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Pomegranate (*Punica granatum* L.) Fruit Quality Attributes in Relation to Aril Browning Disorder

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ABSTRACT

Aril Browning (AB) is a physiological disorder in pomegranate fruit that critically decreases fruit quality and market acceptability. This experiment was carried out in order to explore the effective pomegranate fruit quality traits associated with the AB disorder and select the suitable resistant cultivar and genotypes. Pomegranate physico-chemical fruit quality attributes were assessed on 238 mature pomegranate genotypes and their correlations with the AB disorder were monitored. About 14.7% of the studied genotypes showed resistance to the AB disorder, but 68.14% showed moderate to severely susceptibility to the incidence. The intensity of the AB disorder symptoms in pomegranate genotypes was strongly correlated with physico-chemical fruit attributes. There was a negative significant correlation between the intensity of AB disorder and fruit size, fruit volume, fruit acidity, and total soluble solids (TSS) to titratable acidity (TA) content.. Among the studied fruit traits, stepwise regression analysis showed that fruit acidity (pH), aril color, fruit volume, and TA content were efficient traits for screening of pomegranate genotypes in relation to the AB disorder.

Keywords*:* Aril color, Genotype screening, Peel color, Physiological disorder, Resistant cultivar.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the valuable horticultural crops, cultivated in the Iranian plateau for a long time (Varasteh and Arzani, 2009). Traditionally, world pomegranate consumers prefer relatively large fruit size, high aril weight, high juice content with deep red color and rich in bioactive compounds (Jafari *et al*., 2014).

Pomegranate is a rich source of polyphenol compounds, anthocyanin, organic acid, vitamins, and mineral nutrients with high antioxidant properties (Mphahlele *et al*., 2014). Major constitute of pomegranate arils is a polyphenol substance such as anthocyanin pigments, which influences the aril color and fruit quality. Several studies demonstrated that the accumulation of bioactive compounds and anthocyanin contents in pomegranate fruit are influenced by multiple factors such as genotypes and cultivars (Zhao *et al*., 2013), environmental conditions (Li *et al*., 2015), orchard management and fertilizer application (Jafari, *et al*., 2014; Kavand *et al*., 2017a and b), water quality (Borochov-Neori *et al*., 2013), harvesting time (Fawole and Opara, 2013; Mphahlele *et al*., 2016), and storage condition (Varasteh *et al*., 2012). Recent evidence suggests that pomegranate fruits under severe water stress exhibited less antioxidant activity, total polyphenol compounds, punicalagin, total anthocyanin and more yellowish juice color compared to the pomegranate under non-

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restricted soil water condition (Mena *et al*., 2013). Anthocyanin pigments are unstable in pomegranate arils and may be susceptible to degradation by the presence of oxidizing enzymes such as polyphenol oxidase (Varasteh *et al*., 2012).

Aril Browning (AB) or aril paleness of the pomegranate fruit is a physiological disorder that has critically decreased fruit quality and market acceptability during recent years in some pomegranate cultivars (Meighani *et al*., 2014; Jalikop *et al*., 2010; Tabar *et al*., 2009). The affected pomegranate fruits have arils with the matte surface, and internal space on its texture, in which oxidation of the polyphenol substances altered the natural aril color to light creamy-brown color (Shivashankar *et al*., 2012). Also, the affected fruit remains free from obvious and external symptoms until the fruit is cut and opened (Meighani *et al*., 2014).The affected pomegranate fruit appeared as over-ripened, so, fruit arils dehydrated, showed waterless with lower fresh weight. In addition, the early and mid-ripening cultivars are more susceptible to the AB disorder compared with the late-ripening fruits (Behzadi Sharbabaki, 2014). In the main Iranian pomegranate planting regions such as Saveh and Ferdows areas, two commercial pomegranate cultivars, namely, 'Malase-e Torsh-e Savah' and 'Shishe-e Kap-e Ferdows' showed more sensitivity to the AB disorder (Tabar *et al*., 2009; Meighani *et al*., 2014). In the past decades, the produced pomegranate cultivars had good fruit quality, but in recent years due to the possible adverse effects of the climate change, the AB disorder in pomegranate fruit has been developed in some commercial orchards and regions (Behzadi Sharbabaki, 2014). In India, the pomegranate cultivars 'Ganesh' and 'Bhagwa' are more susceptible to the AB disorder (Shivashankar *et al*., 2012). It has been reported that various factors such as cultivar, orchard location, high air temperature during fruit maturation, water quality, fruit thinning, harvesting time, and chemical fruit traits may have an impact on degree and extension of the AB disorder in pomegranate fruit (Shivashankar *et al*., 2012; Jalikop *et al*., 2010; Behzadi Sharbabaki, 2014). In the affected fruit of the pomegranate cultivar 'Shishe-e Kap-e Ferdows' fruit weight, juice percentage, phenolic compounds, monomeric anthocyanin, and titratable acidity decreased, whereas polymeric anthocyanin and total soluble solid increased significantly compared to the intact fruits (Tabar *et al*., 2009). In addition, it has been reported that the respiration rate and Peroxidase (POX) and and polyphenol oxidase (PPO) activity of the affected arils were significantly higher than the healthy arils of the pomegranate cv. `Malase-e Torsh-e Saveh' (Meighani *et al*., 2014). It has been reported that severity of AB disorder among the 153 studied pomegranate progenies was related to fruit traits such as peel, aril color and total soluble solids (Jalikop *et al*., 2010). The consideration of the plant genetic resources under the possible climate change is essential for improving fruit quality for sustainable production (Govindaraj *et al*., 2015). It has been reported that Iran is one of the centers of origin for pomegranate with great diversity (Varasteh and Arzani, 2009; Sarkhosh *et al*., 2007). The AB disorder problem in the Saveh pomegranate orchards and possible solutions using different cultural practice systems has been reported by Kavand *et al*. (2017a, b). The objective of the present research was to explore the effective pomegranate fruit traits associated with the AB disorder in order to screen and select the resistance genotypes among the major rich Iranian pomegranate germplasm at Saveh collection orchard.

