Journal of Plant Molecular Breeding (2016) 4(2): 9- 16
DOI: 10.22058/jpmb.2016.25530

RESEARCH ARTICLE

Assessment of Iranian Apricot Cultivars Resistant, Susceptible and Mutant to Late Spring Frost

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Abstract: Apricot is grown in a wide range of climatic conditions in Iran, however, it is frequently damaged by late spring frost. In this case, identification of new genotypes tolerant to cold stress is indispensably needed. The objective of this study was to evaluate the genetic population and relationships among 27 apricot accessions (*Prunus armeniaca*) by 30 microsatellite markers and 11 morphological traits. Based on the PIC values, the SSR loci (UDP96001, UDP96003, UDP98412 and UDP98411) were the most informative markers. The morphological traits were categorized into three components which explained 91.23% of total variation. The two-dimensional PCA plot exhibited that the highest degree of fruit quality and quantity belonged to the susceptible cultivar of Shahrood 48 which showed to be the favorable parent for the production of resistant mutants with high value of fruit traits to late spring frost. Moreover, the close relatedness of Shahrood 48 and its mutants according to the molecular analyses (including a Bayesian clustering approach and a Partial repeated bisection) confirmed the results of fruit traits analysis. The findings suggest that the wide diversity present in Iranian apricot genotypes could be used as a genetic resource for conservation and development of new cultivars resistant to late spring frost and for designing further apricot breeding programs. The promising new mutant genotypes tolerant to cold stress will be evaluated based on morphological markers in further breeding studies.

Keywords: *Prunus*, Cold stress, SSR marker, Late spring frost

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is an ecologically and economically important tree species around the world, mostly in China, the Irano-Caucasian region (Turkey and Iran), Central Asia, Europe and North America [14]. Iran is one of the main apricot producer countries as being the second after Turkey due to its suitable climatic and geomorphological condition. One of the greatest limiting factors which influences on apricot growing in Iran is the early flowering of native genotypes and coincidence of their flowering times with a cold spring [26]. Introduction of a rapid and reliable method to precise detection and discrimination of apricot genotypes in relation to cold

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Received: 10 January 2016 / Revised: 30 May 2016
Accepted: 15 August 2016

stress tolerance is extremely important for scientific and economic reasons. Classical methods based on phenological, pomological and morphological characteristics such as tree vigor and growth habit [5-6], fruit quality features [24, 32], stone [22], flower [30-31, 39], stigma and stylus [35] and pollen [3-4, 9] have been used for germplasm collection and characterization of apricot genotypes. However, these phenotypic data are often difficult to be evaluated because of their dependency on environmental conditions and production practices. In contrast with morphological traits, molecular markers are stable, reliable and independent of environmental factors and can be used for genetic diversity analysis in *P. armeniaca* genotypes. Among different molecular markers, microsatellite markers have been widely used for genetic diversity studies. The population structure of various apricot cultivars owing to their desirable characteristics including high numbers of polymorphisms, wide genomic distribution, co-dominant inheritance, and high degree of reproducibility [1, 21, 36-37, 41-42]. In every breeding program, it is suggested to compare and combine molecular and morphological information of germplasm collections in order to evaluate diversity analysis more precisely. In the case of *Prunus*, several works have been performed based on molecular and morphological data on apricot [17, 33], plum [2] sweet cherry [16, 23], peach [8], and almond [12, 15, 34, 40]. Furthermore, the evaluation of genetic structure of germplasm collection can be useful for effective utilization of *Prunus* genetic resources [13, 19].

The objectives of this study were 1) to evaluate the phenotypic variation of 30 morphological traits and their relationships on seven susceptible and resistant apricot genotypes to late spring frost 2) to identify the most desirable susceptible parental genotype for generating mutants in terms of fruit traits and 3) to assess the genetic relationship and structure of 27 Iranian apricot genotypes including resistant, susceptible and their mutants to late spring frost using 30 SSR markers.

MATERIALS AND METHODS

Morphological traits

This study was carried out on seven commercial apricot genotypes, three resistant and four susceptible, as well as 20 mutants to cold stress from Iran. The genotypes were maintained at an experimental orchard located at the Kamal Shahr Research Station of Seed and Plant Improvement Institute in Karaj, Iran.

