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Efficiency of AFLP markers to detect genetic variation in *Phthorimaea operculella* (Lepidoptera: Gelechiidae) offspring irradiated males

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: AFLP technique was used to evaluate the genetic variation among normal and partially sterilized potato tuber moth males. Mating experiments were carried out to obtain partially sterilized males and their descending offspring. Then, 316 AFLP bands were amplified using eight primer combinations of which 33.8 were polymorphic 85.5%, which varied from 68.57% to 100%. The UPGMA dendrogram generated for the AFLP data revealed that irradiated and unirradiated male samples were clustered into two groups, and the offspring of F_1 and F_2 of unirradiated parents were clustered into one group. Moreover, the progeny of F_1 and F_2 of irradiated parents clustered into three groups. No specific DNA marker could identify the irradiated males; however, there was a clear genetic variability between examined individuals. Thus, the AFLP technique could be utilized to study genetic variations among individuals of the same line. The AFLP markers could enhance the monitoring system of mass-released insects program when inherited sterility technique is applied against potato tuber moth.

1. Introduction

The potato tuber moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) is a cosmopolitan pest on potato crop, causing an annual yield reduction of 50 to 100% in some country around the world (Ahmed *et al.*, 2013). Insecticides are widely used to control this pest, but these methods have many drawbacks like high cost, nonselective and environmentally unfriendly. Moreover, insects could develop resistance to insecticides (Harba and Idris, 2018). Therefore, more environmentally friendly methods are required. The inherited sterility technique (IST) was suggested as an alternative control method to compact *P. operculella* (Makee and Saour, 1997; Larraín *et al.*, 2009). Because of no-practical methods are available to separate the adult moths by gender, the males and females are mass-reared, irradiated with low sterilizing doses of gamma radiation, then released within the targeted area (Eyidozehi *et al*., 2015). Moths irradiated with low doses live longer, stronger fliers and mate more frequently than moths irradiated with higher radiation doses

(Vreysen *et al*., 2016). However, a dose of 400 Gy induced almost 90% sterility in irradiated males while, a complete sterility in *P. operculella* females was achieved by 200 Gy dose (Makee and Saour, 2004). Furthermore, the costs of using IST program are likely to be more acceptable in terms of monetary expenditures and efficacy, as reported by Edgington and Alphey (2017), when they released dominant-lethal strain *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes. The cost-effective improvements to the IST programs are required by applying modern genetic methods (Leftwich *et al.*, 2018). RAPD, AFLP, microsatellites and ESTs are popular DNA marker systems used in insect genetic research (Singh *et al.*, 2017). They are used as monitoring systems of insects mass-release programs to improve the application of IST against insects (Oliva *et al.*, 2012; Edgington and Alphey, 2018). In this study, AFLP technique was employed to investigate the genetic variation among the offspring of partially sterilized males of *P. operculella.*

2. Materials and Methods

Inherited sterility experiment

P. operculella insects used in this study were obtained from our laboratory stock cultures. They were reared on wax coated potato slices, maintained at a constant temperature of 25±1˚C, with 70±5% relative humidity, and 12 hour light-darkness cycle as described by Makee and Saour (2004). Fifty couples of females and males were placed in 350 ml transparent plastic boxes with filter papers as an oviposition site. A 10% sucrose solution was provided as food source. Both females and males were kept together until death. The eggs were removed daily, counted, and left until hatching. From the 50 reared couples only two were chosen depending on their fecundity (number of eggs per female), and fertility (percentage egg hatch). All the newly hatched larvae of two couples choosing were reared on small-waxed potato pieces, and the pupae were collected. The couple, with most pupae, was chosen to be the first family for tracking to the F_1 and F_2 progeny. Males were divided into two groups, the first male group was used as a control (♂ N x N ♀), and the second group was irradiated with a 150 Gy in a gamma cell supplied with a Co-60 source rounded the cylindrical (15x25 cm^2) irradiation chamber (Isslcdo-vatel Gamma Irradiator, Techsnabexport Co. Ltd. USR). The average dose rate at the time of irradiation was approximately 40.12 Gy/min with a factor of homogeny (max:min dose ratio) of about 1.05 and the absorbed dose was calibrated with Fricke solution. During this treatment, adult females were kept individually in small plastic tubes inside the irradiation source. The second males group was individually mated with normal virgin females (♂ T x N ♀). All F_1 and F_2 generations were reared on small waxed potato pieces as mentioned above. Fecundity and fertility of the F_1 and F_2 generation were recorded. Adult male parents were kept for DNA extraction and AFLP analysis.

