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# GENETIC STABILITY OF THREE CULTIVARS OF POTATO AT MICROTUBERS PRODUCTION STAGE

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**Abstract**

Random Amplified Polymorphic DNA (RAPD) technique was applied to study the genetic stability using 5 random primers (A15, D2, F8, F18 and H9) in microtuber production stage of three cultivars of potato (Arnova, Rivera and Provento) *in vitro* propagated for 3 sub culture) ( sub culture was repeated every 4 weeks) and interaction with medium (0.0 and 1.0 mg $L^{-1}$ IAA) and growth regulators (4 mg $L^{-1}$ BA, 4 mg $L^{-1}$ Kin-1 and 4 mg $L^{-1}$ BA + 4 mg $L^{-1}$ Kin). Cultures were incubated at a temperature of 25 ± 2 °C and 16 hours of illumination $day^{-1}$ for 10 days, then the cultures were incubated at a temperature of 17 ± 2 °C in the dark for three months. Data were taken on the number of microtuber, their diameters and weights. The results showed the superiority of Riviera plants grown in the medium supplemented with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ Kin in the number of microtubers at rate 2.0 tuber $plant^{-1}$, and the Provento cultivar plants grown in the medium with 1 mg $L^{-1}$ IAA. + 4 mg $L^{-1}$ Kin in the diameter of the microtubers and their weights at rates 0.666 cm and 0.440 g, respectively. All primers (A15, D2, F8, F12 and H9) produced a number of monomorphic bands 21, 19 and 17 bands for all plants (*in vitro* propagated and mother plants) of the cultivars of potato Arnova, Riviera and Provento, respectively, where the percentage of bands similarity was reached 100 %, which indicated its genetic stability and no difference from the mother plants and thus the efficiency of obtaining plants identical to the original plant by using the plant tissue culture technique.

**Key words: Potato, microtubers, genetic stability and RAPD**

## Introduction

Potato *Solanum tuberosum* L. is the fourth most strategic and economic crop in the world after wheat, corn and rice (Wang et al., 2017), Where there is a need to pay attention to propagation of this crop on a large scale using modern methods of breeding for the purpose of obtaining seeds with desirable specifications and free of pathogens, especially viral (Das et al., 2001), Obtaining high-quality tubers is very important in all potato production areas (Morais et al., 2018) because tubers are a source of viral infection that causes low yields and threatens the conservation of genetic resources.

Plant tissue culture technique has been considered one of the biotechnologies that have the ability to rapidly multiply the genotypes identical to their mothers (true-to-type) and a means of

preserving plant resources (morais et al., 2018) and its ability to produce large quantities of disease-free plants for commercial production in a short periods of time and in a specific area without being restricted to place and time.

*In vitro* clonal propagation is used for the potato crop in the production of microtubes and minitubers, which must be identical to the mother plant after sub cultures. Previous studies indicated that *in vitro* culture was accompanied with genetic stability problem, which occurs due to genetic and epigenetic variations when regenerating plants, and this may lead to phenotypic changes in *in vitro* propagated plants ( Deverno et al., 1994; Jaligot et al., 2000; Vazques and Linacero, 2010) Which requires periodic monitoring of its genetic stability (Panda et al., 2007), so the checking of genetic stability of plants at an early stage is important in plant tissue culture technique. As the genetic stability of the *in vitro* propagated plants is affected by several factors, including the type of growth regulators and their concentration in the medium, sub culture and the time period between them (Vasil, 1979, Alatar et al., 2017).

Molecular markers are one of the effective markers in distinguishing between cultivars , determining genetic fingerprint, genetic stability, and detecting genetic variations, similar to phenotypic, enzymatic, protein, and cellular markers by comparing with the original plants. Randomly Amplified Polymorphic DNA-RAPD techniques using primers with universal sequences are widely used to determine the genetic stability of *in vitro* propagated plants for many crops (Joshi and Dhawan, 2007; Lakshmanan et al., 2007). These marker are simple, fast and effective as they are a reliable source as they require only a small amount of DNA samples and do not need any prior sequence information for the primers used. It is also very useful for establishing a breeding system to produce genetically identical plants before they are released for commercial purposes (Bhatia et al., 2010). Therefore, the aim is to study the effect of subculture, medium, and growth regulators on the genetic stability of three cultivars of potato ( Arnova , Riviera and Provento ) *in vitro* propagated and its reflection on the continuity of vegetative propagation and production of microtubes on a commercial scale using the Random Amplified Polymorphic DNA technique.

**Material and methods**

The experiment was conducted in the Plant Tissue Culture Laboratory - Genetic Engineering Department - Food and Biotechnology Center in the Agricultural Research Department / Ministry of Science and Technology for the year 2021/2022. *In vitro* propagated plants of Potato cultivars (Arnova, Riviera and Provento) were subcultures for three times (3 Sub culture, each Sub culture is conducted every 4 weeks, Fig. 1) in MS medium (Murashige and Skooge, 1962) imported by Indian H Medium at 4.4 $g.L^{-1}$ without growth regulators and supplemented with sugar at 30 $g.L^{-1}$and agar 6 -7 $g.L^{-1}$for the solidified medium. The cultures were incubated at a temperature of 25 ± 2 °C and an illumination of 16 h $day^{-1}$. In order to study the genetic stability of *In vitro* propagated plants were subcultures for three times and interaction with medium (0.0 , 1.0 $mgL^{-1}$ ) and growth regulators ( 4mgL-1 BA , 4mgL-1 Kinetin , 4 mg L-1 BA + 4 mg L-1 Kinetin) compared with mother plants. *in vitro* propagated plants were cut into stem cuttings at length 1-1.5 cm with removal the shoot tip and cultured in test tube (with an average of 1 stem cutting test

