Egypt. J. Chem. **Vol. 65**, No. 6 pp. 477 - 487 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

In vitro Screening and Molecular Genetic Markers Associated with Fungal Pathogenic Toxin Filtrate Tolerance in Potato

Ismael A. Khatab[1] and Almoataz Bellah Ali El-Mouhamady[2*]

[1] *Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, 33516, Egypt.*

[2] *Department of Genetics and Cytology, Biotechnology Research Institute, National Research Centre, 33 El Buhouth ST., Postal Code 12622, Dokki, Cairo, Egypt.*

Abstract

Potato is the fourth important most food crop in the world and Egypt as well after rice, wheat and corn. Cultivated potatoes are tetraploid and highly heterozygous, making conventional breeding difficult, their vegetative propagation causing degeneration and accumulation of diseases. Callus induction and plant regeneration of some potato varieties (Cara, Diamont, King-Edward, Nicola and Hermes) were conducted. Stem segments, leaf disc and root tips explants were evaluated on the basis of their growth and ability on callus induction and regeneration media. The impacts of PGRs, cultivars and explant exporters on callus induction media were differ, and Nicola cultivar on CM4 (MS supplemented with 5 mg / L NAA + 1 mg/ L KIN) produced 100% callus induction with highest callus weight 750.5 mg using stem segments explant followed by Diamint 90% on CM4. The produced calli from stem segments occurred with high percentage 70 % for Diamont on RM1 (MS+ 2.25 mg/ L BA + 5 mg / L GA3). Steam segments of Diamont potato cultivars were used for callus induction and plantlets regeneration under culture toxin filtrate stress as *in vitro* selection of tolerance/resistance to *F. oxysporum* and*A. solani*toxin filtrate at 0, 5, 10, and 15 % on the callus induction on CM4 with the selection pressure and plant regeneration on RM1. No any plantlets produced under selection with *A. solani*. In most cases, under selection of *F. oxysporum* increasing toxin filtrate in the tissue culture medium resulted in reduction in plantlet growth potential in erratic degrees, the highest percentage of regenerated calli and number of the obtained 15, 12 and 3 somaclones were obtained from the calli that were produced under 5, 10 and 15 % and no any stress on regeneration phase. However, using 10% toxin filtrate for produced calli under stress with 5, 10 and 15% of filtrate the regeneration decreased and were 5, 4 and 1 somaclones, respectively.RAPD and SRAP primers generated 48and 50 bands in total respectively with highly polymorphic patterns among the original plant and its somaclones. The actual investigation signalized that the use of RAPD and SRAP – DNA markers were vigorous to disclose genetic diversity at the molecular grade between potato cultivars and their regenerates and somaclones besides, could be allows detecting changes due to *in vitro* selection to pathogens.This is the new in the process of genetic improvement to raise the resistance degree of potato for various diseases that threaten its final yield.

Keyword: Potato, *In vitro* selection, somaclonal variation, Pathogenic Toxin Filtrate, RAPD and SRAP markers.

1. Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world and Egypt as well after rice, wheat and corn. The global production in 2018 reached 368.2 million ton **[1].** Egypt shares in export value 6.25% from world production in 2020 with export value, 217.55M USD **[2].** Potato is one of the most important food crops in the world especially in Egypt. Cultivated potatoes are tetraploid 2n = 4x = 48, and highly heterozygous, making conventional breeding difficult. Potato is propagated by vegetative means causing degeneration and accumulation of

*Corresponding author e-mail: moaatazelmohamady@gmail.com.; (Almoataz Bellah Ali El-Mouhamady).
Received date 17 January 2022; revised date 12 February 2022; accepted date 13 February 2022
DOI: 10.21608/EJCHEM.2022.116899.5279

diseases. Traditional methods of potato growth and breeding are fairly slow, opening the door for recent approaches to breeding at the cellular, expression and DNA levels, with biotechnology helping hurry up proliferation. Potato has an ability to multiply and grow in controlled *in vitro* conditions under synthetic nutrient medium. Therefore, it helps to large scale production of potato cultivar without harmful pathogens, it is multiplied by different methods, but nodal cuttings are broadly used **[3].**One of the fundamental tools of plant science research is plant tissue culture, it has widely employed in the improvement, production and conservation of plant genetic resources. Moreover, *in vitro* selection and somaclonal variations are the most researched and applied issues in tissue culture for vegetative crops such as potato. Somaclonal variations were defined as variations/changes that originating in cell and calli cultures by Larkin and Scowcroft**[4].** Somaclonal variations in plant tissue culture are an effective technique for selecting new somaclones and it offers an unlimited scope for breeders. Tissue culture techniques and somaclonal variation have proven to be excellent approaches for obtaining mutants or cell lines/somaclones to solve this problem. In tissue culture techniques, *in vitro* selection/screening is one approach for obtaining useful genetic variation which offers enormous ability for detecting somaclonal variants for disease tolerance/resistance. Studies including *in vitro* selection procedures use pathogen toxin filtrates **[5].** The selected cells are afterward regenerated into somaclones and resistant plants **[6 , 7],** toxic filtrates are one of the arms used by the microbe inducing disease in sensitive crops. Some of the workers have used *P. cactorum* culture filtrate to screen for apple resistant and their regenerants plantlets **[8].** Yet, reports on the selection of somaclonal variations with tolerance/resistance to pathogen toxin filtrate are rare. The results of some studies which used many kinds of stress in the selective medium, callus proliferation and in vitro regeneration capacity were reduced in tomato **[9]** and potato **[10 , 11]** were used PEG, and pathogen toxin filtrate in mustared **[12]** and apple **[8].** Many fungal diseases especially early blight and *Fusarium* wilt cause a decrease in potato quantity and quality. Modern potato production systems depend on the production of nuclear stock of plants free from diseases especially fungal diseases and multiplying it *in vitro* selection **[13].**Yet, an *in vitro* techniquewas used by many researchers to investigate the effects of fungal toxins filtrates on different plant species. *Fusarium* wilt is vascular diseases affect many vegetative crops especially potato, caused by the soil/tuber fungus *Fusarium oxysporum.*This pathogen remained in the soil for long periods of time, use of resistant cultivars considered only an effective control methods. *Alternaria* is a fungus affect plants and produce severe harm in economically important crops, including, vegetables, and other crops, itis able to producing host-selective toxins during the growth on plant surfaces **[14].**Produced somaclonal variants could be tolerant to stress and pathogen toxin filtrates which were selected and utilized as non-conventional breeding method for the improvement of crop against biotic and abiotic stress. *In vitro* selection is now used for obtaining new variants with complex agronomic characters in important vegetative crops such as potato, which has achieved new variants with increased yild and other traits **[15].**Mutation breeding is increasingly considered to be a great and alternate method for genetic variations for many plant breeding; tissue culture offers the opportunity for large populations in a restricted spaceto get the food needed for their live and decreasing the time consumed until the selection of a genotype could be performed. Tissue culture offers the opportunity of making an early selection of somaclones **[16].** Moreover **[17-19]** use tissue culture procedure for *in vitro* selection in potato and sugarcane under different species of *Alternaria* and *Fusarium* respectively. Somaclonal variations are a new variants in plant tissue culture giving rise to the production of new cultivars which are produced in potato, such as plant height, , fruit shape, flower color and habit are improved in selected variants. Cultivars resistance / tolerance to abiotic (salinity, drought, water logging, toxins) and biotic (insects, disease) stress are developed from *in vitro* genetic variations **[15].** Detecting morphological and biochemical changes in plants could be useful in many studies, but there is limited diversity and trait may be influence by environmental conditions. Meanwhile, DNA markers providing valued tools in numerous analyses. Molecular markers using (RAPD) and (SRAP) primers are often preferred over traditional phenotypic, cytological and biochemical analysis, and usually detect any small variations in the

