

Genetic Variability and Heritability of Seed Protein Content in *Vigna unguiculata* L. Walp. Genotypes Grown in Two Agro-Ecological Environments of Chad

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Abstract

Cowpea is an important pulse crop grown in sub-Saharan Africa and in parts of Asia and the Americas. Cowpea seed is an affordable source of nutrients rich in protein with number of essential amino acids. Dehulled seeds of eight improved cowpea lines grown in N'Djamena and Bebedjia (Chad) were analyzed for seed protein content in order to assess the variability, the heritability and the effect of environment interaction. In each locality, the experimental design was a triplicated randomly complete block design. The results showed a wide variability among genotypes for crude seed protein content. The mean value of crude seed protein content was 25.55% with varieties TN-27-80, TN-985-61399 and TN-5-78 showing highest values. High heritability in broad-sense ($h^2 = 0.74$) and moderate genetic advance ($GA = 14\%$) estimated for this character indicated the scope for improvement through selection. The effects of genotype (83.1% of the total sum of square), location (3.5%) and their interaction (13.4%) were highly significant ($P < 0.01$), but the protein content was slightly influenced by the environment. High potential for breeding programs is expected as genetic factors are believed to account for the main variation in protein content. This research could provide information for breeders to develop cowpea cultivars with higher protein content.

Keywords: *Vigna unguiculata*, Chad, seed protein, variability, heritability, genotype x environment interaction.

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1. INTRODUCTION

In Chad, under-nutrition continues to be a major problem with chronic protein energy malnutrition prevalent in children. The main reason of under-nutrition is inadequate access to protein dense foods to meet the daily requirement of poor population. Pulses form a major source of plant protein and are valuable for cropping system in maintaining the productivity of soils due to nitrogen fixation ability (Bala *et al.*, 2012). Grain legumes are often referred to as poor man's meat, owing to their uses as primary cheapest protein sources (Goulet, 2002). From the most cultivated legumes, cowpea (*Vigna unguiculata* L. Walp.) is an important, high-quality staple food that provides large amounts of protein, calories, vitamins and essential mineral micronutrients to the diets of people in most African, Asian and Latin American countries (Hall *et al.*, 2003; Vasconcelos *et al.*, 2010; Goncalvez *et al.*, 2016).

Cowpea has its origin on the southern African country but has spread and it is now cultivated in more than 100 countries between 40°N and 30°S latitudes (Vasconcelos *et al.*, 2010). The total world production of cowpeas in 2019 was 8.9 million metric tons (FAO, 2021).

The environmental advantage of cowpea arises from its ability to be drought tolerant and to grow in the dry savannah regions of the West and Central Africa with low input requirements (Hall *et al.*, 2003). In Chad, and many other African countries, even if millet and sorghum constitute the staple food of the populations, cowpea is of considerable importance as a nutritious leguminous providing an alternative source to animal protein (Nadjiam, 2021). Cowpea plant is a multipurpose crop, being consumed for its leaves, green pods, green beans, dry seeds or processed into paste or flour and used as food ingredients (Affrifah *et al.*,

2021). Cowpea has found utilization in various ways in traditional and modern food processing in the world (Goulet, 2002). Traditionally, cowpeas are mostly consumed as boiled vegetable using whole or dehulled dry seeds taken as a relish with cereal staples (Oyeleke *et al.*, 1985; Olabandji *et al.*, 2018). Cowpeas are also used in the formulation of simple weaning blends, which are relatively cheap for poor rural to afford (Goulet, 2002; Affrifah *et al.*, 2021).