MATERIALS AND METHODS

The experiment was carried out in Saveh Pomegranate Collection Orchard (SPCO) located at (Longitude: 34º 59' 31.93" E) and (Latitude: 50º 13' 8.5" N), Saveh, Iran. The climate condition of this region is semi-arid, with 194.1 mm annual rainfall, 2,725 mm

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potential evapo-transpiration, the average annual temperature of 18ºC, and the average relative humidity and temperature of the warmest months (July and August) as 26% and 30.9ºC, respectively.

The pomegranate genotypes were planted in 2×3 m spacing, on a sandy loam soil (sand 61%, silt 29%, and clay 10%) and trees were irrigated weekly during the growing season (Water EC= 3,256 μS cm^{-1}). Five fruit samples at commercial maturity stages were picked from each 238 pomegranate cultivars and genotypes. Fruit samples were transferred to the SPCO laboratory for observation of the possible AB disorder and to the Pomology Lab at Tarbiat Modares University (TMU) for further supplemental assessments. The fruit length (mm), diameter (mm), and weight (g) were measured on fruit samples and the average was used for data analysis. The fruit Volume (cm^3) was calculated based on fruit diameter using equation (V= 4/3 πr^3) (Wetzstein *et al*., 2011). The pomegranate fruit sorted by peel color based on three scores including score 1 with cream to yellow–orange color, score 2 with pink to reddish pink color, and score 3 was red to deep red color. In addition, the arils color was assessed according to the three scores: score 1 referred to fruit with white to cream color aril, score 2 was pink to dark pink color aril and score 3 was red to deep red color. All fruit samples from each studied genotypes were cut in half, then the intensity of the AB disorder through each fruit was visually evaluated based on five given scores: 1- Severely affected fruit, in which the arils have a very dark surface, deformed texture, and creamy to brown color; 2- Relatively highly affected fruit, in which the arils showed the opaque surface, very injured texture, and light creamy to brown color; 3- Moderately affected fruit, in which the arils showed the semi-translucent surface, injured texture, tiny dot or interior space in the texture and reddish color; 4- Slightly affected fruit, in which the arils appeared with a shiny surface, solid texture, and red color, and 5- Non-affected fruit, in which the arils showed a bright surface, solid texture, and deep red color. In addition, the Intensity of AB disorder index (AB index) was calculated. Generally, the un-affected healthier fruits and severely affected genotypes showed the higher and lower AB index, respectively. The AB index was calculated using the following equation:

$$AB\ index = \frac{\sum(n \times b) \times 100}{\sum(N \times Max b)},$$

Where, the AB index is the index showing the aril browning disorder intensity, n is the number of fruits of each genotype that has the same visually recorded score in the AB disorder, N is the total Number of fruits for each genotype, b is visually recorded score for the intensity of AB disorder, Max b is the highest score for non-affected fruit, which equals 5. The juice of the fruits for each genotype was obtained by hand pressure, then stored at freezer (-20ºC) for chemical analysis. The fruit juice was centrifuged at 10,000 rpm for 10 minutes at 4ºC. Then, TSS, pH, EC, Titratable Acidity (TA) content of fruit juice and juice color absorbance were measured. To measure the juice color absorbance (which is directly related to the total anthocyanin), about 5 mL of fruit juice was diluted with 15 mL distilled water, afterward, the absorbance of diluted extract was measured at 510 nm by UV spectrophotometer in three replicates (Fawole and Opara, 2013). To measure the blank, the plate was filled with distilled water. In order to measure TA, 5 mL of fruit juice was diluted to 45 mL using distilled water, then, the pH was increased to 8.1 using 0.1N of NaOH, so, titrable acidity was calculated and the obtained results were expressed as percentage of citric acid. The TSS, pH, and EC of fruit juice were measured by the digital refractometer (G-won Korea), pH meter (827 pH lab, metrohm) and EC meter (Sensodirect con 200. Loviband Co.). Fruit taste was monitored as the TSS to TA ratio.

Statistical Analysis

Analysis Of Variance (ANOVA) was performed on the obtained data. In addition, Pearson's correlation, multiple linear regression, and Principal Component

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Analysis (PCA) of the data were carried out using SPSS statistical package.

RESULTS

Distribution of the Aril Browning and Fruit Attributes

The results indicated considerable variation in the studied pomegranate genotypes in relation to AB disorder symptoms (Table 1). Our study indicated that the severity of the AB disorder was strongly dependent on some physico-chemical fruit attributes of each genotype. According to the severity of the AB disorder symptoms, the pomegranate genotypes were classified into 5 groups. Group 1 included 35 non-affected pomegranate genotypes, the AB index was close to 100 and the fruits did not show any of AB disorder symptoms. Group 2 consisted of 40 genotypes with relatively slightly affected fruit, with AB index in the range of (84-96) and the fruits showed little sign of the AB disorder. Note that the group 1 and 2 aril showed bright and shiny surface in color with solid texture. In contrast to the severely affected genotypes, the red color arils were prominent for the fruits of these groups: about 21.43% of their fruits showed red color arils, 7.14% pink color arils, and 2.94% creamy color arils. The group 3 includes 120 pomegranate genotypes with moderately affected fruit; the AB index was in the range of (44-80) and fruit in this group showed intermediate sign of AB disorder. Also, group 4 included 29 relatively highly affected genotypes with AB index in the range of 24 to 40 and fruits showed 80% of AB disorder symptoms. Group 5 included 14 severely affected genotypes with AB index close to 20, and fruits showed high AB disorder symptom. Groups 4 and 5 arils attribute showed very dark surface in color with injured texture, and deformed structure. The results indicated that the creamy color for the arils is prominent for the fruit of the highly and severely affected groups, so, about 7.14% of

