



Estimation of Some Genetic Parameters in Faba Beans (*Vicia faba* L.) Affected by Nitrous Acid Mutagen

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Abstract

To estimate some of the genetic parameters and genotypic phenotypic coefficient of variation and to find the interaction between varieties and generations in the morphological traits and yield, yield components. Three varieties of Faba beans were treated by nitroso acid mutagen, field experiments were conducted during 2010-2013. The results showed that variances values were different among traits, and the values of genetic and phenotypic variance were greater than the environmental variance values for most of the traits. Heritability in the broad sense has reached the highest value in most of the studied traits for M1 and M2 generations. Heritability in the broad sense varied among the traits. Highest heritability in most of the traits in the M1 generation because of the high values of genetic variation and lower values in the protein percentage and yield protein. Also, in the M2 generation was the highest heritability in most of the studied traits due to the high genetic variation and low environmental variance ratio and less values in the traits, number of seeds/pod and protein percentage were 59.39 and 18.39, respectively. It also shows that the values of the coefficient of phenotypic variance and coefficient of genetic was convergent slightly for all the traits of the M1 generation and the M2. This leads to increase the coefficient of phenotypic variance over genetic in number of pods/plant and number of seeds/pod of two generations, protein percentage and protein yield in the M1 generation. it was shown that the coefficient of genotypic varaince and the coefficient of phenotypic varaince in M1 generation was low values in most studied traits except in number of branches per plant, yield seed and protein yield, wheares protein percentage reduced in coeffecient of phenotypic variance and coefficient of genotypic variance in M2 generation. Zaina variety was superior in the M1 generation traits such as flowering early, plant height, leaf area index, number of branches/plant, number pods/plant, seed weight, seed yield and protein yield. It can be conclude that M1 generation was that best in heritability in the broad sense, coefficient of genetic variance and coefficient of phenotypic variance in most studied traits.

Keywords: Genetic Parameters, Nitrous Acid Mutagen, Faba beans

تقدير بعض المعالم الوراثية المباقلاء (Vicia faba L.) بتأثير حامض النتروز المطفر

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الخلاصة

لتقدير بعض المعالم الوراثية ومعامل الاختلاف الوراثي والمظهري ومعرفة التداخلات بين الاصناف والاجيال في الصفات المورفولوجية والحاصل ومكوناته لثلاثة اصناف من الباقلاء بتأثير حامض النتروز المطفر نفذت تجارب حقلية خلال عام 2010-2013. أظهرت النتائج بان قيم التباينات قد اختلفت بين الصفات المدروسة ، وكانت قيم التباين الوراثي والمظهري أكبر من قيم التباين البيئي لأغلب الصفات . أما نسبة التوريث بالمعنى الواسع فقد وصلت أعلى قيمة في معظم الصفات المدروسة للجيلين الأول والثاني بعد التطفير ، تبايئت قيم التوريث بالمعنى الواسع بين الصفات المدروسة . كانت أعلى نسبة توريث في معظم الصفات المدروسة في الجيل المطفر الأول M1 وذلك لارتفاع قيم التباين الوراثي واقل قيم في النسبة المئوية للبروتين وحاصل البروتين اما في الجيل المطفر الثاني M2 كانت أعلى نسبة توريث في اغلب الصفات المدروسة بسبب ارتفاع التباين الوراثي وانخفاض نسبة التباين البيئي واقل قيم في عدد البذور /قرنة و النسبة المئوية للبروتين كانت 59.39 و 18.39 على التوالي، كما يظهر أن قيم معامل الاختلاف المظهري ومعامل الاختلاف الوراثي كانت متقاربة بقليل لجميع الصفات في الجيلين الأول والثاني بعد التطفير اذ ارتفع معامل الاختلاف المظهري عن الوراثي في عدد القرنات /نبات وعدد بذور /قرنة للجيلين ونسبة البروتين وحاصل البروتين في الجيل المطفر الأول. لوحظ انخفاض قيم معامل الاختلاف الوراثي والمظهري في الجيل المطفر الثاني عن الجيل المطفر الاول في معظم الصفات عدا عدد الافرع /نبات وحاصل البذور وحاصل البروتين، أما نسبة البروتين فقد انخفض في معامل الاختلاف المظهري والوراثي في الجيل المطفر الثاني. تميز الصنف Zaina في الجيل الأول بعد التطفير بالصفات التالية التبكير بالتزهير وارتفاع النبات والمساحة الورقية وعدد الفروع /نبات وعدد قريات ووزن البذرة وحاصل البذور وحاصل البروتين . يمكن الاستنتاج بأن الجيل الأول بعد التطفير اعطى افضل قيم في نسبة التوريث بالمعنى الواسع و معامل الاختلاف الوراثي والمظهري في معظم الصفات المدروسة.

