Genetic Profiling of a Representative ‘Kerman’ x ‘Peters’ Pistachio (*Pistacia vera* L.) Orchard

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Background

- The common edible pistachio *(Pistacia vera L.)* is a member of the Anacardiaceae or Cashew family and consists of at least 11 species.
- ‘Kerman’ and ‘Peters’ are the most widely used cultivars in California’s pistachio orchards.
- Current observations suggest significant phenotypic variation within the same ‘Kerman’ orchard and among different ‘Kerman’ orchards.
- The amount of genetic variability present in the field and the likely existence of superior/inferior ‘Kerman’ is not well understood at present.
- Few studies have been conducted to identify potential genotypic variation in Pistachio cultivars using molecular markers.
- RAPD markers are marginally more informative than Inter Simple Sequence Repeat (ISSR) in the assessment of genetic diversity in Iranian pistachio cultivars (Ayda et al., 2010).

Motivation

Genetic profiling is important to preserve existing germplasm and serves as the foundation for successful pistachio breeding programs.

Overall Objectives

- To study and determine the amount of genetic variation present in a representative pistachio orchard using RAPD markers.

This Stage

- Optimizing protocols for high quality genomic DNA isolation using extraction kits
- Identify RAPD markers for genetic variability study
- Optimize RAPD PCR reactions

Methodology

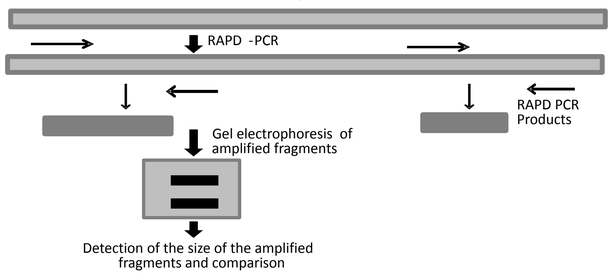

Figure 1. The principle of RAPD-PCR technique. Arrows indicate primer annealing sites (Arif et al., 2010)

Total Genomic DNA extraction from leaf samples using modified DNeasy Plant Mini Kit (QIAGEN Inc., Valencia Ca, USA)

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Quantification of DNA products using Nano Drop 2000 spectrophotometer

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RAPDs identification by Polymerase Chain Reaction (PCR) using random primers

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Separate amplified fragments by gel electrophoresis in agarose

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Score each band for length or incidence of polymorphism and calculate % polymorphism

Figure. 2 Schematic of research methods

Aerial view of Fresno State Pistachio orchard

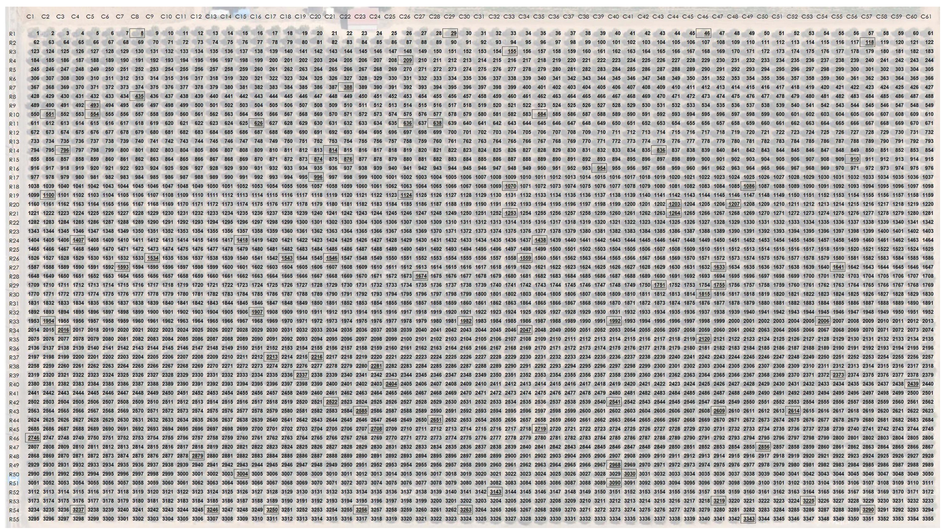

Figure 3 California State University, Fresno Pistachio orchard, overlay tree map was retrieved from Google maps. Each number from 1 to 3355 represents a tree, leaves from 100 trees (in rectangular boxes) were randomly selected.