genome. The main aims of this study were to evaluation of callus induction and regeneration for some potato cultivars, to study regeneration ability of produced calli under *in vitro* selection with pathogenic fungal filtrates and detect of somaclonal variation and *in vitro* plantlets (somaclones) under stress using genetic markers RAPD and SRAP as serious attempts in the path of genetic improvement to resist potato diseases and give the highest output of them, in light of the global food crisis that threatens the vast majority of people.

2. Materials and Methods

Potato cultivars and Micropropagation

The studied five potato varieties, Cara, Diamont, King-Edward, Nicola and Hermes were used in this investigation. Sprouts were sterilized by immersing in 70% ethanol for 2 min, washed with distilled sterilized water three times to remove the traces of Ethanol, then sterilized in 20% (v/v) Clorox , then washed three times with sterilized distilled water. Disinfested sprouts were put on sterilized filter paper and cultured on MS medium [**20].** The cultured shoot tips were incubated for four weeks in the incubation room at 25±2ºC with 16:8 h photoperiod and 2000 – 4000 lux, as a light intensity.

Callus induction and plant regeneration

Three explants; stem segments, leave discs and root tips were cultured on MS medium supplemented with different combinations and concentrations of plant growth regulators (PGRs), 2,4 - Dichlorophenoxy acetic acid (2,4- D), Naphthalene acetic acid (NAA), Benzyladenine (BA), Gibberelic acid (GA_3), Zeatin and Kinetin (KIN.). Four callus induction callus media CM (CM1, CM2, CM3 and CM4) were used for callus induction.
CM1: MS + 2.4 mg / L KIN. + 0.8 mg/ L NAA.
CM2 : MS + 5 mg / L 2,4- D + 1mg / L KIN.
CM3: MS + 5 mg / L 2,4 - D + 1mg/ L KIN. + 0.5 mg/ L NAA
CM4 : MS + 5 mg / L NAA + 1 mg/ L KIN.
The used explants were cultured on different callus induction media in complete dark for four weeks at 25 ± 2ºC. The formed calli were transferred to the same new fresh callus induction media every 21 days for more proliferation. Callus induction percentages, callus fresh weight and morphological appearance were determined. Well-developed calli were cultured on four different regeneration media RM (RM1, RM2, RM3 and RM4) for shoot regeneration and kept at 25 ± 2ºC with photoperiod of 16 h of light using Phillips cool white fluorescent tubes (1500 Lux) and plantlets regeneration percentage was determined.
RM1: MS+ 2.25 mg/ L BA + 5 mg / L GA3.
RM2: MS + 2.25 mg/ L BA + 10 mg / L GA3.
RM3: MS + 0.5/ L BA + 0.2 mg/ L GA3 + 0.1 mg/L KIN.
RM4: MS + 1mg/ L KIN + 0.2 mg/L NAA

Preparation of pathogen toxin filtrate

*Alternaria solani***,** the causal agent of early blight and *Fusarium oxysporum*, the causal agent of *Fusarium* wilt were obtained from the Agriculture Research Center. Pure cultures of fungal were maintained for 15–20 days at 25ºC until uniform mycelial growth was obtained. The small bits (2.0 mm^2) of mycelium were inoculated separately in potato dextrose broth and incubated for 23–25 days at 26+2ºC in stationary conditions until the mycelial growth. These cultures were used for preparation of the culture filtrate after centrifugation at 10,000 rpm, passing through a double disc of Whatmann filter paper and finally through a Millipore filter (0.20 µm pore size). The sterilized culture filtrates of the two isolates were kept in the growth room to check for any types of suspension left. The selective media were prepared by mixing culture filtrate in concentrations of 5, 10 and 15% (v/v) with CM4 medium.

In vitro screening

Four-week-old healthy regenerated shoots were cultured on selective media and incubated similarly as mentioned before. calli tolerant to high concentration of culture filtrate were isolated and subjected to 10% of RM. Continuous selection was performed by using critical concentration of culture filtrate one after the other cycle to further select the tolerant regenerants. In discontinuous selection approach, a pause on culture filtrate free medium was given to the tolerant regenerants to obtain growth. Growth and multiplication rate of regenerants survived three selection cycles were observed. All the treatments were compared with control, calli and shoot induction were determined.

