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Research Article
Impact of Dodder (*Cuscuta* spp.) Infestation and Gamma Radiation on Fahl Ecotype of the Egyptian Clover

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Abstract
Background and Objective: Dodder infestation causes a significant loss for yield and seed quality of the Egyptian clover. Therefore, the aim of this study was to measure differences between healthy Fahl plants, Fahl infested dodder and dodder at molecular level and to induce some desired mutations of Fahl ecotype for more tolerance/resistance to dodder infestation using gamma irradiation. **Material and Methods:** In the first experiment, 100 seeds of Fahl ecotype were planted with different dodder rates. The growth parameters were recorded at the harvest time. The random amplified polymorphic DNA (RAPD) analysis using ten primers was performed to investigate the relationship between Fahl healthy plants (control), infested Fahl with dodder and dodder at the molecular level. In the second experiment, series of Fahl ecotype (100 seeds each) were irradiated with gamma-ray doses of radioactive cobalt 60 (^{60}Co) and infested with different levels of dodder DSPP. The isozymes analysis was used to measure variation in Fahl treated with gamma-ray. **Results:** There were a negative correlation between level of dodder infestation and the growth parameters. The RAPD analysis showed unique markers in Fahl infested by dodder with primer OP-R03, OP-Z10, OP-B20, OP-B06, OP-A08 and OP-A012. The isozymes fractionation showed variation among irradiation doses especially for glutamic oxidase transferase, peroxidase and malate dehydrogenase. **Conclusion:** These results showed that growth parameters of Fahl ecotype has been decreased with increasing the level of dodder infestation. RAPD analysis showed some interaction between Fahl and dodder infestation. There were no significant differences among gamma ray doses used in this study. Therefore, Further investigation is needed, including different doses of gamma radiation to induce mutation in the Egyptian clover Fahl ecotype that provides more tolerance/resistance to the dodder infestation.

Key words: Egyptian clover, RAPD, Isozymes analysis, gamma-ray, growth parameter, dodder, mutation, resistance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Egyptian clover (Berseem) is a critical crop for fodder and soil fertility maintenance in Egypt and west and south Asia as well as many countries with mild winter conditions. Berseem is widely used in large scale livestock systems in the region with mild winters in Europe, the USA and Australia[1]. Parasitic weeds such as dodder suppress the growth of Egyptian clover. Dodder (*Cuscuta* spp.) is an annual parasitic weed parasitizing on Egyptian clover (*Trifolium alexandrinum* L.)[1]. Dodder seeds could maintain their viability for several years. Dodder attacks about 10-20% of the Egyptian clover area which reaches up to 1.2 million hectares infested with dodder causing significant yield losses of forage of clover as well as seed contamination infestation. A survey in Gharbia, Menofia and Fayum governments for Egyptian clover infested with dodder showed that infestation start with small spot which increase rapidly in size with time to cover most of the infested fields[2]. Meanwhile, Abd El-Wahed[3] studied the site of parasitism attachment between dodder plants coiling around clover start contacts stem at the site of the host, then penetrating and expanding reaches the vascular cylinder. In addition, the infestation started as coiling or twining of the parasite stem around host stem and host leaf forming haustoria for penetration[1,4]. The haustoria absorb nutrients from different host tissues xylem/phloem or both making connection between tracheary elements of parasites and vascular bundles of hosts[4].

Abd El-Hamid and El-Khanagry[5] surveyed 260 clover fields in Menofia governorate during May 2005 and showed that 11.1% of surveyed fields were infested with *Cuscuta planiflora* and *Cuscuta pedicellate* mixture appeared as scattered patches ranged from 0.52-22 m^2 in its size. They found that 87% of seeds samples from farmers were contaminated with the dodder seeds and about 85% of farmers were aware of the dodder problem in clover. They indicated that clover seeds are the primary source of infestation, most farmers can control dodder by moving or pulling. Furthermore, El-Nahrawy[6] investigated the performance of 100 berseem genotypes, 96 farmers' seed lots and four commercial cultivars, for resistance/tolerance to dodder infestation and found that moderate genotypes behaved differently, the parasite was scarcely able to pierce the epidermis but lignification of host pericycle and starch sheath had observed incompatible interaction between clover genotypes and types and *C. planiflora*, while the sensitive genotypes were in contrast compatible with susceptible clover accessions. The infestation developed normally with intrusive cells reaching the center cylinder and the host vascular tissues[1]. El-Refaey *et al.*[7] reported that dodder infestation has negative impact on the fresh and dry yield as well as chlorophyll content in Egyptian clover at all rates of infestation and growth stage.

Several molecular markers techniques were used to investigate the genetic diversity among different clover genotypes such as random amplified polymorphic DNA (RAPD)[8], inter-simple sequence repeats (ISSR)[8,9], sequence-related amplified polymorphism (SRAP)[10] and simple sequence repeats (SSRs)[11]. The information acquired from those studies is essential in understanding the relationships among species that may further assist in developing breeding strategies. By using these approaches, Abdel-Fatah and Bakheit[12] were able to map some QTLs for different traits and also reported that the correlation among these traits and co-location could indicate a common genetic control. Also, Zayed *et al.*[9] found that the Fahl monocut ecotype had 29 present bands, three absent bands in total of 32 bands, among those there were two unique bands by using ISSR.

