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Investigation of Aril Characteristics of Some Autochthonous Pomegranate (*Punica granatum L*) Varieties in Macedonia

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Abstract

The individual aril characteristics of eight autochthonous pomegranate varieties (Lifanka, Bejnarija, Karamustafa, Ropkavac, Valandovska, LC, Zumnarija and Hicaz) from R. Macedonia were investigated using computer vision software methods. The great differences in the dimension of aril at the varieties were determined. The variety Lifanka is characterized with the greatest length (12.6 mm), width (9.5 mm) and mass (0.5 g) of the aril. Cross section aril area (61.1 mm^2) and perimeter (31.6 mm) indicate that the variety 'Hicaz' is characterized with the smallest aril. Investigation of the aril shape showed different level of asymmetry depending on the variety. Generally, the variety Hicaz is characterized with the most asymmetrical aril. Based on CIELab system of coloration, great differences in the aril colour were noted among the investigated pomegranate varieties. The variety Hicaz had the darkest coloration (L* 19.3; a* 13.1; b* 5.3). High correlation dependence between coloration and shape and mass was determined.

Key words: *Punica granatum L.*, variety, aril, CIELab coloration

Introduction

Neglected and underutilized species (NUS) represent a particular group of domesticated crops to which is paid little attention and such crops are mainly ignored by agricultural science, breeders or the appropriate institutions (IPGRI, 2002).

Those are wild or partially domesticated species, which are adapted to some, usually, local needs, such as food, remedy or material. Due to the genetic erosion, some of them even now can be considered extinct, because of their growing in isolated conditions near inhabited places. Lately, the international support to the importance and role of the sustainable agricultural systems in exploitation of the less utilized crops and species has increased. This includes more targets such as: increased agrobiodiversity, partial response to the climate changes, contribution to the maintenance of the ecosystem stability, more varied food offer with exceptional bioactive components important to human health and also opening new market perspectives and opportunities (Padulosi et al, 2006).

The fruit of pomegranate is many-celled and many-seeded. It is grown for limited local markets in Turkey, Spain, Tunis, Egypt, Russia and in other countries (Stower & Mercure, 2007). In R. Macedonia, it is mainly presented as a spontaneous shrub or it was introduced in the past through Povardarie, and also in the southern parts (Gevgelija, Valandovo, Dojran). The pomegranate is characterized by tolerant to drought fruit shrub. On the contrary, in too humid areas, the pomegranate suffers from root rotting given the suitable condition for the development of many fungi caused diseases (Gadže et al, 2011). It cannot tolerate freezing winter temperatures below -12 ^{0}C (Ozguven & Yilmaz, 2000). The pomegranate is characterized by remarkable diversity. There are at least 500 pomegranate varieties registered in the world, from which only 50 are cultivated (Holland et al, 2009). A large population of seedless varieties can be found in Kandahar (Afghanistan). In Dashnabad (Uzbekistan) the genotypes have been selected which tolerate very low winter temperatures (Levin, 2006).

The pomegranate varieties are characterized by significant differences in dimension and other fruit characteristics which make it possible to categorize them in several groups (Gadže et al, 2011). Also, the differences are noted in the number and in the size of arils in the pomegranate berries (Durgaç et al, 2008). Those differences are conditioned by cultivation methods, but also by genetic characteristics of the varieties. There is surprisingly little literature data for the investigation of the pomegranate arils, considering that the pomegranate fruit is technically a leathery-skinned berry containing many seeds, each surrounded by a juicy, fleshy aril.

The pomegranate population in Macedonia belongs to the primary geographical gene pool (Still, 2006). In the paper, the population in Valandovo area has been investigated, which, according to preliminary information, has the highest diversity of local or domesticated types and varieties.

Materials and Methods

The investigation of some pomological characteristics was performed during 2015-2016. The trials included eight autochthonous pomegranate (*Punica granatum* L.) varieties from the Valandovo area, located in the south of R. Macedonia. The fruits of varieties were harvested and kept at a temperature of 4 ^{0}C. Three repetitions by 15 randomly taken fruits from the tree crown were investigated. The characteristics of 50 arils per fruit in three repetitions were investigated. The parameters used in the analysis include aril length (AL), aril width (AW), mass of aril (AM), cross section area of aril (AA), cross section perimeter of aril (AP), aril shape asymmetry characteristics in different categories, such as: Obovoid (Ob), Ovoid (Ov), Vertical asymmetry (V), Horizontal asymmetry - Obovoid (HOb), Horizontal asymmetry - Ovoid (HOv), and aril colour characteristics by the CIELab colour system.

