



Evaluations on Large Berry Varieties in the Turkish Grapevine Field Gene Bank and Their Usability in Breeding

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How to cite: Ergönül, O., Zeybek, B., Uysal, T., Polat, A., Tokyol, A. (2025). Evaluations on Large Berry Varieties in the Turkish Grapevine Field Gene Bank and Their Usability in Breeding. Viticulture Studies (VIS), 5(2): 53 – 65. https://doi.org/10.52001/vis.2025.29.53.65

Article History:

Received: 08.07.2025 Accepted: 28.07.2025 First online: 31.07.2025

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Keywords

Table Grape Berry Size Grape Seed Breeding

Abstract

One of the most important characteristics in grapevine breeding studies is berry size. In table grape evaluation, consumers prefer grape varieties with large berries. For growers, grapes with large berries can be sold commercially at higher prices. Although grapevine breeding is a very active field of study, no molecular markers can be integrated into grapevine breeding. Therefore, many breeders want to understand the segregations of the traits they will work on in the varieties they will use as parents. This study evaluated grape varieties with large berries in the Turkiye Grapevine Field Gene Bank, which contains 1459 grape varieties in Tekirdağ Viticulture Research Institute. As stated in many publications, the evaluations used the positive correlation between berry weight and seed. They were designed to identify varieties that can reach the highest berry weight with the lowest seed presence. As a result of the evaluations, varieties with low seed numbers and seed weight values that can produce relatively large berries were identified in the Turkiye Grapevine Field Gene Bank. Local varieties such as Antep Şamı, İt Karası, Gadöv, Beyaz Çavuş and Akhisar Razakısı have emerged as promising candidates and it is recommended that these varieties be used as parents in breeding studies to develop new large-grape varieties.

Introduction

One of the most desired characteristics, particularly in table grape breeding programs, is berry size. Whether seeded or seedless, a new candidate variety is desired to have large berries. According to research conducted, the primary fruit feature determining the quality of table grapes is taste/flavour (26.7%), followed by skin thickness (15.8%), seed status (10%), berry size (8.9%) and expectations regarding the textural qualities of the berry (5.1%). Based on these features, consumers reported that they prefer grapes that are sweet and not overly acidic, thinskinned, have a few seeds or are seedless, and are large, crisp and juicy. (Piva et. al., 2006, Sivritepe and Parlak, 2015). Additionally Özer et al. (2014) stated that the desirable characteristics

of table grape varieties are to have large berries and loose bunches. Also, they mentioned that an attractive appearance and unique taste are important characteristics. It was also emphasized that high yield, solid structure, and early or late ripening are other factors that increase the chance of competition, and the differentiation criterion in table grapes is seedlessness. Although there is a demand for table grapes with different characteristics in world markets, it is stated that there is a much higher demand for varieties that are seedless, have large berries, have a hard fruit flesh, have a unique aroma and are tolerant/resistant to diseases. The priorities of grapevine breeding studies carried out in the world to achieve the above-mentioned goals are focused on yield and quality, seedlessness, resistance and earliness traits (Sabır et. al., 2006). Among these, berry size is an important parameter among yield and quality priorities. In recent years, in Europe and America, studies have been carried out on developing grape varieties with crisp flesh that ripen in different seasons, have large berries, have a unique aroma, are rich in health, are resistant to diseases, have low cultivation costs. have different flavours and fruit characteristics in the sector, are grower-friendly, are suitable for post-harvest storage, have low production costs, require less pesticides, have a short production cycle, and have crisp fruit flesh. In Far Eastern countries, quality rather than yield is prioritised, and in the breeding studies, efforts are made to develop very large berry, seedless varieties with a unique aroma (Atak, 2024).

There are three types of berry formation in grapevines: stenospermocarpic seedless, parthenocarpic seedless and seeded berries (Ağaoğlu, 2002). Seeded grape varieties form large berries, while seedless grape varieties form small berries (Fanizza et al., 2005). The control of berry growth and development depends on many factors, and there is a positive correlation between seed presence and berry size. (Barış and Gürnil, 1991, Boselli et. al., 1995, Ağaoğlu, 2002, Walker et. al., 2005, Mejia et. al., 2007, Costantini et. al., 2008, Ojeda et. al., 2015, Leao et. al., 2023).

