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MUTATION BREEDING FOR EARLY BLIGHT RESISTANCE IN POTATO (*Solanum tuberosum* L.)

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ABSTRACT

Ethyl methane sulphonate (EMS) at different concentrations, viz., 0, 1, 2 and 3 mM/l, were used to induce genetic variability in three cultivars of potato, namely, Atlas, Nicola and Simon, for selecting mutants with increased level of resistance against early blight. Two hundreds and seventy plants per each cultivar were produced from M_0V_1 mutant generation. Each plant was evaluated, in terms of resistance to early blight, as compared to their original cultivar. EMS treatments induced genetic variation in potato genome which lead to new genotypes that displayed high levels of tolerance to *Alternaria solani*, and these genotypes may be considered new clones. Nine clones were selected; four from cv. Atlas, two from cv. Nicola and three from cv. Simon .Most of the selected clones were induced from 2 mM/l EMS treatment. These clones are very important for disease tolerance and breeding programs due to their distinct features. DNA fingerprinting was done on the original cultivars and the selected clones using the RAPD technique. Genetic differences were detected between selected clones and the original cultivars.

Keywords: *Solanum tuberosum L., Ethyl methane sulphonate (EMS) and Alternaria solani.*

INTRODUCTION

Potato *(Solanum tuberosum* L.*)* Belongs to the family *Solanaceae*. It is one of the important crops all over the world after wheat (*Triticum aestivum*) , maize (*Zea mayz*) and rice (*Orisa sativa*). It is considered as one of the most important vegetable crops grown in Egypt.

Early blight, caused by the *Alternaria solani* (Sorauer) fungus, is one of the main diseases of potato. It occurs in most potato-growing regions world-wide, especially at tropical climates, where potato is grown under irrigation, causing yield-losses through defoliation of the plants. The fungicides used to control the disease are expensive and frequently inefficient (Rodreguez *et al., 2007*). There exists also a growing consumer concern about the high dependency on fungicides in the potato crop, where 10 or more applications per season are necessary to control foliar disease. Developing new potato cultivars with resistance to early blight may reduce losses in the field and in storage, and lessen the need for fungicide applications(Christ and Haynes, 2001).

In foliage, early blight appears as target-like leaf spots on older, often senescing foliage. As the disease progresses, these spots may expand, eventually coalesce and, in severe cases, leaves drop off the plant. Young plants are resistant to early blight but older, more mature plants are much more susceptible (Pelletier and Fry, 1989), and early-maturing cultivars are more susceptible to early blight than later-maturing cultivars. In resistant cultivars as well as in young leaves, the incubation period is long and there are small numbers of lesions, suggesting that resistance mechanisms may

act during the early stages of the infection process. (Christ and Haynes, 2001& Dita *et al.*, 2007).

Induction of mutation in potato and other crops have been used to induce variability for tolerance to biotic and a biotic stresses and produced new cultivars (Sonnino *et al.*, , 1986; Sonnino and Ancora, 1988; Li and Chao, 1994; Ahiabu *et al.*, 1995; El-Fiki, 1997; Das *et al.*, 2000; Asbah, 2007 and Ibraheem, 2008).

Improvement of potato cultivars by mutagenesis offers several advantages over conventional methods of plant breeding which are laborious and time consuming (Das *et al.*, 1999). Potato improvement by traditional breeding methods is slow and unpredictable (Bajaj, 1987).

Application of mutation techniques, using chemical and physical mutagenes, for early blight and disease resistance have been used by a number of researchers, (Love *et al.*, 1993; Gosal *et al.*, 1998; Bhagwat and Duncan, 1998; Das *et al.*, 1999 and Rodreguez *et al.*, 2007).

Rodreguez *et al.* (2007) selected and subsequently evaluated 45 lines, derived from irradiated callus of potato, under conditions of natural infection of *A. solani* in the greenhouse. Six lines showed lesser degrees of early blight infection than the cv. Desiree (control). The six lines selected retained lower degrees of infection during 2 years of field trials. The average infection scores were significantly lower than cv. Desiree.

