Genetic stability of transgenic potato expressing *cry1Aa7* gene

(Received: 08.12.2002; Accepted: 30.12.2002)

E. A. Metry, M. A. Ibrahim, S. A. Moustafa and T. M. Nasr El-Din
Agricultural Genetic Engineering Researcher Institute, AGERI, ARC, Giza, Egypt.

ABSTRACT

Potato (Solanum tuberosum L.) is seriously infested with potato tuber moth (Phthorimaea operculella Zeller). Accordingly, genetically modified (GM) potato (cv. Desiree) expressing cry1Aa7 gene from a local Bacillus thuringiensis isolate (Bt-C12) was previously generated and analyzed. The aim of the present study was to evaluate Bt-transgenic potato lines D8, D9, D26, D27 and D37 at cytological, morphological and molecular levels for possible mutations and chromosomal aberrations that could lead to somaclonal variation. The results indicated that all lines under investigation, except D37, exhibited similar phenotypic appearance and tuber yield compared to control. In line D37, number of tubers per plant (31.8) was significantly higher than the other tested lines (23.7). SDS-PAGE, on the other hand, revealed that protein banding patterns of leaves collected from transgenic and non-transgenic lines were comparable. In addition, polymerase chain reaction (PCR) and fifteen arbitrary 10-mer primers, randomly selected, were used to generate random amplified polymorphic DNA (RAPD) markers. The size of the generated DNA fragments ranged from 200 to 2500 base pairs. Identical banding profiles with eleven of the fifteen primers (73%) were observed and a total of nine polymorphic bands (11.5%) were detected and used as RAPD markers. It is concluded that non-significant genetic variation in potato plants expressing the cry1Aa7 gene based on cytological and morphological examination as well as protein profile and RAPD analysis.

Key words: *Cry1Aa7, Bacillus thuringiensis, Potato cultivar (Desriee), RAPD.*

INTRODUCTION

In Egypt, potato yield is greatly reduced due to infestation with insect pests such as potato tuber moth (PTM), *Phthorimaea operculella* Zeller. Currently, *Bacillus thuringiensis* (Bt)-based biopestcide is frequently used against PTM as an environmentally safe alternative to chemical pesticides. In addition, potato plants harboring different crystal *(cry)* protein genes isolated from many Bt strains have been generated, (Hudy, 1998; Ibrahim *et al.*, 2002; Davidson *et al.*, 2002). This strategy would give the transgenic potato self-protection and many advantages over topical applicate of Bt-biopesticide. In addation to potato, genetically altered tomatoes, squash, canola, cotton, maize and soybean have been in the market since 1994. However, there are centaverse views concerning transgenic food crops (Ruibal-Mendieta and Lints, 1998).

Many reports demonstrated that callus induction and regeneration might be responsible for chromosomal aberration and genetic changes Rietveld *et al.* (1987). For

instance, Fish and Karp (1986) stated that regenerated plants through protoplasts showed 50% abnormal chromosome breeding clones and inter specific hybrids. In addition, Pijnacker *et al.* (1989) reported that numerical and structural chromosome variation arising during *in vitro* culture, might contribute to variation in genotype, type of culture, culture medium and culture age. Fleming *et al.* (1992) studied ploidy doubling during callus of potato dihaploid leaf explants and detected variation in regenerated plants. Recently, many techniques have been developed detect and identify genetic variations (Ruibal-Mendieta and Lints, 1998). Molecular techniques such as random amplified polymorphic DNA (RAPD), for instance, is a quick and reliable method that could significantly detect small genetic changes in transgenic plants, (Williams *et al.*, 1990). In addition, RAPD analysis has been successfully used for linkage map studies in tomato (Klein-Lankhorst *et al.*, 1991) and in cultivar identification in brassica, (Hu *et al.*, 1995) and in faba bean and soybean (Hussein *et al.*, 2000; 2001) and in *Phaseolus vulgaris*, El-Fiky *et al.*, 2002). Moreover, Xu *et al.* (1993), reported that the identification of somatic hybrids between *Solanum tuberosum* and *Solanum brevidens* can be carried out using PCR-RAPD technique. Morphological and molecular analysis together with cytological examination were also used to study the genetic instability of monohaploid, dihaploid and tetraploid genotypes of potato (Karp *et al.*, 1984; Sree-Ramulu *et al.*, 1986).

The aim of this work was to elucidate the genetic stability of potato lines harboring *cryIAa7* gene, which regenerated via *Agrobacterium* mediated-transformation system. Accordingly, morphological and cytological analyses together with RAPD-typing and protein finger printing were investigated.

MATERIALS AND METHODS

Plant materials

Leaves drived from five Bt-transgenic potato lines (D8, D9, D13, D26 and D37) and non-transgenic control were collected from *in vitro* regenerated Desiree plants conserved at Micropropagation Technology Laboratory (AGERI/ARC) and used as a source for DNA and protein purification.