Introduction

Broad Bean is one of the important crops grown in Iraq frequently in different areas. It is consumed as pods during February, March and April, and it is one of the important crops for human consumption, which is an essential source of protein for the human body. It also contains a number of amino acids, vitamins and fatty substances [1]. It is grown in abundance to meet the needs of the people for food. Nitrite oxide (NO) is a signaling molecule in a variety of physiological processes during plant growth and development, and also is an important modulator of disease resistance [2]. The regulatory roles of NO have been reported at different stages of crop development and have been especially found beneficial in promoting seed germination and seedling stage of most plant species [3]. Pharmacological studies using mammalian nitric oxide syntheses (NOS) inhibitors along with biochemical and indirect genetic studies have suggested that plants also synthesize NO using an arginine-dependent enzyme similar to mammalian NO[4-6]. They showed that nitrous acid has also been used to produce mutants of plant viruses e.g. cowpea chlorotic mottal virus [7]. Faba beans varieties differ in many morphological traits [8-10]. The difficulties faced by researchers in breeding programs are they determine of genetic variations for important traits such as yield and yield components that can be used in breeding programs. Heritability is the degree of transmission of genetic value from parents to offspring. It represents the proportion of genetic variation to phenotypic variation. Heritability in the broad sense is defined as the ratio posed by genetic variance $\sigma^2 G$ to the phenotypic variance $\sigma^2 P$ for such status [11]. Estimates of mean, genotypic and phenotypic coefficients and path analysis revealed that they were the mean values of different quantitative characters increased in most of mutants when used in his study of chemical mutagen [12]. The understanding and knowledge of genetic variation and genetic similarities present within individuals or populations are useful for the efficient use of genetic resources in breeding program [13,14]. To evaluate performance of F2 hybrids and their parents to identify promising hybrids and estimating genotypic and phenotypic variation, heritability, Broad sense heritability was higher for : plant height , leaf area index , no. of days to flowering , pod setting , no. of pods per plant , 100 seed weight , biological yield and seed yield which is due to the high variability among these traits [15]. Breeding programs depend on the knowledge of key traits, genetic systems controlling their inheritance, and genetic and environmental factors that influence their expression. To plan an efficient development program, it is necessary to understand the breeding systems coupled with statistical analysis of inheritance data [16,17]. Therefore, this study was conducted to estimate the degree of heritability of morphological traits and yield, yield components of three varieties of Faba beans.

Materials and Methods

Nitrous Acid preparation: The acid is unstable ,weakly acidic compound and decomposes rapidly, therfore it has been prepared immediately in cold form. It included tow mixture, the first was prepared by adding 600 ml of HCl slowly to 600 ml of distilled water, in ice bath to get mix, its volume about 1200 ml. The second mixture was prepare by diluting 160 g of Sodium nitrite (NaNO₂) with 400 ml of distilled water. Then, first mixture was added to the second mixture in ice bath to get nitrous acid solution as following equation: HCl + NaNO₂ ------ HNO₂ + NaCl. Seeds were soaked in nitrous acid for 24 hrs and kept in ice bath [18]. The experiment was conducted in a clay loam soil. Treated seeds and control (untreated) were sowed in the filed during winter 2010-2013, at the Experimental Station of Biology Dep. College of Sciences, University of Baghdad. Factorial experiment was arranged in randomized Complete Block Design (RCBD) with three replicates. The area of experimental unit was 10 m^2 with five lines. The spaces between lines were 0.70m and 0.30m between plants. The superphosphate fertilizer (P2O5 45%) at a rate of 80 kg/ha was added to the soil before sowing [19], and urea fertilizer (N 46%) at rate of 50 kg/ha was applied before the first irrigation [20]. The other required practices for faba bean growing were followed as recommended. The parameters were studied during the growth period of faba bean: days from planting to 50% flowering, plant height (cm), leaf area index (LAI), number of branches/plant, number of pods/plant and number of seeds/pod. Seed weight (g), seed yield (kg/ha), protein percentage% and protein yield (kg/ha) were studed after harvesting.