tube$^{-1}$) containing 30 ml of the MS medium supplemented with 80 g $L^{-1}$ sugar and 7 $L^{-1}$ agar with 5 replicates (test tube ) for each cultivar. The cultures were incubated under the previous conditions for 10 days. Then incubated at a temperature 18± 2 °C in the dark for three months. Data were taken on the number of microtubers, their weights and diameters (cm, determined using the Vernier foot).

**statistical analysis**

Factorial experiments were conducted using a Completely Randomized Design (CRD) with five replicates, and the data were analyzed statistically using the statistical program Genstat. Means were compared using the Least Significant Difference (LSD) test at a probability level of 5%.

**Genetic stability**

**Random Amplified Polymorphic DNA (RAPD) Technique**

Isolation of total DNA from leaves of *in vitro* propagated plants in microtuberization stage from the best treatments and comparing them with mother plants of three cultivars of potato, As shown in Table (1) using CTAB in the manner described by Borges et al., 2009 with some modifications.

Table 1. Plant samples and their numbers in the microtubers production stage

| No. | plant samples |
|---|---|
| 1 | *In vitro* propagated plants (3 sub cultures) of Arnova cultivars planted in free hormone medium |
| 2 | *In vitro* propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mg$L^{-1}$IAA |
| 3 | *In vitro* propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mg$L^{-1}$IAA+ 4 mg $L^{-1}$ BA |
| 4 | Mother plants of Arnova cultivar |
| 5 | *In vitro* propagated plants (3 sub cultures) of Riviera cultivars planted in free hormone medium |
| 6 | *In vitro* propagated plants (3 sub cultures) of Riviera cultivars planted in MS medium with 1 mg$L^{-1}$IAA |
| 7 | *In vitro* propagated plants (3 sub cultures) of Riviera cultivars planted in MS medium with 1 mg$L^{-1}$IAA+ 4 mg $L^{-1}$ BA |
| 8 | Mother plants of Riviera cultivar |
| 9 | *In vitro* propagated plants (3 sub cultures) of Provento cultivars planted in free hormone medium |
| 10 | *In vitro* propagated plants (3 sub cultures) of Provento cultivars planted in MS medium with 1 mg$L^{-1}$IAA |
| 11 | *In vitro* propagated plants (3 sub cultures) of Provento cultivars planted in MS medium with 1 mg$L^{-1}$IAA+ 4 mg $L^{-1}$ BA |
| 12 | Mother plants of Provento cultivar |

The DNA concentrations were measured and their purity was estimated by the (Nanodrop) DNA. The reaction was conducted using the PCR® AccuPower kit PreMix using 5 random primers as shown in Table (2). The samples were placed in the Thermal Polymerase chain Reaction apparatus (PCR), and the serial program was carried out, which starts with the initial denaturation of the DNA strand, one cycle for 4 minutes at a temperature of 94°C. Denaturation 35° cycles multiply, Each cycle includes one minute at 94 °C, DNA template primers annealing for one minute at 36 °C, then elongation for one minute at 72 °C with a final cycle of 10 minutes at 72 °C for final elongation). The reaction products were electrophones with Ladder DNA 100 bp (Korea Pioneer Company) via a garose gel (1% concentration and stained with 30 ng ethidium bromide) and in the presence of TBE buffer (1x) in a electrophoresis for 0.45 hours. DNA was vitualed with ultraviolet rays. The number of bands was calculated and their molecular weights were determined using the Photocapt computer program. The monomorphism percentage of primer and the percentage of efficiency of each primer, were calculated using the following equations (Al-Judy and Majeed, 2013):

**Monomorphism %** = ( the number of monomorphic bands of the random primer / the total number of bands of the same primer) x 100

**Efficiency of each primer %** = ( the total number bands of random primer / the total number bands of all the random primers ) x 100

The results (bands) that appeared in the gel were converted into characterization tables by setting 1 is present and zero is absent. Then the data was analyzed using the Past program and based on the similarity Jacard index scale to draw the Genetic affinity tree (Dendogram).

Table (2) Random primers with their sequences

| No. | primer name | 5 ——3 primers sequence |
|---|---|---|
| 1 | A15 | TTCCGAACCC |
| 2 | D2 | GGACCCAACC |
| 3 | F8 | GGGATATCGG |
| 4 | F18 | ACGGTACCAG |
| 5 | H9 | TGTAGCTGGG |

