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BIOCHEMICAL AND MOLECULAR MARKERS ASSOCIATED WITH *IN VITRO* GERMPLASM CONSERVATION OF FOUR POTATO GENOTYPES

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ABSTRACT: The purpose of the present study was to develop an effective and dependable protocol for *in vitro* regeneration of potato genotypes Lady Rosetta, Hermes, Cara, and Spunta. For the regeneration of potato genotypes, young shoots were collected, sterilized with varying concentrations of Clorox and 0.1% (w/v) $HgC1_2$, and then cultured on MS medium. For germplasm preservation and synthetic seed production, in vitro-grown plants were harvested. All genotypes were sterilized with 20% Clorox and 0.1% $HgCl_2$, which resulted in high survival rates. Several parameters, such as the culture medium, were modified to reduce growth. For long-period conservation, potato node cuttings stored in MS medium fortified with ABA 0.50 and 0.75 mg/l at 4 °C for 12 months without subculture gave 100% regrowth in MS medium containing 1 mg/l KN and normal growth conditions. This study showed the effect of storage potato shoots in a tissue culture medium containing ABA on DNA instability of potato genotypes, using the molecular markers RAPD and SSR. All RAPD and SSR primers showed differences in DNA bands. The use of single-stem cutting for synthetic seeds provides maintenance and transport advantages. In a 5% alginate gel, stem cuttings were encapsulated. As the concentration of ABA increased from 0.25 mg/l to 0.75 mg/l, the percentage of synthetic seeds that germinated and were converted into plantlets decreased gradually. The protocol can be used for exchanging potato germplasm and preparing synthetic seeds.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important vegetable crop worldwide. It is one of the most economically significant annual Solanaceae vegetable crops. In 2014, four million and eight hundred thousand (4800000) tonnes of potato tubers were harvested from 439855.8 feddans of potatoes grown in Egypt (FAO, 2015). Potato is, according to the FAO, a staple food of the world. It plays a significant role in the development of food security and the eradication of poverty, as it contributes to the reduction of the proportion of deaths caused by malnutrition, and it also helps to increase investment in agriculture in developing countries. It has several uses in food production, industry, and animal husbandry (Feustel, 1987). More than one billion people consume potatoes worldwide, of which half reside in developing nations. Potatoes provide an exceptionally high yield and produce more edible energy and protein per unit of land and time than most of other crops. While the developed nations make the most diverse use of potatoes as food, feed, and raw material for processed products, starch, and alcohol, the

developing nations are increasingly adopting potato cultivation as a food crop only. Rapid multiplication of these disease-free clones through micropropagation in conjunction with conventional multiplication techniques is now an integral component of seed production in several nations (Donnelly *et al.,* 2003). Synthetic seed production is a viable technique for plant multiplication and preservation, particularly for the propagation of non-seed-producing plants and other plants that require asexual propagation to maintain superior characteristics (Saiprasad, 2001). In vitro conservation of potato, germplasm is regarded as an effective method for its preservation (Gopal and Chauhan, 2009). Ghanbarali *et al.* (2016) observed that synthetic seed technology is a convenient alternative to traditional potato multiplication. Kamińska, *et al.* (2021) demonstrated that ABA inhibited the growth of encapsulated shoot tips during cold storage as well. Therefore, better and clonal plants could be propagated similarly to seeds; preservation of rare plant species for extending biodiversity, more consistent and synchronized harvesting of essential agricultural crops, ease of handling, potential for long-term storage, and low cost of production and subsequent propagation are among the benefits. The purpose of the work is to preserve *in vitro* four potato genotypes, Lady Rosetta, Hermes, Cara, and Spunta for future generations, to improve global food security, and to use the promising clones in future breeding programs considering changing environmental conditions.

MATERIALS AND METHODS

Plant materials and sterilization:

This research was conducted at the Laboratory Tissue Culture and Genetic Engineering Center of the Genetic Engineering and Biotechnology Research Institute of the University of Sadat City, Egypt. Potato *Solanum tuberosum* cv. is virus–free. Lady Rosetta, Hermes, Cara, and Spunta were generously supplied by the greenhouse. As initial plant material, the shoot tips were used. Within 15 days, the tubers planted in the 30-cm pots containing a mixture of peat moss, sand, and perlite will sprout shoot buds. Four genotypes were then trimmed their shoot tips to a length of approximately 5 mm. These removed shoot tips were washed for approximately 30 minutes under running water to remove soil. As described below, the surface of the shoot tips was sterilized.

Effect of 20% Clorox and 0.1 g/l Mercuric Chloride on sterilization:

From the previous experiment, the best concentration produced the highest survival percentage was obtained by 20% Clorox for sterilization of potato followed by emersion for 3 sec without and with 0.1 g/l mercuric chloride. Explants were cultured in MS medium containing 1 mg/l KN, 30 g/l sucrose and 7 g/l agar then cultured in 350 ml jars. Data were taken after two weeks of the explants' survival.

Micropropagation:

Micropropagation was carried out using Murashige and Skoog (MS) medium supplemented with 1 mg/l KN (Hajare *et al.,* 2021). *In vitro* developed plants after 3-4 weeks of growth were propagated by subculturing nodal cuttings on this media and the cultures were incubated in a growth room for mass production of *in vitro* developed plants for further study.

Cold storage:

1. Effect of potato varieties and ABA concentrations on survival percentage after 12 months from storage:

Shoots with nods cuttings of four potato genotypes from the survival of sterilization stage were cultured on MS medium supplemented with different concentrations of ABA (0.0, 0.25, 0.50, 0.75 mg/l), 30 g/l sucrose and 7 g/l agar. The five shoots were cultured and kept in 250 ml jars containing 30 ml of the medium under cold storage at 4 °C for 12 months. After storage, the explants were cultured on MS basal medium supplemented with 1 mg/l KN to check their viability and growth percentage.

2. Molecular experiment:

Using the i-genomic Plant DNA Extraction Mini Kit (iNtRON Biotechnology Inc., Korea), DNA was extracted from the small leaves of the normal and conserved potato cultivars Cara, Spunta, Hermes, and Lady Rosetta. Adjusting the concentration of the extracted DNA solutions to 25ng/l and storing them at -20 °C until use.

This study distinguished between conserved (on a nutrient medium containing 0.5 mg/l ABA) and non-conserved potato cultivars using twelve RAPD 10-mer random primers (Table, 1) and nine SSR primer pairs (Table, 2). The primers were chosen to be related to stress (Eldemery *et al.,* 2022).