Statistical analysis and genetic parameters:

Statistical analysis was performed for each trait at any generation using analysis of variance of (RCBD). The treatment means were compared at 5% level of significant using least significant differences test (LSD). To deduce the differences between the arithmetic averages of treatments . M0= plants of untreated

M1=Plants treatment of nitrous acid mutagen (First generation) M2= Plants after mutagenesis (second generation) Genetic parameters estimated as follows:[21,22 and 23]

$$\delta^{2}G = \frac{msv - mse}{r}$$

$$\delta^{2}E = mse,$$

$$\delta^{2}p = \delta^{2}G + \delta^{2}E$$

$$h^{2}_{b.s} = \frac{\delta^{2}G}{\delta^{2}p} \times 100$$

Where:

 $\sigma^2 \mathbf{E} = \text{Environmental variance}$ $\sigma^2 \mathbf{G} = \text{Genotypic variance}$ $\sigma^2 \mathbf{P} = \text{Phenotypic variance}$ $\mathbf{h}^2 \mathbf{b.s} = \text{Broad sense heritability}$ $\mathbf{\% G.C.V} = \mathbf{\sigma G / x}^- * 100 = \text{Genotypic. Coefficients of Variance}$ $\mathbf{\% P.C.V} = \mathbf{\sigma P / x}^- * 100 = \text{Phenotypic. Coefficients of Variance}$ Statistical combined analysis between varieties and generations was used.

Results and Discussion

Table(1) shows variances values of heritability in the broad sense among traits. M1 has the highest heritability in most traits because of the high values of genetic variation. This leads to crop improvement could be possible by simple selection. High heritability coupled with high genotypic variation revealed the presence of an additive gene effect [24], and lower values in the traits of the protein percentage and yield protein were 25.85 and 24.49%, respectively, whereas number of seeds/pod is not estimated because the value of the genetic variation (δ^2 G) was negative. In the M2 generation was the highest heritability in most of the studied traits, that was due to a high genetic

variation and a low environmental variation, the proportion of heritability was high as possible as considered a standard electorally in improved productivity, and lower values in the number of seeds/pod and protein percentage were 59.39 and 18.39, respectively. As related with heritability of number of pod/plant was not estimated, because of the genetic variation (δ^2 G) was negative value. It also shows that the values of the coefficient of phenotypic variance and coefficient of genetic variance were convergent slightly for all the traits of the M1 generation and M2. So that it caused increasing the coefficient of phenotypic variance more than genetic in the traits number of pods/plant, number of seeds/pod of two generations, protein percentage and protein yield in the M1 generation. It observed there were low values of the coefficient of genetic variance and phenotypic in the M2 generation as compared with of the M1 generation in most traits except number of branches/plant, seed yield, protein percentage and yield protein but the latter decreased in phenotypic coefficient of variance in the M2 generation. Heritability in the broad sense was high in most traits which means that the majority of the phenotypic variation was due to the genetic variation and small part was due to the environmental variation.

Traits	Generation	h ² b.s	%G.C.V	%P.C.V
50% flowering	M1	97.72	21.64	21.8
	M2	90.12	7.72	7.95
plant height	M1	97.54	18.67	18.81
	M2	92.78	15.65	16.36
leaf area index	M1	73.1	23.77	46.26
	M2	95.18	22.18	23.39
number of	M1	86.36	34.48	35.07
branches/plant	M2	92.18	41.21	42.09
number of	M1	79.28	16.78	20.09
pod/plant	M2		9.06	18.57
number of	M1		18.83	38.14
seed/pod	M2	59.37	14.56	24.81
seed weight	M1	97.8	37.12	38.14
	M2	98.03	31.92	33.37
seed yield	M1	98.83	21.61	22.04
	M2	97.48	23.54	23.87
protein	M1	25.85	10.04	12.66
percentage	M2	18.39	7.74	7.99
protein yield	M1	24.49	13.02	16.34
	M2	90.18	26.41	27.15

Table 1- Estimate the proportion of heritability in the broad sense and coefficient of genotypic variance and coefficient of phenotypic variance.

