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SHORT COMMUNICATION

Development and characterization of microsatellite markers in pomegranate (*Punica granatum* L.)

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Abstract The pomegranate (*Punica granatum* L.) is a temperate climate species requiring high temperatures for proper and complete ripening. The species is consumed as a fresh fruit, but also can be used to obtain transformed products such as juice, jam, or preserve. It is a fruit tree species with a high degree of diversity, but the identification of cultivars by morphological traits is very difficult. Thus, the characterization of genotypes through molecular markers is of great value for germplasm preservation, genetic studies, and plant breeding. The number of simple sequence repeat (SSR or microsatellite) markers developed for this genus is too low, so in this work we report the development of 117 microsatellite loci from a CT/AG-enriched pomegranate genomic library. In order to check their utility, eleven accessions were analyzed. The polymorphism information content (PIC) value across all loci ranged between 0.09 and 0.71, with an average of 0.37. These markers will facilitate genetic diversity studies, mapping, and genotyping of pomegranate.

Keywords SSR markers · Enriched library · Cultivar identification

The pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and is native from Iran to the Himalaya in northern India. It has been cultivated and naturalized over the whole Mediterranean region since ancient times. Pomegranate is well adapted to many different climates and soils and very often grows in poor soils. Normally the pomegranate is consumed as a fresh fruit, but it can also be used to obtain transformed products such as juice, jam, or preserve. The plant also has interest as an ornamental, especially old specimens with twisted trunks and branches. Moreover, in recent years the pomegranate has shown great importance for human health because of the high antioxidant content of its juice and peel, and its properties which prevent cancer and cardiovascular diseases (Shishodia et al. 2006; Fuhrman and Aviram 2006).

For pomegranate breeding purposes and for the protection of the plant breeder's rights, correct identification of genotypes is required. Pomegranate shows high diversity of pomological traits, but the identification of cultivars through those traits remains

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difficult and time-consuming. The most recent studies of genetic diversity have been carried out using molecular markers such as random amplification of polymorphic DNA (RAPD) (Ercisli et al. 2007; Zamani et al. 2007; Durgaç et al. 2008; Masoud et al. 2008; Narzary et al. 2009; Hasnaoui et al. 2010a), restricted fragment length polymorphism (RFLP) (Melgarejo et al. 2009) and amplified fragment length polymorphism (AFLP) (Yuan et al. 2007; Jbir et al. 2008; Awamleh et al. 2009) and SSR (Koohi-Dehkordi et al. 2007; Currò et al. 2010; Hasnaoui et al. 2010b; Pirseyedi et al. 2010). Here, we report the development of 117 microsatellite (simple sequence polymorphism, SSR) markers from an enriched genomic library. Despite the high cost of development, these markers have many advantages such as high reproducibility, high level of polymorphism, random distribution throughout the genome, and codominancy.

