Agronomy Department

MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION OF SOME FABA BEAN GENOTYPES

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5- SUMMARY AND CONCLUSION

The present study was carried out in Sakha, Agricultural Research Station Farm, ARC, Kafr El- Shikh Governorate during the two successive seasons of 2017/2018 and 2018/ 2019. The objectives of this investigation were to identify 8 promising lines (from G1-G8) G1- Nubaria1× Determinate, G2- Giza 40×Ohishima- Zaira, G3- Santamora, G4- (Giza716 ×Atona)×Atona, G5-Giza716×Sakha1,G6- Sakha1× Ohishima- Zaira, G7- Sakha 2× Atona, G8- Sakha1× Sakha 2, and 4 varieties: Roomy, Peter15, Misr1(Giza3×123A/45/76) and Giza 843 (561/2076/85×461/845/83) by using morphological (quantitative and qualitative) characters. The need for genotypes identification or verification of varietal identify arises as exacting requirements in breeding programs, varieties registration, seed production, processing of the harvested grain and marketing. The area of each plot was 10.5m² (3.5m long and 3m wide). Each plot consisted of 5 rows (3.5m long and 3m wide with 60 cm between rows and 20 cm between hills). Soil perpetration, seeding, fertilization and other agriculture practice were applied properly as recommended for faba bean cultivation. Biochemical analysis were carried out at laboratories of the Seed Technology Research Section, Field crop Research Institute, Agricultural Research center, Ministry of Agriculture, Giza, Egypt to study the quantitative and qualitative characters for seed and seedling. Also, protein fractionation and PCR in DNA of seed of faba bean genotypes.

- **<u>The following parameters were determined:</u>**

I- Field experiments:

The morphological identification was conducted usually using the International Union for the Protection of New Varieties of Plants (UPOV) descriptors (2015) for twelve genotypes of faba bean (*vicia faba*, L.)

during the two seasons of 2017-2018 and 2018-2019.

1- Quantitative characters:

1.1. Stipule characters:

Stipule length(cm) , Stipule width (cm) and Stipule length/ width ratio: was measured at 59 days age.

1.2. Leaves characters:

Leaflet length (cm) Leaflet width (cm), Leaflet length/width ratio and Number of leaflet/leaf: was measured at 61 days age.

1.3. Flowering characters:

Number of inflorescence on the main axis, Number of flowers/inflorescence, Flower length (cm), Flower width (cm), Flower length/width ratio, Flag leaf length (cm) and Flag leaf width (cm): were measured at 61 days age.

1.4. Stem characters:

1.4.1. Plant height (cm): was measured at physiological maturity from the soil surface to the tip of the plant at harvest.

1.4.2. Stem thickness of the main stem (cm): was measured at early podding stage using pocket thickness Gauge. Measured as width of one side of stem at mid height of plant.

1.4.3. Height of the lowest nod (cm): was measured at 61 days age.

1.4.4. Number of nods on the main stem: was measured at 71 days age.

1.4.5. Number of branches/ plant: (including tillers more than half the length of medium the main stem)

1.5- Pod characters:

1.5.1. Pod length (cm) and pod width (cm): were measured at 61 days age.

1.5.2. No of pods/ nod: was measured at 71 days age.

1.6- Seed characters:

1.6.1. No of seed/pod: was measured at 81 days age.

1.6.2. Seed length (cm), Seed width (cm), Seed thickness (cm) and 100 seed dry weight (g): were measured at 89 days age.

2- Qualitative characters:

2.1- Stem characters:

2.1.1. Intensity of anthocyanin coloration: was measured at 61 days age.

2.2-Leaves characters:

2.2.1- Position of maximum width and Leaflet shape: was measured at 61 days age.

2.3-Flowers characters:

Extent of anthocyanin coloration, Flower ground color , Wing melanin spot and Color of wing melanin spot: were measured at 61 days age.

2.4. Pod characters:

2.4.1. Intensity of green coulor, Degree of pod curvature and Angle of pod

with main axis (on second or third pod –bearing node): were measured at 71 days age.

2.4.2. Pod color at maturity: was measured at 89 days age.

2. 5-Seed characters:

Dry seed shape, Colour of testa and Black pigmentation of helium: were measured at 89 days age.