The results of table-2, show the interaction between varieties and generations. Studied traits at any generation is considered interaction as a clear indicator about possibility of variety in future breeding programs. Superiority of Zaina variety of the M1 generation was in the following traits, early flowering 73.66 days, plant height 100.1cm, leaf area index 2.91, number of branches/plan 17, number pods/plan 28.5, seed weight 1.73 g, seed yield 4997.07 kg/ha and protein yield 929.94 kg/ha, and did not differ significantly with local variety in plant height and number of pods/plant. The **Aguadulce** variety gave the highest protein percentage in the M1generation of 20.89%, may be due to the soaked seed in nitrous acid mutagen solution contains a nitric oxide NO that act as a bioactive molecule and involved in many physiological processes in plants, such as growth and development of plant tissue. Also, NO operates the auxin signals transduction. Auxin causes reducting in the flower abscission percentage and then producing highest number of pods setting and yield components and thus affecting on the seeds yield [25]. From the result above, the M1generation gave the highest value of heritability in the broad sense and genetic coefficient of variance and phenotypic may be there were more segregation taken place.

Traits	generation	M0	M1	M3	LSD
	varieties				
50% flowering	Zaina	80	73.66	90	2.45
	Aguadulce	83.66	93.1	98	
	Local	84	91.33	92	
plant height (cm)	Zaina	67	100.1	48	2.31
	Aguadulce	73	82.3	54	
	Local	71	99.6	57.5	
leaf area index	Zaina	1.5	2.91	1.89	0.39
	Aguadulce	1.9	2.25	2.42	
	Local	1.35	2.4	2.33	
number of	Zaina	7	12.2	10.5	1.32
branches/plant	Aguadulce	5.3	8.5	13.5	
	Local	6.1	12.4	17	
number of pod/plant	Zaina	22	28.5	16.5	2.24
	Aguadulce	18.5	24.2	15	
	Local	26.7	29	15.2	
number of seed/pod	Zaina	3.2	2.9	2.85	0.99
	Aguadulce	2.9	3.2	3.23	
	Local	3	3.6	3.36	
seed weight (g)	Zaina	1.4	1.73	1.04	0.20
	Aguadulce	1.3	1.31	1.14	
	Local	1.21	1.15	1.47	
seed yield(kg/ha)	Zaina	3116.37	4997.09	3001.01	219.4
	Aguadulce	2550.31	4111.12	2998.99	
	Local	3092.64	4003.97	3766.03	
protein percentage	Zaina	18.5	18.61	18.1	1.54
	Aguadulce	20	20.89	19.02	
	Local	19	19.62	18.99	
protein yield(kg/ha)	First	576.53	929.94	542.6	72.51
	second	510.06	858.36	570.2	
	third	587 58	785 31	715 51	

Table 2- The interaction between varieties and generations using statistical combined analysis.

References

- 1. Maurizio, M.; Francesco M.; Aldo P.; Giorgio F.; Mauro M. and G. Piva .2005. Raw pea (*Pisum sativum*), rawfaba bean (*Vicia faba var. minor*)and raw lupin (*Lupinus albus var.multitalia*) as alternative protein sources in broiler diets. *Ital.J.Anim.Sci.* Vol. 4, pp:59-69.
- 2. Bethke, P.C., Libourel, I.G., Aoyama, N., Chung, Y.Y., Still, D.W. and Jones, R.L. 2007. The *Arabidopsis* aluron layer responds to nitric oxide gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. *Plant Physiology*, 143, pp:1173-1188.
- **3.** Libourel, I.G., Bethke, P.C., De Michele, R. and Jones, R.L. **2006**. Nitric oxide gas stimulates germination of dormant *Arabidopsis* seeds use of a flow through apparatus for delivery of nitric oxide. *Planta*, 223, pp: 813-820.
- 4. Lamattina, L.; Garcia- Mata, C.; Graziano, M. and Pagnussat, G. 2003."Nitric oxide: the versatility of an extensive signal molecule"; *Annu. Rev. Plant Biol.*, 54, pp:109 136,.
- 5. Neill, S.; Bright, J.; Desikan, R.; Hancock, J., Harrison, J.; and Wilson, I. 2008. "Nitric oxide evolution and perception"; *Journal of Experimental Botany*, 59, pp:25-35.
- 6. Corpas, F.J.; Palma, J.M.; Del Rio, L.A. and Barroso, J.B. 2009. "Evidence supporting the existence of L- arginine-dependent nitric oxide syntheses activity in plants"; *New Phytol.*, 184, pp:9-14.
- 7. Bancroft, J. B.; Mclean, G.D.; Rees, M.W. and Short, M.N. 1971. "The effect of an arginyl to a cysteinyl replacement on the uncoating behavior of a spherical plant virus"; *Virology*, 45, pp:707-715.