The aim of this investigation was to study the efficiency of the mutagene ethyl methane sulphonate (EMS) in the process of mutation in three cultivars of potato, namely, Atlas, Nicola and Simon, as well as, to induce genetic variability for selecting mutants with increased level of resistance against early blight.

MATERIALS AND METHODS

This work was carried out at the Experimental Farm, Faculty of Agriculture, Cairo University, during the period from 2006 to 2009 to produce potato genotypes more resistant to early blight disease (caused by *A. solani* fungi) by treating sprouted tubers with the chemical mutagen (EMS) and selection.

Plant source

Potato tubers (*Solanum tuberosum* L.) of cvs. Atlas, Nicola and Simon were obtained from Potato Research Division, Horticulture Research Institute, Agricultural Research Center, Cairo, Egypt.

Preparation of potato tubers for sprouting

Potato tubers were washed under running tap water. The surface of tubers were well dried. Potato tubers were stored in dark condition at room temperature 25±1C° for 10-15 days until sprouting. If the tuber was in the dormancy stage, breaking this dormancy was done by soaking tubers for one hour in 100 mg/l GA_3 and maintained in dark for 10-15 days at 25±1C° until sprouting.

EMS treatments

Sprouted tubers of potato cultivars were soaked in 0, 1, 2 and 3 mM/l water solution of EMS for 3 hours.

Tubers treated with EMS were sown (one tuber/hole) in Summer growing season (December-January) of 2006-2007 (M_0V_1). In the next evaluation and selection seasons, tubers were sown in Faull growing season (September) of 2007 (M_1V_2) and in Summer growing season (January-February) of 2008 (M_1V_3). Agricultural practices were done as it recommended, fungicides application were not used at the evaluation seasons for early blight resistant (M_1V_2 and M_1V_3).

Pathogen isolation:

For isolating the early blight pathogen (*A. solani*), naturally infected potato leaves showing the early blight symptoms were collected. The pathogenic fungi were isolated using potato dextrose agar (PDA) medium in Petri dishes and were incubated at 24°C in the dark. After that, the *A. solani* were identified in Plant Pathology Department, Agricultural Research Centre, Giza, Egypt.

Fungal inocula preparation and plants inoculation

Mycelium suspension of *A. solani* were prepared by inoculated sterilized PDA with disk of fungal growth (6 mm diameter) taken from 10 day old cultures of *A. solani*. Isolate was grown on PDA medium. Mycelium suspension of *A. solani* were prepared by mixing every Petri dish with 10 ml water. Potato plants were inoculated at age of 40 days after planting (DAP).

Early blight was assessed four times with 10 days period between each assay, first one was done at the age of 50 DAP, the second, the third and the fourth were done at 60, 70, 80 DAP, respectively. Recording the intensity of infection was measured according the scale described in Table 1. Clones with early blight infection scores lower than the controls were selected.

Plants originating from the tubers treated with EMS as well as tubers produced by these plants were designated mutant clonal generation one (M_0V_1). Inoculation and selection for early blight resistance began in the M_1V_2 . In the M_0V_1 tubers harvested from each plant were evaluated as a separate entity, to segregate the majority of sartorial chimeric events.

In M_1V_2 generation 270 plant per each cultivar under study were evaluated, 30 plant for every treatment of EMS with the control. Evaluation and selection were done depending on single plant selection at M_1V_2.

Table 1. Scale for evaluation of potato cultivars for *A. solani* infection (Rodreguez *et al.*, 2007).

Rating of infection	Description of symptoms
10	Spots on lower leaves.
20	Spots on most of the lower leaves.
30	Spots on all lower and some of the middle leaves.
40	Clearly developed blight lesions in lower leaves.
50	Blight lesions in lower leaves spread to some middle leaves .
60	Blight lesions developed in all inferior and most of the middle leaves.
70	Blight lesions developed in all lower and middle leaves.
80	Blight lesions developed in all lower and middle leaves and spread to upper leaves.
100	Total blight (death of the plant).