Microsatellite isolation was performed from the cultivar Mollar using the enrichment method reported by Aranzana et al. (2002), slightly modified in the hybridization process. Labelling was made by digoxigenin instead of radioactivity and the bacterial colonies grown onto a nylon membrane. Selection of clones was made by dot blot, following Soriano et al. (2006). Genomic DNA was isolated following the protocol described by Doyle and Doyle (1987) and later digested with *Eco*RI and *Rsa*I. Recombinant colonies were selected based on blue/white screening, grown onto a nylon membrane (Hybond N+, Amersham Biosciences) placed on LB plus ampicillin plates and hybridized with a digoxigenin-labelled $(AG)_{15}$ oligonucleotide probe. PCR screening using M13 primers was carried out to verify the presence of the insert in the clones. Positive clones carrying an insert were sequenced using an ABI PRISM 377 DNA Sequencer (Applied Biosystems) and the BigDye Terminator version 1.1 Cycle Sequencing Kit (Applied Biosystems). Primers flanking the repeated motif were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/). Eleven pomegranate accessions were used to assess microsatellite variability; six of them were selected from the Spanish varietal groups Mollar de Elche and Valencianas (Mollar, M-49, M-2, M-3, M-25, and V-1), two accessions came from Israel (CG and ISR), one from USA (Wonderful), one from India (IND), and the last one from Turkey (ARB3). SSR amplifications were performed in a GeneAmp® PCR System 9700 thermal cycler (Perkin-Elmer, Freemont, CA, USA) in a final volume of 20 µl, containing 75 mM Tris–HCl, pH 8.8; 20 mM $(NH_4)_2SO_4$; 1.5 mM $MgCl_2$; 0.1 mM of each dNTP; 20 ng of genomic DNA and 1 U of *Taq* polymerase (Invitrogen, Carlsbad, CA, USA). Each polymerase chain reaction was performed by the procedure of Schuelke (2000) using three primers: the specific forward primer of each microsatellite with M13(-21) tail at its 5′ end at 0.4 µM, the sequence-specific reverse primer at 0.8 µM, and the universal fluorescent-labeled M13(-21) primer at 0.4 µM. PCR conditions were performed using the following temperature profile: 94°C for 2 min, then 35 cycles of 94°C for 30 s, the optimized annealing temperature (Table 1) for 60 s and 72°C for 1 min 30 s, finishing with 72°C for 7 min. Allele lengths were determined using an ABI Prism 3130 Genetic Analyzer with the aid of GeneMapper software, version 4.0 (Applied Biosystems). In order to evaluate the informativeness of the microsatellites employed, the number of alleles per locus and the polymorphism information content (PIC) were calculated. PIC was calculated according to Weir (1990) based on allele frequencies of all of the varieties analyzed as: $PIC_i = 1 - \sum P_{ij}^2$, where P_{ij} is the frequency of the *j*th allele for the *ith* marker locus and summation extends over *n* alleles. 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).

From a total of 542 recombinant colonies, 408 were selected on the basis of blue/white screening; 204 resulted positive after the hybridization with the $(AG)_{15}$ probe. After PCR confirmation of the cloned inserts with M13 universal primers of the cloning vector, 192 were sequenced. These sequences were aligned using CAP3 software (Huang and Madan 1999) revealing the presence of 141 unique sequences. From them, 29 were not suitable for primer development and were discarded (11 did not contain the repetition and 18 contained the repetition at one end). From the remaining 112 sequences, five of them presented two microsatellite sequences. Thus, a total of 117 primer pairs were designed flanking the repeated sequences. Altogether, 66 out of the 117 primers pairs developed were polymorphic, 38 were monomorphic and 13 did not yield clearly defined amplified products (Table 1). The number of alleles from the 66

Table 1 List of the 117 SSR primer pairs isolated from pomegrante SSR-enriched library, showing locus code, repeat motif, primer sequences, melting temperature (T_m), length, number of alleles, and PIC value