Cytological analysis

Roots from transgenic and non-transgenic potato lines were taken from *in vitro* culture and examined for cell division and chromosomal aberrations using the aceto carmine sqush method described by Grant (1982). Basically, root tips were cut at a certain time of the day and immediately fixed in ethanol and glacial acetic acid solution (3:1, v/v) for 2 hr, washed with H_2O and stored in 70% ethanol at 4°C until microscopically examined. Otherwise, it was treated with 1N HCl for 5-8 min at 60°C directly before slide preparation.

Morphological examination

Adaptation and acclimatization of transgenic and non-transgenic potato lines were carried out in the Bio-containment greenhouse facilities at AGERI. Plantlets (3-5 cm), obtained by *in vitro* culture technique, were washed to remove phytagel traces and soaked in fungicide solution (2 g/l of Benlate) for 15-20 minutes. Washed plantlets were cultured in plastic pots (15 cm in diameter) containing a mixture of peat moss and sand (2:1, v/v), and watered immediately. Plantlets were transferred to plastic pots (23 cm in diameter) after 3 weeks from adaptation, irrigated with water containing 1g/l Kristalon (N.P.K./19:19:19) every 10 days up to maturity (120 days). Observations on morphological characters such as plant height and number of leaves were recorded after 90

days from adaptation. Tuber yield was evaluated after full maturation of the plants. Ten individual plants from each line were examined and the number and weight of tubers were calculated after harvesting. The statistical analysis was carried out using SAS computer programme according to Snedecor and Cochran (1980) using L.S.D. test.

Protein analysis

SDS-polyacrylamide gel electrophesis (SDS-PAGA) was investigated for the total soluble proteins of five transgenic lines as well as non-transgenic control plants according to the method described by Laemmli (1970). One hundred mg of leave tissue were collected from *in vitro* culture and macerated in 100 l extraction buffer (1M Tris-HCl pH 6.8, 10% SDS, 1M glycerol and 1 mM DTT) using glass rod. Soluble materials were collected by brief centrifugation and equal volume of 2x protein breakage buffer (150 mM Tris-HCl, pH 6.8, 1.2% SDS, 15% ß-mercaptoethanol, 1.8 mg bromophenol blue and 30% glycerol) were added. Protein samples were boiled at 95°C for 3 min, briefly centrifuged and analyzed on 15% SDS-PAGE.

DNA extraction and RAPD analysis

DNA extraction was carried out according to Edwards *et al.* (1991) with minor modification. Basically, a small piece of leaf tissue (100 mg) was macerated in 400 µl extraction buffer (100 mM Tris-HCl pH 8, 500 mM NaCl, 50 mM EDTA, 0.7 µl/ml ß-mercaptoethanol). Soluble materials were collected by centrifugation at 13,000 rpm for 5 min, then 4 µl RNase A (10 mg/ml) were added and the tube was incubated at 37°C for 30 min. DNA was precipitated by adding 1 volume isopropanol, washed twice with 70% ethanol, dried and dissolved in 100 µl sdH_2O.

Fifteen 10-mer primers (A07, A09, A10, A17, A19, B01, B07, B09, B19, C20, E15, F06, O06, O12 and O13) were selected randomly and synthesized on a DNA synthesizer (model 380 A; Applied Biosystems, CA, USA) and used for RAPD analysis. The nucleotide sequences of the primers are listed in Table (1).

Table (1): Nucleotide sequences of the random primers used for RAPD analysis.

Primer code No.	Sequence 5' to 3'	Primer code No.	Sequence 5' to 3'
A-07	GAAACGGGTG	B-19	ACCCCCGAAG
A-09	GGGTAACGCC	C-20	ACTTCGCCAC
A-10	GTGATCGCAG	E-15	ACGCACAACC
A-17	GACCGCTTGT	F-06	GGGAATTCGG
A-19	CAAACGTCGG	O-06	CCACGGGAAG
B-01	GTTTCGCTCC	O-12	CAGTGCTGTG
B-07	GGTGACGCAG	O-13	GTCAGAGTCC
B-09	TGGGGGACTC		

PCR amplification was carried out in 1X reaction buffer containing 0.2 m dNTPs, 50 pmol primer, 0.5 unit of Taq polymersae, 50 ng of genomic DNA. All components were mixed and sdH_2O was added to a final volume of 25 l. The PCR reactions were carried out in a Gene Amp PCR system 2400 (Perkin-Elmer Corp.) that was programmed for an initial denaturation step of 94°C for 3 min, then 40 cycles of 94, 36 and 72°C for 40, 60 and 90 sec, respectively. Ten µl from each sample was analyzed in 1.5% agarose gel in 1xTAE buffer and gels were photographed under UV light.