Molecular analysis

Produced plantlets subjected to RAPD and SPAP analysis using nine and six primers respectively as listed in Table 1. DNA was extracted from fresh Diamont somaclones leaves and their original by Cetyltrimethyl Ammonium Bromide (CTAB) according to Doyle and Doyle**[21].** RAPD was

performed using 10 random and nine SRAP primers, (Table 1). Polymerase Chain Reaction (PCR) was carried out in presence of 1X Taq DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2), 100 µM dNTPs, 5 picomole single random primer, 25 ng template DNA, 0.5 unit of *Taq* DNA polymerase in a total volume of 25 µl. PCR amplification was performed in automated thermal cycler (MJ-Mini, Bio Rad) programmed as follow, 95ºC for 4 min followed by 35 cycles of 1 min for denaturation at 94ºC, 30 sec for annealing at 37ºC and 1 min for polymerization at 72ºC, followed by a final extension step at 72ºC for 7 min. For SRAPPCR cycling was carried out as the following program; initial denaturation at 94 °C for 4 min, followed by five cycles comprising for 1-min denaturation at 94 °C, 1-min annealing at 35 °C, and 30 s of elongation at 72 °C. In the following 30 cycles, denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 72 °C for 30 s were carried out, ending with an elongation step for 10 min at 72 °C. The amplification products were resolved by electrophoresis in 1.2 % agarose gels in 1 X TAE buffer and documented on Gel Documentation UVITEC, UK.

Table 1. List of multiplexing sets of the used RAPD and SRAP primers and their sequences

RAPD 5′--3′			
OPA-04	**AATCGGGCTG**	**OPA-14**	**TCTGTGCTGG**
OPA-06	**GGTCCCTGAC**	**CA-1**	**AGGTCACTGA**
OPA-11	**CAATCGCCGT**	**CA-2**	**AAGGATCAGA**
OPA-12	**TCGGCGATAG**	**CA-3**	**CACATGCTTC**
OPA-13	**CAGCACCCAC**		
SRAP 5′--3′			
me1+em1	**TGAGTCCAAACCGGATA**	**GACTGCGTACGAATTAAT**	
me1+em2	**TGAGTCCAAACCGGATA**	**GACTGCGTACGAATTTGC**	
me1+em3	**TGAGTCCAAACCGGATA**	**GACTGCGTACGAATTGAC**	
me2+em1	**TGAGTCCAAACCGGAGC**	**GACTGCGTACGAATTAAT**	
me2+em2	**TGAGTCCAAACCGGAGC**	**GACTGCGTACGAATTTGC**	
me2+em3	**TGAGTCCAAACCGGAGC**	**GACTGCGTACGAATTGACs**	

3. Results

Plant breeding using the tissue culture technique is one of the most important scientific methods for the propagation of crops, as well as the production of plant lines that are tolerant to biotic and a biotic stresses in a short time and this is a global demand in light of the large and escalating food crisis coinciding with the increase in the world's population.Among the most important biological obstacles are diseases that affect all plant parts with various kinds, such as fungal, viral and insect diseases.Where this method helped in the process of creating molecular genetic changes that led to the production of somaclones under *in vitro* conditions that differed from the original varieties derived from it and outperformed them in resisting diseases, especially the fungal pathogenic toxin filtrate in Potato. This is what we will present in detail in this context. The effects of studied five varieties, three explant sources and four combinations and interaction of PGRs on the callus induction are presented in Table 2. There were an enormous gauge of callus induction percentage (%) and callus weight (mg) relying on different concentrations of auxins and cytokinins, explant source and varieties. The results displayed that there was a notable and clear variation among calli based on PGRs used. The data showed that the majority of stem segments explant (S) over all tested cultivars consistently produced higher percentage of callus induction using all tested CIM media. The influences of PGRs, cultivars and explant sources on callus induction were differ, and Nicola cultivar on CM4 (MS supplemented with 5 mg / L NAA + 1 mg/ L KIN) produced 100% callus induction with highest callus weight 750.5 mg using stem segments explant followed by Diamint 90% on CM4 as shown in (Tables 2 & 3 and Fig 1a). When the root tips were used as explant the tested cultivars no calli produced from Cara cultivar on CM2, CM3 and M4. The same findings were obtained from diamont and Nicola when cultured on CM3. Out of the above recorded data regarding the interaction between cultivars /callus induction media / explant, callus induction percentage and callus fresh weight varied dramatically. However, steam segments proved to be superior explant over leaf disc and root tips, Moreover, CM4 exhibited the highest callus induction and callus fresh weight 650.5mg for Nicola followed by Diamont and King-Edward recorded 510.0 mg and 370.8mg, respectively.

Table 2. Callus induction percentage of the five potato varieties cultured on four induction media using three explants

Cultivar / Media	Cara			Diamont			King-Edward			Nicola			Herms		
	S	R	L	S	R	L	S	R	L	S	R	L	S	R	L
CM1	60	20	70	70	60	70	30	10	80	50	10	20	80	70	80
CM2	40	0	0	60	20	0	60	40	50	70	20	0	50	40	10
CM3	30	0	30	60	0	20	40	40	80	50	0	10	70	70	60
CM4	60	0	70	90	30	10	60	30	60	100	30	60	80	80	60

