Genetic markers for cultivar identification in potato in relation to insect tolerance as reveald by RAPD analysis

(Received: 11.05.2006; Accepted: 5.06.2006)

Reda E. A. Moghaieb* and Sanaa A. M. Ibrahim**
*Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt
**Plant Protection Institute, Agriculture Research Center, Giza, Egypt

ABSTRACT

In Egypt losses of agricultural products by mole cricket are abundant. However the genetic information on tolerance to mole cricket is limited.Therefore, in the present study the yield of four potato cultivars (Alfa, Scort, Desrea and Diamond) were compared under natural mole cricket infestation. The tuber yield decreased by this insect infestation, and the reduction differed significanly among cultivars. The cultivar Alfa prooved to be the one of choice under the soil that has infection with mole cricket. The infestation of potato by mole cricket reduced the carbohydrate concentration in the infected compared to the non-infected tubers. For RAPD analysis, seven arbitrary 10-mer primers were used. A total of 62 scoreable bands was detected of which 30 bands were polymorphic(48.4 %). Based on the data obtained by RAPD analysis, it was possible to discriminate between the four potato genotypes used in the present study. The genotype–specific marker indicates that six markers distinguish the cultivar Alfa (OPB-09-800, OPB-09-650, OPD-04-1970, OPD-04-475, OPD-04-317,OPE-13-890), three markers distinguish Diamond (OPA-06-1250, OPD-04-1000,OPD-04-600) one marker for Scort (OPD-04-580) and one marker for Desrea (OPA-15-2190). The cultivar-specific markers represented 17.7% of the total markers and 36.6 % of the polymorphic markers. These markers can help in marker-assisted selection breeding programs for improving mole cricket tolerance in potato plants.

Key words: *Potato cultivars, mole cricket, RAPD.*

INTRODUCTION

The cultivated potato *Solanum tuberosum* sub spp. *tuberosum* is one of the most important world food crops. It is nutritious, highly productive, and is an effective cash crop. It is well known that the appearance of fresh fruits and vegetables is a primary criterion in making purchasing decisions. According to Kays (1999), the product appearance is characterized by size, shape, color, condition and absence of defects. The infestation of potato fields by several soil insects including mole-cricket causes remarkable damage in plant yield and quality due to insect feeding on the tubers, leading to reducing its economic value.

Losses due to pests and diseases have been estimated at 37% of the agricultural production world-wide, with 13% due to insects (Gatehouse *et al.*, 1992). Mole cricket, *Gryllotalpa* spp. is a subterranean insect and constitutes one of the largest economic important pests. They cause tremendous damage to seedlings and tuber crops every year by direct feeding on roots (Walker, 1985).

DNA fingerprinting using molecular markers is a tool for plant cultivar identification used to assist in the application of Plant Breeders' Rights (Morell *et al.*, 1995). Molecular markers have also been used to estimate genetic diversity and examine genetic relationships between cultivars in a range of horticultural crops (Bradley *et al.*, 1996; Graham *et al.*, 1996). Random amplified polymorphic DNA (RAPD) analysis (Welsh and McClelland, 1990; Williams *et al.*, 1990) has previously been used in genetic studies of potato, for the differentiation and identification of cultivars and clonal variants (Sosinski and Douches 1996; Ford and Taylor, 1997) and somatic hybrids (Baird *et al.*, 1992; Rasmussen and Rasmussen, 1995), and in the assessment of genetic diversity and relationships of cultivated and wild potato species (Demeke *et al.*, 1996; del Rio *et al.*, 1997 Paz and Veilleux, 1997). RAPD analysis is a fast, simple, cost-effective method and can give high resolution for DNA fingerprinting and genetic relationship studies in potato and other crops.

The present study was carried out to compare the degree of tolerance of four potato cultivars to high infection by mole-cricket in terms of changes in tuber production and carbohydrate concentration. The genetic variation between the potato cultivars used was resolved using the random amplified polymorphic DNA (RAPD) markers, and thereby the genotype-specific markers for each cultivar were determined.

MATERIALS AND METHODS

Four potato cultivars namely, Alfa, Scort, Desrea and Diamond were grown under natural infestation with mole-cricket at Behiera Governorate, Egypt during late summer plantation of potato (Augst-December, 2005).