Locus/genbank accession no.	Repeat	Primer sequence (5′–3′)	T_m (°C)	Length (bp)[a]	No. of alleles	PIC
PGCT001	$(TC)_{18}$	F:AGCTCCGATTGAGAGCAGAT	59	101	3	0.37
FN677521		R:TTGGAGCAATTGGAGAGAGA				
PGCT002	$(CT)_{16}$	F:AAACCCACCATCTCTCACTC	55	116	2	0.17
FN677522		R:CCTCTCTTCTTCAGTACTCCTCT				
PGCT003	$(TC)_{16}$	F:CATTAGTCTGGAAAGATGAG	50	150	1	–
FN677523		R:GAACTTCCAGTATAATATATCAGAG				
PGCT004A	$(CT)_{13}$	F:AACCCAGGGGATACCAAAAG	60	204	–	–
FN677524		R:ACGAAATGGGAAATGCAGAG				
PGCT004B	$(CT)_{11}$	F:CTCTGCATTTCCCATTTCGT	60	116	1	–
FN677524		R:GGGGACGTAGAACTCAGCAA				
PGCT005	$(CT)_{15}$	F:TCCGTGTGTGAAGAAGACCA	59	117	2	0.46
FN677525		R:GGTTTGGATTTCTTGGGTTTT				
PGCT006[b]	$(CT)_{16}$	F:TTGAATTGATGTAACGCTTG	55	173	7	–
FN677526		R:GAGGAAAGTCGTTTGAAGTG				
PGCT007	$(GA)_{15}$	F:ACTCTGATTGAGCCCTACTG	52	150	1	–
FN677527		R:GACTACCTTACACACCTCTCTC				
PGCT008	$(CT)_{16}$	F:ATTCAGCAGATTTTCAGGTC	53	206	1	–
FN677528		R:GATGAGGTGTGAGTTTGATG				
PGCT009	$(CT)_{17}$	F:TTTACTTTACCCTCTTCCCC	55	112	2	–
FN677529		R:TAAACCAAAGCTACCAAGGA				
PGCT010	$(CT)_{17}$	F:ACCGACACACAAACCCGC	57	153	1	–
FN677530		R:AGGAGAGGTGGAGGAGGAT				
PGCT011	$(TC)_{19}$	F:GGCCCCCTCCTTCCTAATAA	60	115	–	–
FN677531		R:CGATTTTCTTGAAAAACCGAGA				
PGCT012	$(CT)_{15}$	F:TCTCTCCCCTTCCACAAAAG	59	107	1	–
FN677532		R:GGGAGGTGCACAGGATATAGAA				
PGCT013	$(CT)_{17}$	F:TCTCACACACACACGCAGAA	59	262	2	0.16
FN677533		R:GAGAAAGAGGAAACCGCAGA				
PGCT014	$(GA)_{17}$	F:ATCTTCACTTAATCCCATGA	51	103	1	–
FN677534		R:AATCAAAACTTCCATTTCTC				
PGCT015	$(CT)_{20}$	F:GACGCCTTTAGTTTGCTCCA	60	161	4	0.58
FN677535		R:CTCGGGACAGGACTTGGAAT				
PGCT016	$(CT)_{14}$	F:ACATTCGCCATAGCTGTTTT	57	187	3	0.48
FN677536		R:GGAATCAGGAAGATCGAGTAGAGA				
PGCT017	$(CT)_{22}$	F:CCCCTAGTAAAGTCCCACCT	57	176	2	0.50
FN677537		R:AGAGGTATTCGCAGGTTTTG				
PGCT018	$(CT)_{17}$	F:ACCGATTGTTTTTGGTCTCG	60	219	1	–
FN677538		R:GGAAGCGGTGAAAGAATGAA				
PGCT019	$(CT)_{10}$	F:ACCCCTTAACCGGCCCC	57	234	2	–
FN677539		R:TCACCTCTAATGGCTTCCTC				
PGCT020	$(CT)_{15}$	F:TTCCTTTCGCTTTCACTCATC	59	152	2	0.17
FN677540		R:CCCGATCATTAAATCCACAAA				