Table 1. Descriptive statistics of various fruit attributes for the studied pomegranate genotypes based on the intensity of the aril browning disorder in the studied Saveh pomegranate accessions.[a]

AB Intensity	Mean of the AB index	Range of the AB index	Percent of total	Fruit weight (gr)	Fruit diameter (cm)	Fruit length (cm)	Fruit volume (cm^3)
Non-affected (H)	100^{a}	100	14.70	142.17^{a}	63.02^{ab}	59.75^{a}	125.09^{a}
Relatively H	86.78^{b}	84-96	16.80	146.57^{a}	61.81^{a}	60.68^{a}	126.30^{a}
Moderately affected	61.15^{c}	44-80	50.42	144.57^{a}	62.38^{a}	60.52^{a}	126.25^{a}
Relatively affected	42.45^{d}	24-40	12.18	173.83^{a}	67.11^{ab}	65.21^{b}	143.35^{ab}
Severely affected	20^{e}	20	5.88	158.39^{a}	65.43^{ab}	61.21^{ab}	157.77^{b}

AB Intensity	Ab	pH	EC (μS cm^{-1})	TSS (°Brix)	TA (%)	Fruit taste (TSS/TA)	The aril fruit color (%)		
							Creamy	Pink	Red
Non-affected (H)	0.43^{a}	3.07^{a}	4.01^{a}	13.06^{a}	2.04^{a}	1.15^{a}	1.7	2.9	10.1
Relatively H	0.35^{ab}	3.16^{a}	3.99^{a}	12.97^{a}	1.45^{b}	1.86^{ab}	1.3	4.2	11.3
Moderately affected	0.29^{b}	3.3^{b}	3.93^{a}	12.85^{a}	1.26^{b}	2.33^{b}	9.7	18.1	22.7
Relatively affected	0.27^{bc}	3.34^{b}	3.90^{a}	12.99^{a}	1.19^{b}	2.03^{ab}	3.8	4.6	3.8
Severely affected	0.15^{c}	3.47^{c}	4.00^{a}	13.01^{a}	0.66^{c}	4.33^{c}	3.4	1.7	0.8

[a] Means, followed by the same letter in column are not significantly different at the 5% level of probability, using Duncan Multiple Range Test. AB= Aril Browning; Ab= Absorbance at 510 nm; pH= Juice fruit acidity; EC= Electrical Conductivity of fruit juice; TSS= Total Soluble Solids of fruit juice; TA= Titratable Acidity of fruit juice.

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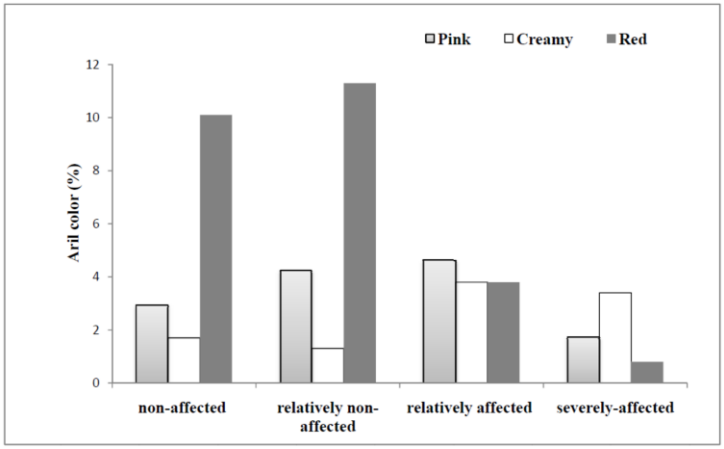

Figure 1. Distribution of pomegranate aril color for non-affeceted and severely-affected genotypes.

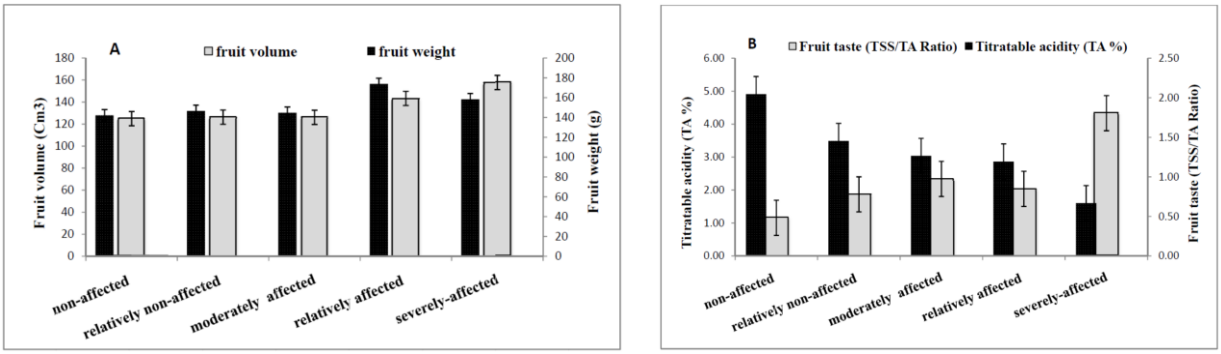

Figure 2. The aril browning intensity of the studied pomegranate genotypes in the Saveh pomegranate collection orchard in relation to a: the average fruit weight and fruit volume and b: the TA content and TSS/TA ratio of fruit juice.

fruits showed creamy color arils, 6.30% pink color arils and 4.62% red color arils (Figure 1). The average fruit weight, volume, length, and diameter for the non-affected genotypes usually were smaller than the severely affected genotypes (Figure 2-A). For instance, the average fruit volume 125.09 (cm^3) for the non-affected genotypes was significantly (P< 0.002) smaller than the average fruit volume of 157.77 (cm^3) for the severely affected genotypes. Also, a significant difference between the TA and TA to TSS ratio of the fruit juice among the non-affected and affected genotypes was detected. Generally, the amount of TA content in fruit juice in non-affected genotypes was significantly (P< 0.001) more than the moderate to severely affected genotypes (Figure 2-B). In contrast, the TSS to TA ratio of fruit juice in the severely affected genotypes was significantly more than the non-affected genotypes. Moreover, the average of fruit acidity (pH) for the non-affected genotypes showed significantly (P< 0.001) lower than the moderately to severely affected genotypes. Also, the amount of juice color absorbance value of the non-affected genotypes was significantly (P< 0.001) more than the moderately to severely affected genotypes (Figure 3).