The examination of the aril characteristics (except their mass) is made using a scanning device for obtaining high resolution images which are processed with the digital image processing method that performs precise analysis of the object dimensions (Markovski & Velkoska-Markovska, 2015) through computer softwares "ImageJ" (IJ) and "Tomato Analyzer" (TA). Flatbed scanner device colour calibration was used with X-rite ColorChecker card for measuring aril colour on the basis of CIELab colour system. In the CIELab colour space L*, a* and b* describe a three-dimensional space, where the values for +L* are lightness direction, values for –L* show darkness direction and consequently, +a* is red direction, -a* is green direction, +b* is yellow direction, -b is blue direction (Ornelas-Paz et al, 2008). Aril shape asymmetry characteristics are analyzed using TA software equations (Rodríguez et al, 2006) for some parameters (Figure 1), such as:

Obovoid – If the area of the fruit is greater below mid-height than above it, then Obovoid is calculated from the maximum width (W), the height at which the maximum width occurs (y), the average width above that height (w1), and the average width below that height (w2), and a scaling function scale *ob* as: Obovoid = 1/2 * scale ob(y) * (1 − w1/W + w2/W), If Obovoid > 0, subtract 0.4. Otherwise, Obovoid is 0.

Ovoid – If the area of the fruit is greater above mid-height than below it, then Ovoid is calculated from the maximum width (W), the height at which the maximum width occurs (y), the average width above that height (w1), and the average width below that height (w2), and a scaling function scale *ov* as: Ovoid = 1/2 * scale ov(y) * (1 − w2/W + w1/W). If Ovoid > 0, subtract 0.4. Otherwise, Ovoid is 0.

Vertical Asymmetry – The average distance between a vertical line (Res_w) through the fruit at mid-width and the midpoint of the fruit's width at each height.

Horizontal Asymmetry Obovoid – If the area of the fruit is greater below mid-height than above it, H. Asymmetry Obovoid is the average distance (Res_h) between a horizontal line through the fruit at mid-height and the midpoint of the fruit's height at each width. Otherwise, it is 0.

Horizontal Asymmetry Ovoid – If the area of the fruit is greater above mid-height than below it, H. Asymmetry. Ov is the average distance (Res_h) between a horizontal line through the fruit at mid-height and the midpoint of the fruit's height at each width. Otherwise, it is 0.
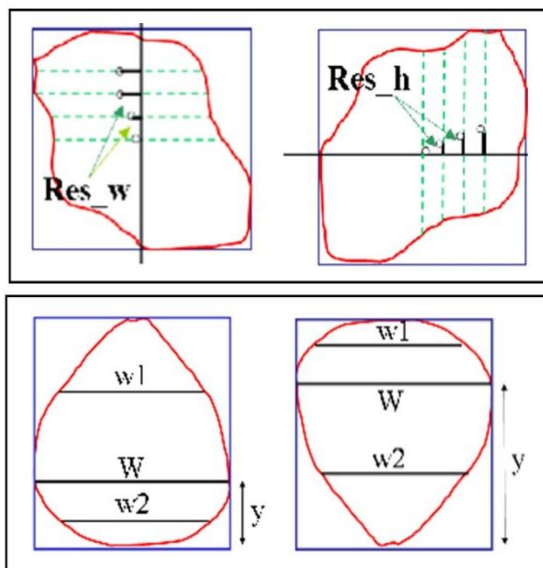

Fig. 1. Tomato analyzer software guide's explanation
for aril shape asymmetry parameters
*Упутство Tomato analyzer софтверског водича за анализу
параметера асиметрије облика арила нара*

Data were statistically analysed by ANOVA and Fisher's multiple comparison testing at a level of 0.05. Clustering of the genotypes into similarity groups with the average linkage method (Unweighted pair-group average with arithmetic mean method) and Pearson correlation matrix were performed using the Minitab and Xlstat statistical software.

Results and Discussion

The results of investigation showed statistically significant differences in the length and width of the arils among the pomegranate varieties' berries. The Lifanka variety is characterized with the largest arils (Figure 2). Also, evident differences in fruit mass were noted on the individual arils between the autochthonous pomegranate varieties from Valandovo region.