Tekirdağ Viticulture Research Institute, founded in 1930, started its activities to reestablish the regional vineyards that were destroyed due to phylloxera pest, producing phylloxera-resistant grapevine rootstocks, and developing regional viticulture. The Institute has ensured the protection of Turkiye's rich grapevine genetic resources with the "Determination, Conservation and Identification of Turkiye's Grapevine Genetic Resources" project, which started in 1965. Tekirdağ Viticulture Research Institute, the primary institution responsible for preserving grapevine genetic resources in Turkiye, has started inventory studies and is carrying out collection activities in this context (Uysal et al., 2024). The main objectives of the project are to identify local grape genotypes grown within the borders of Turkiye, to create their inventories, to preserve these genotypes as live plants in the Grapevine Field Gene Bank located on the institute's field, and to carry out characterisation studies (Uysal et al., 2023). Today, 1459 genotypes are preserved in the Grapevine Field Gene Bank

(Uysal et. al., 2024).

Although the integration of markerassisted selection studies into classical breeding studies is frequently used today, there are some limitations in the using this technique in grape breeding. Although there are effective markers that can detect traits such as disease resistance and seedlessness at an early stage, useful markers that can detect earliness, yield and berry size have not been developed. This situation has led grapevine breeders to take other paths and encourage them to make observations and inferences regarding the berry size segregation in their combinations. There is a need to continue increasing breeding studies for berry size, develop useful markers for this trait, and present findings that will enable appropriate parent selection.

Here, detailed evaluations of berry weight and seed data were made in 65 grape varieties with large berries in the Turkiye Grapevine Field Gene Bank within the Tekirdağ Viticulture Research Institute. In addition, other berry and cluster data are presented for these varieties. The findings will contribute to parent selection in grapevine breeding studies to develop largeberry and seedless varieties.

Material and Methods

In the study, 65 grape varieties with large berries from the Turkiye Grapevine Field Gene Bank and 2 grape varieties with large berries of foreign origin (Red Globe and Velika) were used as a control. Measurements were made in the laboratory of Tekirdağ Viticulture Research Institute.

All observations and measurements were carried out in 2023 and when the varieties reached harvest maturity. In grape varieties, berry color observations were made according to OIV 225, berry shape observations were made according to OIV 223, and cluster density observations were made according to OIV 204 descriptive scales. Berry width and berry length measurements were made using a ruler, and average berry weight measurements were made using a precision scale on 10 berries. In the seed measurements, seeds obtained from 10 berries were evaluated, and the measurements were carried out based on the fresh weight measurements of the seeds that were kept at room conditions for one day. Single seed fresh weight (SFW); was obtained by dividing the total seed weight (TSW) by the number of seeds (NS), berry weight per seed (BWPS); was obtained by dividing the average berry weight (BW) by TSW, and berry weight per 1 g seed (BWPGS); was obtained by dividing BW by NS.

Statistical analyses were performed using the R software version 4.5.0 (The R Foundation for Statistical Computing, Vienna, Austria). Pearson correlation value (R) were estimated between each pair of variables.

Results and Discussion

The grouping of the studied varieties according to berry shape is given in Figure 1. According to berry shape, the majority of the varieties were those with broad ellipsoid (45%), followed by those with narrow ellipsoid at 21%. Memecik and Memki Eyşo varieties were found to have finger-shaped berries. Among the studied varieties, no varieties with horn-shaped, obovoid and obloid berry shapes were detected. By measuring the berry width and berry length, which determine the berry shape, the berry width/berry length ratios of the varieties were calculated. Accordingly, in parallel with the berry shape observations, varieties such as Şam Üzümü, Nuribey, Devegözü, which are the varieties with values close to each other (close to 1), were found to be globose. Similarly, in previous studies, Devegözü variety was reported to have a slightly obloid berry shape (Çoban and Küey, 2006). The lowest berry width/length ratio was found in Memecik (0.40), Salamur (0.55) and Meyane (0.59) varieties, respectively. Of these varieties, Memecik is characterized as fingershaped, while Salamur and Meyane varieties are characterized as narrow ellipsoid. Similar findings regarding the berry shapes of the

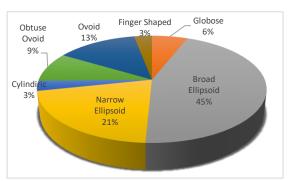


Figure 1. Distribution of Varieties According to Berry Shape.

varieties given here were also found in the Turkiye Grapevine Genetic Resources publication (Boz et. al., 2012).

