ORIGINAL ARTICLE

EFFECT OF ENVIRONMENT ON PROTEIN, MINERAL AND FAT COMPOSITION OF FABA BEAN

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ABSTRACT

BACKGROUND: ***Faba bean (Vicia faba L.) is one of the most important high land pulse crops of Ethiopia. It is an excellent source of protein supplement for the majority of the population, used in various popular Ethiopian dishes. It also contains fat and appreciable amount of minerals. Data on the effect of environment on the protein, mineral matter and fat content of faba bean grown in southwestern agro-ecological zone of Ethiopia is lacking. This study was designed to determine the effect of environment on the protein, mineral and fat contents of advanced lines of faba bean genotypes grown in Southwestern agro-ecological zones of Ethiopia.***

METHODS: ***The effect of environment on the nutritional compositions of improved varieties of faba bean and one local variety was investigated between August 2000 and January 2001. Seed samples were obtained from Holetta Research Center and grown in three locations namely, Dedo, Gera and Yebu in South-western Agro -ecological zones. The crude-protein content was determined using micro-Kjeldahl method. Data on crude fat and mineral matter contents were obtained using standard procedures described in Association of Official Analytical Chemists. Data were collected and descriptive statistics was employed to examine findings. Analysis of variance was computed and statistical variations were determined as significant at $P<0.05$ using MSTATC procedure version 5.1 Soft ware.***

RESULTS: ***The Genotypes investigated gave different percentage protein, mineral matter and fat ranges for all sites. Variations were statistically significant for protein content at Gera and Yebu and significant variation for mineral matter contents was observed at Gera only.***

CONCLUSION: ***The results obtained confirm that those genetic variations were lower than those caused by the environment. In addition, variations in nutritive values do exist among cultivars. It is important to make a more detailed examination at amino acid level and specific mineral content to determine the nutritional variation among the faba bean genotypes.***

KEY WORDS: **Agro-ecological zone, Vicia faba, faba bean genotypes, altitude**.

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INTRODUCTION

Faba bean (*vicia faba*) is one of the most important High land pulses of Ethiopia. It is most widely cultivated in Ethiopia in the mid altitude (1800-2200 meters, average annual rainfall of 740 mm and mean daily temperature of 18-22^{o}c), and high altitude (>2200 meters, average annual rainfall of 900mm and mean daily temperature of 18-20^{o}c). It is grown from June to December in rotation with cereal (1). Of the cultivated grain legumes (pulses) in Ethiopia, faba bean ranks first in terms of area coverage and production (1-2).

Faba bean is an important source of fat and mineral (3). Above all faba bean is an excellent source of protein supplement for the majority of the population, used in various popular Ethiopian dishes such as curry and injera (a pancake bread) (1). It has also a relatively higher content of the essential amino acids lysine and threonine (4). Protein has been recognized as a dietary essential for a century. Proteins are the most important of all nutrients, no life possible without protein (5).

Grain protein content can be considered as the amount of protein per seed or unit of weight of grain. It is directly controlled by the plant's capacity to take up and transfer nitrogen from roots and leaves to the seed (6). In spite of the high production level of seed protein by the faba bean, breeders continue their attempts to improve the quality of this major seed component. However, knowledge of the range in variation of seed components such as protein is per amount importance for improving the nutritional quality of the faba bean. Some screening investigations on the protein content of faba bean have been carried out and several workers reported different protein values (3, 8-9) (22.7-29.6, 25.4, 26.33%, respectively).

In Southwestern Ethiopia the very few chemical studies on faba bean were made on market samples that are usually mixtures of different genotypes and generally lumped according to seed colour, shape & size (7). Addisie (10-13) has analyzed faba bean samples obtained from research centers like Holetta, Kulumsa and Bekoji. The results obtained by him ranged from 6.8 to 25.8% of total protein (on dry weight basis) with an average of 18.83%. Senait and her co-workers have reported similar result (14) and this was calculated from percent nitrogen using a conversion factor of 6.25. The conversion factor 6.25 is obtained by assuming that protein contains 16% nitrogen. The Ethiopian Nutrition Survey Team has used the conversion factor of 5.83 (7). So it must be obvious that by using the same conversation factor for all protein sources significant error will be introduced (15). However, it was recommended that the factor 5.7 to be used as a standard N to protein conversion factor for reporting protein content in all products of plant origin (16).