DNA extraction and AFLP analysis

Six DNA isolation protocols of *P. operculella* males from adult stage were used to obtain a good quality and quantity of DNA for AFLP analysis (M1: Beye and Raeder, 1993; M2: Blanchetot, 1991; M3: Favia *et al*., 1994; M4: Harrison *et al*., 1987; M5: Marchant, 1988; M6: Moeller *et al*., 1992) (Reineke *et al*., 1998). The M5-modified protocol was the most appropriate to produce a high quality and quantity of DNA from one adult moth. From each adult moth of 4-5 mg, an 8 to 12 µg pure genomic DNA was obtained.

The AFLP protocol was carried out as reported by Shoaib *et al*. (2008). DNA from all samples was digested with *Eco*R1 and *Mse*I restriction enzymes (0.125 U/µl). Selective amplification reactions were performed using eight primer combinations and the amplified fragments were separated by gel electrophoresis. The sequences of eight primers combinations and adapters used in this study are presented in Table 1. AFLP data analysis for each primer pair, the numbers of polymorphic and monomorphic bands were determined. Each gel from the AFLP experiments was scored as presence (1) or absence (0) of a specific band for every sample. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. Allelic polymorphic information content (PIC) was calculated using the formula of Botstein *et al*. (1980). Data for all the 8 primer combinations were used to estimate the genetic distances among analyzed individuals on the basis of the number of shared amplification products by using the Nei and Li, (1979) method. A dendrogram was generated using the Unweighted pair group of arithmetic means (UPGMA) by Statsoft program (2003).

3. Results

The data revealed that the first and the second

Table 1 - Sequences of oligonucleotide adapters and primers used in the pre amplification step and the selective AFLP primers combinations

Name	Reaction	Code	Sequence
EcoRI adapter	Ligation		5¢-AATTGGTACGCAGTCTAC3¢
			3¢- CCATGCGTCAGATGCTC-5¢
MseI adapter	Ligation		5¢-TACTCAGGACTCAT-3¢
			3¢-GAGTCCTGAGTAGCAG-5¢
EcoRI	Preamplification	E	5¢-GACTGCGTACCAATTC3¢
MseI		M	5¢-GATGAGTCCTGAGTAA3¢
EcoRI +A	Selective amplification	E-A	5¢-GACTGCGTACCAATTCA-3¢
EcoRI +G		E-G	5¢-GACTGCGTACCAATTCG-3¢
EcoRI+ C		E-C	5¢-GACTGCGTACCAATTCC-3¢
EcoRI+ T		E-T	5¢-GACTGCGTACCAATTCT-3¢
MseI + C		M-C	5¢-GATGAGTCCTGAGTAAC-3'
MseI + T		M-T	5¢-GATGAGTCCTGAGTAAT-3'
MseI + A		M-A	5¢-GATGAGTCCTGAGTAAA-3'
MseI + G		M-G	5¢-GATGAGTCCTGAGTAAG-3'

* Families selected for AFLP analysis.

