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DEDUCED TWO BROAD BEAN CULTIVARS BY EXPERIMENTAL MUTAGENS IN IRAQ

M. A. Al-Hamdany A. H. Kadhem*
A. A. Nefel H. A. Abas

ABSTRACT

Gamma rays 30 and 40 Grays with 0.2% of the chemical mutagen Ethyl methane sulfonate (EMS) and their combinations were used on the dry seeds of both Ekwadelgi and Kebrisii broad bean cultivars. As a result of recultivation of M1-M3 generations in the same field, heavy wilt incidence was observed on M3 progeny. The most pathogenic wilt causal agent (*Fusarium avenacium*) was identified and used for soil infestation during M4-M6 generations. The selected mutant lines from M6 progeny were thoroughly evaluated for productivity and some agronomic traits during M7-M9 generations. Two mutants namely K762 and E357 were developed to new broad bean cultivars (Tuwaitha and Taqa 357 respectively). The second cultivar was characterized by having terminated growth (Topless inflorescence). The productivity of these new cultivars was investigated in M11-M14 in comparison trails with the registered cultivar (Babil) and the local cultivar. Results indicated that Tuwaitha was surpassed Babil cultivar. However, both Babil and Tuwaitha cultivars were outyielded the local cultivar and were surpassed the local cultivar in many agronomic traits such as weight of 100 seeds, number of pods/plant, pod lengths and wilt resistance. Both cultivars were registered by The National Committee of Registration and Release of Agricultural Cultivars in Iraq/Ministry of Agriculture (No. 16 on 10/6/2001)

INTRODUCTION

Although broad bean (*Vicia fabae* L.) is very important legume crop in Iraq, this crop has not been considered for along time in any breeding program for crop improvement. Yield potential and yield instability of broad bean could represent a limiting factor for acreage and cultivated areas in the world (9). In Iraq, this crop is annually suffered from many agents particularly the causal agents of wilt and root rot diseases which characterized by difficult control (5). Due to the absence of any resistance source in Iraq, using physical and or chemical mutagens to create host diversity could offer a good chance for crop improvement (14, 18).

Mutation induction programs in different field crops reported numerous successful achievements in developing many induced mutants to new cultivars in different countries (14 and 15). Statistical reports of The International Agency of Atomic Energy (IAEA), mentioned that more than 30 legume cultivars had been developed from induced mutants either by gamma rays or and chemical mutagens (15). The nuclear techniques also offered good opportunities for screening and selection programs.

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Numerous variants in broad bean V. *faba var. minor* were selected following seed treatment with different mutagens and one of them has a resistant reaction to stem blight (*Sclerotinia sclerotiorum*) (12). Other but limiting successful results were achieved in soybean against mosaic disease (13), and against bacterial blight and leaf rust in bean (8 and 19).

In Iraq, one induced mutant in broad bean was developed to new cultivar (Babil), which was registered and released in 1994 (3). Similar achievement was reported in soybean (7).

This report represented a part of crop improvement program in broad bean aimed to induce useful mutants in broad bean by gamma rays and EMS.

MATERIALS AND METHODS

Dry seeds of Ekwadelgi and Kebrisii broad bean cultivars were treated with 30 and 40 Grays of gamma rays and 0.2% of Ethyl methane sulfonate (EMS) alone and in combinations. Treated and non-treated seeds (800 seeds/treatment) were planted in the field in 2m rows with 70 and 15cm apart between the rows and the seeds respectively. Seeds of the first three pods from each survival M1 plant were obtained and planted in the same field for M2 generation. During M2 season, many plants from different treatments were excluded due to wilt symptoms. The same field was again used for M3 generation. All M3 plants were screened for wilt disease, which was in a high incidence. Wilt causing fungi from all diseased M3 plants were isolated and tested for their pathogenicities (4). The survival plants were selected and coded as lines in the following generations.

Seeds of selected M3 plants were planted in new field, which had never been planted with any vegetable or legume crop. The seeds were planted in lines with 40cm apart inside 4X6 m field plots. In order to create favorable conditions for wilt disease, the seeds of selected plants were planted early (Sept. 15th) instead of October 20th. Spore Suspension ($4x10^5$) of *Fusarium avenacium* was used for soil infestation at sowing time. Twenty days later, the crown region of each plant was injected with 20 ml. of F. *avenacium* suspension. The second challenge was conducted to avoid any disease escape phenomenon which is a very common in soil borne diseases.

The same procedures were also applied in M5 and M6 seasons. During the three mentioned seasons, the plants were thoroughly screened for wilt resistance. Therefore, the selected plants from M4 were only raised in M5 and M6 respectively.