DNA fingerprints

DNA fingerprinting was done to the control and the selected plants. DNA was isolated using CTAB method (Doyle and Doyle, 1987). For RAPD analyses, PCR amplification (polymerase chain reaction) was performed in 0.01 ml reaction mixture containing 20 ng template DNA, 0.5 unit taq polymerase, 200 µM each of dATP, dCTP, dGTP, dTTP,10 p mol random primer (10 mer) and appropriate amplification buffer. The mixture was assembled on ice, overlaid with a drop of mineral oil. Amplification was performed for 45 cycles at 92°C for 3 min, 45 cycles at 92°C for 30 sec., and 35°C for 60 sec. and 72°C for 2 min. for denaturation, annealing and extension, respectively. Reaction was finally incubated at 72°C for 10 min. and farther 10 min. at 62°C. The amplification products were analyzed by electrophoresis in 2% agarose in TAE buffer, stained with 0.2 µg ml^{-1} ethidium bromide and photographed under UV light. Seven random primers were used in this study, viz., OPB-03, OPB-05, OPB-07, OPB-09, OPC-05, OPC-08 and OPC-11.

Experimental design

The experimental design used was the randomized complete block design with three replicates. All selected clones and the original cvs. were randomly distributed in each block. Data were tested by analysis of variance. Duncan's multiple range test was used for the comparisons among the treatment means (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

1. Evaluation of three potato cultivars and selection to *A. solani* resistance

In M_0V_1 generation 90 plants per cultivar were evaluated, 30 plants for every treatment of EMS with the control. Evaluation and selection was done depending on single plant selection at M_1V_2. For next generation evaluation and selection were done depending on clonal evaluation and selection.

Two hundreds and seventy plant per each cultivar were produced from M_0V_1 mutant generation. Each one was evaluated, in terms of their resistance to early blight inoculation, with their original cultivars. Nine clones were selected from all obtained and evaluated cultivars; four from Atlas, two from Nicola and three from Simon .

The mutagenized populations showed variation in susceptibility to *A. solani* infection. Clones showing lower affectation rates have been selected for M_1V_3 evaluation. Data presented in Table 2 show the progress of early blight foliar disease on selected clones from M_1V_2 for the three cultivars. The data indicated that most of the selected clones were from 2 mM/l EMS treatment. At 80 DAP evaluation the lowest value of early blight scoring in the selected clones was 20 for Atlas cv. (A1) and it was 30 for Nicola (N1) and Simon (S1) cvs., while it was 74,74,72 for the control plants of cv. Atlas, cv. Nicola and cv. Simon, respectively.

Table 2 . First year evaluation of selected clones from M_1V_2 in the field (progress of early blight foliar disease).

Cultivar	EMS Treat. (mM/l)	Selected clones	Early blight scoring			
			first evaluation 50 DAP	second evaluation 60 DAP	third evaluation 70 DAP	fourth evaluation 80 DAP
Atlas	2	A1	0	10	20	20
	2	A2	10	20	30	30
	2	A3	0	10	20	30
	3	A4	20	30	40	50
	Co.	Co.	26	42	56	74
Nicola	2	N1	0	10	20	30
	2	N2	10	20	30	40
	Co.	Co.	28	44	60	74
Simon	1	S1	0	10	20	30
	2	S2	10	20	30	40
	2	S3	20	30	40	50
	Co.	Co.	28	42	60	72

DAP: Days After Planting

It is clear from Fig.1 that the nine clones selected of M_1V_2 generation and evaluated in the M_1V_3 generation were significantly more tolerance to *A. solani* with lower disease progress curves and significantly decreased mean infection ratings. There were significant differences in early blight scoring between the selected clones and the control at all evaluation times in the three cultivars. The clones which gave the lowest value of early blight scoring - the highest level of tolerance to *A. solani* - in the three cultivars was the clones namely: A1, N1 and S1 for cv. Atlas, cv.Nicola and cv. Simon, respectively, at all times of evaluation. While the highest value of early blight scoring was noticed by control plants of cvs. Atlas, Nicola and Simon at all times of evaluation. Further field evaluations are necessary to confirm the stability of the selected clones.

The present results seemed to agree with those of Love *et al.* (1993), Gosal *et al.* (1998), Bhagwat and Duncan, (1998), Das *et al.* (1999), Asbah, (2007) and Rodreguez *et al.* (2007).