RESULTS AND DISCUSSION

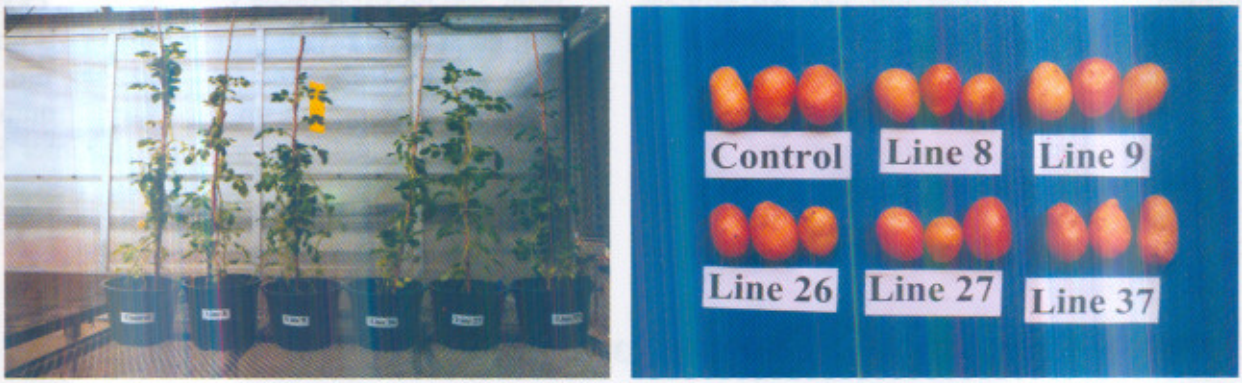
Gene modification (GM), is a recent development which allows specific genes to be identified, isolated, and inserted into other hosts with a high level of specificity and select for desirable qualities (Gasser *et al.*, 1989; Beck and Ulrich, 1993). Potential benefits of GM crops include increased nutritional value and enhance pest and disease-resistance features. Although GM crops sounds acceptable, the introduction of GM food to the human diet has caused considerable debates. There is a concern that gene insertion and regeneration may cause mutations or chromosomal aberrations (somaclonal variation) that could have safety implication and may affect other genes.

Cytological and morphological examination

Previously, we have reported the production of Egyptian transgenic potato expressing *cry1Aa7* gene derived from a local Bt isolate (Ibrahim *et al.*, 2002). To confirm the stability of transgenes during subsequent regeneration and selection, PCR using *npt-II* and *cry1Aa*-specific primers were carried out on total DNA extracted from Bt-transgenic potato leaves. Results showed amplified DNA fragments (0.41 and 2.1 kb) corresponding to *npt-II* and *cry1Aa7* genes, respectively (data not shown). Subsequently, root tips were collected from transgenic and non-transgenic potato lines for cytological examination. It has been previously reported that the use of *in vitro* cultures leads to genetic instability expressed as chromosomal disturbance in regenerated plants (Grant, 1982). Results from the present study indicated that there was no genetic variation in the transgenic potato lines under investigation. The cytological data emphasized that there was no detectable chromosomal abnormalities occurred during regeneration and transformation steps. Similar findings were reported by Koga-Ban *et al.* (1998) who stated that transgenic cucumber plants expressing rice chitinase had no chromosomal aberrations or any characteristic difference to non-transgenic cucumber except expression of rice chitinase gene and resistance to fungal disease. The chromosomal instability, that detected in other reports may be attributed to variation in regeneration protocols or to differences in plant genotypes used in their experiments.

For morphological examination, criteria such as plant hight (cm), average number of leaves and tubers per plant, were investigated in transgenic and non-transgenic potato plants under greenhouse conditions (Figure 1). Data were taken after 90 days from planting date and were statistically analyzed (Table 2). Results indicated that there were no significant morphological differences between transgenic and non-transgenic lines. The average number and weight (gr) of tubers per transgenic potato line were 26 and 94.6 gr, respectively. D9, D26 and D27 lines produced almost the same number of tubers as control, while D37 produced 31.8 tubers/plant. Although number of tubers of D37 was significantly high, the weight of these tubers (86.8 gr) was the lowest among all tested lines (Figure 1 & Table 2). Regenerated potato plants through tissue culture technique has been morphologically evaluated in a similar way (Cassells *et al.*, 1983, Rossignol *et al.*, 1984; Berlijak, 1991). Some investigators were not able to identify any phenotypic variation among potato plants derived from tissue culture (Cassells *et al.*, 1983; De-Block, 1988). On the other hand, others (Weeb *et al.*, 1983) repoted that the morphological responses were found to differ according to cultivars under investigation. Additionally, Conner *et al.* (1994), morphologically exmined 13 transgenic potato expressing *npt-II* gene. They observed that all lines developed unexpected changes in the phenotypic appearance of shoots, and/or poor

tuber yield (generally involving reduced number of small tubers). Moreover, their results reveald that each independently selected transgenic line showed distinctly different changes in phenotypic appearance or yield performance. They suggested that these changes were attributed to either epigenetic or genetic events occurring during tissue culture phase of transformation. We are postulating that this contradiction migh be due to using different potato cultivars (llam Hary, lwa and Rua) rather than Desiree in their study.