Correlation between Aril Browning Disorder and Various Pomegranate Fruit Traits

Results of the Pearson's correlation indicate that there was a negative and significant correlation between AB index and the fruit weight (r= -0.160*), fruit

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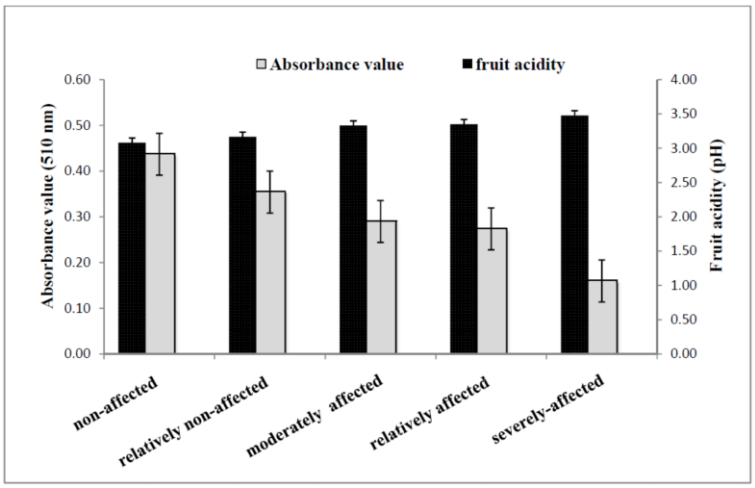

Figure 3. The aril browning intensity of the studied pomegranate genotypes in the Saveh pomegranate collection orchard in relation to the pH content of fruit juice and juice color absorbance value at 510 nm.

diameter (r= -0.193**) fruit length (r= -0.162**), and fruit volume (r= -0.226**). In other words, by increasing the pomegranate fruit size, the intensity of the AB disorder symptom is increased and vice versa. Also, a positive and significant correlation was observed between the AB index and juice color absorbance value (r= 0.282**) and TA (r= 0.362**) content. It means that with increasing juice color intensity, the severity of the AB disorder symptoms decreased among the studied pomegranate genotypes. A negative and significant correlation was observed between the AB index and fruit acidity (pH content; r= -0.527**). This means that, with an increase in fruit acidity, the intensity of the AB disorder among the studied pomegranate genotypes decreased (Table 2).

Multivariate Analysis

The result of stepwise regression showed that pomegranate four traits including fruit acidity (pH), red color aril, pink color aril, fruit volume and TA content of juice simultaneously had a significant effect on AB disorder symptom in pomegranate genotypes. Therefore, the regression model is represented as follows:

Y (The AB index)= $187.42-39.91x_1+28.49 x_2 +19.13 x_3-0.11 x_4+3.71x_5$

Where, the x_1, x_2, x_3, x_4 and x5 are for the fruit acidity (pH), red color aril, pink color aril, fruit volume and TA content of fruit juice, respectively. According to the Beta coefficient, red color aril (Beta= 0.61) had more influence on AB disorder, such that the severity of AB disorder in pomegranate genotype with red color aril was less than the cream color aril. The fruit acidity (pH) (Beta= -0.40) is the second fruit trait that has more influence on AB disorder in pomegranate fruit genotype such that by increasing in fruit acidity (pH) the severity of AB disorder in pomegranate fruit was decreased. Regression model showed that pink color aril (Beta= 0.37) is the third fruit trait that simultaneously affected the severity of AB disorder. The severity of AB disorder in pomegranate fruit with pink color aril was less than the cream color aril. Fifth and sixth pomegranate fruit traits that had direct effect on the AB disorder were fruit volume (Beta= -0.22) and TA content (Beta= 0.15) of fruit juice. AB disorder symptom was decreased by increasing fruit volume and decrease in TA content. The stepwise regression showed that fruit acidity (pH), red color aril, pink color aril, fruit volume, and TA content of juice had the greatest effect on screening of

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Table 2. Person's correlation analysis between the intensity of the aril browning disorder and different pomegranate fruit quality traits in the studied Saveh pomegranate accessions.[a]

	AB	FW	FL	FD	FV	Abs	pH	EC	TSS	TA	TSS/TA
AB Index	1										
FW	-0.160*	1									
FL	-0.162*	0.693**	1								
FD	-0.193**	0.759**	0.756**	1							
FV	-0.226**	0.676**	0.796**	0.844**	1						
Abs	0.282**	0.024	-0.005	0.017	-0.018	1					
pH	-0.527**	-0.057	-0.017	0.003	0.032	-0.247**	1				
EC	0.103	0.084	0.044	0.095	0.070	.222**	-0.257**	1			
TSS	0.006	0.088	0.010	0.034	0.035	0.127	0.007	0.183**	1		
TA	0.362**	-0.002	-0.030	-0.053	-0.097	0.163*	-0.467**	0.204**	0.039	1	
TSS/TA	-0.327**	0.042	0.025	0.078	0.085	-0.097	0.457**	-0.102	0.209**	-0.589**	1

[a] AB: Aril Browning index; FW= Fruit Weight; FL= Fruit Length; FD= Fruit Diameter; FV= Fruit Volume; Ab= Absorbance value; pH= Fruit juice acidity; EC= Electrical Conductivity of fruit juice; TSS= Total Soluble Solids of fruit juice; TA= Titratable Acidity of fruit juice.

