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Full Length Article

Evaluation of Elite Pomegranate Genotypes of Balochistan Based on Morphological, Biochemical and Molecular Traits

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Abstract

Characterization of Pomegranate germplasm is necessary for patency, expansion of supply window and variety improvement programs. Morphological, chemical and molecular variation and relatedness among 11 pomegranate genotypes of Balochistan were determined. The highest fruit weight (372 g) was recorded in MG-I; however, MK-IV had lowest WPI with high juiciness, while MG-III & MG-IV had highest WPI with less juice percentage. Significant variation was recorded for TA, Vit. C, total sugar, TPCs, $IC_{50,}$ SODs and PODs, whereas no difference was observed for TSS, protein and CAT contents among selected pomegranate genotypes. Narrow genetic base was noted in selected pomegranate genotypes as shown in average PIC value (0.282), however, SSR marker (POM_AGC11) produced the highest values for PIC, HZ and GD (0.373, 0.909 and 0.496), respectively; however, average allele frequency was 0.739 in amplified DNA fragments of 11 pomegranates with the highest value (0.954) recorded in SSR marker tagged as PGCT075b. Molecular variance was 11% among two populations (Quetta and Mustang) with 16% among individuals of these populations and remaining 73% variation recorded within the genotypes. Fruit size and juiciness could be improved through utilizing genetic variations in pomegranate genotypes. The genotype "MG-I" had the highest fruit size; however, MK-IV proved the best for white sweet arils, containing least WPI. Moreover, commercial genotypes (Q-I, Q-II and MK-III) were red in fruit and aril color, sweet to sour in taste with less juiciness. Genetic base of selected genotypes was narrow with massive flow of gene pool within the region. © 2018 Friends Science Publishers

Keywords: Fruit characterization; Punicaceae; Germplasm; Domestication; Antioxidants; Genetic base
Abbreviations: Wood portion index (WPI), Titratable acidity (TA), Total phenolic contents (TPCs), Antioxidant activity at 50% (IC_{50}), Super dismutase oxide (SODs), Per oxide (PODs), Total soluble solids (TSS), Catalase (CAT), Polymorphic information contents (PIC), Simple sequence repeats (SSR), Heterozygosity (HZ) and Genetic difference (GD)

Introduction

Pomegranate (*Punica granatum* L.) is a deciduous large shrub belonging to family Punicaceae with three species in genus *Punica* (Mercure, 2007; Melgarejo *et al.*, 2009). There are more than 500 pomegranate genotypes with only a few marketable in the world (IPGRI, 2001). Plant is distributed from Eurasia to Himalayas (Levin, 2006), proving its high range of adaptability and diversity (Holland *et al.*, 2009). Its domestication was started in Neolithic age (Still, 2006), for its nutritional, medicinal and pharmaceutical importance. Largest pomegranate producer in world are India Iran and USA; however, Spain is its greatest exporter in the world (Jaime *et al.*, 2013). Demand of pomegranate fruit and juice is increasing for medicinal purposes (Sturgeon and Ronnenberg, 2010), while nutritionists preferentially recommend it to improve human diet. Germplasm improvement program in pomegranate was based on morpho-chemical and molecular characterization (Jalikop, 2010; Hasnaoui *et al.*, 2011a). Morphological and biochemical analysis provide basic information to compare genotypes for breeding or to evaluate growth under different climatic conditions (Dafny-Yalin *et al.*, 2010; Al-Maamari *et al*., 2016; Mahmood *et al*., 2017). High level of variation in fruit weight, shape, density, juice contents, sweetness, fruit and aril color, and Wood Portion Index (WPI) was recorded in wild and cultivated pomegranate genotypes (Ercisli *et al.*, 2007).

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Estimation of antioxidant activity, TPCs, organic acid and sugar contents in pomegranate germplasm is required in variety improvement programs and diversification of trading around the globe (Dafny-Yalin *et al.,* 2010). In addition to morphological studies, molecular analysis is used for characterization and variety improvement programs through qualitative trait linked analysis (Hasnaoui *et al.,* 2011a; Pirseyedi *et al.,* 2010; Islam *et al*., 2016). Moreover, these studies provide accurate information of genotype polymorphism and linkage of cultivars with parents (Hasnaoui *et al.,* 2011b). Following above morpho-molecular studies on indigenous and exotic pomegranate germplasm, Spain, Iran, India, Israel, USA and China are producing excellent quality pomegranates in diverse climatic conditions on sustainable basis. China has a repository of 238 pomegranate cultivars for its sustainable supply in the country (Fang *et al.,* 2006). Genetic diversity estimation and association analysis are successfully used to reduce bacterial blight and fruit cracking in Indian pomegranates (Xue *et al.,* 2006; Jalikop, 2010).