Six per cent of the body weight is made up of as many as 60 different mineral elements. Approximately 21 of these have proved essential in human nutrition (5). Faba bean contains appreciable amount of mineral (3). Some screening investigation by different workers on faba bean revealed that the range of minerals in the seeds of the crop was 1.42 to 7.1% (3, 8, 9, 17, 18).

Until recently, nutritionists considered the fat components of the diet only important as a concentrated source of energy and little else. It is now recognized that at least one component of the fat is a dietary essential and has important health implications (5). In Ethiopia, there is little or no information on the fat content of faba bean. Agren and Gibson reported 1.4 per cent fat content in faba bean (17). Similar results reported by other researchers (8, 9) (1.5%, 2% in that order).

The effect of environment on gene expression is evident. It has from high to little or no influences on gene expression. In addition, environment has influence on nutritional composition of faba bean. For example, the protein content of the faba bean seeds grown in a location with high N-content could be higher compared to the protein content of faba bean seeds grown in a location with soil that has low N-content.

The protein, mineral and fat contents of faba bean are of major nutritional composition of the crop. In Ethiopia some efforts have been made to determine the chemical composition of the crop grown in some parts of the country. However, the studies made to date are not exhaustive. Data on the effect of environment on the nutritional composition of faba bean genotypes produced in some major faba bean growing regions of Ethiopia such as South-western Ethiopia is lucking.

The present study aimed at eliciting the effects of environment on protein, mineral and fat contents of faba bean genotypes grown in different locations of Southwestern Agro-ecological zone of Ethiopia. It is hopped that the knowledge obtained through this study will serve as part of important information required for future research activities.

MATERIALS AND METHODS

Seed samples

The study was conducted between August 2000 and January 2001. Six advanced lines of faba bean and one local check that represent the range of possible variation in *Vicia faba* so far identified morphologically, on yield basis or otherwise, were used in this study. Seed samples were obtained form Holetta Agricultural Research Center of the Ethiopian Agricultural Research Organization (EARO). The seed samples were grown in southwestern agro-ecological zone of Ethiopia. The locations selected for the field experiments were Dedo (2400 meters), Gera (2300 meters) & Yebu (2200 meters). In addition, the sites were also varying in soil, temperature, rainfall amount, *etc*. The plants were grown with no additional supplement of fertilizers. In all location, the randomized complete block design was used with a plot size of 4m x 1.6m in four replications. Various agronomic data were recorded. Crop management practices such as weeding, were applied as per the National Highland Pulse Team recommendation.

Crude protein determination

In this study, seed samples from each plot were cleaned by hand and grounded. Flour samples were digested by micro-Kjeldahl method. Faba bean flour samples of 0.5 gm were weighed in tarred scoop and transferred to boiling tubes.

A catalyst tablet, selenium (mixture of sodium sulfate and copper sulfate in the ratio of 10:1) was dropped into each tube and about 25ml of concentrated sulfuric acid was added. The tubes were then placed in a heater set at 200^{o}C and heating was controlled automatically. Mixture was heated until the color changes to light blue. For samples which have digest color of light brown or yellow, the digestions were repeated two or more times. Thirty ml of distilled water was added into the digestion tube carefully. During these events, the organic matter of faba bean flour is oxidized, and the protein nitrogen is converted to ammonium (19, 20).