During two years, eleven morphological characters (five qualitative and six quantitative) were studied as follows: fruit length (FL), fruit width (FWi), fruit width stomach (FWiS), fruit weight (FWe), stone weight (SWe), total soluble solids (TSS), pH, fruit quality (FQ), fruit taste (FT), panel test (PT) and frost (Fr) (Table 3). Morphological data analyses including PCA and 2D PCA plot were performed by SPSS software version 16.0.0 (SPSS 2007). In PCA, factor loadings > 0.80 were regarded as significant.

Molecular evaluation

Total DNA was extracted using the procedure described by Doyle [10]. The quality of the extracted DNA was verified on 1.5 % agarose gel and the quantity of DNA was checked by NanoDrop®ND-1000 Spectrophotometer. We used 30 SSR primer pairs to evaluate polymorphism among Iranian apricot genotypes (Table1). PCR amplification was performed in a 25 µl reaction containing 2.5 µl of 10 X buffer, 2 mM $MgCl_2$, 0.2 µM of each primer, 0.1 mM dNTPs, 1 U *Taq* DNA polymerase, and 90 ng genomic DNA. Amplification included an initial denaturation at 95◦C for 5 min, followed by 36 cycles of 30 s denaturation at 95°C, 45 s annealing at 57°C, and 2 min extension at 72°C. A final extension step of 7 min at 72°C was added. Amplified PCR products were separated by electrophoresis on 6.5% acrylamide using BioRad model GS-800 imaging densitometer. Amplified products were scored as present (1) or absent (0) to form a binary matrix. To examine different variability parameters including the number of alleles (A), major allele frequency, expected heterozigosity (*He*), observed heterozigosity (*Ho*) and polymorphism information content (PIC), Power Marker v.3.25 [20] and Popgenev.3.2 [38] were used.

To assess genetic relationship among the samples, a partial repeated bisection (RB) analysis was carried out using GCluto v. 1.0 [29]. A Bayesian clustering approach was applied to infer genetic structure of the apricot germplasms which are implemented in STRUCTURE v.2.3.4 [28]. A burnin of 50,000 steps followed by 50,000 Markov Chain Monte Carlo (MCMC) iterations were used and all other parameters were set at their default values. Bayesian analysis was conducted with three independent runs for each value of *K*.

Table 1. Genetic diversity among the 27 apricot genotypes using SSR analysis.

Marker	Major allele frequency	(A)[1]	(*He*)[2]	(*Ho*)[3]	(PIC)[4]
UDP96001	0.37	6.00	0.71	1.00	0.66
UDP96003	0.46	4.00	0.63	0.84	0.56
UDP96005	0.69	4.00	0.48	0.38	0.44
UDP96008	0.50	2.00	0.50	1.00	0.38
UDP96010	0.71	5.00	0.55	0.47	0.45
UDP96013	0.45	4.00	0.35	0.57	0.44
UDP96015	0.44	3.00	0.44	0.04	0.51
UDP96018	0.50	2.00	0.50	1.00	0.38
UDP96019	0.32	3.00	0.51	0.49	0.44
UDP97401	0.66	5.00	0.66	0.32	0.38
UDP97402	0.76	4.00	0.40	0.08	0.37
UDP97403	0.56	4.00	0.47	0.54	0.48
UDP97408	0.45	2.00	0.49	0.44	0.39
UDP98024	0.67	3.00	0.64	0.61	0.53
UDP98405	0.50	2.00	0.50	1.00	0.38
UDP98406	0.81	5.00	0.32	0.07	0.30
UDP98407	0.49	5.00	0.55	0.07	0.42
UDP98409	0.50	2.00	0.49	0.88	0.37
UDP98411	0.50	4.00	0.61	0.63	0.54
UDP98412	0.43	4.00	0.62	0.89	0.54
Pchgms1	0.73	3.00	0.46	0.78	0.53
Pchgms2	0.87	3.00	0.41	0.66	0.40
Pchgms3	0.68	4.00	0.58	0.51	0.42
PS7a2	0.87	4.00	0.42	0.77	0.44
PS9F8	0.49	4.00	0.59	0.69	0.52
PS12e2	0.57	5.00	0.57	0.58	0.50
BPPCT017	0.63	4.00	0.54	0.07	0.44
BPPCT020	0.69	4.00	0.66	0.04	0.41
BPPCT006	0.77	5.00	0.60	0.21	0.46
Mean	0.59	3.75	0.52	0.53	0.45

[1]A: number of alleles;
[2]H_e: expected heterozigosity;
[3]H_o: observed heterozigosity;
[4]PIC: polymorphic information content

RESULTS AND DISCUSSION

Phenotypic variation

There were significant differences among samples regarding the morphological features (Table 2). As for fruit dimensions, the genotypes had a range of 31.41 to 56.08 mm for FL, 29.21 to 47.73 mm for FWi and 26.55 to 41.03 mm for FWiS. In addition, SWe ranged from 1.76 gr to 3.36 gr as well as fruit weight varied from 17.57 gr to 66.41gr. Previous studies on apricot also reported a high variability among accessions regarding this parameter [27, 33].