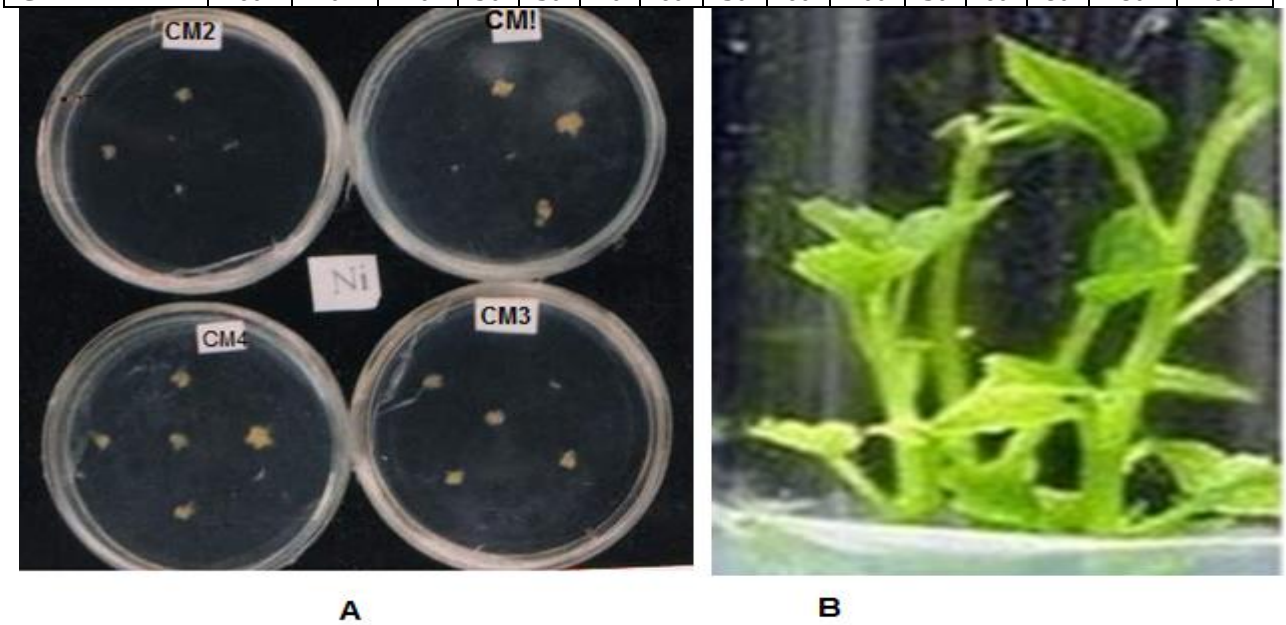

Fig 1. A: Differential response to callus induction using Nicola stem segment on different callus induction media and

B: Plant regeneration of diamond cultivar on RM1

Table 3. Callus fresh weight (mg) of the five potato varieties cultured on four induction media using three explants

CultivarMedia	Cara			Diamont			King-Edward			Nicola			Herms		
	S	R	L	S	R	L	S	R	L	S	R	L	S	R	L
CM1	37.5	0.5	180.0	316.0	98.5	5.5	150.7	0.8	25.8	420.5	0.5	2.0	105.0	98.5	138.5
CM2	98.1	0.0	0.0	122.3	89.8	0	250.9	2.0	112.3	480.6	0.5	0	350.5	20.6	128.9
CM3	129.9	0.0	150.0	185.6	0.0	0.8	180.5	35.6	119.5	490.8	0	5.6	250.0	75.4	298.8
CM4	260.0	0.0	250.0	510.0	2.0	3.4	370.8	50.6	150.4	650.5	8.0	150.0	320.0	150.8	220.5

Plant regeneration

The ability of plant regeneration of calli tested for different RM and no any regeneration occurred from root tip in all cultivars, similarly for cultivars Cara and Herms using the three explants. The regeneration occurred only for Diamont, King Edward and Nicola using stem segments and leaf disc. The produced calli from stem segments occurred with high percentage 70 % for Dimont on RM1 as shown in (Fig. 1b)followed by 50% for Nicola on RM1. Since regeneration of potato from calli is considered an important for potato improvement and affected with many factors especially PGRs and source of explant, this is clearly found in our data.

Table 4. Plant regeneration percentage of the three potato varieties cultured on four induction media using stem segments and leaf disc explants

Cultivar / Media	Diamont		King-Edward		Nicola	
	S	L	S	L	S	L
RM1	70	30	10	5	60	0
RM2	50	10	0	0	30	10
RM3	20	0	10	0	0	0
RM4	10	0	0	0	0	0

In vitro selection

Steam segments of Diamont potato cultivars were used for callus induction and plantlets regeneration

under culture toxin filtrate stress as in vitro selection of tolerance/resistance to *F. oxysporum* and*A. solani*toxin filtrate at 0, 5, 10, and 15 % on the callus induction on CM4 with the selection pressure and plant regeneration on RM1.

Table 5. The callus induction percentage of Diamont stem explant on CM4 under different concentrations of *F. oxysporum* toxin filtrate

Toxin filtrate concentration	*F. oxysporium*	*A. solani*
0	92.6	92.6
5	85.6	45.6
10	45.6	15.3
15	20..0	10.3

Data in table (5) showed that the percentage of callus induction of Diamont stem explants on CM4 it noticed that the callus induction was declined by increasing of the fungal filtrate concentration. It was noticeable that the callus induction percentage decreased greatly following the treatment with different concentrations of *F. oxysporum* and *A. solani*toxin filtrate. The decrease for *A. solani* higher than *F. oxysporium.* To study the regeneration ability of the obtained calli under stress of the *F. oxysporum* and *A. solani* toxin filtrate doses, all the obtained calli of stem explants were transferred onto the regeneration medium RM1suplimented with 10% of *F. oxysporum* toxin and *A. solani* filtrate as intermediate dose of *F. oxysporum* toxin filtrate. There was no any regeneration under *A. solani.* Table 6 presents the number of plated calli and regenerated plantlets obtained following different either during callus induction or regeneration phases of steam segments of Diamont under different concentrations of *F. oxysporum*. Also, the results showed that the highest percentage of the regenerated calli and number of the regenerated shoots/obtained plantlets. The data in table 6 indicated that the highest percentage of regenerated calli and number of the obtained 15, 12 and 3 somaclones were obtained from the calli that were produced under 5, 10 and 15 % and no any stress on regeneration phase. However, using 10% toxin filtrate for produced calli under stress with 0, 5, 10 and 15% of filtrate the regeneration decreased and was 14, 5, 4 and 1 somaclones, respectively. Results in this investigation proved that the culture comprising 15% pathogenic Fusarium toxin filtrate was fit for selecting potato somaclones resistant to toxic culture filtrate of the fungus.