The fruits from Lifanka variety are characterized with statistically different greatest mass of the aril. It is interesting to note, according to the local people claims that the Lifanka variety is brought from abroad in the past from the time of the Ottoman conquest of the Balkans. The variety with similar name "Lefan or Lifani" and with similar aril characteristics is mentioned in the inventory of the autochthonous Turkish pomegranate varieties in literature data (Ozguven & Yilmaz, 2000).
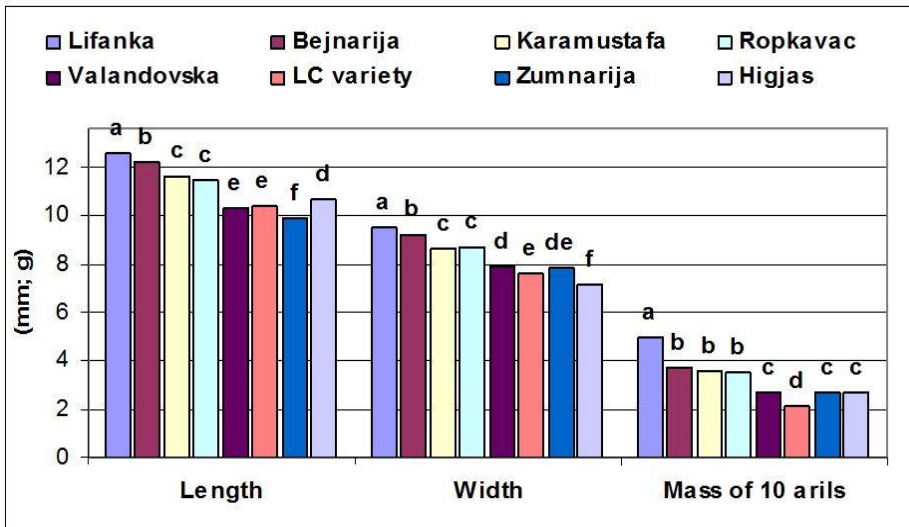
*The means followed by the same letter in different columns are not significantly different at P ≤ 0.05

Fig. 2. Aril characteristics of pomegranate varieties
Особине арила различитих сорти нара

The Lifanka variety dominated with the mass of the arils compared to the other varieties. The varieties Bejnarija, Karamustafa and Ropkavac are almost equal according to aril mass, while the LC variety is characterized with statistically smallest aril mass.

Using the advantages of ImageJ software tools the cross section area and the perimeter of the arils are determined, where statistically the greatest values for these parameters have characterized the arils of the Lifanka variety. The variety Hicaz is characterized with statistically smallest cross section area of the arils, while according to perimeter this variety is equated with the varieties Zumnarija and LC (Table 1).

Very small statistically insignificant differences according to the obovoid shape of arils from the pomegranate varieties or more exactly the small differences in bellow mid-height of shape are noted. Also, the very small or statistically insignificant differences are determined at the category of ovoid shape of arils between the varieties. Only in variety Hicaz a higher and statistically significant deviation is noted and its arils are characterized with the greatest ovoid asymmetry compared with other investigated varieties (Table 1).

Tab. 1. Aril characteristics of pomegranate varieties
Особине арила различитих сорти нара

Variety	Dimension		Shape asymmetry					Colour CieLab colouring		
	Area (mm^2)	Perimeter (mm)	Obovoid	Ovoid	Vertical	Hor. ob.	Hor. ov.	L*	a*	b*
Lifanka	95.3[A]	40.4[A]	0.083[A]	0.088[ABC]	0.077[BC]	0.064[A]	0.030[BC]	34.4[AB]	18.1[C]	13.1[E]
Bejnarija	88.5[B]	38.1[B]	0.088[A]	0.090[ABC]	0.066[BCD]	0.038[AB]	0.042[BC]	25.9[DE]	30.9[A]	17.4[A]
Karamustafa	79.4[C]	36.0[C]	0.083[A]	0.087[ABC]	0.072[BC]	0.045[AB]	0.043[BC]	24.5[E]	30.3[A]	16.3[B]
Ropkavac	79.3[C]	35.9[CD]	0.095[A]	0.089[ABC]	0.081[B]	0.047[AB]	0.042[BC]	26.6[CD]	24.2[B]	12.6[E]
Valandovska	65.1[D]	34.6[D]	0.099[A]	0.078[BC]	0.053[D]	0.038[AB]	0.030[C]	35.1[A]	19.9[C]	15.3[C]
LC variety	62.8[DE]	31.9[E]	0.069[A]	0.113[AB]	0.073[BC]	0.039[AB]	0.055[AB]	27.7[C]	31.0[A]	17.6[A]
Zumnarija	61.3[E]	31.9[E]	0.081[A]	0.071[C]	0.061[CD]	0.030[B]	0.040[BC]	33.3[B]	23.2[B]	14.6[D]
Hicaz	61.1[E]	31.9[E]	0.074[A]	0.123[A]	0.111[A]	0.050[AB]	0.077[A]	19.3[F]	13.1[D]	5.3[F]
Average	74.1	35.1	0.084	0.093	0.075	0.043	0.046	28.4	23.9	14.1