Himalayan range of Pakistan is considered as 2nd source of origin of pomegranate (Nasir and Ali, 1972; Diganta *et al.,* 2009). However, it is a minor fruit crop with fewer registered cultivars (Nafees *et al.,* 2015). It is commercially grown in warm tropical to subtropical, arid to semiarid regions and desert zones of the country (Nasir and Ali, 1972); however, due to less production and inferior fruit quality, local market demand is fulfilled through import from Iran and Afghanistan. In this manuscript various morphological, biochemical and molecular studies were done in some pomegranate genotypes of Balochistan to assess their trade potential and to use this information in advanced breeding program.

Materials and Methods

Pomegranate Materials

Fully ripe fruit samples of 11 pomegranate (*Punica granatum* L.) genotypes were collected on October 10, 2012, from trees (12 years old) commercially growing in Quetta (Barkhan) and Mustong (Gulab Bag and Kari Kucha), Baluchistan. Newly emerged leaf samples were also collected and stored in cold cabinet for DNA extraction to check amplification with 30 selected SSR markers for molecular studies.

Evaluation of Fruit Morphological Traits

Various commercial quantitative traits like fruit and rind weight, 100 arils and seeds weight was recorded, Length and width of fruit, aril, seed and crown height were measured using digital vernier calliper (KBD-MT 0014). A commercial fruit trait, wood portion index (WPI) was also recorded to access juiciness.

Stock Solutions for Chemical Analysis of Pomegranate Aril Juice (PAJ)

Water dissolved stock solution of PAJ was used to measure total sugar and ascorbic acid contents using spectrophotometry (Razzaq *et al.,* 2013). Phosphate buffer stock solution was prepared by measuring the specific activity of enzymes (SODs, PODs, CAT and Protease) and soluble protein contents (Naqvi *et al.,* 2011). Methanol stock solution was prepared by following to measure TPCs (Razzaq *et al.,* 2013).

Chemical Analysis

Juice characteristics: A digital refractometer (ATAGO RX 5000) was used to measure TSS (°Brix) in aril juice of pomegranate. AOAC (2000) titration method was used to measure titratable acidity (TA) on the basis of percentage of acetic acid. Total soluble sugars were determined with antherone reagent (Khan *et al.,* 2011). Bradford (1976) protocol was followed by taking 1 mL Bradford reagent and phosphate buffer stock solution of PAJ to calculate soluble protein contents.

Ascorbic acid (AsA): AsA contents were measured with stock solution prepared using dichlorophenolindophenol (D-CIP), Na_2CO_3, meta-phosphoric acid, glacial acetic acid and methanol dissolved stock solution of PAJ. Absorbance of the samples was taken at 515 nm using spectrophotometer for measuring the absorbance at 515 nm (Bozin *et al.,* 2006). Standard curve at $R^2 = 0.9855$ was used to measure AsA.

Total phenolic contents (TPC) and antioxidant activity: Ainsworth and Gillespie (2007) method was used to measure TPCs. Antioxidant activity was measured at 25, 50, 75 and 100%, using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in buffer stock solution. Antioxidant activity (IC_{50}) was recorded using with equation of scatter graph ($R^2 = 0.9968$).

Enzymatic activity: Antioxidant enzymes activity was determined in phosphate buffer dissolved stock solutions of PAJ and calculated as IU/mg of proteins. Peroxide (POD, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6) was measured using the method of Liu *et al.* (2009) by adding PODs reagent of phosphate buffer, H_2O_2 (40 mm) and Guaiacol to record PODs, while CAT concentration was measured by dissolving phosphate buffer in H_2O_2 (5.9 mm). Super dismutase oxides (SOD, EC1.15.1.1) were assayed through Stajner *et al.* (2009) method by adding SODs reagent in phosphate buffer. Protease was assayed using caseins digestion assay, taking 2 mL casein (1%), TCA (10%) in phosphate buffer (Liu *et al.,* 2009).

DNA extraction and SSR analysis: CTAB method of Doyle and Doyle (1987) was followed to extract DNA from pomegranate genotype and quantified using spectrophotometer (NanoDrop 2000, Thermo Science, Wilmington, DE 19810 USA) to dilute DNA stock of genotypes to 30 ng per 50 µL of triple distillated water for

its use in PCR analysis. Highly polymorphic SSR primers (29) were used in for amplification of DNA of selected pomegranate genotypes in PCR reaction to record the level of molecular variance, polymorphism and intergeneric relationship following the protocols of Ebrahimi *et al.* (2010), Pirseyedi *et al.* (2010), Soriano *et al.* (2011) with some modification. The resolution of the PCR products was visualized on 6% denaturing polyacrylamide gel (PAGE) followed by staining in ethidium bromide.

