Indentification of some broad bean - vicia faba, l.- varieties using morophological, chemical and biotecnological tecchniques

Rehab Ttawdy Behariy

The objectives of this investigation were to actual identify and characterize of ten faba bean (Vicia faba) using morphological, biochemical methods and biotechnological techniques. The necessity for such information to assist in characterizing and identifying these varieties for its quality control and certification tests. Consequently, the morphological characteristics, gross chemical composition, protein fraction, electophoretic patterns of seed protein, some isozymes and RAPD-PCR were determined and analyzed.Two field experiments were carried out in Moshtohor agricultural research station, Egypt during 2002/2003 and 2003/2004 growing seasons. Experimental units were setup in Randomized Complete Block Design (RCBD)in three replicates. Chemical and biotechnological analysis were carried out at Seed Technology Research Section laboratories, Field crop Research Institute, Agricultural Research center,Minstry Of Agriculture, Giza.The obtained results could be summered as following:-1- Morphological Characteristics:--Tannin in seed of all faba bean cultivars was absent.-Plant height, faba bean varieties could be classified into three groups as follows: 1) low group include Sa. land Sa. 2 varieties; 2) Medium group include G.717, G.3 Im G. 643, G. 641, M.1,G.843 and Nu.1 and 3) High group include Y.S. variety .-Plant: number of stems (including tillers more than half the length of medium the main stem) of all faba bean varieties was medium.-Number of nodes was medium in all varieties under study. -Anthocyanin coloration was absent in Sa.1 while in the other varieties was present.-Intensity of anthocyanine coloration was medium in M.1 but was slight for the rest of varieties.-Color of foliage was green in all cultivars of faba bean. -Intensity of green color in foliage was medium in all of faba bean varieties under study.-M.1 and Nu. lvarieties were short in leaflet while the other varieties were medium.-Width of leaflet, faba bean varieties could be classified into three classes of follows: - 1) Narrow class include Nu.l ;2) Medium class include Sa.1,Sa. 2 G. 717, G. 643, G.461, M.1,G. 843 and Y.S varieties and 3) Long class include G.3 Im- Floding of leaflet was medium in all varieties under study. - Number of flowers was medium in all faba bean cultivars.- Time of flowering of faba bean varieties was in three categories as follows:-1) Early category included Y.S variety;2) Medium category included Sa.1,Sa. 2,G. 717, G. 3 Im, G. 643 and G.843 varieties and 3) Late category included Nu. lvariety.- Length of flower was medium in all the varieties of faba bean. - Melanin spot in wing in all cultivars under study was present.- Melanin spot in standard was present in all faba bean varieties under study.- Anthocyanin coloration in standard was absent in all the of cultivars of faba bean.- Extent of anthocyanin coloration standared was slight in all of the studied varieties.- Number of pods of Y.S. variety was many while Sa.l ,Sa.2, G.717, G.3 Im, G. 643, G.843 and Nu.1 varieties were medium. - Attitude of pod of faba bean varieties could be classified into three groups as follows:- 1) Erect group included Sa.l and G.843 varieties; 2) Semi-erect group included Sa.2, G.717, G. 3 Im, G.643 and G.843, M.1 and Y.S. varieties and 3) Semi pendulous group included Nu.1 variety.- Length of pod was medium in all of the studied faba bean cultivars.- Melanin width of pod was broad in Nu.1 variety but in the other varieties was medium.- Degree of curvature of pod faba bean cultivars could be classified into four classes as follows:- 1) Slight included Sa.1, G.717, G.3Im, G. 461 and G.843 varieties; 2) Medium class included Sa.2 and Y.S. varieties ;3) Strong class included M.1 and Nu.1