Table 6. Plant regeneration of Diamont stem explant under *F. oxysporum* toxin filtrate

Somaclons	Stress (%) treatment CM 4	RM1	No. of plate calli	Regenerated calli No.	%	No. of plantlets
1	0	0	20	14	70.0	20
2	5	0	20	3	15.0	15
3	10	0	16	2	12.5	12
4	15	0	15	1	6.6	3
5	0	10	25	3	12.0	14
6	5	10	15	2	13.3	5
7	10	10	10	1	10.0	4
8	15	10	10	1	10.0	1

Molecular analysis

Molecular markers are considered one of the most important modern techniques in genetics, which have made fruitful attempts in various sciences, especially agriculture, with the aim of genetic improvement of various crops to tolerate environmental stresses such as high salinity, toxicity of heavy elements and water stress along with resistance to widespread diseases that affect various crops and destroy the final output.From this, this work focused on the use of two types of molecular genetic markers (RAPD and SRAP) primers for trying to compare and differentiate between the original potato varieties under investigating and the most susceptible to pathogens and their somaclones resistant to toxic culture filtrate and resulting from *in vitro* selection to pathogens.This is what will be listed in the following results in this regard. Nine RAPD and six SRAP primers were tested for their capability to amplify the genomic DNA of the original micropropagated potato plants its regenerants and somaclones. Primers show different DNA banding patterns for and it regenerants in comparison with sonaclones produces from *in vitro* selection under pathogenic toxin filtrate. RAPD primers generated 48 band in total, out of them 27 band were polymorphic, resulting polymorphic rate with values between 33.3% in case of OPA 4 and 80.0% for primer OPA6. Primer OPA 6 generated the highest no., of bands (10 bands).The number of DNA

bands ranged from 6 to 10 based on the primer sequences and the source of DNA plantlets with a mean value of 8 bands per primer (Table 7). Similarly, using SRAP primer combinations generated 50 bands, out of them 28 band were polymorphic, resulting polymorphic rate with values between 0.0% in case of me2+em3 as a monomorphic primer and 90.9% for primer me2+em1. Lane 8 which produced under stress with 10% of toxin filtrate in both callus and regeneration phases showed extra bands not recorded in original plant or regenerants with band size 600, 350 and 300bp for RAPD and bands with molecular sizes 1050 and 300bp which were not detected in the control and other somaclones. These primers could be used as a marker to distinguish potato somaclones resistance/tolerance to *Fusarium* toxin filtrate.

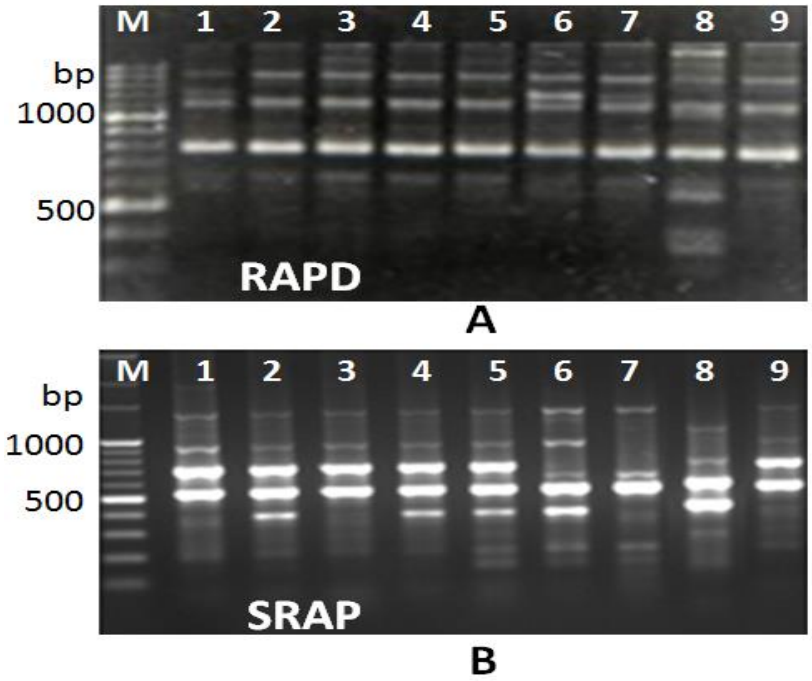

Fig 2. Amplification DNA banding patterns of the RAPD primer OPA-6 (A) and SRAP primer combination me2+em1 (B) of potato original plant lane 1, regenerated plantlets without any stress lane 2 and somaclones under pathogenic toxin filtrate stress lanes 3-9

Table 7. Polymorphism of potato and its somaclones using RAPD and SRAP primers

No.	primers	Number of bands		Polymorphism (%)
		Total No of bands	Polymorphic	
1	OPA 4	6	2	33.3
2	OPA 6	10	8	80.0
3	OPA 11	7	4	57.14
4	OPA 13	8	4	50.0
5	OPA 14	9	6	66.6
6	CA2	8	3	37.5
Total		48	27	-
7	me1+em1	10	5	50.0
8	me1+em2	9	7	77.7
9	me1+em3	6	2	33.3
10	me2+em1	11	10	90.9
11	me2+em2	10	4	40.0
12	me2+em3	4	0	00.0
Total		50	28	