With respect to the value of cowpea seeds as a source of proteins, values in the range 203 – 394 g Kg⁻¹ have been reported, which is close to the content of the major legumes with exception of soybean protein (Goncalvez *et al.*, 2016). In cowpea seeds, protein types comprise globulins (49%), albumins (27%), glutelins (22.5%) and prolamins (1.5%) (Noubissié *et al.*, 2011). Nutritionally, the amino-acid composition of cowpea seed proteins shows a prevalence of glutamate, aspartate, phenylalanine, tyrosine as well as a secondary prevalence of arginine, leucine, lysine, valine and proline, while methionine, cysteine and tryptophan are limiting amino acids (Giami, 2005; Vasconcelos *et al.*, 2010; Mune *et al.*, 2013). Although cowpea protein is deficient in sulfur-containing amino acids for infants, it satisfies the requirements suggested for young children and adults (Affrifah *et al.*, 2021). As cowpeas are excellent source of lysine, they are used extensively to fortify cereal-based foods. Bioactive peptides with antioxidant activity are obtained from enzymatic proteolysis of cowpea proteins indicating their potential as functional food ingredients (Oyeleke *et al.*, 1985; Goncalvez *et al.*, 2016).

Because of the importance of cowpea in the nutrition of Africans, efforts have been directed towards the breeding and improvement of cowpea cultivar for nutritional attributes including seed protein content. Advanced lines of cowpea have been developed and released for cultivation by the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria) and some national institutes of agronomic research (Fashakin and Ojo, 1988; Hall *et al.*, 2003). Therefore, the selection of cowpea cultivars presenting adequate concentration of protein appears to be the simplest and most effective method to improve nutritional value of this legume (Fashakin and Ojo, 1988). It was shown that the seed protein content of cowpea exhibit a wide variability revealing the possibility of breeding (Nielsen *et al.*, 1993; Giami, 2005; Ajeigbé *et al.*, 2008; Vasconcelos *et al.*, 2010; Noubissié *et al.*, 2011; Ubini *et al.*, 2016; Weng *et al.*, 2019). The variability in the levels of cowpea protein are dependent on the cultivar, locations and the interaction with the environment where it is grown, an area of research that has received little attention (Oluwatosin, 1997; Goncalvez *et al.*, 2016; Ravelombola *et al.*, 2016). In Chad, little effort has been made to ascertain the quality attributes of improved cowpea genotypes including protein content. The present study, therefore, seeks to understand the

genetic variability and the heritability on cowpea improved genotypes grown in two locations, with the aim to developing a strategy for improving the quality of the seeds in order to fulfil the basic nutritional requirements of the people.

2. MATERIALS AND METHODS

2.1. Testing environments

The research was carried out during the year 2020 rainy season at the experimental farms of Chadian Institute of Agronomic Research for Development (ITRAD) in N'Djamena (12°6'59''N, 15°4'20''E, altitude 298m) and Bebedjia (08°40' 34''N, 16°54'65''E, altitude 382m). These test locations, selected to sample climatic and edaphic conditions, vary in latitude, rainfall, soil types, temperature and other agro-climatic factors (Asrangar *et al.*, 2023). Bebedja (Department of Nya, Logone oriental region in Southern Chad) belongs to the savannah Sudano-Guinea belt with an annual average rainfall ranges between 950 to 1300 mm. The climate is tropical semi-humid with a single rainy season that ranges from May to November. The mean annual temperature is between 25 - 30°C, while the annual humidity is about 60%. The soil is sandy-clay with 8.2 mg.kg⁻¹ of organic matter and pH of 5.5 (Pias, 1972). The vegetation is a clear forest tree savannah (Nadjiam, 2021).

N'Djamena (capital of Chad) located in the south-west of the country at the confluence of Logone and Chari rivers, belongs to the Sahelian belt with an annual average rainfall ranges between 400 to 700 mm (Vivien, 2006; Asrangar *et al.*, 2023). The climate is tropical hot semi-arid, with a short rainy season ranges from July to September. Based on annual temperatures, N'Djamena is one of the hottest major cities on the planet. The mean annual temperature is about 29°C, while the annual humidity is about 43%. The soils of the experimental site are ferruginous, characterized by a hard sandy-loam texture and a pH of 6.5 (Pias, 1972).