Among the morphological traits studied, frost percentage is the main feature for identifying different apricot genotypes in terms of tolerance to late spring frost. As we expected, frost percentage ranged from 10 (for resistant cultivars of BN-KB576, BN-KB24 and Jahangiri) to 90 (for susceptible cultivar of Shahrood 48). TSS content is an important quality attribute, influencing notably the fruit taste. The most amount of TSS in the present study was shown in the resistant cultivars of BN-KB576, BN-KB24 and Jahangiri than susceptible cultivars. It is considered the mean values of TSS over 10% were the minimum value for consumer acceptance of apricots, which is the case in our cultivars [30].

The results of PCA analysis showed the characteristics that correlated best with fruit attractiveness, for example fruit dimensions (fruit length, fruit width, fruit width stomach), fruit weight, fruit taste, and stone weight [6], had the highest loadings in the first two components. This indicates that these traits are not only useful for assessment of diversity but also for characterization of apricot germplasm.

In 2D PCA plot, the samples were distributed based on the level of qualitative and quantitative fruit characters (Figure 1). Since the sample of Shahrood 48 showed the highest measure of fruit traits amongst other susceptible genotypes, it was known as the desirable parental cultivar. The Jahangiri and BNKB24 resistant genotypes which had the closest level of fruit attributes were introduced as the high-grade of resistant cultivars. The results showed that the measured fruit features including FL, FWi, FWiS, FWe, FQ, SWe, FT, PT, Fr% and pH are suitable for characterization of apricot germplasm related to cold stress. This finding was in agreement with those of [5, 7, 40] who revealed that morphological evaluation is an efficient tool for characterization of apricot germplasm and species distinction.

Molecular assessment

In correspondence with molecular analysis, the maximum level of heterozygosity and the number of alleles obtained from this study were in agreement with another study reported on Turkish apricot germplasm [1]. The highest polymorphic information index PIC values belonged to primers UDP96001, UDP96003, UDP98412 and UDP-98411 which showed the potential of the SSR loci for genetic identification of Iranian apricot genotypes tolerant

Table 2. The principle components (PCs) and the mean values of the 30 morphological traits for seven apricot cultivars.

Traits	Components			Accession code						
	1	2	3	BNKB576	BNKB24	Jahangiri	Royal	BNKB18	BNKB511	Shahrood 48
FL	0.914	-0.223	-0.116	40.89	41.54	42.42	38.57	31.41	56.08	39.83
FWi	0.892	0.423	-0.147	29.21	36.90	42.09	37.24	33.62	47.73	39.11
FWiS	0.812	0.565	0.073	26.55	34.03	39.95	34.14	29.38	41.03	36.53
FWe	0.947	0.123	-0.134	17.57	36.50	38.25	27.97	20.96	66.41	40.81
FQ	0.200	0.892	-0.324	40	50	60	70	70	60	60
SWe	0.802	0.420	0.197	1.76	2.20	2.85	3.36	2.12	1.83	2.92
FT	0.114	0.961	-0.204	30	60	70	80	60	60	60
PT	0.165	0.932	0.135	40	70	70	70	60	60	60
Fr%	0.138	0.223	-0.898	10	10	10	80	50	80	90
pH	0.161	0.072	0.917	4.48	5.25	4.41	4.94	4.07	4.31	4.31
TSS%	-0.554	-0.192	0.667	23	25	20	17	10	15	16
Variance%	38.75	31.07	21.41							
Total	91.23									

cold stress. In another study, the highest value of PIC was 0.47 based on RAPD markers among apricot genotypes which were lower than the results from the present study [25].