Ammonium in the digestion mixture was determined by distillation and titration (20). The digestion tube was placed on to the Tecator steam distillation apparatus. The distiller was set and the digestion tube was inserted in the system and 150ml distillate was collected in the receiver flask containing 50ml 4% boric acid. Then the distillate was titrated against a standard acid (0.1N 10%HCl). The total Nitrogen was converted to crude or total protein by multiplying N% by the factor 5.7 as given by Tkachuk (16).

Mineral matter and fat determination

The percent of mineral matter & crude fat were determined using procedures described in Association of Analytical Chemists (AOAC) (20). Mineral matter was determined after igniting the sample at 500^0C to burn off all organic material. Finally, the percentage of mineral matter was calculated according to the formula given in Association of Analytical Chemists (20).

In fat determination, floor samples (0.5gm) of faba bean were weighed in a pre-weighed thimble and introduced in to the soxhelet. Solvent beakers were dried in an oven at 105^0C for 30 minutes, cooled in a desiccator to room temperature, and weighed. Ether was poured through the condenser at the top by means of funnel. Water was then turned on to cool the condenser. The hot plates were raised until they are in contact with the beaker and heaters were turned on. Careful check was made for ether leaks after the ether started to boil and condense and the apparatus was left (with repeated checks), for 8 hours until extraction was complete. Then the heater was lowered and the thimble was allowed to drain empty. The samples were removed and glass ether reclaiming tubes were placed under the condenser. Then beaker was replaced, hot plate was raised and ether was distilled into the reclaiming tubes. Then after beakers were removed from hot plates and they were allowed to remain in an open air under a hood to completely dry the ether. Finally the ether extracts (ether extracts + beaker) were dried in a forced-draft oven at 105^0C for 30 minutes and were cooled in a desiccator at a room temperature and weighed. Statistical analysis was made using the procedure of version 5.1- MSTATC software.

RESULTS

Protein content of seven genotypes of faba bean as determined by Kjeldahl procedure is given in Table 2. Cultivars gave different percent protein contents for all locations, and ranks of varieties varied considerably. At Dedo varieties Mesay and CS 20DK gave the highest (25.78%) and the lowest (22.36%) values, respectively. At Gera and Yebu the local check and NC 58, and Tesfa and Kuse-2-22-33 gave the highest (26.9% and 25.73%) and lowest values (20.18 and 23.85%), in the mentioned order.

The over all mean for mineral content ranged between 1.7 and 15% (Table 2). The highest mean (15%) for mineral content was obtained at Yebu from the local check, the lowest (1.7%) from NC 58 at Dedo and Gera. Variation for the mean crude fat contents (1.5-7%) of the genotypes is given in Table 2. The highest value (7.4%) for fat content was recorded at Dedo for local check, where as the lowest fat content (1.5%) was obtained at Yebu for Bulga genotype.

Variation among genotypes was considerable & it was statistically significant ($P<0.05$) at Gera and Yebu for protein content. But a mineral matter content of the genotypes is significant at Gera only.

Table 1. Genotypes used in field experiment, Jimma, August 2000-January 2001.

Nº	Genotypes	Altitude (m)
1	CS 20DK	2100-2700
2	Bulga	> 2200
3	Kuse - 2-22-33	2100 - 2700m
4	Mesay	> 2200
5	Tesfa	> 2200
6	NC 58	1900 - 2100
7	Local check	1900 - 2400

Table 2. Percentage protein, percent mineral matter and percent fat content of seven faba bean genotypes grown at three locations on a dry weight basis. Percent protein content, Jimma, August 2000-January 2001.