## Results and discussion

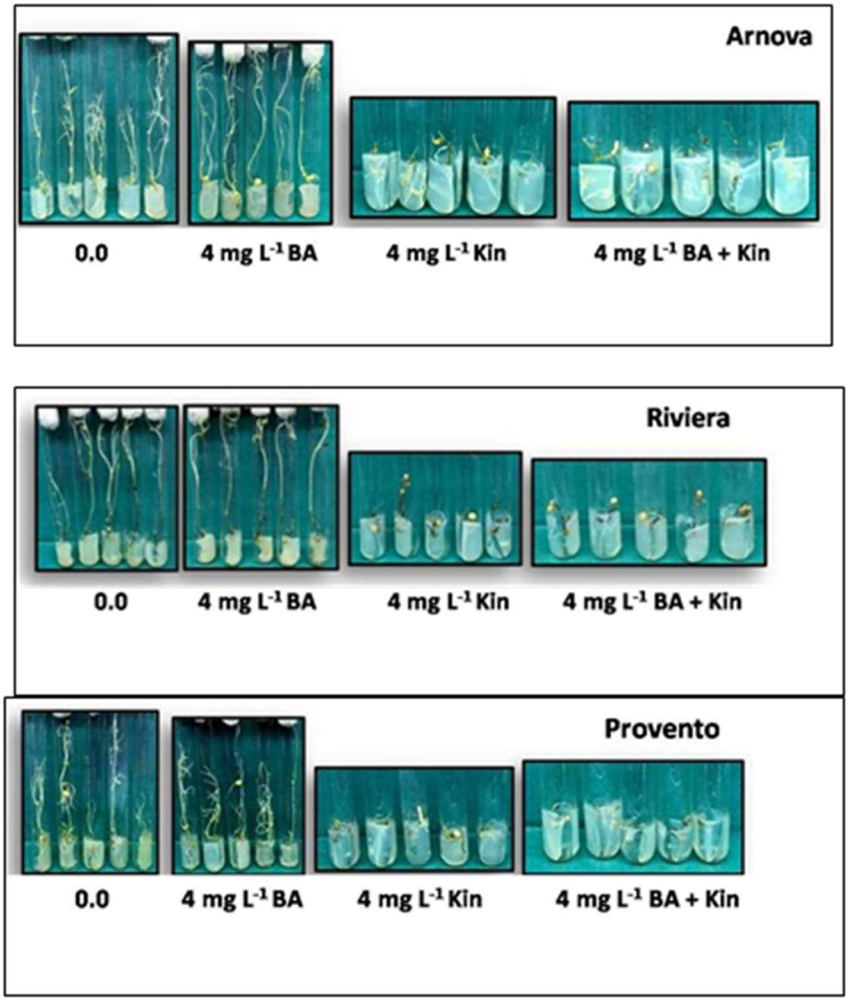
**Effect of growth regulators and medium on the phenotypic traits of three cultivars of potato**. The results of Table 3 indicate that the interactions of cultivars, medium, and growth regulators were significant in the number of microtubes. Whereas, the Riviera cultivar, followed by the Provento cultivar grown in the medium supplemented with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ Kin, gave an average of 2.0 and 1.80 microtuber $plant^{-1}$, respectively, which did not differ significantly from the Provento cultivar grown in the medium supplemented with 4 mg $L^{-1}$ Kin and the Arnova cultivar grown in the medium supplemented with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ BA they differed from the rest of the interactions (Figures 1). As for the lowest average number of microtubers, it was 0.20 microtubers $plant^{-1}$ for the Provento cultivar grown in the medium supplemented with 4 mg

$L^{-1}$ for each Kin and BA and the Riviera cultivar was grown in the medium with 4 mg $L^{-1}$ BA and the medium with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ BA, respectively. Whereas, the failure of the two cultivars, Rivera and Provento, appeared to give tubers in the free medium (comparison medium) and the Rivera cultivar in the medium with 1 mg $L^{-1}$IAA without growth regulators. As for the diameter of the tubers, there was also a significant effect of the triple interaction in this trait, where the Provento cultivar excelled and grown in the medium with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ Kin. It was followed by the Rivera cultivar grown in the free medium (free hormone) with average of 0.666 cm and 0.564 cm, respectively, which did not differ significantly from the Provento cultivar grown in the medium with 1 mg $L^{-1}$ IAA and the medium supplemented with 4 mg $L^{-1}$ Kin and the Arnova cultivar grown in the medium prepared with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ BA and the medium with 4 mg $L^{-1}$ BA, and the Riviera cultivar grown in the medium prepared with 1 mg $L^{-1}$ IAA + 4 mg $Kin^{-1}$ and differed from the rest of interaction. Also, the Rivera and Provento cultivars failed to produce tubers in free medium (control) and the Rivera cultivar grown in the medium 1 mg $L^{-1}$ IAA free from the addition. Also, not all of the triple interactions between cultivars, medium and growth regulators differed significantly in the average weight of the microtubers, except the provento cultivars grown in the medium supplemented with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ kin, which was significantly excelled in this trait with an average of 0.440g.