In 15 l volumes, 50 ng of template DNA (2 l of 25 ng/l), 1.5 l of the primer, 4 l nuclease-free ddH_2O, and 7.5 l of master mix solution were used for RAPD-PCR reactions (i-Taq, iNtRON Biotechnology Inc., Korea). Thirty-five cycles of PCR amplification at 95 °C for 1 minute, 32 °C for 1 minute, and 72 °C for 1 minute were performed. 5 minutes of denaturation at 95 °C was followed by 5 minutes of elongation at 72 °C. On 1.5% ethidium bromide-stained agarose gels, the PCR products were separated and photographed using a gel documentation system.

SSR analysis was performed using a 15 l reaction mixture containing 7.5 l of 2× PCR Master mix solution (i-Taq, iNtRON Biotechnology Inc., Korea), 1.5 l of each primer (10 M/ml), 2 l of DNA, and 2.5 l of nuclease-free ddH_2O. 32 cycles of the following PCR steps were performed: denaturation at 95 °C for a minute and fifty seconds, annealing at 48 °C for forty seconds, and extension at 72 °C for one minute. Five minutes of denaturation at 95 °C were followed by three minutes of extension at 72 °C in the previous PCR protocol. After the PCR reaction was completed, samples were separated on 1.5% agarose gel electrophoresis and a digital photograph was taken.

The total and polymorphic number of amplified fragments from both RAPD and SSR analyses were computed, as well as the polymorphic information content (PIC), which was computed according to Anderson *et al.* (1993) using the simplified formula below. PICi= 1- Σpij 2, pij represents the frequency of the jth allele for the SSR primer, whereas it is the frequency of all alleles for the locus. Similarity coefficient matrices were computed for the RAPD and SSR markers using the DIC and simple matching similarity algorithms, respectively (Sokal and Sneath, 1963). The UPGMA method (Unweighted Pair-Group Method with Arithmetic Algorithms Averages) was utilized to construct the phylogenetic dendrogram (Sneath and Sokal, 1973) Using NTSYS PC2.1, all of the aforementioned analyses were performed (Rohlf, 2000).

Synthetic seeds production:

1. Effect of potato genotypes and different sodium alginate concentrations on synthetic seeds' formation:

Shoot tips were encapsulated in 3, 4, and 5% sodium alginate gel (prepared in MS) as well as 100 mM $CaCl_2$ solution (prepared in double-distilled water). For encapsulation, isolated shoot tips were suspended in a 3, 4, and 5% sodium alginate solution for 2 minutes before being individually dropped through a Pasture pipette into a $CaCl_2$ solution and kept on a shaker for 30 minutes to harden. Calcium alginate gel is the result of the reaction between sodium alginate and calcium chloride. This reaction polymerizes the gel and hardening around the explants begins in thirty minutes and lasts for forty-five minutes. The residue of $CaCl_2$ was removed from the surface of encapsulated explants by washing them for 5 minutes in sterile distilled water. Under aseptic conditions, each step of the encapsulation procedure was completed. Four weeks later, the percentage of synthetic seed germination was measured.

2. Effect of ABA on germination of synthetic seeds:

This study was conducted to determine the effect of adding ABA at concentrations of 0.0, 0.25, 0.50, and 0.75 mg/l to MS basal

Table 1. RAPD primer pairs polymorphism and their polymorphic information content used in ABA-conserved of potato genotypes.

Primer name	Sequence (5-3)	Amplified bands			PIC
		Total	Polymorphic	% Polymorphism	
OPA 11	CAATCGCCGT	8	4	50	0.69
OPB 06	TGCTCTGCCC	6	4	66.7	0.78
OPC 15	GACGGATCAG	8	5	62.5	0.78
OPD01	ACCGCGAAGG	9	5	55.6	0.74
OPD02	GGACCCAACC	7	5	71.4	0.78
OPE15	ACGCACAACC	7	6	85.7	0.86
OPH17	CACTCTCCTC	12	9	75	0.79
OPM16	GTAACCAGCC	11	9	81.8	0.88
OPM20	AGGTCTTGGG	13	12	92.3	0.89
0PN03	GGTACTCCCC	9	6	66.7	0.79
OPN09	TGCCGGCTTG	4	1	25	0.38
OPN10	ACAACTGGGG	12	10	83.3	0.89
Total and average		106	76	71.7	0.77

Table 2. SSR primer pairs polymorphism and their polymorphic information content used in ABA-conserved potato genotypes.

Primer name	Forward Sequence (5-3)	Reverse Sequence (5-3)	Amplified bands			PIC
			Total	Polymorphic	% Polymorphism	
BARC11	GCGATGCGTGTAAAGTCTGAAGATGA	GCGTCCATGGAGCTCTGTTTTATCTGA	11	9	81.8	0.89
GWM165	TGCAGTGGTCAGATGTTTCC	CTTTTCTTTCAGATTGCGCC	6	6	100	0.83
GWM617	GAT CTT GGC GCT GAG AGA GA	CTC CGA TGG ATT ACT CGC AC	14	13	92.9	0.91
GWM190	GGAGTGTCGAGATGATGTGGAAAC	CGCAGACGTCAGCAGCTCGAGAGG	7	6	85.7	0.45
GWM111	TCTGTAGGCTCTCTCCGACTG	ACCTGATCAGATCCCACTCG	11	9	81.8	0.64
WMC121	GGCTGTGGTCTCCCGATCATTC	ACTGGACTTGAGGAGGCTGGCA	7	6	85.7	0.82
WMC396	TGCACTGTTTTACCTTCACGGA	CAAAGCAAGAACCAGAGCCACT	10	8	80	0.88
GWM525	GTTTGACGTGTTTGCTGCTTAC	CTACGGATAATGATTGCTGGCT	8	6	75	0.41
GWM537	AAGAGATAACATGCAAGAAA	TTCAAATATGTGGGAACTAC	6	2	33.3	0.50
Total and average			80	65	81.3	0.70

nutrient medium supplemented with 30 g/l sucrose and 7 g/l agar at full strength. Capsules of cultured shoot tips were incubated at a daytime and nighttime temperature of 25 °C in the dark. Synthetic seed germination and survival rates were recorded as percentages.

