Genetic Diversity Among Commercial Potato Cultivars Using RAPD Analysis

Abdullah Alaklabi
Department of Biology, College of Science and Arts, Albaha University, Baljurashi, Saudi Arabia

Corresponding author: Abdullah Alaklabi, e-mail: alaklabia@yahoo.com

ABSTRACT: Potatoes (*Solanum tuberosum* subsp. *tuberosum*) are one of the most important vegetable crops worldwide and contain hundreds of cultivars with similar morphological parameters that are commercially produced. The identification of such genetic diversity represents an important factor in crop breeding and improvement as well as market pricing. This study genotyped twelve potato commercial cultivars using six RAPD primers. The results showed high polymorphism among all cultivars. The primers produced 61 bands in total. The number of bands per cultivar ranged from 7-14. The genetic distance among the cultivars ranged from 0.114^ – 0.3934. The closest cultivars were Disree and Lady Balfour and the furthest one, were Ospery and Winston. Primers 5 and 6 produced the highest number of genetic polymorphic bands with numbers (14&12; respectively) and were able to identify and differentiate all examined cultivars. In conclusion, RAPD primers might be useful in commercial potato cultivar identification.
Key words: Potato, RAPD, polymorphic, genetic diversity

INTRODUCTION

Potatoes belong to the family Solanaceae and are one of the most important vegetable crops in the world with a global production of 385 million tons; about 500000 tons of them are produced in Saudi Arabia (FAOSTAT, 2016). Potato is a tetraploid plant that has a basic number of 12 chromosomes (x = 12). In the local Arabian markets there are dozens of potato cultivars that are grown and consumed fresh or processed and like any other crop, the assessment of such genetic variability may help in crop improvement programs. Further, agricultural companies produce many cultivars and varieties each year to fulfill consumer expectations regarding taste and color as well as grower expectations. Such large variation urge continuous effort to discriminate among cultivars.

The identification of potato cultivar is usually based on morphological traits, such as tuber and leaf shapes and color as well as flower color (Lopez-Vizcón and Ortega, 2012; Rosa *et al*., 2010) which are time consuming, difficult to assess and are influenced by environmental factors. Several methods had been applied on potato cultivars such as morphological, physiological and biochemical parameters as well as molecular markers such as Simple Sequence Repeats in France (Moisan-Thiery *et al*., 2005), microsatellites in brazil (Rosa *et al*., 2010) and RAPD in Pakistan (Abbas *et al*., 2008), however for our knowledge, no molecular markers using RAPD were applied on Saudi Arabia grown potato cultivars were performed.

Variety identification is essential to certify the identity and purity of genotypes hence might be useful in the analysis of genetic variability among cultivars, determining distinct parental combinations to produce segregating progenies that capture maximum genetic variability to enhance selection options among progeny.

The objective of the current study, was to evaluate a set of RAPD markers for varietal identification and characterization of recently introduced potato cultivars in Saudi Arabia and elucidate the genetic relationship among potato cultivars and also, simply cultivar identification.

MATERIAL AND METHODS

Plant material

Potato (*Solanum tuberosum*) cultivars Winston, Desiree, Oceania, Argos, Fridor, Lady Balfour, Ospery, Nicola, Hermas, Valor, Xcaliber and Belini were selected as major potato cultivars in the region.

DNA extraction

Young leaves were taken from each plant and washed thoroughly with water then ethanol to remove dust and other contaminants. The DNA was extracted according for Štorchová *et al.* (2000) with little modifications. Briefly, about 200 mg of fresh shoot tissue was ground in liquid nitrogen, transferred to the extraction buffer (0.34M sorbitol, 0.1M Tris–HCl pH 7.6, 5mM EDTA, 0.2% (v/v) 2 mercaptoethanol) and centrifuged at 13 000 rpm for 10 min. The pellet was suspended in the extraction buffer, the same volume of the lysis buffer (0.2M Tris–HCl pH 7.6, 2M NaCl, 0.05M EDTA, 2% CTAB) was added, then chloroform extraction, isopropanol precipitation and washing with 80% ethanol were performed. The DNA was quantified spectrophotometrically and by electrophoresis on 0.8% agarose gels.