A B

Fig. (1): Morphological examination of transgenic and non-transgenic potato lines. A: Transgenic and non-transgenic potato lines grown under biocontainment greenhouse conditions. B: Minitubers of transgenic and non-transgenic potato lines harvested from pot experiment at biocontainment greenhouse after 120 days.

Table (2): Statistical analysis of plant hight, number of leaves, number and weight of tubers per plant for genetically- modified and non-modified potato line (control).

Lines	Characters			
	Plant hight (cm)	No. of leaves / Plant	Weight of tubers / Plant (gram)	No. of tubers / Plant
Control	78.1 *	21	94.9	24.2
D 8	77.5	20.5	88	25.3
D 9	80.6	20	99.1	24.1
D 26	77 3	18.8	100	23.8
D 27	79.9	20.4	98.5	23.7
D 37	78.5	20	86.8	31.8
L.S.D	11.62	3.88	23.96	9.24

L.S.D. at .0.5%

* Each datumrepresents the average of ten individual plants from one independent transgenic line.

Protein profile and RAPD analysis

The electrophoretic protein banding patterns for five transgenic lines compared to the non-transgenic line (control) are shown in Figure (2). SDS-protein patterns exhibited nine major bands, none of which were polymorphic and all lines showed identical SDS-protein patterns. This finding may suggest that the protein banding profiles were not sufficient to detect variations among transgenic and non-transgenic lines. Accordingly, DNA-based analysis was applied due to its high sensitivity and accuracy in detecting single base changes. Badr (2000), reported that RAPD markers

were used to charcterize and detect somaclonal variations in calli of five commercial potato cultivars. Their results reveald that somaclonal variations were found to be genotype dependent. In the present study, fifteen, randomly selected, Operon primers (Table 1) were used and each tested primer produced distinct banding profiles Fig. (3). All primers used in this study resulted in the appearance of DNA fragments varied in numbers and sizes. Of the fifteen primers tested, eleven (73%) produced identical banding patterns as the non-transgenic lines. A total of seventy eight DNA bands were detected across the fifteen random primers (Table 3), nine of which (11.5%) were polymorphic. For phenogram demonstration and relationships among five Bt-transgenic and non-transgenic plants, bands were scored and computer analyzed (Table 3). Intensive bands were considered positive (+), while weak bands were considered negative (-). The size of the amplified fragments ranged from 200 bp for primers C20, E15 and O06 to as high as 2500 bp for primer B09. All primers used, except B01, B09, B19 and F06, did not show any polymorphism. The largest number of amplified bands was scored for primer B19 (9 bands), while the lowest was scored for primer O12 (3 bands).