Statistical analysis:

Data collected in this study were statistically analyzed using the generalized linear models (GLM) of SAS (2003). Separation among means was achieved using Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Effect of Clorox and Mercuric chloride treatments on survival percentage of sterilization of four potato genotypes:

Results in Table (3) and Fig. (1) show that the addition of mercuric solution ($HgCl_2$) to sodium hypochlorite (NaOCl) was found more effective than NaOCl alone. All the explants of four potato genotypes of our study, treated with NaOCl solution alone, resulted in less survival percentage (75%). While $HgCl_2$ gave positive results in Lady Rosetta, Hermis, Cara and Spunta explants with a high survival rate of 100%. Our results were higher than those reported by other authors. Yasmin *et al.* (2003) reported that for surface sterilization, isolated sprouts of potato were first sterilized with 70% (v/v) ethanol for a few seconds. The sprouts were then rinsed twice with sterile distilled water then immersing in 0.1% $HgCl_2$ solution for 2 min then washed several times with sterile distilled water. (Kanwal *et al*., 2006; Badoni *et al*., 2010, Liljana *et al*., 2012). Xhulaj (2018) found that the suitable sterilization protocol giving high percentages of survived individuals was that of 1% $HgCl_2$.

Effect of the addition of abscisic acid (ABA) on mean percentage survival of shoots cultures from four potato genotypes stored at 4 °C for 12 months:

Slow growth storage (also known as medium-term conservation or minimal growth storage) is based on reducing the metabolic activity (i.e., growth rate) of *in vitro* shoot cultures by maintaining them under "modified culture conditions.

The growth of the four potato genotypes was inhibited by both ABA treatment and low temperature. Induction of controlled ABA for a delay conservation period was the objective of the treatments utilized in this study. The growth rate decreased as the ABA concentration increased from 0 to 0.75 mg/l. Maximum survival rate was recorded for Lady Rosetta, Hermes, and Spunta, as shown in Table (4) and Figs. (2 and 3). The genotypes did not influence the success of conservation. The addition of 0.50 and 0.75 mg/l of ABA to culture media allows for a decrease in the growth rate of potato germplasm and an increase in its survival rate. In the case of prolonged conservation with no immediate need for multiplication, media can be used to reduce costs and store potato material for longer periods as slow-growth storage. After 12 months of storage, the addition of 0.50, 0.75 mg/l ABA and 0.50, 0.75 mg/l ABA can reduce the growth of shoot cultures by 50% and 75%, respectively, and produce 100% survival for all potato genotypes. Westcott (1981 a) demonstrated that a temperature reduction from 22 to 6–12 °C increased the subculture duration from 4 weeks to 12 months. Westcott (1981 b) demonstrated that the addition of ABA at a concentration of 5.0 mg/l extends the subculture duration to 12 months, resulting in 96% efficient survival of plants cultured for 6 months and 66% survival of plants cultured for 12 months. The internodes of ABA-treated plants were diminished. The storage temperature is dependent on the species' sensitivity to cold. kiwi shoots are stored at 8 °C. (Monette, 1986). The optimal temperature range for cold-tolerant plants in cold storage depends on their ecology and geographic origin but is typically between 0 and 5 °C (Ashmore, 1997). The CIP gene bank maintains 4062 potato accessions in vitro under slow growth conditions; the in vitro plantlets can be kept for approximately two years without subculturing (Niino and

Table 3. Effect of potato genotypes and 20% Clorox without or with MC on sterilization survival percentage.

Genotypes	Clorox conc. 20%		
	Without MC	With MC	Mean
Lady rosette	80.00	100.00	90.00
Hermes	70.00	100.00	85.00
Cara	76.66	100.00	88.33
Spunta	76.66	100.00	88.33
Mean	75.00	100.00	
LSD at 0.05			
A		1.975	
B		2.793	
AxB		3.950	

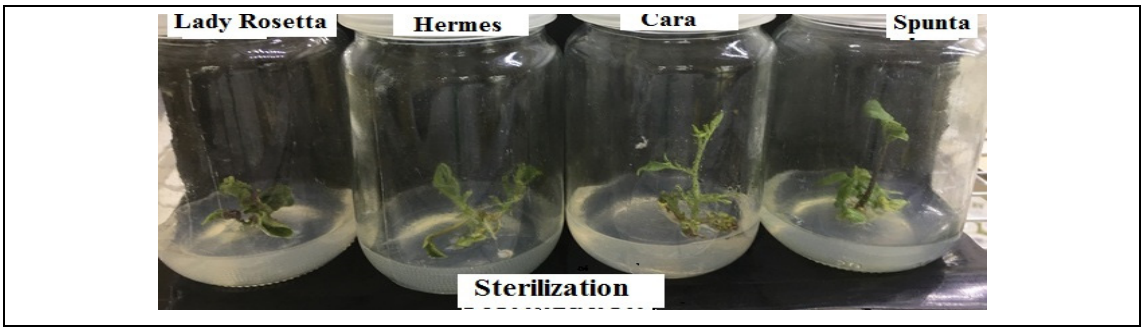

Fig. 1. Initial sterilization of four potato genotypes.

Table 4. Effect of potato genotypes and ABA concentrations on survival % after 12 months from conservation at 4 °C.

Genotypes	Survival % of conserved explants after 12 months				
	ABA conc. mg/l				Mean (A)
	Control	0.25	0.50	0.75	
Lady rosette	53.33	70.00	100.00	100.00	80.833
Hermes	50.00	63.33	100.00	100.00	78.333
Cara	40.00	53.33	100.00	100.00	73.333
Spunta	60.00	63.33	100.00	100.00	80.833
Mean (B)	50.83	62.49	100.00	100.00	
LSD at 0.05					
A			3.499		
B			3.499		
AxB			6.998		

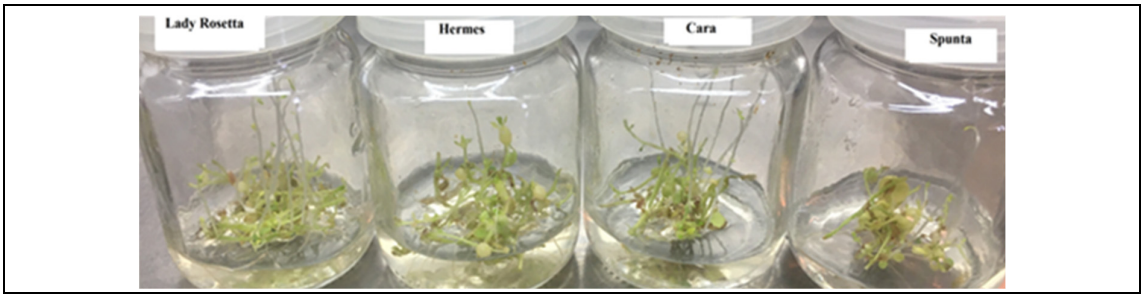

Fig. 2. *In vitro* storage of four potato genotypes on MS supplemented with 0.50 mg/l ABA at 4 ˚C for 12 months.