Table 3. Multi linear regression analysis (Stepwise) for the aril browning disorder and various pomegranate fruit quality traits in the studied Saveh pomegranate accessions.[a]

Independent traits	B	Std Error	Beta	t	Sig	Ad R2	F
Constant	187.419	19.105		9.810	.000		
Fruit acidity (pH)	-39.910	5.325	-0.390	-7.495	.000	0.29	
Red color aril	28.492	2.732	0.609	10.428	.000	0.42	58.76**
Pink color aril	19.131	2.970	0.371	6.442	0.00	0.51	
Fruit Volume	-0.114	.023	-0.222	-4.883	.000	0.57	
TA	3.711	1.244	0.154	2.983	.000	0.58	

[a] TA: Titratable acidity of fruit juice. ** Predictors: Constant; fruit acidity (pH); red color aril; pink color aril; fruit volume,

pomegranate genotypes in relation to AB disorder (Table 3).

Principal Component Analysis

To better understand the overall relationship between the fruit traits and the AB disorder among the 238 pomegranate genotypes, the Principal Component Analysis (PCA) was performed. The factor loading, eigenvalue, percent of the variation, and percent of total variation for each factor are shown in Table 4. The PCA indicated that the fruit size, fruit acidity (pH), and fruit taste were the most efficient factors that influenced the severity of the AB disorder in the studied pomegranate genotypes (Figure 4). These traits took out under the first three un-correlated factors that explain about 65.83% of the total variation. The first factor (named fruit size) included fruit volume, fruit diameter, fruit length, and fruit weight with the positive Eigenvalue representing 30.19% of the total variation. The second factor or (fruit acidity) includes fruit acidity (pH), TA, TSS to TA ratio and aril browning index, which represents 22.46% of the total variation. The third factor or (fruit taste) includes TSS, EC, and juice color absorbance value, which represents 13.25% of the total variation. Other studies including

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Table 4. Factor loading, eigenvalue, present of variance, and present of cumulative variance for the first three factors among the studied genotypes in the Savah pomegranate accessions.

Fruit traits[a]	FAC1 (Fruit size)	FAC2 (Fruit acidity)	FAC3 (Fruit taste)
Fruit weight	**0.857**	-0.003	0.085
Fruit diameter	**0.925**	-0.042	0.049
Fruit volume	**0.909**	-0.078	-0.004
Absorbance value	-0.056	**0.296**	**0.582**
Fruit acidity	-0.077	**-0.768**	-0.258
EC	.119	0.230	**0.603**
TSS	0.010	-0.215	**0.743**
TA	0.003	**0.772**	0.107
Aril browning index	**-0.280**	**0.645**	**0.254**
Fruit taste	0.001	**-0.822**	**0.224**
Fruit length	**0.891**	0.009	-0.033
Eigenvalue	3.36	2.57	1.30
Variance	30.19	22.46	13.25
Cumulative variance	30.19	52.58	65.83

[a] EC= Electrical Conductivity of fruit juice; TSS= Total Soluble Solids of fruit juice, TA= Titratable Acidity of fruit juice. The bold number of studied traits in each column is more efficient for each factor against AB discrimination among studied pomegranate accessions.

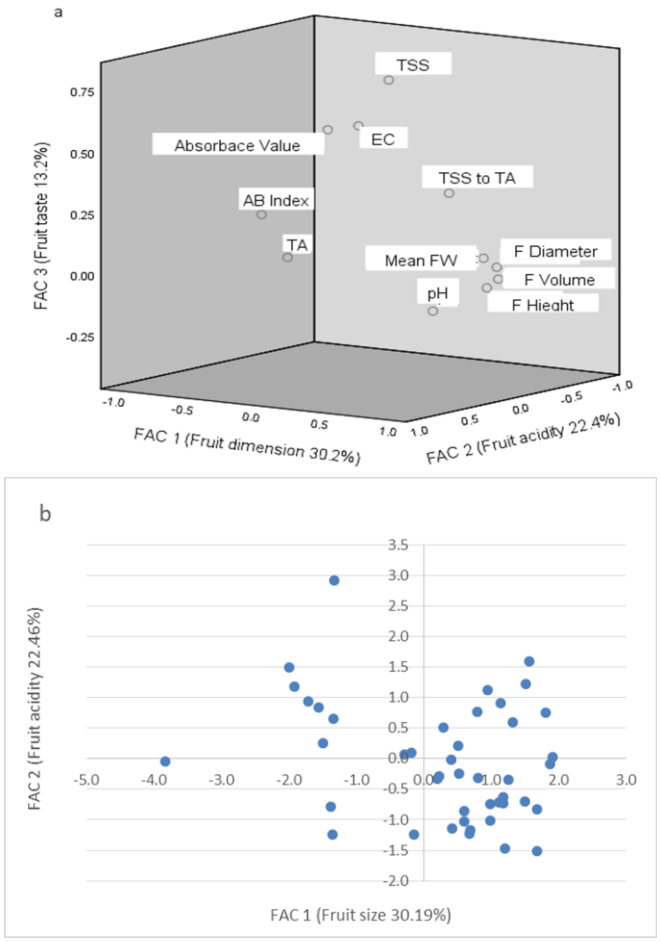

Figure 4. The Principal Component Analysis (PCA): **(A)** The first three-factor for the variables and **(B)** Scatter biplot of (FAC1 and FAC2) for the non-affected and affected genotypes of AB disorder in pomegranate genotypes.

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pomegranate (Beaulieu *et al*., 2015), apricot (Leccese *et al*., 2010), and peach (Nikolić *et al*., 2010) also used the PCA technique to evaluate the important traits of germplasm. Schwartz *et al*. (2009) revealed by PCA analysis that pomegranate genotypes that have a weak genetic background are more influenced by climate condition than the strong genetic accessions. The PCA technique clearly separated the pomegranate genotypes with desirable positive and negative factors related to the AB disorder, for example, the biplot for non-affected and severely affected genotypes for the two components (FAC1 and FAC2) are shown in Figure 4-B. In summary, the PCA showed that various fruit traits that related with fruit size, fruit acidity (pH), and fruit taste are the most important and efficient fruit traits for genotype screening in relation to the AB disorder among the studied pomegranate genotypes.

