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GENETIC VARIATIONS OF FOUR POTATO VARIETIES *SOLANUM TUBEROSUM* L. PROPAGATE *IN VITRO* USING RAPD-PCR

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ABSTRACT
RAPD markers were used to assess genetic relationships of four potato cultivars: Lusa, Arizona, Ambio and Riviera, variations among in vitro regenerated plants. 6 arbitrary 10-base primers were successfully used to amplify DNA extracted from *in vitro* plants. Of these, five primers showed the specific characteristic. Compared with the ability to propagation the close related varieties (Lusa, Arizona, Ambio) showed more ability for propagation.

KEY WORDS: RAPD-PCR, potatoes propagation, genetic variation.

INTRODUCTION

Over the years, potato has become an important crop for both farmers and consumers worldwide. It is the most important crop by volume of production, high yield, and high nutritive value and gives high returns to farmers [1]. The recent advances in tissue culture and the flexibility of organ development in potato allows for alternative methods of propagation through *In vitro* techniques. Tissue culture offers an excellent technique for the rapid propagation of potato plant. The methods used depend on a synthetic growth medium tissue culture which stimulates the growth of axillary buds. *In Vitro* propagation is the alternative to conventional of potatoes production[2], by using meristem tips, nodal cuttings and micro tubers are more reliable for maintaining genetic integrity of the multiplied clones since de-differentiation and the subsequent organogenesis/ embryo genesis with the accompanying genetic changes have been reported[3,4]. Node culture provides a reproducible and economically viable method for producing virus free plants[5]. PCR based Random amplified polymorphic DNA (RAPD) analysis[6,7] has previously been used in genetic studies of potato, for differentiation and identification of cultivars and clonal variants[8] and somatic hybrids[9] and in the assessment of genetic diversity and relationships of cultivated and wild potato species[10].Yasmin *et a.*[11] successfully used RAPD-PCR method to study the genetic diversity of 4 cultivars of potato. Aghaei *et al.*[12] used this method to confirm distinct polymorphism between salt sensitive and salt tolerant cultivars of potato. SimilarlyBiswas *et al.* [13] used RAPD technique as a tool for assessing genetic diversity and varietal relationships among ten varieties of *Solanum* sp.[14]. This study was aimed to describe the genetic variation in four most popular potato varieties in Iraq according to the response for in vitro propagation

Material and Methods

The plant material

In vitro cultures of four potato cultivars *Solanum tuberosum* L.: Lusa, Arizona, Ambio and Riviera, were maintained by optimizing a regeneration protocol. Nodes segments were cultured on MS basal media [15].

Genomic DNA isolation and analysis

The plant DNA extraction attempt using (Genomic DNA Mini Kit (Plant), Geneaid) isolation from the plant samples propagated *in vitro***.**

RAPD analyses

DNA concentrations in the working solution of approximately 30ng/µL in d.H2O were confirmed by spectrophotometer. For RAPD analysis[7], 1X PCR buffer, 200 µM of each dNTPs, 10 pMol random primers and 1.25 U of *Taq* DNA polymerase, concentration of genomic DNA and $MgCl_2$ were optimized. The 10-base oligonucleotide primers obtained from (Operon Technologies, Alameda, CA, USA) were used (Table 2). DNA amplification reactions were performed in a thermal cycler (GeneAmp 9700, ABI). The PCR profile was: one cycle of 94°C for 5 min, 40 cycles of 94°C for 1 min, 38°C for 1 min, and 72°C for 1 min, and a final extension for 10 min at 72°C.

TABLE 1. Primers used in RAPD analysis

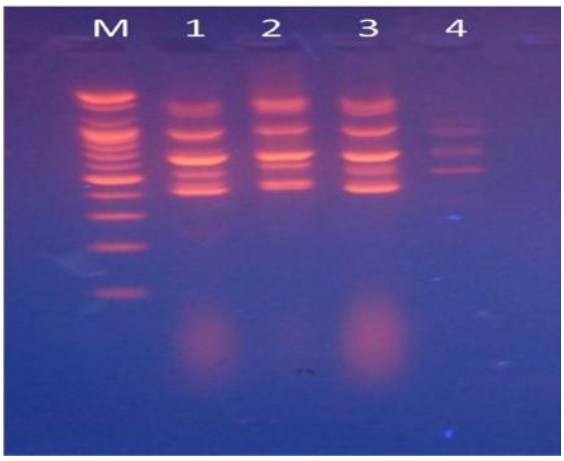
AACGGCGACA	OPT-08	OPERON
GGCATGACCT	OPL-01	OPERON
AGGGGTCTTG	OPA-05	OPERON
GGTGCGGGAA	OPE-02	OPERON
GCCAGACAAG	5	(16)
TGGTTCCCGA	6	(16)

