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Seed and Germination Characteristics of Different Hybrids Belonging to *Vitis* Species

Vitis Türlerine Ait Farkli Hibritlerin Tohum ve Çimlenme Özellikleri

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SEED AND GERMINATION CHARACTERISTICS OF DIFFERENT HYBRIDS BELONGING TO *VITIS* SPECIES

ABSTRACT

In this research, some grape cultivars are belonging to *Vitis vinifera*, *Vitis labrusca,* and interspecific grape cultivars were used for crossbreeding. The aim of the cross-breedings was to obtain individuals that could be used as a table grape, female flower type genotypes, and would be resistant to disease and cold hardy grapes. In the research, berry set (seeded and seedless berry number), seed weight (g), seed width and length (mm), floating empty seed rate (seed viability, %), seed germination rates (%), seedling survival rates (%) and shoot length (cm) were calculated in the seeds obtained as a result of cross-breeding. In this research, as a result of crossbreeding with different *Vitis* species and hybrids, the germination rate was significantly higher, especially when using 'Çeliksu', 'Red Globe' and 'Cayuga White' cultivars as maternal parents. Although the germination rates of the seeds obtained after cross-breeding are low, the use of 'Çavuş' grape cultivar with female flower form in cross-breeding facilitates the work since emasculation is not necessary. It would be appropriate to increase the number of grapevines with female flowers type in breeding studies.

Keywords: Cross-breeding, Emasculation, Seed Germination, *V. labrusca*, *V. vinifera*, Interspecific Grapevine.

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VİTİS TÜRLERİNE AİT FARKLİ HİBRİTLERİN TOHUM VE ÇİMLENME ÖZELLİKLERİ

ÖZ

Bu araştırmada; *Vitis vinifera*, *Vitis labrusca* ve türler arası bazı üzüm çeşitlerinde melezlemeler yürütülmüştür. Melezlemelerin amacı; melezlemelerde kolaylık açısından dişi çiçek tipi genotipe sahip yeni bireylerin elde edilmesinin yanı sıra hastalıklara ve soğuğa dayanıklı yeni bireyler ile anaç olarak kullanılabilecek bitkiler elde etmektir. Araştırmada; tane tutumu (çekirdekli ve çekirdeksiz tane sayısı), tohum ağırlığı (g), tohum genişliği ve uzunluğu (mm), yüzen boş tohum oranı (%), tohum çimlenme oranları (%), çimlenen bitki yaşama oranları (%) ve sürgün uzunluğu (cm) hesaplanmıştır. Bu araştırmada, farklı *Vitis* tür ve melezleriyle yapılan melezleme sonucunda, özellikle 'Çeliksu', 'Red Globe' ve 'Cayuga White' çeşitleri ana ebeveyn olarak kullanıldığında, çekirdeklerde çimlenme oranı önemli ölçüde artış göstermiştir. 'Çavuş' üzüm çeşidinden elde edilen tohumların çimlen-

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me oranları düşük olmasına rağmen, dişi çiçek formuna sahip çeşitlerin melezleme çalışmalarında kullanılması emaskülasyona gerek olmadığından melezlemeyi kolaylaştırmaktadır. Islah çalışmalarında dişi çiçek tipine sahip üzüm çeşitlerin kullanılmasıyla daha fazla sayıda tohum ve bitki elde edilebilecektir.

Anahtar Kelimeler: Melezleme, Emaskülasyon, Tohum Çimlenme, *V. labrusca*, *V. vinifera*, Türlerarası Asma.

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1. INTRODUCTION

Today, more than 10.000 grape cultivars belonging to the *Vitis* genus are grown for different purposes all around the world (Khan et al., 2020). People's consumption habits show vary from year to year. For example; the people, especially children, prefer seedless, colorful, crunchy, medium bunch or exotic grapes (Peña-Neira et al., 2023; Atak and Şen, 2021). In recent years, the breeding studies focused on to obtain new grapes as cold hardy, drought and disease resistant, seedless, medium bunch, large berries and having unique aroma (Atak and Kahraman, 2012; Akkurt et al., 2019; Verma et al., 2019; Atak, 2022). In addition, many new varieties have been registered and transferred to growers from different breeding studies carried out in Turkey in the last 20 years (Atak et al., 2022).

Successful breeding in different grape varieties requires many of different grape genetic resources (Atak, 2023). Breeding works is nowadays predominantly carried out to improve grape quality, including increasing the resistance of plants to biotic and abiotic stresses (Parmar et al., 2017). Different plant species may not reach the desired level of viability during seed or seed formation, depending on many factors during their development following flowering and subsequent pollination. Among these factors, genetics plays an important role. For this reason, differences in viability rates may occur depending on the parents used. This situation is thought to be particularly related to embryo development (Atak and Şen, 2021; Jovanović-Cvetković et al., 2022).

The germination power of the seeds plays an important role in the success of cross-breeding studies carried out to obtain new varieties and combine good characteristics. Since the number of plants obtained from seeds with poor germination power is low, the probability of the emergence of vines with the desired characteristics is therefore low. Today, the aim of breeding studies carried out for various purposes is to germinate the hybrid seeds obtained at the highest possible rate and to obtain healthy plants from them (Goldy, 1992; Rieseberg et al., 1998; Kuligowska et al., 2016; Demirkaplan and Gübbük, 2023). The seeds obtained after cross-breeding are important aspects of the breeding process (Uzun et al., 2019).