The following amount of fertilizers (130 kg ha^{-1} N and 150 kg ha^{-1} P_2O_5 and 180 kg ha^{-1} K_2O) were applied uniformely at a basal dressing to the field. The experimental design used was a randomized complete block design with four replications. Each plot consisted of 3 rows spaced 90 cm apart with 60 cm between hills. One potato tuber was planted per hill. Fifteen plants were grown per plot. Data on tuber yield, number and weight of infested tubers and non-infested tubers, infestation percentage in each cultivar were scored from 2000 tubers harvested randomly from each cultivar. The data were statistically analyzed according to Snedecor (1956).

The differences in carbohydrate concentration of the intact and infected tubers from each cultivar were determined according to the methods described in A.O.A.C., (1980).

DNA extraction and random amplified polymorphic DNA (RAPD) analysis

Total genomic DNA was isolated using the method descried in Rogers and Bendich (1985). PCR reactions were conducted using arbitrary 10-mer primers (Operon Technology, Inc., Alameda, CA, USA). The names and sequences of the primers that gave reproducible bands are listed as fellows:

Primer code	primer sequence
OPA-06	GGTCCCTGAC
OPA-15	TTCCGAACCC
OPB-09	TGGGGGACTC
OPD-04	TCTGGTGAGG
OPD-07	TTGGCACGGG
OPE-07	AGATGCAGCC
OPE-13	CCCGATTCGG

The reaction conditions were optimized and mixtures (25 µl total volume) were composed of DNA (50 ng), 1x reaction buffer (Promega), 1 unit Tag DNA polymerase (M1861, Promega), 3 mM $MgCl_2$, 0.2 mM of each dNTP, and 20 pM primer. Amplification was carried out in a PC708 Program Temp. Control System (ASTEC, Japan) programmed for 45 cycles as follows, 94 °C/ 4 min (1 cycle). 94°C /1min., 35°C / 1 min, 72°C / 2 min followed by one additional cycle at 72°C /6 min. The amplification products were separated in 1% (w/v) agarose gel in 1xTBE buffer and visualized by staining with ethidum bromide. Reproducibility of DNA profiles was determined by replicating all RAPD reactions for three times. Variations among potato genotypes across the primers used in the present study were evaluated from pairwise comparison for the proportion of shared bands amplified (Nei, 1987).

RESULTS AND DISCUSSION

The Egyptian farmers used to grow potato at three plantations, early summer, late summer and in winter. Potato planted during late summer (Nili) plantation has been suffered more severely from mole-cricket infestation compared with the early summer and winter plantations Hemeida *et al*. (1994).

In the present study, four potato genotypes were compared for their tolerance to mole-cricket infestation in terms of changes in yield quantity and quality. The yield of the cultivar Alfa was higher than the others, followed by Desrea, then Scort and the least in Diamond (Table 1).

The infestation percentage was the highest in the cultivar Diamond, followed by Scort then by Deserea and least in Alfa (Table 1). The reduction in average tuber weight by infestation was the highest in the cultivar Diamond (48%), followed by Scort (27.8%), then by Desrea (19.4%) and least in Alfa (16.6%) (Table 1). These data indicate that the tolerance in the cultivar Alfa is not due to the reduction of tuber weight but to the reduction of frequency of tubers infested by mole cricket. This is probably due to the production of glycoside compounds as previouly reported by Shehata (1986). These results are consistent with Ibrahim (1993) and Hemeida *et al*. (1994).

The infestation of potato by mole-cricket not only adversely affected the yield but also reduced the tuber quality. The data indicated that the cultivars differed significantly for the yield per plot (Table 2).

The results of this study reflects the differential response of cultivars to mole-cricket infestation, suggesting that the cultivar Alfa is more tolerant and the cultivar Diamond is highly susceptible to mole-cricket infestation. This result is consistent with the findings reported by Matsuura and Echizaki, 1985 and Ibrahim (1993) in which the cultivar Alfa was found tolerant to mole-cricket infestation. Shehata (1986) reported that the tubers of Alfa contain more glycoside compounds, which are not favorable for mole-cricket, therefore the insects do not prefer to attack the tubers from this cultivar. The carbohydrate concentration in the infected tubers was decreased compared to the non-infected tubers (Fig. 1).

Table (1):The effect of mole-cricket on tuber weight and infestation % in four potato cultivars under natural infestation.