2.2. Genotypes

Eight cowpea homozygous cultivars adapted to the sudano-sahelian zone conditions and cultivated in Chad were used for the study (Asrangar *et al.*, 2023). Seeds were obtained from the Chadian Institute of Agricultural Research for Development (ITRAD). The tested materials comprised registered genotypes IT81-D994, IT99-K573-1-2 and Vita 5 from the International Institute of Tropical Agriculture (IITA, Nigeria), TN5-78 (Dan Louma), TN-27-80 (Dan Matarawa) and TN-985-61399 selected by the National Institute of Agronomic Research of Niger (INRAN), Melakh obtained by the Senegalese Institute of Agricultural Research (ISRA) and, popular cultivar K VX30-309-6G (Dan Bobo) from the Institute of Environment and Agronomic Research of Burkina-Faso (INERA) (Cissé *et al.*, 1997; Asrangar *et al.*, 2023).

2.3. Experimental plots

In each location, the seeds of entries were sown in a 185 m² (14 m length x 13.2 m broad) experimental plot arranged in a randomized complete block design (RCBD) with three replications. Each plot unit consisted of one row of 3m length x 1m broad, spaced 1m apart. Three seeds were sown at an intra-row spacing of 30 cm and thinned to two per hill, 20 days after sowing (DAS). The plots were manually weeded 20, 40 and 60 DAS. At flowering stage, plots were sprayed with a standard insecticide formulation, Cypermethrin + Dimethoate at the rate of 30 g + 250 g a.i/L, to control pod borers and flower midges. At maturity, harvesting was done at five-day intervals and seeds were separated to dry pods.

2.4. Determination of seed protein content

The biochemical analyses of cowpea seeds were carried out in the Laboratory of Food Sciences and Nutrition, National School of Agro-industrial Sciences, University of Ngaoundéré, Cameroon. To determine the protein content, a random sample of 100 seeds per genotype was taken from a bulk sample of seeds from each replication for the production of flour. Cowpeas seeds were decorticated manually after soaking in water during five hours. Dehulled seeds were separately ground to a fine powder using a Culatti grinder (Polymix, France) fitted with a 1.5 mm mesh sieve and stored in polyethylene bags at 4°C until analysis. The crude seed protein content was estimated by Lowry *et al.* (1951) procedures, after extraction of 0.5 g flour finely crushed to the SDS 1% in 0.1% NaOH under agitation for 24h (AOAC, 2002).

2.5. Statistical and genetic analysis

All biochemical analyses were done in triplicate. For the genotypic variability, data obtained from the eight pure lines were subjected to analysis of variance (ANOVA) using STATGRAPHICS PLUS version 3.0 (Manugistics 1997). The genotypic means were compared using least significant difference at 5% level of probability (LSD 5%). Environmental means were compared using t-Student test. The combined analysis of variance was done using the GEST 98 microcomputer program (Ukai, 2000).

The broad-sense heritability (h^2) was estimated as the ratio of genetic variance of pure lines (σ_g^2) in the phenotypic variance between parents (σ_p^2) as outlined by Xu *et al.* (2009):

$$h^2 = \sigma_g^2 / \sigma_p^2 = (\sigma_p^2 - \sigma_E^2) / \sigma_p^2,$$

where, σ_p^2 = the total phenotypic or inter-varietal variance from the eight varieties; σ_E^2 = the environmental or intra-varietal variance estimated by the average of the phenotypic variance of each pure line; and σ_G^2 = genetic variance derived from the difference between the total phenotypic variance and the environmental variance.

The genetic advance (GA) was calculated as per the following formula given by Allard (1960):

$$GA = K \times \sigma_p \times h^2,$$

Where, K = the selection differential in standard units in the present study and it was 1.75 at 10% level of selection as outlined by Allard (1960);

σ_p = standard deviation of the phenotypic variance and, h^2 = heritability in broad-sense.

The genetic advance expressed as percentage of mean (GA%) was measured by the following formula:

$$GA\% = (GA \times 100) / X_0;$$

Where X_0 = average of the population.

The combined analysis of variance across locations was done by using the Hardwick and Wood (1972) model with genotypes being considered as fixed effects and replications within environments being random model in order to evaluate the effect of difference between genotypes, across locations, and also to determine whether their interaction was significant. Genotype and environment interaction (GEI) was quantified using pooled analysis of variance, which partitioned the total variance into its component parts namely genotype, environment, GEI and pooled error.

3. RESULTS AND DISCUSSION

3.1. Genotypic Variability

Analysis of variance for protein content of seeds from the eight tested pure lines showed the presence of highly significant differences among the genotype grown in Bebedjia and in N'Djamena ($p < 0.05$) (Table 1).