varieties and 4) Slight medium class included G.643 variety.- Intensity of green color pod was light in Nu.1 variety while it was medium in all of the other varieties.- Number of ovules in all varieties of faba bean under study were medium.- Thickness of pod wall was thin in G.461 and Y.S. varieties while medium in the rest of varieties under study .- Shape of median longitudinal section of seed ,faba bean varieties could be classified into three groups as follows:- 1) Oblong group include G.717 variety;2) Square group include M.1 variety and 3) Ovate group include Sa.1,Sa.2, G.3 Im, G. 643, G.843, Nu.1 and Y.S. varieties- Shape of cross section of seed was broad elliptic in Sa.land Nu.1 varieties and was elliptic in all of the other varieties under study.- 1000 seed weight, faba bean cultivars could be classified into three categories as follows:-1) Medium category included G.717, M.1 and Y.S. varieties; 2) Large category included Sa.1, G.3Im, G. 643, G.461 and G.843 varieties and 3)Very large category included Sa.2 and Nu.1.- Color of testa was beige in all the varieties of faba bean except for G.717 which was semi beige green.- Black pigmentation of hilum was present in all of varieties under study.- Time of all full development of pod of faba bean varieties could be classified into three classes as follows:-1) Early as Y.S. variety; 2) Medium as Sa.1, Sa.2, G.717, G.3Im, G.643, G.461, M.1 and G.843 varieties and 3) Late Nu.1 variety.2- Chemical analysis of faba bean seeds showed the following:The variety Sa.1 contained the highest value of protein content (30.60%), while variety M.1 gave the lowest value (23.81 %). The highest value of fat content was obtained from variety G.461 (2.21 %). The G.3 Im variety contained the highest values (8.58 %) whereas G.461 variety had the lowest value (5.81%). The highest ash content was recorded for variety Y.S. (4.45%). Carbohydrate content was 57.57% and 64.70 % for Y.S and G.843 varieties, respectively.3-Protein fractions:Seeds crude proteins obtained from seeds of faba bean varieties under investigation were fractionated into five fractions (Albumins, globulins, prolamins, glutulins and non-soluble proteins) depending upon the solubility in the different solvents. The highest albumin content (17.10%) was found in G.717 variety. While the highest globulin content was found in M.1 variety (38.50%). The variety G.843 contained the highest prolamins (6.22%) and glutelins (13.11%) content. The variety G.3 Im had the highest non-soluble proteins content levels (38.%) while the lowest content was observed in variety G.843 (31.57 %).The ratios of globulins/albumins and globulins/prolmins were calculated and the results of these ratios can easily be used to characterize faba bean varieties successfully.4-Electrophoresis analysis for SDS-protein fraction:-Total number of bands ranged from 8 in variety G.643 to 12 in G.461 and Sa.2 varieties. There was three common bands that were found in all varieties, which have molecular weight of 84.742, 51.620 and 38.554 kda. Some varieties showed some specific bands which could be used to characterize them among others. Each of Sa.1, M.1, Nu.1, G.843 and G.717 varieties had only unique band, with molecular weight of 223.041, 192.156, 141.056, 90.412 and 10.421 kda, respectively. Band for MW about 30.746 kda is found in all varieties except G.643, G.843 and Sa.1, which could be considered as a negative unique marker (NUM).5- Isozymes:On the isozymes level, bands of three isozyme systems (peroxidase Prx, glutamate oxaloacetate transaminase GOT and esterase Est.) were determined for the identification and characterization faba bean varieties based on polyacrylamide gel electrophoresis. Using the number of bands and the Rf values of the bands of the isozymes under study can be used to identify and characterize these varieties. The number of GOT bands indicated that the varieties G.717 and Nu.1 contained only four bands .To characterize between these varieties depending upon RF values of these bands (0.125, 0.281, 0.346, 0.408 and 0.118, 0.232, 0.285, 0.355), respectively. While, the other varieties comprised from 2 to 3 bands were differ in their Rf values and can be distinguish between them depending on the difference of Rf values.Esterase bands of various varieties showed that the G.3 Im variety showed seven bands only led which to characterize this variety from the others . Also, Nu.1 variety contained only five bands. Also, M.1, G.717, G.843, Sa.1 and Sa.2 varieties comprised six bands. The RF values for the second band of EST for these varieties were different, therefore, can be characterizing between these varieties depending on these RF values. Peroxides bands of varieties demonstrate that the G.717 variety comprised 5 bands only which characterize it from the others. While, the other varieties comprised from 2 to 3 bands were different in Rf values and can be distinguish between them depending on the difference of their Rf values.6- RAPD-PCR:The RAPD analysis for the ten faba bean varieties utilizing five random 10-mer primers was as follows:-The number of bands for

each primer ranged from one (A 12) to 12 (B 10).- The molecular size of the bands for each primer ranged from 85 by (B 01) to 10744 by (B 20).- Primer A 12 gave a maximum of 7 bands ranged from 260 to 4710bp. One specific band was exhibited for G.717 faba bean variety with 2901 bp. This band could be used to characterize this variety from the others.- Primer A 07 exhibited 5 amplification product at the molecular size ranged between 510 and 4385 bp. Single common band at 2035 by found in all varieties. A specific band was exhibited for Y.S. variety of 4385 bp, this band could be used to distinguish this variety .Primer B 20 gave a maximum 16 bands ranged from 275 to 10744 bp. Some varieties had some specific bands which could be used to identify them among the others. For instance, five specific bands for variety Y.S. with 10744 bp, 3360 bp, 1160 bp, 390 by and 275 bp. Also, Nu.1 variety contained three specific bands of 2153 bp, 1015 by and 820 by- Primer B 10 gave amaximum12 bands ranging from 460 by to 3635bp. Six common bands were observed in all varieties with 3635bp, 2695 bp, 1440 bp, 1265 bp, 800 by and 605 bp.The fragment with the molecular size of 1850 was present in all varieties except for Y.S.- Primer B 01 gave a maximum of 15 bands ranged between 85 and 8080bp. Variety Sa.2 could be distinguish from the others varieties by the presence of unique fragments at 8080 bp. Also, two specific bands which were found in Y.S. variety with 4985bp and 5445bp. These could be used to characterize and identify this variety among others (as a positive specific marker).