DISCUSSION

The occurrence of the AB disorder in some pomegranate production areas of Iran has been the result of the adverse effects of the climate change in recent years (Behzadi Sharbabaki, 2014). In the present research, a significant variation in the AB disorder among the studied pomegranate genotypes showed that the severity of the AB disorder strongly depended on some physico-chemical properties of the pomegranate fruit (Tables 1 and 2). Some fruit traits lead to the reduction, but some other may cause increase in AB disorder. The intensity of AB disorder symptoms in the pomegranate genotypes with bigger fruit size was relatively more than the genotypes with smaller fruit size (Figure 2-A). Previously reported results showed the increase in AB disorder symptoms at the time of fruit maturation process (131 days of fruit set) for pomegranate CV. 'Shishe-e Kap-e Ferdows' and 'Malas-e-Torshe-e-Saveh' (Kavand *et al*., 2017a; Meighani *et al*., 2014), might be due to the maximum physical, chemical, and physiological changes during maturation process (Fawole and Opara, 2013). At maturation process, the amount of TSS, total sugar and reducing sugar contents significantly increased whereas the amount of antioxidant activity, ascorbic acid, total phenolic and acidity content of pomegranate fruit significantly decreased (Fawole and Opara, 2013; Kulkarni and Aradhya, 2005). It is known that the bigger fruits require more water, carbohydrate, and mineral nutrients, especially Ca cation. It is clear that the pomegranates are grown on the long shoots with thin spur. By increase in the fruit weight, the spur is bent and protected by fewer leaves area against direct sunlight. As a result of the climate condition (direct sunlight, high air temperature, and low RH), the internal temperature of the larger fruit might be increased to above 40˚C (Kavand *et al*., 2017a). High temperature increases production of the Reactive Oxygen Species (ROS) in the plant cells (Wahid *et al*., 2007).Therefore, the interaction effects of the high internal temperature, the presence of ROS and PPO, reduction of Ca concentration and acidity of the arils tissue from the larger fruit lead to the breakdown of the cellular membrane and arils texture. As a result of the fraction of the aril tissue, the polyphenol substances are transferred from the vacuole membrane to the cytosol and internal space of the cell. Finally, the polyphenol substances are oxidized to produce the brown colored quinones in the aril tissue (Varasteh *et al*., 2012). The reported higher AB disorder in the pomegranate genotypes with larger fruits support our findings that showed a positive correlation between fruit size and dimension with AB disorder (Table 2). One interesting finding was that by increasing the juice color intensity, the severity of the AB disorder was decreased (Figure 3). Also, a positive and significant correlation was observed between the AB intensity and juice color absorbance value (Table 2). Most of the non-affected genotypes had fruits with red color arils (Figure 1). Jalikop *et al*. (2010), reported the negative correlation (-0.41)

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between the aril color and intensity of the AB disorder in pomegranate progenies. The pomegranate genotypes with red color arils have more anthocyanin, polyphenol, and antioxidant capacity than the genotypes with pink or creamy skin color (Tzulker *et al*., 2007). Anthocyanin pigment is very unstable and may be altered by the presence of light, oxygen, UV, pH and presence of the PPO (Cabrita *et al*., 2000). Anthocyanin is a water soluble pigment, which is conserved from the oxidative enzymes by vacuoles membranes. The results of the AB disorder are destruction in aril texture, oxidation of polyphenolic substances, and discoloration in aril color (Jaiswal *et al*., 2010). According to our results, by increase in acidity of the fruit juice, the severity of the AB disorder was decreased (Figure 3). Also, a negative and significant correlation was observed between the AB intensity and fruit acidity (Table 2). It has been reported that fruit acidity (pH) of AB disordered pomegranate fruit was significantly higher than unaffected fruit (Shivashankar *et al*., 2012; Meighani *et al*., 2014). It seems that the major organic acids in pomegranate fruit are citric and malic acids, and the amount of citric acid in the sour cultivars was 15 times more than sweet cultivars (Legua *et al*., 2012). Also, the main role of organic acid in pomegranate fruit is antioxidant activity (Gil *et al*., 2000). Therefore, the polyphenol substances and anthocyanin pigments under lower pH of fruit juice are more stable and the PPO activity decreases (Cabrita *et al*., 2000; Joas *et al*., 2005). These reported results support our findings in which the intensity of AB disorder in the sour pomegranate genotypes was significantly less than the sweeter genotypes.

CONCLUSIONS

The aril browning disorder is a serious problem in some pomegranate production areas of the world as well as in Iran. The severity of this disorder is possibly affected by the degree of climate change in the production regions. The main cause of the AB disorder is destruction in aril texture and changes in anthocyanin components altered the red color to the creamy-brown color of the aril. This physiological injury in pomegranate critically decreased fruit quality and market acceptability. In our study, considerable diversity in the AB disorder was observed among the studied pomegranate genotypes. This diversity created good breeding potential to select the suitable AB resistant cultivars or genotypes within the Iranian rich fruit germplasm. The present research results showed that the diversity of the pomegranate genotypes in relation to the AB disorder strongly depended on the fruit attributes. For pomegranate breeders interested in AB disorder, the fruit weight, fruit dimension, aril color, anthocyanin content and fruit juice acidity are the most effective traits for genotype discrimination. About 14.7% of the studied genotypes had non-affected fruit without any disorder symptom. Usually, these pomegranate genotypes had smaller fruit weight and fruit dimensions, higher intensity of juice color, higher TA, and lower pH compared to the severely affected fruits. Among the non-affected genotypes, nine genotypes had good fruit quality and were suitable for use in the future breeding programs in order to improve the commercial cultivars susceptible to AB disorder.