RAPD

Total Genomic DNA of each cultivar was diluted in sterile double distilled water to a concentration of 10 ng/ml for RAPD analysis. PCR was performed in PEQLAB thermocycler, Germany in a 25 ul reaction volume containing 200 uM of each dNTP (MBI Fermentas), 3.0mM $MgCl_2$, 0.48 uM primer, magnesium-free reaction buffer and 1U *Taq* DNA polymerase (Promega, USA). After initial heating for 5 min at 94 ◦C, samples were PCR amplified using 40 cycles (94 ◦C, 20 s; 42 ◦C, 20 s; 72 ◦C, 1 min) followed by a final extension of the PCR products for 4 min at 72 ◦C. The products of amplification were analyzed by electrophoresis in 2.0% agarose gels with 1× TAE running buffer, visualized by ethidium bromide staining, and photographed under UV light with a digital Canon power shot G7 camera. Each reaction was repeated twice and negative controls accompanied the reactions without adding DNA for increasing the fidelity of the data. Six RAPD primers were used (Ready-To-Go RAPD Analysis Beads, GE Health Care, UK).

Table (1). RAPD primers used in the study.

Primer	Sequence5`-3`
RAPDA1	GGTGCGGGAA
RAPDA2	GTTTCGCTCC
RAPDA3	GTAGACCCGT
RAPDA4	AAGAGCCCGT
RAPDA5	AACGCGCAAC
RAPDA6	CCCGTCAGCA

Data analysis

All visible and unambiguously scorable fragments amplified by the primers were scored by visual observation. Amplification profiles (band in each position) were scored as present (1) or absent (0). The scores obtained using all the primers in the RAPD analysis were then joined and used to estimate polymorphic locigenetic distance and to construct an UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using a computer program, PAUP4 (Swofford, 2000).

RESULTS

Polymorphic information content

RAPD analysis of twelve cultivars of potatoes revealed that all primers were polymorphic, produced 61 bands in total. The number of bands per cultivar ranged from 7-14. Data in Figure (1) showed the gel electrophoreses of the RAPD-PCR product of primers 3 and 4. The profiles of 12 cultivars showed that primer 3 is more useful in cultivar identification than primer 4. Figure (2) showed that primer 6 profiles the 12 cultivars of Potato and that primer can easily identify the cultivars Oceania, Argos and Xcaliber. Figure (3) showed that primer 1 can use easily to identify Belini, Xcaliber, Valor, Nicola. In general the primers 5 and 6 produced the highest number of polymorphic bands (14&12). The remaining primers showed lower polymorphism.

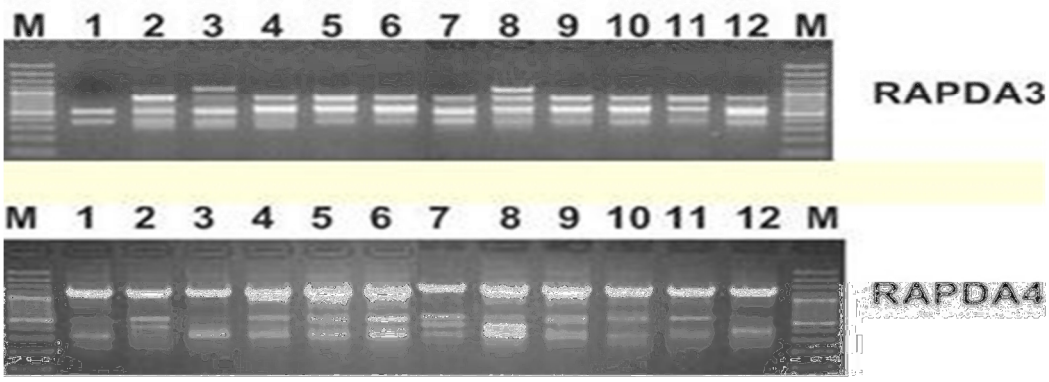

Figure (1). RAPD3, RAPDA4 profiles of 12 cultivars of Potato cultivars. 1) Winston, 2) Desiree, 3) Oceania, 4) Argos,5) Fridor, 6) Lady Balfour, 7) Belini, 8) Xcaliber, 9) Valor 10) Hermas 11) Nicola 12) Ospery, M)Molecular weight marker.