Table 1 continued

Locus/genbank accession no.	Repeat	Primer sequence (5′–3′)	T_m (°C)	Length (bp)[a]	No. of alleles	PIC
PGCT021	$(CT)_{16}$	F:GATGGCGAAGTGTGTCCTCT	59	158	2	0.50
FN677541		R:TTGGGACTGTGTTGACTGCT				
PGCT022	$(CT)_{18}$	F:GCGCGTTATTTCGATAATTC	55	220	3	0.38
FN677542		R:GGCTCGAACATCATTACATC				
PGCT023	$(CT)_{17}$	F:ATCTCTCATCTCTGCTTCCC	56	145	2	0.40
FN677543		R:GCACACTTTCCTCCCTATGT				
PGCT024	$(CT)_{15}$	F:GATGGACTGCTCGTTTAGAA	55	170	2	0.30
FN677544		R:TTCCTCGTCATAGCAGAAAG				
PGCT025	$(CT)_{14}$	F:AGTAGCCCGTTTAAGATGCT	55	130	2	0.48
FN677545		R:GCAGAGGAAAGAGAAAGAGG				
PGCT026		F:TTGGGACCGCATAGAGACTG	59	219	2	0.16
FN677546		R:CTTCGTCCCCCAAATTAACA				
PGCT027	$(TC)_{10}$	F:CGTTATCTTCCTCCCCTTCC	60	230	1	–
FN677547		R:TCGGCCATTTCTTGACTTTC				
PGCT028	$(CT)_{15}$	F:AAAAGCTGGCACTCAAACTC	57	215	3	0.54
FN677548		R:GGCATTACTTCCAGGACAAC				
PGCT029	$(CT)_{12}$	F:AGCCACACCTCACCATCAA	59	162	–	–
FN677549		R:TGGTGATGTTGTGAGGAAATG				
PGCT030	$(CT)_{15}$	F:CCTCATGTCAGATTGTTTGG	56	152	2	0.35
FN677550		R:GTTATGAGAGGGAGGCAGGA				
PGCT031A	$(GA)_{20}$	F:AGTTTGATCGACTGAGGAATG	56	208	2	0.43
FN677551		R:CACTCGAGAAGCTCTGTGAA				
PGCT031B	$(CTT)_8$	F:AGCTTCACAGAGCTTCTCG	56	105	1	–
FN677551		R:TTCCCTTCAACGGACAGC				
PGCT032	$(CT)_{16}$	F:TCTGAAGCCGATCTCGAAGT	59	103	3	0.52
FN677552		R:GTCAAGCCAAGCATTCACAG				
PGCT033[b]	$(TC)_{22}$	F:TAATAAGCTGCCCCGAAGTC	59	103	3	–
FN677553		R:CGGTGATGTCCCTATTGGAG				
PGCT034	$(CT)_{15}$	F:ATTTCGTGCTCTGTGCCTCT	60	195	1	–
FN677554		R:GTGTTGGGAAGAACGGAAAA				
PGCT035A	$(TC)_{16}$	F:GTAGCTTCCCCTTCCGTCCT	59	160	1	–
FN677555		R:CTTGGGAGGAGCAGTTATGG				
PGCT035B	$(CT)_{15}$	F:AAAACGACACCACCAATTCT	57	180	2	0.16
FN677555		R:CATTTGAAGGGAGAGAGGAA				
PGCT036	$(CT)_{11}$	F:CCACACAAACTCACATCACC	57	167	1	–
FN677556		R:ACTGCTAATGATCGCCATTT				
PGCT037A	$(CT)_{16}$	F:GTTTCGATTGGCCGCTTTA	61	100	2	0.50
FN677557		R:TGTCTTGCCTTCAAGCACCT				
PGCT037B	$(CT)_{13}$	F:CCGCGACACTTTGCTAGAAC	58	244	–	–
FN677557		R:CACAGCAAAAGAGAAGAAAAGG				
PGCT038[b]	$(GA)_{21}$	F:CGTGCCAAATGGGTAAATAA	57	262	3	–
FN677558		R:AGAACTCCACGACCCATAAA				
PGCT039	$(TC)_{16}$	F:TCCGACGATATAATCCCAAT	57	158	1	–
FN677559		R:ATTGCTTTCTTTTGCACCTC				