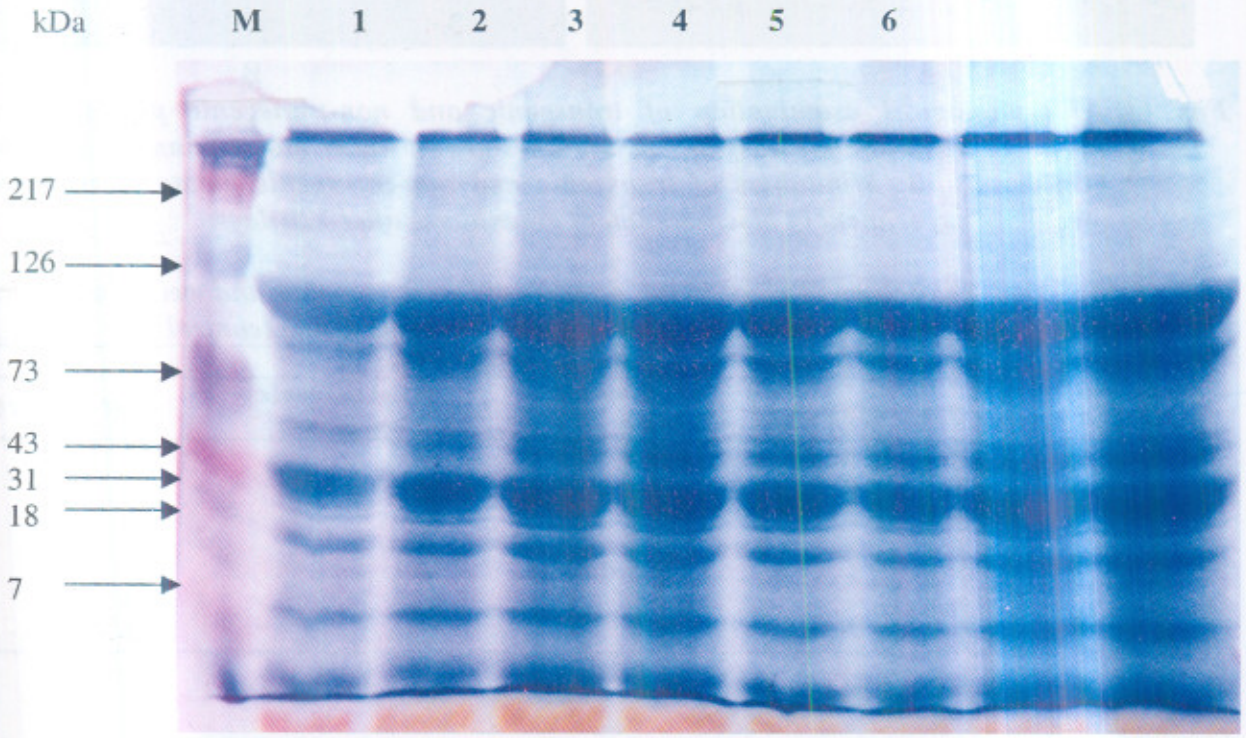


Fig. (2): SDS-PAGE of total protein extracted from transgenic and non-transgenic potato lines. Lanes 1-6 are control, D8, D9, D26, D27 and D37, respectively. M represents the protein marker (217, 126, 73, 43, 31, 18 and 7 kDa).