The genetic relatedness based on RB analysis divided the samples into three main clusters (Figure 2). The larger group contained all 20 mutant genotypes and their parental cultivar as Shahrood 48. The second larger group included all three resistant apricot genotypes and the BNKB511 susceptible one. Finally, the third group consisted of only susceptible genotypes of BNKB18 and Royal. The Bayesian clustering approach using STRUCTURE software showed that the most likely value of *K* was three (Figure 3).

The different groups showed three different colors as red (mutant), green (resistant) and blue (susceptible). Almost all 27 samples showed a clear relation to each cluster based on a high tendency to cluster by tolerance to late spring frost while two samples (Shahrood 48 and its mutant) were categorized as admixture (Figure 3). Due to the admixture of the two colors as blue and red, it could be concluded that the close relationships between the mutants and their susceptible parent resulted from the common genetic source of those samples.

The results from model-based method completely confirmed the grouping that we observed in the RB analysis. Similarities were found in the results of previous studies on different *Prunus* species emphasizing that the molecular and morphological results were in the same direction [8, 15, 17, 19, 30]. In contrast, some studies showed low correlation between morphological assessment and different molecular approaches [11, 40]. In relation to structure analysis, Wang *et al.* [2014] reported on the genetic and structure diversity of Siberian apricot populations in China using 31 nuclear SSR loci. The structure analysis clustered all of the populations into four genetic clusters also there was no significant difference between the wild and semi-wild groups, indicating that recent cultivation practices have had little impact on the genetic diversity of Siberian apricot.

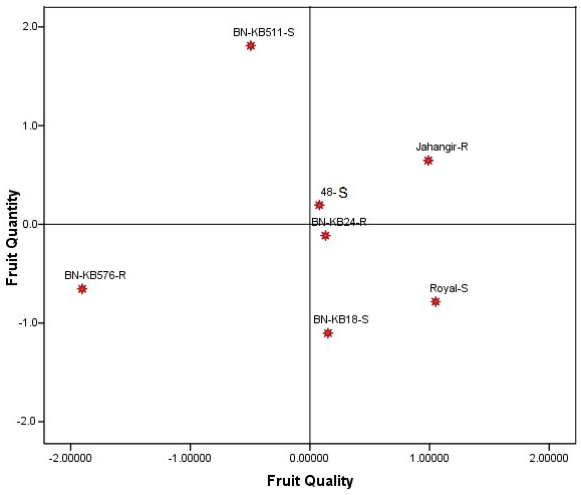

Figure 1. Two-dimensional (2D) PCA plot based on the first two components and according to the combined morphological data from 2 years of study.

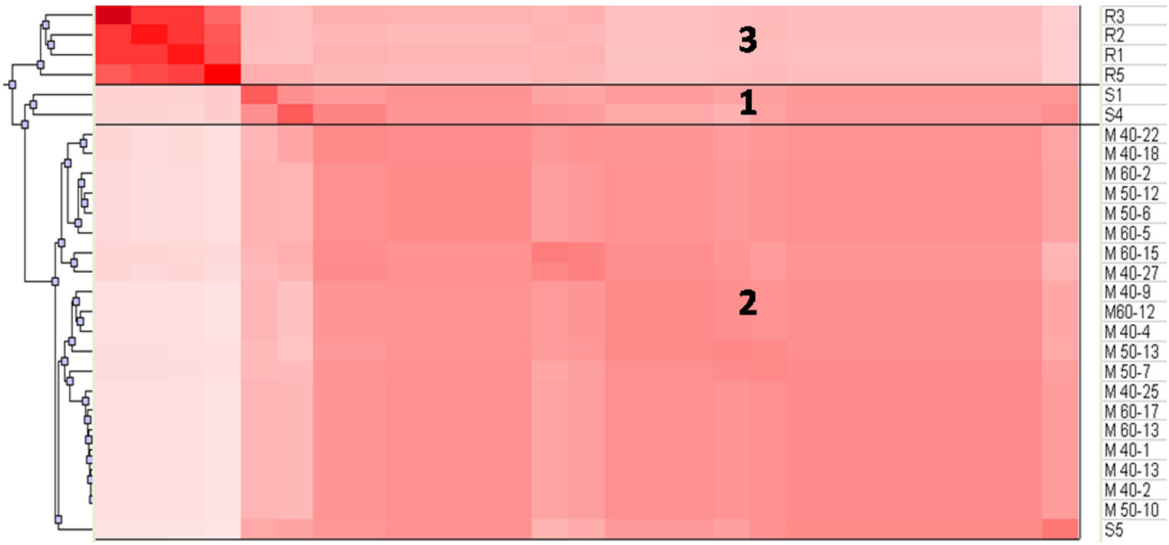

Figure 2. The classification of 27 apricot accessions using gCluto v. 1.0. Software.