Creating new genetic variation in crops by inducing mutations was expansively used in addition to classical methods. Gamma rays have been used as an economical and practical approach for the improvement of various plant species compared to other radiation methods such as laser radiation, electric field, microwave radiation and magnetic field because of its availability and the power of penetration[13]. Gamma rays have impact on plant growth and development by generally induce cytological, biochemical, physiological and genetically changes in the cells and tissues depending on the levels of irradiation[14]. It is imperative to determine the right dose to control these changes. Yassein and Aly[15] reported that low doses of gamma rays increased seed germination while high doses decrease seed germination. Akshatha *et al.*[14] attributed the effects of gamma rays on germination to the activation of RNA synthesis and increased enzymatic activation. Ibrahim[16] utilized the gamma irradiation to improve Egyptian clover seed quality, she irradiated clover seeds with gamma-ray doses (0, 50, 100, 150 and 200 Gy) of radioactive cobalt (^{60}Co) and studied the impact on seed viability. Meanwhile, she exposed, aged clover seed to gamma rays (150 Gy) and reported seed germination improvement compared with other treatments.

Finally, the aims of this study were to assess the effect of dodder infestation on growth and development of the Egyptian clover Fahl ecotype and measure differences between Fahl ecotype healthy plants, infested Fahl plants with dodder and dodder at the molecular level and to induce some desired variability for agronomic traits and molecular characteristics to measure the correlation between gamma rays doses and Fahl tolerance/resistance to dodder infestation.

MATERIALS AND METHODS

Experiment I: Effect of dodder infestation level on Fahl ecotype:

Plant material and seeds germination: Seeds of the Egyptian clover Fahl cultivar were obtained from Forage Crops Department, Field Crops Research Institute, Agriculture Research Center, Egypt. This experiment was carried out during the season 2017/ 2018 at Weed Research Laboratory greenhouse, Agricultural Research Center, Egypt and Cell Study Research Department, Field Crops Research Institute, to determine the interaction between Fahl ecotype and dodder. The Fahl seeds were sown in 3 replicates with experimental pots of diameter 50 cm and depth 50 cm following the recommended agronomical practices. Fifteen days after sowing, the pots were infested by dodder seeds at four different levels of infestation, 10, 20, 30 and 40 dodder seeds/pot (DSPP). The seeds of Fahl and dodder levels of infestation were sown in completely randomized design (CRD) experiment.

Forage yield and traits components: The growth parameters were recorded at the harvest of single cut Fahl ecotype of the Egyptian clover. The recorded parameters were fresh and dry yield of Fahl and Fahl yield was calculated. Simultaneously, plant height (cm), leaf to stem ratio (L/S), number of leaves and stem diameter (mm) were also recorded.

DNA isolation and RAPD analysis: The RAPD markers experiment was carried out to demonstrate the relationship between Fahl, dodder and infested Fahl by dodder at the molecular level. Genomic DNA was extracted from healthy plants, infested plants and dodder according to Dellaporta *et al.*[17]. To remove RNA contamination, DNA samples were treated with RNase A (10 mg mL^{-1}) and incubated at 37°C for 30 min. Concentration and the quality of the DNA in the samples were performed using NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific®, Waltham, MA, USA). The DNA samples were diluted to the concentration of 20 ng µL^{-1} in distilled water and used for further analysis or stored at -80°C. The polymerase chain reaction (PCR) for RAPD analysis was done in a reaction volume of 25 µL. The reaction contained 40 ng template DNA, 400 nM primer, 20 mM TRIS-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 nM each dNTP and 1.0-unit of *Taq* DNA polymerase (Thermo Fisher Scientific®). All primers listed in Table 1 were ordered from Operon Technologies, Alameda, CA. The PCR amplicons were separated on 1.2% agarose gels. The RAPD patterns were stained with Ethidium bromide (0.5 µg mL^{-1}), visualized with ultraviolet light, photographed and the presence or absence bands were recorded.

Experiment II: Effect of gamma irradiation on Fahl/dodder interaction: Groups of 100 seeds each of Fahl ecotype were exposed to different doses (Control, 100, 150, 200, 250 and 300 Gy) of gamma rays using a gamma cell 200 apparatus equipped with a ^{60}Co γ source with average dose rate of 0.7 Gy min^{-1} at Atomic Energy Authority, Egypt. The irradiated seeds were sown in 3 replicates into an experimental pots of diameter 50 cm and depth 50 cm following the recommended agronomical practices. Dodder seeds were added to all irradiated Fahl seeds 15 days after sown at 10, 20, 30 and 40 DSPP. The seeds were sowing in split-completely randomized design (Split-CRD) in three replicates to measure variation among irradiation doses and control and to investigate the interaction between doses of gamma ray and dodder.

