Genetic diversity among pomegranate germplasm assessed by microsatellite markers

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Abstract

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and is native from the region between Iran to the Himalaya in Northern India. It has been cultivated and naturalized over the whole Mediterranean region since ancient times. Normally consumed as a fresh fruit, pomegranate can also be used to obtain transformed products such as juice, jam or preserves. Moreover, in recent years the pomegranate has shown great importance for human health because of the high antioxidant content of its juice and peel. Improvement of the crop and fruit quality are the main goals in breeding programs. Pomegranate species shows high diversity of pomological traits, which is very interesting for improvement of the crop by breeding. One of the main tools for breeding are the germplasm resources and their genetic diversity. In this study, 96 accessions belonging to the IVIA's collection were analysed using 18 microsatellite markers developed from pomegranate. The plant material come from nine countries and included some hybrids and accessions from unknown origin. A total of 111 alleles were obtained. They were used for addressing the molecular genetic diversity and population structure of the collection. Knowledge of the substructure and diversity of the collection will allow us to manage the genetic resources as well as planning the incorporation of new materials in the future. The goal is to select the best plant material for the pomegranate breeding program.

Key words: pomegranate, microsatellites, diversity, germplasm, *Punica*

INTRODUCTION

The pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and is native from Iran to the Himalaya in northern India. It was cultivated and naturalized over the whole Mediterranean region since ancient times. Pomegranate is well adapted to many different climates and soils and grows very often in poor soils. This species is mainly consumed as a fresh fruit, but it can also be used to obtain different transformed products as juice, jam or preserves. The plant has also interest as ornamental, especially old specimens with twisted trunks and branches. Moreover in the last years the pomegranate is revealing a great importance for the human health because of the high antioxidants content of its juice and peel, related with the prevention of cancer and cardiovascular diseases (Seeram et al, 2006; Sestili et al, 2007). The cultivars grown today are the result of human selection from natural intra-species variation or achieved by seedling selection or hybridization followed by selection (Jalikop 2010). However, in a scenario of a high demanding market, the pomegranate breeding programs should make an effort to accomplish the characteristics demanded by the market, as for instance darker skin, red peel, early ripening, higher antioxidant content and soft seeds. Plant genetic resources are the most important tool for plant breeders, but knowing their variability is essential to be useful. Pomegranate species

shows high diversity of pomological traits, which is very interesting for improvement of the crop by breeding. However, the morphological diversity studies are difficult and time consuming and in some cases it is not possible to address the homonyms and synonyms present in the collections. In this sense, studies of genetic diversity based on pomological traits have been completed by molecular markers. In this study we selected microsatellites markers developed by Soriano et al. (2010) for genotyping a germplasm collection belonging to the IVIA.

MATERIALS AND METHODS

Plant material

Ninety-six pomegranate accessions (Table 1) were used to assess microsatellite variability; 30 of them were selected from the Turkmenistan, 18 from Russia, 14 from United States, 13 from Spain, 5 from Iran and Japan, 2 from Israel, 1 from Greece and Italy and 6 with unknown origin The collection belongs to the IVIA and the EEE .

Table 1: Accessions analysed and country of origin.

Accession	Origin	Accession	Origin
Ermioni	Greece	Syunt	Turkmenistan
Alk	Iran	Koinekasyrskii	Turkmenistan
Mahali Dezful	Iran	Gissarskii	Turkmenistan
Shirin	Iran	Kara-Kalinskii	Turkmenistan
Entek	Iran	Pamyati	Turkmenistan
Tabestani malas	Iran	H x K	Turkmenistan
Dorosht 5	Iran	Vishnevyi	Turkmenistan
isr-8	Israel	White Flower	Turkmenistan
isr-9	Israel	Machtumkuli	Turkmenistan
Palermo	Italy	Molla Nepes	Turkmenistan
Ki-zakuro	Japan	Desertnyi	Turkmenistan
Haku-taka	Japan	Sverkhranniy	Turkmenistan
Nochi-shibori	Japan	Sirenevyi	Turkmenistan
Haku-botan	Japan	Azadi	Turkmenistan
Toryu-shibori	Japan	Podarok	Turkmenistan