Table 1 continued

Locus/genbank accession no.	Repeat	Primer sequence (5′–3′)	T_m (°C)	Length (bp)[a]	No. of alleles	PIC
PGCT040	$(GAA)_{10}$	F:CTACTGGGTTTTGAGCTATT	51	234	1	–
FN677560		R:TTTACTTCCCAATTCAAATC				
PGCT041	$(TC)_{14}(AC)_{19}$	F:TGACACGGAACAGAGCTGAA	60	192	2	–
FN677561		R:GGGGAGGAAACGAAGAAGAA				
PGCT042	$(CT)_{16}$	F:GTTGTTGTGGCGCATGTTAG	60	240	2	0.09
FN677562		R:GGAGGGAGACAGAGGGAGAC				
PGCT043	$(CT)_{19}$	F:ACCACCGCACATAATAACTTC	56	232	3	0.31
FN677563		R:TTGAATACGCCTGTTGTTCT				
PGCT044	$(GA)_{21}$	F:GGGGCAACACAAAGAAGAAC	59	141	–	–
FN677564		R:CTCCCCCGAATTTACACAGA				
PGCT045	$(CT)_{12}$	F:TGCTTTCTTCCACACTCACC	58	124	1	–
FN677565		R:GAGGGCGTAAGAGTGAGACC				
PGCT046	$(CT)_{13}$	F:ACATCCCTTCTCTCTCTTTC	52	245	2	0.50
FN677566		R:GCATTCCTCCTCGTCTTC				
PGCT047	$(GA)_{16}$	F:TTCAGCTTCCTTAGCTTCTCC	57	184	2	0.30
FN677567		R:AGCAGAATCACCATCCTCAC				
PGCT048	$(CT)_8$	F:AGCTCTTAACCATGATCCCAGT	59	151	1	–
FN677568		R:CAAAAGGCGCTTCAACAAA				
PGCT049	$(CT)_{10}$	F:CTGCCTCCTCTACCTCGAAT	57	111	1	–
FN677569		R:CGATGCAGAACAAGAGAACA				
PGCT050	$(TC)_{23}$	F:GTCGGTCGTTCCAGTGAAGT	60	250	2	0.16
FN677570		R:AACACCAGACATCGGCAAAC				
PGCT051	$(CT)_{16}$	F:CTAATGGCTGCTTGGCTTGT	59	204	2	0.23
FN677571		R:TTTCACCGAAATTCCCAAAC				
PGCT052	$(CT)_{12}$	F:CACTGAGCTTGATCGCAAAA	59	130	1	–
FN677572		R:CGCCAGAAAACCCTATGAAC				
PGCT053	$(AG)_{14}AA(AG)_{11}$	F:CCTTCACCTCCCCACATAGA	59	177	–	–
FN677573		R:TCGACCGGTTCATCTCTTTC				
PGCT054	$(CT)_{21}$	F:GATCATGAATTTTTGCCCATC	59	100	–	–
FN677574		R:TTCAGTTTCCAGAAGAGAGAGAGA				
PGCT055	$(GA)_{17}$	F:CAACAGAACACCACCCACAC	59	235	–	–
FN677575		R:CCCCCTGGAAGAAAATTGTA				
PGCT056	$(TC)_{17}$	F:TCACTAACTCTCACTTCCACTCA	56	207	1	–
FN677576		R:TGAGAGAATATATGAATTTACCTCTTG				
PGCT057	$(CT)_8$	F:ACCCATAGTCTCACCCATTC	56	161	3	0.49
FN677577		R:GCGATCCCTAGAGAGAGAAA				
PGCT058	$(CT)_{17}$	F:GGGAACCGTTTTCATTGTT	56	144	–	–
FN677578		R:TTTTTGGTTGAAAGTTTAGAGAGA				
PGCT059	$(TC)_{19}$	F:ATACCTGCTCGCTAATCTGG	57	155	2	0.48
FN677579		R:GTGGACAAACAGAGGGAGAG				
PGCT060	$(CT)_{21}$	F:CACGCCCAGAGAGAAGAAGA	59	159	2	0.16
FN677580		R:CTACCCGACACGACACAAAA				
PGCT061	$(CT)_{19}$	F:GAATAAGGCGTCCCTCTCTC	58	155	4	0.38
FN677581		R:CTCCTCCTCGTAATCCCAAC				

