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Full Length Research Paper

Protein content variation in cowpea genotypes (*Vigna unguiculata* L. Walp.) grown in the Eastern Cape province of South Africa as affected by mineralised goat manure

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Nutrients availability of cultivated soils in the Eastern Cape region of South Africa is generally low, and recognizing that manure application has been one of the most effective methods to improve soil fertility and crop yield in tropical African countries, we assessed the effect of manure application on the leaf crude protein content of six cowpea genotypes (Vegetable cowpea, Ivory grey, Okhalweni, Fahari, Fahari dark, and 97 K-1069-8) a green house experiment. Fresh leaves were collected from each cowpea plant 21 days after planting and ground in liquid nitrogen for protein extraction and quantification. The results showed that Fahari had the highest concentration of crude protein (46.51 mg/ml) while vegetable cowpea (24.41 mg/ml) had the lowest without the influence of manure application. However, upon application of manure (goat), Fahari dark had the highest crude protein concentration (53.53 mg/ml) while vegetable cowpea had the lowest (29.08 mg/ml). Fahari, 97K-1069-8, Ivory grey, and Okhalweni contained 51.79, 49.03, 44.83, and 38.33 mg/ml crude protein concentrations, respectively. This study demonstrated that genotypes as well as manure application significantly influenced cowpea yields in terms of its leaf crude protein content.

Key words: Cowpea, genotypes, crude protein, manure.

INTRODUCTION

Grain legumes have been described as one of the most important crops in many countries providing about one-quarter of the world's dietary protein. They are an essential source of protein for about 700 million people (Nagl et al., 1997), particularly in the developing countries of South America, Africa, and Asia, where plants provide 83% of total protein in the average diet (Mahe et al., 1994). Cowpea is a grain legume cultivated in the savannah areas of the tropics and sub-tropics. The crude protein from the seed and leaves ranges, respectively between 23 and 32% (Diouf, 2011) and between 13 and 17% in the haulms on a dry weight basis with high digestibility value and high fibre level (Tarawali et al., 1997; Bressani, 1985; Nielsel et al., 1997). Cowpea seed pods and leaves are consumed in fresh form as green vegetables in some African countries (Ghaly and Alkoaik, 2010), while the rest of the cowpea plant after the pods have been harvested and it serves as a nutritious fodder

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for livestock (Abebe et al., 2005). Its nutritive value makes it an extremely important protein source to vegetarian and people who cannot afford animal protein.

Poor soil fertility has been reported as one of the main limiting factors to efficient crop production in arid areas owning to low organic matter, high temperature and low rain fall (Abebe et al., 2005). Manure application has been recognised as one of the most effective methods to improve soil fertility and crop yield in tropical African countries (Kihanda et al., 2004); and it serves as a source of all essential nutrients for improved crop production and soil sustainability.

Most of the soils in former homelands of South Africa have very low fertility status (Laker, 1976). Mandiringana et al. (2005) reported that nutrients availability of cultivated soils in the Eastern Cape region of South Africa is generally low due to low soil organic matter content and low geological reserves of phosphorus (P), potassium (K), and calcium (Ca). Generally, nutrient supplies for crop productions by small-scale farmers in the Eastern Cape Province depend on livestock manure, because of poor access to inorganic fertilizers (Mandiringana et al., 2005; Mnkeni and Mkile, 2006). Although, cowpea is known to be a low management crop that can grow in poor soils, its yields could be improved by application of manure (Abebe et al., 2005).

Ghaly and Alkoaik (2010) reported that nutrients deficiencies such as sulphur, phosphorus, potassium, and magnesium known to affect plant growth may influence protein content and decrease protein yields in plants. Abebe et al. (2005) also argued that manure from confined animal feeding is very valuable for crop production and soil sustainability as a source of essential nutrients and provides an excellent source of organic matter when added to soil. Meanwhile, cowpea plant responds very well to fertilizer application.