In the berry color evaluations made according to the OIV 225 descriptive scale, 40 varieties were determined as green-yellow, 11 varieties as rose-coloured, 8 varieties as blueblack, 6 varieties as dark-red violet and 2 varieties as grey. No variety with red berry colour was detected (Figure 2). In addition, in the bunch density evaluations of the varieties, genotypes with medium dense bunches, 5 with loose bunches, 16 with dense bunches and 3 with very dense bunches were identified (Table 1). The graph of varieties with a berry weight above 7 g is given in Figure 3. The varieties with the highest berry weight were determined as Velika 10.9 g, Emirali 10.09 g, Red Globe 9.97 g, Akhisar Razakısı 9.63 g, Antep Şamı 9.33 g. Similarly, Ergönül et. al., (2018) stated in their study that the berry weight of Emirali varied between 7.3 g and 9.6 g in different years. Gargin and Altındişli (2020) reported that the berry weight of the Red Globe varied between 9.4-10.2 g.

The number of seeds per berry according to varieties is given in Figure 4. The varieties with the least number of seeds were 973-08 (1.4), Meyane (1.6), Parmak (1.7) and Gadöv (1.7). Although the berry weight of the Gadöv variety is high (8.5 g), it is noteworthy that the number of seeds is low. The varieties with the highest number of seeds in their berries were 948-27 (4.3), Sofra Üzümü (4), 900-42 (3.8), Devegözü (3.8) and Red Globe (3.7), respectively. Despite the high seed number of the 948-27 variety, the berry weight remains relatively low (6.8 g). The seed numbers of Kızılbanki, Hönüsü (synonym Mahrabaşı) and Azezi varieties were found to be consistent with Polat et al. (2023). In their study,

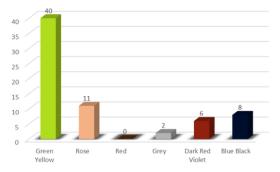


Figure 2. Distribution of Varieties According to Berry Color.

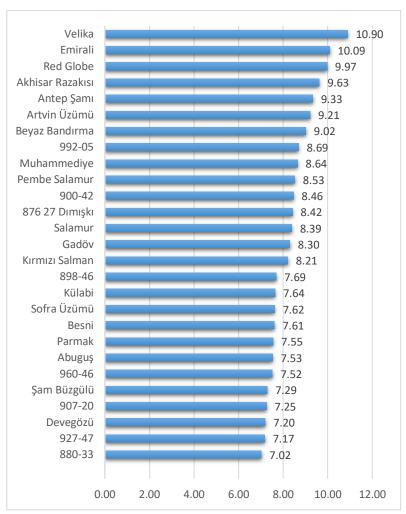


Figure 3. Berry Weights of Varieties (g).

Köse et al. (2024) stated that the number of seeds of the Red Globe variety varied between 2.5 and 3. In the same study, the number of seeds in the Çavuş variety varied between 2 and 2.5. Similarly, in our study, the number of seeds in the Beyaz Çavuş variety was determined to be 2.5.

Apart from molecular methods, one of the methods used to determine seedlessness is seed weight measurements. There are no seedless genotypes in the Türkiye Grapevine Field Gene Bank, except for Sultanina and Round Seedless. Therefore, all varieties used here are seeded varieties and their single seed fresh weight (SFW) measurements were made. According to SFW measurements, Meyane variety (100.1 mg) gave the highest value (Figure 5). Şam x Çavuş (24.4 mg), İt karası (27.9 mg) and Kırmızı Şam (30.8 mg) are the varieties with the lowest SFW values. Similar to our findings, the SFW data of the Beyaz

Çavuş variety (34 mg) and the SFW data of the Çavuş variety found in Köse et al. (2024) (27-37 mg) are consistent with each other.