Isozymes fractionation: Acid phosphatase, alkaline phosphatase, glutamic oxidase transferase, peroxidase and malate dehydrogenase were analyzed using the native polyacrylamide gel electrophoresis (native-PAGE) 10% according to Pan *et al.*[18]. Isozymes were extracted from 20 clover leaves (about 0.3 g fresh leaves samples) as described by Anderson *et al.*[19]. Leaves were homogenized in 0.01 M sodium phosphate buffer (pH 6.0) using mortar and pestle as 2 mL buffer g^{-1} fresh weight. The extract was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was kept at -20°C until use as the enzyme source. A volume of

Table 1: List of ten RAPD primers and their nucleotides sequence

Primers	Sequences (5'············3')	Primers	Sequences (5'············3')
OP-A08	GTGACGTAGG	OP-B20	GGACCCTTAC
OP-A12	TCGGCGATAG	OP-D04	TCTGGTGAGG
OP-B06	TGCTCTGCCC	OP-R03	ACACAGAGGG
OP-B07	GGTGACGCAG	OP-R09	TGAGCACGAG
OP-B10	CTGCTGGGAC	OP-Z10	CCGACAAACC

RABD: Random amplified polymorphic DNA

40 µL extract was mixed with 20 µL sucrose solutions and 10 µL of bromophenol blue and then a volume of 50 µL from this mixture were applied to each well of the gel. Electrophoretic run was performed at 10°C in a BioRad vertical electrophoresis unit (BioRad® Laboratories, Hercules, CA, USA) at 150 volt until the bromophenol blue dye reached the separating gel and then the voltage was increased to 200 volt. After electrophoresis, the gels were stained with the appropriate substrate and chemical solutions, incubated at room temperature in dark for complete staining according to Aboshosha *et al.*[20] and Soltis and Solits[21]. The gels were scanned for the isozyme bands and analyzed.

Statistical analysis: The obtained data were subjected to statistical analysis of variance (ANOVA) for CRD with three replications for each dodder infested level as well as the control (non-infested) using SPSS program version 17 (SPSS Inc. Chicago, USA) and the means were compared by the least significant differences test (LSD) at 0.05 levels. Correlation and regression analysis were performed using Microsoft Office Excel 2016.

RESULTS AND DISCUSSION

The two experiments were mainly conducted to study the dodder and its relationships to Fahl ecotype of the Egyptian clover. Fahl ecotype was chosen because of its unique characters (mono cut). The second experiment was conducted as a step to initiate a breeding program for Fahl ecotype that tolerance/resistance to the dodder and the selection as it's known occur in the following generation after first and even second generation. This study was conducted in first generation to identify the stimulation and the indicator that need to be consider in the breeding program for Fahl ecotype.

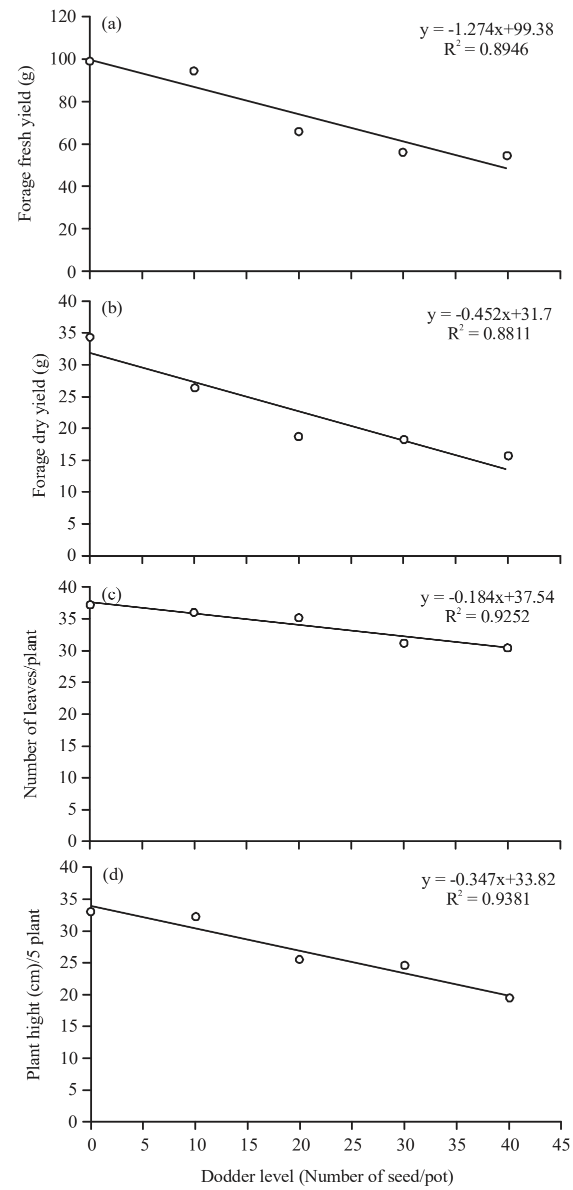

Fig. 1(a-d): (a) Correlation between dodder doses and the fresh forage yield, (b) Forage dry yield, (c) Number of leaves/plant and (d) Plant height

Effect of dodder level on Egyptian clover Fahl ecotype forage yield and traits components: The results in Table 2 and 3 and Fig. 1a indicated that the forage fresh yield was reduced from the highest yield in control (healthy) to lowest yield at the dodder infestation level of 40 DSPP. The fresh yield of Fahl ecotype was ranked as control >10 DSPP >20 DSPP >30 DSPP >40 DSPP. Similarly, dry weight differs between the control and different level of dodder infestation (Table 3 and Fig. 1b). As shown in Table 2 fresh weight was the least significant difference among dodder infestation levels. There were significant differences between the control and 20, 30 and 40 DSPP, while, there were no significant differences at infestation level of 10 DSPP. Also, there were significant differences between dry weight of Fahl ecotype and dodder infestation levels, the most significant differences were between the control and infestation levels of 20, 30, 40 DSPP (Table 2). The number of leaves/plant did not show any differences between the control and dodder levels of infestation (Table 2). The number of leaves/plant showed a slight small negative correlation with the infestation level of dodder (Fig. 1c). Weight of stem was significant differences between the control and the level of dodder

