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INDUCTION OF HIGH PERFORMANCE GENOTYPES FROM PEA MUTANTS

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ABSTRACT : The stable selected mutants of two pea cultivars Master B and Lincoln, from M_2 were studied in M_3 in a private farm at Sharkia Governorate during winter seasons 2004 and 2005. Previously, such mutants were detected under the predictable low dose " one kr " of gamma rays, at M_1 . Selection in M_2 and M_3 was carried out from some the characters i,e number of branches, pods, seeds per plant and the dry weight of 100 seeds.

The following results were obtained.

1.At M_3, the stable selected mutants exceeded significantly their respective parents for the studied agronomic characters.

2.Heritability estimates at M_3 were high for number of branches, pods and seeds per plant as well as dry 100 seed weight that refer to insure that selection for high yield is more effective if based on these characters and the importance of additive genetic action.

3.The intergeneration correlation coefficients (M_2 / M_3) were positive and almost approaching unity indicating the extent of similarity of the selected mutants at M_2 and their progenies at M_3 content in seeds.

4. Elevation in both individual and total amino acid was observed at the stable M_3 mutants as compared with their parents.

5.The electrophoretic studies showed that the stable selected mutants at M_3 were distinguished with highly band number with heaviest darkness and high intensities genotypes that should be introduced as compared with their respective parent.

6.The selected mutants at M_3 generation could be used as new genotypes for agriculture or in breeding programs. One out of the mutants was distinguished with the values (7.00, 65.73, 346.51, 33.86 g and 3.75 mlg and the other had, the values (8.00, 65.68, 364.65, 27.58 g and 3.33 mlg for number of branches, pods, seeds per plant, dry weight of 100 seed and total amino acids, respectively.

Key words: *Pisum sativum,* mutants, genotype, induction, amino acids

INTRODUCTION

Peas, *Pisum sativum L.* occupy a position of considerable importance among the grain legumes for their valuable uses. Vasileva *et al* (1984) mentioned that the varieties differ in their response to the mutagen in isolating economic mutations with higher frequency of genetic variations. Useful mutations derived from low doses of gamma rays are of utmost importance if they will be related to some agronomic characters. Eman (2000), Kharkwal (2000), Mihov *et al* (2001) and Azza (2004) obtained some mutant genes with favorable effects through selection in M_2 and M_3 with higher quality. Mick *et al (1990)* and FAO/LAEA (1991) recorded mutations from 1363 cultivars having valuable and economic characters and mentioned that more than 90% of lines are based on mutations induced by gamma rays.

Induction new genotypes and cultivars for Egyptian agriculture is a glorious phase for agriculture progress. It's the time, we are looking forward to seeing all the cultivars are Egyptian in origin.

The aim of this investigation is to induce pea stable mutations as new genotypes having high yielding capacity and higher contents of amino acids.

MATERIALS AND METHODS

El Ghareeb, exposed the seeds of Master B and Lincoln under 1,2,3 and 4 Kr gamma rays and obtained the M_1 for both two cultivars during winter season 2003 and was published in 2006, at Atomic Energy Station, City Nasr, Cairo. Seeds from the plants derived from the predictable low dose of gamma rays "one Kr" were gathered and isolated for the M_2 planting. More than one hundred and fifty mutations were recorded at M_2. Induced morphological the mutants were classified according to the main alternation brought about through a mutational event for screening and isolated of mutants at M_2. Mutants affecting number of branches, pods, seeds per plant and the weight of 100 dry seed were obtained at M_2. The stable and selected mutants at M_2 generation were studied at M_3 generation. Seeds of both M_2 and M_3 generations were sown in hills, 25 cm a part at the private farm in Sharkia Governorate during two winter seasons 2004 and 2005. Common agricultural practices were done according the guidance of Agriculture Ministry in Egypt.