4. Discussion

Herein we success in this a protocol to regenerate of potato plants by using different explant *in vitro* depends on many factors, the most important of which are genotypes, explant source, PGRs concentrations and combinations**.** Thus, this study is interested with finding the most appropriate PGRs combinations to obtain a high percentage of callus induction and plant regeneration to use the best one for *in vitro* selection with toxin filtrate of *F. oxysporium* and *A. solani*. Kumaly and Ercisli**[22]** studied the effect of many combinations and concentrations of PGRs on callus induction, shoot proliferation and root regeneration of three potato cultivars by using (MS) medium containing benzyl amino purine (BAP) and naphthalene acedic acid (NAA) **[20]**. Stem segments proved a better performance for callus induction compared to leaf disc. Shoot promordia when sub-cultured on MS media supplemented with different concentration of 2.0 mg/ L and BAP 0.25 mg/ L GA3. Additionally, BAP confirmed as a better response in terms of shoots regenration, shoot length and number of leaves and nodes in different potato cultivar **[23].**Dhital et al.**[24]** Summarized that the internode stem explants produced calli and shoot regeneration higher than leaf disc the reason for this is referred to the mature and more vascular internodes tissue. Moreover, when it is added GA3 to MS medium, it plays a relatively comprehensive spectrum role for cell development and cell division. GA3 stimulates shoot elongation and node enlargement. It is well known that cytokinins motivate plant cell division, in the stimulation of adventitious buddevelopment **[25].**Ideal concentration and exact ratios of PGRs (auxins and cytokinins) are essential for efficient callus induction and plant regeneration. PGRs also favorably increase the amount of division in cells which already have genetic abnormalities. High concentration of cytokinins involved increasing chromosome number in produced somaclones in many crops **[26 , 27].** Meanwhile, high rate of auxins increase genetic changes by increasing DNA-methylation rats **[28].** Similarly, using 2,4-D or NAA that is commonly used in callus induction has been associated as cause of somaclonal variation and causing many of genetic aberrations such as polyploidy and the stimulation of DNA synthesis **[29].** Synthetic PGRs have been associated with somaclonal variation **[30].** Somaclonal variation may be an important tool to increase the rate of genetic variation in many crops, and many new cultivars have been recorded using *in vitro* selection and somaclones breeding schemes for potato with various aims in mind for salinity tolerance and the late blight disease in potato using various approaches. Cultivars, source of explant and PGRs combinations play a vital role in callus induction and plant regeneration as well in transformation efficiency among potato varieties. Observed that Nicolla and Diamont cultivars showed over all highly callus induction percentage and fresh weight on CM4, **[13 , 31]**. They reported that the callus induction from potato explants depends largely on cultivar, explant and callus induction media and hormones combination. Where, the combination of NAA and Kin PGRs on CM4 was reported by many studies and produced many good and healthy calli. This result is in support of the results obtained by **[32 , 33].** Among all the growth regulators used NAA was found to be the most effective growth regulator for potato callus induction either when used alone or in combination with cytokines. Stem segments explants were inoculated on various induction media with different combinations and concentrations of NAA and KIN and the shoots were subjected to proliferation medium with different combinations of NAA and BAP for regeneration **[34].** Somaclonal variations are important in plant tissue culture and give escalation to the production of new varieties. Most of traits were improved in selected variants, which were tolerance to abiotic (salinity, drought, water logging, toxins) and resistance biotic (disease, insects) stress **[15].** Moreover, banana developed from *in vitro* selection and genetic variations are the changes selected having resistance to tolerance to water logging and *Fusarium* wilt **[15].** Similarly, Suthar et al.**[35]**used *in vitro* selection of resistant somaclones using culture filtrates is tried which could lead to the development of new cultivars resistant Cumin to *Fusarium*. Potato protoplasts resistant to *Alternaria solani* were regenerated from 'Rssset Burbank' and 'Bintje' cultivars **[36, 37].** This method has been used to select rice resistance to brown spot disease **[38].***In vitro* mutation induction on *F. oxysporum* culture filtrate (FCF) were evaluated and screened at molecular level using RAPD and SRAP techniques have been successfully used by many researchers to authorize genetic uniformity and to detect banana somaclonal variants **[39 , 40],** and RAPD in potato **[41 , 42].** At the molecular level, differences in somaclones resulting from indirect changes in the DNA. Technically, this may be done at an early stage prior to the significant time and expense of achieving full regeneration. Currently, there are many kinds of molecular markers are available to detect DNA variation between closely related plants including changes among original plants and their regenerants or somaclones. These markers include the use of DNA markers which are beneficial in comparing the different samples due to genetic variation by identifying polymorphisms and

DNA banding patterns DNA molecular markers are able to recognize a particular DNA sequence **[43].** Somaclonal variation is often prompted by the composition of the culture media and subculturecycles. RAPD and SRAP, a total of 48 and 50 amplicons were amplified with nine and six primers respectively. Our results indicated that a RAPD and SRAP markers allow discriminating of the produced somaclones for induction of somaclonal variation. This conclusion is similar to the results obtained by Mangolin et al.**[44]** regarding analysis of genetic variation in selected calli. Likewise, Mangolin et al.**[44]**and Bordallo at al.**[45]**found a high genetic variation in calli obtained by 2,4-D and kinetin. Larkin**[46]** reported that somaclonal variationscould be to increase after *in vitro* selection. Likewise, RAPD marker had been applied to detect markers associated potato resistance to *F. oxysporum***[41, 42].**Williams et al.**[47]** detected that DNA variations among genotypes could rise through DNA sequence change that prevented amplification by deletion or insertion of a priming site and mismatch at one priming site. These results indicated that SRAP and RAPD markers could be efficaciously applied to micropropagated plants and to identify cultivars and their somaclones**[48].** The present study indicated that selected primers (OPA-6 and me2+em1**)** from use of RAPD and SRAP markers were useful to discover genetic variability at the level of DNA sequences among potato varieties and their somaclones or regenrants plantlets, and also allows the finding of variations after *in vitro* selection under stress via *F. oxysporium* toxin filtrate.