2. Laboratory experiments:

2.1. Germination test results:

The studied character was conducted usually using the rules of International Seed Testing Association, ISTA (1985).

1- Germination %.

2- Speed of germination.

3- Shoot length (cm).

4- Root length (cm).

5- Seedling dry weight (g).

2- Inter simple sequence repeat (ISSR).

3- Protein fractionation:

SDS – polyacrylamide gel electrophoresis (SDS – PAGE).

4- Isozymes.

- **The main results that obtained from this study could be summarized as follows:**

I- Field experiments:

A- Quantitative characters:

1- Sowing **G1** promising line produced shortest stipule length, stipule width and minimum number of inflorescence in both seasons while, it gave maximum values in leaflet width, stem thickness, number of bod /node and 100 seed wight in both sesons , while it gave maximum flower width in second season only .

2- Planting **G2** produced maximum flag leaf length, and seed thickness. While it gave maximum flag leaf width in second season. while recorded lowest number of branches/plant.

3- Planting **G3** gave the lowest flag leaf width in the second season. On contrary, G3 exhibited the highest number of leaflet/leaf in both seasons.

4- Planting **G4** attained the highest stipule length/width ratio in the second season, flower width and flag leaf width in the first season. While, gave highest leaflet length, leaflet width and flower length/width ratio.

5- Planting **G5** attained the highest flag leaf width in the first season.

6- Planting **G6** produced lowest stipule length/width ratio in the second season and flower width in the first season.

7- Planting **G7** exhibited the highest number of seeds/pod in both seasons. However, it gave lowest stipule length in the first season.

8- Planting **G8** produced highest leaflet length, leaflet length/width ratio, , plant height, number of nodes, height of lowest node.

9- Planting **Roomy** genotype produced the highest stipule width, number of inflorescence, flower length, flower length/width ratio, number of branches/plant, pod length, pod width, seed length and seed width while it gave lowest flag leaf width in the first season.

10- Planting **Giza843** gave highest number of flowers/inflorescence and number of branches/plant. While, it exhibited statistically the lowest values of stipule length/width ratio, leaflet length/width ratio, flag leaf width.

11- Planting **Peter15** produced the highest number of pods/node. Moreover, it produced lowest number of leaflet/leaf, flower length, flower width , flag leaf length,seed length, seed width, seed thickness, number of nodes, number of seeds/pod, pod length, pod width and 100 seed weight .

12- Planting **Misr1** attained the highest stipule length. Also it gave highest stipule length/ width ratio in the first season. However, it gave lowest number of flowers/inflorescence, plant height, stem thickness and height of lowest node.

B- Qualitative characters:

1- G1 was identified by absent anthocyanin coloration of stem, position of maximum width at middle, elliptic shape of leaflet, medium extent of anthocyanin coloration of flower, white flower ground color, wing melanin spot present, black color of wing melanin spot, medium green color of pod, strong curved pod, black pod color at maturity, acute pod angle with main axis, light brown testa and elongated seed shape, color of helium is black.

2- G2 was identified by medium anthocyanin coloration of stem, position of maximum width at middle, elliptic shape of leaflet, medium extent of anthocyanin coloration of flower, white flower ground color, wing melanin spot present, black color of wing melanin spot, medium green color of pod, weak curved pod, brown pod color at maturity, prependicular pod angle with main axis, yellow green testa, helium color is black and elongated seed shape.

3- G3 was identified by medium anthocyanin coloration of stem, position of maximum width at middle, trullate slightly elongated shape of leaflet, large extent of anthocyanin coloration of flower, wing melanin spot present, black color of wing melanin spot, white flower ground color, light green color of pod, weak curved pod, brown pod color at maturity, obtuse pod angle with main axis, medium brown ,testa helium color is black and elongated seed shape.

4- G4 was identified by absent anthocyanin coloration of stem, position of maximum width at middle, trullate slightly elongated shape of leaflet, small extent of anthocyanin coloration of flower , white flower ground color, wing melanin spot present, black color of wing melanin spot, medium green color of pod, absent to weak curved pod, dark brown pod color at maturity, obtuse pod angle with main axis, yellow green testa, testa helium color is black and elongated seed shape.