Statistical Analysis of M_3

Means and the analysis of variance was performed according to the method of Singh and Shoudhary 1977. Means and standard errors were estimated. Heritability (h^2) was estimated as the ratio between genotypic and phenotypic variance. The intergeneration correlation coefficients between selected mutants M_2 / M_3 was estimated

Biochemical Studies

Determination of amino acid content:

The individual amino acids were determined in the seed flour of the two cultivars seeds "Master B and Lincoln" and their superior selected mutations. The used technique according to the procedures described by Block 1958 was used. The two solvent systems of absolute n- butanol: acetic acid: distilled water in the ratios 4:1:5 (v/v/v) and 4:l:l(v/v/v) were applied .

Chromatograms were air dried and the spots were developed using 0.2 % ninhydrin in acetone (Smith 1958). The spots were eluted by 50 % methanol solution. Then, the eluted coloring spots were measured Spectrophotometrically at 570 mu. Using standard curves of each amino individual acid. The concentration of each amino acid was estimated as mgs per gram dry seed weight.

Protein electrophoresis

This investigation was carried out at laboratory of Genetic Engineering Dept. Genetics, Fac. of Agric., Ain Shams University. Sodium dodocyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli 1970 after being modified by Studier 1973.

Sample preparation

M_3 seed samples from two superior mutants belong each character and the two pea cultivars "Master B and Lincoln" were used. Seeds were pressured by a drill to release their contents. Samples of 0.5 gram of each genotype with 5 ml of sample buffer was homogenized and used for SDS-PAGE.

Gel preparation

Polyacrylamide standard gel at PH 8.9, consisted of 150 ml monomer solution (8.55 Acrylamide, 0.45 Biscarylamide in 0.150 M Tris-Borate buffer). Then the following were added without delay: 300 mgs sodium sulphate (dissolve completely), 0.40 ml TEMED (tetramethlendiamine), and 40 ml ammonium Prcsulphate (2%) freshly prepared, 200µ extract of each sample was mixed with 50 µ

glycerol and 50 µ bromophenol blue.

Gel incubation and agitation were carried out at room temperature until the bands appear in clear background. Then the gel was washed with distilled water and photographed.

RESULTS AND DISCUSSION

Table 1 shows significant and highly significant variations between M_3 mutant lines for number of branches, pods and seeds per plant as well as the dry weight of 100 seed. These results confirm that the mutagen treatment "one kr" of gamma rayes permits to select and improve yield and quality characters. Rajput (2001b) and Azza (2004) reported the same trend of results.

Heritability estimates in broad sense Table 1 were high for the studied characters and differed from (61.12% - 78.66%) as the character in question. Such high heritability might indicate the presence of genetic action for these characters and proved that selection of high yielding capacity is more effective, if based on these, parameters. These results might confirm that the genetic control play an important role in the inheritance of these characters. Same trend was obtained by Kumari 1996, Geetha and Vaidyanathan 1998, Nandi *et al.*, 1998 and Azaa 2004.

Means and standard errors for the stable mutants at M_3 are presented in Tables 2. From this Table, it could be noticed that all stable M_3 selected mutants exceeded their respective parents for the characters, number of branches, pods, seeds per plant and even dry weight of 100 seed, whereas highly significant values were obtained for all selected mutants over their respective parents. Many rescarchers obtained mutants superior in agronomic characters, i.e. Fayza and Mahasen 1989 for number of branches per plant, Nandi *et al.*, 1998 for number of pods per plant, Hodson and Hezky 1994 in number of seeds per plant, Mehetre *et al.*, 1998 for weight of 100 seeds per plant, Rajput (2001 a and b) for yield. Furthermore Azza 2004 for faba bean obtained mutants superior for many characters as, number of branches, number of pods and seed yield.