- 8. Bahy, R. Bakheit ; Abdou, R. F. and A. M. Eissa. **1991**. Genetical studies of some Egyptian and imported varieties of Faba beans. Assuit. *J. of Agric. Sci.*, 22 (1), pp: 33-48.
- **9.** Loss, S. P., and K. H. M. Siddique.**1997**. Adaptation of faba bean (*Vicia faba* L.) to dry land Mediterranean type environment, 1-seed yield and yield components. *Field crops. Res.* 52, pp:17-28.
- **10.** Link, W., Abdelmula, A. A., and E. Kittlitz.**1999**. Genotypic variation for drought tolerance in (*Vicia faba* L.) *Plant breeding* 118, pp:477-483.
- 11. Allard, R. W.1960. Principle of Plant Breeding. John Wiley and Sons, Inc., U.S.A, pp:485.
- 12. Khan, I.A., 1985. Correlation and path coefficient analysis of yield components in mung bean (Phaseolus aureus roxb). *Bot. Bull. Academia Sinica* 26, pp:13-20.
- 13. Ertugrul, Y., Özcan, S. and Açık, L. 2006. Analysis of genetic relationships among Turkish cultivars and breeding lines of Lens culinatis Mestile using RAPD markers. *Genet. Resour. Crop E*.vol. 53, pp:507-514.
- 14. Safavi, S.A., S.S. Pourdad, M. Taeb and M. Khosroshahli, 2010. Assessment of Genetic Variation among Safflower (*Carthamus tinctorius* L.) Accessions using Agro-morphological Traits and Molecular markers. *Journal of Food Agriculture and Environment*, 8(3&4), pp:616-625.
- **15.** Al-Shakarchy, W. Y. R. **2010**. Estimation of some genetic parameters, correlations and path coefficient analysis for F2 generation in faba bean (*Vicia faba L.*). *Tikrit, J. Agri. Sci*.10(1), pp:50-61.
- 16. Yap, T.C. and B.L. Harvey 1972. Inheritance of yield components and morphological traits in barley (Hordeum vulgare L.). *Crop Science*, 12, pp: 283-288.
- **17.** Srivastava, J.P. and A.B. Dhamania. **1989**. Use of collections in cereal improvement in semi arid areas. Cambridge University, Cambridge., pp :88-104.
- **18.** Strnadova, K. **1976**. "A method of preparation and Application of nitrous acid as a mutagen in *Claviceps purpurea*"; *Folia Microbiol.*, 21:455-458.
- **19.** Aguilera-Diaz, C.; and Recald, M. L. **1995**. "Effect of plant density and inorganic nitrogen fertilizer on field bean (*Vicia faba* L)"; *J. Agric. Sci., Camb.*, 125 (1), pp:87-93.
- **20.** Mady, M. A. **2009**. "Effect of foliar application with yeast extract and Zinc on fruit setting and yield of faba bean (*Vicia faba* L)"; *J. Biol. Chem. Environ. Sci.*, 4(2), pp:109-127.
- **21.** Das,P.K.**1972**. Studies on selection for yield in wheat: An application of genotype and phenotypic correlation, path coefficient analysis and discriminate function *J: gric.Sci.*49, pp:238-234.
- **22.** Al Aseel, A. S. M. **1998**. Phenotypic and Genetic Correlation and Path coefficients Field of traits in wheat bread, Doctoral Dissertation. College of Agriculture-University of Baghdad.
- **23.** Abudlgaffor, A. H, Nawfal. A. S. and Ahmed. A. M.**2011**. Variances estimation and genotypic, phenotypic correlation and broad heritability percentagein mazie (*Zea mays L.*). *Diala, Jour. Agri.Sci.*3(1), pp:206-217.
- 24. Noor, F., Ashaf. M. and Ghafoor A. 2003. Path analysis and relationship among quantitative traits in chickpea (*Cicer arietinum* L.). *Pak. J. Biol. Sci.*, 6, pp: 551-555.
- **25.** Hayat, S.; Hasan, S.A.; Mori, M.; Fariduddin, Q. and Ahmed, A. **2010**. "Nitric oxide: Chemistry, Biosynthesis, and Physiological role"; In: Hayat, S., Mori, M., Pichtel, J. and Ahmed, A. (eds). *Plant physiology*. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.