According to berry weight per seed (BWPS), it can be seen that varieties with low berry weight, such as Nuri Bey and Memecik, also have low BWPS measurements (0.14 g and 0.14 g, respectively). On the other hand, it is noteworthy that varieties with high BWPS measurements, such as Akhisar Razakısı (0.51 g), Velika (0.50 g) and Gadöv (0.49 g) also have high berry weights (Figure 6).

In the measurements of berry weight per 1 g of seed (BWPGS), Memecik (2.71 g), 897-17 variety (2.92 g), Kızılbanki (2.98 g) varieties gave the lowest values. The highest BWPGS were given by Antep Şamı (9.98 g), İt Karası (9.25 g), Gadöv (9.22 g) and Beyaz Çavuş (8.20 g) varieties. The BWPGS value of the Red Globe variety, one

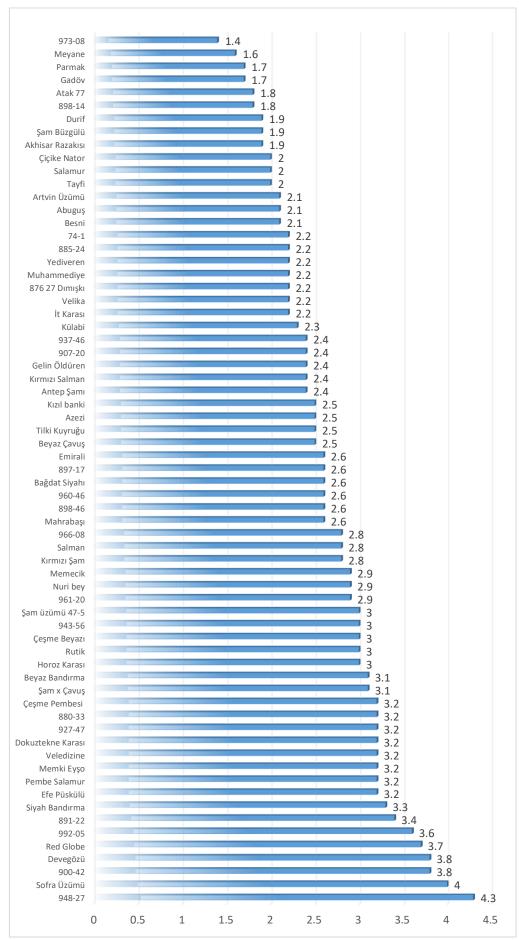


Figure 4. Number of Seeds of Varieties.

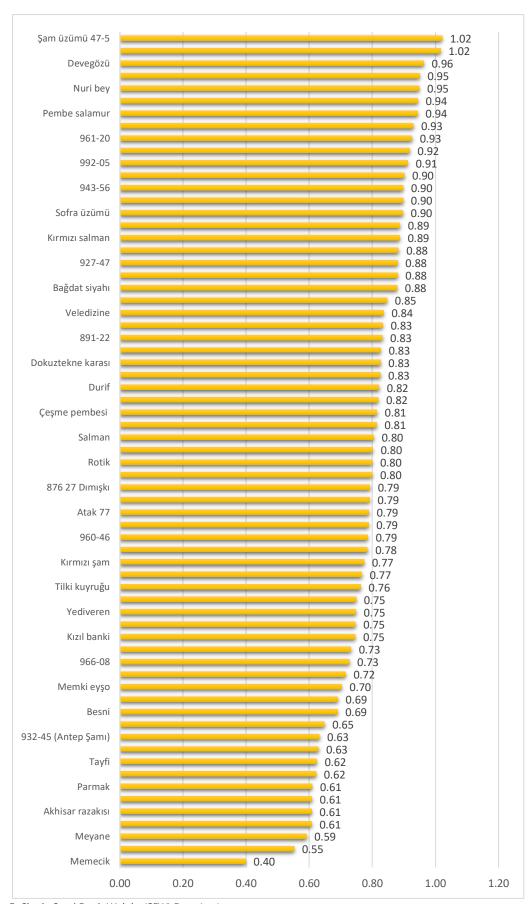


Figure 5. Single Seed Fresh Weight (SFW) Data (mg).