Table 2: Mean of fresh forage yield reduction and traits components at different levels of dodder infestation

Dodder	Fresh weight (g)	Dry weight (g)	Number of leaves	Weight of stem (g)	Weight of leaves (g)	Plant height (cm)	Dry weight (g)	Stem diameter (mm)
Control	99.0	34.3	37.0	16.2	16.7	32.9	5.3	2.0
10	94.4	26.3	36.0	14.6	16.0	32.2	4.8	1.0
20	65.5	18.7	35.0	13.0	15.0	25.4	4.8	1.0
30	56.2	18.3	31.0	11.3	14.0	24.5	4.6	1.0
40	54.4	15.7	30.3	10.0	13.0	19.4	4.5	0.8
LSD 0.05	20.3	7.1	8.4	1.1	1.0	3.4	0.6	0.7

Data presented is average of 5 plants, LSD: Least significant differences

Table 3: Analysis of variance of forage fresh and dry yield and their traits component

Source of variation	Degrees of freedom	Fresh weight	Dry weight	Number of leaves	Weight of stem	Weight of leaves	Plant height	Dry weight	Stem diameter
Replications	2	289.01	00.087	0.42	7.32	0.47	0.08	35.27	0.13
Dodder	4	1358.35**	27.27	18.43**	96.07**	6.57**	0.25	175.00**	0.68
Error	8	192.57	33.37	0.58	5.56	0.47	0.18	23.85	0.23
Mean		73.91	33.87	13.03	26.87	14.93	4.79	22.67	1.16
CV%		0.19	00.17	0.06	0.09	0.05	0.09	0.22	0.41

Data presented is average of 5 plants, CV: Coefficient of variation

infestation especially at 40 DSPP (Table 2). Weight of leaves was influenced by the level of dodder infestation where there was a significant difference between the control and the level of dodder infestation (Table 2). Results for plant height showed that the higher the level of dodder infestation, the lower of the plant height, where the level of infestation at 40 DSPP recorded the lowest average of plant height followed by level infestation of 30 DSPP and then 20 DSPP (Table 2 and Fig. 1d). The results of dry weight indicated in Table 2 showed that the lowest mean was at level of dodder infestation at 40 DSPP followed by 30 DSPP while the remaining levels of dodder infestation at 10 and 20 DSPP did not have any effect on the dry weight. Regards to the stem diameter, there were significant differences between the control and the levels of the dodder infestation and the lowest diameter of the stem was at level of dodder infestation of 40 DSPP, this means that the diameter of the stem is affected by all levels of dodder infestation (Table 2). It is clear from the above data that all crop traits were affected by dodder infestation. It is also evident that the higher the infestation levels of dodder, the lower the mean trait. Thus, there was an inverse relationship between the mean trait and the level of dodder infestation (Fig. 1).

The results were in agreement with Soliman *et al.*[22], who stated that *C. planiflora* led to decreased fresh and dry yield of Egyptian clover and caused a great reduction in total chlorophyll contents which was estimated by 63.3%. Moreover, Dawson[23] reported a higher reduction rate of 57% in forage yield of alfalfa. On the other hand, Soliman[24] revealed that, dodder caused a great reduction in plant height and fresh weight of berseem as well as total chlorophyll content, where seed yield losses reached to 82.9%. Parker[25] indicated that yield losses of over 50% have been recorded in alfalfa. Mean squares of reduction percentage (R%) for fresh forage yield due to dodder infestation at four level had presented in Table 3. Moreover, highly significant differences of the interaction for all traits except number of leaves, weight of stem and dry weight of the plant (Table 2 and 3) and (Fig. 1).

RAPD analysis: RAPD markers used to investigate the molecular differences between the infested and non- infested Fahl plants and also dodder using ten primers to differentiate between those plants. The primers produced multiple bands with a number of amplified DNA bands ranging from 250-2000 bp (Table 4). The number of bands was ranged from 4 bands with primer A12-14 bands with primer R03 (Table 4). The total number of the reproducible bands amplified by 10 RAPD primers reached 81 bands, from which 44 bands were polymorphic, which indicated level of polymorphism (50%) as shown in Table 5 and Fig. 2. Nine out of 10 primers were amplified bands to distinguish between Fahl, infested Fahl by dodder and dodder (Table 4). Only primer OP-B07 did not show any polymorphic among the tested material (Table 4). The highest number of unique markers was observed in Fahl infested by dodder (10 bands) followed by Fahl (9 bands) and dodder (8 bands) (Table 4 and 5). The unique bands markers were scored in Fahl infested by dodder with primer OP-R03, OP-Z10, OP-B20, OP-B06, OP-A08 and OP-A012. Thus, at the molecular level, there were differences between healthy and infested Fahl (Table 4 and 5).