Analyses of RAPD data

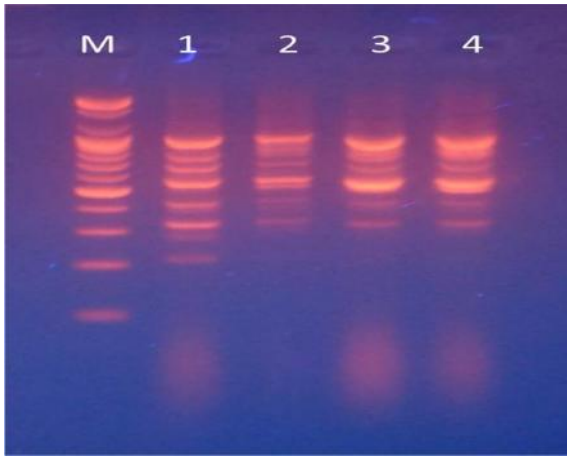
The RAPD fragments were analyzed by electrophoresis on 1.5% agarose gels with ethidium bromide (10 ng/100 mL of agarose solution in Tris borate EDTA buffer). The bands were counted by starting from the top of the lanes to the bottom. All visible and unambiguously scorable fragments amplified by the primers were scored under the heading of total scorable fragments. Amplification profiles of the six genotypes were compared with each other, and bands of DNA fragments were scored as present or absent. The data of the primers were used to estimate genetic similarity (Table1) on the basis of Jacard Index depending on number of shared amplification products[17]. The equation used was: No. of shared amplification products = 2 X (No. of common bands between any two lanes) / (Total No. of bands in the same two lanes). Genetic relationship among the genotypes was estimated with the dendrogram (Figure 7) constructed using unweighted pair group of arithmetic means UPGMA[18].

RESULTS & DISCUSSION

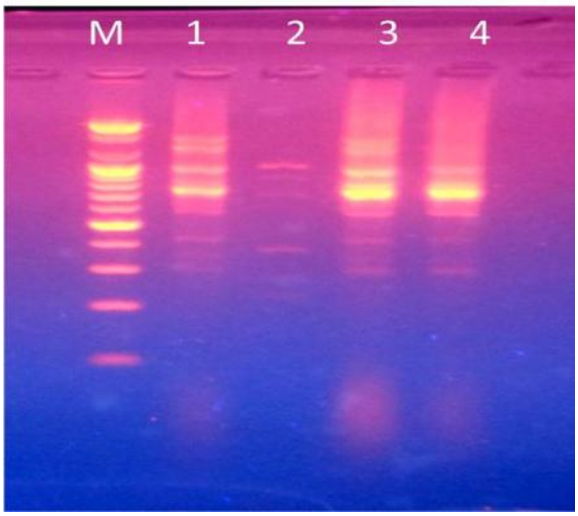
RAPD-PCR was used for detection of DNA profile changes in four potato varieties Lusa, Arizona, Ambio and Riviera, to characterize the relation between the ability of *in vitro* propagation and the genetic variation. Totally, 6 primers of 10-mer priming oligonucleotides were used in RAPD and five of them were selected for their stable results and produced the stable patron number of bands. Fig 1 presents all RAPD bands of selected 6 primers. Each primer generated 3–11 bands with an average of 6.25 bands per primer. The number and size of the DNA fragments were strictly dependent upon the sequence of the primer Reactions were repeated from two to three times to check the consistency of the amplified products; only easily resolved and bright DNA bands were counted. All the four potato varieties could be identified with a single primer. These results suggest that RAPD markers provided substantial information for the identification of potato genotypes. Among the four potato genotypes, Riviera2 produced the lowest number of DNA-amplified fragments. According to the results, RAPD profiles showed significant differences Furthermore, some primers resulted in alteration of a few amplification products. Primer OPT-08 show a highly homology between three varieties (Lusa, Arizona and Ambio) in band profile, obvious different band profile were noticed with Riviera variety, same band profile difference were obtained using the rest of primers, primer OPA-05 show one ban td in all varieties so it is excluded from band score.