Table 1 continued

Locus/genbank accession no.	Repeat	Primer sequence (5′–3′)	T_m (°C)	Length (bp)[a]	No. of alleles	PIC
PGCT062	$(CT)_{14}$	F:CATTTTCTGTTACCCCTTGG	56	206	2	0.50
FN677582		R:TCTCGACATGCTTAGTCTCG				
PGCT063	$(GA)_{15}$	F:GAAGAAAACCGAACCAGTCG	59	247	1	–
FN677583		R:TTCACCTCCATTGCCACAG				
PGCT064	$(GA)_{12}$	F:CCAACAGACACCTGAGCAAA	58	536	1	–
FN677584		R:GTTGTAGAGGAAGATCACGGAG				
PGCT065	$(CT)_{20}$	F:GAAACAGAAGAAGCGCAAAA	58	201	3	0.24
FN677585		R:AGGCGATCACACTCAACAAG				
PGCT066[b]	$(CT)_{19}$	F:CGAGGAGTGGTCCAGGTTAG	59	112	4	–
FN677586		R:AACAGACGACAAGGGGAATG				
PGCT067	$(CT)_8CA(CT)_{12}$	F:GAGCGAAACAGAGGAAAACG	58	185	3	0.31
FN677587		R:AACAGGTTGAACACGACAGC				
PGCT068	$(CT)_{11}$	F:ACAGTCTCCTAATACTAATTCCTC	52	215	2	0.16
FN677588		R:TCCTTATTTCTTTTGTCACC				
PGCT069	$(CT)_{11}$	F:TCTTGTTACGCCTTGTTGGT	58	238	2	0.09
FN677589		R:CACAAATCCTCAAGCAGACC				
PGCT070	$(CT)_{20}$	F:TAACAACCATGCCCCTTAAT	56	210	2	0.43
FN677590		R:CCAATTAAAACGCCTCATCT				
PGCT071	$(CT)_{15}$	F:AAACCCAGAAGAAGAACGAG	56	229	2	0.23
FN677591		R:AAGAGAGAAACAGAGGAGGAAG				
PGCT072	$(TC)_9$	F:GACTTGAGGAAGAGGAATTGG	57	182	–	–
FN677592		R:ACGTGTATCGGCGAATTAAA				
PGCT073	$(CT)_{15}$	F:GGAGGAGAGGATGAAAGGTC	57	132	–	–
FN677593		R:TTTGGCTTTGGTTTCTGTTT				
PGCT074	$(AG)_{16}$	F:CAATGCAAGCAGAAAGACGA	60	123	1	–
FN677594		R:ACCCCATCTTTCCCATCTTC				
PGCT075[b]	$(TC)_{17}$	F:GGCGAGCTTCTGCTACTTCT	59	229	6	–
FN677595		R:TCTGTCCCCAGATCATCAAA				
PGCT076	$(CT)_{13}$	F:TTATCGCCTTCCTCTTCTCC	58	148	2	0.09
FN677596		R:CCCGAGAAATGCTAGACAGA				
PGCT077	$(CT)_{14}$	F:CCCGAGCTGAAGACAGAAAC	57	191	1	–
FN677597		R:AAGTGAAGAGAGCGACAAGG				
PGCT078	$(CT)_{14}$	F:CCTCCATTGTTGTTCCTCCT	59	241	1	–
FN677598		R:TGAGATGCCCAAACAAAGAA				
PGCT079	$(CT)_9$	F:CCATTGCAGCATTCTTCCTC	60	171	1	–
FN677599		R:TATGTGAAGTGTGCGGTGCT				
PGCT080	$(GA)_{17}$	F:TGAGTGGAAGGGAAATAGGA	57	230	2	0.50
FN677600		R:TCACCCTCTCCAAAATCAAA				
PGCT081	$(CT)_{21}$	F:AAAACCCTAATCGCCCACTT	59	158	1	–
FN677601		R:GAATGGAAGCCCAATGAAAA				
PGCT082	$(TC)_7N_{21}(TC)_{10}$	F:CCACTCATCAACCTCACCAC	58	201	1	–
FN677602		R:CAATGCAGAGAAGCTGGAAC				
PGCT083	$(CT)_{17}$	F:TTCGGTTGCATTGATTTCCT	59	127	3	0.51
FN677603		R:AGCTCAGTGGAGAGGACTGG				