*The means followed by the same letter in each column are not significantly different at P ≤ 0.05

The vertical asymmetry of aril shows the greatest values for the Hicaz variety, which statistically significantly differs from the other varieties, with the exception of the variety Ropkavac (Table 1). The variety Valandovska has the statistically significant vertically most uniform arils, compared with the other varieties with the exception of Zumnarija and Bejnarija. The arils of Lifanka variety are characterized with the statistically greatest obovoid horizontal asymmetry. Among the other varieties, there are no statistically significant differences in horizontal obovoid asymmetry (Table1). The Hicaz variety has statistically significantly larger ovoid horizontal asymmetry of arils compared with the other varieties with the exception of *LC* variety. Also, the statistically important difference is not recorded between the other varieties in the ovoid horizontal asymmetry of arils (Table 1).

Statistically, Valandovska is characterized with the lightest arils (L* 35.1) compared with the other varieties, where the aril basis has significantly greater pale coloration, and generally gives the visually red-white impression of the aril coloration. The Hicaz variety is characterized with statistically darkest arils (L* 19.3). The varieties LC, Bejnarija and Karamustafa are characterized with statistically greatest values for a* (31.0, 30.9, 30.3) coloration. These three varieties have exceptionally uniform red coloration and from our point of view are characterized with the most attractive arils among the investigated varieties. The variety Hicaz has again the statistically lowest values for a* (13.1), which means also the weakest red coloration, and besides, the arils visually have a dark red colour. Even lower are the values of the aril for b* coloration or for yellow coloration (5.3). LC variety is characterized with statistically greatest value for b* coloration, which gives a characteristically red-orange coloration of the arils (Table 1).

A great number of aril measurements were used to make dendrogram generated from the average linkage cluster analysis and to classify all autochthonous varieties into 2 main groups (Fig. 3).
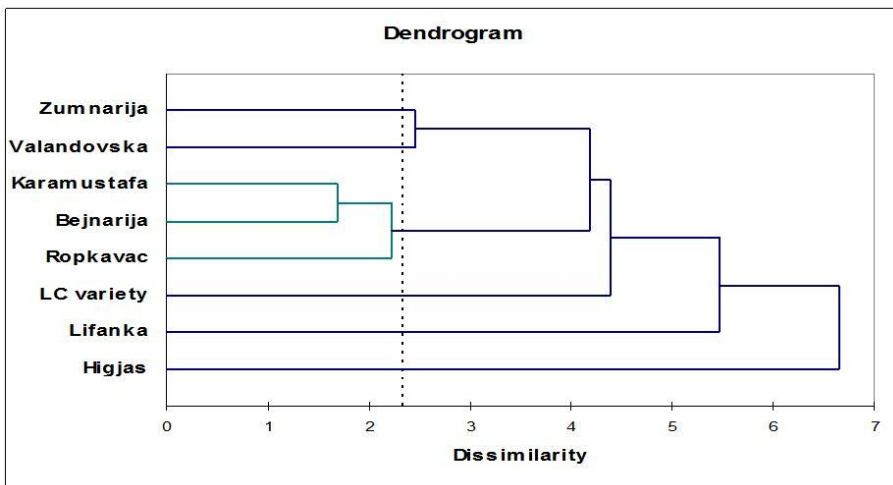

Fig. 3. Aggregation of eight pomegranate genotypes by investigated traits into different cluster groups (different line colour)
Распоред осам генотипова нара у различите кластере према анализираним особинама (различита боја линија)

The first group includes the varieties (Karamustafa, Bejnarija and Ropkavac) or 37.5 % of genotypes from investigated population. It has average parameters of AL, AW, AM, AA, AP, Ov, V, Hov, Hob, L*, b* and high values of Ob and a*.