The ten primers with three samples produced unique bands (fingerprint) that could be used for the evaluation of

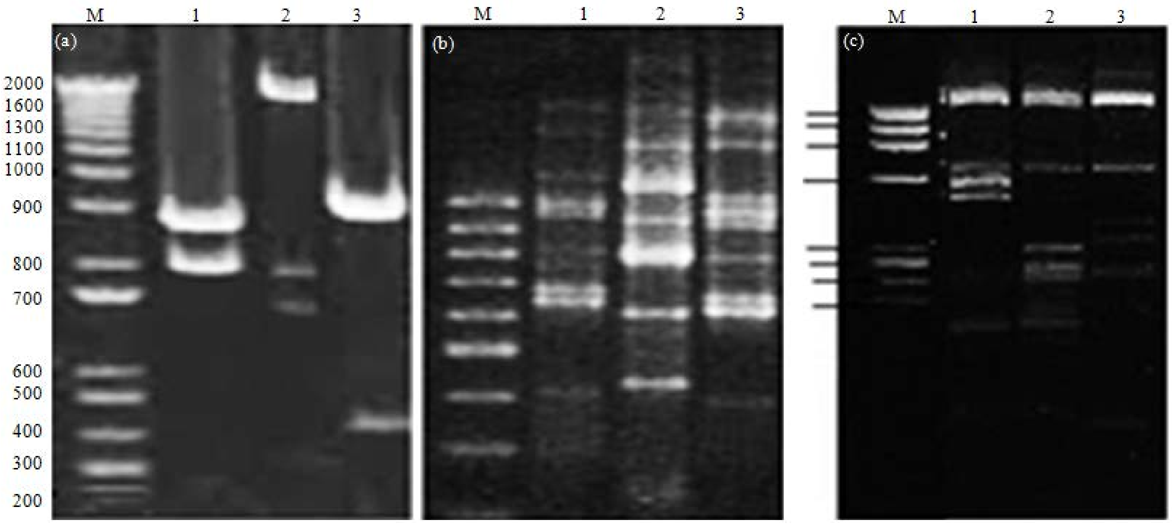

Fig. 2(a-c): RAPD analysis carried out for the Fahl ecotype of the Egyptian clover with different primers (a) OP-B20 primer, (b) OP-R3 primer and (c) OP-Z10 primer

1: Fahl, 2: Fahl infested by dodder, 3: Dodder, M: DNA1 kb ladder

Table 4: Detected amplified bands by ten RAPD primers

M (bp)	Fahl	Fahl infested with dodder	Dodder	M (bp)	Fahl	Fahl infested with dodder	Dodder	M (bp)	Fahl	Fahl infested with dodder	Dodder
R03				**Z10**				**B10**			
250	0	1	0	250	0	1	1	300	1	0	0
300	1	0	0	300	0	1	0	400	1	0	0
400	1	0	1	400	0	0	1	650	1	1	1
420	0	1	0	650	0	0	1	700	1	1	1
600	1	1	1	700	0	0	1	750	1	1	1
650	1	0	1	750	0	0	1	800	1	1	1
750	1	0	1	800	1	1	0	950	1	1	1
800	1	1	1	950	1	1	1	1000	1	1	1
950	1	1	1	1400	1	0	0	1100	1	1	1
1000	1	0	1	2000	1	1	1	1400	1	1	1
1100	0	0	1	**R09**				2000	1	1	1
1150	1	1	0	250	0	0	1	**B07**			
1400	1	1	1	300	0	1	1	650	1	1	1
2000	1	1	1	1100	1	0	1	700	1	1	1
P04				1400	1	1	1	750	1	1	1
250	1	1	1	2000	1	1	0	800	1	1	1
300	1	1	1	**B20**				950	1	1	1
400	0	1	1	300	0	0	1	1000	1	1	1
650	1	1	1	600	0	1	0	1100	1	1	1
700	1	0	0	800	1	1	0	1400	1	1	1
750	1	1	1	1000	1	0	1	2000	1	1	1
800	1	1	1	2000	0	1	0	**A08**			
950	1	1	1	**B06**				300	0	0	1
1000	1	1	1	250	0	0	1	400	1	0	0
1100	1	1	1	300	1	0	0	800	0	1	0
1400	1	1	1	400	0	1	0	950	0	1	0
2000	1	1	1	650	0	1	0	1000	1	0	1
A12				700	1	0	1				
300	1	0	0								
400	1	0	1								
650	0	1	0								
700	1	1	1								

1: Present band, 0: Absent band, RABD: Random amplified polymorphic DNA, M: Marker

Table 5: Levels of polymorphism based on RAPD analysis for Fahl, Fahl infested by dodder and dodder

Primers	Number of total bands	Polymorphic band	Monomorphic band	Polymorphism (%)	Unique bands Fahl	Fahl infested by dodder	Dodder
OP-R03	14	9	5	64.3	2	2	1
OP-P04	12	2	10	16.7	1	0	0
OP-B07	9	0	9	-	0	0	0
OP-B10	12	2	10	16.7	2	0	0
OP-Z10	10	9	1	90.0	1	1	4
OP-R09	5	4	1	80.0	0	0	1
OP-B20	5	4	1	80.0	0	2	0
OP-B06	5	5	0	100.0	1	2	1
OP-A08	5	5	0	100.0	1	2	1
OP-A12	4	3	1	75.0	1	1	0
Total	81	43	38	50.0	9	10	8

RABD: Random amplified polymorphic DNA

Table 6: Analysis of variance of yield and its traits component of irradiated Fahl ecotype with 5 doses of gamma-ray and infested with four levels of dodder