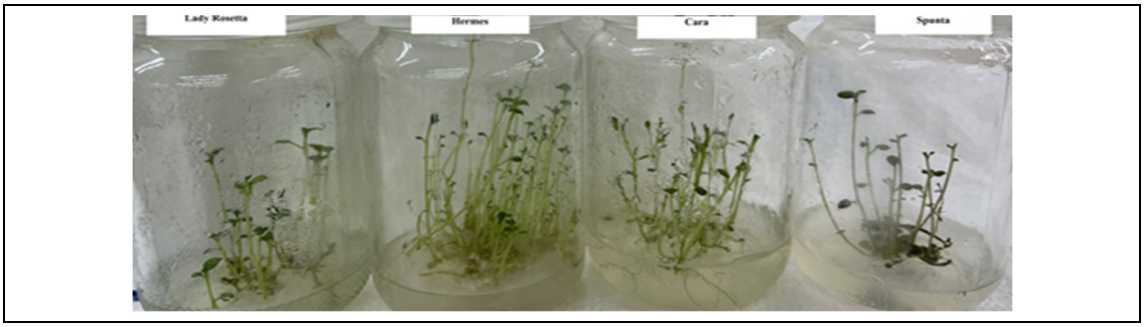

Fig. 3. Regrowth and survival shoots on MS supplemented with 1 mg/l KN from cold storage at 4 ˚C on MS supplemented with 0.50 mg/l ABA for 12 months of four potato genotypes.

Arizaga, 2015). Numerous gene banks preserve potato collections *in vitro* for three-month and three-year durations (Machida-Hirano and Niino, 2017). *In vitro* techniques involving meristem and shoot tip culture are utilized at CIP to preserve potato germplasm for international exchange (Machida-Hirano and Niino, 2017). Muthoni *et al.* (2019) discovered that *in vitro* techniques can be used to conserve potato genetic resources and the status of potato conservation in major gene banks.

Molecular pattern:

The amplified fragments of RAPD primers were separated on 1.5% agarose gel electrophoresis (Fig., 4). All of the amplified primers showed differences in the amplified fragments. Some primers amplified fragments discriminating specific cultivars. For example, the primer OPA11 discriminates against the cultivar Lady Rosetta (amplified fragment at about 550 bp), the primers OPM16 and OPN10 discriminate against the cultivar Cara (amplified fragment at about 370 and 1200 bp, respectively). On the other hand, some fragments disappeared from the amplified pattern of some cultivars compared to the other cultivars. For example, the primer OPM03 discriminates the cultivar Hermes (disappearing amplified fragment at about 950 bp), (Fig., 4).

The amplified fragments of SSR primers were separated on 1.5% agarose gel electrophoresis (Fig., 5). Most of the amplified primers showed differences in the amplified fragments. The differences in the pattern could be noticed especially in the pattern of the primer pairs BARC11, GWM111, GWM617, GWM525, GWM165 and WMC121 (Fig., 5). While the amplified pattern of the primer pair BARC11 showed different amplified fragments in all samples, some amplified fragments characterized specific cultivars in some primer pairs amplified patterns. For example, an amplified fragment at a molecular weight of about four hundred bp characterized the cultivar Spunta in the pattern of the primer pair GWM111. The same thing was noticed for the cultivars Hermes (at about 1000 bp in the pattern of the primer pair GWM617), Lady Rosetta (at about 250 bp in the pattern of the primer pair GWM525), Lady Rosetta (at about 270 bp in the pattern of the primer pair GWM165), Spunta (at about 250 bp in the pattern of the primer pair GWM165) and Lady Rosetta (at about 200 bp in the pattern of the primer pair WMC121), (Fig., 5).

Primers' polymorphism:

The result of RAPD marker revealed that 106 fragments were amplified from twelve primes. The total number of amplified fragments from each primer varied and ranged from four fragments (for primer

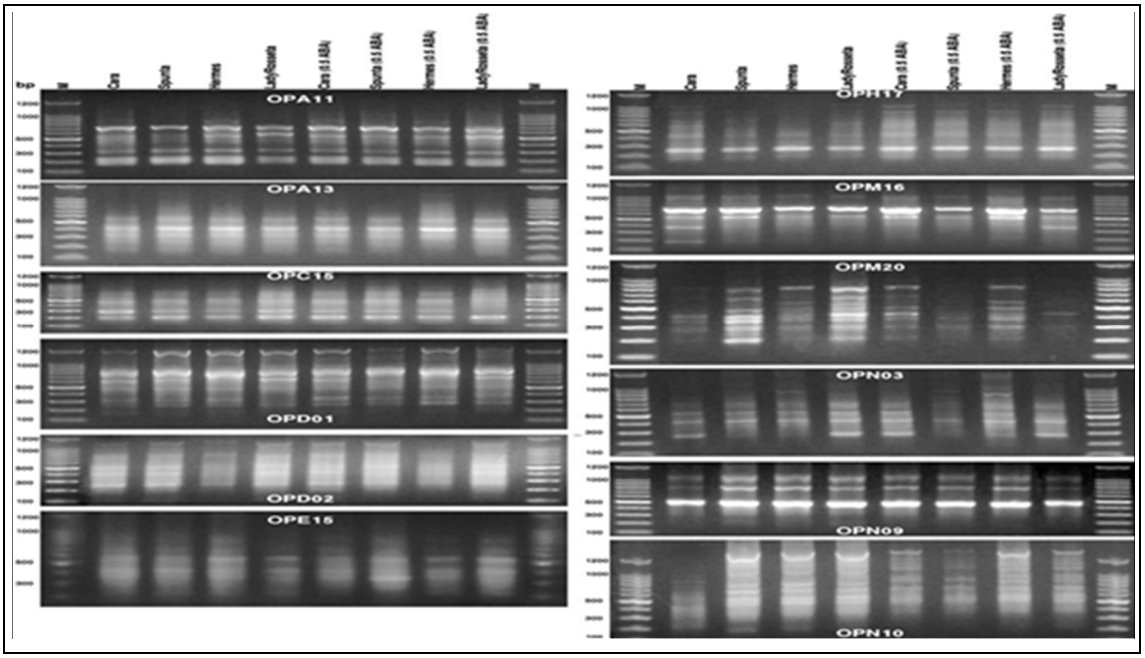

Fig. 4. PCR products of twelve RAPD primers of four potato cultivars (conserved on nutrient medium containing 0.5 mg/l ABA along with control) separated on 1.5% agarose gel electrophoresis.