Total protein content of the seeds in N'Djamena varied from 22.34% DM for Vita 5 to 30.71% DM for TN-985-61399 (average = 26.09 % in N'Djamena) with lines TN-985-61399, TN-27-80 and TN-5-78 showing the highest values (Table 1). In Bebedjia, the seed protein content ranged from 21.09 22.34% DM (Melakh) to 28.63 % DM (TN-27-80) with an environmental mean of 25.02% DM (Table 1). The richest genotypes in Bebedjia for seed protein level were TN-27-80, TN-985-61399, TN-5-78 and Vita 5. Globally, TN-27-80 had the highest seed protein content, with 29.11% dry seed weight; TN-985-61399 was second highest (28.97%); TN-5-78 was the third highest (28.26%); and the three lines were not significantly different each other, but they had total protein content significantly higher than other genotypes. Melakh had the lowest seed protein content with 21.94% dry seed weight. The comparisons of environmental means of N'Djamena and Bebedjia showed globally a significant difference ($p < 0.05$) for this biochemical trait.

Significant difference observed between the eight pure lines tested for seed protein in decorticated seed indicates a large genetic variability for this biochemical trait. In the present study the mean value of crude seed protein content is 25.55% DM with a 10.41% coefficient of variation indicating the seed protein content had large variation in the eight cowpea genotypes. Wide variation observed in protein content is reported in cowpea elsewhere (Nielsen *et al.*, 1993; Giami, 2005; Ajeigbé *et al.*, 2008; Vasconcelos *et al.*, 2010; Boukar *et al.*, 2011; Noubissié *et al.*, 2011; Ubini *et al.*, 2016; Olabandji *et al.*, 2018; Verma *et al.*, 2019). Improved popular cowpea varieties grown in Nigeria were found to contain seed protein ranging from 21.3 to 29.9% DM (Ajeigbe *et al.*, 2008). A study performed by Boukar *et al.* (2011) on cowpea germplasm from International Institute of Tropical Agriculture (IITA) Genetic Resources Unit, revealed that the protein content averaged 24.7%.

Ubini *et al.* (2016) also screened a large population (101 varieties) from the Genetic Resources Unit of IITA Ibadan and noted that 20 genotypes had protein content greater than 30%, which were considered high protein lines, whereas 73 genotypes were medium protein content (20 to 30% protein) and 8 genotypes were low protein (less than 20%). Crude protein values reported by Verma *et al.* (2019) in eight Indian cowpea varieties ranged from 23.74 to 29.69%.

In improved and local cowpeas, Olabandji *et al.* (2018) noted that the seed protein content ranged from 16.19 to 36.75%. Amounts of protein close to this study have been reported by Ravelombola *et al.* (2016) and Weng *et al.* (2019) in decorticated cowpea seeds from USA. Weng *et al.* (2019) highlighted that the protein content of 173 USDA cowpea accessions ranged from 22.8% to 28.9% with an average of 25.6%. According to Weng *et al.* (2019), seed-coat color was related to protein content with cream and pinkeye expressed higher protein content than other seed-coat colors.

Cowpea varieties TN-27-80, TN-985-61399 and TN-5-78, all selected by the National Institute of Agronomic Research of Niger, showed higher protein content and could be selected for formulating added, affordable, and culturally acceptable products to help combat persistent protein malnutrition (Oyeleke *et al.*, 1985). These genotypes had protein content less than 30%, suggesting a need for more breeding effort to increase the proportion of high protein genotypes in Chad. As highlighted by Ajeigbe *et al.* (2008), and Affrifah *et al.* (2021), the high protein content with hardly any anti-nutritive factor represents a major advantage in the use of cowpea in nutritional products for infant and children's food and cowpea could be a good source of protein for industrial product manufacturing.