The selected plants from M6 progeny were introduced in a comparison test with their origins and the French broad bean cultivar (Long Grain Ekwadelgi) in M7. The productivity (gm. seeds/plant/line) and natural incidences of both mosaic and wilt diseases were included in the test. However the productivity and agronomic traits were investigated during two consecutive a generations (M8 and M9). Wilt resistance under artificial soil infestation in a greenhouse was evaluated in M9 selected

lines. Protein percentages in the dry seeds of all selected lines were also measured.

Productivity of the selected mutants was investigated in four consecutive generations (M11-M14). Two selected mutants were applied for registration and released by The National Committee of Registration and Release of Agricultural Cultivars/Ministry of Agriculture.

RESULTS AND DISCUSSION

Regardless of germination percentages or number of survival plants in M1 generation, seeds of the first three pods of every plant were harvested and used for M2 generation. Slight wilt incidence was observed on M2 plants, therefore diseased and abnormal plants were excluded during the season. Due to recultivation of M3 progeny in the same field, an epidemic wilt disease was dominated M3 plants because of inocula accumulation of wilt causing fungi in the soil. Isolation, purification, identification and pathogenicity test of all wilt-causing fungi from M3 diseased plants indicated that *Fusarium avenacium* proved to be the most dominant, virulent and aggressive causal agent (4).

The epiphytotic form of wilt disease occurred on M3 plants create a good opportunity for preliminary selection. Off 1067 of Ekwadelgi and 11050 plants of Kebrisii, 593 and 304 plants were individually selected respectively (Table 1). Thus all the selected plants showed wilt resistance or having disease escape trait.

Table 1: Preliminary selection of M3 broad bean progeny following seed treatment with Gamma rays and Ethyl methane sulfonate (EMS) under natural epiphytotic form of wilt disease (1)

Cultivars	Treatments	Total no. of Plants	No. Selected Plants [2]
Ekwadelgi	30 Grays of Gamma Rays	4014	152
	40Grays of Gamma Rays	1629	80
	0.2% EMS	2659	144
	30Gr.+ EMS	1074	68
	40Gr. +EMS	1295	149
	Total	10671	593
Kebrisii	30 Grays of Gamma Rays	2534	72
	40 Grays of Gamma Rays	2904	67
	0.2% EMS	2960	65
	30Gr.+EMS	1665	39
	40Gr.+EMS	987	61
	Total	11050	304

1-Seeds were planted for three consecutive generations (M1-M3) in the same field.
2-Wilt resistance and or disease-escaped plants were selected only.

In the following three-generations (M4-M6), sowing the seeds in *F.avenacium* heavy infested soil, numerous lines were completely lost due to the susceptible reaction of these lines. Differences in the number of lost lines in the three generations were observed with highest number in M4 plants, which obviously reflected disease escape phenomenon from the natural infested soil during M3 season. This is quite true since few mutant lines were lost in the second generation. Thus a high level of stability in selection program on M6 progeny was achieved which reflected the successfulness of the screening procedure used in this program (Table 2). In the end of M6, 51 and 39 mutants were selected from Ekwadelgi and Kebrisii treatments respectively.

Table 2: Screening program for wilt resistance in M4-M6 generations of broad bean under artificial soil infestation with *Fusarium avenacium* (1)

Cultivars	Treatments	Selected Numbers of Wilt Resistant Mutants In [2]			
		M3	M4	M5	M6
Ekwadelgi	30 Grays	152	22	11	11
	40 Grays	80	25	14	14
	0.2% EMS	144	22	14	11
	30Gr.+EMS	68	6	6	6
	40Gr.+EMS	149	16	9	9
	Total	593	91	54	51
Kebrisii	30 Grays	72	10	7	7
	40 Grays	67	23	18	18
	0.2%EMS	65	3	1	1
	30Gr.+EMS	39	1	–	–
	40Gr.+EMS	61	22	18	13
	Total	304	59	44	39

1-Soil was infested with spore suspension of *Fusarium avenacium* at sowing time and the plants were also inoculated after 20 days by injection method.
2-Wilt resistance was considered only in the selection.

During M7, the natural incidence of wilt disease observed on Ekwadelgi mutants ranged from 1.6 to 6.7% compared to 20.7 and 17.8% in the origin and in the French cultivar respectively. For mosaic disease, the natural incidence ranged from 0.1 to 3.7% compared to 5.3 and 2.3% in the origin and in the French cultivar respectively. Meantime, wilt incidence on Kebrisii mutants ranged from 2.1 to 7.94% compared to 21.28% in the origin and from 0.6 to 7.69% for mosaic incidence in comparison to 7.95 in the origin.

The selected mutants were highly surpassed their origins and the French cultivar. However, the following agronomic criteria were highly considered in selection program at the harvesting time of M7:

1- Good agronomic traits.
2-Growth uniformity.
3- Wilt resistance.
4- Lodging resistance (Erect growth).
5- High number of branches/plant.
6- Pod Quality.
7- Number of seeds/pod.