Zubejda	Russia	Chandyr	Turkmenistan
Mejhos 6269	Russia	Sogdiana	Turkmenistan
Saartuzski	Russia	Shirin Zigar	Turkmenistan
Nikitski ranni	Russia	Girkanets	Turkmenistan
Surh-anor	Russia	Medovyi Vahsha	Turkmenistan
Al-sirin-nar	Russia	Dotch Legrelley	Turkmenistan
Kunduzski	Russia	Shainakskii	Turkmenistan
Bala Miursal	Russia	Myatadzhy	Turkmenistan
Kara bala	Russia	Parfyanka	Turkmenistan
Afganski	Russia	Sumbarskii	Turkmenistan
Apseronski	Russia	Vkusnyi	Turkmenistan
Sakerdze	Russia	H x G	Turkmenistan
Salavatski	Russia	Sumbar	Turkmenistan
Kazake	Russia	Cranberry	United States
Kaj-acik-anor	Russia	Purple Heart	United States
Kaim-anor	Russia	Ambrosia	United States
Apseronski krasnyj	Russia	Loffani	United States
Sejanec 2-5/8	Russia	Phoenicia	United States
agrio2	Spain	Green Globe	United States
casta del reino	Spain	Chico	United States
piñon tierno de Ojós	Spain	Golden Globe	United States
agrio1	Spain	NM	United States
olivares	Spain	Crab	United States
moll-7	Spain	Wonderful	United States

elx-6	Spain	Eve	United States
elx-7	Spain	Elf	United States
elx-8	Spain	Mae	United States
elx-9	Spain	Wonderful	Unknown
elx-10	Spain	Balegal	Unknown
elx-11	Spain	Ink	Unknown
elx-12	Spain	Orange	Unknown
Agat	Turkmenistan	Cana	Unknown
Ariana	Turkmenistan	How Sweet	Unknown

Methods

DNA isolation:

DNA was extracted from 50 mg of young leaves following the method described by Doyle and Doyle (1987). DNA quantification was performed by NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and integrity was checked on 1% agarose gel.

Microsatellite Analysis

Eighteen microsatellite markers (Table 2) were selected according to their informative content from Soriano et al (2010). The PCR conditions used were those described by Soriano et al. (2010). Each reaction was performed with three primers: the specific forward primer of each microsatellite with an M13(−21) tail at its 5 end, the sequence-specific reverse primer and the universal fluorescent-labeled M13(−21) primer (Schuelke, 2000). Allele lengths were determined using an ABI Prism 3130 Genetic Analyzer with the aid of GeneMapper software, version 4.0 (Applied Biosystems).

Data analysis
For each microsatellite the number of alleles and their size range were calculated. In order to determine the relationship of the accessions used, a factorial correspondence analysis (FCA) was carried out using the Genetix program (Belkhir et al. 1996).

Table 2. Microsatellites used in the study, number of alleles obtained and size.