5- G5 was identified by absent anthocyanin coloration of stem, position of maximum width at middle, elliptic shape of leaflet, large extent of anthocyanin coloration of flower , light purple flower ground color, medium green color of pod, strong curved pod, black pod color at maturity, obtuse pod angle with main axis, medium brown testa testa helium color is black, and elongated seed shape.

6- G6 was identified by strong anthocyanin coloration of stem, position of maximum width at middle, elliptic shape of leaflet, small extent of anthocyanin coloration of flower, beige flower ground color, light green color of pod, weak curved pod, black pod color at maturity, acute pod angle with main axis, light brown testa, testa helium color is black and with angles seed shape.

7- G7 was identified by weak anthocyanin coloration of stem, position of maximum width toward base, trullate slightly elongated shape of leaflet, medium extent of anthocyanin coloration of flower, white flower ground color, medium green color of pod, medium curved pod, black pod color at maturity, obtuse pod angle with main axis, light brown, testa helium color is black testa and elongated seed shape.

8- G8 was identified by weak anthocyanin coloration of stem, position of maximum width at middle, lanceolate shape of leaflet, small extent of anthocyanin coloration of flower, white flower ground color, medium green color of pod, weak curved pod, black pod color at maturity, acute pod angle with main axis, yellow green testa and elongated seed shape.

9- Roomy genotype was identified by absent anthocyanin coloration of stem, position of maximum width at middle, trullate slightly elongated shape of leaflet, large extent of anthocyanin coloration of flower, beige flower ground color, medium green color of pod, medium curved pod, black pod color at maturity, acute pod angle with main axis, light brown testa and elongated flattened seed shape.

10- Giza 843 genotype was identified by absent anthocyanin coloration of stem, position of maximum width to ward base, ovate shape of leaflet,

small extent of anthocyanin coloration of flower, white flower ground color, medium green color of pod, medium curved pod, black pod color at maturity, acute pod angle with main axis, brown testa and elongated seed shape.

11- Peter15 genotype was identified by absent anthocyanin coloration of stem, position of maximum width to ward base, lanceolate shape of leaflet, small extent of anthocyanin coloration of flower, white flower ground color, medium green color of pod, medium curved pod, black pod color at maturity, acute pod angle with main axis, dark brown testa and spherical seed shape.

12- Misr1 was identified by weak anthocyanin coloration of stem, position of maximum width at middle, elliptic shape of leaflet, large extent of anthocyanin coloration of flower, white flower ground color, medium green color of pod, weak curved pod, black pod color at maturity, acute pod angle with main axis, dark brown testa and elongated seed shape.

II- Laboratory experiments:

1- Germination test results:

The highest germination% were given by genotypes G1, G2, G3, G4, G5, G7 and G8. G6 was highest germination% in the first season. While Giza843 and Peter15produced the lowest germination%. The maximum speed of germination produced by G4 and G6 in the first season while G1, G2, G3, G4, G5, G6, G7and G8 surpassed the maximum speed of germination in the second season. While the minimum speed of germination were given by roomy in the first season and peter15 in second season. Thereupon, although there are some differences between the genotypes in these characters, but may not be useful in genotypes identification.

The highest shoot length were given by germinating G8, while the lowest shoot length were produced from sowing Misr1 in both seasons. The highest root lengths were obtained from sowing G8. On the other side, the lowest root lengths were produced by planting Peter15 in both seasons. The heaviest seedling dry weights were resulted from planting G8, while the lowest ones were produced from sowing Misr1 genotype in both seasons.

2- Inter simple sequence repeat (ISSR):

ISSR primer 44B succeeded in targeting its repetitive motif and generated 19 amplicons with molecular sizes ranged from 269 to 1923 bp (Fig. and Table), from which 9 amplicons were polymorphic (90% polymorphism), while one amplicon was presented in all genotypes which are considered as common fragment at molecular size of 269 bp. Two unique positive markers were appeared at 1932 and 666 bp in genotypes 2 and 6 respectively that could be used to distinguish between faba

genotypes.