Statistical Analysis

Fruit samples of eleven pomegranate genotypes were collected for various fruit, aril and seed morphological and biochemical studies. Data were analyzed using Statistix8.1, under Completely Randomized Design to record significant difference among genotypes for studied characters, and Least Significant Difference (LSD) among mean values was done in Tukey's test to record significant difference among genotypes. Genomic DNA fragments of selected 11 pomegranate genotypes, amplified in 29 SSR primers were scored in binary format (0 for absent, 1 for present) for allele(s) at a respective locus. The efficacy and degree of polymorphism in pomegranates was analyzed in Power Marker (Liu and Muse, 2005); however, Analysis of Molecular Variance (AMOVA) and cluster analysis was performed in GenAlEx 6.1 (Peakall and Smouse, 2006) and PopGEN (Yeh *et al.*, 1999) statistical program, respectively.

Results

Fruit Morphological Traits of Pomegranate Genotypes

Coordinates of growing regions and qualitative traits of selected pomegranate genotypes showed that some of selected genotypes were sour in taste and not produced for commercial cultivation (Table 1). A few genotypes like Q-I, Q-II, MG-I, MK-III & MK-IV are commercially produced for their sweet to sour taste and soft to semi soft seeds with good digestively. A high fruit weight (372 g) was recorded in cv. MG-I (Mustong, Gulab Bag) followed by cv. MK-III (236 g), which was at par with Quetta cvs. (Q-I and Q-II) and MG-II ($p \geq 0.05$). A high fruit height (80.94 mm) was recorded in cv. MG-I, similar with all other selected genotypes except MK-II and MG-II (Table 2). Fruit diameter was similar in all genotypes (78.64 mm) except MK-II, showed a lower value of this character (56.13 mm). Significant different was recorded in crown height (CH) with highest value (27.50 mm) in cv. Q-III followed by 13.11mm in cv. MK-III. Least fruit CH (7.35 mm) was recorded in cv. MK-IV. Fruit rind weight was significantly different among the genotypes with highest value (123.92 g) in MG-I, followed by 78.64 g in cv. MK-III as shown in Table 2. There was high variation ($P \leq 0.05$) in aril dimensions with highest aril length (10.90 mm) in cv. MG-II, similar with Q-I, MG-I, MK-I and MK-III; however, least aril length (6.68 mm) was recorded in MG-IV. Highest aril width (6.95 mm) was recorded in cv. MG-II, similar with Q-I, MG-I and MG-III, however, its least value (3.44 mm) was observed in cv. MG-IV. The highest 100 aril weight (83.41 g) was weighed in cv. Q-I, at par with MG-III, MG-IV, MK-I, MK-III and MK-IV, whereas, its least value (14.69 g) was recorded in cv. Q-III (Table 2). Highest 100 seed weight (5.73 g) was recorded in MG-IV, similar with MG-III; however, its least value (0.83 g) was recorded in cv. Q-III. Wood Portion Index (WPI) was significantly different in pomegranate genotypes with highest value 9.38% in cv. MG-IV, similar with MG-III followed by 5.7% in cv. Q-III, similar with Q-II, MG-I and MK-II. Lowest WPI (2.48%) was recorded in cv. MK-IV (white aril sweet cultivar) which showed more juiciness in this genotype (Table 2).

Pomegranate Fruit Biochemical Traits

Non-significant different ($p \geq 0.05$) was recorded among all selected pomegranate genotypes for TSS, however, its highest and lowest value (15.52 and 13.22°Brix) was measured in cvs. MK-III and MK-II, respectively. Significant different was recorded for fruit titratable acidity (TA) with highest value (1.11%) in cv. MK-III, at par with cv. Q-II, however, least TA contents (0.11%) were recorded in cv. MG-I (Table 3). Ascorbic acid contents (Vit. C) varied with highest and lowest value (0.64 and 0.21%) in cvs. MK-III and MG-III, respectively. Significant different ($p \leq 0.05$) was recorded in total sugar contents in genotypes with highest and lowest value (20.44% and 5.54%) in cvs. MG-III and MK-II, respectively.