Table (3) Effect of cultivars and interaction with medium and growth regulators on the morphological characteristics of microtubers

| Weight (g) | Diameter (cm) | NO. Microtubers | Cultivars | Growth regulators 4 mg $L^{-1}$ | Medium |
|---|---|---|---|---|---|
| 0.014 | 0.096 | 0.4 | **Arnova** | **0** | **0** |
| 0 | 0 | 0 | **Riviera** | | |
| 0 | 0 | 0 | **Provento** | | |
| 0.128 | 0.534 | 1 | **Arnova** | **BA** | |
| 0.01 | 0.06 | 0.2 | **Riviera** | | |
| 0.06 | 0.32 | 0.4 | **Provento** | | |
| 0.016 | 0.104 | 0.6 | **Arnova** | **Kin** | |
| 0.116 | 0.564 | 1 | **Riviera** | | |
| 0.07 | 0.52 | 1.4 | **Provento** | | |
| 0.012 | 0.092 | 0.6 | **Arnova** | **BA+ Kin** | |
| 0.04 | 0.208 | 0.4 | **Riviera** | | |
| 0.002 | 0.042 | 0.2 | **Provento** | | |
| 0.026 | 0.151 | 0.4 | **Arnova** | **0** | **1** |
| 0 | 0 | 0 | **Riviera** | | |
| 0.074 | 0.467 | 0.6 | **Provento** | | |
| 0.13 | 0.37 | 1.6 | **Arnova** | **BA** | |
| 0.016 | 0.082 | 0.2 | **Riviera** | | |
| 0.022 | 0.202 | 0.8 | **Provento** | | |

| | | | | |
|---|---|---|---|---|
| 0.078 | 0.293 | 0.8 | **Arnova** | **Kin** |
| 0.193 | 0.545 | 2 | **Riviera** | |
| 0.44 | 0.666 | 1.8 | **Provento** | |
| 0.044 | 0.246 | 0.6 | **Arnova** | **BA+ Kin** |
| 0.125 | 0.538 | 1 | **Riviera** | |
| 0.034 | 0.31 | 1 | **Provento** | |
| **0.235** | **0.277** | **0.71** | | **LSD 0.05** |




The process of producing microtubules is a complex physiological process that is affected by the level and type of endogenous hormones and the nature of the cultivar (Al Taweel, 2004). The clear difference appears in the response of cultivars to the formation of microtubules . This may explain the difference in their content of natural hormones as well as differences in genotype and its inherent genetic susceptibility (Bachem et al., 2000; Gargantini et al., 2009) or it appears the effectiveness of the growth regulators in the presence of indole acetic acid in the production of microtubres if compared control treatment (free hormone). This may be due to the active role that

auxins play in the processes of stimulating cell division and elongation, in addition to the activity of BA and Kin, which also contribute to cell division and stimulate growth. These results agreed with some studies that showed the effectiveness of hormones (Yunhai and Xianming, 1992) and differed with some others (Al-Taweel et al., 2004; Ghazal et al., 2006).

**Genetic stability of *in vitro* propagated and mother plants using RAPD DNA markers.**

The concentration of the plant samples ranged between 95.1 and 572.1 ng μmol$^{-1}$ and the purity ranged between 1.92 and 2.09 (Table 4).Where the quantities of DNA isolated from the plant samples appeared the efficiency of the method and materials used in the extraction, where the quantities and the purity were appropriate for the subsequent steps.

Table 4. Concentration and purity of DNA extracted from leaves from plant samples of different cultivars of potato .

| Plant samples | DNA concentration (ng μl-1) | Purity |
|---|---|---|
| *In vitro* propagated plants (3 sub cultures) of Arnova cultivars planted in free hormone medium | 159.7 | 2.00 |
| *In vitro* propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL$^{-1}$IAA | 242.7 | 2.00 |
| *In vitro* propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL$^{-1}$IAA+ 4 mg L$^{-1}$ BA | 572.1 | 2.03 |
| Mother plants of Arnova cultivar | 100.4 | 1.92 |
| *In vitro* propagated plants (3 sub cultures) of Riviera cultivars planted in free hormone medium | 250.5 | 2.08 |
| *In vitro* propagated plants (3 sub cultures) of Riviera cultivars planted in MS medium with 1 mgL$^{-1}$IAA | 261.0 | 2.06 |
| *In vitro* propagated plants (3 sub cultures) of Riviera cultivars planted in MS medium with 1 mgL$^{-1}$IAA+ 4 mg L$^{-1}$ BA | 95.1 | 2.09 |
| Mother plants of Riviera cultivar | 119.2 | 1.98 |
| *In vitro* propagated plants (3 sub cultures) of Provento cultivars planted in free hormone medium | 256.0 | 1.98 |
| *In vitro* propagated plants (3 sub cultures) of Provento cultivars planted in MS medium with 1 mgL$^{-1}$IAA | 315.2 | 2.03 |
| *In vitro* propagated plants (3 sub cultures) of Provento cultivars planted in MS medium with 1 mgL$^{-1}$IAA+ 4 mg L$^{-1}$ BA | 345.0 | 2.04 |
| Mother plants of Provento cultivar | 101.6 | 1.94 |

Five random primers were used to study the genetic variations between the *in vitro* propagated and the mother plants. All primers (A15, D2, F8, F12 and H9) proved their effectiveness in giving