Using these above mentioned selection criteria, 30 promising mutants were successfully chosen. These selected mutants approved their yield potential during M8 and M9 seasons (Table 3). The protein percentages in the dry seeds of many mutants exceeded 30%. The wilt resistance was confirmed at growth room under artificial soil infestation and favorable conditions for infection establishment and development (Table4).

Table3: Productivity limits of broad bean induced mutants during M7-M9 generations

Cultivar	Treatments	Weight of Dry Seeds/Plant (gm) In		
		M7 [1]	M8	M9 [2]
Ekwadelgi	30 Grays of Gamma Rays	99.2-161.8	114.0-171.2	120.0-169.8
	40 Grays of Gamma Rays	91.0-152.8	140.0-156.9	138.0-150.2
	0.2% EMS	151.8	121.2	129.3
	30Gr. +EMS	94.0-196.0	137.3-145.0	129.5-153.0
	40Gr. +EMS	98.3-141.9	129.2-173.2	131.6-169.5
	Control	98.5	90.1	92.3
Kebrisii	30 Grays of Gamma Rays	96.0-182.7	103.0-152.4	118.9-148.9
	40 Grays of Gamma Rays	119.0-190.1	103.2-162.0	126.0-157.3
	40Gr.+EMS	110.0-158.7	82.3-163.8	117.3-152.1
	Control	83.4	80.3	84.5

1-Thirty mutants were selected from M7 generation.
2-Weights of dry seeds per plant represented the means of 30 plants in 3 replicates during M8 and M7 generations.

Table 4: Protein percentages in dry seeds and wilt incidences of broad bean mutants selected from M7 generation

Cultivars	Treatments	No. of Mutants [1]	Protein % [2]	Wilt % [3]
Ekwadelgi	30 Grays of Gamma Rays	3	26.5-30.7	6-10
	40 Grays of Gamma Rays	4	23.6-27.0	4-20
	0.2% EMS	1	25.0	12
	30Gr.+EMS	2	22.4, 28.0	12, 14
	40Gr.+EMS	5	21.4-31.5	6-12
	Control	-	25.5	54
Kebrisii	30 Grays of Gamma Rays	4	24.5-31.0	12-20
	40 Grays of Gamma Rays	4	24.5-32.9	4-12
	40Gr.+EMS	7	21.0-28.4	4-12
	Control	-	25.5	40

1-Mutants were selected from M7 generation.
2-Protein was measured in the whole dry seeds of each mutant.
3-Fifty seeds of each mutant were planted in *Fusarium avenacium* infested soil under greenhouse conditions. Readings were taken after 60 days.

Although most mutants were outyielded their origins and the French cultivar, two mutants namely K762 and E 357 were selected. Forty Grays of gamma rays was used to induce K762 from Kebrisii cultivar while the second mutant (E357) was induced from Ekwadelgi by 0.2% of EMS. The second mutant characterized by having terminated growth (Topless inflorescence) that might be suitable for mechanical harvestation. The two mutants were named as Tuwaitha and Taqa 357 respectively. Yield trails of the two promising selected mutants during four consecutive seasons (M11-M14) revealed that Tuwaitha cultivar (K762) was significantly surpassed Babil, Local, and Taqa 357 (E375) cultivars (Table 5).

Table 5: Yield trails of the induced broad bean cultivars (Tuwaitha and Taqa 357) in four consecutive generations compared with Babil and local cultivars

Cultivars	Tons/h. of Dry seeds in [1]				Means Tons/h.
	M11	M12	M13	M14	
Tuwaitha	3.12 b	3.35 b	3.65 a	3.59 a	3.42
Taqa 357 [2]	2.32 e	2.42 de	2.51 cde	2.56 cde	2.45
Babil [3]	2.68 cd	2.75 c	3.14 b	3.22 b	2.95
Local	1.64 f	1.64 f	1.60 f	1.71 f	1.65

1-Means of three replicates of 250, 450, 1000 and 1000 m^2/replicate in the consecutive generations respectively.

2-Topless inflorescence cultivar.

3-Babil cultivar (registered and released in 1994) was developed from the mutant E194.

Data of yield trails were significantly confirmed the yield potential of Tuwaitha cultivar, which significantly surpassed Babil in many seasons. Meantime, Taqa 357 significantly surpassed the local cultivar. The two new cultivars however, surpassed the local cultivar in many agronomic traits such as branches/plant, pods/plant, seeds/pod, pod lengths, protein percentages in the dry seeds, and finally wilt resistance (Table 6). Weights of 100 seeds of Tuwaitha and Taqa 357 cultivars were 175 and 167 gm respectively compared to 109.2 gm in the local cultivar.