There was no ($p \geq 0.05$) difference in pomegranate genotypes for soluble protein contents; a highest and a lowest value (0.35 & 0.18%) was recorded in cv. Q-II and MK-III, respectively. The fruit TPC concentration was different ($p \leq 0.05$) in the genotypes with highest value (481.63 g/100 mL) in cv. MK-II followed by 319.33 g/100 mL in MG-I; a lowest TPCs (133.39 g/100 mL) was recorded in cv. Q-II (Table 3). Antioxidant activity (IC_{50}) was variable with a highest value (471.13 µ/mL) in cv. MK-II followed by 423.9 µ/mL in cv. Q-III with lowest value (192.07 µ/mL) recorded in cv. Q-I. Enzymatic activity as Super dismutase (SOD, EC 1.15.1.1) in pomegranate fruits was different ($p \leq 0.05$) with highest value (1493.93 IU/mg of proteins) in cv. MK-IV followed by 1450.97 IU/mg of proteins in cv. Q-II, however, its least value (854.16 IU/mg of proteins) was recorded in cv. MG-I. Peroxidase (POD, EC 1.11.1.7) concentration was also significantly different in all pomegranate fruits with highest value (34.52 IU/mg of proteins) followed by 31.31 IU/mg of proteins in cv. MG-I, however, its least value (7.19 IU/mg of proteins) was recorded in cv. MK-III. Catalase (CAT, EC 1.11.1.6) activity in genotypes was not much different ($P \geq 0.05$) with highest and lowest value (12.48 and 10.36 IU/mg of proteins) in cv. Q-III and MK-III, respectively.

Table 1: Qualitative features of selected pomegranate genotypes

Districts of collection	Collection site	Genotype name and code	Characteristics of genotypes	Latitude	Longitude	Elevation (m)
Quetta	Barkhan	Kandhari Red (Q-I)	Red fruit & aril color with sweet to sour in taste, soft seed wood	71.41	34	1686
		Qabli (Q-II)	Red fruit & red to pink strips on arils, sour in taste, hard seed wood			
		White Kandhari (Q-III)	Red fruit with white arils containing red strips, sweet to sour in taste, hard seed wood			
Mustong	Gulab Bag	MG-I	Red fruit and aril color, sour in taste with hard seed wood	66.85	29.8	1682.8
		MG-II	Red greenish fruit with red arils, bitter in taste with hard seed wood			
		MG-III	Pinkish red fruit with red arils bitter in taste with hard wood seeds			
		MG-IV	Red fruit & arils, sour in taste with hard seed wood			
	Kari Kucha	MK-I	Red to pinkish fruit with red arils, sour in taste with soft seed wood			
		MK-II	Green to red fruit with red arils, sour sweet in taste & semi hard seed wood			
		MK-III	Red fruit and aril color with sour taste & soft seed wood			
		MK-IV	Green red fruit and white arils, sweet in taste and very soft seed wood			

Table 2: Fruit morphological commercial trait based importance of pomegranate genotypes

Genotypes	Fwt. (g)	FH (mm)	FD (mm^2)	CH (mm)	Rind wt. (g)	AL (mm)	AW (mm)	100Awt. (g)	100Swt.(g)	WPI
Q-I	229.01±30.84b	71.76±2.26ab	70.99±3.42ab	8.75±1.69c	62.73±14.04bcd	10.25±0.78ab	6.02±0.59abc	83.41±37.56a	3.11±1.13b	3.91±0.47d
Q-II	225.61±34.32b	70.48±2.96ab	75.92±4.21ab	10.88±3.37bc	54.62±16.52cd	8.50±0.7c	4.76±0.73cd	35.84±18.19de	1.80±0.77cd	5.25±0.76bc
Q-III	169.31±40.66cd	74.48±4.9ab	77.09±7.51ab	27.50±6.23a	28.03±8.55e	8.92±0.87bc	4.46±0.56de	14.69±3.54e	0.83±0.17d	5.70±0.67b
MG-I	372.90±48.38a	80.94±5.06a	78.64±8.58a	10.29±1.63bc	123.92±11.55a	10.11±0.92ab	6.31±1.06ab	57.58±10.29bcd	2.84±0.32bc	4.99±0.42bc
MG-II	198.89±17.39bc	57.49±4.57b	59.17±8.73ab	8.30±1.25c	67.01±2.15bcd	10.90±1.09a	6.95±0.93a	48.39±9.08cd	2.18±0.29bc	4.55±0.42cd
MG-III	165.88±17.84cd	70.99±4.44ab	68.03±3.95ab	7.91±0.26c	68.30±4.17bc	8.94±0.81bc	6.04±0.54abc	61.76±7.46abc	5.19±0.59a	8.41±0.34a
MG-IV	174.36±18.70de	65.87±7.45ab	64.48±6.78ab	10.56±1.89bc	64.80±7.89bcd	6.68±1.03d	3.44±1.15e	61.15±11.63abcd	5.73±1.09a	9.38±0.58a
MK-I	148.50±27.92de	60.04±5.75ab	58.31±7.21ab	7.78±0.24c	67.55±6.38bcd	10.06±0.54ab	5.29±0.62bcd	60.72±17.36abcd	2.22±0.87bc	3.69±1.30d
MK-II	143.97±26.25de	56.27±2.97b	56.13±8.12b	8.09±0.29c	76.38±23.18b	9.22±0.98bc	4.02±0.98de	51.53±15.71bcd	2.32±0.49bc	4.68±0.81bcd
MK-III	236.68±34.10b	73.57±6.99ab	74.71±5.93ab	13.11±3.57b	78.64±4.29b	9.60±0.65abc	4.48±0.49de	61.69±15.35abc	2.28±0.49bc	3.77±0.66d
MK-IV	118.59±12.02e	52.91±1.72ab	54.31±5.92ab	7.35±0.57c	50.93±2.87d	9.29±1.14bc	4.91±1.16cd	77.02±9.64ab	1.90±0.31c	2.48±0.41e

