J. Agric. Sci. Mansoura Univ., 33 (6): 4609 - 4617, 2008

IDENTIFICATION OF SOME FABA BEAN (*Vicia faba* L.) VARIETIES USING MORPHOLOGICAL AND CHIMICAL METHODS

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ABSTRACT

This study includes seven faba bean (*Vicia faba L*.) varieties to identify it using morphological and chemical methods. Morphological identification is carried out by observation through recording a range of morphological characters as reported in the internationally recognized descriptor of the International Union for Protection Of new Varieties (UPOV1994). According to the UPOV guideline could use about nine characteristics such as plant height, anthocyanin coloration, pod attitude and seed black pigmentation of hilum can be used to distinguish between the varieties. The chemical composition analysis showed that the varieties seeds contained average percentage as follows, protein (23.52- 28.92 %), crude fat (1.21- 1.51%), carbohydrate (58.63- 64.47 %) and crude fibers (6.10-8.35%). The crude protein of different varieties were fractionated into five fractions (albumins, globulins, prolamins, glutelins and non soluble proteins) depending upon the solubility in different solvents. The ratio of globulins\ albumins and globulins\prolamins were calculated and results of these ratios can be use to identification of some varieties under study.

INTRODUCTION

Identification of cultivars among plant species is becoming increasingly important and developing reliable technology for analysis is imperative. Morphological description may be expensive and time consuming process, and some certain characters are continuous and their expression are affected by the environmental factors. To supplement the morphological description and chemical methods were conducted chemical composition of seeds is basically determined by genetic factors as sources for different raw materials. Modern varieties of faba bean have been developed for higher content of proteins which represent significant improvement over earlier varieties.

Varieties identification is generally accomplished by evaluating several morphological characteristics such as seed, seedling and plant morphology traits. Determine the chemical composition and protein fraction is one of the the most useful data for identification of the different varieties.

Faba bean considered as important diet for people in developing countries. They are consumed in the form of immature tender green pods, green mature seed or as dry seeds after slow boiling cooking.

World cultivated area of faba bean for 2005 was 6,364 million fed. and the production was 4,343 million ton (FAO,2005) .

In Egypt, Faba bean seeds are among the major nutritional source of plant proteins. Its seeds contain. Faba bean seeds vary greatly in size

depending on the variety. The colour of seeds may be oblong reddish brown, greenish brown and purple to black. It is widely grown as a winter season crop in subtropical and tropical climates and as a spring season crop in temperate regions. Mudzana *et al*. (1995*)* discrimination in 12 faba bean varieties using visual assessment of plant morphology and biochemical character. These assessments included seed characters, plant characters, stem characters, flower characters and pod characters. Hammam (1996) studied the gross composition of mineral content and functional properties of five genotypes broad bean. He found little differences among the ecotypes varieties in their chemical composition ranging between 23.4 to 26.45% for protein, 1.58 to 1.88 for fat and 68.32 to 71.88% for total carbohydrates

Mohamed (1999) fractionated crude proteins of faba bean into five fractions. Which were Albumins, globulins, prolamins, glutelins and non-soluble proteins depending upon the solubility in different solvents. Some protein fractions had the same values for certain faba bean germplasms. Therefore, the ratios of globulins / albumin and globulins / prolamins were calculated and the data of these ratios can easily be used to characterize the germplasms belonging to each crop.

Naguib (2000) evaluated morphological characters for identification of some faba bean varieties using qualitative characters like pod color, seed coat color and hilum color also the used quantitative characters like leaflet characters, number of flowers, plant height and pod characters. This study indicated that these characters are important descriptor for discrimination among different studied faba bean genotypes.

Sayed (2006) investigated morphological characters for identification of eight soybean genotypes using UPOV descriptor. The obtained result indicated that some qualitative characteristics could be used for soybean genotypes identification.

MATERIALS AND METHODS

Varieties seed samples:

Seed samples of seven faba bean (*Vicia faba L*.) varieties were used in this study: { Giza Blanka (G.B.), Giza 716 (G.716), Giza 717 (G.717), Masr 1 (M.1), Masr 2 (M.2), Giza 3 (G.3) , Giza 643 (G.643)}.Samples were obtained from the Leguminous Crops Research Department (LCRD), Field Crops Research Institute, Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt.

Morphological characteristics:

The morphological identification was measured and recoded using the recommended scales as reported the International Union for Protection Of new Varieties (UPOV1994) descriptors. The decimal code for the growth stage of legumes according to Tottman (1987) was also used to standardize the growth stage of varieties during morphological description and identification.

Chemical composition:

Untreated seed samples were randomly taken to determine moisture, crude protein, crude fat, total carbohydrate, crude fibers and ash according to A.O.A.C (1990).