bands that varied in their positions based on the type of primers when multiplied with the DNA samples and migrated on agarose gel. As the random primers produced 21, 19 and 17 bands for the cultivars Arnova, Rivera and Provento, respectively, the number of monomorphic bands reached 21, 19 and 17 bands for the same cultivars (table 5), and the primers varied in the number of bands and the lowest number of sites was 2 bands in the primer D2 and H9, The highest number of sites for the bands appeared in the A15 primer was 8, 6 and 6 sites in the cultivars Arnova, Rivera and Provento, respectively. When calculating the percentage of similar bands and the efficiency of primers, it was found that the percentage of similar bands for all primers and for all cultivars reached 100%, and the highest efficiency was 38.10% and 35.29% for primers A15 for the two cultivars Arnova and Provento, respectively, and 31.58% for the primers F12 for the Riviera cultivars. While the D2 and H9 primers gave the lowest efficiency of 9.52% for Arnova cultivar. The differences in the number of bands resulting from the primers may explain to the compatibility linkage with the plant genome and the components of each primer from the nitrogenous bases, because the difference in the sequence of the primers bases may lead to different sites of association with the genome of the plant and thus lead to the variation in the number of bands (Vos et al., 1995). It was pointed out by Brar and Jain, 1998 that any change in the nucleotide sequence occurs as a result of insertion, or deletion, or rearrangement of nucleotides in the genome of the studied samples for any reason that would lead to a change in the primer binding sites, causing a change in the size of the amplified segments and thus the appearance of different bands, or their absence in specific locations on the gel.

Table 5. Monomorphism and their percentage and efficiency of the primers

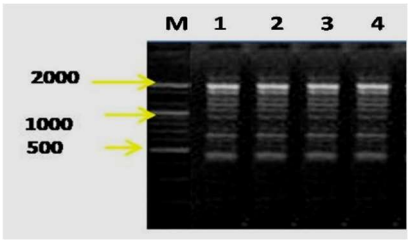
| **Arnova** | | | | |
|---|---|---|---|---|
| **primer symbol** | **Total number of bands** | **The number of Monomorphic bands** | **Monomorphic bands %** | **primers efficiency %** |
| **A15** | 8 | 8 | 100 | 38.10 |
| **D2** | 2 | 2 | 100 | 9.52 |
| **F8** | 4 | 4 | 100 | 19.05 |
| **F12** | 5 | 5 | 100 | 23.81 |
| **H9** | 2 | 2 | 100 | 9.52 |
| **Sum.** | **21** | **21** | | |
| **Riviera** | | | | |
| **A15** | 6 | 6 | 100 | 31.58 |
| **D2** | 2 | 2 | 100 | 10.53 |
| **F8** | 3 | 3 | 100 | 15.79 |
| **F12** | 6 | 6 | 100 | 31.58 |
| **H9** | 2 | 2 | 100 | 10.53 |
| **Sum.** | **19** | **19** | | |
| **Provento** | | | | |

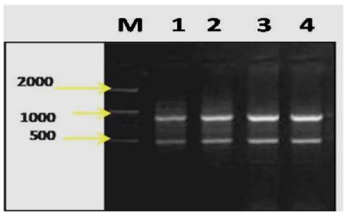
| | | | | |
|---|---|---|---|---|
| **A15** | 6 | 6 | 100 | 35.29 |
| **D2** | 2 | 2 | 100 | 11.76 |
| **F8** | 3 | 3 | 100 | 17.65 |
| **F12** | 4 | 4 | 100 | 23.53 |
| **H9** | 2 | 2 | 100 | 11.76 |
| **Sum.** | **17** | **17** | | |

The PCR-RAPD technique was applied with the presence of 5 random primers based on the method of analyzing the results of the study of genetic variations on the presence or absence of the bands resulting from amplified of certain segments of the genome of the samples used and on the molecular weights of those while neglecting the very light bands (Swoboda and Bhalla, 1997). The products of the reaction showed a number of similar bands that appeared in both *in vitro* propagated samples and the mother plants for the same cultivar, which indicates that they are genetically stable and not different from the mother plant. The number of identical bands was 21, 19 and 17 in the cultivars Arnova, Rivera and Provento, respectively, whose molecular weights ranged between 250-2000 base pair. The primers were distinguished many similar bands of different molecular weights in *in vitro* propagated and mother plants within the same cultivar. In the cultivar Arnova, the primer A15 distinguished the highest number of similar bands ( 8) with molecular weights ranged from 490-2000 base pair, it was found in *in vitro* propagated plants from the third sub cultures and grown in free medium ( control treatment), medium supplemented with 1 mg $L^{-1}$ IAA, medium supplemented with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ BA and mother plants. As for the primer D2 and H9, they distinguished lowest number of bands (2) with molecular weights of 900, 500, 600 and 500 base pairs, respectively. The primers F8, F12 distinguished 4 and 5 bands with molecular weights ranging between 1800 and 280 base pairs (Table 5, Figure 2). As for the cultivar Rivera, the A15 primer distinguished 6 similar bands with molecular weights ranging from 400-2000 base pairs found in *in vitro* propagated plants from the third sub cultures and grown in free medium ( control treatment), medium supplemented with 1 mg $L^{-1}$ IAA, medium supplemented with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ Kin and mother plants, as well as the primer D2 and H9 distinguished two bands with molecular weight 600, 1000, 400 and 600 base pairs, respectively, and the primers F8, and F12 distinguished 3 and 6 bands with molecular weights between 1100 and 280 base pairs (Table 5, Fig. 3). In the Provento cultivar, the A15 primer was able to distinguish 6 similar bands with molecular weights (490-2000 base pairs), and the primers D2 and 9H gave 950, 600 and 2000, 600 bands in the studied plant samples, respectively. The primers F8 and F12 were distinguished 3 and 4 bands whose position varied (900-250) base pairs (Table 5 Figure 4) . The reaction results also specifically for H9 showed the intensity of the resulting bands, it may be explained by several explanations, including that it may be the result of the appearance of some amplified bands together with the same molecular weight, so they appear in the form of one thick bands, which is originally more than one band that may be the result of the homozygote, or it can be explained as that the increase in the concentration of template DNA leads to the repetition of the number of DNA copies and thus it is multiplied more than once, and

because it is difficult to determine the exact concentration of DNA because it is affected by many factors, so the difference in the thickness of the bands is not considered as a measure of genetic variation, especially in the RAPD marker, which follow complete dominance (Al-Hasani, 2002). This result is in agreement with Vogt et al., 1997 and Al-Asimi, 2022.