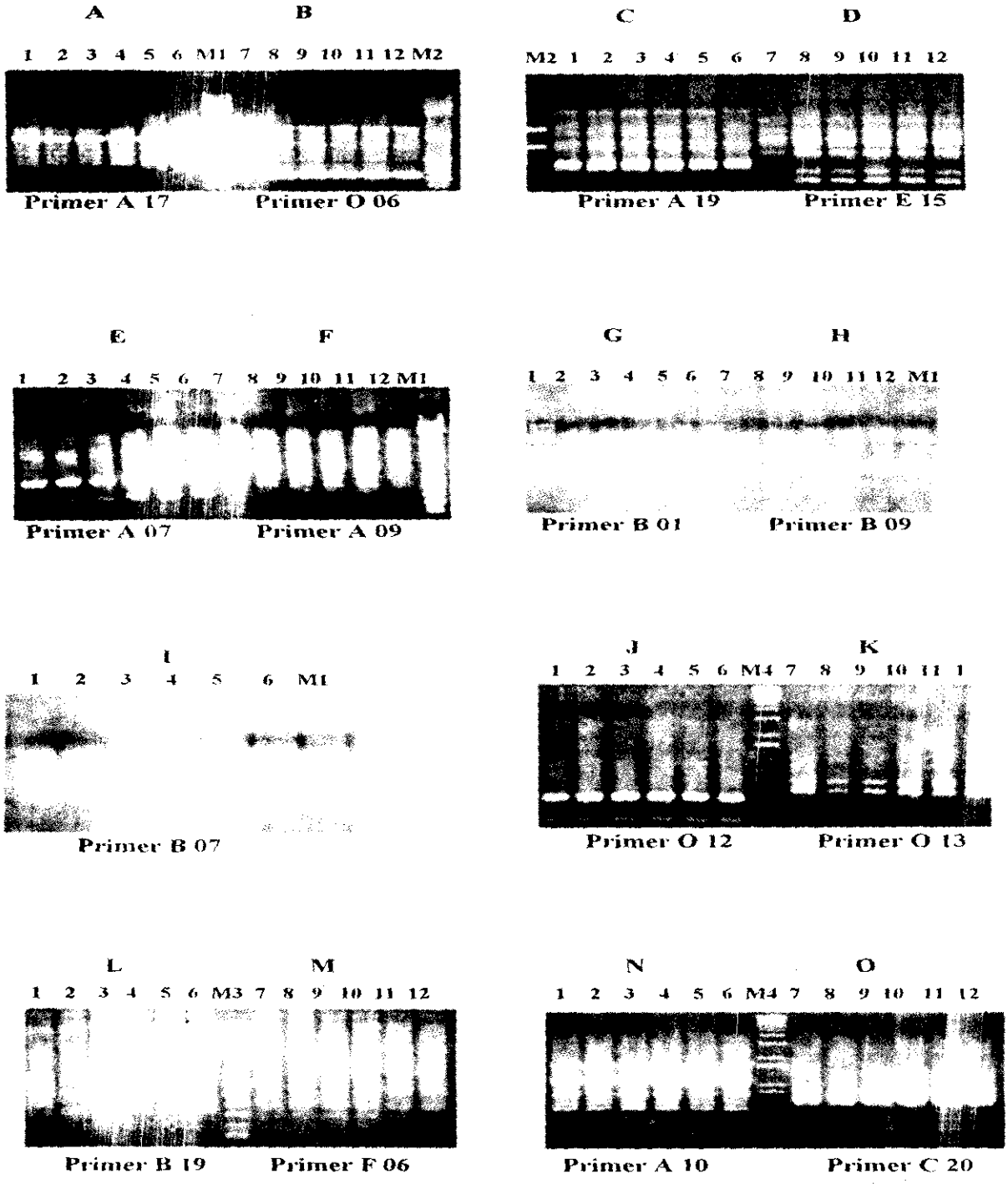


Fig. (3): RAPD analysis demonstrating polymorphisms among six transgenic and non-transgenic potato lines. Control (1), D8 (2), D9 (3), D26(4), D27 (5) and D37(6) using fifteen random 10-mer primers (A-O). M1, M2, M3 & M4 refer to DNA markers.

Table (3): Survey of RAPD-PCR markers in the five transgenic lines (D8, D9, D26, D27 & D37) and control (C) using fifteen random primers. The presence and absence of corresponding bands are represented by (+) and (-) signs, respectively.

Primers	MW (bp)	C	D8	D9	D26	D27	D37	Primers	MW (bp)	C	D8	D9	D26	D27	D37
A 07	2000	+	+	+	+	+	+	B 07	1100	+	+	+	+	+	+
	1900	+	+	+	+	+	+		1000	+	+	+	+	+	+
	1400	+	+	+	+	+	+		750	+	+	+	+	+	+
	1300	+	+	+	+	+	+		550	+	+	+	+	+	+
	600	+	+	+	+	+	+								
	300	+	+	+	+	+	+								
A 09	950	+	+	+	+	+	+	B 09	2800	+	+	+	+	+	+
	800	+	+	+	+	+	+		1750	+	+	+	+	+	+
									690	-	+	+	-	+	+
									600	+	-	-	+	-	-
									500	+	+	+	+	+	+
A 10	1650	+	+	+	+	+	+	B 19	2000	+	+	+	+	+	+
	1200	+	+	+	+	+	+		1300	-	+	+	+	+	+
	900	+	+	+	+	+	+		1200	+	+	+	+	+	+
	750	+	+	+	+	+	+		1000	-	+	+	+	+	+
	600	+	+	+	+	+	+		750	+	+	+	+	+	+
	500	+	+	+	+	+	+		650	+	-	-	+	+	+
									570	+	+	+	+	+	+
									450	+	+	+	+	+	+
									320	-	+	+	+	+	+
A 17	1300	+	+	+	+	+	+	C 20	900	+	+	+	+	+	+
	1000	+	+	+	+	+	+		700	+	+	+	+	+	+
	750	+	+	+	+	+	+		650	+	+	+	+	+	+
	610	+	+	+	+	+	+		600	+	+	+	+	+	+
	500	+	+	+	+	+	+		490	+	+	+	+	+	+
	420	+	+	+	+	+	+		390	+	+	+	+	+	+
									210	+	+	+	+	+	+
A 19	1500	+	+	+	+	+	+	E 15	900	+	+	+	+	+	+
	1100	+	+	+	+	+	+		800	+	+	+	+	+	+
	950	+	+	+	+	+	+		700	+	+	+	+	+	+
	850	+	+	+	+	+	+		600	+	+	+	+	+	+
	750	+	+	+	+	+	+		400	+	+	+	+	+	+
	650	+	+	+	+	+	+		300	+	+	+	+	+	+
	500	+	+	+	+	+	+								
	450	+	+	+	+	+	+								
B 01	2200	+	-	-	-	-	-	F 06	2100	+	-	-	+	+	-
	1250	+	+	+	+	+	+		1400	+	+	+	+	+	+
	1000	+	+	+	+	+	+		1300	-	+	+	+	+	+
	900	+	+	+	+	+	+		1000	+	+	+	+	+	+
	720	+	+	+	+	+	+		900	-	+	+	+	+	+
	600	+	+	+	+	+	+		600	+	+	+	+	+	+
	410	+	+	+	+	+	+		400	-	+	+	+	+	+
O 13	800	+	+	+	+	+	+	O 06	1500	+	+	+	+	+	+
	700	+	-	+	+	+	-		800	+	+	+	+	+	+
	600	+	+	+	+	+	+		250	+	+	+	+	+	+
								O 12	600	+	+	+	+	+	+
									310	+	+	+	+	+	+

In conclusion, our results confirmed genetic stability of transgenic potato lines expressing *cry1Aa7* gene.