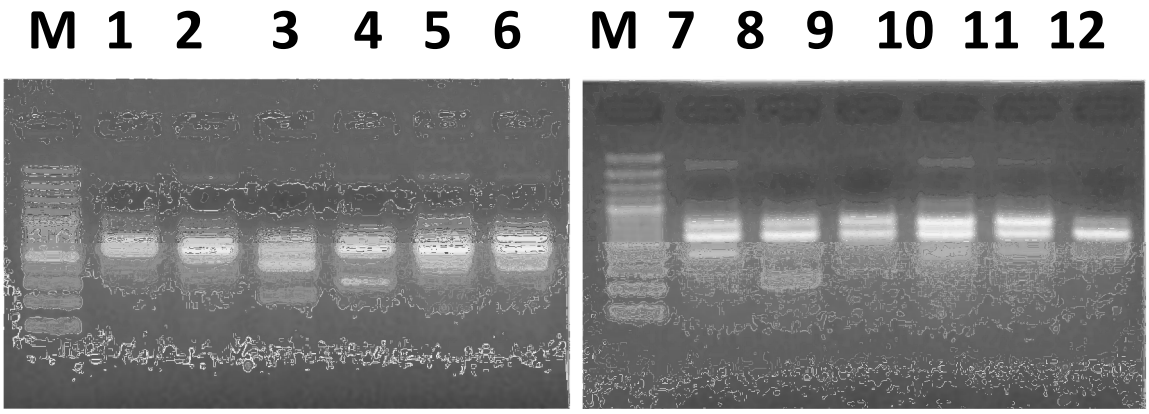

Figure (2). RAPDA6 profile of 12 cultivars of Potato cultivars. 1) Winston, 2) Desiree, 3) Oceania, 4) Argos,5) Fridor, 6) Lady Balfour, 7) Belini, 8) Xcaliber, 9) Valor 10) Hermas 11) Nicola 12) Ospery, M)Molecular weight marker.

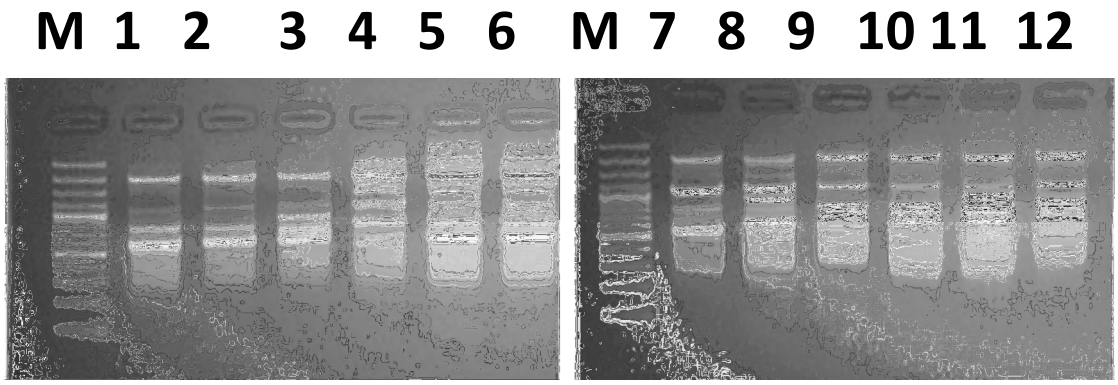

Figure (3). RAPDA1 profile of 12 cultivars of Potato cultivars. 1) Winston, 2) Desiree, 3) Oceania, 4) Argos,5) Fridor, 6) Lady Balfour, 7) Belini, 8) Xcaliber, 9) Valor 10) Hermas 11) Nicola 12) Ospery, M)Molecular weight marker.

Genetic distances

The genetic distance among the cultivars ranged from 0.11475 – 0.39344 and the closest cultivars were Disree, Lady Balfour and the furthest were Ospery and Winston as shown in Table (2).