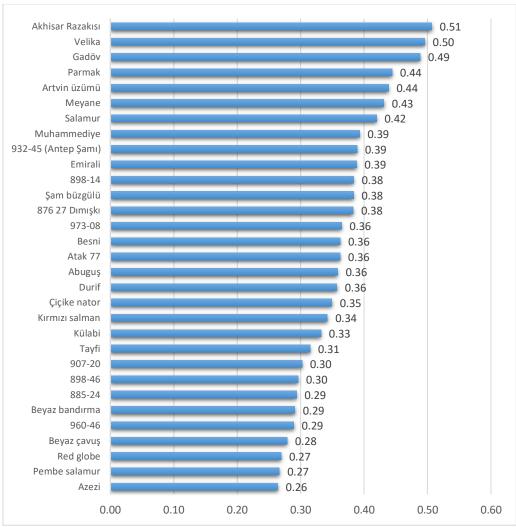


Figure 6. Berry Weight Per Seed (BWPS) of Varieties (g).

of the standard varieties, quite low, at 4.03 g. This is because the Red Globe produces many seeds in its berries (3.7 seeds per berry), and it forms large seeds (the average fresh weight of a single seed is 66.8 mg). Velika gave relatively high values in both BWPS and BWPGS measurements. It is thought that the Velika's very high berry weight (10.9 g) and low number of seeds (2.2 seeds per berry) are responsible for this situation. It was remarkable that the Gadöv was at the forefront in both BWPS and BWPGS measurements (Figure 7). In the literature review, not many studies were found, especially regarding BWPS and BWPGS. In a study conducted considering single seed dry weight (Barış, 1975), it was determined that Hönüsü (Synonymous: Mahrabaşı), Gelibolu Çavuşu and Gemre varieties produced a high amount of berry weight per unit seed. Similar to the findings of the Gelibolu Çavuş variety in this study, the findings of the Beyaz Çavuş variety in our study gave high values in this respect. Barış (1975) also stated that varieties with long berries contain fewer seeds than round ones, and that the possibility of obtaining seedless individuals may be higher by crossbreeding varieties with fewer seeds and seedless varieties.

The presence and development of seeds in grape berries are of great importance for berry growth and development. Although it is stated that the seed weight constitutes up to 10% of the berry weight (Winkler et al., 1974), in our study, it was determined that the seed weight constitutes 1-4.28% of the berry weight. In terms of the ratio of seed weight to berry weight, Antep Şamı (1%), İt Karası (1.08%), Gadöv (1.08%) and

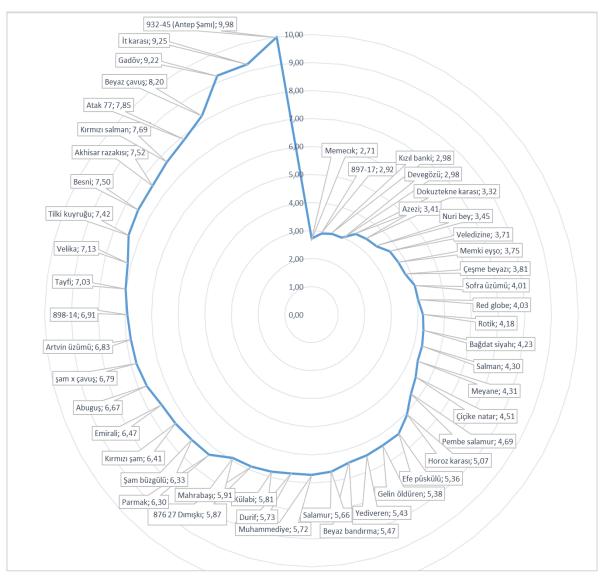


Figure 7. Berry Weight Per Gram Seed (BWPGS) of Varieties (g).