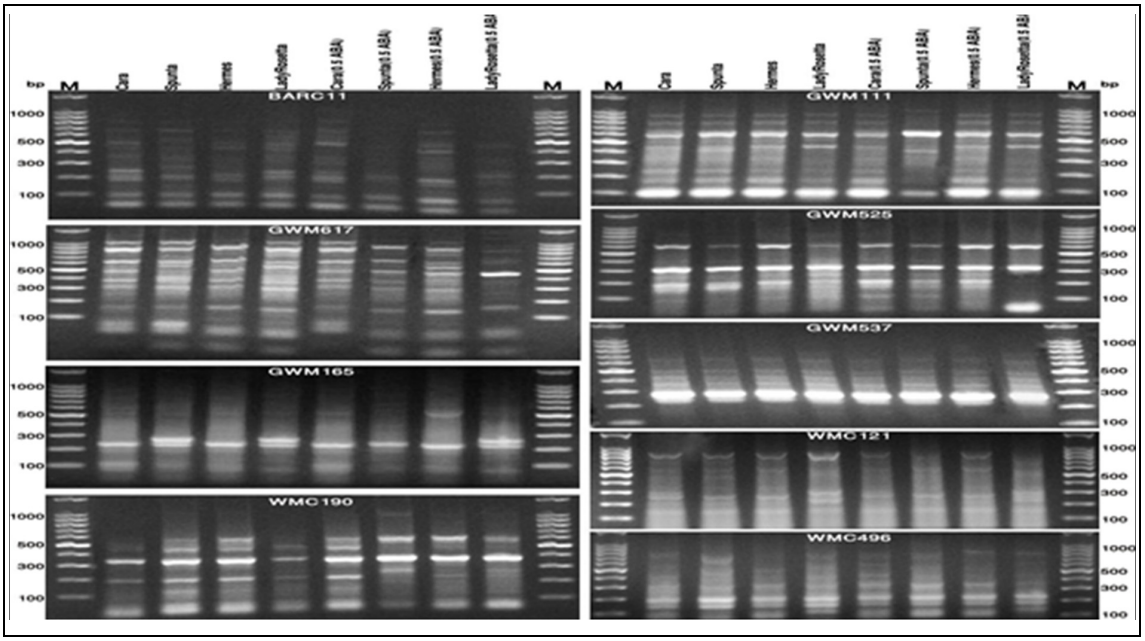

Fig. 5. PCR products of nine SSR primer pairs of four potato cultivars (conserved on nutrient medium containing 0.5 mg/l ABA along with control) separated on 1.5% agarose gel electrophoresis.

OPN09) to thirteen fragments (for primer OPM20, Table, 1). The total number of polymorphic bands for the RAPD primers was seventy-six fragments and the number for each primer ranged from one fragment (for primer OPN09) to 12 fragments (for primer OPM20) and the polymorphic information content (PIC) ranged from 0.38 (for primer OPN09) to 0.89 (for primer OPM20) with an average of 0.77 overall the RAPD primers.

The total number of amplified fragments of SSR marker was eighty fragments from nine primer pairs and the number for each primer pair ranged from six fragments for the primer pairs GWM165 and GWM537 to 14 fragments for the primer pair GWM617. The polymorphic fragments for SSR markers were sixty-five fragments and ranged for each primer pair from two fragments for the primer pair GWM537 to 13 fragments for the primer pair GWM617 (Table, 2). The percentage of polymorphism for the SSR markers ranged from 33.3% for the primer pair GWM537 to 100% for the primer pair GWM617 and the polymorphic information content (PIC) ranged from 0.41for the primer pair GWM537 to 0.91 for the primer pair GWM617 with an average of 0.7 (Table, 2).

Cluster analysis of molecular markers:

According to the RAPD data cluster analysis, the eight potato genotypes were divided into two groups at 75% similarity coefficient percent. The first group contained the potato genotypes before treating with ABA (before conservation or the control samples) while the other group contained the cultivars after treating with ABA (Fig., 6). The most related genotypes according to this cluster analysis were Spunta and Hermes cultivars (at a similarity level of 86%).

According to the RAPD molecular analysis, the potato genotypes before ABA treatment were separated from the genotypes after storage indicating that storage of potato genotypes on ABA affected their DNA structure. Roy and Das (2017) mentioned that increasing ABA level in plant cells inhibits DNA replication and cell division, causing plant growth retardation. They detected phosphorylation of histone H2AX in wild-type (Col-0) and DSB repair gene mutants after ABA treatment, indicating the activation of DNA damage response due to ABA treatment and they suggested a possible effect of ABA on DNA repair machinery in plants and indicated potential functional involvement of HR pathway in repairing ABA-induced DNA damage in Arabidopsis. Our results strongly supported this assumption in potato genotypes. Many aspects of this DNA damage or change developed from plant tissue culture remain unknown according to Bednarek and Orłowska (2020). These changes are produced because of nutrient components in the artificial nutrient medium and environmental factors.

There was no discrete segregation of potato genotypes according to the SSR cluster analysis. The results of cluster analysis of SSR data showed that potato genotypes were distributed into four clusters (from up to down of the dendrogram) at a level of similarity 57%. The first cluster (from the above) consisted of genotypes Cara, Hermes, Spunta, Cara 0.5 mg/l ABA, and Hermes 0.5 mg/l ABA (Fig., 7). Each of the other three clusters containing only one genotype (i.e., Lady Rosetta 0.5 mg/l ABA for the second cluster, Lady Rosetta for the third cluster and Spunta 0.5 mg/l ABA for the fourth cluster) (Fig., 7).