couples were the best. The fecundity and fertility of the two couples were (111/103) and (95/88) (total eggs/ hatched eggs), respectively (Table 2). The first couple (89 pupae, no. of males and females 37 ♂/ 35 ♀) was selected to be the first family. Table 2, 3 show the F_1 and F_2 generations of irradiated and unirradiated males that resulted from seventeen males of this family, which were irradiated with 150 Gy dose and seven males were kept as a control. Table 2, 3 show the families of irradiated (T) and unirradiated (N) males which were selected based on the fecundity and fertility of F_1 and F_2 generations, and presenting in a marker (*). All purified genomic DNA of *P. operculella* samples submitted to AFLP analysis (Table 4). Eight primer pairs successfully amplified DNA fragments from the genomic of 17 samples. However, 316 fragments were scored with an average of 85.5% polymorphic bands per primer combination. The percentage of polymorphism detected by individual primer combination ranged from 68.57% for E-AAG/ M-CTA primer combination to 100% for E-AAC / M-CTG primer combination (Table 5). The ratio of number of fragments produced by primer pairs were 39.5.

The UPGMA dendrogram generated for the AFLP data shows that irradiated and unirradiated males samples were clustered into two groups. Hence, the offspring of F_1, and F_2 of unirradiated parent clustered into one group. While, the progeny of F_1 and F_2 of irradiated parent clustered into three groups. The first group include female parent, the second include the male parent, and the third one include all F_2 progeny that were produced from irradiated male parents (Fig. 1).

Table 2 - Inherited sterility technique experiments and the families of F_1 generations selected for AFLP analysis

Tow couples were chosen from 50			
No. of families	No. of eggs	Eggs hatching	No. of pupea
*1	111	103	89
2	95	88	77
Irradiated F_1 males (♂ N/ ♀ N)			
No. of families	No. of eggs	Eggs hatching	No. of ♂\♀
1	41	25	1\6
2	Death	-	-
3	4	0	0/0
4	29	14	2\1
5	11	2	1\1\
6	48	37	8\1
7	Death	-	-
8	6	3	1\1
9	48	20	5\1
10	38	26	14\1
11	3	3	2\1
12	204	140	45\12
*13	131	70	22\8
14	25	4	2\1
15	5	3	0\1
*16	206	146	61\17
*17	45	14	6\2
Unirradiated F_1 males (♂ N/ ♀ N)			
*18	127	67	8\11
19	Death	-	-
20	34	18	2\5
21	26	19	0\0
22	23	5	0\0
*23	138	92	38\47
*24	153	153	22\18

* Families selected for AFLP analysis.

Table 3 - F_1 progressed studied families and the families of F_2 generations selected for AFLP analysis

The F_1 progressed studied families		
No. of F_1 families	No. of couples studies	No. of couples sustained
12	6	1
13	1	0
*16	8	6
18	6	4
*23	11	8
24	5	2
Irradiated F_2 males		
No. of F_1 families	No. of cross	No. of eggs	Eggs hatching
*16	2	2	0
	3	52	0
	4	7	0
	5	7	0
	7	7	0
	8	3	0
12	6	23	0
Unirradiated F_2 males			
No. of F_1 families	No. of cross	No. of eggs	Eggs hatching
18	1	17	12
	2	11	45
	3	1	0
	6	8	0
*23	1	23	1
	2	35	29
	3	44	35
	4	28	11
	7	153	63
	8	8	7
	9	166	65
	11	58	27
24	3	80	37
	5	121	67

* Families selected for AFLP analysis.

Table 4 - DNA samples for AFLP analysis

Extraction from	No. of Samples
Female	1
Male	2
Irradiated males of F_1	3-apr
Unirradiated males of F_1	5-giu
Mix samples of DNA 8-9-11-12	7
Unirradiated males of F_2	8-9-11-12
Mix samples of DNA 14-15-16-17-18	13
Irradiated males of F2	14-15-16-17-18

Table 5 - Percent polymorphism, band numbers and polymorphic bands produced by eight primer combinations