Abbreviations: FWt: Fruit weight; FH: Fruit height; FD: Fruit diameter; CH: Crown height; AL: Aril length; AW: Aril width; Awt: Aril weight; Swt: seed weight; WPI: Wood Portion Index

Table 3: Fruit biochemical commercial traits based importance of pomegranate genotypes

Genotypes	TSS	TA	Vit. C	T. Sugars	Protein	TPCS	IC50	SOD	POD	CAT
Q-I	13.56±0.47a	0.17±0.01c	0.62±0.10a	7.5±0.06bcd	0.21±0.04a	154.29±2.00h	192.07±1.42i	912.69±2.53j	34.52±1.26a	11.65±1.76a
Q-II	15.33±0.58a	1.04±0.14a	0.36±0.02cd	8.13±0.34bc	0.35±0.08a	133.39±1.36i	268.31±2.18f	1450.97±1.69b	24.32±0.92bc	12.26±1.85a
Q-III	15.07±0.55a	0.16±0.01c	0.46±0.05b	6.47±0.02cd	0.26±0.06a	210.24±0.91e	423.90±2.9b	1098.77±1.4g	23.28±0.57bc	12.48±1.88a
MG-I	14.32±0.66a	0.11±0.01c	0.22±0.03e	8.26±0.29bc	0.25±0.07a	319.33±0.91b	235.31±1.69g	854.16±1.48k	31.31±2.17a	11.52±1.74a
MG-II	13.59±1.37a	0.39±0.05b	0.42±0.02bc	7.77±0.62bc	0.21±0.04a	179.99±1.82g	391.40±1.1d	1410.68±2.12c	8.03±1.14e	11.41±1.72a
MG-III	13.39±0.93a	0.42±0.13b	0.21±0.01e	20.44±1.61a	0.21±0.04a	213.87±0.90e	293.00±2.31e	1286.79±0.88d	21.06±2.03c	11.46±1.73a
MG-IV	13.99±0.82a	0.48±0.37b	0.32±0.02d	20.15±1.32a	0.21±0.04a	267.08±1.36c	208.55±1.95h	1016.16±1.57h	16.74±1.14d	11.47±1.73a
MK-I	13.61±0.91a	0.33±0.07b	0.4±0.015bc	8.53±0.26bc	0.22±0.04a	180.24±1.81g	391.21±2.65d	982.89±2.41i	10.09±0.69e	11.70±1.77a
MK-II	13.22±0.5a	0.14±0.04c	0.43±0.03bc	5.54±0.37d	0.21±0.04a	481.63±1.36a	471.13±1.56a	1164.17±1.19f	8.14±0.34e	11.37±1.71a
MK-III	15.52±0.41a	1.11±0.04a	0.64±0.04a	6.66±0.04cd	0.18±0.05a	229.93±1.04d	237.90±0.60g	1257.52±2.43e	7.19±0.06e	10.36±1.75a
MK-IV	14.69±0.44a	0.14±0.04c	0.32±0.02d	9.19±0.89b	0.21±0.05a	195.69±0.90f	411.06±0.93c	1493.93±1.54a	25.31±1.75b	11.05±1.74a

Abbreviations: TSS: Total soluble solids; TA: Titratable acidity; TPCs: Total phenolic contents; IC50: Antioxidant activity at 50%; SOD: Super dismutase oxides; POD: Per oxides; CAT: Catalase

Analysis of Molecular Variance (AMOVA)