Table 1 continued

Locus/genbank accession no.	Repeat	Primer sequence (5′–3′)	T_m (°C)	Length (bp)[a]	No. of alleles	PIC
PGCT084	$(CT)_{19}$	F:GCCAGTCAGGAGTTCTTTGC	60	209	1	–
FN677604		R:GCGGCAGGAGAACAGATAAG				
PGCT085	$(CT)_{10}$	F:GCACAGATGTTTTGGAGTCA	57	154	2	0.09
FN677605		R:ATTGTTGGAAAGGAAAGCTG				
PGCT086[b]	$(GA)_{19}$	F:TGGTGATTCGTGTTGTTTTC	57	180	4	–
FN677606		R:CAACAACCTCCTCTGCTCTC				
PGCT087	$(CT)_{22}$	F:TCCTCCGACCCTTTCTTATC	58	237	3	0.69
FN677607		R:CCCTATCATCCTTCCCATTC				
PGCT088	$(CT)_{20}$	F:TCTCTCTCTACCCCGACACC	59	150	4	0.58
FN677608		R:TAGCGTCAAGATTGTGAAAAGG				
PGCT089[b]	$(CT)_{24}$	F:TGCATCCTTCCCCTACTCTC	59	120	5	–
FN677609		R:AGCTCATGTAATGCGTCGTG				
PGCT090	$(AG)_{21}$	F:TACAGGCTACCACAGGTTGA	56	155	3	0.25
FN677610		R:ATTGCCACCACATCACTGT				
PGCT091[b]	$(CT)_{12}TT(TC)_9$	F:ATCAGAATTGGAATCGGAAC	56	186	5	–
FN677611		R:ACCGAGGTCATCGAACTAAA				
PGCT092	$(AG)_8AA(AG)_8$	F:TTTCCCGTCTGCTCTCTGTT	60	192	1	–
FN677612		R:GCGACCATCAACCTCATCTT				
PGCT093A	$(CT)_{18}$	F:GTAGCCACTTTAGGGCGAGA	58	230	4	0.54
FN677613		R:CGTCTAAAAGCGACAGCAAG				
PGCT093B	$(CT)_{24}$	F:GCCTTTTCCTGCTTTCCTTT	60	181	4	0.61
FN677613		R:CATACAGCGGACCACAACAC				
PGCT094	$(GA)_{16}$	F:GGCTTTTGCTTATGACACTGC	59	198	2	0.16
FN677614		R:CCTCGATTTGGTAGCTTTCG				
PGCT095	$(GA)_{16}$	F:GGGGTGGAAACAGAACTTACA	59	212	1	–
FN677615		R:CTTCTTCCTCCTCCTCCCTC				
PGCT096[b]	$(CT)_{18}$	F:CAGACCCTGCGCTCGCT	59	184	5	–
FN677616		R:TTATGGAGAGCGGGAGAAAC				
PGCT097	$(CT)_{17}$	F:TCCCATAAACATAGCAGGAA	55	152	2	0.46
FN677617		R:GGCATCTCAGGGGTAATAAA				
PGCT098[b]	$(GA)_{16}$	F:ATCAACCAAACCGCACAGAC	60	120	6	–
FN677618		R:CCATTTCATTCTCCCCCTCT				
PGCT099	$(CT)_{14}$	F:CTGCTGCTGCTTCCTATTCC	60	193	2	0.24
FN677619		R:AAGAGGAGAGACGGTGACGA				
PGCT100	$(CT)_{15}$	F:AAGACCTAAAGCCGTGTCGT	58	153	–	–
FN677620		R:GGAAGCTAGGGTCTTTGACG				
PGCT101[b]	$(GA)_{24}$	F:GAACGCCAAATTCAAGAACC	59	273	3	–
FN677621		R:GACGATTCTTTCCTGCCTTG				
PGCT102	$(CT)_{17}$	F:AACCTATGGTGCCTTCCTTC	57	159	1	–
FN677622		R:AAATCGCAGACACACTCACA				
PGCT103	$(CT)_{13}$	F:GCTTGCTCTCGATTGAACCT	59	112	1	–
FN677623		R:TCCCTCCCTGTGTCAAAATC				
PGCT104	$(GA)_{18}$	F:CGCCCAAAACAGGAAATAGA	59	192	2	0.50
FN677624		R:GCTGGGATCTGGAAGAAATG				

Table 1 continued

Locus/genbank accession no.	Repeat	Primer sequence (5′–3′)	T_m (°C)	Length (bp)[a]	No. of alleles	PIC
PGCT105	$(CT)_{24}$	F:AGGAGGTGAGGTTGGTGATG	58	220	3	0.14
FN677625		R:TTGCGTCGTTTTGAATTTTT				
PGCT106	$(GA)_{13}$	F:CCGAATTCCATGTATTTTGTAG	56	159	1	–
FN677626		R:AGCGGAAGTCTATCTTTGCT				
PGCT107	$(CT)_{19}$	F:CTCTGCTCTACCCCCTCTCC	60	118	–	–
FN677627		R:TCAACCAAGTGTGCTGCTTC				
PGCT108	$(GA)_{13}$	F:GCAGAAAACTTGACGATGAG	55	231	–	–
FN677628		R:CGAATGGACGATGATTAGAC				
PGCT109	$(GA)_{16}$	F:CCACTTCCCTCCTACCTTCC	60	188	2	0.65
FN677629		R:ACGTCTGCTTGCACCTCTTT				
PGCT110	$(GA)_{22}$	F:GAGCCATTGTAGAGACAAGA	52	103	4	0.71
FN677630		R:GACTGCTGACAACTTTCTTT				
PGCT111	$(AG)_{19}$	F:TATCTGTCGCAGGAAGGATG	58	235	4	0.69
FN677631		R:GAAGCCAATTCCTCAAAGATG				
PGCT112	$(TC)_{19}$	F:CAGCCAATTACGGCAACTAA	58	181	2	0.51
FN677632		R:GTCCCTCGCAAACACCTAAA				