In spite of the large mutant number that obtained in M_2, twelve mutants only derived under the optimum dose "one kr" of gamma rays were only stable and gave the same features to be used at M_3 as useful

Table 1. Analysis of variance and heritability (h^2) estimates for some agronomic parameters at M_3 selected mutants of some irradiated pea cultivars

Traetments	Number of branches /plant			Number of pods / plant			Number of seeds / plant			Dry weight of 100 seed / plant (g)		
	df	Ms.	h^2	df	Ms.	h^2	df	Ms.	h^2	df	Ms.	h^2
Master B												
Control												
Between lines	25	0.34		25	386.14		25	922.27		25	15.16	
Within lines	20	0.34		20	252.13		20	812.34		20	14.72	
Between mutant lines	21	0.53	62.57	21	712.12**	76.06	20	1218.63*	78.66	20	37.14**	61.12
Within mutant lines	20	0.42		20	363.43		13	1123.65		9	11.82	
Lincoln												
Control												
Between lines	24	0.78	-	21	614.12	-	21	4371.41	-	20	62.21	-
Within lines	20	0.46	-	20	581.23	-	20	4121.62	-	21	57.19	-
Between mutant lines	20	5.69**	67.49	20	789.37**	69.7	23	5327.16**	68.5	21	73.80**	68.75
Within mutant lines	21	2.58		13	397.41		20	2105.27		20	35.43	

*, ** = sig. at 0.05 and 0.01, respectively

Table 2. Mean and standard error of stable selected superior mutants at M_3

Treatments	No. branches/plant	No. pods/ plant	No. seeds / plant	Dry weight of 100 seed / plant (g)
		Master B		
Control	1.58 ± 0.37	9.85 ± 2.10	88.69 ± 6.17	15.51 ± 1.82
1	8.00 ± 0.24	58.12 ± 5.16	346.73 ± 12.16	28.14 ± 2.15
2	7.00 ± 0.42	51.55 ± 3.17	343.32 ± 11.51	40.18 ± 6.28
3(A)	7.00 ± 0.35	65.73 ± 3.44	346.51 ± 11.65	33.86 ± 3.69
4	6.00 ± 0.22	63.49 ± 2.89	344.80 ± 11.32	39.88 ± 5.75
5	6.00 ± 0.32	60.24 ± 3.56	344.29 ± 11.58	37.02 ± 6.02
		Lincoln		
Control	1.97 ± .22	14.94 ± .90	128.56 ± 4.67	12.85 ± .37
1	6.00 ± 1.10	59.90 ± 2.10	345.51 ± 12.17	25.59 ± 1.66
2	7.00 ± 1.19	63.53 ± 2.68	367.91 ± 14.25	32.64 ± 2.10
3(B)	8.00 ± 1.32	65.68 ± 2.41	364.65 ± 13.71	27.58 ± 1.87
4	6.00 ± 1.10	61.31 ± 2.18	359.15 ± 14.37	31.95 ± 2.12
5	6.00 ± 1.12	65.34 ± 2.48	362.68 ± 13.52	33.68 ± 2.15
6	7.00 ± 1.18	62.21 ± 2.46	358.19 ± 14.46	32.96 ± 2.08
7	7.00 ± 1.19	58.65 ± 2.91	366.22 ± 14.78	34.13 ± 2.27

3A and 3B= desirable mutants

mutations. Only two out of the twelve mutants were chosen as promising line mutants for high yield. Table 2 shows mean stable selected and superior mutants concerning number of branches number of pods, number of seeds per plant and dry weight of 100 seeds at M_3 selected for Master B and Lincoln and indicated that the mutants 3A and 3B were superior having highest and surprised values for most the studied characters. It is well known that yield and some of it's components are quantitative characters and large affected by polygenes in addition the environmental conditions. Abd El-Raheem *et al.*, 1988 mentioned that the fitness of yield characters was affected by mutational events showing a response to environment in peanut. The same trend has been found by Rajput 2001a and Azza 2004.

Intergeneration M_2/ M_3 Correlation Coefficient

The extent of similarity in the expression of various yield characters, and number of branches per plant was measured by the intergeneration M_2/M_3 correlation coefficient Table 3. In addition of highly significant positive intergeneration correlations coefficients, the (r) values were specially high in magnitude and almost approaching unity. This means that the selected mutants of M_2 and their M_3 progenies gave the same features for number of branches per plant and some yield attributes. Rajput (2001a and b) and Azza 2004 mentioned that the same features of the stable selected mutants at M_3 were previously observed at M_2.