Cultivars	Healthy tuber number	Infected tuber number	Infestation %	Healthy tuber weight (kg)	Infected tuber weight (kg)	Reduction% in tuber weight
Alfa	1680	320	16	300	50	16.6
Desrea	1500	500	25	180	35	19.4
Scort	1200	800	40	215	70	27.8
Diamond	1050	950	48	250	120	48.0

Table (2):Analysis of variance of yield / plot of four potato cultivars under natural infestation by mole-cricket.

S.V	d.f	S. S	M.S	F-cal	F 0.05
Replications	3	6	2	0.9	3.85
Cultivars	3	45	15	6.73*	
Error	9	20	1.2		
Total	15	71			

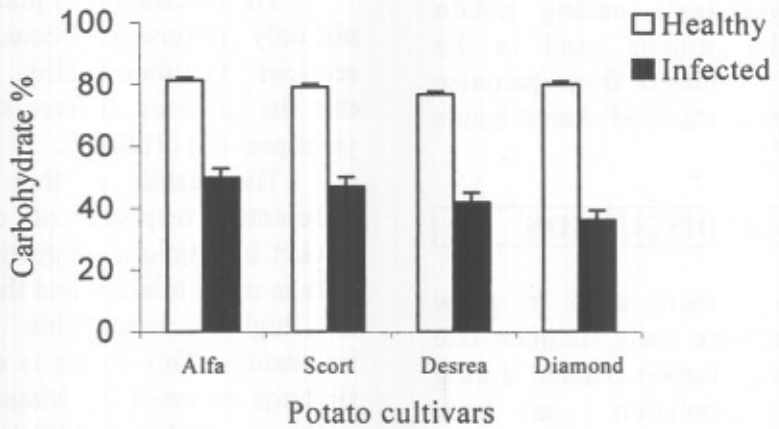

Fig. (1): Total carbohydrate % of the infected and intact potato tubers from four potato cultivars grown under natural infestation by mole-cricket (The bars indicate the ±SE).

In the present study, in order to investigate the genetic differences between the cultivars used, the random amplified polymorphic DNA (RAPD) analysis was performed. All primers used in the present study resulted in the appearance of PCR products with a variable number of bands. A total of 62 DNA markers was detected among the four potato cultivars, 30 bands out of them were polymorphic (48.4%) and can be considered as useful RAPD markers for the four potato cultivars used in the present study. The largest number of RAPD bands was detected for primers OPD-04 (14 band) and OPE-07 (11 bands), while the lowest was scored for OPA-06 (6 bands) (Fig. 2 and Table 3). The data presented in Table (4) show the polymorphic bands generated from each primer.

Table (3): Primers used in RAPD analysis and their number of bands and size range.

Primer code	No. of scorable bands (bp)	Size range of scorable bands (bp)	No. of Polymorphic bands
OPA-06	6	690-1490	3
OPA-15	9	790-2190	7
OPB-09	8	650-2200	4
OPD-04	14	317-1970	9
OPD-07	7	229-1400	2
OPE-07	11	270-1917	1
OPE-13	7	785-2100	4
Total	62		30
Polymorphism %			48.4 %

Similarity indeces for any pair of cultivars used in the present study are shown in Table (5). The specific RAPD markers for the different potato cultivars used are listed in Table (6). Eleven out of the 62 generated RAPD markers were found to be genotype-specific (17.7 %). The largest numbers of RAPD specific markers were scored for Alfa (6 markers), then Diamond (three markers) while Scort and Desrea (one marker each). In the meantime, the largest number of RAPD genotype-specific markers was generated for primers OPD-04 (six markers) and OPB-09 (two marker). From these results, one may conclude that these RAPD-bands can be considered as genetic markers for detecting mole-cricket tolerance in the potato genotypes.

Considering all the results, it is concluded that the infection of potato cultivars by mole-cricket reduces the tuber yield and quality. The differences in tuber yield between cultivars is expected due to the difference in the genetic background between them. The cultivar Alfa prooved to be the one of choice under the soil that has infection with mole cricket.

Based on the data obtained by RAPD analysis, it was possible to discriminate between the four potato genotypes used in the present study. The genotype–specific markers indicate that eleven markers distinguish the cultivar Alfa, Diamond, Scort and Desrea respectively (Table 6).These bands can be verified as being RAPD markers associated with mole-cricket tolerance in the potato genotypes. Subsequent experiments need to be achieved to determine the linkage between these RAPD markers and gene(s) for pest tolerance in the potato cultivars used in the present study.