No.	Primers combination	Total No. of bands	Polymorphic bands	Polymorphism %
1	E-ACT x M-CTG	49	46	93.87
2	E-AAG x M-CTA	35	24	68.57
3	E-ACG x M-CAC	51	42	82.35
4	E-ACG x M-CTA	47	42	89.36
5	E-ACA x M-CAT	45	36	80
6	E-AAC x M-CAC	41	34	82.92
7	E-AGG x M-CTC	24	23	95.83
8	E-AAC x M-CTG	24	24	100
Total		316	271	
Average		39.5	33.8	85.5

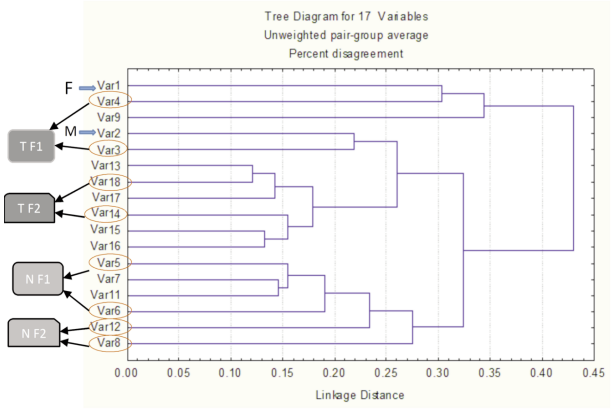

Fig. 1 - UPGMA dendrogram showing genetic relationships among 17 DNA samples of unirradiation and irradiation of P. operculella. Samples are: 1. Female, 2. Male, 3-4. F1 irradiated males, 5-6. F1 unirradiated males, 8-12. F2 unirradiated males, 7. Mix DNA samples of F2 irradiated males 8-12, 18-14, 13. Mix DNA samples of F2 irradiated males 14-18.

4. Discussion and Conclusions

Potato tuber moth, like most of Lepidoptera moths, when exposed to substerilizing doses of gamma rays undergo several physiological, biochemical and genetic changes (Makee and Saour, 2004; Hallman *et al.*, 2013, Sachdev *et al.*, 2017). However, some of the DNA damages due to irradiated male parents are inherited by their progeny (Steinitz *et al.*, 2015). Although, inherited sterility did not occur in *P. operculella* females but infertility of irradiated males and females is irreversible (Makee and Saour, 1997, 1999; Idris *et al.*, 2019). Thus, the sterility in F_1 progeny was more than in its irradiated male parents when IST applied against *P. operculella* (Makee and Saour,

2004). However, the majority of the inherited deleterious effects are expressed in the F_1 generation (Saour, 2014). The potential use of the AFLP technique to discriminate irradiated offspring of partially sterilize males of *P. opercullella* from the unirradiated was the aim of this investigation. Thus, the AFLP-technique using as fingerprinting tools to determine the genetic population structure of potato tuber moth (Medina *et al.*, 2010). Our AFLP data that were obtained from this study shown that no specific DNA marker could distinguish irradiated males from the unirradiated ones, but there was a clear DNA polymorphism between in F_1 and F_2 generations of partially sterilized males of irradiated and unirradiated male parents. Induced DNA damage could have significantly begun at 20 Gy and higher doses as reported by Hambarde *et al*., 2013 on Sf9 Lepidoptera cells. Consequently, it is known, that DNA damages caused by irradiated males at 150 Gy are irreversible and randomly inherited to their offspring (Makee and Saour, 2004; Vreysen *et al.*, 2016). Thus, the DNA damages inherited randomly in F_1 and F_2 generation are not stable when the males exposed to the partially sterility irradiation doses (Sauor, 2014; Kheirallah *et al.*, 2017). Based on these facts, we suggest that DNA changes in F_1 and F_2 generations between irradiated and unirradiated were adequate to be detected by AFLP technique. Additionally, the high percentage of polymorphism between male samples of irradiated and unirradiated reflected the vast diversity genetic level in *P. operculella* males due to a gamma radiation applied doses.

In conclusion, the AFLP-technique revealed to be powerful for studying genetic variation between incest species or between individuals of the same line, which have biological differences induced by several factors such as irradiation. Thus, using AFLP technique in tracking the genetic variation in offspring of partially sterilized males may enhance the effectiveness of the monitoring system in mass-released insects programs when, IST applied against potato tuber moth.