Biochemical Studies

Amino acids

Table 4 indicated increases in amino acids contents as compared with the parents. Higher levels of amino acids were determined for Aspartic acid that recorded the highest levels, followed by Arginine, Serine, Glutamin, Glycine, Alanine and Leucine. While the lower levels of amino acids were, the sulpher amino acid "Methionine" as well as Aspargine and Glutamine. These results agree with that were reported by Staikov *et al.*, 1985 who mentioned that low irradiation by gamma rays doses increased total amino acids as the increase of specific change in catalase and peroxidase activities and Hassan 1997 who reported that the amino acid contents varied according to species. These results elucidate that two selected mutation were superior in amino acid contents.

Electrophoretic studies

b-Electrophoresis banding patterns (SDS-PAGE) of extracted protein from dry seeds of two pea

Table 3. Intergeneration correlation coefficient(r) between M_2/M_3 of Master B and Lincoln cultivars for the studied characters

Characters	No. branches/plant	No. pods/ plant	No. seeds / plant	Dry weight of 100 seed / plant (g)
	Master B			
Number of branches / plant	0.98**			
Number of pods /plant		0.99**		
Number of seeds / plant			0.97**	
Dry weight of 100 seed (g.)				0.93**
	Lincoln			
Number of branches / plant	0.97**			
Number of pods /plant		0.99**		
Number of seeds / plant			0.97**	
Dry weight of 100 seed / plant (g)				0.87**

*, ** = sig. at 0.05 and 0.01, respectively

Table 4. Amino acids composition and their standard error ($\overline{X} \pm SE$) at two selected superior M_3 (mg) derived from irradiated pea cultivars

Amino acids	Master B		Lincoln	
	Control ($\overline{X} + SE$)	Mutant (A) ($\overline{X} + SE$)	Control ($\overline{X} + SE$)	Mutant (B) ($\overline{X} + SE$)
Arginine	0.150 ± 0.001	0.320 ± 0.015	0.140 ± 0.002	0.220 ± 0.003
Aspargine	0.050 ± 0.022	0.030 ± 0.001	0.005 ± 0.001	0.091 ± 0.001
Glutamine	0.008 ± 0.001	0.010 ± 0.001	0.005 ± 0.001	0.091 ± 0.001
Serine	0.140 ± 0.001	0.003 ± 0.016	0.160 ± 0.002	0.270 ± 0.004
Aspartic acid	0.280 ± 0.012	0.550 ± 0.022	0.270 ± 0.004	0.450 ± 0.007
Glutamic acid	0.330 ± 0.021	0.390 ± 0.0108	0.410 ± 0.005	0.520 ± 0.014
Therionine	0.190 ± 0.003	0.230 ± 0.011	0.170 ± 0.003	0.210 ± 0.011
Glycine	0.220 ± 0.011	0.370 ± 0.017	0.240 ± 0.004	0.310 ± 0.012
Alanine	0.210 ± 0.010	0.350 ± 0.015	0.140 ± 0.002	0.160 ± 0.009
Methionine	0.010 ± 0.001	0.030 ± 0.001	0.080 ± 0.001	0.030 ± 0.001
Valine	0.210 ± 0.010	0.250 ± 0.011	0.190 ± 0.009	0.220 ± 0.010
Phenylalanine	0.170 ± .002	0.180 ± 0.009	0.140 ± 0.007	0.150 ± 0.001
Isoleucine	0.140 ± 0.001	0.170 ± 0.008	0.110 ± 0.006	0.120 ± 0.001
Leucine	0.240 ± 0.011	0.50 ± 0.15	0.220 ± 0.012	0.200 ± 0.004
Histidine	0.090 ± 0.001	0.110 ± 0.001	0.080 ± 0.001	0.090 ± 0.001
Lysine	0.150 ± 0.001	0.210 ± 0.011	0.130 ± 0.001	0.150 ± 0.001
Tyrocine	0.110 ± 0.001	0.170 ± 0.001	0.120 ± 0.001	0.140 ± 0.001
Total	2.798 ± 0.175	3.750 ± 0.228	3.010 ± 0.152	3.330 ± 0.226