Protein deficiency has been reported as one of the main source of nutritional problems in the developing world (FAO, 1997). According to Ghaly and Alkoaik (2010), the most disastrous consequences occur in children where protein malnutrition shows itself in forms of two notorious diseases: marasmus and kwashiorkor. However, about one billion people are still reported to be suffering from protein deficiency and malnutrition worldwide (WHO/FAO, 2007). Breeding high yielding varieties with quality trait such as high protein contents will contribute to food security and improve income generation to alleviate poverty.

Previous reports showed that in most of the developing countries, where majority of the people are vegetarian, the cost of animal protein is too high such that most families cannot afford it (Ghaly and Alkoaik, 2010). Hence, there is an urgent need to increase current agricultural practices into marginal lands in order to bring a lasting solution to the menace of protein deficiency and world food shortage.

It has also been reported that the leaves of cowpea are used as a special delicacy in many African countries including Nigeria, Zaire, Zimbabwe, and Zambia where they are cooked fresh together with immature pods or may be dried and conserved for later use due to their high nutritive value (Ghaly and Alkoaik, 2010). Protein synthesis through the process of photosynthesis remains the only non depletable protein source which can also provide some essential amino acids as well as supply adequate nitrogen in the diet for production of non-essential amino acids (Ghaly and Alkoaik, 2010).

The report of Ghaly and Alkoaik, (2010) also revealed that leafy vegetable protein is about half the vegetable protein present in the human diet and probably amounts more to the world total protein than do fish, whereas less attention is given to it. Furthermore, only about 8 to 20% of the plant protein consumed by animals is recoverable as protein for human nutrition. Thus, more efficient ways of utilizing plant protein must be found. Interestingly, several studies have revealed nutritional differences among genotypes of different species (Nielson et al., 1993; Hall et al., 2003).

South Africa recently acquired some selected genotypes of cowpea for agronomic and nutritional characterization (Rhandzu, 2007). Evaluation study on the performance of this species under different microclimates of the country is needed for the ultimate introduction of these genotypes into the farming systems of the country. Meanwhile, several previous researches on the protein contents of cowpea were accessed on the seed (Sharawy and El-Fiky, 2002; Tshovhote et al., 2003; Kokiladevi et al., 2005) whereas the percentage of crude protein in cowpea leaves is higher than the amount found in the seed (Tarawali et al., 1997, 2002). Evaluating the protein contents is the first step at improving the protein quality of any crop. However, there is currently no evaluation study on the leave protein contents and the most appropriate time to introduce accessions of cowpea into the farming systems of the Eastern Cape province of South Africa. This study therefore aimed at assessing the nutritional value of the leave crude protein content of six cowpea genotypes as affected by manure application and also to determine the feasibility of using them as a protein supplement in the Eastern Cape province of South Africa.

MATERIALS AND METHODS

Plant

Six genotypes of cowpea (*Vigna unguiculata* (L). Walp.), namely vegetable cowpea, Ivory grey, Okhalweni, Fahari, Fahari dark, and 97K-1069-8 were used in this study. These genotypes were obtained from the Agricultural Research Council, Vegetable and Ornamental Plant Institute, Pretoria, South Africa and their origins are as shown in Table 1. The six accessions were planted in two separate groups in a plastic pot in the green house of the University of Fort Hare, Alice, South Africa. Dried goat manure was applied to

Table 1. Names and origin of the eight cowpea genotypes used.

Genotype	Origin
Vegetable cowpea	South Africa
Ivory grey	South African Cultivar
Okhalweni	Land race from KwaZulu Natal, South Africa
Fahari	Land race from AVRDC Arusha Tanzania
Fahari dark	Land race from AVRDC Arusha Tanzania
97K-1069-8	IITA, Nigeria
IT93K-73h	IITA, Nigeria
129-3	IITA, Nigeria

IITA: International Institute of Tropical Agriculture

Table 2. Some chemical and physical characteristics of the soil and manure used.