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References

AHMED A.A.I., HASHEM M.Y., MOHAMED S.M., KHALIL S.S., 2013 - *Protection of potato crop against* Phthorimaea operculella *(Zeller) infestation using frass extract of two noctuid insect pests under laboratory and storage simulation conditions*. - Arch. Phytopathology Plant Protect., 46(20): 2409-2419.

BEYE M., RAEDER U., 1993 - *Rapid DNA preparation from bees and %GC fractionation.* - BioTechniques, 14(3): 372-374.

BLANCHETOT A., 1991 - *Genetic variability of a satellite sequence in the dipteran* Musca domestica*.* - EXS, 58: 106-112.

BOTSTEIN D., WHITE R.L., SKOLNICK M., DAVIS R.W., 1980 - *Construction of a genetic linkage map in man using restriction fragment length polymorphisms*. - Am. J. Hum. Gen., 32(3): 314-331.

EDGINGTON M.P., ALPHEY L.S., 2017 - *Conditions for success of engineered underdominance gene drive systems.* - J. Theor. Biol., 430: 128-140.

EDGINGTON M.P., ALPHEY L.S., 2018 - *Modeling the mutation and reversal of engineered underdominance gene drives*. - bioRxiv., 257253: 1-14.

EYIDOZEHI K., FANOOJ M.A.H., MOKHTARI A., 2015 - *The sterile insect technique and inherited sterility in Lepidoptera*. - Biol. Forum., 7(1): 1871-1874.

FAVIA G., DIMOPOULOS G., LOUIS C., 1994 - *Analysis of the* Anopheles gambiae *genome using RAPD markers.* - Insect Mol. Biol., 3(3):149-157.

HALLMAN G.J., ARTHUR V., BLACKBURN C.M., PARKER A.G., 2013 - *The case for a generic phytosanitary irradiation dose of 250 Gy for* Lepidoptera *eggs and larvae*. - Radiat. Phys. Chem., 89: 70-75.

HAMBARDE S., SINGH V., CHANDNA S., 2013 - *Evidence for involvement of cytosolic thioredoxin peroxidase in the excessive resistance of Sf9 Lepidopteran insect cells against radiation-induced apoptosis*. - PLoS One, 8(3): 58261.

HARBA M., IDRIS I., 2018 - *The effect of host density and viability on superparasitism behavior of* Trichogramma cacoeciae *and* T. principium *females*. - Agric. Forestry Fish., 7(1): 11-18.

HARRISON R.G., RAND D.M., WHEELER W.C., 1987 - *Mitochondrial DNA variation in field crickets across a narrow hybrid zone*. - Mol. Biol. Evol., 4: 144-158.

IDRIS I., HUSSIAN K., ALALI N., IHKTIAR A., 2019 - *Irreversible fertility of irradiated* Phthorimaea operculella *(Lep., Gelechiidae) females*. - J. Agri. Sci. Technol., (In press).

KHEIRALLAH D., EL-SAMAD L., FAHMI N., OSMAN, W., 2017 - *Ultrastructure alterations induced by gamma irradiation in spermiogenesis of the ground beetle,* Blaps sulcata*: reference to environmental radiation protection*. - Environ. Sci. Pollut. Res. Int., 24(27): 22102-22110.

LARRAÍN S.P., GUILLON M., KALAZICH J., GRAÑA F.,

VÁSQUEZ C., 2009 - *Effect of pheromone trap density on mass trapping of male potato tuber moth* Phthorimaea operculella *(Zeller) (Lepidoptera: Gelechiidae), and level of damage on potato tubers*. - Chilean. J. Agri. Res., 69(2): 281-285.