Table 1: Mean seed protein content of eight cowpea lines in two agroecological sites of Chad

Genotypes	Protein content across environment		
	Bebedjia	N'Djamena	Genotypic mean
IT81-D994	23.62 ± 1.11 ^c (6)	24.95 ± 1.82 ^{cd} (5)	24.29 ± 0.93 ^b
IT99-K573-1-2	23.86 ± 0.92 ^c (5)	23.81 ± 0.33 ^{de} (6)	23.83 ± 0.64 ^b
KVX-30-30966	22.28 ± 1.28 ^d (7)	25.40 ± 0.88 ^c (4)	23.83 ± 2.05 ^b
Melakh	21.09 ± 1.39 ^d (8)	22.78 ± 1.00 ^{ef} (7)	21.94 ± 0.69 ^c
TN-5-78	27.41 ± 0.98 ^b (2)	29.10 ± 1.13 ^b (3)	28.26 ± 1.66 ^a
TN-27-80	28.63 ± 1.11 ^a (1)	29.59 ± 1.53 ^{ab} (2)	29.11 ± 2.07 ^a
TN-985-61399	27.24 ± 1.22 ^b (3)	30.71 ± 1.11 ^a (1)	28.97 ± 2.17 ^a
Vita 5	26.95 ± 0.55 ^b (4)	22.34 ± 1.30 ^f (8)	24.20 ± 1.86 ^b
Environmental Mean	25.02 ± 2.85 ^B	26.09 ± 2.91 ^A	25.55 ± 2.66
LSD 5%	0.97	1.26	1.04
CV (%)	11.39	11.15	10.41

Means of varieties with the same subscript within the same column do not differ significantly ($p > 0.05$); LSD: Least significant difference at 5% level of probability; CV: Coefficient of variation; Number in parenthesis denote ranking of varieties in each environment

3.2. Heritability and genetic advance

Table 2 summarizes the genetic parameters of the seed protein content of the eight varieties in the two locations. Broad-sense heritability (h^2) is a commonly used parameter in plant breeding (Allard, 1960). The estimates of h^2 represent the proportion phenotypic variance which is due to genetic effects (Xu *et al.*, 2009). In the present study, the heritability of seed protein content was 0.74, which was high, indicating that the protein content can be inherited and can be selected for in the progeny (Table 2). In general, the

genetic variance was higher in magnitude than the environmental variance, suggesting that the environment had little effect in the expression of this character. Genotypic and phenotypic variances make available the information of variability only but the heritable portion of this variation is determined by the estimation of heritability. The higher phenotypic variance as compared to environmental variance (Table 2) would indicate that the phenotypic variance was less influenced by environment. High broad sense heritability values recorded in both environments are

within the values reported from several published studies for seed protein content in cowpea (Emebiri, 1989; Kabas *et al.*, 2007; Noubissié *et al.*, 2011; Santos *et al.*, 2012; Weng *et al.*, 2019). In Burkina Faso, Sombié *et al.* (2021) reported broad-sense heritability of 0.97 and a genetic advance as percent of mean of 8.73% for protein content in cowpea. Ajeigbe *et al.* (2008) also recorded very high estimates (0.86) of broad-sense heritability for protein content in Nigeria. In the United States of America, Weng *et al.* (2019) noted that the broad-sense heritability of seed protein among 173 cowpea genotypes was 0.51. In addition, range of moderate (0.48) to high (0.68) heritability for grain protein has been found by Noubissié *et al.* (2012) in common beans. Those results indicate that the estimate of broad-sense heritability dramatically varies by environments and among cowpea genotypes and, the 0.74 noted in this study was reasonable. However, heritability values depend on the extent of genetic variability analyzed, unpredictable environment variation and experimental design.

In the present investigation, high heritability estimates ($h^2 = 0.74$) combined with moderately high value of genetic advance as percent of mean (GA = 14.60%) was noted for protein content. The estimate of genetic advance is more useful as a selection tool when considered jointly by heritability estimates. The estimates of genetic advance help in understanding the type of gene action involved in the expression of various polygenic characters (Allard, 1960). High values of genetic advance are indicative of additive gene action whereas low values (GA < 10%) are indicative of non-additive gene action (Sombié *et al.*, 2021). This result is crucial for breeding programs because additive variance, which depends only on the contribution from homozygotes, can be fixed by selection, and is the most important component in gain prediction expressions (Allard, 1960). Therefore, the protein content in cowpea is controlled by additive and non-additive genes and a limited scope for improvement in this trait is expected in earlier generations. Improved methods to predict genetic gain and evaluate these quantitative traits without the environmental influence are also needed.

