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Changes in Seed and Leaf Characteristics of Common Bean (*Phaseolus vulgaris* L.) Cultivars Treated With Co-60

HATICE BOZOGLU*, HÜSEYIN ÖZÇELIK† and ERKUT PEKSEN
Department of Field Crops, Faculty of Agriculture
Ondokuz Mayis University, Samsun, Turkey
Fax: (90)(362)4576034; Tel: (90)(362)3121919/1175
E-mail: hbozoglu@omu.edu.tr

The aim of this study was to create a variation in bean which is an important plant Black sea region of Turkey by using physical mutation and to introduce a plant material which can complete the deficient characteristics of cultivars are grown in this region. Four cultivars were used in this study. Different Co-60 γ-rays (50, 100, 200, 300 Gy doses) were used as mutation source. The influences of γ-irradiation on leaf and grain characteristics of cultivars were investigated in M_2 population. Higher variation coefficients were obtained in doses of 50, 100, 200, 300 Gy compared to control group, but mutant plant count was lower for 300 Gy dose due to this dose's higher influence. For this reason, variation coefficients were found lower compared to control. It was determined that mutagen application with different doses in different cultivars created variation in various seed and leaf characteristics of bean. According to t-test results, change of seed characters was lower than leaf characters.

Key Words: Bean, Leaf, Mutation, Seed.

INTRODUCTION

Although Turkey is homeland of many leguminosae plants, bean has came in Turkey. Especially, it spreaded out over wide areas in Black-Sea region. In terms of production area in Turkey[1], bean ranks third after chickpea and lentil with 173 000 ha. Samsun is one of the leading cities in Turkey in terms of bean agriculture. But, yield is below country average yield due to differences in production technique and faulty applications. Not only lack of suitable production technique but also lack of high-yield cultivars affect bean agriculture negatively.

Plant breeding, which is a controlled evolution event, is achieved by manipulating of spontaneous mutations, crossbreedings and natural selections by plant breeders. The occurrence rate of spontaneous mutations is

†Black Sea Agricultural Research Institute, Samsun, Turkey.

too low. For this reason, artificial mutations are used. Mutation is a useful and preferable method for improving characteristics which show simple inheritance pattern and directed by one gene in self-germinated plants. It is worthwhile to create mutation in a recessive gene. Transport of this gene *via* back crossing is a successful method even if this gene is present in agricultural cultivars[2].

Donini *et al.*[3] reported that traits such as plant morphology, flowering and maturity period might be changed by using mutation breeding in many plants.

In a study carried out in New Delhi, India, 500 healthy and uniform seeds of exotic cowpea genotypes were treated with 3 doses of γ-rays (15, 30 and 45 kR). The γ-ray doses of 15 and 20 kR were the most effective for generating useful polygenic variability through induced mutagenesis in cowpea genotype[4].

Dry seeds of mung bean (*Vigna radiata*) were treated with 10, 20, 30 and 40 kR γ-irradiation in India to evaluate the extent of macro and micro mutations appearing in the M_2 generation. Significant changes ($p < 0.05$) with wide range of variations had been identified in yield and its contributing characters. The selection of individual plants in the M_2 generation can be studied to observe the spectrum of variation for quantitative characters[5].

The aim of this study is to attain parents which can produce productive and high quality lines by creating variation with physical mutation in bean cultivars which are poor in some characteristics and grown in this region. The influence of mutation on agronomic characteristics was investigated in many studies. It was aimed in this study to determine whether mutation could be created in M_2 by observing the changes occurred in seed and leaf characters of plant.

EXPERIMENTAL

This study was conducted for 2 years in Samsun province in Middle Black Sea region which was found in the north of Turkey. This region is characterized by humid and rainy climate characteristics.

In this study, Yalova-5, Sahin-90, Karacasehir-90 and ABA-58 cultivars were used. These cultivars are classified as nanus type according to the plant height and oblangus and elipticus types according to the grain shape.