Comparing the dendrogram of both RAPD and SSR molecular analyses, it could be noticed that SSR dendrogram was more precise in the separation of potato genotypes. The RAPD dendrogram separated the used potato genotypes into two main general groups (i.e., the genotypes before and after storage) while SSR dendrogram separated the genotypes into four clusters. In this regard, the potato genotypes before storage were aggregated in one cluster except for the cultivar Lady Rosetta (Fig., 7). This cultivar was distinguished in its response to regrowth after ABA storage. Even the DNA pattern of

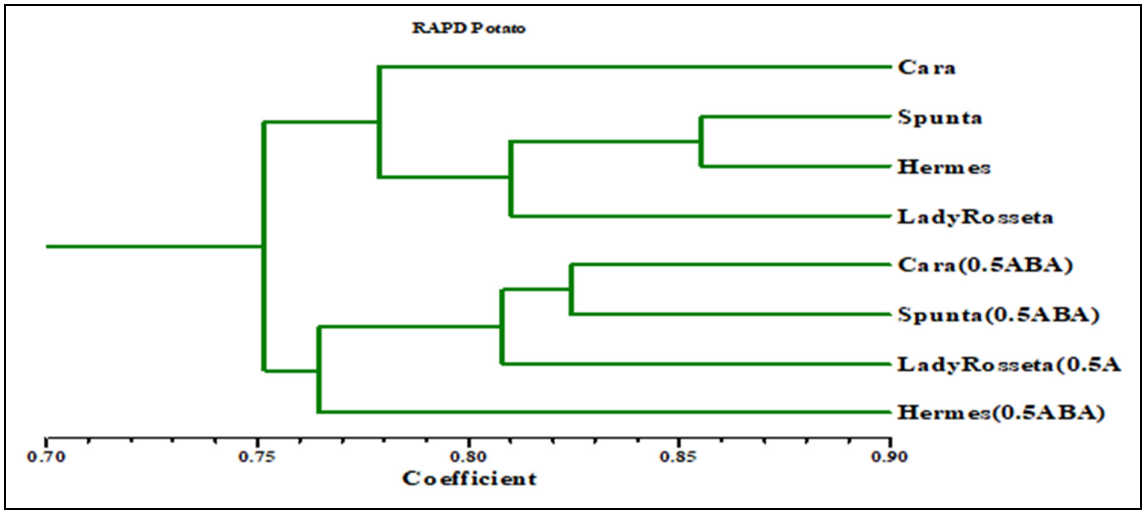

Fig. 6. Cluster analysis of potato cultivars (conserved and non-conserved) using DIC coefficient of RAPD data and UPGMA clustering method.

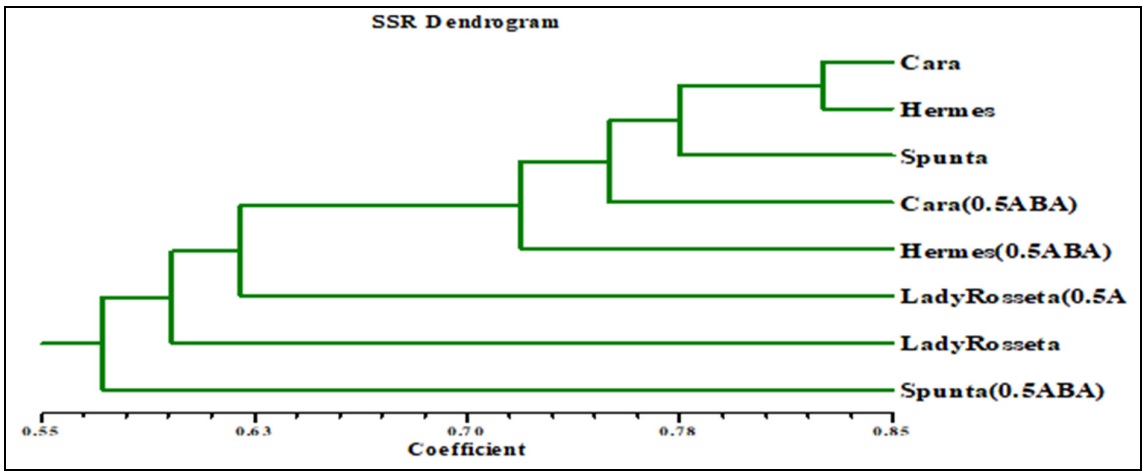

Fig. 7. Cluster analysis of potato cultivars (conserved and non-conserved) using simple matching coefficient of SSR data and UPGMA clustering method.

cultivar Lady Rosetta in SSR markers was also different from the pattern of the other cultivars. That means SSR markers are more efficient than RAPD markers in differences detection. Liao and Guo (2014) reported that SSR markers are good tools for discriminating potato cultivars because of their high polymorphism and good resolution of the primers. They studied the genetic diversity of potato cultivars collected from Yunnan Province (China) was evaluated using twenty-four pairs of SSR markers. The efficiency of SSR markers was proved in many plant species such as wheat (Abouzeid *et al.,* 2013; Eldemery *et al.,* 2022) and fig (Abdelsalam *et al.,* 2019).

Synthetic seeds production:

1. Effect of potato genotypes and different sodium alginate concentrations on formation of synthetic seeds:

Calcium alginate bead hardening is influenced by the concentrations of sodium alginate, calcium chloride, or calcium nitrate, and can also vary with complexation time. Generally, a higher texture corresponds to greater protection during transport and manipulation, but a greater difficulty for the

