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INDUCED RESISTANCE IN ALFALFA AGAINST RUST DISEASE BY GAMMA RAYS

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Abstract:

A programe was carried out that aims at inducing new source of rust disease resistance in alfalfa . Dry seeds of local cultivar were treated with 0, 50, 100 and 150 krads of gamma rays at a dose rate of 45.0 red/sec . The radiosensitivity of alfalfa was significantly affected by gamma doses . Resistant plants were selected during series of artificial inoculations of M_2 and M_3 progenies . Data of screening procedure indicated that repeating the inoculation was very necessary to confirm highly plants. All resistant plants selected after the fourth inoculation improved their resistance at the last inoculation . In the field, all selected plants remained highly resistant to the disease and most of them showed considerable increment in number of tillers and the fresh weight .

INTRODUCTION

Alfalfa (Medicago sativa L.) is a vigorous, productive and important forage crop in Iraq. Like all farm crops, however, alfalfa is subject to withstand various damage caused by many pathogens . Rust incited by Uromyces striatus Schroet became one of the major diseases occuring continuously with two disease outbreaks every year (1). The epidemic form of this disease usually occur on all plants held for seed production . This disease reported to be as a limiting factor in alfalfa seed

production (2). Therefore this crop should be protected by all possible means from this disease .

Breeding for disease resistance represents the most visible and economic method to counteract the pathogens. However, new approaches and more efficient methods are needed for producing resistant cultivars or lines. Using ionizing radiations to induce mutations or variants is now being developed as a complimentary tool in breeding for disease resistance in crop plants (3) . There were numerous successful examples proved that mutations induced by radiations or chemical mutagens can alter the host-parasite interaction so that the host plant is less damaged by the parasite (4, 5) .

Evaluation of M_3 generation of chickpea (Cicer arietinum L.) following gamma irradiation resulted in selecting 8 mutant lines with substantial improvement of resistance to root rot disease (6) . Moreover, in soybeans, Lu (7) failed to find any good resistance source of leaf rust in over 300 cultivars, but then, after treatments of three cultivars with gamma rays, he found many plants with no pustules in M_2 progency . Other investigators obtained different mutants in many crops with improved disease resistance following experimental mutagens (8,9) .

The main purpose of the study was to induce and select rust resistant plants in alfalfa after gamma irradiation .

MATERIALS AND METHODS

Dry seeds of alfalfa local cultivar were exposed to Co^{60} gamma rays, 50, 100, and 150 krads at a dose rate of 45.0 rad/sec . At the Iraqi Nuclear Center . Samples from treated and non-treated seeds were planted in ironic tubs (30 cm wide x 52 cm long x 25 cm deep) and maintained at 25°C with 14 hr photoperiod . Meanwhile, the rest of the seeds were raised in isolated field plots for M_1 generation .

Radiosensitivity of alfalfa was measured by counting the survivals after 35 days in growth room .

In the field, seed from M_1 plants were harvested for each dose and grown for M_2 generation . For inoculation tests, seeds from M_1 and M_2 plants were planted in ironic

tubs to obtain M_2 and M_3 alfalfa generations respectively . Twenty days after sowing, all the seedling were inoculated with urediospore suspension of U. striatus ($8.92\text{-}9.89 \times 10^5$ spores/ml) . Immediately after inoculation, all the seedlings were covered for 16 h to establish rust infection . The inculum was prepared and increased periodically by repeating the inoculation of detached alfalfa leaves (10) using 20 ppm of benzimidazole .

Fourteen days after inoculation, all infected plants were counted and removed before the seconed inoculation of the healthy plants. Inoculation - screening procedure was repeated every 15 days on all escaped plants from the previous inoculation . Therefore, plantages at the time inoculation were 20, 35, 50, 65 and 80 days for 1st-5th inoculations respectively . All resistant plants after the 5th inoculation were transplanted in the field .

During one year (March 1984-1985), with two disease outbreaks, the plants were checked every time for rust infections . Later, number of tillers and fresh weight of individual plant were measured . Cutting from certain plants will be obtained for further study .

RESULTS AND DISCUSSION

Survival percentage of alfalfa was significantly affected by gamma rays . The radiosensitivity of alfalfa revealed that 10.88, 28.27, and 41.31% of survivals were reduced when the seeds exposed to 50, 100 and 150 krads respectively . Such reduction in either germinations or survivals is a common phenomenon in M_1 progeny of many crops when physical or chemical mutagens were used (11) .

The inoculation-screening procedure on M_2 and M_3 alfalfa plants exerted a heavy selection pressure for disease resistance . Thus repeating the procedure five times was sufficient enought to confirm rust resistant plants since alfalfa is continuously exposed to inoculum units of U. striaus (1). For M_2 progeny, following the first inoculation, there were 69.97, 57.06 and 58.05% as susceptible plants at 50, 100, and 150 krads respectively (Table 1) . The cumulative percentages were increased to some extent when the heathly plants received the second challange of spore suspension . Considering plant age at

the time of inoculation, it seems that the majority of susceptible plants at seedling stage (20-35 day) were discarded at the first inoculation . Data of third inoculation on 50 days old plants (flowering stage) indicated that the susceptibility of alfalfa increased sharply at flowering or adult stage . Therefore, the slight increment in cumultive percentages of susceptible plants after the fourth inoculation along with the stability of resistant reaction following the 5th. inoculation were successfully improved the screening procedure (Table 1) .

Table 1: Percentage of rust susceptible plants in M_2 - alfalfa gamma irradiated materials following five inoculations .