Co-60 γ-ray was used as mutagenic agent and radiation process was made in Turkish Atomic Energy Institution. 750 Number seeds from each of the cultivars was exposed to γ-rays of 50, 100, 200 and 300 Gray (Gy). Radiated seeds were sown in 28 may together with their controls at 60×5 cm density with 15 rows at each parcel and control parcels containing 2 rows were inserted between these parcels. M_1 population was grown in standard culture conditions without fertilization. In M_1 population, emergence ratio, survival, kimera, number of pods and seeds per plant and seed yield on plant were investigated. These characters were considered and seeds were selected for M_2. In second year, seeds of selected plant from M_1 were sown at 60 × 5 planting density as an offspring row on 15 may. Control materials were sown as a single row after each 10 offspring rows. In M_2 generation, plants which phenologically differ from control during vegetation period, were marked as macro mutant types according to the macro mutation method[6]. Total plant counts for each dose were increased to 100 by adding plants selected randomly. The widths, lengths and thicknesses of at least 10 seeds from each doses were measured with caliper. Width, length of middle leaflet of leaf and leaflet petiole on same node of plants were measured in 10 plants.

Averages ($\bar{x}$), standard deviations (S), standard error of mean ($S\bar{x}$), ranges (R) and variation coefficients (CV) related to traits examined in M_2 generation were calculated by using MSTAT-C pocket programme. T-test was used to determine the differences between treatments. T-test value and significantly were given in text.

RESULTS AND DISCUSSION

There are lots of studies related to mutation applications in grain legume crops. Some significant agronomic characteristics such as plant heights and pods number of plant, 100 seed weight were investigated in many of these studies. In the M_2 generation of mung bean genotypes, irradiation with γ-ray provided significant changes wide range of variations had been identified in yield and its contributing characters like number of pods/plant, 100 seed weight and seed yield per plant, cluster of pods/plant, number of leaves/plant, primary and secondary branches/plant, internode length and plant height[5].

Yaqoob and Bashir *et al.*[7] studied the effects of induced mutation by γ-irradiation (100, 200, 300, 400 and 500 Gy) on various agronomic traits of five mung bean. A wide range of variability was determined for all the characters except plant height.

In this study, the effects of mutation on two important part of plant *i.e.*, leaf where photosynthesis occurs and seed where organic material accumulate, were investigated.

Leaf characters: Averages, ranges and variation sources related to leaflet length were given in Table-1. Averages of control groups were found higher than those of all the cultivars' mutant populations. With respect to ranges, control groups constitute upper limits and mutant populations constitute lower limits. Ray application led to a decrease in leaflet length. There were statistical differences between Sahin 50 Gy and its control (t =

4.620**), between Yalova 50 Gy and Yalova 300 Gy (t=3.42 **) and between ABA 100 and ABA 200 Gy (t = 4.2**).

The higher variation coefficients for mutant populations indicate that mutagens are effective in obtaining significant variation in examined traits. The 50 and 100 Gy populations of Karacasehir and 200 and 300 Gy populations of Yalova cultivar had lower variation compared to the control groups. Ranges of 200 and 300 Gy populations of Yalova were found as 3.7-6.2 and 3.8-7.9, respectively (Table-1). This can be attributed to scarcity of mutant plant due to harmful influence of higher doses of γ-radiation. Ardelean *et al.*[8] founded that the LD50 for all the bean cultivars ranged from 22.8 to 32.2 Kr.

The highest leaflet width was found for ABA cultivar. Averages for leaflet width of control groups were found higher than mutant populations. Control groups and mutant populations had similar range values (Table-1). Higher variation coefficients in mutant populations indicate that mutagens led to great variations on examined traits. Variation coefficients were found higher for all mutant populations compared to control groups except for 50 and 100 Gy doses of Karacasehir and 200 and 300 Gy doses of Yalova. Prashant *et al.*[9] reported that the leaf area of faba bean was almost the same in the mutant (γ-irradiation at 5, 10, 15, 20 or 30 kR) and control.

Average leaflet petiole lengths were found higher in M_2 populations exposed to radiation compared to control groups of all cultivars (Table-1).

There were statistical differences between control of Sahin and 50 G dose (t = 5.14**), between control of Sahin and 200 Gy dose (t = 6.68 **), between control of Karacasehir and 50 Gy dose (t = 5.35**), between control of Yalova and 50 Gy dose (t = 3.28**), between control of ABA and 50 Gy dose (t = 3.0*).