**A15**

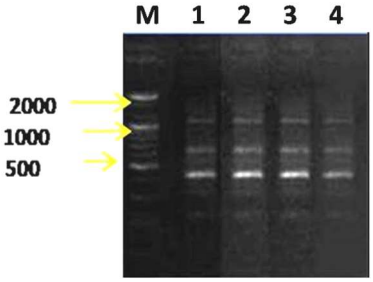


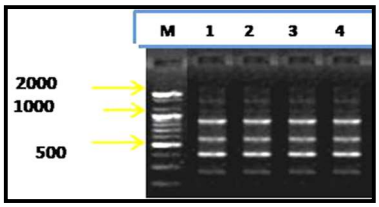

| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 2000 | 1 | 1 | 1 | 1 |
| 2 | 1900 | 1 | 1 | 1 | 1 |
| 3 | 1500 | 1 | 1 | 1 | 1 |
| 4 | 1400 | 1 | 1 | 1 | 1 |
| 5 | 1100 | 1 | 1 | 1 | 1 |
| 6 | 900 | 1 | 1 | 1 | 1 |
| 7 | 650 | 1 | 1 | 1 | 1 |
| 8 | 490 | 1 | 1 | 1 | 1 |

| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 900 | 1 | 1 | 1 | 1 |
| 2 | 500 | 1 | 1 | 1 | 1 |

**F8**



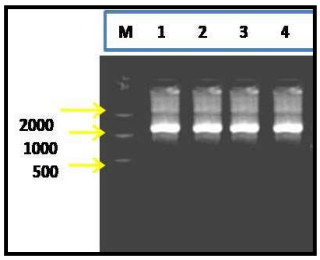

**F18**




| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 1800 | 1 | 1 | 1 | 1 |
| 2 | 900 | 1 | 1 | 1 | 1 |
| 3 | 600 | 1 | 1 | 1 | 1 |
| 4 | 400 | 1 | 1 | 1 | 1 |
| 5 | 280 | 1 | 1 | 1 | 1 |

| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 1200 | 1 | 1 | 1 | 1 |
| 2 | 800 | 1 | 1 | 1 | 1 |
| 3 | 400 | 1 | 1 | 1 | 1 |
| 4 | 350 | 1 | 1 | 1 | 1 |

**H9**




| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 600 | 1 | 1 | 1 | 1 |
| 2 | 500 | 1 | 1 | 1 | 1 |

0 : Absent band 1: Presence band

Figure 2 The PCR products with primers with their molecular weights for plant samples of Arnova cultivar which electrophoresed on 1% agrose gel.

M: DNA ladder 100 bp M: DNA ladder 100 bp

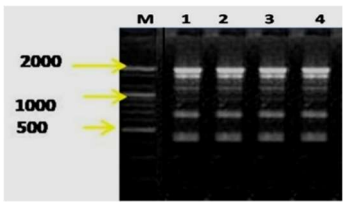
1: In vitro propagated plants (3 sub cultures) of Arnova cultivars planted in free hormone medium

2: In vitro propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL-1IAA

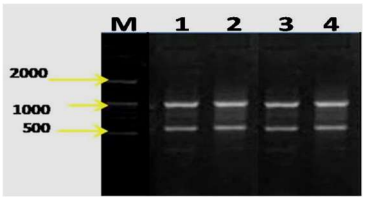
3: In vitro propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL-1IAA+ 4 mg L-1 BA

4: Mother plants of Arnova cultivar

**A15**



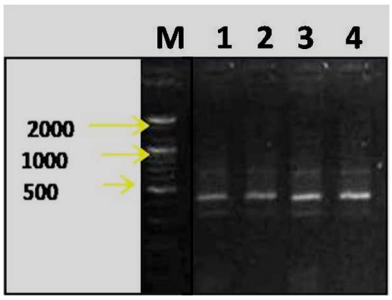

**2**




| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 1000 | 1 | 1 | 1 | 1 |
| 2 | 600 | 1 | 1 | 1 | 1 |

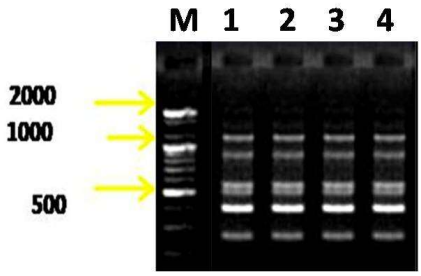
| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 2000 | 1 | 1 | 1 | 1 |
| 2 | 1900 | 1 | 1 | 1 | 1 |
| 3 | 1400 | 1 | 1 | 1 | 1 |
| 4 | 900 | 1 | 1 | 1 | 1 |
| 5 | 700 | 1 | 1 | 1 | 1 |
| 6 | 400 | 1 | 1 | 1 | 1 |