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REFERENCES

1. Beaulieu, J. C., Lloyd, S. W., Preece, J. E., Moersfelder, J. W., Stein-Chisholm, R. E.

[DOR: 20.1001.1.16807073.2020.22.4.7.9]

and Obando Ulloa, J. M. 2015. Physicochemical Properties and Aroma Volatile Profiles in a Diverse Collection of California-Grown Pomegranate (*Punica granatum* L.) Germplasm. *Food Chem.,* **181:** 354-364.

2. Behzadi Sharbabaki, H. 2014. *The Pomegranate Heritage for The Desert (Cultivated, Management and Harvesting)*. Agricultural Research, Education and Extension Organization (AREEO), 443 PP. (in Persian)
3. Borochov-Neori, H., Lazarovitch, N., Judeinstein, S., Patil, B. S. and Holland, D. 2013. Climate and Salinity Effects on Color and Health Promoting Properties in the Pomegranate (*Punica granatum* L.) Fruit Arils. In: "*Tropical and Subtropical Fruits: Flavors, Color, and Health Benefits*". American Chemical Society, PP. 43–61.
4. Cabrita, L., Fossen, T. and Andersen, Ø. M. 2000. Color and Stability of the Six Common Anthocyanidin 3-Glucosides in Aqueous Solutions, *Food Chem.,* **68(1):** 101-107.
5. Fawole, O. A. and Opara, U. L. 2013. Changes in Physical Properties, Chemical and Elemental Composition and Antioxidant Capacity of Pomegranate (cv. Ruby) Fruit at Five Maturity Stages. *Sci. Hortic.,* **150:** 37–46.
6. Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M. and Kader, A. A. 2000. Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing. *J. Agric. Food Chem.,* **48(10):** 4581–4589.
7. Govindaraj, M., Vetriventhan, M. and Srinivasan, M. 2015. Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances: An Overview of Its Analytical Perspectives. *Genet. Res. Int.,* 1–14. https://doi.org/10.1155/2015/431487
8. Jafari, A., Arzan, K., Fallahi, E. and Barzegar, M. 2014. Optimaizing Fruit Yeild, Size, and Quality Atribute in Malase Torshe Saveh Pomegranate throught Hand Thining. *J. Am. Pomol. Soc.,* **68:** 89–96.
9. Jaiswal, V., Dermarderosian, A. and Porter, J. R. 2010. Anthocyanins and Polyphenol Oxidase from Dried Arils of Pomegranate (*Punica granatum* L.). *Food Chem.,* **118:** 11–16.
10. Jalikop, S. H., Venugopalan, R. and Kumar, R. 2010. Association of Fruit Traits and Aril Browning in Pomegranate (*Punica granatum* L.). *Euphytica,* **174:** 137–141.
11. Joas, J., Caro, Y., Ducamp, M. N. and Reynes, M. 2005. Postharvest Control of Pericarp Browning of Litchi Fruit (*Litchi chinensis* Sonn cv. Kwaii Mi) by Treatment with Chitosan and Organic Acids: I. Effect of pH and Pericarp Dehydration. *Postharvest Biol. Technol.,* **38:** 128–136.
12. Kavand, M., Arzani, K., Barzegar, M. and Mirlatifi, M. 2017a. Orchards Management for Reducing of the Pomegranate Aril Vrowning Disorder. Abstracts Book, *First International Horticultural Science Conference of Iran (IrHC2017),* September 4-7, Tarbiat Modares University (TMU), Tehran, Iran, 63 PP.
13. Kavand, M., Arzani, K., Barzegar, M. and Mirlatifi, M. 2017b. Identification of the Tolerant Pomegranate Genotypes for the Aril Browning or Aril Paleness Disorder. Abstracts Book, *First International Horticultural Science Conference of Iran (IrHC2017),* September 4-7, Tarbiat Modares University (TMU), Tehran, Iran, Page 264.
14. Kulkarni, A. P. and Aradhya, S. M. 2005. Chemical Changes and Antioxidant Activity in Pomegranate Arils during Fruit Development. *Food Chem.,* **93:** 319–324.
15. Leccese, A., Bureau, S., Reich, M., Renard, M. G. C. C., Audergon, J. -M., Mennone, C., Bartolini, S. and Viti, R. 2010. Pomological and Nutraceutical Properties in Apricot Fruit: Cultivation Systems and Cold Storage Fruit Management. *Plant Foods Hum. Nutr.,* **65:** 112–120.
16. Legua, P., Melgarejo, P., Abdelmajid, H., Martínez, J. J., Martínez, R., Ilham, H., Hafida, H. and Hernández, F. 2012. Total Phenols and Antioxidant Capacity in 10 Moroccan Pomegranate Varieties. *J. Food Sci.,* **77(1):** 115–120.
17. Li, X., Wasila, H., Liu, L., Yuan, T., Gao, Z., Zhao, B. and Ahmad, I., 2015. Physicochemical Characteristics, Polyphenol Compositions and Antioxidant Potential of Pomegranate Juices from 10 Chinese Cultivars and the Environmental Factors Analysis. *Food Chem.,* **175:** 575–584.
18. Meighani, H., Ghasemnezhad, M. and Bakshi, D., 2014. Evaluation of Biochemical Composition and Enzyme Activities in Browned Arils of Pomegranate Fruits. *Int. J. Hortic. Sci. Technol.,* **1(1):** 53–65.