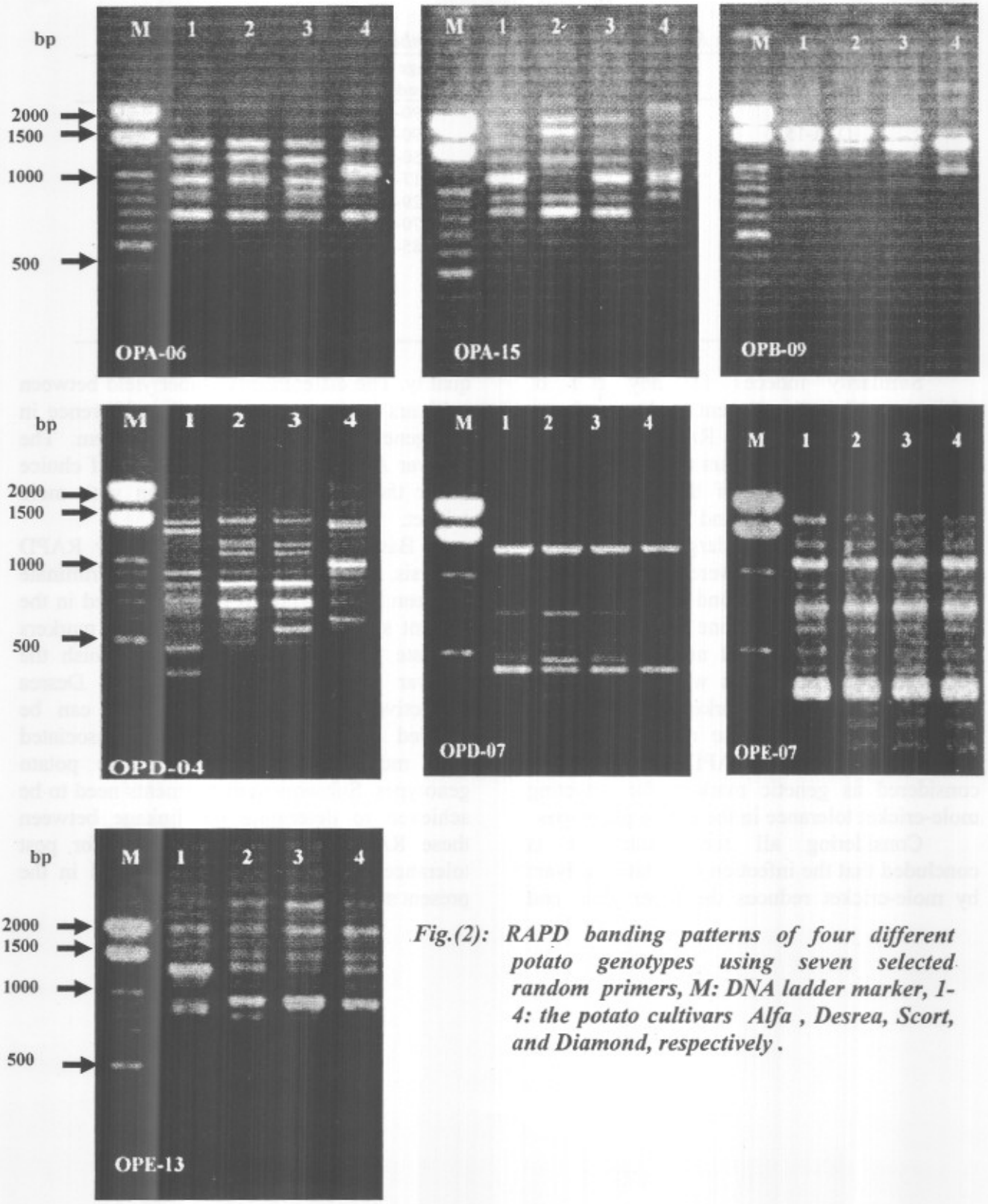

Fig.(2): RAPD banding patterns of four different potato genotypes using seven selected random primers, M: DNA ladder marker, 1-4: the potato cultivars Alfa , Desrea, Scort, and Diamond, respectively .

Table (4): RAPD polymorphic bands generated by seven different primers in four different potato genotypes.