**F8**



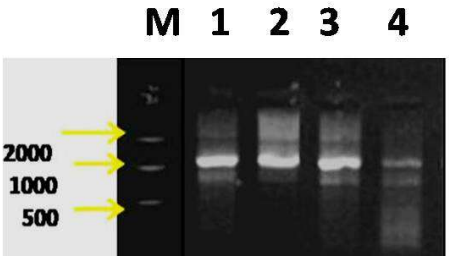

| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 700 | 1 | 1 | 1 | 1 |
| 2 | 400 | 1 | 1 | 1 | 1 |
| 3 | 350 | 1 | 1 | 1 | 1 |

**F18**




| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 1100 | 1 | 1 | 1 | 1 |
| 2 | 900 | 1 | 1 | 1 | 1 |
| 3 | 600 | 1 | 1 | 1 | 1 |
| 4 | 500 | 1 | 1 | 1 | 1 |
| 5 | 400 | 1 | 1 | 1 | 1 |
| 6 | 280 | 1 | 1 | 1 | 1 |

**H9**




| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | 600 | 1 | 1 | 1 | 1 |
| 2 | 400 | 1 | 1 | 1 | 1 |

0 : Absent band 1: Presence band

Figure 3 The PCR products with primers with their molecular weights for plant samples of Riviera cultivar which electrophoresed on 1% agrose gel.

M: DNA ladder 100 bp M: DNA ladder 100 bp

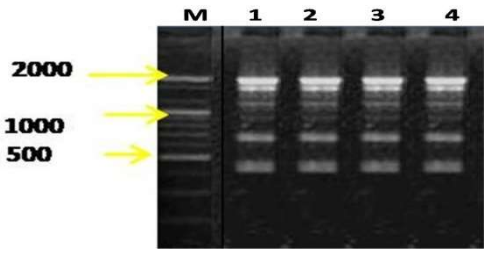
1: In vitro propagated plants (3 sub cultures) of Arnova cultivars planted in free hormone medium

2: In vitro propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL-1IAA

3: In vitro propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL-1IAA+ 4 mg L-1 Kin

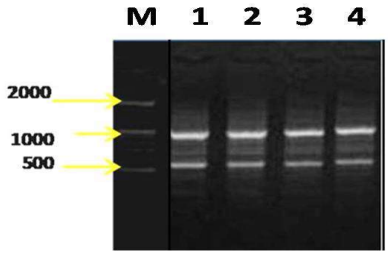
4: Mother plants of Arnova cultivar

**A15**



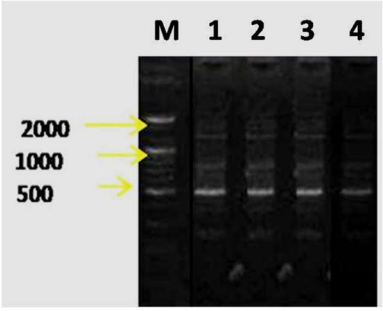

| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | **2000** | **1** | **1** | **1** | **1** |
| 2 | **1900** | **1** | **1** | **1** | **1** |
| 3 | **1400** | **1** | **1** | **1** | **1** |
| 4 | **900** | **1** | **1** | **1** | **1** |
| 5 | **650** | **1** | **1** | **1** | **1** |
| 6 | **490** | **1** | **1** | **1** | **1** |

**D2**



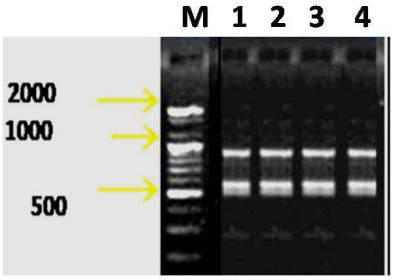

| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | **950** | **1** | **1** | **1** | **1** |
| 2 | **600** | **1** | **1** | **1** | **1** |

**F8**



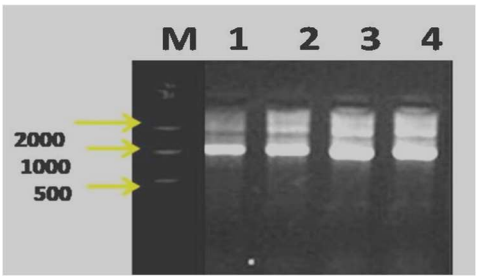

| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | **500** | **1** | **1** | **1** | **1** |
| 2 | **350** | **1** | **1** | **1** | **1** |
| 3 | **250** | **1** | **1** | **1** | **1** |

**F18**




| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | **900** | **1** | **1** | **1** | **1** |
| 2 | **600** | **1** | **1** | **1** | **1** |
| 3 | **500** | **1** | **1** | **1** | **1** |
| 4 | **280** | **1** | **1** | **1** | **1** |

**H9**




| NO. | Molecular Weight (bp) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | **2000** | **1** | **1** | **1** | **1** |
| 2 | **600** | **1** | **1** | **1** | **1** |

0 : Absent band 1: Presence band

Figure 4 The PCR products with primers with their molecular weights for plant samples of Provento cultivar which electrophoresed on 1% agrose gel.
M: DNA ladder 100 bp M: DNA ladder 100 bp
1: In vitro propagated plants (3 sub cultures) of Arnova cultivars planted in free hormone medium
2: In vitro propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL-1IAA
3: In vitro propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL-1IAA+ 4 mg L-1 Kin
4: Mother plants of Arnova cultivar