Results

S/no	Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	Q2614	28.9	ng/µl	0.578	0.344	1.68	5.41	DNA	50
2	Q1815*	26.2	ng/µl	0.523	0.315	1.66	0.68	DNA	50
3	Q2609	27.5	ng/µl	0.55	0.369	1.49	0.44	DNA	50
4	Q2856	15.9	ng/µl	0.319	0.218	1.46	0.37	DNA	50
5	Q2373	15.6	ng/µl	0.312	0.218	1.43	0.56	DNA	50
6	Q3256*	16.2	ng/µl	0.324	0.21	1.54	0.63	DNA	50
7	Q636	16.4	ng/µl	0.327	0.232	1.41	0.37	DNA	50
8	Q3225	11.4	ng/µl	0.229	0.154	1.49	0.55	DNA	50
9	Q1543	11.5	ng/µl	0.231	0.145	1.59	0.78	DNA	50
10	Q3143*	18.4	ng/µl	0.369	0.185	2	0.03	DNA	50
11	Q3237	16.6	ng/µl	0.332	0.207	1.6	0.92	DNA	50
12	Q910	17.8	ng/µl	0.357	0.231	1.55	0.29	DNA	50
13	Q796	10.1	ng/µl	0.202	0.12	1.68	0.55	DNA	50
14	Q3209*	21.7	ng/µl	0.435	0.289	1.5	2.52	DNA	50
15	Q155*	8.7	ng/µl	0.174	0.11	1.57	0.48	DNA	50
16	Q1822*	16.8	ng/µl	0.336	0.199	1.69	0.28	DNA	50
17	Q1203	33.9	ng/µl	0.678	0.438	1.55	0.71	DNA	50
18	Q584	12.5	ng/µl	0.249	0.156	1.6	3.08	DNA	50
19	Q1407*	13.5	ng/µl	0.271	0.16	1.69	0.82	DNA	50
20	Q1633	6.2	ng/µl	0.124	0.078	1.58	0.41	DNA	50
21	Q1559*	12.3	ng/µl	0.247	0.142	1.73	0.31	DNA	50
22	Q1100*	15.4	ng/µl	0.308	0.204	1.51	0.8	DNA	50
23	Q8*	11.5	ng/µl	0.23	0.143	1.61	0.02	DNA	50
24	Q2879*	11.1	ng/µl	0.223	0.15	1.48	0.02	DNA	50
25	Q1418*	15.7	ng/µl	0.314	0.197	1.6	0.59	DNA	50

Table 1. DNA concentration and purity index (A260/A280) of extracted DNA measured by NanoDrop 2000 spectrophotometer. Twelve samples marked (*) were examined for genetic polymorphism(s).