5. Conclusion

Given the great nutritional importance of the potato crop and its strategic importance locally and globally as a vegetable crop, this study was launched with great effectiveness to discuss the most important pathogens that afflict this vital crop and affect its productivity, as well as its great impact on human health, including the most important scientific and laboratory methods to raise and stimulate the degree of resistance for these pathogens under Egyptian conditions. Also, the established system in the current study for tissue culture of potato could be getting enough callus and plant regeneration efficiency to perform transgenic plants. Moreover, as the potentiality of regeneration regenerates may be characterized by somaclonal variation and giving birth to pathogen resistance/tolerant. Efficient protocol for *in vitro* selection of regenerant plantlets of potato somaclones by using pathogenic toxin filtrate to increase the tolerant degrees of somaclones against F. oxysporium occasioned Fusarium wilt has been consolidated. Molecular markers using SRAP and RAPD primers are considered the most fruitful and fertile method to test regenerants and somaclones for comparing and finding molecular genetic differences among them. Thus, this investigation succeeded with great form in the process of genetic improvement of disease resistance in the potato cropand tries to reduce the biological challenges that hinder its final production.

Conflict of interest

There is no conflict of interest

6. References

[1] FAO, Food and Agriculture Organization Database. Retrieved from www.fao.org/faostat (2020).

[2] Tridge, (https://www.tridge.com/intelligences/potato/EG/season (2020).

[3] R. Rishi, L. C. Diengdoh, A. K. Srivastava et al, Efficiency of different nodal segments for potato micro-propagation. Environ Ecol 30(3) (2012) 594–597.

[4]P. Larkin, W. Scowcroft, Somaclonal variation-a novel source of variability from cell cultures for plant improvement. Theor Appl Genet (60) (1981)197–214.

[5]F. Hammerschlag, D. Ritchic, D. Werner, G. Hashmil, L. Krusberg, R. Meyer, R. Huettel, *In vitro* selection of disease resistance in fruit trees. Acta Hortic (392) (1995) 19–26.

[6] L. Svabova, A. Lebeda, *In vitro* selection for improved plant resistance to toxin producing pathogen. J Phytopathol (153) (2005) 52–64.

[7] S. Kumar, S. P. Negi, J. K. Kanwar, *In vitro* selection and regeneration of chrysanthemum (*Dendranthema grandiflorum* Tzelev) plants resistant to culture filtrate of *Septoria obese* syd. In Vitro Cell Dev Biol Plants (44) (2008) 474–479.

[8] S. Verma, M. Modgil, S. Patidar, *In vitro* screening of apple rootstock MM106 somaclones with *Phytophthora cactorum* culture filtrate. *J Plant Pathol* (103) (2021) 231–240.

[9] M. A. Aazami, M. Torabi, E. Jalil, In vitroresponse of promising tomato genotypes for tolerance to osmotic stress. Afr. J. Biotechnol. (9) (2010) 4014–4017.

[10]J. Gopal, K. Iwama, In vitro screening of potato against water stress mediated through sorbitol and polyethylene glycol. Plant Cell Rep(26) (2007) 693–700.

[11] H. Mirkarimi, A. Abasi-Moghadam, J. Mozafari, Assessment on early blight of potato in order to compare the two methods *in vitro* using

pathogenic fungi *Alternaria solani*. *Natural Science* (5) (2013) 1189-1192.

[12] V. Kumari, A. Kumar, H. K. Chaudhary, R. Prasad, S. Jambhulkar, S. Sharma, In vitro screening method: An efficient tool for screening Alternaria blight resistance/tolerance during early generations in Ethiopian mustard (*Brassica carinata* A. Braun). African Journal of Agricultural Research, 9(1) (2014) 137-143.

[13]M. Khalafalla, G. A. Khadiga, S. M. Rasheid, Callus formation and organogenesis of potato (*Solanum tuberosum* l.) cultivar Almera. Journal of Phytology (2) (2010) 40-46 .

[14] M. Wang, X. Sun, D. Yu, J. Xu, K. Chung, H. Li, Genomic and transcriptomic analyses of the tangerine pathotype of Alternaria alternata in response to oxidative stress. Sci. Rep (1) (2016) 32437.

[15]R. P. Rajan, G. Singh, A Review on Application of Somaclonal Variation in Important Horticulture Crops. Plant Cell Biotechnology and Molecular Biology, 22(35-36) (2021) 161-175.

[16] M. Pérez-Jiménez, O. Pérez-Tornero, Improved salt-tolerance in Citrus macrophylla mutant rootstocks. *Sci Hortic (259*) (2020) 108815.

[17]A. Saito, N. Nakazawa, M. Suzuki, Selection of mutants resistant to Alternaria blotch from in vitro cultured apple shoots irradiated with X- and γ-rays. J. Plant Physiol (158) (2001) 391–400.

[18]N. V. Rodríguez, B. Kowalski, L. G. Rodríguez, L. B. Caraballoso, M. A. Suárez, M. O. Pérez, C. R. Quintana, N. González, R. Q. Ramos, In vitro and ex vitro Selection of Potato Plantlets for Resistance to Early Blight. J. Phytopathol (155) (2007) 582-586.

[19] T. Mahlanza, R. S. Rutherford, S. J. Snyman, M. P. Watt, In vitro generation of somaclonal variant plants of sugarcane for tolerance to Fusarium sacchari. Plant Cell Rep (32) (2013) 249-262.

[20]T. Murashige, T. F. Skoog, A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant (15) (1962) 473-479.

[21]J. J. Doyle, J. L. Doyle, Isolation of plant DNA from fresh tissue. Focus (12) (1990) 13-15.

[22]A. M. Kumlay, S. Ercisli, Callus induction, shoot proliferation and root regeneration of potato (*Solanum tuberosum* L.) stem node and leaf explants under long-day conditions. Biotechnology and Biotechnological Equipment, 29 (6) (2015) 1075-1084.

[23]R. H. Sarker, B. Mustafa, Regeneration and Agrobacterium mediated genetic transformation of two indigenous potato varieties of Bangladesh. Plant Tissue Cult (12) (2002) 69-77.

[24]S. P. Dhital, H. T. Lim, H. K. Manandhar, Direct and efficient plant regeneration from different explant sources of potato cultivars as influenced by plant growth regulators. Nepal J Sci Technol (12) (2010) 1-6.

[25] A. Kumlay, Combination of the auxins NAA, IBA, and IAA with GA3 improves the commercial seed-tuber production of potato (Solanum tuberosum L.) under in vitro conditions. BioMed Res Int (014) (2014) 439259.