explant to break the coating after sowing. The involucres of explants must be washed in distilled water multiple times to remove the residual chloride and sodium ions, which are toxic. After being washed, encapsulated explants can be stored before being transferred to a sowing substrate to stimulate vegetative growth in the enclosed plant material. *In vitro* culture relies heavily on the encapsulation technique used to create synthetic seeds. Therefore, 2, 3, and 4% Na-alginate were utilized. Regarding the complexation step, alginate-coated explants are immersed in a calcium chloride solution for a limited amount of time to give involucres their rigidity. During this time, ion exchange occurs, resulting in the substitution of Na^{+} with Ca^{++} and the formation of calcium alginate. Maximum plant growth. The varieties Cara and Spunta contained 75% and 77% of synthetic seeds, respectively. Low concentrations of sodium alginate (3%) do not solidify well, resulting in fragile capsules and enabling the regrowth of more explants and a quicker regrowth. In turn, high concentrations (4 or 5%) would result in excessively hard capsules that are difficult to break up, thereby delaying bud regrowth as shown in Table (5) and Fig. (8). The shoot regeneration response of the beads, regardless of sodium alginate concentration or potato variety, was significantly higher at 3% sodium alginate than at other concentrations of sodium alginate. Results indicated that the concentration of sodium alginate affected the physical properties of the beads, with concentrations of 4% and 5% proving to be optimal for satisfactory bead formation and subsequent germination response of artificial potato seeds. The bead capsule and bead shape are significantly affected by the concentrations of sodium alginate, which facilitate the completion of ion exchange between Na^{+} and Ca^{2+}. Because *in vitro* tuberization is a time-consuming and labor-intensive process, Sarkar and Naik (1997) demonstrated that synthetic seed production is a promising alternative to in vitro tuberization. Sarkar and Naik (1998) studied a system that enables the use of potato single node cuttings encapsulated in an alginate matrix as a new propagulum type concurrent to microplants and microtubers. This novel approach under favorable conditions allowed for a survival rate of 57%. Using shoot tips encapsulated in hollow calcium alginate beads, Patel *et al*. (2000) created the first synthetic seeds of the potato cultivar 'Priwal' After four weeks on MS medium, 13 of 16 shoot tips had matured into full plants. Due to its moderate viscosity, low spin ability of solution, lack of toxicity to explants, low cost, and biocompatibility, Na-alginate is the most commonly used chelating agent. In addition, sodium alginate is utilized because, depending on its concentration (typically ranging from 2% to 5% w/v), level of viscosity or commercial type, and the complexation conditions, it offers superior protection against mechanical damage to the encapsulated explants (Standardi, 2012). To ensure seed-to-plant development, the concentration of sodium alginate is one of the most important variables influencing encapsulation and conversion (Sharma and Shahzad, 2012), sugar beet (Rizkalla *et.al.*, 2012) encapsulation of potato explants in 3% sodium alginate with 1% $CaCl_2$ and full-strength MS culture medium, followed by regrowth in solid MS culture medium and transfer to coco peat (coir fibre pith) for transformation into plantlets, as described by Ghanbarali *et al.* (2016). In accordance with Kamińska, *et al*. (2021), after cold storage and ABA treatment, synthetic potato seeds are capable of effective regrowth under optimal growth conditions.

2. Effect of ABA on germination and development of synthetic seeds:

Potato storage in the form of synthetic seeds under slow-growth conditions with ABA treatment is one of the ex-situ methods of potato protection.

In general, synthetic seeds (without ABA treatment) germinated after 1 to 2 weeks of culture. In contrast, synthetic seeds treated with ABA germinated in three to four weeks,

Table 5. Effect of different concentrations of sodium alginate and four genotypes of potato on germination of synthetic seeds after six weeks.

Genotypes	Germination % of synthetic seeds Sodium Alginate Conc.			Mean (A)
	3%	4%	5%	
Lady Rosetta	100.00	41.67	25.00	55.55
Hermes	100.00	50.00	33.33	61.11
Cara	100.00	83.33	41.67	75.00
Spunta	100.00	83.33	50.00	77.77
Mean (B)	100.00	64.58	37.500	
LSD at 0.05				
A		13.445		
B		11.644		
AxB		23.288		

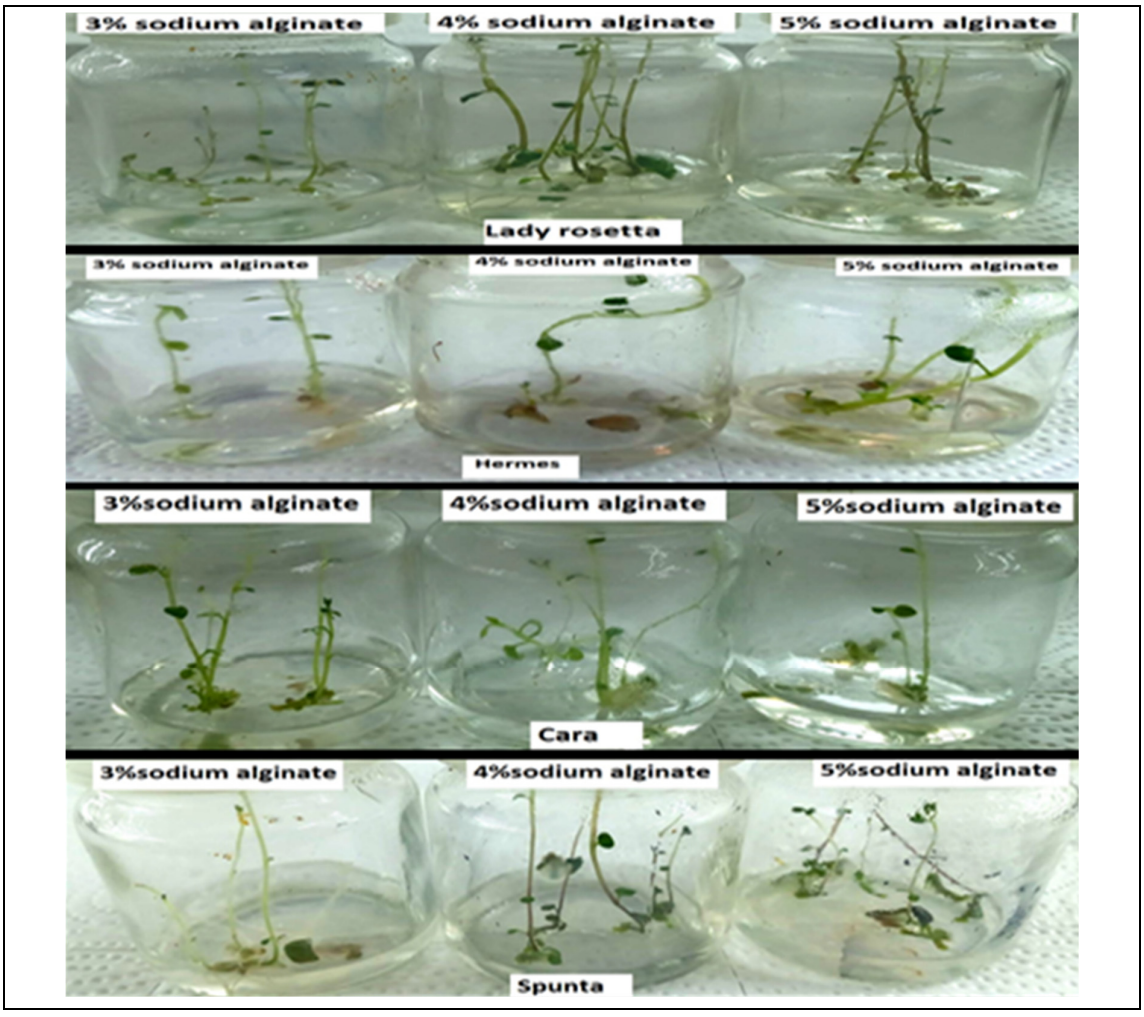

Fig. 8. Development of synthetic seeds on different concentrations of sodium alginate after six weeks.