Simple Sequence Repeat (SSR) markers (29) successfully amplified DNA fragments of 11 pomegranate genotypes of Balochistan (arid zone of Pakistan). Molecular data analysis showed narrow genetic base of selected genotypes with average polymorphic information intents (PIC) of 0.282 amplified by 29 SSR marker, however marker (POM_AGC11) produced highest PIC value (0.373) and its least value (0.076) was amplified in PGCT075b (Table 4). Average molecular heterozygosity (HZ) was 0.306 with a highest value (0.909) amplified by POM_AGC11, however its lowest level (0.045) was recorded in SSR markers PGCT061 and PGCT086b. There was narrow genetic diversity (GD) in the genotypes with mean value 0.353%; however, maximum and minimum GD values as 0.496 and 0.083% were amplified by POM_AGC11 and PGCT075b, respectively (Table 4). Average value of major allele frequency (0.739) was high in selected pomegranate genotypes with highest and lowest values (0.954 and 0.545) were amplified by PGCT075b and POM_AGC11 SSR primers, respectively.

Molecular Variance and Genetic Relationship in Pomegranate Genotypes

Based on allelic distance matrix for F-Statistics analysis, total molecular variance among populations based on molecular data was distributed as (Fig. 1):

Table 4: Degree of polymorphism of SSR primers for selected pomegranate genotypes

Primer	Forward/Reverse primer	Annealing temperature (°C)	Allele length	Major allele frequency	Genetic diversity	Heterozygosity	Polymorphic information content
PGCT006	TTGAATTGATGTAACGCTTG GAGGAAAGTCGTTTGAAGTG	55	100-300	0.879	0.209	0.121	0.186
PGCT015	GACGCCTTTAGTTTGCTCCA CTCGGGACAGGACTTGGAAT	60	100-200	0.727	0.397	0.364	0.3180
PGCT061	GAATAAGGCGTCCCTCTCTC CTCCTCCTCGTAATCCCAAC	58	150-200	0.659	0.424	**0.045**	0.331
PGCT066b	CGAGGAGTGGTCCAGGTTAG AACAGACGACAAGGGGAATG	59	150-430	0.894	0.186	0.091	0.166
PGCT075b	GGCGAGCTTCTGCTACTTCT TCTGTCCCCAGATCATCAAA	59	200-250	**0.954**	**0.083**	0.091	0.076
PGCT086b	TGGTGATTCGTGTTGTTTTC CAACAACCTCCTCTGCTCTC	57	150-250	0.704	0.390	**0.045**	0.310
PGCT088	TCTCTCTCTACCCCGACACC TAGCGTCAAGATTGTGAAAAGG	59	110-400	0.742	0.371	0.152	0.300
PGCT089b	TGCATCCTTCCCCTACTCTC AGCTCATGTAATGCGTCGTG	59	150-200	0.750	0.374	0.227	0.304
PGCT091b	ATCAGAATTGGAATCGGAAC ACCGAGGTCATCGAACTAAA	56	170-250	0.818	0.287	0.182	0.241
PGCT093A	GTAGCCACTTTAGGGCGAGA CGTCTAAAAGCGACAGCAAG	58	200-330	0.697	0.421	0.424	0.333
PGCT093B	GCCTTTTCCTGCTTTCCTTT CATACAGCGGACCACAACAC	60	200-250	0.591	0.483	0.455	0.367
PGCT096b	CAGACCCTGCGCTCGCT TTATGGAGAGCGGGAGAAAC	59	200-250	0.591	0.479	0.727	0.364
PGCT098b	ATCAACCAAACCGCACAGAC CCATTTCATTCTCCCCCTCT	60	150-200	0.795	0.324	0.227	0.271
PGCT110	GAGCCATTGTAGAGACAAGA GACTGCTGACAACTTTCTTT	52	100-300	0.614	0.465	0.773	0.356
PGCT111	TATCTGTCGCAGGAAGGATG GAAGCCAATTCCTCAAAGATG	58	100-300	0.773	0.314	0.273	0.254
POM_AAC1	GGGTCTTCCTAATTCTCTGG TACAACTTCGGACTCACTTGC	55	100-200	0.591	0.479	0.273	0.364
POM_AAC2	TGTTGTATCCCATCTTCTTCC TTTCCACCGCCATTTACTTC	55	100-250	0.727	0.393	0.364	0.315
POM_AGC5	TTCGATATTGTTTATTGTGTCG CAACGAACTAGACGACACAC	55	100-150	0.864	0.198	0.182	0.159
POM_AGC11	CGTCATCCCTTATGTTCTTC CTGGGGAAGTCGACGAAG	55	150	**0.545**	**0.496**	**0.909**	**0.373**
POM_AAC3	TGATGAAACCATGTAACTCG CTCCGATAACGTCTCCAAGC	55	100-250	0.704	0.415	0.227	0.329
POM_AAC7	GCCTGGACATCTAACGCTCTC GCCGAACAAAGTCCTGAAAC	55	200	0.727	0.397	0.364	0.318
POM_AAC13	TCTCCCGACAACAAATCAC CCCGACACAACACATACTTCAG	53	150-300	0.682	0.415	0.576	0.327
POM_AAC14	CGAGAACCGTTAGTCATGC AGTGACGGCAGGACAAGAAC	55	150	0.682	0.434	0.636	0.340
ABRII-MP07	GATTAACAGCAAAGCCTAGAGG AGTAGCTGCAACAAGATAAGG	60	150-250	0.750	0.366	0.227	0.297
ABRII-MP12	TTGAGTCCCGATCATATCTC TCAATCTGTCAGGAACAACA	60	100-340	0.704	0.407	0.136	0.323
ABRII-MP26	TTTCTCGAAGAATTGGGTAA CTGAGTAAGCTGAGGCTGAT	55	160-240	0.682	0.368	0.273	0.291
ABRII-MP28	ATCCTCTGTCTTTGTGTTCG TGAGTAATTCCGGTCAGAAG	56	100-300	0.841	0.217	0.227	0.170
ABRII-MP39	AGTCTCTGAAGTTTGTCGGA CCTGAGTAAAGCATCTCACTG	60	200	0.909	0.165	0.182	0.152
ABRII-MP30	CCCAGTTTGTAGCAAGGTA	60	200	0.818	0.298	0.101	0.253
Average		-	-	**0.739**	**0.353**	**0.306**	**0.282**