Primer HB9 succeeded in targeting its repetitive motif and generated 19 amplicons with molecular sizes ranged from 173 to 2166 bp (fig. and Table). All the 19 amplicons were polymorphic with (100% polymorphism). Three positive unique markers were found at the molecular sizes 2166, 359 and 522bp in genotypes 6, 11 and 2 respectively.

ISSR primer HB10 succeeded in targeting its repetitive motif and generated 6 amplicons with molecular sizes ranged from 274 to 1088 bp (fig. and Table), from which 5 amplicons were polymorphic (83.3% polymorphism), while one amplicon was presented in all genotypes which are considered as common amplicon at molecular size of 329 bp.

Primer HB15 succeeded in targeting its repetitive motif and generated 9 amplicons with molecular sizes ranged from 275 to 1082 bp as shown in (fig. and Table), all the 9 amplicons were polymorphic with 100% polymorphism. One positive unique marker was found at molecular size of 700 bp in genotype 4, which could be used for distinguish between studied faba bean genotypes.

Primer HB12 succeeded in targeting its repetitive motif and generated 7 amplicons with molecular sizes ranged from 326 to 1016 bp (fig. and Table), from which 6 amplicons were polymorphic (85.7% polymorphism), while one amplicon was presented in all genotypes which are considered as common amplicon at molecular size of 809 bp. Two unique positive markers were appeared at 1016 and 326 bp in genotypes 2 and 10 respectively that could be used to distinguish between faba genotypes.

3-Cluster analysis:

The 12 studied genotypes were grouped in to four clusters namely A, B, C and D according to the truncated line at a coefficient of dissimilarity=0.544. G1 formed cluster A meanwhile, G2, G4, G5, G6, G7 and G9 formed cluster B and four genotypes namely G3, G8, G10 and G11 formed cluster C finally G12 formed cluster D.

4- Electrophoresis analysis for SDS-protein fraction:

Results of SDS-PAGE patterns of leave protein fraction of the studied faba bean genotypes indicated distinct differences between them. Electrophoretic analysis of leaves proteins exposed a total of 19 protein bands with variable intensity and molecular weights across the twelve faba bean genotypes. The protein bands ranging from 12.884 to 226.393KDa .Out of the 19 detected protein bands, 7 were polymorphic (showed variations), while 11 was monomorphic and one unique genotype with 42.105 % polymorphism.

5- Isozymes analysis:

On the isozymes level, bands of two isozyme systems (peroxidase PX and polyphenyle oxidase) were determined for the identification and characterization faba bean genotypes based on polyacrylamide gel electrophoresis. Using the density of bands and the relative mobility values of the band of the isozymes under study can be used to identify and characterize these genotypes. The density of PX bands indicated that Roomy contained three high density bands in all relative mobility levels. While, G3 and G7 showed low density bands. On the other hand, results of polyphenyle oxidase isozyme illustrated four levels of relative mobility (0.1, 0.5, 0.6, and 0.8). G1 and G4 were high density bands in relative

mobility (0.1). Whereas G1, G3, G4, Roomy, Peter15 and Misr1 were showed high density bands in relative mobility (0.6). Finally, PPO4 indicated one unique moderate density band in G6 which could be used to distinguish this genotype.

❖ CONCLUSION

From this study it can be concluded that the identification of studied faba bean genotypes under the environmental conditions of Kafr-El Shikh Governorate is shown in following Tables:

Table 29: Field identification (quantitative and qualitative characters).

Characters \ Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	Roomy	Giza843	Peter15	Misr1
1- Stipule length									Tall			
2-leaflet length								Long	Long			
3-leaflet width	broad			Narrow					Broad			
4- Plant height							Short	Tall				
5- Flower length									Long			
6- Pod length									Long		short	
7- Seed length									Long		Short	
8- Seed width									Broad		Narrow	
9- 100 seed weight									High		Low	
10- Anthocyanin coloration of stem						Strong						
11- leaflet shape										Ovate		
12-Flower ground color					Light purple	Beige						
13- degree of pod curvature	Strong			Absent to weak	Strong							
14- pod angle		prependicular										
15- pod color at maturity	Light brown	Yellow green	Medium brown	Yellow green	Medium brown	Medium brown	Light brown	Yellow green	Light brown	Medium brown	Dark brown	Yellow green
16- pod intensity of green color			Light									