Vitis vinifera is the most widely cultivated grape species in the world due to its economic value. V. vinifera grapes are also still widely used worldwide as the main material for breeding new grape varieties (Alleweldt et al., 2005; Wang et al., 2021). For this purpose, in order to obtain a large number of new hybrid plants, it is expected that the germination properties of the seeds obtained by cross-breeding should be strong. In an effective breeding study, high germination and seedling formation rates of intraspecific and interspecific hybrid seeds are expected in *V. vinifera* in order to obtain larger seedling populations in a short time and to achieve faster grape breeding (Wang et al., 2022). The formation of seeds to seedlings is one of the most important issues of breeding efficiency. The germination rate of grape seeds is typically low (30-50%) and the final seedling rate is even lower (Lin et al, 2009; Zhang et al., 2009). Low germination rates may be due to parental selection, an incompletely developed seed embryo of the female parent, or a hard seed coat that is not easy to break (Ma et al., 2014).

Interspecific hybrid cultivars, obtained from crosses with *V. vinifera*, including many of North American and Asian grape species, are important to obtain new genotypes resistant to biotic and abiotic stress condition (Töpfer et al., 2011; Eibach and Töpfer, 2015; Lisek and Lisek, 2019; Foria et al., 2022). Many researchers suggested that three months of stratification at 4°C gave the best results for grape seed germination (Conner, 2008; Sabir, 2011; Seget et al., 2020; Atak and Şen, 2021; Hawezy and Sharif, 2023). If the stratification period is too long or too short, phenolic compounds in seeds can inhibit the germination rate (Gao at al., 2014; Weidner et al., 2014; Orrù et al., 2023). Previous studies have shown that grape seed germination and seedling formation rates vary between different populations and cultivars (Ledbetter and Ramming, 1989; Ristic and Iland, 2005; Gao et al., 2014).

Germination rates of hybrid seeds may vary due to some environmental factors such as light, temperature, humidity, soil conditions (Çelik, 2001; Humphrieset al., 2018; Travlos et al., 2020) and polen sources (Sabır, 2011; Sabır, 2015; Atak and Şen, 2021; Atak, 2022; Alzohairy et al., 2023). On the other hand, Ellis et al. (1983) reported that 25°C is the best temperature for grape seed germination. When choosing hybrid combinations, it is very important seeded berry set, seed number and germination rate of the mother parentage (Singh et al., 1984; Sabır, 2011; Wang et al., 2022). Sabir (2011) was found the highest rate of berry set was obtained from Italia x 1103 P (44.1%) and Italia x 140 Ru (43.2%), while the lowest percentage was noted in self-pollinated plants (36.4%). Germination rates of seeds obtained after cross-breeding shows differences according to the combinations (Akkurt et al., 2013; Ergönül et al., 2023; Atak et al., 2019; Uzun et al., 2019). For example; Ergönül et al. (2023) determined the lowest seed germination rates in 'Trakya İlkeren' (2.6%) and 'Cardinal' (0-2.9%) grapes and the highest rate in 'Yalova İncisi' (70.8-79.1%) in hybrid combinations.

The aim of this research was obtained berry set after cross-breeding, seed specifications and germination characteristics of some crossbreeding combinations are belonging to *Vitis vinifera*, *Vitis labrusca* and interspecific grapes.

2. MATERIALS AND METHODS

2.1. Plant Materials

Cross-breeding studies were carried out in the vineyards and greenhouses of Ondokuz Mayıs University, Faculty of Agriculture. Berry set, viability and germination rate was obtained with 13 different combinations. In cross-breedings, five grape cultivars belonging to the *Vitis vinifera* species ('Çavuş', 'Yalova İncisi', 'Trakya İlkeren', 'Prima', 'Red Globe'), two grape cultivars belonging to the *Vitis labrusca* species ('Çeliksu', 'Ülkemiz'), three interspesific cultivars ('Valiant', 'Marechal Foch', 'Cayuga White') and 1103 Paulsen rootstock were used. 'Çavuş' grape has morphological hermaphrodite, physiological female type and not need to emasculate. The Cross-breeding combinations carried out in the research are given in Table 1.

Table 1. Crossbreeding combinations

	Breeding Combinations	
Ülkemiz x Marechal Foch	Çavuş x Yalova İncisi	Red Globe x Valiant
Cayuga White x Marechal Foch	Çavuş x Trakya İlkeren	Trakya İlkeren x Valiant
Çeliksu x Marechal Foch	Çavuş x Marechal Foch	Çeliksu x Valiant
Red Globe x Ülkemiz	Çavuş x Prima	Cayuga White x Valiant
	Çavuş x 1103P	

In the study, the origin and characteristics of the grape cultivars used in Cross-Breedings are given in Table 2.