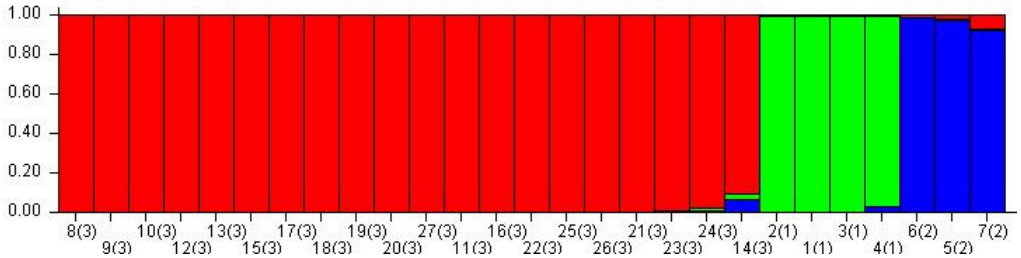

Figure 3. Model-based clustering approach for each of the 27 apricot germplasm examined based on 30 microsatellite markers. Each individual bar represents a genotype. Different color bars refer to three different genetic groups.

Concerning the positive correlation between the SSR markers and morphological data, it seems that the SSR markers with the characteristics of being multi-allelic and co-dominant may be a suitable choice for marker-trait association genetic studies of various apricot germplasms [33].

ACKNOWLEDGEMENTS

The authors would like to appreciate the experimental orchard of Kamal Shahr Research Station of Seed and Plant Improvement Institute in Iran for maintaining the germplasm collection. We are also thankful for the efforts of the Agricultural Biotechnology Research Institute of Iran (ABRII) for funding this study.

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ارزیابی ارقام زردآلوی ایرانی مقاوم، حساس و موتانت به سرمای دیررس بهاره

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چکیده

زردآلو با قابلیت رشد در شرایط آب و هوایی مختلف ایران، در معرض خطر سرمای دیر رس می باشد. با توجه به خطر سرمای دیررس بهاره، معرفی ژنوتیپ های متحمل به تنش سرما ضروری است. هدف از این مطالعه، بررسی تنوع فنوتیپی و ساختار ژنتیکی ۲۷ رقم زردآلوی مقاوم، حساس و موتانت به سرمای دیر رس بهاره با استفاده از ۳۰ نشانگر ریزماهواره و ۱۱ نشانگر مورفولوژیکی بود. بر اساس نتایج بدست آمده از آنالیز های مولکولی، نشانگرهای UDP96001، UDP96003، UDP98412 و UDP98411 بیشترین میزان PIC را نشان دادند. صفات مورفولوژیکی مورد بررسی با میزان تنوع ۹۱/۲۳٪ از تنوع کل در سه گروه دسته بندی شدند. نتایج PCA نشان داد که از میان ارقام مقاوم و حساس مورد مطالعه تنها رقم حساس شاهرود ۴۸ بالاترین درجه کیفیت و کمیت میوه را دارا بود. و از این رقم به عنوان رقم مادری به منظور تولید ارقام موتانت مقاوم به سرمای دیر رس بهاره با کیفیت و کمیت بالای میوه استفاده شد. بعلاوه، روشهای آنالیزی بایسین و دوبخشی تکراری نسبی، ارتباط نزدیک رقم مادری شاهرود ۴۸ با موتانت های بدست آمده را تایید کردند. یافته های حاصل از تحقیق حاضر، بیانگرحضور تنوع ژنتیکی وسیع در ژنوتیپ های زردآلوی ایرانی می باشد که می توان از این ذخایر غنی ژنتیکی در مطالعات اصلاحی آینده با هدف حفظ و توسعه ارقام جدید مقاوم به سرمای دیررس بهاره استفاده کرد. بعلاوه، ژنوتیپ‌های امید بخش جهش یافته متحمل به سرما بر اساس صفات مورفولوژیکی در مطالعات اصلاحی بعدی مورد ارزیابی قرار خواهند گرفت.

کلمات کلیدی: پرونوس، تنش سرما، نشانگر SSR، سرمای دیررس بهاره