Table 2: Broad-sense heritability and genetic advance for seed protein content of cowpea grown in two agroecological sites of Chad

Parameter	Environnements		Mean
	Bebedjia	N'Djaména	
Environmental variance	2.07	2.16	2.12
Phenotypic variance	8.13	8.46	8.30
Broad-sense heritability	0.74	0.74	0.74
GA (K = 1,75)	3.69	3.77	3.73
GA (%)	14.75	14.44	14.60

GA: Genetic advance at 10% level of selection; GA (%): Genetic advance as percent of mean

3.3. Genotype x environment interaction effect

The combined analysis of variance for seed protein content (Table 3) showed that there are highly significant differences ($p < 0.01$) for cowpea genotypes (F ratio = 301.76), environments (F ratio = 88.81) and their interaction (F ratio = 48.67). Seed protein content was highly significantly affected mainly by genotype which explained 83.10% of the total variation, while GEI and environment captured respectively 13.40% and 3.50% of the total sum of square. The significant effects of genotype, location and genotype by environment interaction were also observed by Ravelombola *et al.* (2016) for total seed protein content in eleven Arkansas cowpea breeding lines grown in three locations. A large sum of square for genotypic effect indicated diversity of the tested lines for protein content. The proportion of variance captured by environmental effect (3.50%) suggested less effect of environment on seed protein content as compared to the effect of genotypes.

In contrast, according to a study conducted by Oluwatosin (1997) in Nigeria, the environment heavily affect the protein content in cowpea, accounting for 71% of the total variability. The comparison of

environmental means of N'Djaména (26.09%) and Bebedjia (25.02%) showed globally significant differences for this biochemical trait. N'Djaména offered better conditions for protein accumulation as compared to Bébedjia. Location effect was detected, indicating that environment affected seed protein content in cowpea, although the genotype had the main effect. Decreases in protein content were observed during water stress depending on cultivars, due to an increase in the proteolytic enzymes, which break down the stored proteins and from the decrease in protein synthesis (Oluwatosin, 1997).

Furthermore, the responses of some cultivars change with environments suggesting the effect of genotype x environment interaction. The proportion of variance recorded by GEI was stronger than those of the environment. When GEI was significant for a particular trait, no valid comparison could be made regarding the relative performance of genotypes over all environments. According to Hardwick and Wood (1972), the GEI has three adverse effects in plant breeding: (i) it reduces the correlation between genotypic and phenotypic values, decreasing the

progress from selection in a wide range of environments; (ii) it decreases heritability and hinders breeding for complex traits; (iii) it masks the potential benefits of exotic materials introgression. Weng *et al.* (2019) also pointed out that both genotype and

genotype x environment interaction significantly influence seed protein content in cowpea. Therefore, safe environments for production of cowpea with high levels of seed protein must be identified through multi-locations trials.

Table 3: Combined analysis of variance for protein content in the study of eight cowpea cultivars in two environments

Source of variation	df	SS	%SS	MS	F-value	P
Genotype (G)	7	324.51	83.10	46.36	301.76	<0.001
Environment (E)	1	13.64	3.50	13.64	88.81	<0.001
Interaction (I)	7	52.34	13.40	7.48	48.67	<0.001
Residual	32	4.92		0.15		
Total	47	390.49				

df: Degree of freedom, SS: Sum of square, %SS: Percentage of the total sum of square, MS: Mean square; P: Level of probability

4. CONCLUSION

Cowpea provides cheap protein for human consumption in sub-Saharan Africa. In this study, significant genotype, location and genotype x environment interaction effects were found for seed protein content in cowpea. The protein content was significantly different among the eight tested lines, and among the two locations. The tested materials are classified as medium protein lines. Together with industrial processes, breeding could improve the quality of cowpea to meet the needs expressed by different users. The understanding of the genes action for these characters and the genotype x environment interaction effects are the key for best selection strategies of genotypes presenting adequate contents.

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