Table (2). Genetic distances based on mean character differences. The dendrogram based on genetic distance Unweighted Pair Group Method of Arithmetic Means (UPGMA)

	Winston	Disree	Oceania	Argos	Fridor	Lady-Balfour	Ospery	Nicola	Hermas	Valor	Xcaliber	Belini
Winston	0	0.1639	0.2787	0.2787	0.1803	0.1475	0.3934	0.3279	0.2623	0.2459	0.3115	0.3115
Disree		0	0.2131	0.2131	0.1803	0.1148	0.2951	0.2623	0.2623	0.2131	0.2459	0.2131
Oceania			0	0.3607	0.2295	0.2295	0.3443	0.3771	0.3443	0.3279	0.3279	0.3607
Argos				0	0.2295	0.1639	0.3443	0.2459	0.2459	0.2623	0.3279	0.2295
Fridor					0	0.1316	0.2787	0.2459	0.2131	0.1639	0.2951	0.2623
LadyBalf						0	0.3443	0.2459	0.2459	0.1967	0.2951	0.2295
Ospery							0	0.2951	0.2951	0.2787	0.3443	0.2131
Nicola								0	0.2623	0.2459	0.3115	0.2787
Hermas									0	0.2131	0.3443	0.2131
Valor										0	0.2295	0.1967
Xcaliber											0	0.2951
Belini												0

Cluster analyses

In this study, it was found that the commercial potato cultivars could be grouped into two major clusters (Figure ٤). Cluster 1 includes (Ospery, Oceania, Xcaliber (X) and Nicola) and Cluster 2 included two groups G1 and G2, where G1 inlcudes (Argos, Fridor, Disree, Lady Balfour and Winston), G2 inlcude (Hermas, Valor and Belini).