Bhagwat and Duncan (1998), Treated shoot apices of in vitro-grown cultures of banana *Musa* spp., AAA Group cv. Highgate. with various concentrations of the mutagens sodium azide, diethyl sulphate, and ethyl methane sulphonate to produce variants tolerant to the fungus *Fusarium oxysporum* f. sp. *cubense*. Twelve weeks after inoculation, 4.6, 1.9 and 6.1% of plants regenerated after sodium azide, diethyl sulphate and ethyl methane sulphonate mutagenesis respectively had less than 10% vascular invasion of their corms with no external symptoms of the disease. These plants were considered to be tolerant.

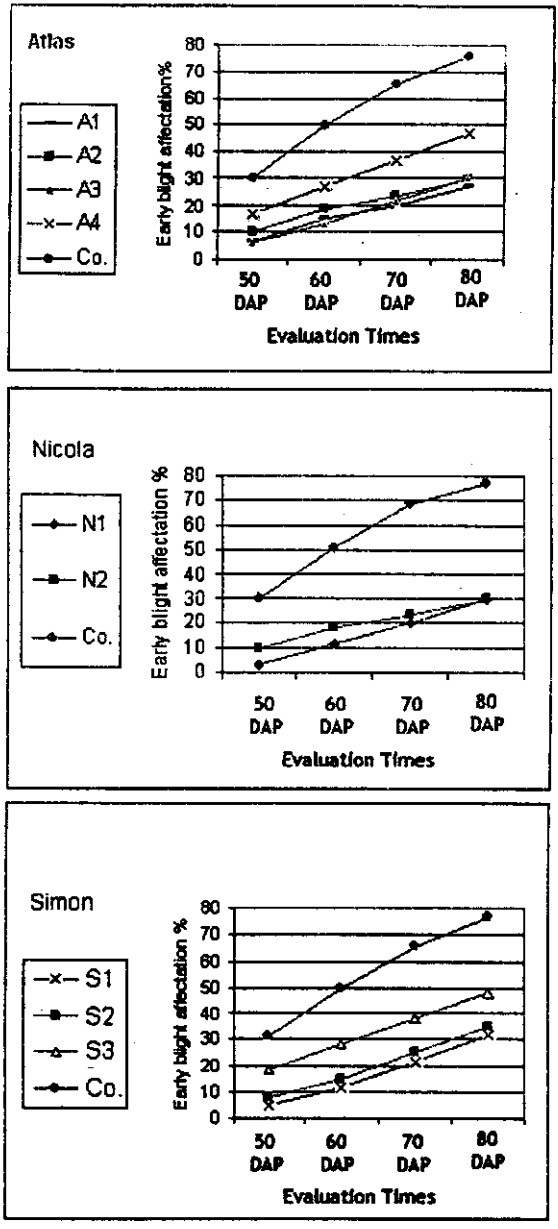

Fig. 1 :Early blight foliar disease progress curve in the second year evaluation of the selected clones (A1, A2, A3, A4, N1, N2, S1, S2, and S3) for cvs. Atlas, Nicola and Simon, respectively with the original cultivars (Co.). DAP: Days after planting.

To induce resistance to late blight caused by *Phytophthora infestans* in potato, Das *et al.* (1999) irradiated plantlets with gamma rays at 20 and 40 Gy. A dose of 40 Gy induced 3 and 2.3 times more resistant plants for cultivars Kufri Chandramukhi and Kufri Jyoti, respectively. Resistant plants *in vitro* were also resistant under field conditions. All the plants obtained from a single tuber were not consistent in the disease resistance, suggesting a segregation of the mutant character.

2- DNA fingerprints

Chemical mutagens cause gene mutation and chromosomal changes, while physical mutagens (gamma irradiation) my cause chromosomal changes rather than gene mutation (Mike and Donnini, 1993 & Chahal and Gosal, 2003).

The genetic variability among the newly developed clones of potato and their original cultivars, based on randomly amplified polymorphic DNA (RAPD) relationships analysis was studied using seven random 10-mer primers. The distribution of the polymorphic bands is shown in Table 3 and figure 2 which reflects the variability among the studied genotypes.

Table 3 . List of seven 10-mer random primers and amplification results with the original cultivars (Aco, Sco, Nco) and the selected clones (A1, A2, S1, S2, N1) for cvs. Atlas, Simon and Nicola, respectively.