Seed characters: One of the characteristics which was taken into consideration in classification of beans is seed shape[10]. Shape is a significant criterion in varying consumption preference among the regions. Seed size index can be determined by multiplying seed width, seed length and seed thickness values[10,11]. Heredity degree of seed size index (0.94) in bean is higher than other morphological characters[12]. In this study, the influence of mutation in seed dimensions was determined. Seed dimensions for 4 cultivars of M_2 populations were given in Table-2.

Average seed lengths that is effect this trait were found higher for mutant populations compared to their control groups. Sahin 300 Gy, Karacasehir 200 Gy, Yalova 200 and 300 Gy, ABA 300 Gy populations had the highest seed lengths. There were no statistical differences between doses except for between control of ABA cultivar and 50 Gy dose (t = 2.74*) and between 50 Gy and 200 doses (t = 2.65*).

TABLE-1
LEAFLET LENGTHS, LEAFLET WIDTHS AND PETIOLE LENGTHS OF BEAN CULTIVARS OF M_2 POPULATION

Populasyon	Leaflet length (cm)			Leaflet width (cm)			Leaflet petiole length (cm)		
	$\bar{x} \pm S_{\bar{x}}$	Range	CV (%)	$\bar{x} \pm S_{\bar{x}}$	Range	CV (%)	$\bar{x} \pm S_{\bar{x}}$	Range	CV (%)
Sahin control	7.642 ± 0.37	5.5-8.3	17.80	5.814 ± 0.22	4.6-7.4	10.73	2.715 ± 0.13	2.1-3.7	12.17
Sahin 50 G	5.328 ± 0.31	4.3-7.9	15.12	4.372 ± 0.28	2.9-6.3	14.69	2.415 ± 0.27	1.5-3.8	21.06
Sahin 100 G	5.177 ± 0.43	4.2-7.8	21.72	4.168 ± 0.36	2.8-7.1	19.71	2.375 ± 0.22	1.4-3.9	25.11
Sahin 200 G	4.873 ± 0.51	3.2-6.7	25.77	3.875 ± 0.24	2.7-5.9	17.13	2.199 ± 0.16	1.3-3.3	22.27
Sahin 300 G	6.019 ± 0.75	3.6-8.2	29.19	4.474 ± 0.51	2.8-7.5	25.66	2.241 ± 0.24	1.6-3.1	19.64
K.sehir control	5.715 ± 0.22	4.9-7.1	9.84	4.711 ± 0.14	3.7-5.9	7.93	2.372 ± 0.09	1.9-3.1	8.74
K.s ehir 50 G	4.913 ± 0.28	3.7-5.8	8.15	3.965 ± 0.17	2.9-4.3	6.15	1.967 ± 0.17	1.4-3.0	15.03
K.sehir 100 G	4.710 ± 0.33	3.4-5.2	7.28	3.782 ± 0.25	3.0-4.4	7.17	2.009 ± 0.19	1.3-3.2	16.94
K.sehir 200 G	4.137 ± 0.41	3.1-5.7	13.64	3.371 ± 0.26	2.3-5.4	14.63	1.912 ± 0.19	1.2-3.1	19.83
K.sehir 300 G	5.003 ± 0.49	3.2-7.0	16.53	3.872 ± 0.33	2.8-5.6	19.74	1.917 ± 0.22	1.4-2.9	14.72
Yalova control	7.157 ± 0.33	5.7-9.4	13.77	5.519 ± 0.16	4.3-7.0	12.63	2.673 ± 0.12	2.1-3.4	9.71
Yalova 50 G	6.344 ± 0.39	4.1-8.3	17.46	4.273 ± 0.22	2.6-5.7	22.71	2.281 ± 0.19	1.5-3.5	21.24
Yalova 100 G	6.149 ± 0.47	3.9-7.6	20.93	4.152 ± 0.33	2.3-5.7	24.69	1.997 ± 0.21	1.3-3.7	25.77
Yalova 200 G	5.917 ± 0.36	3.7-6.2	11.62	3.997 ± 0.25	2.8-4.7	12.01	2.366 ± 0.15	1.4-3.2	9.19
Yalova 300 G	5.793 ± 0.43	3.8-7.9	13.52	4.073 ± 0.31	2.9-4.1	9.88	2.414 ± 0.16	1.5-2.9	8.63
ABA control	7.743 ± 0.33	5.9-9.7	15.33	5.929 ± 0.18	4.8-7.3	13.83	2.933 ± 0.07	2.3-3.5	10.94
ABA 50 G	6.671 ± 0.29	4.0-8.7	20.20	4.733 ± 0.21	2.7-6.6	20.17	2.567 ± 0.12	1.7-3.6	20.88
ABA 100 G	6.557 ± 0.39	4.3-8.7	21.59	4.600 ± 0.37	3.0-7.5	28.85	2.500 ± 0.19	1.7-3.8	28.60
ABA 200 G	5.219 ± 0.37	3.1-7.6	28.57	4.119 ± 0.22	2.8-5.6	21.58	2.362 ± 0.15	1.3-3.6	26.16
ABA 300 G	6.025 ± 0.62	3.7-9.3	28.93	4.550 ± 0.54	2.9-7.3	33.49	2.237 ± 0.21	1.4-3.3	26.60