The second group includes two varieties (Zumnarija and Valandovska) or about 25 % of the varieties. This group has below average to average values of aril dimensions and mass and high value of light (L*) coloured arils. Three varieties remain (Lifanka, LC variety and Hicaz) which are not included in any of previous groups by the investigated characteristics. Lifanka stands out from all investigated varieties with the highest values of aril dimension and it has the highest obovoid asymmetry of the arils. LC variety has a high value of coloration and ovoid asymmetry of arils (a* and b*), and Hicaz has greatest ovoid, vertical and horizontal ovoid asymmetry and darkest coloration (L*, a*, b*) of the arils.

According to the estimated Correlation matrix, the great correlation dependence among the aril dimensions is determined, especially between the width of arils and cross section arils area and perimeter (Table 2).

Tab. 2. Correlation matrix (Pearson (n))
Корелациони матрикс (по Пирсону (n))

Variables	AA	AP	AL	AW	AM	L*	a*	b*	Ob	Ov	V	HOb
HOv	-0.503	-0.607	-0.288	-0.650	-0.491	-0.839	-0.225	-0.620	-0.666	0.870	0.821	0.008
HOb	0.592	0.600	0.686	0.453	0.723	-0.086	-0.456	-0.444	-0.063	0.304	0.531	
V	-0.086	-0.176	0.133	-0.269	0.020	-0.757	-0.494	-0.840	-0.484	0.803		
Ov	-0.248	-0.331	-0.023	-0.428	-0.297	-0.730	-0.173	-0.490	-0.665			
Ob	0.311	0.439	0.190	0.414	0.295	0.403	-0.056	0.171				
b*	0.236	0.230	0.081	0.370	0.000	0.443	0.854					
a*	0.191	0.084	0.128	0.272	-0.104	-0.058						
L*	0.125	0.260	-0.094	0.269	0.201							
AM	0.935	0.937	0.903	0.902								
AW	0.977	0.962	0.898									
AL	0.969	0.925										
AP	0.975											

*Values in bold are different from 0 with a significant alpha level = 0.05

The high positive correlation between a* and b* coloration of the arils is noted. On the other hand, a correlation between L* and a* coloration of the arils from varieties is not found. According to matrix, the pomegranate varieties with greater Horizontal obovoid asymmetry of the arils (Hob) are characterized by greater mass of the arils (AM). L* coloration is in negative correlation with aril asymmetry (Ov, V, Hov) or when the value of L* is bigger (lighter arils) the arils are more symmetrical. Vertically more symmetrical arils (V) are characterized by lower values of b* coloration (Table 2).

Conclusion

The investigation covers one part of the pomegranate varieties presented in the southern area of Macedonia and these show great differences in some pomological characteristics among the pomegranate varieties. Obtained data allow us to select the varieties which are characterized with superior characteristics in relation to dimensions and mass of aril, such as the variety Lifanka. On the other hand compared to the attractiveness of the aril, very interesting are the varieties LC, Bejnarija and Karamustafa. The Hicaz variety with the dark red coloration of the arils can have importance in food processing production such as juice, cider production etc. All of these parameters indicate the presence of some polymorphism level in *Punica granatum L* population in Macedonia, which is the base for a more comprehensive selection process for appropriate cultivation of this fruit culture.

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Испитивање карактеристика арила код неких аутохтоних сорти нара (*Punica granatum* L) у Македонији

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Сажетак

Испитиване су карактеристике појединачних арила код осам аутохтоних сорти Нара (*Лифанка, Бејнарија, Карамустафа, Ропкавац, Валандовска, ЛЦ, Зумнарија* и *Хиџас*) из Р. Македоније, помоћу коришћења компјутерско визуелних софтверских метода. Утврђене су велике разлике у димензијама арила код сорти Нара. Сорта *Лифанка* се карактеризује највећом дужином (12,6 mm), ширином (9,5 mm) и тежином (0,5 g) арила. Површина (61,1 mm^2) и периметар попречног пресека арила (31,6 mm) указује да најмањи арил поседује сорта *Хиџас*. Испитивање облика арила показује различите нивое асиметрије зависно од сорте. Углавно, најасиметричнији арил поседује сорта *Хиџас*. Према CIELab систему колорације, велике разлике у боји арила су забележене међу испитиваним сортама Нара. Најтамније обојен арил је код сорте *Хиџас* (L* 19,3; a* 13,1; b* 5,3). Велика корелациона зависност је забележена између обојености арила са једне стране и облика и тежине арила са друге стране.

Кључне ријечи: *Punica granatum L.*, сорта, арил, CIELab обојеност

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