Primers	No. of bands							
	A1	A2	ACo	S1	S2	SCo	N1	NCo
OPB-03	8	8	8	8	6	7	8	7
OPB-05	8	8	6	6	6	7	5	4
OPB-07	6	5	5	8	7	7	7	6
OPB-09	8	7	7	5	4	3	3	4
OPC-05	6	5	7	12	10	11	13	11
OPC-08	7	5	5	6	6	8	6	8
OPC-11	10	8	7	10	10	10	7	9
TOTAL	53	46	45	55	49	53	49	49

Aco, Sco and Nco: the original cultivars, A1, A2, S1, S2 and N1 the selected clones for cvs. Atlas, Simon and Nicola, respectively.

DNA fingerprinting was done for the original cultivars as well as for the selected clones (A1, A2, N1, S1, S2) which showed high levels of tolerance to *A. solani* fungi. The amplification results of the potato genotypes, using the selected primers, were analyzed and summarized as follows:

Table 3 and Fig. 2 shows a list of seven 10-mer random primers and amplification results with the original cultivars (control) and the selected clones (A1, A2, S1, S2, N1). It indicated that the molecular weights of PCR products, for example generated by primer OPB-03, ranged from 472 bp to 1903 bp. It gave a maximum of nine amplification products. It is also noted that there were identified common bands among the studied clones for each cultivar with all primers. In this connection, S1,S2 and SCo. Simon clones had 8,6,7 fragments, respectively, but only the fragments No. 3,5,7 and 8 were present in all of them, while the others were present in some of them and absent in others.