**Genetic affinity tree**

The analysis of the affinity tree (Dendogram) depends on the genetic range in which the main groups are linked, where the cultivars are arranged based on similarity values ( El Kichaoui, et al., 2013; Khierallah, et al., 2011 ).It appears from Figure 5 the separation of *in vitro* propagated plants cultured on different medium and mother plants of the potato cultivars into two main groups, the first main group included two sub-groups. The first sub-group included the plants of the cultivar Arnova resulting from the third sub culture and grown in the free medium and the plants grown in the medium supplemented with 1 mg $L^{-1}$ IAA. The second sub-group included the plants of the same cultivars grown in the medium supplemented with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ BA and mother plants. The second main group was divided into two sub-groups. The first sub-group included two sub-groups. The first sub-group included the Provento cultivar plants resulting from the third sub-culture and grown in free medium and plants grown in the medium supplemented with 1 mg $L^{-1}$ IAA. under the second sub culture. The Provento cultivar grown in the medium was combined with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ Kin and mother plants. The second sub-group included two sub-groups The first sub-group included plants of the cultivar Rivera resulting from the third sub-culture and grown in free medium, and plants are grown in the medium supplemented with 1 mg $L^{-1}$ IAA. As for the second sub-group, included Rivera cultivar plants grown in the medium supplemented with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ Kin and mother plants. The presence of plants of the same cultivar within a particular group referred to the extent of the genetic similarity of those plants within that group (Askari, et al., 2003 ; Eissa, et al ., 2009 ; Elmeer, et al., 2011)

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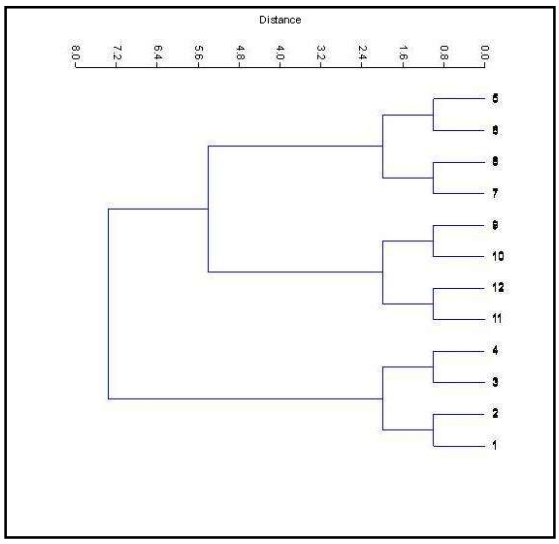


Figure 5. Dendogram to cleairfy of Genetic relationship between of *in vitro* propagated and mother plants of three cultivars of Potato.

1: *In vitro* propagated plants (3 sub cultures) of Arnova cultivars planted in free hormone medium

2 *In vitro* propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL-1IAA

3: *In vitro* propagated plants (3 sub cultures) of Arnova cultivars planted in MS medium with 1 mgL-1IAA+ 4 mg L-1 Kin

4: Mother plants of Arnova cultivar

5: *In vitro* propagated plants (3 sub cultures) of Riviera cultivars planted in free hormone medium

6: *In vitro* propagated plants (3 sub cultures) of Riviera cultivars planted in MS medium with 1 mgL-1IAA

7: *In vitro* propagated plants (3 sub cultures) of Riviera cultivars planted in MS medium with 1 mgL-1IAA+ 4 mg L-1 Kin

8: Mother plants of Riviera cultivar

9: *In vitro* propagated plants (3 sub cultures) of Provento cultivars planted in free hormone medium

10: *In vitro* propagated plants (3 sub cultures) of Provento cultivars planted in MS medium with 1 mgL-1IAA

11: *In vitro* propagated plants (3 sub cultures) of Provento cultivars planted in MS medium with 1 mgL-1IAA+ 4 mg L-1 Kin

12: Mother plants of Provento cultivar

is clear from this that the reaction products showed a number of similar bands that appeared in all the plant samples ( *in vitro* propagated plants from the third sub cultured grown in the free medium (control treatment ), medium supplemented with 1 mg $L^{-1}$ IAA , medium with 1 mg $L^{-1}$ IAA + 4 mg $L^{-1}$ kin or BA and mother plants within the same cultivar. In our studies, RAPD analysis showed absence of genetic variation of the 5 RAPD primers used for genetic stability of tissue – culture derived and mother plants, it was confirmed by the study of genetic variation by drawing a Genetic affinity tree for all the cultivars (Fig. 5) Which refers to its genetic stability and not being different from the mother plant, and thus gives a perception that propagation by plant tissue culture technique is a safe and efficient way to obtain plants identical to the original plant . Hence, it can be concluded that the micropropagation protocol developed in this study is suitable for micropropagation of this economically important food value crop and it is an effective method in preserving genetic resources without changing their genetic composition as a result of sub culture and the use of different growth regulators. These results agreed with some studies that indicated the efficiency of the tissue culture technique in obtaining plants identical to their mothers ( Tiwari et al., 2013 ; Mallaya and Ravishankar, 2013 ; Al-Asimi, 2022 ).

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