Protein fractions:

Fractionation of seed proteins was conducted according to the method of Skoch *et al.* (1970). Samples were subjected to successive extraction processes using the following solvents: distilled water for the extraction of albumines, sodium chloride (5%,w/v) for the extraction of globulins, ethanol (80% v/v) containing sodium acetate (0.2%w/v) for prolamins and sodium hydroxide (0.2%,w/v) solution for glutelins.

RESULTS AND DISCUSSION

1-Morphological characteristics:

1- Data in Table (1) indicated that the plant height of faba bean are medium in height except for G.716 variety which was the shortest.
2- The intensity of anthocyanin coloration in stem was medium which detected in M.1 variety but was slight in the rest of the tested varieties.
3- Concerning pod medial width, G.B., G.716 and M.2 faba bean varieties have broad melanin width of pod whereas all the other studied varieties having medium melanin width.
4- Data listed in Table (1) clarified that G.B. and M.2 faba varieties were semi-pendulous while the other varieties were of semi-erect pod.
5- Pod length without beak was medium in the all varieties under studied except G.B. while G.716 faba been varieties were the longest.
6- Seed shape of medium longitudinal section was square for G.B., G.716 and M.1 faba bean varieties, oblong for G-717 and ovate for the rest of the tested faba bean varieties.
7- Weight of 1000 – seed of the studied faba bean varieties were medium for G.B, G.717 and M.1; large for G.716, G.3 and G.643 and very large for M.2 faba bean variety.
8- All of the studied faba bean varieties were of the black pigmentation of hilum in seed except G.B. variety was absent.
9- G.B. and M.2 faba bean varieties were the only one which recorded to be the latest varieties of first developed pods but the other varieties were recorded to be of medium time.

Results of Morphological characteristics of faba bean varieties under study are in agreement with many authors which studied different morphological characteristics for faba bean varieties identification. As instance, Mudzana *et al*. (1995) and El-Emery and El-Rabie (1996) identified of some faba bean varieties using visual assessment of plant morphology and biochemical character. These assessments included seed, plant, stem, flower and pod characters. Moreover, Naguib (2000) evaluated the morphological characters to identify some faba bean varieties by using qualitative characters and quantitative characters. This study indicated that these characters are important descriptor for discrimination among different studied faba bean genotypes.

Table (1): Morphological characteristics of faba bean varieties under study.

Characteristics	Degree	G.B	G.716	G.717	M.1	M.2	G.3	G.643
1-Plant: height	Very low (1) Low(3) Medium(5) Height (7) Very High(9)	5	3	5	5	5	5	5
2-Stem: intensity of anthocyanin coloration	Slight(3) Medium(5) Much(7)	3	3	3	5	3	3	3
3-Pod: Medial width	Very narrow(1) Narrow(3) Medium(5) Broad(7) Very broad(9)	7	7	5	5	7	5	5
4-Pod: attitude	Erect (1) Semi-erect(3) Horizontal(5) Semi-pendulous(7) pendulous(9)	7	3	3	3	7	3	3
5-Pod: Length (without Beak)	Very short(1) Short(3) Medium(5) Long(7) Very long(9)	7	7	5	5	5	5	5
6-Seed: shape of Median longitudinal section	Elliptic(1) Broad elliptic (2) Circular(3) Oblong(4) Square(5) Ovate(6)	5	5	4	5	6	6	6
7-1000 seed weight	Very small(1) Small(3) Medium(5) Large(7) Very large(9)	5 (678)	7 (786)	5 (712)	5 (685)	9 (912)	7 (774)	7 (862)
8-Seed:black pigmentation of hilum	Absent(1) Present(9)	1	9	9	9	9	9	9
9-Time of full development of pod(first developed pods)	Early(3) Medium(5) Late(7)	7	5	5	5	7	5	5

2-Chemical Composition

The gross chemical composition analysis (on dry matter basis) of seven faba bean varieties under study is given in Table (2).

Moisture content ranged from 9.81 to 10.52 %. The highest value was 10.52% while the lowest one was 9.81 % for G.643 and G.3 varieties, respectively. Moisture content of the other genotypes ranged from 9.81 to

10.52 %. Such results of moisture content were affected by many factors as location, variety, maturity and storage were reported to effect on the moisture content of seeds (Rosario *et al.,* 1980)

Result in Table (2) showed the crude protein content of faba bean for the varieties under investigation. Results indicated that the variety M.2 had the highest protein content 28.92 %, while variety M.1 gave the lowest value 23.52 %. However, protein content behaved in a similar trended which was 28.11 and 28.75 % for G.643 and G.717 varieties, respectively. Whereas, levels of crude protein of other genotypes ranged from 23.52 to 28.92 %.