regardless of the ABA concentration. However, ABA concentration affected the proportion of response. The percent germination and plantlet development of synthetic seeds grown on MS medium supplemented with 0.75 mg/l ABA was marginally lower than the control. The synthetic seeds of Lady Rosetta, Hermes, Cara, and Hermes did not differ significantly in terms of germination response, with a large proportion of seeds either dying or exhibiting arrested germination. As the concentration of ABA increased from 0.25 to 0.75 mg/l, the percentage of germination and plantlet formation decreased gradually. Synthetic seeds of Lady Rosetta, Cara, and Hermes were stored in MS medium at the control and exhibited maximum germination of one hundred percent. However, the interaction of these factors had no significant effect on the measured parameter, and at 0.25 mg/l ABA, Hermes and Cara exhibited 100% germination without significant differences from other treatments, as shown in Table (6). The addition of ABA to the medium reduces the growth rate of potato seeds grown from synthetic material. The findings concur with Badr-Elden (2013) and Ghanbarali *et al.* (2016) observations, that synthetic seed technology is a practical alternative to conventional potato multiplication. Kamińska *et al*. (2021) demonstrated that ABA inhibits the growth of encapsulated shoot tips during cold storage as well.

Table 6. The effect of ABA concentrations on germination percentage of synthetic seeds of four potato genotypes after 6 weeks.

Genotypes	Germination percentage of synthetic seeds				
	ABA conc. mg/l				Mean (A)
	Control	0.25	0.50	0.75	
Lady Rosetta	100.00	83.33	75.00	41.67	75.00
Hermes	83.33	100.00	75.00	33.33	72.91
Cara	100.00	100.00	75.00	25.00	75.00
Spunta	100.00	75.00	58.33	25.00	64.58
Mean (B)	95.83	83.33	77.08	31.25	
LSD at 0.05					
A			18.497		
B			18.497		
AxB			36.995		

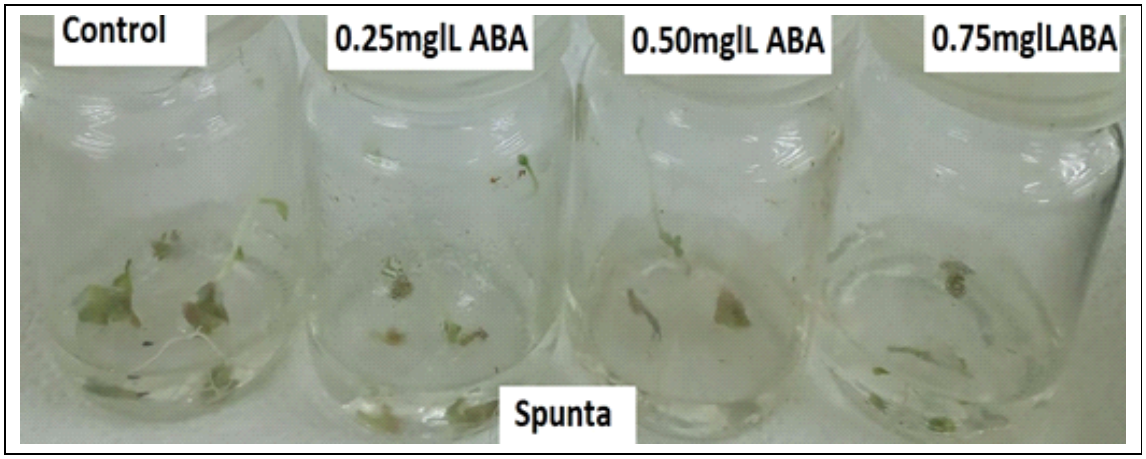

Fig. 9. Germination of synthetic seeds and appearance of shoots from the synthetic seeds of Spunta.

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الواسمات البيوكيميائية والجزيئية المرتبطة بحفظ الأصول الوراثية في المعمل لأربعة طرز وراثية من البطاطس

فاطمة أمير العطار، إبراهيم عبد المقصود إبراهيم ، عواطف محمود بدر الدين ، كمال عبد اللطيف ، أمال محمد زويل
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أجريت هذه الدراسة لتطوير بروتوكول فعال وموثوق به للإكثار في المعمل للأنماط الجينية للبطاطس: ليدي روزيتا، هيرميس، كارا وسبونتا. تم جمع براعم حديثة من التراكيب الجينية للبطاطس وتعقيم الأسطح بتركيزات مختلفة من الكلوراكس و ٠,١٪ كلوريد الزئبق وزرعت على بيئة موراشيج وسكوج للإكثار. وتم استخدام النبيتات المنزرعة لحفظ الأصول الوراثية وإنتاج البذور الصناعية. كان استخدام تركيز التعقيم الذي أعطى نسب عالية من البقاء على قيد الحياة ٢٠٪ كلوروكس و ٠,١٪ كلوريد الزئبق لجميع الأنماط الجينية. وتم الحد من النمو كوسيلة للحفظ عن طريق تعديل المعاملات المختلفة، مثل وسط الزراعة. والحفظ لمدة طويلة، أدى تخزين الأجزاء النباتية للبطاطس في بيئة موراشيج وسكوج المضاف إليها تركيزات ٠,٥٠ و ٠,٧٥ مجم/لتر حامض الأبسيسيك عند ٤ درجات مئوية لمدة ١٢ شهرًا دون تجديد الزراعة وتم إعادة النمو للنبيتات المخزنة بنسبة ١٠٠ ٪ على بيئة موراشيج وسكوج المحتوية على ١ مجم/لتر كاينيتين في ظروف النمو العادية. أظهرت دراسة تأثير تخزين القمم النامية للبطاطس في زراعة الأنسجة في بيئة تحتوي ABA على عدم ثبات الحمض النووي لأصناف البطاطس وذلك بإستخدام الواسمات الجزيئية RAPD و SSR. أظهرت جميع بادئات RAPD و SSR اختلافات في حزم ال DNA. يوفر استخدام برعم واحد للبذور الصناعية مزايا في الحفاظ عليها ونقلها بسهولة. تم تغليف البراعم في ٥٪ ألجينات الصوديوم. مع زيادة تركيز حامض الابسيسيك من (٠,٢٥ إلى ٠,٧٥ ملي لتر) ، انخفضت نسبة إنبات البذور الصناعية وتحويلها إلى نبيتات تدريجياً. ويمكن استخدام هذا البروتوكول لتبادل الأصول الوراثية للبطاطس وإعداد البذور الصناعية.