[DOR: 20.1001.1.16807073.2020.22.4.7.9]

19. Mena, P., Galindo, A., Collado-González, J., Ondoño, S., García-Viguera, C., Ferreres, F., Torrecillas, A. and Gil-Izquierdo, A. 2013. Sustained Deficit Irrigation Affects the Color and Phytochemical Characteristics of Pomegranate Juice. *J. Sci. Food Agric.*, **93:** 1922–1927.
20. Mphahlele, R. R., Fawole, O. A. and Opara, U. L. 2016. Influence of Packaging System and Long Term Storage on Physiological Attributes, Biochemical Quality, Volatile Composition and Antioxidant Properties of Pomegranate Fruit. *Sci. Hortic. (Amsterdam)*, **211:** 140–151.
21. Mphahlele, R. R., Fawole, O. A., Stander, M. A. and Opara, U. L. 2014. Preharvest and Postharvest Factors Influencing Bioactive Compounds in Pomegranate (*Punica granatum* L.): A Review. *Sci. Hortic.*, **178:** 114–123.
22. Nikolić, D., Rakonjac, V., Milatović, D. and Fotirić, M. 2010. Multivariate Analysis of Vineyard Peach (*Prunus persica* L.) Batsch. Germplasm Collection. *Euphytica*, **171:** 227–234.
23. Sarkhosh, A., Zamani, Z., Fatahi, R. and Ebadi, A. 2007. Genetic Relationships among Pomegranate Genotypes Studied by Fruit Characteristics and RAPD Markers. *J. Hortic. Sci. Biotechnol.*, **82(1):** 11–18. doi:10.1080/14620316.2007.11512192
24. Schwartz, E., Tzulker, R., Glazer, I., Bar-Ya'akov, I., Wiesman, Z., Tripler, E., Bar-Ilan, I., Fromm, H., Borochov-Neori, H., Holland, D. and Amir, R., 2009. Environmental Conditions Affect the Color, Taste, and Antioxidant Capacity of 11 Pomegranate Accessions' Fruits. *J. Agric. Food Chem.*, **57:** 9197–9209.
25. Shivashankar, S., Singh, H. and Sumathi, M. 2012. Aril Browning in Pomegranate (*Punica granatum* L.) Is Caused by the Seed. *Curr. Sci.*, **103:** 26–28.
26. Tabar, S. M., Tehranifar, A., Davarynejad, G. H., Nemati, S. H. and Zabihi, H. R., 2009. Aril Paleness, New Physiological Disorder in Pomegranate Fruit (*Punica granatum*). *Hortic. Environ. Biotechnol.*, **50:** 300–307.
27. Tzulker, R., Glazer, I., Bar-Ilan, I., Holland, D., Aviram, M. and Amir, R. 2007. Antioxidant Activity, Polyphenol Content, and Related Compounds in Different Fruit Juices and Homogenates Prepared from 29 Different Pomegranate Accessions. *J. Agric. Food Chem.*, **55:** 9559–9570.
28. Varasteh F. and K. Arzani 2009. Classification of Some Iranian Pomegranate (*Punica granatum*) Cultivars by Pollen Morphology Using Scanning Electron Microscopy. *Hort. Environ. Biotechnol.*, **50(1):** 24-30.
29. Varasteh, F., Arzani, K., Barzegar, M. and Zamani, Z. 2012. Changes in Anthocyanins in Arils of Chitosan-Coated Pomegranate (*Punica granatum* L. cv. Rabbab-e-Neyriz) Fruit during Cold Storage. *Food Chem.*, **130:** 267–272. doi:10.1016/j.foodchem.2011.07.031
30. Wahid, A., Gelani, S., Ashraf, M. and Foolad, M., 2007. Heat Tolerance in Plants: An Overview. *Environ. Exp. Bot.*, **61(3):** 199–223.
31. Wetzstein, H. Y., Zhang, Z., Ravid, N. and Wetzstein, M. E. 2011. Characterization of Attributes Related to Fruit Size in Pomegranate. *HortScience*, **46(6):** 908–912.
32. Zhao, X., Yuan, Z., Fang, Y., Yin, Y. and Feng, L. 2013. Characterization and Evaluation of Major Anthocyanins in Pomegranate (*Punica granatum* L.) Peel of Different Cultivars and Their Development Phases. *Eur. Food Res. Technol.*, **236**: 109–117.

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ارزیابی صفات کمی و کیفی میوه ژنوتیپ‌های انار در ارتباط با عارضه قهوه‌ای شدن آریل دانه انار

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چکیده

عارضه فیزیولوژیکی قهوه‌ای شدن آریل دانه انار به شدت کیفیت و بازارپسندی میوه انار را کاهش داده است. به منظور مطالعه صفات موثر در شدت عارضه قهوه‌ای شدن آریل دانه انار و شناسایی ژنوتیپ‌های متحمل به عارضه، برخی از صفات فیزیکی و شیمیایی میوه انار مربوط به ۲۳۸ ژنوتیپ انار موجود در ایستگاه ملی تحقیقات انار ساوه مورد ارزیابی قرار گرفتند. نتایج نشان داد، در حدود ۱۴/۷٪ از ژنوتیپ های مورد مطالعه دارای میوه سالم و در حدود ۶۸/۱۴٪ ژنوتیپ ها دارای میوه با علائم متوسط تا شدید عارضه را نشان دادند. بین شدت عارضه قهوه ای شدن آریل و برخی از صفات فیزیکی و شیمیایی میوه انار همبستگی قوی و معنی داری وجود داشت. به طوری که با افزایش اندازه و حجم میوه در هر ژنوتیپ بر شدت بروز علائم عارضه قهوه‌ای شدن آریل افزوده شد. همچنین ژنوتیپ‌های با میوه‌های دارای اسیدیته (pH) بیشتر، شدت عارضه قهوه‌ای شدن آریل کمتری داشتند. تجزیه رگرسیون به روش گام به گام نشان داد که صفات اسیدیته میوه(pH)، رنگ قرمز آریل، رنگ صورتی آریل، حجم میوه و میزان اسیدیته قابل تیتراسیون (TA) از صفات موثر و کلیدی در غربال ژنوتیپ‌های انار در ارتباط با عارضه قهوه‌ای شدن آریل است.

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