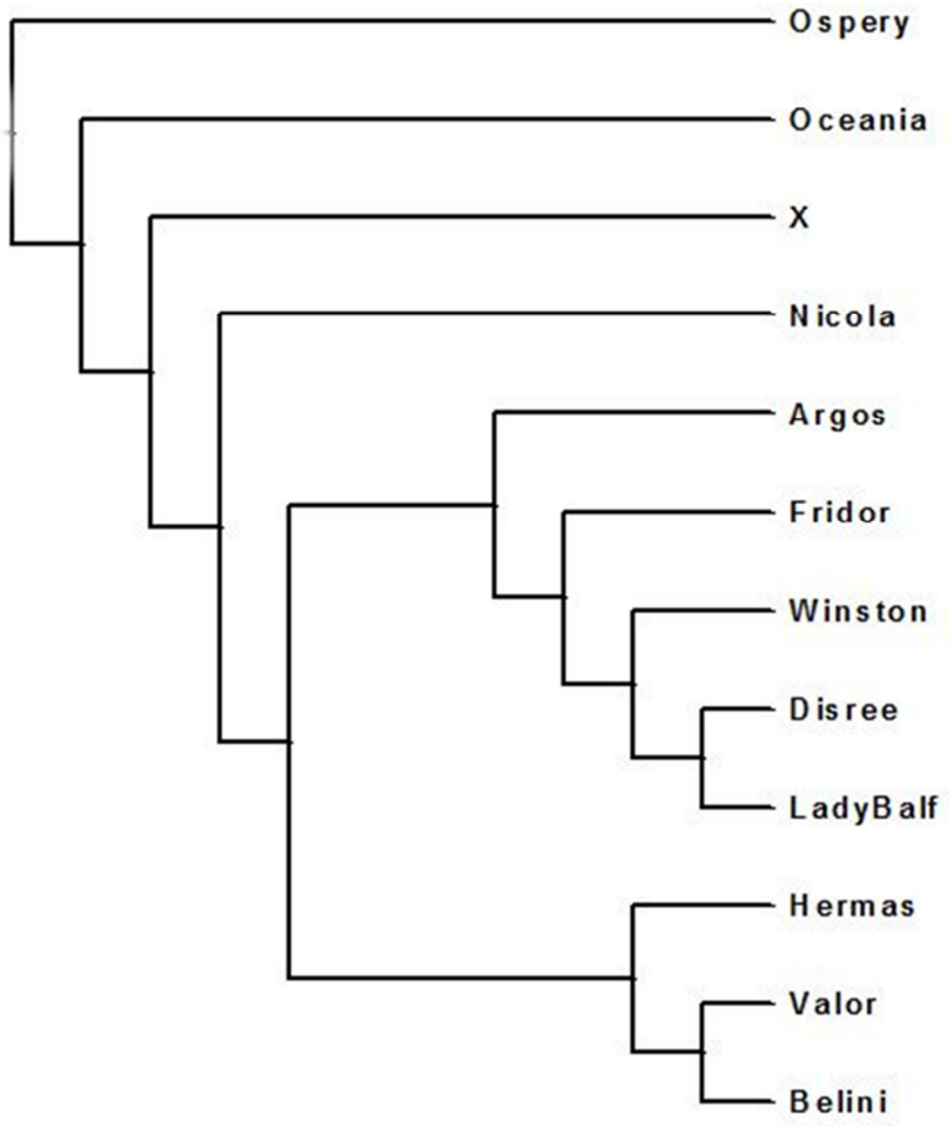

Figure (4). Dendrogram of the 12 potato cultivars based on UPGMA analysis. Two clusters were observed. Cluster 1 include Ospery, Oceania, Xcaliber(X) and Nicola. Cluster 2 devided into 2 groups, G1 include Argos, Fridor, Winston, Disree and Lady Balfour, G2 include Hermas, Valor and Belini.

DISCUSSION

The high polymorphism among potato cultivars found in this study is in agreement with previous investigations on potato worldwide (Solano *et al.,* 2013; Abbas *et al*., 2008). In a previous study on RAPD markers Abbas *et al*. (2008) reported that 26.3 alleles per genotype were amplified using RAPD primers and mean genetic distance estimated ranged from 17% to 55%. However, in this study we reported similar range to that reports in other countries. Solano *et al*. (2013) studied the diversity of nine commercial potato cultivars in Chile as well as native accessions and found that those commercial cultivars cluster according to their breeding programs in Chile and Europe also they found that native accessions showed higher genetic diversity than commercial cultivars. In this study, it was found (Figure 2) that the commercial potato cultivars could be grouped into two major clusters. Cluster 1 includes (Ospery, Oceania, Xcaliber (X) and Nicola). Cluster 2 include two groups G1 and G2, where G1 inlcudes (Argos, Fridor, Disree, Lady Balfour and Winston), G2 include (Hermas, Valor and Belini). In the potato cultivars grown in Pakistan (Abbas *et al*., 2008) there were six potato varieties and 11 primers, the genotypes were grouped into three clusters which indicate that potato culivars usually group accourding to genetic similariy using RAPD technique and that RAPD primers might be useful in breeding programs in potatoes in Saudi Arabia. In the current study primers A1,A6 showed highly polymorophism among potato cultivars and this results are in agreement with previous investigations on other horticultural crops that these molecular markers are capable to discriminate the genetic diversity among species and subspecis as well as cultivars (Elansary and Elansary, 2013; Elansary *et al*., 2011).

This study is useful in cultivar identification in Saudi Arabia because cultivars should be labelled with their variety name accourding to European Union rules for example (EU Directive 2003/89/EC). Potato producers and consumers have the right to know which cultivar they are buying, growing and consuming. Further more, globally most potato varieties are prized and classified accouring to their morphological characteristics only and end-use. Using RAPD technique for the identification of potato cultivars may assist in the accurate identification of potato in the Saudi market.

REFERENCES

Abbas, S. J., G. Rasool, S. R. U. Shah and A. Iqbal (2008). Analysis of genetic diversity in Pakistani potato cultivars by using Randomly Amplified Polymorphic DNA (RAPD) primers, Am.-Eurasian J. Sustain. Agric., 2(1): 50-53.

Elansary, H. O. and D.O. Elansary (2013). Genetic diversity and biochemical activity of leaves and fruits of main *Ficus* Sp. grown in Egypt. Journal of Horticultural Science & Ornamental Plants 5:30–36.