OPA-06					OPA-15				
M	Alfa	Desrea	Scort	Diamond	M	Alfa	Desrea	Scort	Diamond
1300	+	+	+	-	2190	-	+	-	-
1250	-	-	-	+	2100	+	-	-	+
970	+	+	+	-	2000	+	+	+	-
					1980	+	+	-	+
					1670	+	-	+	-
					1220	+	-	+	+
					790	+	+	+	-

OPB-09					OPD-04				
M	Alfa	Desrea	Scort	Diamond	M	Alfa	Desrea	Scort	Diamond
1000	+	-	+	+	1970	+	-	-	-
900	+	-	+	-	1710	-	+	+	-
800	+	-	-	-	1385	-	+	+	-
650	+	-	-	-	1315	-	+	+	-
					1000	-	-	-	+
					600	-	-	-	+
					580	-	-	+	-
					475	+	-	-	-
					17	+	-	-	-

OPD-07					OPE-07				
M	Alfa	Desrea	Scort	Diamond	M	Alfa	Desrea	Scort	Diamond
590	+	+	+	-	270	-	+	+	+
229	+	+	+	-					

OPE-13				
M	Alfa	Desrea	Scort	Diamond
2100	-	+	+	+
900	-	+	+	+
890	+	-	-	-
785	-	+	+	-

Table (5): Similarity index (%) of each pair of examined potato cultivars.

Potato genotypes	Alfa	Desrea	Scort	Diamond
Alfa	100			
Desrea	70.9	100		
Scort	75.8	88.7	100	
Diamond	69.3	72.6	70.9	100

Table (6):Genotype-specific RAPD markers of the potato cultivars Alfa, Desrea, Scort and Diamond respectively .

Genotype	RAPD marker	Total markers
Alfa	OPB-09-800, OPB-09-650, OPD-04-1970, OPD-04-475, OPD-04-317,OPE-13-890	6
Desrea	OPA-15-2190	1
Scort	OPD-04-580	1
Diamond	OPA-06-1250, OPD-04-1000,OPD-04-600	3
Total		11

REFERENCES

A.O.A.C.,(1980). Official Method of Analysis of the Association of Official Analytical Chemists. Washington D.C., 3rd Ed.

Baird, E., Cooper-Bland, S., Waugh, R., DeMaine, M. and Powell, W. (1992). Molecular characterisation of inter- and intra-specific somatic hybrids of potato using randomly amplified polymorphic DNA (RAPD) markers. Molecular and General Genetics 233: 469–475.

Bradley, K.F., Rieger, M.A. and Collins, G.G. (1996). Classification of Australian garlic cultivars by DNA fingerprinting. *Australian Journal of* Experimental Agriculture 36: 613–618.

del Rio, A.H., Bamberg, J.B., and Huaman, Z. (1997). Assessing changes in the genetic diversity of potato gene banks. 1. Effects of seed increase. Theoretical and Applied Genetics 95: 191–198.

Demeke, T., Lynch, D.R., Kawchuck, L.M., Kozub, G.C., and Armstrong, J.D. (1996). Genetic diversity of potato determined by random amplified polymorphic DNA analysis. Plant Cell Reports 15: 662–667.

Ford, R., and Taylor, P.W.J. (1997). The application of RAPD markers for potato cultivar identification. Australian Journal of Agricultural Research 48: 1213–1217.

Gatehouse, A.M.R., Hilder, V.A., and Boulter, D., (1992). Potential of plant-derived genes in the genetic manipulation of crops for insect resistance, in: Biotechnology in Agriculture No 7: Plant Genetic Manipulation for Crop Protection, CAB International, p 155-181

Graham, J., McNicol, R.J., and McNicol, J.W. (1996). A comparison of methods for the estimation of genetic diversity in strawberry cultivars. Theoretical and Applied Genetics 93: 402–406.

Hemeida, I., Salama, R. A. K., Khatab, A., and Ibrahim, S. (1994). Effects of plantation date and cultivar on the infestation of potato tubers by the mole cricket in Giza, Egypt. Bull. Ent. Soc. Egypt., 72: 237-243

Ibrahim, S. (1993). Studies on the mole cricket in Egypt. MSc. Thesis, Fac. of Agric. Cairo Univ. Egypt

Kays, S. J., (1999). Postharvest factors affecting appearance. Postharvest Biol. And Technol., 15: 233-247

Matsuura, H., and Echizaki, H., (1985). Damage of Chinese yem by African mole-cricket *Gryllotalpa africana* and its control by chemicals. Jp. J. Appl. Entomol. Zool., 79: 36-40

Morell, M.K., Peakall, R., Appels, R., Preston, L.R. and Lloyd, H.L. (1995). DNA profiling techniques for plant variety

identification. Australian Journal of Experimental Agriculture 35: 807–819.