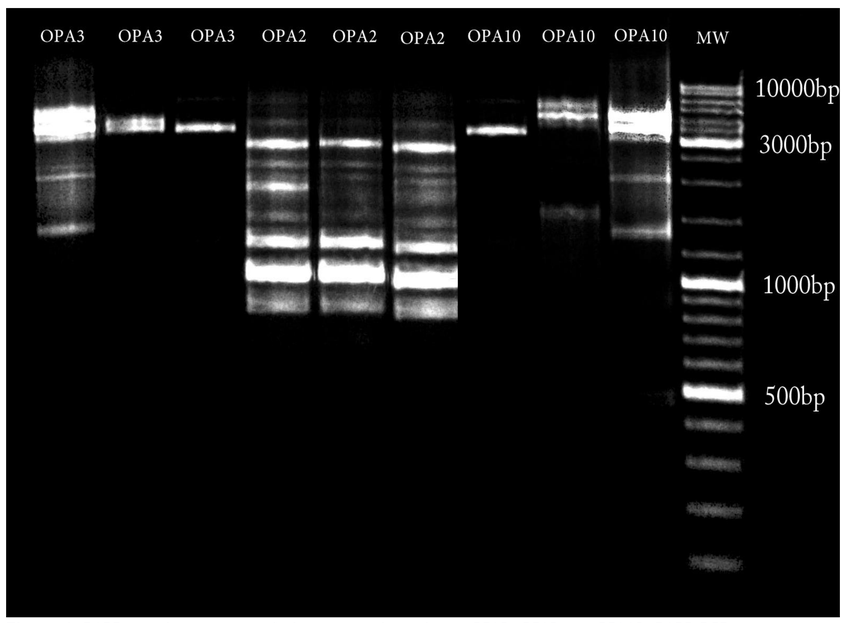

Figure 4. RAPD banding patterns of nine trees using primers OPA3, OPA2 and OPA10. RAPD PCR products were stained in 6X GelRed and visualized under a UV light. MW is molecular weight standards; the size of fragments are in base pairs. Overall, primers OPA10 and OPA3 showed polymorphic banding pattern (33%) in 12 randomly selected trees from the ‘Kerman’ population at Fresno State.

Genetic Polymorphism(s)

SN	RAPD Primers	Sequence (5'-3')	Number of polymorphic bands	Total bands	Polymorphism %
1	OPA-2*	TGCCGAGCTG	4	12	33
2	OPA-3*	AGTCAGCCAC	3	12	25
3	OPA-4	AATCGGGCTG	-	-	-
4	OPA-10*	GTGATCGCAG	5	12	41.6
5	OPA-13	CAGCACCCAC	-	-	
6	OPA-14	CAAACGTCGG	-	-	-
7	OPC-3	GGGGGTCTTT	-	-	-
		Average	**4**	**12**	**33**

Table 2. Primers used for RAPD analysis and number of DNA polymorphic bands produced. Primers marked with (*) produced polymorphic bands

Discussion

Conventional DNA extraction methods can be time consuming, laborious, yield low quality DNA and produce many hazardous wastes (Oboh et. Al., 2009). Our results showed that a modified DNeasy Plant Mini Kit Quick Start protocol produced a sufficient amount of DNA. DNA products from this kit were high quality as confirmed by a purity index using the ratio of absorbance at A260/A280nm (1.41 to 2.0).

We screened 7 random primers for polymorphism(s) in 12 randomly selected trees (Table 1). Only two primers (OPA3 and OPA10) resulted in polymorphic bands (Figure 4). PCR products were stained with 6X GelRed dye and visualized under UV light. GelRed was our choice staining dye because it is sensitive, stable and environmentally safe relative to the widely-used mutagenic ethidium bromide.

RAPD bands were scored as either one or zero for presence or absence of polymorphism. Twelve out of 36 scorable bands were polymorphic (Table 2). Preliminary data from 12 random selected trees in the orchard suggest the presence of polymorphism (33%). Ayda et al. (2010) reported 67% polymorphism among 19 Iranian pistachio cultivars using RAPD markers.

The difference in polymorphism reported in the current study and that of Ayda et al. is likely due to differences in the tested genotypes, selected primers, and or due to random mutagenesis that occurred during scion clonal propagation (budding) in the United States over the past 50 years.

Future Directions

We are screening an additional 88 trees in this orchard for evidence of further genetic variation.

Application

These data are widely applicable to California’s rapidly expanding pistachio industry; specifically to preserve existing germplasm and serve as the foundation for successful pistachio breeding programs. This is important as pistachio acreage is increasing in California since agricultural water use is increasingly being restricted, and this species is considered a low-water use crop.

Acknowledgements

We appreciate the financial support from California Pistachio Research Board and Fresno State Plant Tissue Culture Laboratory.

References

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