cultivars, Master B and Lincoln and their selected mutations at M_3 are presented in Fig. 1. Four major regions are detected for both two cultivars, Master B and Lincoln. R_1 region contains three major bands, are dark and one is faint. R_2 region consists of one major band . Such major band is distinguished with the increase of its density in Master B more than Lincoln cultivar. Three major bands were observed in the third region R_3. As for R_4 region which indicated three bands, the two cultivars also were differed. From the previous conclusion, it could be noticed that, large differences were observed for the major protein banding patterns of the two cultivars, Master B and Lincoln. Such differences in size and density make one assumes that the variation in banding patterns are genotypically and evolutionary different. This was substantiated by the facts that some of the sub fraction of a particular protein either slightly disappeared or were reduced in size and mobility. Such quantitative variations in the two cultivars banding patterns could be found if one assumes that the genes responsible for these metabolic phenomena are different in their action. A reasonable explanation that could be forward, is that these cultivars are of different origins and they have gone through completely different paths during evolutionary processes. Same results were obtained. Matsumoto *et al.*, 1997, Amer *et al* 1999, Ismail and El.Ghareeb 2000 and El.Ghareeb *et al* 200.

In spite of having the four regions of banding patterns that were found in their respective cultivars, they contained heavy darkly stained banding patterns. Thus it could be concluded that the selected M_3 generation containing the selected characters i, e number of branches, number of seeds, pods per plant and weight of 100 seeds, had highest dark bands and intensity.

Comparing selected M_3 with their respective cultivars it was found that M_3 had higher band number as compared with the parents. Fig. 1 showed that, the appearance of highly band number and intensities is nearly equal with slight differences among the selected parameters, i,e. number of branches, pods and seeds per plant as well as dry weight of 100 seed. The previous results confirm the importance of SDS-PAGE technique in foundation the characterized pattern for discriminating high and low yielding plants under gamma rays. These results agreed with El. Demerdash 1993 who found that the results of SDS-PAGE could be used to follow the technique in

protein patterns after gamma irradiation. Abd El.Tawab *et al*, 1993 confirmed that SDS-PAGE was a highly successful technique in cultivar identification. These results confirm the results obtained from intergeneration correlation coefficients between the selected M_2 mutations and their M_3 stable Mutations, indicating the extent of similarity at M_3. It is worth noting that the similarity of banding patterns number and intensity for the selected mutations elucidated that these M_3 selected mutations are of similar origins and have gone through completely similar paths during evolutionary processes. Thus selection would be efficient if based on the previous parameters. Generally, these results were in agreement with those obtained by Cooke 1990 and Hassan 2004.