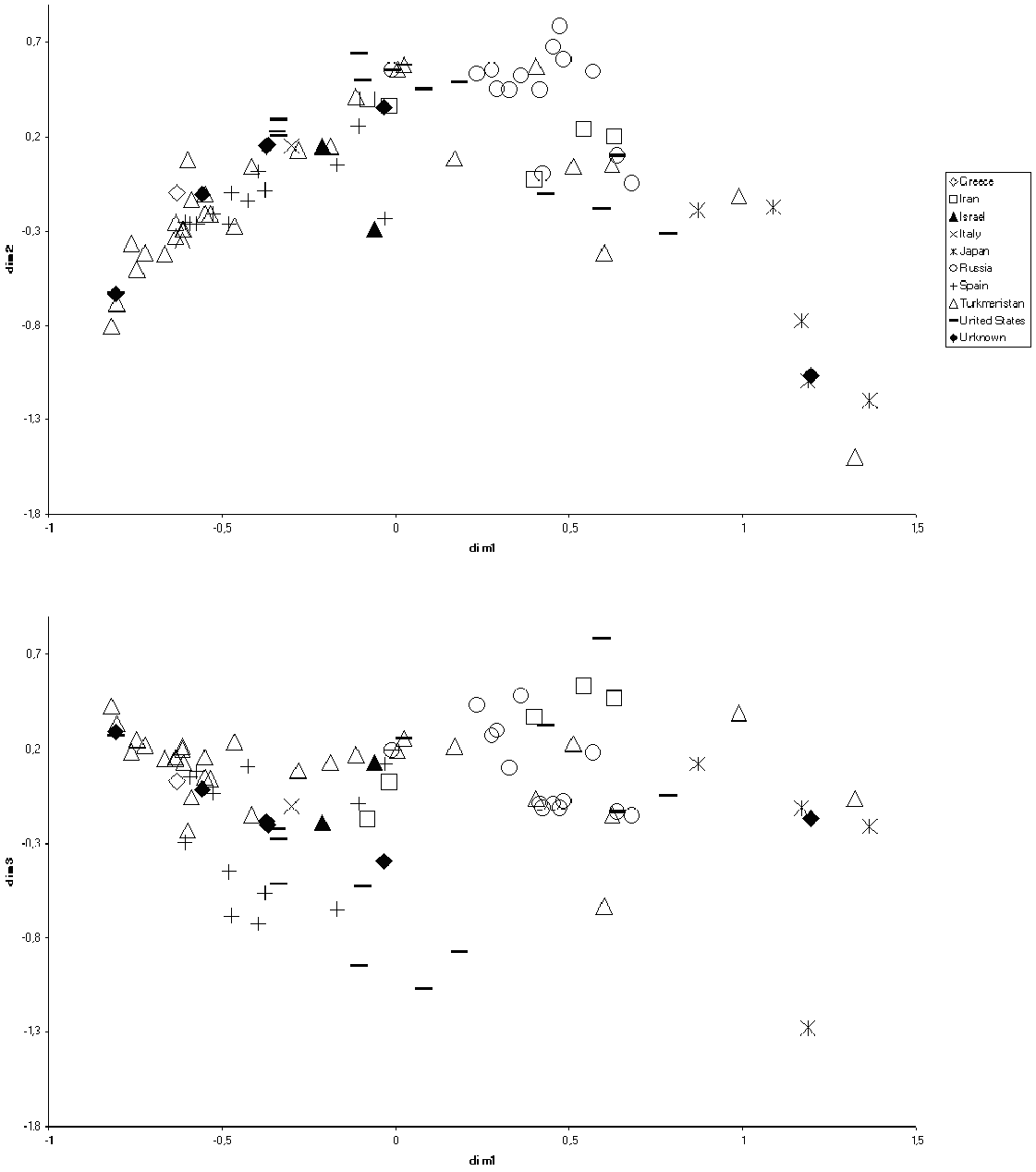
Locus Name	Alleles	Size Range (bp)	Locus Name	Alleles	Size Range(bp)
PGCT015	7	164-228	PGCT087	5	253-263
PGCT016	3	196-204	PGCT088	5	148-169
PGCT022	4	238-243	PGCT089	7	134-154

PGCT028	4	228-240	PGCT091	8	203-223
PGCT032	3	123-129	PGCT093A	7	231-254
PGCT038	5	265-284	PGCT093B	6	182-208
PGCT057	3	178-182	PGCT098	4	114-146
PGCT066	3	124-131	PGCT110	12	106-188
PGCT083	5	131-143	PGCT111	6	206-248

RESULTS AND DISCUSSION

In a previous work, we reported the development of 117 microsatellite (simple sequence polymorphism, SSR) markers from an enriched genomic library from the cultivar Mollar (Soriano et al., 2010). From them, 18 microsatellites were screened in 96 accessions from IVIA's germplasm collection. The number of alleles ranged from 3 to 12, with a total of 97 different alleles, and their sizes varied from 114 to 248 bp (Table 2).
The factorial correspondence analysis (FCA) showed the relationship of the accessions used (Figure 1). Under this analysis, the first three principal dimensions accounted for 24.91% of the total variance. An accession from India appeared clearly differentiated from the rest and was eliminated from the analysis. According to the first and second dimensions, the accessions from Turkmenistan and USA appeared more dispersed, showing more genetic diversity. Moreover, the Japanese accessions used in this study appear separated from the rest fo the materials. The results allowed to identify a putative duplicates from Iran, as accessions Entek and Dorosht5 have the same pattern for all the microsatellites analyzed. This point should be confirmed with more detail using more markers. Regarding the third dimension, some Spanish and USA accessions appear together that could suggets a relationship between them.

Figure 1



In conclusion, results indicate that the microsatellite markers are a useful tool for diversity studies in pomegranate. In fact, they showed a high level of variation in *P. granatum,* proving to be a powerful tool for genetic diversity studies in germplasm collections as well as for cultivar identification.

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