Parameter	Soil (sandy loamy)	Manure (goat)	Mixture
Organic carbon (%)	2.23 ± 0.23	7.36 ± 0.32	6.40 ± 0.35
Total nitrogen (%)	0.05 ± 0.02	0.45 ± 0.06	0.71 ± 1.26
Phosphorus (%)	0.67 ± 0.76	2.04 ± 0.55	1.09 ± 0.06
Potassium (ppm)	0.80 ± 0.07	9.63 ± 0.83	0.76 ± 0.12
Magnesium (ppm)	0.03 ± 0.02	2.19 ± 0.15	0.09 ± 0.05
Sodium (ppm)	0.35 ± 0.07	0.55 ± 0.06	0.26 ± 0.06
Calcium (ppm)	0.54 ± 0.04	9.15 ± 0.80000	0.47 ± 0.02
C:N	45:1	16:1	9:1

Mean of Three replicates.

one group, while the other group was raised without manure application. The plants were cultivated in pots with 5 kg soil capacity containing a mixture of two parts of soil and one part manure. Fresh leaves were collected from each cowpea plant 21 days after planting and were ground in liquid nitrogen.

Extraction of crude protein

The extraction of proteins from cowpea leave was carried out following the method of Mirkov et al. (1994). 100 mg of finely grounded leaves flour were extracted in the extraction buffer (10 mM Tris-HCL [pH 7.5], 500 mM NaCl, 1% 2-mercapto-ethanol, 0.1% Triton-X-100, 2 mM phenylmethylsulphonyl fluoride [PMSF] [1 ml/ml sample]) by homogenization followed by incubation at 4°C for 1 h, and then centrifuged at 15,000 rmp for 15 min at 4°C. The supernatant was collected and stored frozen in aliquots.

Quantification of leave crude protein

The protein content in the extract was quantified using a Pierce® BCA Protein Assay Kit following the manufacturer's procedures (www.piercenet.com). A protein standard was prepared using bovine serum albumin (BSA) and absorbance was taking at 562 nm using a microtitter plate reader from which a standard curve for BSA was constructed. The samples' absorbances were also read at 562 nm and their duplicate concentrations were determined by interpolation from the BSA standard graph. Data were analyzed using analysis of variance (ANOVA) in the Statistical Package for Social Sciences (SPSS) version 13 at $P \leq 0.05$

Physicochemical analysis of the soil and manure used

The soil and goat manure used for this study were collected from the University of Fort Hare farm. The samples were obtained from the top 0 to 20 cm depth using a spade and were air-dried and passed through a 2 mm sieve for characterization at the Department of Soil Science, University of Fort Hare. Organic carbon content was determined by the Walkely-Black procedure as described by Nelson and Sommers (1996). Total Ca, Na, K, and Mg in both soil and manure were estimated following wet digestion with sulphuric acid and hydrogen peroxide (Okalebo et al., 2002). The total nitrogen and phosphorus were determined calometrically as described by Okalebo et al. (2002).

RESULTS AND DISCUSSION

The result of the physicochemical properties of the soil and manure used is presented in Table 2. Previous report has shown that macronutrients available in the soil should be in the range of N (0.1 to 0.5%), P (0.08 to 0.5%), and K (1.5 to 3.0%) in other to produce a good yield (Dutta, 2005). The total soil nitrogen was 0.05%

Table 3. Leave crude protein concentrations of six cowpea genotypes showing the effect of manure application.

Cowpea genotype	Crude protein concentration (mg/ml)	
	With manure	Without manure
Vegetable cowpea	29.08 ± 0.91	24.41 ± 4.96
Ivory grey	44.83 ± 21.4	42.72 ± 1.03
Okhalweni	38.33 ± 17.4	37.70 ± 1.42
Fahari	51.79 ± 3.25	46.51 ± 4.40
Fahari dark	53.53 ± 18.76	39.85 ± 1.08
97K-1069-8	49.03 ± 5.45	38.51 ± 0.81

F- LSD (Least significant different) 1.3 ($P \leq 0.05$).