REFERENCES

Badr, E.A. and Mabrauk, Y.M. (2000). Identification of potato cultivars and somaclonal variations by RAPD. Arab. J. Biotech, 3: 181-198.

Beck, C.I. and Ulrich, T. (1993). Biotechnology in the food industry. Bio/Technology, 11: 895-902.

Berlijak, J. (1991). Variation in plants regenerated from potato somatic cells. International Symposium on Plant Biotechnology and its Contribution to Plant Development. Palexpo, Geneva, Switzerland, 19-20 April 1991.

Cassells, A.C., Goetz, E.M. and Austin, S. (1983). Phenotypic variation produced from lateral buds, stem explants and single-cell derived callus of potato. Potato Research, 26: 367-372.

Conner, A.j., Williams, M.K., Abermethy, D.J., Abermethy, P.J., Fletcher, P.J. and Gent, R.A. (1994). Field performance of transgenic potatoes. New Zealand Journal of Crop and Horticultural Science, 22: 361-371.

Davidson, M.M., Jacobs, J.M., Reader, J.K., Butler, R.C., Christina, M., Markwich, N.P., Wratten, S.D. and Conner, A.J. (2002). Development and evaluation of potatoes transgenic for a *cry1Ac9* gene confreeing resistance to potato tuber moth. J. Amer. Soc. Hort. Sci., 127 (4): 590-596.

De-Block, M. (1988). Genotype independent leaf disc transformation of potato (*Solanum tuberosum*) using *Agrobacterium tumefactions*. Theor. Appl. Genet., 76: 767-774.

Edwards, K., Johnstone, C. and Thompson, C. (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res., 19: 260-266.

El-Fiky, Z.A., Hussein, M.H., Mohamed, E.M. and Hussein, H.A. (2002). Biochemical and molecular genetic studies using SDS-proteins isozymes and RAPD-PCR in some common bean (*Phaseolus vulagris* L.) cultivars. Arab J. Biot., 5: 249-262.

Fish, N. and Karp, A. (1986). Improvements in regeneration from protoplasts of potato and studies on chromosome stability. Theor. Appl. Genet., 72: 405-412.

Fleming, M.L., De-Maine, M.H., Powell, M.J. and Warwick, W. (1992). Ploidy doubling by callus culture of potato dihaploid leaf explants and the variation in regenerated plants. Annals of Applied Biology., 121: 183-188.

Gasser, C.S. and Fraley, R.T. (1989). Genetically engineered plants for crops improvement. Science., 244: 1293-1299.

Grant, F.W. (1982). Chromosome aberration assays in Allium. A report of the U.S. Environmental protection agency. Mutation Res., 93: 161-168.

Hu, J., Eysden, J.V. and Quuiros, C.F. (1995). Generation of DNA based markers in specific genome regions by two primers RAPD reaction. PCR Methods and Applications., 4: 346-351.

Hudy, P.S. (1998). Analysis of engineered (*cry1Ac*-Bt-transgenic) and natural resistance mechanisms in potato (*Solanum Spp.*) for the control of potato tuber moth (*Phthorimaea operculella* Zeller). M. S. Thesis Michigan State University, East Lansing.

Hussein, M.H., Mahfouz, H.T., Domyati, F.M., Fikery, Z.A. and Hussein H.A. (2001). Molecular fingerprinting of newly developed soybean (*Glycine max* L. Merr) cultivars. Arab J. Biotech., 4: 63-74.

Hussein, M.H., Saker, M.M. and Husssein, H.A. (2000). DNA fingerprints of faba beans tolerant to broomrape (*Orobanche crenata*, Forsk). Arab. J. Biotech., 3: 189-198.

Ibrahim, M.A., Metry, E.A., Osman, Y.A., Nasr El-Din, T.M. and Madkour, M.A. (2002). Genetically modified potato (*Solanum tuberosum* L.) resistant to potato tuber moth (*Phthorimaea operculella*). Arab J. Biotech., 5: 1-10.

Karp, A., Rissiott, R., Jones, M.J.K. and Brigh, S.W.J. (1984). Chromosome doubling in monohaploid and dihaploid potatoes by regeneration from culture leaf explants. Plant Cell, Tissue and Organ Culture., 3: 363-373.

Klein-Lankhorst, R., Vermunt, A., Weide, R., Liharska, T. and Zabel, P. (1991). Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor. Appl. Genet., 83:108-114.

Koga-Ban, Y., Tabei, Y., Ishimoto, M., Nishzawa, Y., Tsuchiya, K., Kayano, T. and Tanaka, H. (1998). Environmental risk assessment of transgenic cucumber introduced rice Chitinase. 5th Int. Sym. The biosafety results of field tests of genetically modified plant and microorganisms. 6-10 Sep. Braunschweig. Germany.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head becteriophage T4. Nature., 227: 680-685.