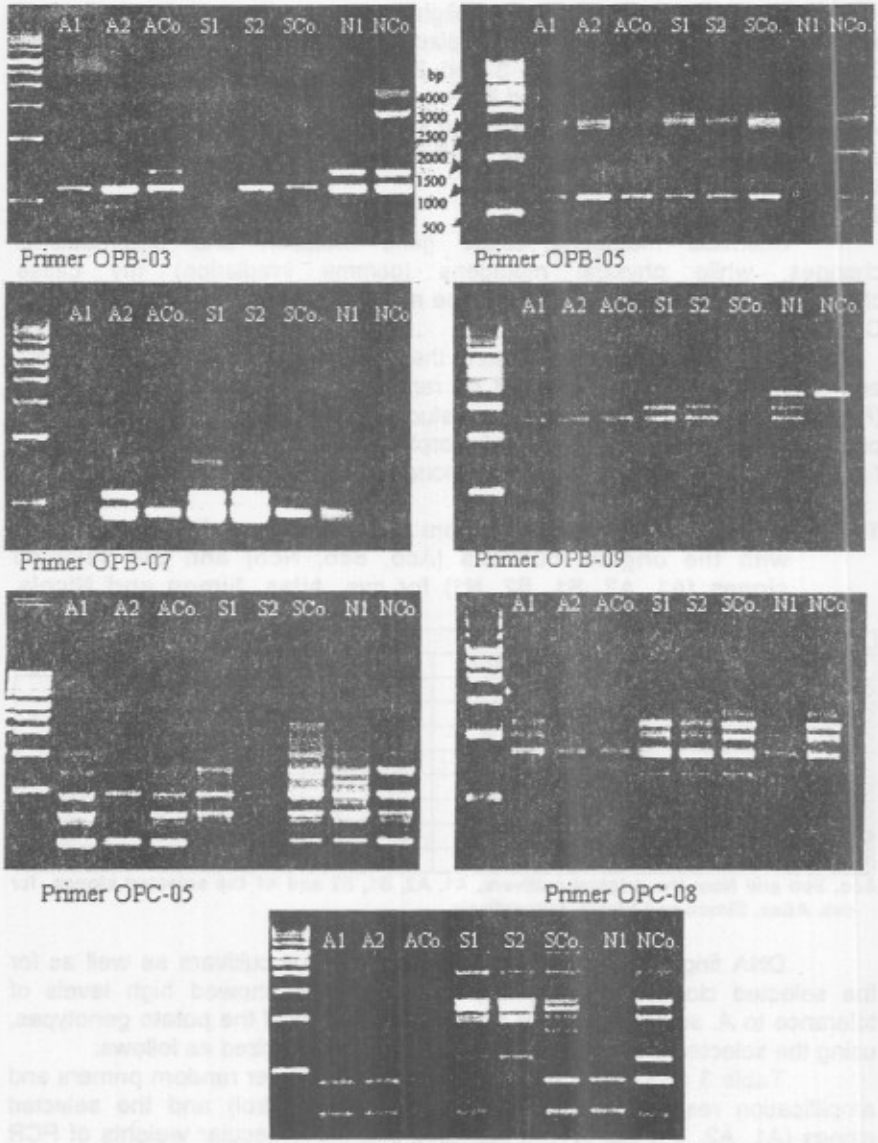

Fig. 2: RAPD patterns of genomic DNA of the original cultivars (ACo, SCo, NCo) and the selected clones (A1, A2, S1, S2, N1) for cvs. Atlas, Simon and Nicola, respectively, using the random primers : OPB-03, OPB-05, OPB-07, OPB-09, OPC-05, OPC-08 and OPC-11.

The bands were polymorphic at the used primers amplification, as they were present in some genotypes and absent in the others. Some genotypes had some specific bands with some primers and could be used to distinguish them; as found in S1 Simon line for example, which could be distinguished from the others by the existence of one unique fragment at the molecular weight of 820 pb with primer OPB-03.

On the other hand, a band with different molecular weight was found in some of genotypes, which indicated that these bands might be considered as specific markers for these genotypes.

The present results seemed to agree with those of other researchers (EL Demerdash, 2000; Stefano, 2001; Atak *et al.*, 2004; Harb *et al.*, 2005; Badawi *et al.*, 2006 and Ibraheem, 2008) whom found that RAPD markers were useful for separating different species, strains and cultivars.

In conclusion, the consensus fingerprint profiling, using the RAPD markers, is a useful and reliable method for establishing genetic identities of the potato cultivars and advanced selections from plants treated with mutagenesis. It provided also an improved discrimination way for evaluating genetic diversity and relatedness.

EMS treatments induced new genetic variation in potato genome which leads to new genotypes, that displayed high levels of tolerance to *A. solani* enabling them to be considered new clones. In the present study, we select nine clones named A1, A2, A3, A4, S1, S2, S3, N1 and N2. These clones are very important for disease tolerance and breeding programs due to their distinct features. It is important to keep and multiply them for other studies and subject them for further evaluation.

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التربية بالطفرات لمقاومة الندوة المبكرة في البطاطس

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استخدمت المادة المطفرة إيثايل ميثان سلفونات (EMS) بتركيزات ٠، ١، ٢، ٣ ملليمول/لتر في ثلاثة أصناف من البطاطس هي أطلس ونيكولا وسيموني ، بهدف استحداث اختلافات وراثية و انتخاب طفرات ذات مستوى عالي من المقاومة لمرض اللفحة المبكرة. تم الحصول على ٢٧٠ نبات من كل صنف ناتجة من المعاملة بالمطفر EMS والإكثار الخضري (M_0V_1). قيمت هذه النباتات مع الأصناف التي نتجت عنها حسب درجة مقاومتها لمرض اللفحة المبكرة. أدى استخدام الـ EMS إلى استحداث اختلافات وراثية نتج عنها طرز وراثية جديدة من البطاطس أظهرت مستويات مرتفعة من التحمل لمرض اللفحة المبكرة، بحيث أمكن اعتبارها سلالات جديدة على درجة كبيرة من الأهمية في برامج التربية لمقاومة المرض. تم انتخاب تسع سلالات، أربعة من الصنف أطلس واثنتان من الصنف نيكولا وثلاثة من الصنف سيموني. وكانت معظم السلالات المنتخبة قد نتجت من المعاملة بتركيز ٢ ملليمول/لتر EMS . تم إجراء اختبار البصمة الوراثية للأصناف المدروسة و للسلالات المنتخبة باستخدام تقنية الـRAPD ، حيث لوحظ وجود اختلافات وراثية بين تلك السلالات المنتخبة مقارنة بالأصناف التي نتجت عنها.