a. 11% was attributed among two populations (Quetta and Mustang),
b. 16% was recorded among individuals of these populations,
c. 73% variation was observed within the individuals (genotypes).

Genetic relationship among 11 pomegranate genotypes from Baluchistan showed that they were successfully clustered in two main groups with five genotypes in group A, whereas, group B consisted of 4 genotypes (Fig. 2). In group A, MK-I and MG-III were directly clustered with each other by sharing most of

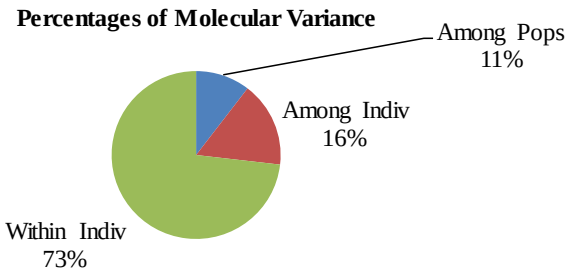

Fig. 1: Molecular variance of pomegranate genotypes as amplified with SSR markers

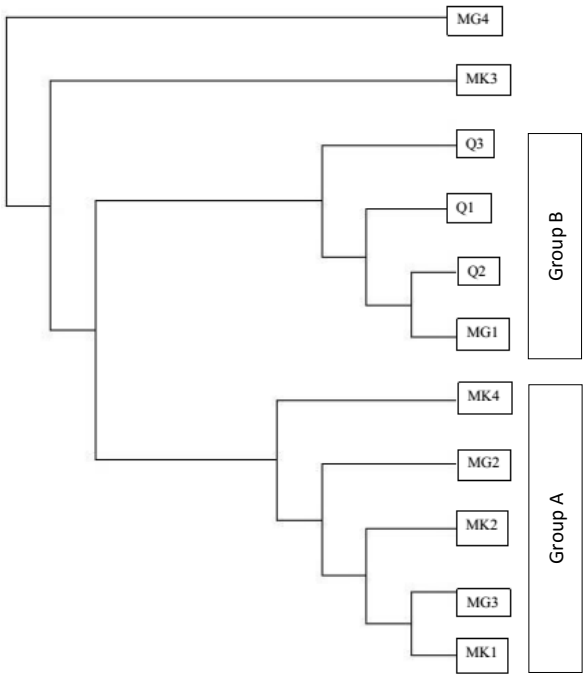

Fig. 2: Genetic relationship among pomegranate genotypes as amplified with SSR markers

their alleles; however, they also showed some allelic relationship with MK-II, MG-II and MK-IV. The genotype MG-I and Q-II also directly clustered in group B, whereas, Q-I and Q-III were indirectly linked with this cluster for some genetic sharing. The genotype MK-III and MG-IV are openly clustered with groups A and B (Fig. 2).