Beyaz Çavuş (1.22%) gave the lowest values, while Memecik (3.69%), Çeşme Pembesi (3.77%) and Siyah Bandırma (4.28%) gave the highest values. It is thought that the low seed weight values in our study are due to the use of mostly table grape varieties. Gombau et al. (2020) found the ratio of seed weight to berry weight as 6.53% in Merlot and 5.82% in Cabernet Sauvignon. In general, the ratio of seed weight to berry weight data was parallel to the BWPGS data.

According to the statistical analysis results, although a moderate positive relationship (r: 0.39) was found between BW and TSW, no significant relationship was found between BW and NS (Table 2). All varieties studied here consist of seeded and large berries.

It is thought that the fact that no selection criterion other than this phenotypic analysis was used resulted in the absence of a significant correlation between NS and TSW and berry size. Similar results were also given by Houel et al. (2013). As is known, seed content can affect berry size through hormonal mechanisms. Apart from this effect, however, cell volume and cell number of ovaries, carpel number in the berry are the factors affecting the berry size.

In this study, varieties with low values in terms of seed number and seed weight, but which can form quite large berries, were identified in the Turkish Grapevine Field Gene Bank.

Table 1. Data of varieties

Genotype	Province	Berry Shape	Berry Color	Bunch Density	Berry Width (cm)	Berry Length (cm)	Berry Width / Berry Length	Average Berry Weight (BW) (g)	Number of Seeds (NS)	Berry Weight Per Seed (BWPS)	Berry Weight Per 1 g Seed (BWPGS)	Single Seed Fresh Weight (SFW) (mg)
932-45 (Antep Samı)	Manisa	2	9	2	2.2	3.4	0.63	9.3	2.4	0.39	9.98	38.96
it karası		4	2	7	1,9	2,9	0,65	5,7	2,2	0,26	9,25	27,91
Gadöv	Siirt	3	-	5	2,5	2,4	1,02	8,3	1.7	0,49	9,22	52,94
Beyaz çavuş		3	-	7	2,2	2,4	06'0	7,0	2,5	0,28	8,20	34,00
Kırmızı salman	Aydın	3	-	6	2,4	2,7	68'0	8,2	2,4	0,34	7,69	44,50
Akhisar razakısı	Manisa	4	-	5	2,1	3,5	0,61	9'6	1,9	0,51	7,52	67,37
Besni	Adiyaman	4	-	2	2,0	2,9	69'0	7,6	2,1	0,36	7,50	48,29
Tilki kuyruğu		9	-	2	2,0	2,6	0,76	5,8	2,5	0,23	7,42	31,40
Velika	Bulgaria	2	2	2	2,2	3,7	0,61	10,9	2.2	0,50	7,13	69,55
Tavfi	Mardin	4	-	2	1,9	3,0	0,62	6,3	2.0	0,31	7,03	44,75
898-14	Bolu	7	-	2	2,1	2,7	0,77	6'9	8,	0,38	6,91	55,56
Sam x cavus		23	-	2	1,9	2,4	0,79	5,1	3,1	0,17	6,79	24,35
Abuguş		m	-	2	2,3	2,6	0,88	7,5	2,1	0,36	6,67	53,71
Kırmızı şam	Manisa	4	2	m	1,9	2,5	0,77	5,5	2,8	0,20	6,41	30,82
Şam büzgülü		9	9	2	2,0	2,6	0,78	7,3	1,9	0,38	6,33	60,53
Parmak	Afvon	4	-	23	1,8	3,0	0,61	2.6	1.7	0,44	6,30	70,47
Mahrabaşı	Kahramanmaraş	m	2	7	2,0	2,5	08'0	5,7	2.6	0,22	5,91	37,38
876 27 Dımışkı	Gaziantep	4	-	7	2,1	2,7	0,79	8,4	2.2	0,38	5.87	65,23
Külabi		23	-	2	2,1	2,3	06'0	7,6	2,3	0,33	5,81	57,17
Durif		9	-	2	2,0	2,5	0,82	6,8	1,9	0,36	5,73	62,37
Muhammediye	Diyarbakır	4	-	2	2,0	3,3	0,61	9,8	2,2	0,39	5,72	68,73
Salamur	Aydın	4	-	2	1,9	3,4	0,55	8,4	2.0	0,42	5,66	74,15
961-20	Denizli	m	2	m	2,2	2,4	0,93	6'9	2.9	0,24	5,65	42,07
Beyaz bandırma		4	-	7	2,1	2,9	0,75	0'6	3,1	0,29	5,47	53,23
Yediveren		4	-	2	1,9	2,5	0,75	5,8	2.2	0,26	5,43	48,18
898-46	Siirt	9	2	2	2,2	2,9	0,75	7.7	2.6	0,30	5,42	54,62
Gelin öldüren	Konya	m	2	6	1,9	2,3	0,83	4,9	2,4	0,20	5,38	38,08
Efe püskülü	Aydın	23	2	5	2,1	2,5	0,85	5,4	3,2	0,17	5,36	31,25
907-20	Denizli	9	2	2	2,0	2,9	69'0	7,3	2,4	0,30	5,33	26,67
960-46	Kahramanmaraş	7	-	2	2,2	2,8	0,79	7,5	2.6	0,29	5,15	56,15
Horoz karası	Gaziantep	4	2	7	1,9	3,0	0,63	6,2	3.0	0,21	5.07	40,67
885-24	Erzincan	m	9	7	2,1		68'0	6,5	2.2	0,29	4,93	59,55
Pembe salamur		3	-	6	2,4	2,5	0,94	8,5	3,2	0,27	4,69	56,75
Çiçike nator	Siirt	4	-	5	1,9	3,1	0,62	7,0	2,0	0,35	4,51	77,50