Source of variation	Degrees of freedom	Plant height	Stem diameter	Number of branches	Number of flowers	Flower weight	Flower length	Number of flowers	Weight	Seeds	Weight seeds
Blocks	2	190.3333	5.3733	0.3333	0.2133	0.0048	0.0065	40.3200	0.2512	101.1733	0.0968
Dodder	4	263.6000**	0.3467	9.5000**	115.4467**	0.2535	0.0677	511.3800**	8.1431**	6940.4133	0.2126**
Main plot error	8	61.6000	0.5067	3.2500	0.5467	0.0101	0.0234	40.3200	1.0500	90.7733	0.0977
Gamma	4	178.9667	0.0133	0.0000	2.2133	0.2112	0.3733	791.5800	0.9751	13.5133	0.0279
Gamma×Dodder	16	443.6917	0.8967	0.0000	14.8300	0.1915	0.0506	384.4050	1.1514	20.9050	0.0189
Error	40	34.4967	0.7800	0.0000	0.3467	0.0011	0.0103	2.5200	0.2844	6.7867	0.0063

Data presented is average of 3 pplants, **p = 0.065

genetic variation between and within species as well as infested Fahl by dodder (Table 4 and 5) and (Fig. 2). These results indicated that there was an interaction between dodder and Fahl based on gene expression in the host and parasite as well as the genes of both were found in the infestation statues. But on the other hand, it needs in-depth work to identify and isolate the genes which associated with the infestation to understand the infestation mechanism.

The results were in agreement with Kim *et al.*[26], who reported molecular methods to investigate genetic variation among the emerging dodder seedlings. On emergence, dodder seedlings were collected and analyzed for DNA sequence diversity in the intron, a noncoding region of chloroplast DNA. Therefore, they reported that the predominant dodder haplotype found in this study may be a close relative of *C. attenuata* and not *C. gronovii,* the common species found in cranberry bogs. Furthermore, differences in species identity between ecotypes may reflect observed differences in herbicide tolerance[4,26,27-30].

Effect of irradiation on Fahl/dodder interaction: The impact of gamma irradiation on Egyptian clover Fahl ecotype and the interaction with dodder infestation was discussed in this study. The effect of gamma radiation on the growth parameters and isozymes pattern were compared with the control plants. Analysis of variance results (Table 6) showed significant differences among the different dodder treatments for all traits except stem diameter, flowers weight, flowers length and seeds. The results are in harmony with Abd El-Hamid and Shebl[31], who investigated the efficacy of 12 weed control treatments against dodder and clover productivity as well as determine yield losses due to dodder infestation. In addition to, the hand combing for controlling dodder weed (*Cuscuta planiflora* Ten.) and their effects on some growth characters and seed yield of clover plants were investigated by Soliman[24] and found that dodder weed caused a great decrease in plant height, fresh and dry weight and seed yield of clover plants. Also, the results indicated that the hand combining treatment was not enough in dodder control but it used only as a factor in control programs. Also, Soliman[24] reported that plants infested with dodder showed the lowest chlorophyll a, b and highest carotene contents. Data also, cleared that different herbicide showed least decreased on chlorophyll a and b and increased carotene content compared to un-infested and untreated plants. Data also, revealed herbicidal treatments slightly decreased protein content of clover plants.

Table 7: Mean of yield and its component traits and 4 level of dodder infestation and 5 doses for gamma-ray, as well as the interaction between them

Doses	Plant height	Stem diameter	Number of branches	Number of flowers	Flower weight	Flower length	Number of flowers	Flower weight	Seeds	Weight seeds
Control	42.33	3.87	2.67	9.40	0.43	1.05	32.20	2.20	51.60	0.43
10	52.33	3.47	2.67	3.07	0.22	0.90	26.40	1.13	60.13	0.12
20	48.27	3.60	3.00	4.33	0.41	1.03	26.20	0.79	96.60	0.22
30	43.27	3.53	2.00	2.93	0.55	1.07	16.00	0.33	46.40	0.20
40	49.13	3.60	1.00	3.00	0.26	1.01	25.80	0.58	44.00	0.20
SE	1.61	0.22	0.19	0.16	0.01	0.03	0.77	0.17	1.18	0.04
100 Gy	50.80	3.67	2.27	4.20	0.33	1.20	29.40	1.43	60.80	0.16
150 Gy	47.13	3.60	2.27	4.60	0.25	0.76	33.60	0.80	59.00	0.24
200 Gy	44.27	3.60	2.27	4.60	0.36	1.01	14.80	1.00	58.60	0.25
250 Gy	50.13	3.60	2.27	5.13	0.33	1.09	27.00	0.82	59.80	0.25
300 Gy	43.00	3.60	2.27	4.20	0.59	0.98	21.80	0.98	60.53	0.26
SE	1.61	0.22	0.19	0.16	0.01	0.03	0.77	0.17	1.18	0.04
0×100	54.33	3.33	2.67	3.00	0.26	1.00	25.00	1.62	60.00	0.11
0×150	39.33	4.00	2.67	11.00	0.47	1.07	34.00	2.80	49.67	0.51
0×200	39.33	4.00	2.67	11.00	0.47	1.07	34.00	2.80	49.00	0.53
0×250	39.33	4.00	2.67	11.00	0.47	1.07	34.00	1.90	49.67	0.51
0×300	39.33	4.00	2.67	11.00	0.47	1.07	34.00	1.90	49.67	0.51
10×100	54.33	3.33	2.67	3.00	0.26	1.00	25.00	1.62	60.00	0.11
10×150	55.00	3.00	2.67	3.00	0.20	0.50	27.00	0.35	60.67	0.12
10×200	65.00	4.00	2.67	2.67	0.33	1.00	15.00	1.24	60.00	0.11
10×250	38.67	3.00	2.67	3.67	0.20	1.10	41.00	1.17	60.00	0.11
10×300	48.67	4.00	2.67	3.00	0.12	0.89	24.00	1.28	60.00	0.11
20×100	48.67	4.00	3.00	9.00	0.50	1.33	55.00	2.80	96.00	0.20
20×150	47.67	4.00	3.00	3.00	0.20	0.75	31.00	0.23	96.00	0.20
20×200	65.00	3.00	3.00	3.33	0.34	1.00	13.00	0.27	96.00	0.20
20×250	51.67	4.00	3.00	3.67	0.22	1.10	26.00	0.26	96.00	0.20
20×300	28.33	3.00	3.00	2.67	0.80	0.96	6.00	0.37	99.00	0.29
30×100	49.33	3.67	2.00	3.00	0.40	1.33	19.00	0.41	46.00	0.18
30×150	41.67	3.00	2.00	3.00	0.20	0.90	25.00	0.10	46.67	0.19
30×200	24.33	4.00	2.00	2.67	0.34	1.00	4.00	0.24	46.00	0.21
30×250	52.33	3.00	2.00	3.67	0.39	1.10	11.00	0.34	47.33	0.22
30×300	48.67	4.00	2.00	2.33	1.40	1.00	21.00	0.53	46.00	0.18
40×100	47.33	4.00	1.00	3.00	0.25	1.33	23.00	0.69	42.00	0.18
40×150	52.00	4.00	1.00	3.00	0.20	0.60	51.00	0.50	42.00	0.19
40×200	27.67	3.00	1.00	3.33	0.33	1.00	8.00	0.43	42.00	0.21
40×250	68.67	4.00	1.00	3.67	0.38	1.10	23.00	0.43	46.00	0.22
40×300	50.00	3.00	1.00	2.00	0.14	1.00	24.00	0.83	48.00	0.22
SE	3.61	0.49	0.42	0.36	0.03	0.06	1.71	0.37	2.63	0.08