TABLE-2
SEED LENGTHS, SEED WIDTHS AND SEED THICKNESS OF BEAN CULTIVARS OF M_2 POPULATION

Population	Seed length (mm)			Seed width (mm)			Seed thickness (mm)		
	$\bar{x} \pm S_{\bar{x}}$	Range	CV (%)	$\bar{x} \pm S_{\bar{x}}$	Range	CV (%)	$\bar{x} \pm S_{\bar{x}}$	Range	CV (%)
Sahin control	14.30 ± 0.24	12.08-15.48	6.32	6.09 ± 0.11	5.59-7.00	6.71	4.83 ± 0.09	4.24-5.56	6.91
Sahin 50 G	14.48 ± 0.11	11.02-16.65	7.40	6.14 ± 0.06	4.99-10.00	9.81	5.02 ± 0.05	3.76-6.70	9.12
Sahin 100 G	14.35 ± 0.11	11.66-17.54	7.67	6.12 ± 0.08	5.14-12.28	13.55	4.90 ± 0.05	3.97-6.38	9.44
Sahin 200 G	14.30 ± 0.11	11.66-17.31	7.95	6.17 ± 0.07	4.47-11.02	11.65	5.03 ± 0.05	3.97-7.19	10.31
Sahin 300 G	13.82 ± 0.15	7.19-16.88	10.69	6.22 ± 0.08	4.76-10.22	12.46	4.98 ± 0.05	3.93-6.63	10.00
K.sehir control	8.28 ± 0.12	7.41-9.44	6.04	4.83 ± 0.09	4.18-5.61	7.97	3.97 ± 0.08	3.55-4.77	8.51
K.sehir 50 G	8.34 ± 0.21	7.41-9.00	6.54	4.65 ± 0.14	4.22-5.20	7.96	3.99 ± 0.09	3.61-4.21	5.91
K.sehir 100 G	8.29 ± 0.07	6.97-12.23	7.98	4.83 ± 0.05	3.73-7.84	10.02	3.95 ± 0.03	2.98-4.77	7.79
K.sehir 200 G	8.22 ± 0.07	6.43-9.64	8.17	4.87 ± 0.05	3.94-7.20	9.67	4.01 ± 0.04	3.17-5.19	9.64
K.sehir 300 G	8.35 ± 0.08	5.65-11.12	9.10	4.97 ± 0.07	4.01-7.77	13.37	3.96 ± 0.05	3.15-5.51	10.65
Yalova control	15.32 ± 0.19	14.12-18.52	7.03	8.85 ± 0.12	6.63-10.15	7.16	5.21 ± 0.10	4.95-7.75	7.85
Yalova 50 G	15.44 ± 0.25	13.12-18.11	7.45	8.97 ± 0.09	6.12-10.55	7.98	5.86 ± 0.12	4.87-8.74	8.14
Yalova 100 G	15.76 ± 0.23	14.54-19.46	8.19	9.13 ± 0.08	5.82-10.17	8.54	5.93 ± 0.07	5.18-8.45	7.81
Yalova 200 G	15.11 ± 0.18	13.99-19.22	8.85	9.19 ± 0.13	5.87-9.86	8.94	5.88 ± 0.14	5.54-9.82	10.14
Yalova 300 G	15.02 ± 0.13	13.36-21.31	9.87	9.15 ± 0.10	6.12-11.13	11.14	6.01 ± 0.16	5.78-9.55	12.15
ABA control	15.03 ± 0.25	13.93-17.56	7.45	7.65 ± 0.23	7.21-9.26	6.88	5.74 ± 0.09	4.35-5.89	6.87
ABA 50 G	15.88 ± 0.36	12.75-18.14	8.14	7.92 ± 0.24	7.12-10.12	7.14	5.78 ± 0.11	4.01-7.14	8.14
ABA 100 G	15.22 ± 0.39	12.55-19.87	8.01	8.01 ± 0.32	6.85-11.13	7.98	5.62 ± 0.08	3.99-7.42	7.19
ABA 200 G	15.44 ± 0.51	12.98-18.45	9.85	8.12 ± 0.24	6.99-10.98	9.54	6.01 ± 0.15	4.12-6.87	9.16
ABA 300 G	14.87 ± 0.23	11.95-17.85	11.14	7.99 ± 0.36	7.01-10.85	11.25	5.96 ± 0.12	4.00-7.12	11.12