Results indicated that the highest value of crude fat content was obtained for variety M.2 (1.51 %). While the lowest value was found in variety G.3 (1.21%). The level of crude fat content of the other genotypes ranged from 1.21 to 1.51 %.

Carbohydrate contents of faba bean varieties under study are presented in Table (2). Data indicated that the variety G.B. contained the highest values of carbohydrate content (64.47%). However the lowest carbohydrate content was obtained in seeds of M.2 variety (58.63 %). Carbohydrate content for seeds of the other genotypes were ranged between the heighest and the lowest values as mentioned before.

Data indicated that the highest value of crude fibers was` 8.35% while the lowest value was 6.10 % for G.3 and G.717 varieties, respectively. Crude fibers content of the rest of the varieties ranged between these tow limits.

Results presented in Table (2) showed the ash content of the listed faba bean seeds. The highest ash content was recorded for faba bean varieties G.643 (4.35%) and M.2 (4.21 %). Whereas, the lowest ash content was found for faba bean seeds variety G.717 (3.42 %).It is generally noticed that the levels of ash content in the other genotypes under study were ranged from 3.42 to 4.35 %.

Results of the chemical composition of faba bean varieties under study are in agreement with those of Welch and Griffiths (1984), Salama (1988), Hussien (1989) and Naguib (2000) where they found that the faba bean seeds contained 8.60% moisture, 24% protein, 55.5% carbohydrates, 9.50% fiber, 3.50% ash, and 1.30% fat. Total crude protein content is also affected by several parameters including soil type, climatic conditions, region, use of fertilizers, and genetic factors {Mosse and Pernollet, (1982) and Deshpande and Damodaran (1990).}

Table (2): Chemical composition of faba bean varieties under study. (On dry matter basis)

Varieties	Moisture	Crude protein	Crude fat	Total carbohydrate	Crude fibers	Ash
G.B.	9.85	24.74	1.25	64.47	7.38	3.82
G.716	9.96	27.66	1.41	62.15	7.67	3.63
G.717	10.11	28.75	1.38	60.24	6.10	3.42
M.1	9.91	23.52	1.45	63.25	6.82	3.65
M.2	10.05	28.92	1.51	58.63	7.46	4.21
G.3	9.81	27.77	1.21	59.13	8.35	3.55
G.643	10.52	28.11	1.32	61.50	6.24	4.35

3-Protein Fractions

Samples of the crude proteins obtained from faba bean seed varieties under investigation were fractionated into five fractions (Albumins, globulins, prolamins, glutulins and non-soluble proteins) depending upon the solubility in different solvents and the results were listed in Table (3).

3-1-Water soluble protein (Albumins)

Data presented in Table (3) clearly recorded the albumins content of faba bean varieties under study. Such results indicated that the highest albumin value was found in G.717 (16.95 %) followed by G.716 (16.20 %), while the lowest value (13.63 %) was found in G.643 variety. Each of the varieties M.2 and G.3 contained approximately similars values which were 14.11 and 14.18 %, respectively.

3-2- Salt soluble protein (globulins)

The globulins content of faba bean varieties under investigation recorded in Table (3). The globulins results showed that the highest globulin content was found in M.1 variety (37.57 %). While, the lowest content was recorded for G.716 variety (31.04 %). The globulins content of other studied genotypes ranged between highest and the lowest levels, as described before.

3-3- Alcohol soluble protein (prolamins)

Data in Table (3) showed that the prolamins contents of the seven faba bean varieties. Results indicated that variety G.717 contained the highest value with an average content of 6.67 % followed by variety G.3 (6.12%). While variety G.716 had the lowest prolamin content of 5.54 %. The other genotypes had intermediate levels prolamins which ranged from 5.54 to 6.76 %.

3-4- Alkali soluble protein (glutelins)

Results presented in Table (3) showed glutelins contents of faba bean seeds varieties under study. The highest glutelins content was recorded in variety G.B. (13.35%). While varieties G.717 and G.3 contained approximately the similar value (9.73 and 9.71 %, respectively). The albumin content of rest genotypes ranged from 9.71 to 13.35 %.

3-5- Non-soluble proteins.

Non-Soluble proteins content of faba bean seeds under investigation recorded in Table (3). Data showed that the varieties G.716 and G.3 had the highest non-soluble proteins content levels which were 36.95 and 36.76 %, respectively. While the lowest value was observed in variety G.717 (31.92 %). The other faba bean varieties contained non-soluble proteins in the range from 31.92 to 36.95 %.

Some of protein fraction had the similar values for some other faba bean varieties. Therefore, it is no feasible to use such levels of different protein fractions to characterize between faba bean varieties under investigation. Therefore, the ratios of glubulin / prolamin (G/P) and globulin/albumin (G/A) were calculated for faba bean varieties and the obtained results are presented in Table (4).