Nei., M. (1987). Molecular Evolutionary Genetics, Columbia University Press, New York.

Paz, M.M. and Veilleux, R.E. (1997). Genetic diversity based on randomly amplified polymorphic DNA (RAPD) and its relationship with the performance of diploid potato hybrids. Journal of the **Rasmussen, J.O. and Rasmussen, O.S. (1995).** Characterization of somatic hybrids of potato by use of RAPD markers and isozyme analysis. Physiologia Plantarum 93: 357–364.

Rogers, S.O.and Bendich, A.J. (1985). Extraction of DNA from milligram amounts of fresh herbarium and mummified plant tissues. Plant Mol. Biol. 5: 69 – 76.

Shehata, S. A., (1986). Physiological studies on solanine promotion in potato *Solanum tuberosum* L. Ph.D. Thesis, Fac. of Agric. Cairo Univ. Egypt

Snedecor, G. W. (1956). Statistical Methods. 5th Ed. Iowa State College Press, Ames, Iowa, U.S.A

Sosinski, B., and Douches, D.S. (1996). Using polymerase chain reaction-based DNA amplification to fingerprint North American potato cultivars. HortScience 31:130–133.

Walker, T. J. (1985). Mole cricket in Florida. Univ. Fla. Agric. Exp. Stn. Bull., 846-850.

Welsh, J., and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213–7218.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers.Nucleic Acids Research 18: 6531–6535.

الملخص العربي

استخدام الواسمات الوراثية الجزينية (RAPD-markers) لتعريف اصناف البطاطس وعلاقتها بصفة تحمل الاصابة بالحشرات

رضا علوانى عبد الحليم مغيب* و سناء عبد القادر محمد ابراهيم **

قسم الوراثة - كلية الزراعة - جامعة القاهرة*

**معهد بحوث وقاية النباتات – مركز البحوث الزراعية- الدقي - الجيزة - مصر

تتسبب حشرة الحفار فى العديد من الخسائر للمنتجات الزراعية فى مصر، و نظرا لقلة المعلومات الوراثية المتاحة عن مقاومة أصناف البطاطس للحفار فقد صممت هذه الدراسة لمقارنة محصول أربعة أصناف من البطاطس تحت ظروف الاصابة الطبيعية بالحفار .

أوضحت النتائج أن محصول الدرنات انخفض نتيجة الاصابة و كان الانخفاض معنوى بين الأصناف . و اتضح من الدراسة أن الصنف ألفا هو الأكثر مقاومة مقارنة بالأصناف الأخرى المستخدمة لذلك ينصح بزراعته فى الأراضى الموبؤة بهذه الأفة . أدت الاصابة بالحفار الى خفض نسبة الكربوهيدرات فى الدرنات المصابة عن السليمة لنفس الصنف. و عند دراسة أنماط شرائط ال RAPD الناتجة باستخدام سبعة من بادئات الRAPD أوضحت النتائج أن عدد الواسمات الوراثية المتحصل عليها كانت 62 واسم جزيئى أظهرت فيما بينها درجة تباين وراثى تقدر بحوالى 48,4% . و طبقا للمعلومات المتحصل عليها عن طريق تحليل الRAPD نجد أنه يمكن التمييز بين أصناف البطاطس الأربعة المستخدمة فى هذه الدراسة . و قد تم تحديد الواسمات الوراثية المعلمة لكل صنف من الأصناف المستخدمة و اتضح أن هناك ستة واسمات تعلم الصنف ألفا (OPB-09-800, OPB-09-650, OPD-04-1970, OPD-04-475, OPD-04-317,OPE-13-890) وثلاثة واسمات تعلم الصنف دياموند (OPA-06-1250, OPD-04-1000,OPD-04-600) و واسم واحد يعلم الصنف ديزرية (OPA-15-2190) وواسم واحد يعلم الصنف سكورت (OPD-04-580). تمثل الواسمات الوراثية المعلمة للأ صناف نسبة17,7 % من عدد الواسمات الكلية المتحصل عليها و36,6 % من اجمالى الواسمات المتباينة . و يمكن استخدام هذه الواسمات فى الانتخاب لصفة المقاومة للاصابة بالحفار بين أصناف البطاطس المختلفة فى برامج التربية .