Inoculations No. *	Percentages of susceptible plants (cumulative) in		
	50 Kr.	100 Kr.	150 Kr.
1st Inoculation **	69.97	57.06	58.05
2nd =	75.08	62.71	66.38
3rd =	98.54	93.53	95.83
4th =	99.75	100.00	98.61
5th =	99.75	---	98.61
No. of tested plants	413	464	360
No. of resistant plants	1	0	5

* Inoculation was carried on by spraying the healthy plants with urediospore suspension every 15 days ($8.92\text{-}9.89 \times 10^5$ spores/ml) .

** Following each inoculation, all susceptible plants were discarded and the healthy ones received the following inoculation and so on .

Meanwhile, M_3 plants react with U. striatus in a similar pattern. The 3rd inoculation caused considerable increment in percentages of susceptible plants (Table 2). Once again all resistant plants (37 plants) after the 4th inoculation resist the pathogen . Regarding the final results, out of 1237, 2231, and 1270 plants which represented M_2, M_3, and parent (origin) progenies 6, 37, and 1 resistant plants were selected respectively . From these results, it was very interesting that the percentages of resistant plants in gamma irradiated materials were higher than the one obtained from untreated seeds .

Similar findings were observed in soybean, rice and

Table 2: Percentage of rust susceptible plants in M_3 - alfalfa gamma irradiated materials following five inoculations .

Inoculations No. *	Percentages of susceptible plants (cumulative) in			
	50 Kr.	100 Kr.	150 Kr.	Control (parent)
1st Inoculation **	40.74	23.41	43.77	67.79
2nd =	63.51	54.11	65.26	79.52
3rd =	91.74	92.75	91.48	97.71
4th =	98.00	98.60	98.42	99.92
5th =	98.00	98.60	98.42	99.92
No. of tested plants	751	717	763	1270
No. of resistant plants	15	10	12	1

* Inoculation was carried on by spraying the healthy plants with urediospore suspension every 15 days ($8.92\text{-}9.89 \times 10^5$ spores/ml) .

** Following each inoculation, all susceptible plants were discarded and the healthy ones received the following inoculation and so on .

Table 3: Number of tillers and fresh weight of alfalfa resistant plants after one year from transplanting in the field.

Plant No.	Gamma dose (Krads)	Generation No.	No. of trillers	Fresh weight (gm.)
1	50	M3	180	1980
2	50	M3	318	2640
3	50	M3	118	2050
4	50	M3	107	1100
5	50	M3	135	1766
6	50	M3	200	2482
7	50	M3	93	1100
8	50	M3	68	519
9	50	M3	22	494
10	50	M3	111	920
11	50	M3	110	847
12	50	M3	105	717
13	50	M3	88	1233
14	50	M3	80	872
15	50	M3	attacked by wilt pathogens	
16	150	M3	262	2556
17	150	M3	283	2265
18	150	M3	100	1536
19	150	M3	145	1785
20	150	M3	225	2624
21	150	M3	136	1460
22	150	M3	175	1760
23	150	M3	265	1040
24	150	M3	88	1135
25	150	M3	50	593
26	150	M3	30	575
27	150	M3	80	620
28	100	M3	170	3060
29	100	M3	330	1562
30	100	M3	50	843
31	100	M3	70	800
32	100	M3	130	1580
33	100	M3	230	1422
34	100	M3	145	2032
35	100	M3	55	932
36	100	M3	45	831
37	100	M3	50	685
38	control	M3	45	630
39	50 Krads	M2	135	1410
40	150	M2	225	2020
41	150	M2	40	1125
42	150	M2	77	1135
43	150	M2	78	725
44	150	M2	70	765
Average			128	1354

spearmint (7, 12, 13) . Moreover, Rawlings and his co-workers (14) found in the comparison of control and irridiated populations for many agronomic characters that irradiation treatments resulted in increased variability in treated materils . In another hand, the frequency of resistant plants in M_3 progeny was higher than those obtained in M_2 progeny which became in a full agreements with other investigations (12, 15) .

Regarding other agronomic triats in these resistant plants, results of tillering and fresh weight were incourging . Out of 43 resistant plants, there were 17 plants surpassed the average of each parameter (Table 3). However, number of tillers and fresh weight of 37 plants were higher than that obtained in the resistant plant of untreated seeds . Therefore, the results reported here might be of great values to the growers since many plants imporved their resistance to rust disease a long with excellent vegetative growth . In the future, in order to get a complet idea on agronomic traits of these plants, cutting from each supperior plant will be obtained for a comparison study .

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استحداث المقاومة في الجت ضد مرض الصدأ باستعمال اشعة كاما

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استلم في 15 تشرين الثاني 1985

المستخلص:

بدأ العمل ببرنامج استهدف استحداث مصدر جديد لمقاومة صدأ الجت ، فقد تم تعريض بذور الصنف المحلي الى الجرعات 0 ,50 ,100 ,150 كيلورواد من اشعة كاما وكان معدل الجرعة 45.0 راد/ثانية . وتشير النتائج الى ان حساسية النباتات للاشعة قد تأثرت تبعا لكمية الجرعة . انتخبت النباتات المقاومة للصدأ من خلال سلسلة من التلويثات الاصطناعية على نباتات الجيل الثاني والثالث . وقد برهنت النتائج لعمليات الغربلة على ان اعادة التلويث كان ضروريا للكشف عن النباتات ذات المقاومة العالية للمرض . ومن هذا فان النباتات المقاومة والمنتخبة بعد التلويث الرابع قد قاومت المسبب المرضي عند التلويث الخامس والاخير . وفي الحقل فقد اثبتت النباتات مقاومة عالية للمرض اضافة الى ان معظم النباتات قد اظهرت زيادة عالية في عدد التفرعات والوزن الخضري .