 Table 1. Data of varieties (continue)

Genotype	Province	Веггу Shape	Berry Color	Bunch Density	Berry Width (cm)	Berry Length (cm)	Berry Width / Berry Length	Average Berry Weight (BW) (g)	Number of Seeds (NS)	Berry Weight Per Seed (BWPS)	Berry Weight Per 1 g Seed (BWPGS) (g)	Single Seed Fresh Weight (SFW) (mg)
937-46	Kahramanmaras	7		7	1.9	2.4	0.80	4.8	2.4	0.20	4.37	45.42
Meyane	Siirt	4	-	5	1,9		0,59	6'9	1,6	0,43	4,31	100,06
Salman	Aydın	3	-	7	2,0	2,5	0,80	5,3	2,8	0,19	4,30	43,93
Bağdat siyahı		3	9	5	2.0	2,3	0,88	5,4	2.6	0,21	4,23	48,85
973-08	Artvin	7	-	5	1,8	2,2	0,83	5,1	1,4	0,36	4,22	86,43
Rotik	Siirt	3	5	5	2,0	2,5	0,80	6,4	3,0	0,21	4,18	50,83
74-1	Adana	3	-	5	1,7	2,1	0,82	4,0	2,2	0,18	4,04	45,45
Red globe	USA	3	5	5	2,4	2,8	0,88	10,0	3.7	0,27	4,03	92'99
Sofra üzümü	Denizli	3	9	5	2,3	2,5	06'0	2,6	4.0	0,19	4,01	47,58
900-42	Konya	3	-	5	2.5	2,6	0,95	8,5	3,8	0,22	3.81	58,42
Cesme beyazı	İzmir	3	2	5	1,9	2,4	0,79	5,9	3.0	0,20	3,81	51,67
Memki eyso	Siirt	10	9	7	1,8	2,6	0,70	5.0	3,2	0,16	3,75	41,88
90-996	Artvin	7	-	5	2,0	2,8	0,73	9'9	2,8	0,24	3,72	63,57
Veledizine	Siirt	3	4	5	2,1	2,6	0,84	6,8	3,2	0,21	3,71	56,97
943-56	Siirt	7	-	2	1,9	2,2	06'0	4,6	3.0	0,15	3,49	44,33
992-05	Amasya	3	-	2	2,4	2,6	0,91	8,7	3,6	0,24	3,48	69,44
Nuri bev	Kütahya	2	2	m	1,9	2,0	0,95	4,0	2,9	0,14	3,45	39,66
891-22	Edirne	7	-	2	2,1	2,6	0,83	6,4	3,4	0,19	3,42	55.00
Azezi	Urfa	7	-	7	1,9	2,6	0,72	9'9	2,5	0,26	3,41	77,52
Dokuztekne karası	Adana	3	5	2	1,9	2,3	0,83	5,9	3,2	0,18	3,32	55.75
948-27	Gaziantep	7	-	2	2,2	2,6	0,83	6,8	4,3	0,16	3,24	48,84
927-47	Mardin	33	4	2	2,2	2,5	0,88	7,2	3,2	0,22	3,19	70,31
Sam üzümü 47-5	Amasya	2	-	2	1,9	1,9	1,02	5,2	3.0	0,17	3,04	26,67
880-33	jel	33	-	7	2,1	2,6	0,81	2,0	3,2	0,22	3,00	73,13
Deveqözü		2	-	7	2,1	2,2	96'0	7,2	3,8	0,19	2,98	63,55
Kızıl banki	Sanlıurfa	3	2	7	1,9	2,5	0,75	5,3	2,5	0,21	2,98	71,60
897-17	Canakkale	2	-	7	1,9	2,0	0,94	4,0	2,6	0,15	2,92	52,69
Memecik		10	-	2	1,4	3,6	0,40	4,0	2,9	0,14	2,71	51,03
Cesme pembesi		33	2	2	2.0	2,5	0,81	5,8	3,2	0,18	2,65	68,28
Siyah bandırma	Muğla	9	9	7	1,9	2,5	0,73	5,0	3,3	0,15	2,34	64,64
Artvin üzümü	Artvin	3	-	2	2,4	2,6	0,93	9,2	2,1	0,44	6,83	64,19
Emirali	Tekirdağ	3	9	3,5	2,5	2,7	0,92	10,1	2,6	0,39	6,47	00'09
Atak 77	Yalova	3	-	5	2,0	2,6	0,79	6,5	1,8	0,36	7,85	46,10