Pijnacker, L.P., Sree-Ramulu, K., Dijkhuis, P. and Ferwerda, M.A. (1989). Flow cytometric and karyological analysis of polysomaty and polyploidization during callus formation from leaf segments of various potato genotypes. Theor. Appl. Genet., 77: 102-110.

Rietveld, R.C., Hasegawa, P.M. and Bressan, R.A. (1987). Genetic variability in tuber disc-derived potato plants in Biotechnology C.F. "In: Agriculture and Forestry" V. 3: Potato (ed. By Y. P.S. Bajaj) Springer-Verlag Berlin Heidelbery, Pp 392-407.

Rossignol, L., Rossignol, M., Ducreux, G., Nozeran, R. and Darpas, A. (1984). Analysis of the variability of plants regenerated *in vitro* from callus in potato (*Solanum tuberosum* L. Variety Bf 15). Lettres Batoniques, 131: 171-190.

Ruibal-Mendieta, N.L. and Lints, F.A. (1998). Novel and transgenic food crops: Overview of scientific versus public perception. Transgenic Res., 7(5): 379-386.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods. 6th ed., The Iowa State Univ. Press., Ames., USA.

Sree-Ramulu, K., Dijkhuis, P., Bokelmann, G.S. and Roest, S. (1986). Genetic instability *in vitro* of monohaploid, dihaploid and tetraploid genotypes of potato. C. F. "Somaclonal Variation and Crop Improvement"., pp. 213-218.

Weeb, K.J., Oslfo, E.O. and Henshaw, G.G. (1983). Shoot regeneration from leaflet discs of six cultivars of potato (*Solanum tuberosum* subsp. *Tuberosum*). Plant Science Letters., 30: 1-8.

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

Xu, Y., M. Clark, S. and Pehu, E. (1993). Use of RAPD markers to screen somatic hybrids between *Solanum tuberosum* and *S. brevidens*. Plant Cell Reports, 12: 107-109.

الملخص العربي

الثبات الوراثي لنباتات البطاطس المعدلة وراثيا والمعبرة لموروث ال *cry1Aa7*

عماد أنيس متري ، محمد احمد إبراهيم ، صلاح على مصطفى ، تيمور محمد نصر الدين
معهد بحوث الهندسة الوراثية الزراعية-مركز البحوث الزراعية-الجيزة-مصر .

يتأثر محصول البطاطس تأثرا ملحوظا نتيجة لإصابته بواسطة فراشة درنات البطاطس وتبعا لذلك فقد تم إكثار نباتات البطاطس صنف ديزيرية والمعدلة وراثيا لجين *cry1Aa7* المعزول من عزلة محلية من بكتريا باسيلس ثيرنوجنسيس (Bt) وكان هدف هذه الدراسة هو تقيم سلالات (د٨، د٩، د٢٦، د ٢٧و د٣٧) المعدلة وراثيا وذلك تبعا للدراسات الخلوية-والمورفولوجية - وكذلك على المستوى الجزيئى لتحديد مدى إمكانية حدوث طفرات وتغيرات كروموسومية والتى قد تؤدى إلى اختلافات مورفولوجية وقد أوضحت نتائج هذا البحث ان كل سلالة ماعدا د٣٧ قد أظهرت نفس المظهر الخارجي وشكل الدرنة مقارنة بالنباتات غير المعدلة وراثيا. بينما د ٣٧قد أعطت عددا اكبر من الدرنات عن السلالات الأخرى. على الجانب الآخر فقد أظهرت تجربة التفريد الكهربائي للبروتين بأن حزم البروتينات المستخلصة من الأوراق المعدلة وراثيا وغير المعدلة كانت متشابهة وتكاد تكون متطابقة. بالإضافة الى ذلك تم استخدام تفاعل البلمرة المتسلسل باستخدام ١٥ بادئة عشوائية (primers) لاستخدامهم فى التكبير العشوائي لمقاطع الحمض النووي المتباينة (RAPD). وقد وجد ان حجم أجزاء الدنا المكبرة عشوائيا يتراوح ما بين ٢٠٠ - ٢٥٠٠ زوجا من القواعد النيتروجنية وقد لوحظ وجود حزم متطابقة ل١١ بادئ من الـ ١٥ بادئ المستخدمة بنسبة ٧٣% أيضا تم الحصول على ٩ حزم متباينة بنسبة ١١,٥% ويمكن استخدامها كواسمات وراثية (Genetic markers). ويمكن تلخيص نتائج هذا البحث بأنة قد أمكن إثبات عدم وجود اختلافات جوهرية فى نباتات البطاطس المعدلة وراثيا الحاملة لجين *cry1Aa7* على أساس الدراسة الخلوية والمورفولوجية وأيضا على المستوى الجزيئى (RAPD analysis and protein profile) .