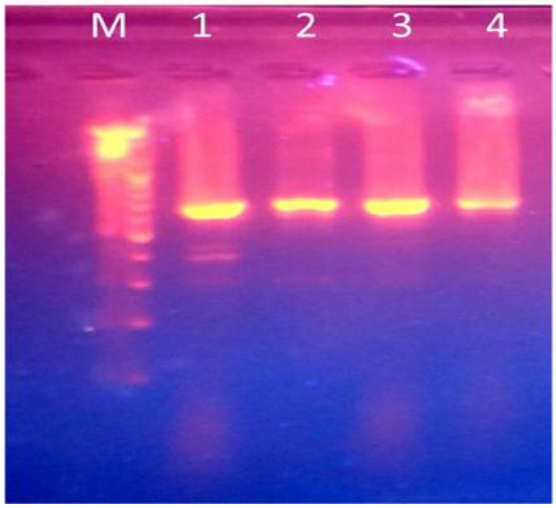
OPT-08



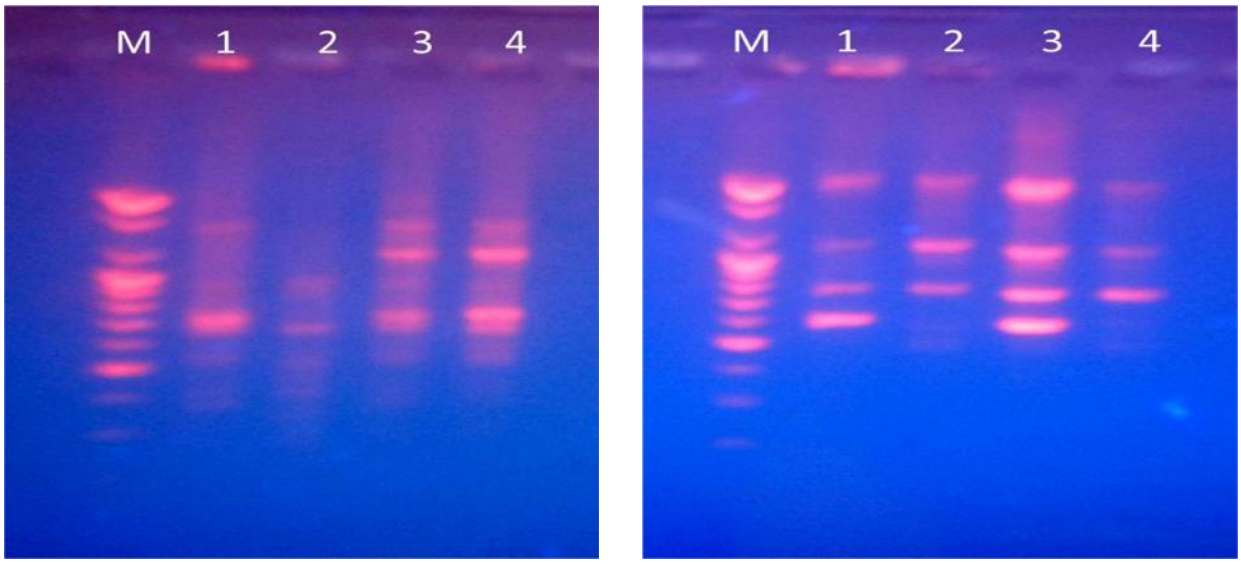
OPL-01



OPA-05



OPE-02



5 6

FIGURE 1: An example of RAPD banding pattern obtained from primer OPB-05 on 2% agarose gel, 5V/cm at 1hr. for genotypes of potato, lane 1: Lusa, lane 2: Arizona, lanne 3: Ambio, 4: river and lane M represented the molecular marker (100bp DNA Ladder Promega).

The distance matrix that analyzed with Jacard index show highly simelarty between Arizona4 and Ambio3 (0.667/1**)** table2**.**

TABLE 2: Similarity Matrices computed with Jaccard coefficient for potato varieties obtained from RAPD-PCR showing the relationship between verities.

	Lusa1	Riviera2	Ambio3	Arizonia4
Lusa1	1	0.314	0.459	0.297
Riviera2		1	0.297	0.273
Ambio3			1	0.667
Arizonia4				1

The Riviera variety shows highly dissimilarity with all other varieties as listed in table were the similarity percent with Lusa, Arizona and Ambio were 0.314, 0.273 and 0.279 respectively. These results indicate the highly genetic difference for Riviera and explain the poor ability to propagated in vitro comparing other varieties**.** The period which spent by the vegetarian part that cultivation in propagation of the growth of a complete plant for both Ambio and Arizona (21 days). For Lusa variety its spend 5 weeks and Riviera variety was very weak to response to the histological cultivation more than 8 weeks.

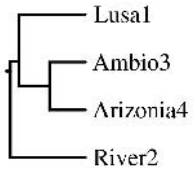


FIGURE 2: Dendrogram of six wheat varieties showing genetic similarity based on RAPD data by using UPGMA cluster analysis, showing the relationship between verities.

The reproducibility of the RAPD technique can be influenced by variable factors, such as primer sequence, template quality and quantity, the type of thermocycler, and polymerase concentration[19]. However, the use of a standardized RAPD protocol can ensure a reproducible. Same result has been obtained for using RAPD-PCR [20] characterized 39 potato cultivars in Japan by RAPD assay using five decamer primers which amplified 15 useful DNA segments either polymorphic or shared among the cultivars. Forty six North American potato cultivars were fingerprinted using RAPD[21]. All cultivars was discriminated with 10 primers. Demeke *et al.*[22] used RAPD to distinguish 30 potato cultivars by a single primer.

The ability of a few random primers to produce RAPDs capable of differentiating the four cultivars examined demonstrates the potentiality of this method for distinguishing and identifying potato cultivars. This study provides information on the molecular basis of polymorphism detected as RAPD profile in potato propagation. The propagation variation shown in our study is in agreement with Yang *et al.*[23] who analyzed

somaclonal variation in cultured cells of rice. More than 50 polymorphic fragments were identified with the five primer tested. Propagation variations were found to in due to variety.

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