Data presented is average of three plants, SE: Standard Error

Effect of gamma radiation on Fahl ecotype and dodder interaction: In respect to gamma ray levels, no significant differences were detected for all traits, which demonstrated that absence effect of gamma ray treatments. The results in this experiment agree with the results of other researchers[14]. In terms of the interaction between gamma ray and dodder levels of infestation, there were insignificant differences for all the traits. However, Akshatha *et al.*[14] concluded that germination, growth and development were enhanced by using low doses of radiation in *T. arjuna*.

Data in Table 7 show the effect of dodder level of infestation on the studied traits in irradiated Fahl ecotype. The mean data showed that the highest value of number of flowers, number flowers, flower weight and seeds weight was for the level of infestation at 10 DSPP. On the other side, the highest value of plant height was obtained by using infestation level of 20 DSPP. Meanwhile, the level infestation at 30 DSPP showed the highest number of branches and seeds. From the above results, the infestation level at 10 DSPP recorded the best values for most traits regardless of the irradiation does.

It is noticed from data in Table 7 that the differences among gamma ray treatment and interaction between dodder and doses for gamma ray did not reach a level of significance. This data was in agreement with Bakheit[32]. The field performance was determined in Egyptian clover by

Table 8: Detected bands of 5 isozymes in Fahl ecotype using different 5 doses of gamma-ray and control

	Fahl					
Isozymes	Control	100 Gy	150 Gy	200 Gy	250 Gy	300 Gy
Acid phosphatase						
1	1	1	1	1	1	1
2	1	1	0	0	1	0
3	1	1	1	1	1	0
4	1	1	1	1	1	1
5	1	1	1	1	1	1
6	1	1	1	1	1	1
7	1	0	1	0	1	1
8	1	0	1	0	0	0
9	0	0	1	1	1	1
10	1	1	1	0	1	1
Alkaline phosphatase						
1	1	0	1	1	1	1
2	1	0	1	1	1	1
3	1	0	1	0	0	0
4	1	1	1	1	1	1
5	1	0	1	1	1	1
6	1	1	1	1	1	1
7	1	1	1	0	0	0
Glutamic oxidase transferase						
1	1	1	1	0	0	0
2	1	1	1	1	1	1
3	1	1	1	1	1	1
4	1	1	1	1	1	1
5	1	1	1	1	1	1
Peroxidase						
1	1	0	1	1	0	0
2	1	0	1	1	0	0
3	1	1	1	1	1	1
4	1	1	1	1	1	1
Malate dehydrogenase						
1	1	0	1	0	1	1
2	1	1	1	1	1	1
3	0	1	0	1	0	0
4	1	1	1	1	1	1
5	1	1	1	1	1	1

1: Present band, 0: Absent band

different factors. Phenotypic and genotypic correlations among traits in Egyptian clover showed that mean plant height was positively correlated with each of seasonal fresh and dry forage yield, mean dry matter percentage and seasonal protein yield but negatively correlated with mean protein percentage. Genetic variance exceeded the environmental variance for all the studied traits and heritability was high for all characters studied. Further, high environmental variation indicates that the varieties exhibit environmental effect[33].