Discussion

Highly significantly variation was recorded in weight of fruits, rind, arils and seeds of collected pomegranate genotypes. Moreover, WPI was also diverse in our genotypes. Ercisli *et al.* (2007), Hasnaoui *et al.* (2011a), Nafees *et al.* (2015) also found high variation in fruit size, rind weight, aril weight and seed hardness in Turkish, Tunisia and Pakistani wild and cultivated pomegranates, respectively. Low WPI value could be utilized in domestic genotypes of other regions to improve juice contents as did by Martinez *et al.* (2006), Sarkhosh *et al.* (2009) in Spanish and Iranian sweet pomegranates, respectively. Moreover, significant difference in WPI and other fruit morphological traits, could be used in variety improvement program as done in Iranian soft-seeded pomegranates (Sarkhosh *et al.*, 2009) and Chinese pomegranate germplasm (Wang *et al.*, 2006) after characterization of existing germplasm.

Non-significant difference of TSS value in selected pomegranate genotypes showed contradiction with Spanish, Saudi Arabian and Portuguese pomegranates (Al-Maiman and Dilshad, 2002; Mjguel *et al.*, 2004; Martinez *et al.*, 2006). This might be due to the reason that collected genotypes were from only two regions with similar climatic conditions. Moreover, in this study, fruits of all genotypes were harvested at the same time, so collapsed the proper time of harvesting. Significantly high variation in vitamin C and titratable acidity of selected pomegranates is in line with the findings of Ozgen *et al.* (2008). Total phenolic contents, antioxidant activities and antioxidant enzymes (super dismutase and per oxidase) concentration was also significantly different, which is as par with the findings of Tezcan *et al.* (2009), Tehranifar *et al.* (2010), Fawole *et al.* (2011), Mena *et al.* (2011) who reported that the concentration of antioxidants highly depend on pomegranate genotype. High level of biochemical variation recorded in pomegranate genotypes of Balochistan, endorsed by Nafees *et al.* (2017) proved wide genetic base in Pakistani pomegranate germplasm (Wild and domesticated) for various fruit biochemical traits.

Selected primer groups in this research are multiallelic in nature for amplifying more than one locus in our pomegranates which is in line with Soriano *et al.* (2011) recorded in Spanish pomegranates. The PIC value in selected primers of POM and PGCT series was high and low, respectively in selected 11 pomegranate genotypes, which is in contradiction with the findings of Nafees *et al.* (2015) who recorded PIC value of 0.37 to 0.55 in 95 pomegranate genotypes (wild and domesticated) of Pakistan. There was a range of PIC (0.076 to 0.373) amplified in our genotypes which was at par with the findings of Basaki *et al.* (2011) who reported 0.01 to 0.46% PIC with an average of 0.34 in Iranian pomegranate cultivars. Low PIC value in our selected pomegranate genotypes of Balochistan might be due to reason that they are from the same growing region and propagated through selection instead of breeding process; moreover, genotypes were cultivated rather wild or domesticated pomegranates. Heterozygosity, genetic diversity and allele frequency of amplified DNA of Balochistan pomegranate genotypes was narrow, which has contradiction with the findings of Parvaresh *et al.* (2012), Nafees *et al.* (2015) who recorded broad genetic base in large collection of wild and cultivated pomegranate germplasm of Pakistan and Tunisia, respectively. Limited and only commercial genotypes, and growing in less diverse climatic region, might be the reasons

of narrow genetic base in studied pomegranate germplasm.

Reported primers in this study were efficient and restored high level of polymorphism. Moreover, morphological and biochemical variation in studied pomegranate genotypes was confirmed in molecular studies. The results are contradicting with the findings of Kumar (1999), Gupta and Rustgi (2004) stated that genetic markers did not influence morphological traits. Selected pomegranates had similarity for various morphological and biochemical traits; however, molecular analysis based cluster, proved high level of diversity as most of genotypes openly clustered in groups. This is in line with the findings of Zamani *et al.* (2013) stated that morphological and biochemical data is poorly correlated (r=0.45) with molecular data to record germplasm variation in pomegranates. Zamani *et al.* (2007), Nafees *et al.* (2015) supported our findings and concluded that SSR and RAPD marker based variation estimation in pomegranate genotypes of Pakistan and Iran had no connection with morphological variation, respectively. Discrepancy of morphological and molecular variation in our genotypes is also supported by Gupta and Rustgi (2004), Mehmood *et al.* (2016). This might be because of the genotypes growing in similar agro-climatic conditions of Balochistan which showed morphological similarities but molecular studies confirmed significant variation among genotypes.

Conclusion

The highest fruit weight was examined in genotype MG-I; however, high juiciness was recorded in MK-IV with soft seeds and white sweet arils. Commercial genotypes (Q-I, Q-II and MK-III) had red fruit and aril color, sweet to sour in taste with low WPI. Inferior genotypes need improvement in fruit weight and juiciness characteristics. Moreover, narrow genetic base of selected genotypes could be utilized with elite genotypes of other regions in variety improvement program.

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