Results of protein fractions of faba bean varieties under study are in agreement with those obtained by Sathe *et al.* (1984) where they based on

the solubility classification, they found that legume storage proteins are primarily globulins (soluble in dilute salt solutions) globulins typically constitute up followed by the albumins (water-soluble proteins).

Mohamed (1999) fractionated crude protein for some faba bean and peanut germplasms into 5 fractions (albumins , globulins, prolamins, glutelins and non soluble proteins) depending upon the solubility in different solvents .Results indicated that some protein fractions and the ratios of globulin / albumin and globulins / prolamins were used to characterize the germplasms belong to each of the studied crops.

Table (3): Protein fraction (%) for seven faba bean varieties under study.

Varieties	Albumins	Globulins	Prolamins	Glutelins	Non soluble proteins
G.B.	14.03	32.00	5.60	13.35	34.57
G.716	16.20	31.04	5.54	10.27	36.95
G.717	16.95	34.76	6.67	9.73	31.92
M.1	13.94	37.57	5.92	10.33	32.24
M.2	14.11	36.00	5.66	9.88	34.35
G.3	14.18	33.23	6.12	9.71	36.76
G.643	13.63	34.53	5.76	11.21	34.87

Table (4): Ratio of certain protein fractions of faba bean varieties under study

Varieties	Globulin /Prolamin	Globulin /Albumin
G.B.	5.71	2.28
G.716	5.60	1.91
G.717	5.21	2.05
M.1	6.34	2.69
M.2	6.36	2.55
G.3	5.42	2.34
G.643	5.99	2.53

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توصيف بعض أصناف الفول البلدى بإستخدام الطرق المورفولوجية والكيميائية

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أجرى هذا البحث بهدف توصيف سبعة أصناف من الفول البلدى بإستخدام الطرق المورفولوجية والكيميائية.حيث تم تحديد الصفات الموفولوجية المميزة للأصناف وذلك تبعا لدليل التوصيف الموفولوجى لمحصول الفول البلدى الصادر عن الإتحاد الدولى لحماية حق المربى (UPOV) مثل طول النبات وكثافة صبغة الأنثوسيانين فى الساق وبعض صفات القرن مثل عرض وسط القرن واتجاه نمو القرن وطول القرن وبعض صفات البذرة مثل شكل البذرة ووزن الألف بذرة ولون السرة.

وتم إجراء تقدير المكونات الكيميائية الرئيسئة فى البذور وتشمل البروتين- الزيت-الكربوهيدرات- الألياف- الرماد. كما تم فصل البروتين الخام إلى خمس مكونات وهى البيومين- جلوبيولين- برولامين- جلوتيلين- بروتينات غير ذائبه وذلك بإستحدام مذيب خاص لكل مكون بروتينى .

وكانت أهم النتائج كالآتى:-

- تم توصيف وتمييز الأصناف المستحدمة فى الدراسة بواسطة تسعة صفات مورفولوجية من بين 32 صفه يحتويها دليل التوصيف المورفولوجى لمحصول الفول البلدى الصادر عن الإتحاد الدولى لحماية حق المربى UPOV). حيث ان الصنف جيزه-716 يتميز بقصر الطول بينما باقى الاصناف كانت متوسطه فى هذه الصفه. وتميز الصنف مصر-1 بكثافة متوسطة فى صفة صبغة الانثوسيانين فى الساق وباقى الاصناف كانت ذات تلوين ضعيف. وتميزت جميع الاصناف تحت الدراسة بوجود الصبغة السوداء فى السره ما عدا الصنف جيزه بلانكا فتميز بغياب هذه الصفه.
- أظهرت نتائج التحليل الكيميائى للبذور ان الصنف مصر-2 يحتوى على اعلى نسبه من البروتين (28.92 %) والزيت (1.51%). اما نتائج تحليل الكربوهيدرات فكانت فى حدود (58ز63-64ز47%) فى الأصناف مصر-2 وجيزه بلانكا على التوالى واعلى نسبة من الالياف وجدت فى الصنف جيزه-3 (8.35%) بينما احتوى الصنف جيزة-643 على اعلى نسبه من الرماد.
- بالنسبة لنتائج فصل البروتين الخام الى خمس مكونات تبين ان الصنف جيزه-717 يحتوى على اعلى نسبة من الالبيومن (16.95%) والبرولامين (6.67%) واعلى نسبة من الجلوبيولين كانت (37.57%) فى الصنف مصر-1.اما الصنف جيزه بلانكا اعطى اعلى محتوى من الجلوتيلين (13.35).كما تم عمل علاقة نسبية بين كلا من نسب الجلوبيولين/الالبيومين ونسب الجلوبيولين/البولامين وذلك لإستحدامها فى توصيف وتمييز الأصناف تحت الدراسه.