Isozymes analysis: The Isozyme analysis data in Table 8 and Fig. 3 explored the differences between gamma-ray doses to ensure the differences in the genetic material treated with different doses of gamma-ray compared to the control. The acid phosphatase explored the differences between Fahl seeds treated with 200 Gy and the control (Table 8, Fig. 3a). Furthermore, Alkaline phosphatase has demonstrated the variation between the control and the Fahl seeds treated with dose of 100 Gy which differs in four bands (Table 8, Fig. 3b). The variation between control, 250 Gy and 300 Gy were given by glutamic oxidase transferase and peroxidase (Table 8, Fig. 3c, d). Peroxidase isozymes pattern has been proved as genetic markers for resistance and susceptibility for fungal disease in sunflower[20] and for cotton leaf worm in soybean[34]. Malate dehydrogenase had shown the differences between all the treatments. Meanwhile, in malate dehydrogenase the first band was absent in 100 Gy and 200 Gy, while the 3rd band was also absent in control, 150 Gy, 250 Gy and 300 Gy (Table 8, Fig. 3e).

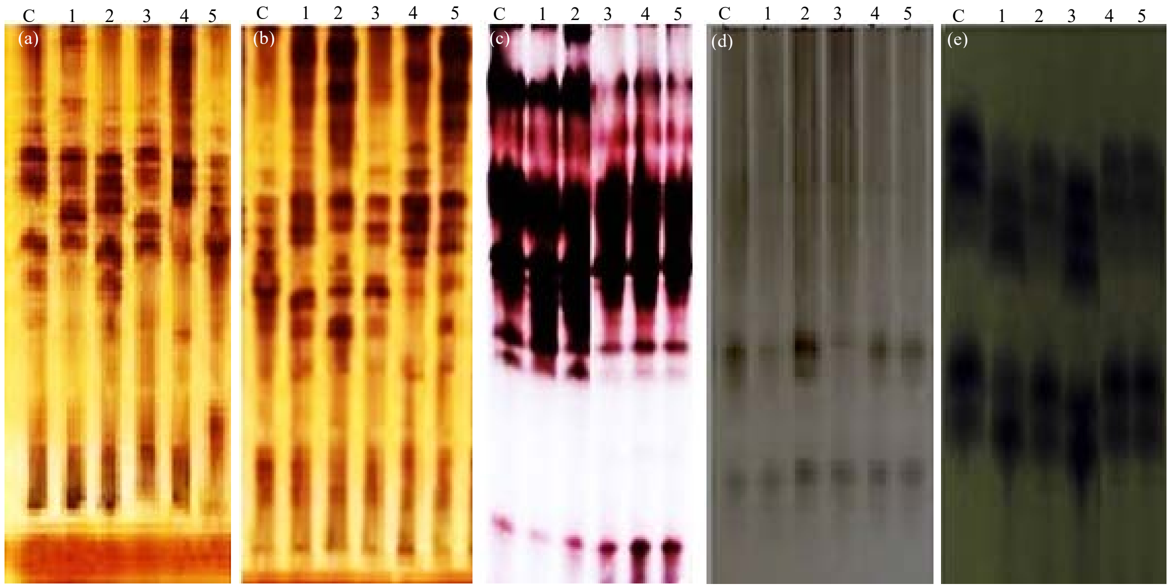

Fig. 3(a-e): Native-PAGE (10%) of isozyme polymorphism of the Fahl ecotype of Egyptian clover, (a) Acid phosphatase, (b) Alkaline phosphatase, (c) Glutamic oxidase transferase, (d) Peroxidase and (e) Malate dehydrogenase
C: Fahl control, 1: Fahl irradiated by 100 Gy, 2: 150 Gy, 3: 200 GY, 4: 250 Gy, 5: 300 Gy

Furthermore, the results in Table 8 showed that there are differences between gamma-ray doses and control. Doses of 200 and 300 Gy recorded 9 bands different from the control in all isozymes, while the dose of 100 Gy recorded 8 bands, which differ from the control in all isozymes. The dose of 250 Gy recorded only 5 bands different from the control in all isozymes, the lowest dose was 150 Gy that showed only 2 bands different from the control in all isozymes. From isozymes results, it is clear that doses 200 and 300 Gy are the most effective doses in the occurrence of physiological responses followed by a dose of 100 Gy (Table 8 and Fig. 3). These results are relatively close to Ibrahim[16], who utilized the gamma irradiation to improve Egyptian clover seed quality.

CONCLUSION

This study was aimed to investigate the relationship between the Egyptian clover Fahl ecotype and different levels of dodder infestation as well as the impact of gamma irradiation on the interaction between Fahl ecotype and dodder infestation. The obtained results showed that growth parameters of Fahl ecotype has been decreased with increasing the level of dodder infestation. RAPD analysis showed some interaction between Fahl and dodder infestation. There were no significant differences among gamma ray doses used in this study. Therefore, Further investigation is needed, including different doses of gamma radiation to induce mutation in the Egyptian clover Fahl ecotype that provides more tolerance/resistance to the dodder infestation because high level of dodder infestation affecting negatively both productivity and quality of the produced forage. Also, generating Fahl ecotype more tolerance/resistance to the dodder infestation could have a significant impact on the environment by reducing the use of herbicide to control dodder infestation.

SIGNIFICANCE STATEMENT

This study showed the relationship between the Egyptian clover Fahl ecotype yield and dodder infestation that can be beneficial for the grower to always use Fahl seeds source that is free of dodder seeds contamination and for the breeder to generate cultivars or mutation that are more tolerant or resistance to dodder infestation. This study will help the researchers to uncover the critical areas of gamma irradiation and its effect to induce more physiological responses of Fahl ecotype that still required for the industry to reduce the loss of the Egyptian clover Fahl ecotype caused by dodder infestation.

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