[a] Length is calculated on the sequenced clone

[b] Multilocus

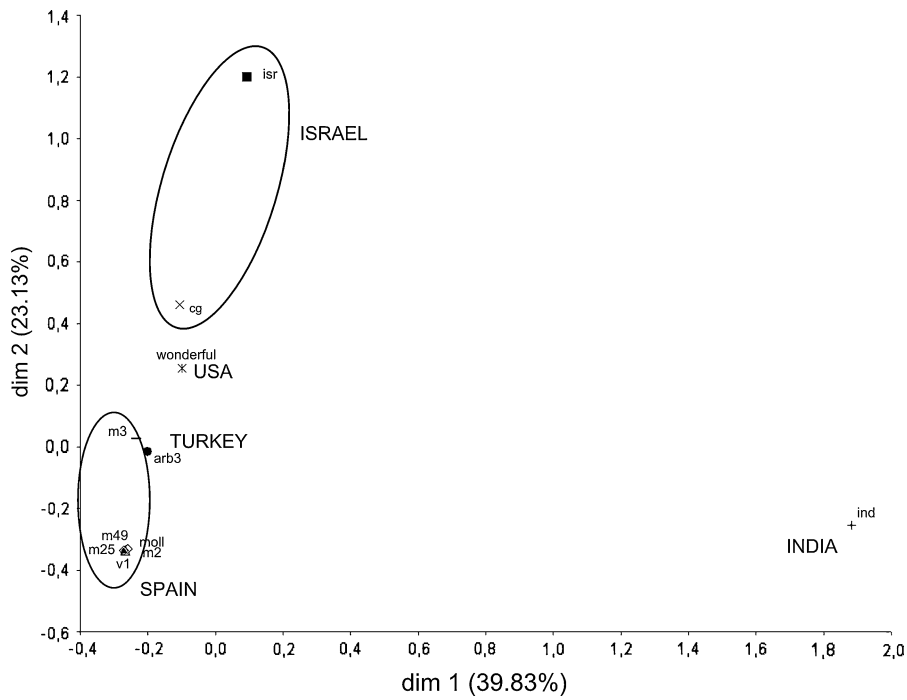

Fig. 1 FCA multivariate analysis based on the microsatellite data of the 11 pomegranate accessions. Only first and second dimensions are shown

polymorphic markers ranged from two to seven. Markers PGCT006, PGCT033, PGCT038, PGCT066, PGCT075, PGCT086, PGCT089, PGCT091, PGCT096, PGCT098, and PGCT101 were judged to be multiloci because they amplified more than two alleles in most of the accessions. This result is in agreement with that one

found by Gisbert et al. (2009) in loquat. For the 55 remaining SSR markers, the average polymorphism information content (PIC) value across all loci was 0.37 with a range of 0.09–0.71. Botstein et al. (1980) define any locus with a PIC $\geq$ 0.5 as highly polymorphic. In our study only 19 out of the 55 loci analyzed (34.5%) met this criterion. These results are in accordance with the type of accessions used in the study since six of them came from varietal groups of the Elche-Orihuela region in Alicante province (Spain). These findings were confirmed by factorial correspondence analyses (FCA) of the 11 accessions (Fig. 1). Under this analysis, the first three principal dimensions accounted for 78.16% of the total variance. As expected, accessions from India (IND), Israel (CG and ISR) and USA (Wonderful) appeared separated from the rest. The Turkish accession (ARB3) appeared closest to the Spanish accessions.

In conclusion, despite the low number of accessions analyzed, these preliminary results indicate that the microsatellite markers developed in this study are a very useful tool for all kinds of genetic studies in pomegranate. In fact, they showed a high level of variation in *P. granatum* even using a low number of accessions, thus proving to be a powerful tool for genetic diversity studies in germplasm collections as well as for cultivar identification.

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