[26]C. Gime´nez, E. de Garcı´a, N. X. de Enrech, I. Blanca, Somaclonal variation in banana: cytogenetic and molecular characterization of the somaclonal variant CIEN BTA-03. In Vitro Cell Dev Biol Plant (37) (2001) 217–222.

[27]M. Siragusa, A. Carra, L. Salvia, A. Puglia, F. De Pasquale, F. Carimi, Genetic instability in calamondin (Citrus madurensis Lour.) plants derived from somatic embryogenesis induced by diphenylurea derivatives. Plant Cell Rep (26) (2007)1289-1296.

[28] F. LoSchiavo, L. Pitto, G. Giuliano, G. Torti, V. Nuti-Ronchi, D. Marazziti, R. Vergara, S. Orselli, M. Terzi, DNA methylation of embryogenic carrot cell cultures and its variations as caused by mutation, differentiation, hormones and hypomethylating drugs. Theor Appl Genet (77) (1989) 325-331.

[29]S. Mohanty, M. Panda, E. Subudhi, S. Nayak, Plant regeneration from callus culture of Curcuma aromatica and in vitro detection of somaclonal variation through cytophotometric analysis. Biol Plant (52) (2008)783-786.

[30]K. Martin, S. Pachathundikandi, C. Zhang, A. Slater, J. Madassery, RAPD analysis of a variant of banana (Musa sp.) cv. grande naine and its propagation via shoot tip culture. In Vitro Cell Dev Biol Plant (42) (2006)188-192.

[31]I. Shahab-ud-din, N. Sultan, M. A. Kakar, A. Yousafzai, I. F., A. Sattar, F. Ahmmad, M. Ibrahim, M. Hassanullah, B. Arif, The effects of different concentrations and combinations of growth regulators on the callus formation of potato (Solanum tubrosum) ex-plants. Current Research Journal of Biological Sciences (3) (2011) 499-503.

[32]A. K. Fiegert, W. G. Mix, K. D. Vorlop, Regeneration of So-lanum tuberosum L. Tomensa cv, Induction of somatic embryo-genesis in liquid culture for the production of artificial seed. Landbau-forschung Volkenrode (50) (2000) 199-202 .

[33]S. Yasmin, K. M. Nasiruddin, R. Begum, S. K. Talukder, Regen-eration and establishment of

potato plantlets through callus formation with BAP and NAA. Asian J. Plant Sci (2) (2003) 936-940.

[34]M. H. A. Othman, A. I. A. Abido, A. A. A. jabal, In vitro Propagation and Ex vitro Acclimatization of Potato (Solanum tuberosum L.) Using Nodal Cutting Explants, J. Adv. Agric. Res. 21(1) (2016) 1-8.

[35]R. Suthar, P. N. Bhatt, D. P. Bhatt, Selection of vascular wilt resistance cumin callus to culture filtrate of Fusarium equiseti and regeneration of plants. Vegetos (34) (2021) 318–324.

[36] J. F. Shepard, D. Bidney, E. Shahin, Potato protoplasts in crop improvement. Science (208) (1980) 17-24.

[37]H. C. J. Burg, K. S. Ramulu, G. M. M. Bredemeijer, S. Roest, P. Dhijkuis, J. J. V. Hoogen, A. Houwing, Patterns of phenotypic and tuber protein variation in plants derived from protoplast of potato (Solanum tuberosum l. cv bintje). Plant Sci (64) (1989) 113-124.

[38] D. H. Ling, P. Vidhyasekharan, E. S. Borromeo, F. J. Zapata, T. W. Mew, In vitro screening of rice germplasm for resistance to brown spot disease using Phytotoxin. Theor. Appl. Genet (71) (1985) 133-135.

[39]M. W. Bairu, A. O. Aremu, J. Van Staden, Somaclonal variation in plants: Causes and detection methods. Plant Growth Regulation (63) (2011) 147–173.

[40]I. S. Khatab, M. S. Mohamed, Micropropagation Assessment of Genetic Stability of in vitro Musa sp. CV. Williams using RAPD and SRAP Markers, Egyptian J of Botany 58(3) (2018) 371-380.

[41] A. D. El-Sawy, S. Bekheet, U. I. Aly, Morphological and molecular characterization of potato microtubers production on coumarin inducing medium. J Agri Biol 9(5) (2007) 675-80.

[42]M. D. Rahman, S. Hasan, H. Ashraful, H. Altaf, G. Dipali, I. Mazadul, *In vitro* Screening and Molecular Genetic Markers Associated with Salt Tolerance in Potato. International Journal of Plant & Soil Science 24(4) (2018) 1-11.

[43]S. A. Gostimsky, Z. G. Kokaeva, F. A. Konovalov, Studying plant genome variation using molecular markers. Russ J Genet (41) (2005) 378–388.

[44]C. A. Mangolin, L. M. M. Ottoboni, M. F. P. S Machado, RAPD markers to evaluate callus tissue of *Cereus peruvianus* Mill (Cactaceae) maintained in different growth regulator combinations. *Biochemical Genetics* (40) (2002) 351-358.

[45]P. N. BORDALLO, D. H. SILVA, J. MARIA, C. D. CRUZ, E. P. FONTES, Somaclonal variation on in vitro callus culture potato cultivars. Horticultura Brasileira, BrasÌlia (22) (2004) 300-304.

[46]P. J. Larkin, Somaclonal variation: history, method and meaning. Iowa State Journal of Research (61) (1987) 393434.

[47] J. G. Williams, A. R. Kubelik, K. J. Livak, J. A. Rafalski, S. V. Tingey, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers.Nucleic Acids Research, 18 (22) (1990) 6531-5.

[48] A. H. Zian, I. S. El-Demardash, A. A. El-Mouhamady, E. El-Barougy, Studies the resistance of lupine for *fuzarium oxyporium* F. sp lupine) Through molecular genetic technique. World Applied sciences Journal 26 (8) (2013) 1064-1069.