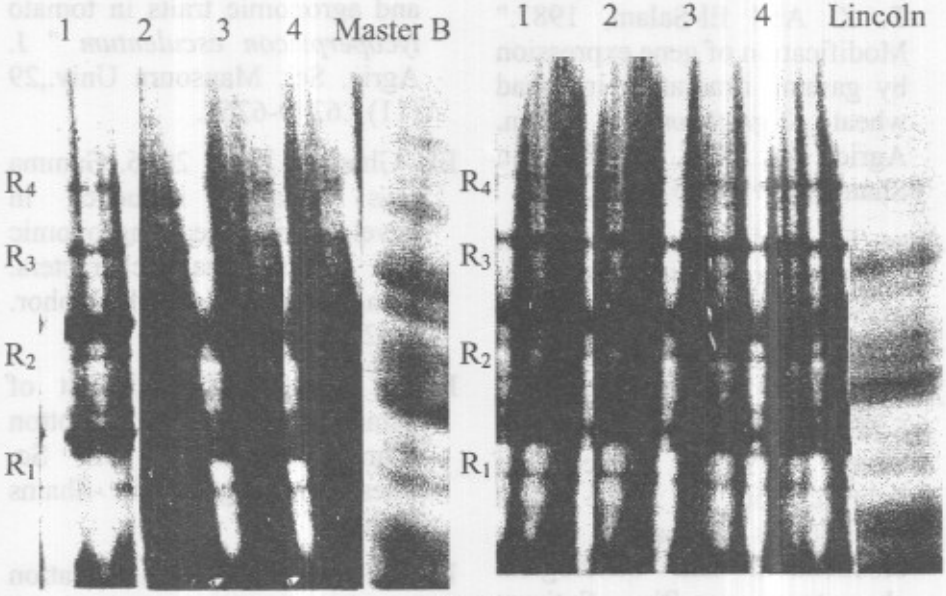

Fig (1)

1-High weight of 100-seeds. 2- High number of branches. / plant
3- High number of seeds. / plant. 4- High number of pods . / plant.

Fig 1. SDS -PAGE Profiles of seed protein banding patterns according to their density and intensity for selected M_3 as useful irradiated mutations in pea cultivars, as compared with their respective parents

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أستحداث بعض التراكيب الوراثية ذات الأداء العالى من طفرات البسلة

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بعض الطفرات المنتخبة الثابتة من الجيل الطفرى الثاني من بعض أصناف البسلة " ماستر بي، لنكولن " تم دراستها في الجيل الطفرى الثالث. وذلك بمزرعة خاصة بمحافظة الشرقية شتاء ٢٠٠٤، ٢٠٠٥

ولقد تم الانتخاب في الجيل الثاني والثالث على أساس الصفات " عد الفروع للنبات وعدد القرون وعدد بذور النبات الواحد وكذلك وزن المائة بذرة جافة. وفي الجيل الثالث تم عمل تحليل التباين كما تم تقدير درجة التوريث والحصول على النتائج الآتية.

١- الطفرات المنتخبة والثابتة في الجيل الثالث تفوقت على الآباء معنويا في الصفات المحصولية والتي كانت أساساً للأنتخاب.

٢- تقديرات درجة التوريث في الجيل الثالث الطفري كانت عالية لصفات عدد الفروع والقرون والبذور في النبات وكذلك وزن المائة بذرة جافة موضحا أهمية الفعل الجيني المضيف. ومؤكدا أن الانتخاب للمحصول المرتفع يكون أكثر تأثيراً إذا أعتمد على هذه الصفات.

٣- معامل الارتباط بين الأجيال (الجيل الثاني / الجيل الثالث) كان موجبا وقريبا من الوحدة موضحا امتداد التماثل للطفرات المنتخبة في الجيل الثاني مع نسلها في الجيل الثالث الطفري.

٤- لوحظ زيادة في محتوى كل من الأحماض الأمينية الفردية والكلية في طفرات الجيل الثالث الثابتة عند مقارنتها بالأباء.

٥- أظهرت دراسات التفريد الكهربي ، أن طفرات الجيل الثالث الثابتة المنتخبة قد تميزت بزيادة عدد الحزم مع زيادة في تركيز وكثافة هذه الحزم مقارنة بالأباء.

٦- الطفرات المنتخبة في الجيل الثالث قد ، تستخدم كتراكيب وراثية جديدة في الزراعة في مصر أو في برامج التربية . أحد هذه الطفرات قد تميز بالقيم (٧ ، ٦٥,٧٣ ، ٣٤٦,٥١ ، ٣٣,٨٦ (جرام)، ٣,٧٥ ملليجرام ومن ناحية أخرى القيم (٨ ، ٦٥,٦٨ ، ٣٦٤,٦٥ ، ٢٧,٥٨ جرام ، ٣,٣٣ ملليجرام) كانت لطفرة أخرى وذلك لعدد الفروع والقرون والبذور للنبات الواحد وكذلك وزن المائة بذرة جافة والمحتوى الكلي للأحماض الأمينية على التوالي.