Table 2. Correlations between berry and seed properties

	Berry Weight (BW)	Number of Seed (NS)	Single Seed Fresh Weight (SFW)	Total Seed
Berry Weight (BW)	1	-0.09	0.39 **	0.26 *
Number of Seed (NS)	-0.09	1	-0.21	0.59
Single Seed Fresh Weight (SFW)	0.39**	-0.21	1	0.66
Total Seed	0.26 *	0.59	0.66	1

^{*, **} indicate significance at p \leq 0.05, 0.01, respectively

Conclusion

As in the breeding studies of other species, examining the characteristics of the varieties to be selected as parents in grapevine breeding studies and scientifically process characteristics is essential. Due to restrictions such as the limited number of molecular markers that can be used in marker-assisted selection in grapevine breeding and the inability to perform early selection with MAS for some traits, breeders are directed to know the segregations of the traits they are interested in and to examine in detail the characteristics of the varieties they will use as parents. Grape berry size is a characteristic increases that market opportunities and increases the sales price of table grapes. Unfortunately, even today, a molecular marker has not been developed to allow early selection of berry size in grapes. As a result, local varieties such as Antep Samı, İt Karası, Gadöv, Beyaz Çavuş and Akhisar Razakısı have come to the fore, and it is recommended that these varieties be used as maternal parents in the development of new large-grape varieties. Using these varieties can increase the effectiveness of breeding programs. It has been observed that the Red Globe, which is frequently used in table grape breeding in the world, remains in the background in terms of relationship between berry size and seed number/seed weight. Apart from all these data, the seeds of the variety to be used as the maternal parents must have high germination ability.

Ethical Approval

Ethics committee certificate is not required

Acknowledgments

The authors would like to thank Tekirdağ Viticulture Research Institute Directorate for providing land and laboratory facilities for this study.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Author Contribution

Author OE were planned and designed the trial. TU, AP, AT and BZ performed the field experiments and labratory studies. OE and BZ made critical revisions of the manuscript for intellectual content. All authors read and approved the final manuscript.

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