Genotypes	Dedo	Gera	Yebu	Mean
Protein content				
CS 20DK	22.36	24.19	25.22	23.92
Bulga	24.89	22.56	25.19	24.21
Kuse - 2-22-33	24.83	24.66	23.85	24.45
Mesay	25.78	26.01	24.58	25.45
Tesfa	24.66	20.18	23.93	22.92
NC 58	24.02	26.23	25.73	25.32
Local check	25.42	26.9	24.41	25.57
F test	NS	*	*	
CV (%)	5.29	13.64	4.39	
Mineral content				
CS 20DK	11.7	3.3	13.5	9.5
Bulga	5.0	6.7	11.5	7.79
Kuse – 2-22-33	3.3	3.3	13.5	6.67
Mesay	5.0	3.3	8.4	5.57
Tesfa	3.3	3.3	10.0	5.53
NC 58	1.7	1.7	13.5	5.63
Local check	3.3	8.4	15.0	8.9
F test	NS	*	NS	
CV (%)	96.96	65.14	37.11	
Fat content				
CS 20DK	4.5	5.0	5.2	4.9
Bulga	6.5	6.7	1.5	4.9
Kuse - 2-22-33	5.5	8.3	3.0	5.6
Mesay	7.0	6.7	2.5	5.4
Tesfa	6.7	5.3	2.5	4.83
NC 58	6.5	5.2	6.9	6.2
Local check	7.4	5.3	6.9	6.53
F test	NS	NS	NS	
CV (%)	17.91	34.84	55.80	

NS = non-significant; * = $P<0.05$; CV= Coefficient of variation

DISCUSSION

Protein content of seven genotypes of faba bean as determined by Kjeldahl method is given in Table 2. Cultivars gave different percent protein contents for all locations, and ranks of varieties varied considerably. The considerable variation in protein may partly be attributed to variation in genetic make up of the genotypes studied. In addition, variation in environmental factors (soil, temperature, *etc*.,) of the study sites may also contribute for the variation in the protein contents of the genotypes. All genotypes studied exhibited protein content exceeding 14.5%. Therefore, these genotypes represented the best potential sources of genes for protein. The mean values were generally higher than that previously reported for faba bean (21). Nevertheless, this finding is in agreement with crude protein contents of advanced lines of faba bean reported by Addise (10-13) as well as Senait and her co-workers (14).

The genotypes have about three times grater amount of protein than cereals (such as maize and sorghum). This finding is consistent with the previous finding (3). Maize and Sorghum together with starchy root crops are the main sources of calorie (more than 80%) in southwestern Ethiopia. The nutritional quality of the diet is usually considered unsatisfactory when more than 80% of the calories are derived from cereals, starchy roots and sugars (21). The protein content of the genotypes raises the quality of the protein and improves the consumers' dietary intake since faba bean has high-quality protein compared with cereals (4, 14). Further, this plant protein is important, particularly in developing countries in general and in Ethiopia in particular, because of the cost and scarcity of animal protein.

The mean mineral content was much lower than the value reported by earlier workers in faba bean (17). However, this observation was comparable with that of Purseglove (8) who noted greater mineral matter content in the dry seeds. The over all coefficient of variation of mineral matter content was the highest mainly due to the variation in mineral matter between individuals of faba bean samples studied (Table 2). Furthermore, high content of mineral matter indicated that soil nutrients might play a role in mineral matter content of the studied faba bean genotypes. Therefore, mineral content of a given cultivar should be determined across location.

The fat content of genotypes exhibited considerable range of variations (from 1.5 to 7.4%). This finding is in a harmony with the finding of Purseglove (8). More recently, the importance of faba bean in relation to nutritional or metabolic diseases (such as blood cholesterol and diabetes) has become evident. Moreover, several studies have shown, in experimental animals and human subjects that most food legumes including faba bean decreases blood cholesterol values. One of the components in faba bean implicated in lowering serum cholesterol is poly-unsaturated fatty acids (23).

This finding seemed to confirm that genetic variation for protein, mineral and fat contents among the seven genotypes is lower than the difference observed due to location. The variation in grain protein content, which can be attributed to genetic difference, is generally smaller in magnitude than the variation caused by environmental influence (22). It was also reported that protein concentration of wheat grain was four times more dependent on growth conditions than on genotypes (24). It would be important to make a more detailed examination on the essential amino acid composition & specific nutrient contents such as iron, calcium, and phosphorus.

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