Zdravkovic *et al.*[13] observed among the bean genotypes examined selected 10 local genotypes in Yugoslavia. Significant differences were the length:width ratios varied between 1.41-2.40. the thickness:width ratio varied between 0.62 and 0.85.

Seed widths were found to increase in treatments exposed to mutation for all cultivars. In Turkey, grain largeness is an important preference criterion for consumers. That is to say, mutation caused by mutagen applications in grain width is generally desired to occur.

Average seed width values for control groups were found higher compared to those for mutant populations except for 50 Gy dose of Karacasehir. Mutant populations had higher range values than control groups in terms of seed width (Table-2). But, there were found differences between 50 Gy and 300 Gy doses of Karacasehir cultivars that is the smallest grains (t = 2.46*) and between ABA control and 200 Gy dose (t = 2.76*).

Seed thicknesses, ranges and variation coefficients for M_2 populations of 4 cultivars were given in Table-2. Average values for mutants of all varieties except for 100 and 300 Gy doses of Karacasehir cultivar and 100 Gy dose of ABA cultivar were found higher than their control groups. Mutants had higher range values compared to control groups in terms of seed thickness. But there were no statistical differences among doses.

Conclusion

Bean, which is one of the most important agricultural products, is consumed in different forms in Turkey. Black-Sea region, especially Samsun, is one of the most important sites in Turkey for bean breeding studies. Because this area is high genetic diversity. Turkey has 19 registered national cultivars and this amount is insufficient. One of the targets is to increase the number of cultivars in Turkey.

In this study, it was aimed to enrich genetic diversity *via* mutation and also to introduce parent lines which can be used in crossing studies. Mutants can be crossed with parents which are not mutant in simple crossing studies and also they can be used as parents in more than one crossing. When the cross combinations and mutant characteristics which were used in crossings were taken into consideration, it can be concluded that use of mutant in crossing studies led to beneficial results compared to direct production of mutant.

The fact that mutagen doses create variations in bean cultivars has been evidenced by the higher variation coefficients observed in mutant populations. Ranges of all the mutant populations were found higher compared to those of control populations. Higher variation coefficients were obtained in doses of 50, 100, 200, 300 G compared to control groups, but mutant plant count was lower for 300 Gy dose due to this dose's higher influence. Generally, mutagen application with different doses created variation in leaf and seed characteristics of different common bean cultivars. According to t-test results, change of seed characters was lower than leaf characters. None of γ doses affected the seed thicknesses. Seed length only showed significant different in ABA cultivar. Statistical differences were defined on Karacasehir and ABA cultivars for seed width. Changes in width of leaflet and leaflet petiole were more than seed length. Studies on confirmation of these mutants in future generations has been continued.

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