which is below the range of most cultivated top soils (0.06 to 0.5%). Also, the concentration of P and K were not sufficient according to Ryan et al. (1996). Exchangeable Calcium in soils should range between 12 and 75% of cation exchange capacity (CEC), while the exchangeable Mg in soils ought to range between 4 and 20% of CEC (Eckert and McLean, 1981). Also, the Ca and Mg concentrations in this study were very much below average confirming the report of Mandiringana et al. (2005) that soils in the Eastern Cape Province contained extremely low macronutrients. The C:N ratio of the soil is far higher than that present in the manure, thereby accounting for the low rate of mineralization seen in the chemical properties of the soil than the manure. Meanwhile, the optimum macronutrients content of the goat manure used is higher than that present in the soil as presented in Table 2.

The results for the protein studies as presented in Table 3 showed that Fahari had the highest concentration of crude protein content (46.51 mg/ml), while vegetable cowpea (24.41 mg/ml) had the lowest crude protein content without the influence of manure application. Ivory grey, Okhalweni, Fahari dark, and 97K-1069-8 yielded significantly different ($p \leq 0.05$) protein concentrations of 42.72, 37.70, 39.85, and 38.51 mg/ml, respectively. However, upon the application of manure (goat), Fahari dark had the highest crude protein concentration (53.53 mg/ml), while vegetable cowpea had the lowest value (29.08 mg/ml). Fahari, 97K-1069-8, Ivory grey and Okhalweni contained 51.79, 49.03, 44.83, and 38.33 mg/ml crude protein content, respectively.

The present study found significant differences ($p \leq 0.05$) among the six different cowpea germplasms for leave crude protein yield when tested on goat manure mixed with sandy loamy soil as well as untreated soil. The differences were probably due to genetic variations among genotypes, environmental factors and climatic conditions (Ali-khan and Youngs, 1973).

The result of the total protein content in this study is much higher than those obtained by Ghaly and Alkoaik, (2010) probably due to the geographical location where the plants were cultivated and season, the optimum harvest age for protein extraction (70 days compared to 21 days) as well as the influence of manure application. These same conclusions were suggested in previous work of Ghaly et al. (2010).

Also, Abebe et al. (2005) reported that the release of essential nutrients upon the decomposition of air dried manure by microbes mainly contributes to the availability of nutrients in soils and plant as well as improvement in the protein content of cowpea. Plants grown on manure treated soils showed higher content of protein than those without manure treatment. Interestingly, as documented in previous study of Abebe et al. (2005), application of dried goat manure influenced an increase in the crude protein concentration as presented in Table 3. For example, Fahari dark contained 53.53 mg/ml of crude protein as compared to 39.85 mg/ml when cultivated without manure application. The same variations in crude protein contents were discovered for all the studied genotypes. The higher content of protein was due to high nitrogen supplied as a result of manure treatment (Abebe et al., 2005). However, the influence of manure application was minimal on the crude protein concentrations in Ivory grey (44.83 mg/ml against 42.72 mg/ml) and Okhalweni genotypes (38.33 mg/ml against 37.70 mg/ml), respectively; an indication that cowpea genotypes react differently to the application of plant nutrients.

This study also agreed with the report of Abebe et al. (2005) that differences in protein level that is shown within species may be as a result of variations in genotypes as well as agronomic practices. This probably explained the variations observed in the crude protein concentrations among the studied genotypes. For instance, Fahari variety has 46.51 mg/ml crude protein, while vegetable cowpea contained 24.41 mg/ml crude protein without the influence of manure treatment. The same scenario was discovered for all the studied genotypes as shown in Table 3. Fahari and Fahari dark outperformed all the other genotypes with crude protein concentrations of 46.51 and 53.53 mg/ml respectively. Thus, these two genotypes could be better alternative sources for cheap and affordable plant proteins for vegetarians as well as to the marginal income bracket communities of the Eastern Cape Province, South Africa. Conclusively, this study demonstrated that genotypes as well as manure application significantly influence cowpea yields in terms of its leave crude protein content.

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