Elansary, H.O., G.G. Mostafa, D. O. Elansary and A. Hussein (2011). Assessment of genetic diversity within the genus Acacia grown in Egypt and studying its relation to leaf tannin and phenolic contents. Proceed, Seventh Pl. Breed. Conf. May 4-5, 2011, Alexandria, Egypt. Egyptian Journal of Plant Breeding 2:243–250 Special issue (2011).

FAOSTAT. (2016). Production metrics of Potatoes http://faostat3.fao.org/browse/Q/QC/E

Lopez-Vizcón,C. and F. Ortega (2012). Detection of mislabelling in the fresh potato retail market employing microsatellite markers. Food Control, 26:575-579.

Moisan-thiery, M., S. Marhadour, M.C. Kerlan, N. Dessenne, M. Perramant, T. Gokelaere and Y.L. Hingrat. (2005). Potato cultivar identification using simple sequence repeats markers (SSR). Potato Research, 48 (3-4): 191-200.

Rosa, P. M., T. de Campos, A. C. B. de Sousa , D. A. Sforça, G. A. M. Torres and A. P. de Sousa. (2010). Potato cultivar identification using molecular markers. Pesquisa Agropecuária Brasileira, 45, 110-113.

Solano, J., M. Mathias, F. Esnault and P. Brabant (2013). Genetic diversity among native varieties and commercial cultivars of *Solanum tuberosum* ssp. *tuberosum* L. present in Chile. Electronic Journal of Biotechnology, 16(6):1-14

Štorchová, H., R. Hrdličková, J. Chrtek, M. Tetera, D. Fitze, J. Fehrer (2000). An improved method of DNA isolation from plants collected in the field and conserved in saturated NaCl/CTAB solution. Taxon, 49:79-84.

Swofford, D. L. (2000). PAUP* 4.0 Beta Version. Phylogenetic analysis using Parsimony (*and other methods) Version 4. Sinauer Associates, Sunderland, MA.

الملخص العربى

إستخدام تكنيك التضاعف العشوائى للمتشابهات فى المادة الوراثية لدراسة التنوع الجينى بين أصناف البطاطس التجارية

عبدالله الأكلبى

قسم الأحياء ، كلية العلوم والأداب بلجرشى ، جامعة الباحة ،المملكة العربية السعودية

يعتبر محصول البطاطس واحدا من أهم محاصيل الخضر على مستوى العالم والذى يضم العديد من الأصناف التى تنتج تجاريا وتتشابة مورفولوجيا ويعتبر التعرف على الأختلافات الوراثية بين أصناف البطاطس من الأمور الهامة لمربى النبات وذلك لتحسين الأصناف من حيث الجودة والسعر. كان غرض الدراسة التعرف على أهم الأختلافات الوراثية بين الاصناف المختلفة فى سبيل التعرف عليها حتى لو كانت متشابهه شكليا أو مورفولوجياً تمت هذه الدراسة للتفرقة وراثيا بين ١٢ صنف تجارى بأستخدام ٦ معلمات عشوائية وأوضحت النتائج المتحصل

عليها أن جميع البوادىء أعطت أختلافات متعددة بين الأصناف المدروسة بأجمالى ٦١ حزمة هذا وتراوح عدد الحزم لكل عينة ما بين ٧-١٤ حزمة . وتراوحت المسافة الوراثية بين جميع الأصناف ما بين ٠.١١٤٧٥ الى ٠.٣٩٣٤٤ موضحة أن اكبر نسبة تشابه بين صنفى الديزيريه والليدى بالفور وأقل نسبة تشابة بين صنفى أوسبرى ووينستون.كما أتضح أن البادىء العشوائى رقم ٥و ٦ اعطى أكبر عدد من الحزم المتعددة الأشكال وكذلك بوادىء ١٤و١٢ على التوالى والذى بالتبعية فرق بين الأصناف المختلفة وعرفها . والخلاصة أن أستخدام البوادىء العشوائية الأكثر تخصصية نجح فى تفرقة وتعريف الأصناف التجارية تحت الدراسة.