Table 6: Evaluations of many agronomic traits in broad bean deduced cultivars Tuwaitha and Taqa 357

Agronomic Traits	Broad bean Cultivars			
	Tuwaitha	Taqa 357	Babil	Local
Plant Heights (cm)	120	80	100	135
Height of the first pod above the ground (cm)	23	60	20	60
Branches/Plant	10	13	9	6-12
Pods/Plant	35	50	28	16-18
Seeds/Plant	4-5	5	6	1-4
Pod Lengths (cm)	20-23	19	26	6-12
Weight of 100 Dry Seeds (gm)	175	167	173.4	109.2
Protein % in Dry Seeds	31.5	26.2	30.5	24.5
Wilt Incidence under soil infestation conditions [1]	4.0	9	8	57
Yield (Tons/h of Dry seeds) in 4 Seasons (M11-M14).	3.42	2.45	2.95	1.65

1-Seeds were planted in *Fusarium avenacium* infested soil at growth room conditions.

The successfulness of both screening and selection procedures adapted for wilt resistance in this program could be attributed to many factors such as soil infestation, plant inoculations, viable inoculum units, and favorable conditions for establishment and development of F. *avenacium* infection. The outcome of this project revealed that mutation induction trend might be very helpful to create host diversity in any crop, if there is no other mean for crop improvement (14). The reports of IAEA documented numerous successful results on broad bean. In Egypt, many broad bean mutants induced from Geiza 1 and 2 showing resistant reactions to brown leaf spot disease and having many agronomic traits were selected (6). Early mature and high protein content mutants were obtained following gamma irradiation on the seeds of broad bean cultivar Kornberger Kleinkornige (small seed group) (16). Yield increment in broad bean cultivar Geiza 2 following gamma irradiation ranged from -13 to +54% in M4 and from 0 to 40% in M5 based on their origin were observed (1). Protein improvement in many broad bean induced mutants was reported (2, 10 and 11). In Soybean, using up to 250 Grays of gamma rays resulted in selection different variants having remarkable increments in oil contents (17).

Finally, since the relationship between protein quantity and productivity in broad bean was not clear enough (1, 11 and 16), high yield potential trait must be highly considered as priority factor in any selection program.

The two new cultivars were registered by The National Committee of Registration and Released of Agricultural Cultivars / Ministry of Agriculture, in 2001(No. 16, on 10/6/2001).

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مجلة الزراعة العراقية مجلد١٣ عدد١ ص١٥٠-١٥٨ كانون الثاني/٢٠٠٨

استنباط صنفي باقلاء بواسطة التطفير التجريبي في العراق

محمد عبد الخالق الحمداني أياد حسن كاظم

عبد الكريم نفل حسن عبد الواحد عباس

الملخص

ضمن برنامج تربية طويل على محصول الباقلاء الغذائية، استخدمت أشعة كاما للجرعتين ٣٠ و٤٠ كري و٠,٢% من المطفر الكيميائي اثيل ميثيل سلفونيت (EMS) والمعاملات المشتركة على البذور الجافة للصنفين أكوادلجي وقبرصي. لوحظ مستوى عال من مرض الذبول في نباتات الجيل الثالث نتيجة لتكرار زراعة الحقل نفسه لثلاث أجيال متعاقبة. أفرزت عمليات العزل والتنقية التي جرت على مسببات مرض الذبول عن تشخيص الفطر *Fusarium avenacium* كمسبب رئيس حيث استخدم في تلويث الألواح الحقلية للأجيال الرابع- السادس. قومت الإنتاجية والمواصفات الزراعية للطفرات المنتخبة في الأجيال السابع- التاسع. انتخبت الطفرتان K762 وE357 من معاملات الصنفين قبرصي واكوادلجي على التوالي. طورت الطفرتان إلى صنفين جديدين وهما تويثة وطاقة ٣٥٧ الذي يتميز بالنمو المحدود. استخدم صنف الباقلاء المعتمد (بابل) والصنف المحلي مع الصنفين المستحدثين في دراسة الإنتاجية عبر أربعة أجيال متعاقبة (١١-١٤). أشارت النتائج إلى تفوق الصنف تويثة بشكل واضح على الصنف المعتمد بابل ويحتل الصنف طاقة ٣٥٧ المرتبة الثالثة. تفوق الصنفان الجديدان على الصنف المحلي في الإنتاجية والمواصفات الزراعية كوزن البذور وعدد القرنات و الفروع في النبات واطوال القرنات وعدد البذور في القرنة وأخيرا مستوى مقاومة مرض الذبول. سجل الصنفان من قبل اللجنة الوطنية لتسجيل واعتماد الأصناف الزراعية في وزارة الزراعة العراقية (رقم القرار ١٦ في ٢٠٠١/٦/١٠).

وزارة العلوم والتكنولوجيا- بغداد، العراق.