/100 g (Golam *et al.*, 2011). For seeds flour of wheat genotypes, Akond Massum *et al.* (2010) observed a TPC of 129 to 1316 mg GAE/100 g while Shao *et al.* (2011b) recorded a range of 42.6-100.7 mg GAE/100 g for TPC in whole rice seeds. Adom and Liu (2002), Connor *et al.* (2002) and Papoulias *et al.* (2009) pointed out that polyphenols accumulation in plants is affected by genetic factors, environmental and cultural conditions and also various stresses like dehydration stress (Kravic *et al.*, 2009), storage and cooking (Manach *et al.*, 2004) and nitrogen fertilization (Nunez-Ramirez *et al.*, 2011). Significant variation of the AOA among varieties was also pointed out for many grains (Adom and Liu, 2002), for wheat flour (Akond Massum *et al.*, 2010) and for common bean (Golam *et al.*, 2011). Heritability values for TPC and AOA didn't vary considerably between crosses. Large heritability estimates in broad sense (h^2), indicated that the TPC and AOA of flours from dehulled seeds were highly heritable in cowpea under the agroecological conditions of Ngaoundéré. These findings get support from previous studies by Warrington *et al.* (2002) and Aladji Abatchoua (2010) in cowpea, Connor *et al.* (2002) in blue berry, Anttonen and Karjalainen (2005) in red raspberry and Shahbazi *et al.* (2009) in bread wheat. Nzaramba (2004) also noted a high value of h^2 (0.87) for AOA of whole seed in cowpea ($h^2 = 0.87$). On *Solanum*

melongena, Prohens *et al.* (2004) noted a moderate value of h^2 (0.50) for TPC, while on *Ribes nigrum* Currie *et al.* (2006) observed that estimates of h^2 for AOA ranged from 0.46 to 0.80. The estimates of h_n^2 suggested that additive genes contribute more to high TPC and AOA than non additive genes. On wheat, Shahbazi *et al.* (2010) noted that h^2 and h_s^2 values for AOA varied between 0.46-0.93 and 0.12-0.62 respectively. Therefore, our results indicated that genetic selection for these traits can be achieved with minimal effort. Breeding efforts to increase TPC and AOA will not require good control over environmental variation. However, a larger population needs to be studied to determine more precise estimates of heritability.

The F_1 hybrids of the various combinations are statistically different from the mid-parent and the best parent for the two measured characters. The heterosis values (H) suggest additive and partial dominance (Mather and Jinks, 1982). The variation among crosses implied that hybrid vigor depends on the choice of the parents. In contrast, for AOA, significant and positive values of heterosis were recorded on wheat (H = 118%) by Shahbazi *et al.* (2010) and on triticale by Gorji *et al.* (2011) due to the importance of dominance in the control of this trait. Similar trends were observed for TPC in spring wheat (Mpofu *et al.*, 2006) and in eggplant (Prohens *et al.*, 2007). On *Capsicum annum*, Mantri (2006), also noted wide range of heterobeltiosis (HB) estimates for TPC (-71.7 to 1160.38%). Negative values of HB might due to absence of parental combinations capable of producing transgressive segregants.

Regarding gene effects, our results showed that, besides the additive and dominance genetic effects, epistatic components have also contributed to the genetic variation of the characters studied. The presence of epistasis has important implications for plant breeding program (Allard, 1960). In such a situation, the appropriate breeding method is one that can effectively exploit the three types of gene effects simultaneously (Mather and Jinks, 1982;

Gorji *et al.*, 2011). Additive (a) and dominance (d) effects were nearly always positive for both TPC and AOA. Among the interaction components, the fixable additive x additive effects were always positive compared to additive x dominance effects which were generally negative for both traits and the dominance x dominance epistasis that was significantly positive for TPC but negative for AOA. Because of the opposite signs of d and dd, the sums of the significant a + aa effects were usually of a greater magnitude than the sums of the significant d + dd for AOA. Nzaramba (2004) also noted that the dominance effects (d) were positive for AOA in most crosses of cowpea while the d x d interactions were negative, suggesting the presence of duplicate gene interactions. Genetic models which assume no epistasis do not accurately describe TPC or AOA in cowpea. This finding is in agreement with Nzaramba (2004) who observed that AOA in cowpea depends on additive genes, dominance effects and epistasis interactions. In contrast, the activity of antioxidant enzymes under drought stress adequately can be described by additive-dominance model respectively on maize (Kravic *et al.*, 2009), bread wheat (Shahbazi *et al.*, 2009) and triticale (Gorji *et al.*, 2011). Based on the results of our study, a recurrent selection scheme in which large populations are carried forward to later generations to allow favorable gene combinations to be in a homozygous state before practicing final selection, would be the most appropriate.

Estimates of the minimum number of effective genes for TPC and AOA by Lande (1981) assumption were about 12 and 9 respectively, confirming that these traits are polygenic. Nzaramba (2004) reported that a minimum of five genes controlled AOA activity in cowpea. The estimates of the number of genes were highly biased by the failure to meet the analysis assumptions of no epistasis and no dominance, because dominance effects were detected and epistasis was significant for both traits. It is assumed that the segregating factors are iso-directionally distributed between the parents and they have equal additive effects.

Consistent with previous observations in soybean (Malencic *et al.*, 2007), maize (Kravic *et al.*, 2009), green asparagus (Papoulias *et al.*, 2009), potato (Rumbaoa *et al.*, 2009), okra (Khomsug *et al.*, 2010), wheat (Akond Massum *et al.*, 2010), common bean (Oomah *et al.*, 2005; Golam *et al.*, 2011) and in rice (Shao *et al.* 2011a; 2011b), correlations studies attested that the antioxidant capacity of the seeds was positively correlated with phenolic compounds content. The polyphenols content of cowpea seeds was considered as important characteristic in selecting breeding lines that showed high antioxidant capacity and its determination might make the screening progress relatively easy. Mokgope (2007) noted that in general, the efficacy of phenolic constituents as antioxidants depends on factors such as the number of hydroxyl groups bonded the aromatic ring, the site of bonding, mutual position of hydroxyls in aromatic ring and their ability to act as hydrogen or electron donating agents and free radical scavengers.

Conclusion

The genetic analysis of the polyphenols contents and antioxidant activity of dehulled seeds of *V. unguiculata* showed significant genetic variability among the seven tested lines. Our study highlighted that the investigated traits are genetically controlled and the values of heritability indicated ample scope for selection. In addition to additive effects, dominant and non allelic interactions were observed, suggesting recurrent selection for improvement of these traits. The positive interrelationship between these two parameters demonstrates that the antioxidant activity depends mainly on polyphenols contents. Furthermore, the antioxidant capacity and others beneficial properties of cowpea may, with further investigations, be harnessed for nutraceutical and functional applications. Moreover, gene tracking can be done using molecular tags that reduce the need for extensive testing over time and space.

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