LEFTWICH P.T., EDGINGTON M.P., HARVEY-SAMUEL T., PALADINO L.Z.C., NORMAN V.C., ALPHEY L., 2018 - *Recent advances in threshold-dependent gene drives for mosquitoes*. - Biochem. Soc. Trans., 46(5): 1203-1212.

MAKEE H., SAOUR G., 1997 - *Inherited effects in F_1 progeny of partially sterile male* Phthorimaea operculella *(Lep., Gelechiidae)*. - J. Econ. Entomol., 90: 1097-1101.

MAKEE H., SAOUR G., 1999 - *Nonrecovery of fertility in partially sterile male* Phthorimaea operculella *(Lep., Gelechiidae)*. - J. Econ. Entomol., 92: 516-520.

MAKEE H., SAOUR G., 2004 - *Efficiency of inherited sterility technique against* Phthorimaea operculella *Zeller (Lep., Gelechiidae) as affected by irradiation of females*. - J. Vege. Crop. Prod., 10: 11-22.

MARCHANT A.D., 1988 - *Apparent introgression of mitochondrial DNA across a narrow hybrid zone in the* Caledia captiva *species-complex.* - Heredity, 60: 39-46.

MEDINA R.F., RONDON S.I., REYNA S.M., DICKEY A.M., 2010 - *Population structure of* Phthorimaea operculella *(Lep., Gelechiidae) in the United States*. - Environ. Entomol., 39: 1037-1042.

MÖLLER E.M., BAHNWEG., SANDERMANN H., GEIGER H.H., 1992 - *A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues.* - Nucleic Acids Res., 20(22): 6115-6116.

NEI M., LI W.H., 1979 - *Mathematical model for studying genetic variation in terms of restriction endonucleases*. - Proc. Nat. Aca. Sci., 76: 5269-5273.

OLIVA C.F., JACQUET M., GILLES J., LEMPERIERE G., MAQUART P.O., QUILICI S., SCHOONEMAN F., VREYSEN M.J., BOYER S., 2012 - *The sterile insect technique for controlling populations of* Aedes albopictus *(Diptera: Culicidae) on Reunion Island: mating vigour of sterilized males*. - PLoS One, 7(11): 1-8.

REINEKE A., KARLOVSKY P., ZEBITZ C.P.W., 1998 - *Preparation and purification of DNA from insects for AFLP analysis*. - Insect Mol. Biol., 7(1) : 95-99.

SACHDEV B., KHAN Z., ZARIN M., MALHOTRA P., SETH R.K., BHATNAGAR R.K., 2017 - *Irradiation influence on the phenoloxidase pathway and an anti-oxidant defense mechanism in* Spodoptera litura *(Lepidoptera: Noctuidae) and its implication in radio-genetic 'F_1 sterility' and biorational pest suppression tactics*. - Bull. Entomol. Res., 107(3): 281-293.

SAOUR G., 2014 - *Sterile insect technique and F1 sterility in the European grapevine moth,* Lobesia botrana. - J. Insect. Sci., 14(8): 1-10.

SHOAIB A., JAWHAR M., ARABI M., 2008 - *AFLP fingerprinting of old, modern and landraces of durum wheat (*Triticum turgidum *var.* durum *Desf.) in Syria*. - Cereal Res. Commun., 36(3): 387-395.

SINGH S., MISHRA V.K., BHOI T.K., 2017 - *Insect molecular markers and its utility-a review*. - Int. J. Agric. Environ. Biotechnol., 10(4): 469-479.

STATSOFT, 2003 - *STATISTICA. Data analysis software system, version 6*. - Statsoft Inc.

STEINITZ H., SADEH A., KLIOT A., HARARI A., 2015 - *Effects of radiation on inherited sterility in the European grapevine moth (*Lobesia botrana). - Pest. Manag. Sci., 71(1): 24-31.

VREYSEN M.J., KLASSEN W., CARPENTER J.E., 2016 - *Overview of technological advances toward greater efficiency and efficacy in sterile insect-inherited sterility programs against moth pests*. - Fla. Entomol., 99(1): 1-12.