Table 2. Characteristics of grape cultivars/genotypes used in crossbreeding studies

Cultivar/genotype/Species	**Specifications**	**Cluster**	**Leaf**
Cayuga White: *Vitis labrusca* hybrids Seyval x Schuyler at Cornell University, New York, 1945 (USA).	Seeded Disease Resistant Moderately Cold Hardy		

Marechal Foch: Millardet et Grasset 101 O.P. x Goldriesling. Eugene Kuhlmann of Alsace, France 1920 (France).	Seeded Disease Resistant Cold Hardy		
Çavuş: Female type Turkish grape variety (*Vitis vinifera*)	Seeded Table grape		
Çeliksu: (*Vitis labrusca*). Selected from Black Sea Region and registrated in 2016 by Ondokuz Mayıs University	Seeded Disease Resistant		
Ülkemiz: (*Vitis labrusca*). Selected from Black Sea Region and registrated in 2016 by Ondokuz Mayıs University	Seeded Disease Resistant		
Yalova İncisi: Hönüsü x Black Gemre crossing. Early ripening, released in 1988 by Atatürk Horticultural Central Research Institute, Turkiye.	Seeded Table grape		

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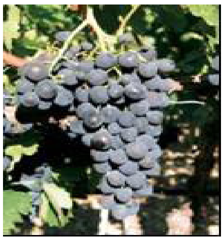
Trakya İlkeren: Alphonse Lavallee x Perlette crossing. Early ripening, released in 1994 by Tekirdağ Viticultural Institute, Turkiye.	Seeded Table grape		
Prima: Lival x Cardinal crossing. Early ripening and it was obtained in 1974 by INRA.	Seeded Table grape		
Red Globe: L 12-80 X S 45-48, (Hunisa x Emperor) x (Hunisa x Emperor x Nocera) crossing. California Agricultural Experiment Station.	Seeded Table grape		
Valiant: Fredonia x South Dakota 9-39	Seeded Disease resistant Extremely Cold Hardy		
1103 Paulsen: Berlandieri Rességuier No. 2 x Rupestris du Lot	Rootstock Male flower type		

2.2. Methods

2.2.1. Polen Supply and Storage

During the flowering period (first week of the June), pollen was obtained by taking clusters from pollinator varieties in full bloom. The clusters of the polliantor cultivars were collected during the full bloom period. Pollen was collected freshly from fully open clusters. Flower clusters kept at room temperature for overnight. The flower clusters were sieved through a fine sieve and the pollen and they were stored at +4 °C until the pollination process (Staudt, 1999; Sabır, 2011).

2.2.2. Emasculation and Cross-Breedings Process

The clusters were checked daily and the emasculation process was initiated approximately 3-5 days before the beginning of flowering (Hardie et al., 1996). In the study, for all crossings; cross-breeding was carried out on 3 vines and 3 clusters of each vine and cultivar. Totally 9 clusters emasculated for each cross-breeding combination. Male organs were emasculated during the flowering period except for 'Çavuş' grape (female flower type). The emasculated clusters were washed with pure water to ensure that no pollen remained. The emasculated clusters were placed in a paper bag, labeled and closed. Emasculated clusters were checked daily, and pollinated with pollen collected from pollinator varieties when sugary liquid was seen on the stigma. Pollination was carried out early in the morning and late in the evening. These processes continued until the upper part of the stigma turned from greenish to brown. After pollination, the clusters were re-bagged and labeled with details of the mother and father varieties and pollination dates. The clusters and vines were subjected to regular cultural practices and spraying with fungicides against fungal diseases.

2.2.3. Harvest of Clusters

Seeds were harvested when the grapes were fully ripe. Fruit harvested had to be at least of 22°Brix. The berries were separated from the cluster skeleton, and the seeds were removed. The seeds were washed with distilled water to separate them from other fruit components and then left to dry on a blotting paper for a week. The seeds were stored in capped plastic tubes at +4**°C** until germination.

2.2.4. Seed Germination Procedures

To separate viable seed from others, the seeds were placed in a small beaker of distilled water. 24 hours waited seed in the water and floating seed were considered non-viable. The following procedure was applied before germination. After the non-viable seeds floating in the water were separated, the remaining seeds, which were considered to be viable, were planted in the viols in 3 replicates. A total of 3300 seeds were sown in the viols and totally 1905 seeds germinated in the research.

The following treatments were applied to the seeds before germination.

1. The seeds were soaked in water for 48 hours.
2. It was kept in 3% hydrogen peroxide (1.5:5 water: hydrogen peroxide) for 24 hours.
3. 1000 ppm GA_3 was applied for 24 hours.
4. It was kept in 6% sodium hypochlorite for 2 minutes.
5. It was scarified in moistened vermiculite at +4°C for 3 months.

Filling the viols and opening the planting pits, the seeds were sown in a plastic viol. Peat was used as germination medium. Seeds were sown in the viols manually at a depth of about 0.5 cm. After sowing, the viols were covered with vermiculite and placed in the climate chamber for germination. In the study, germination rates were evaluated on a total of 50 viable seeds for each application. The viols were germinated by keeping them in a growth room with controlled temperature and humidity at ±26°C, 85% humidity and a 12 h photoperiod. The viols were checked frequently, and when the root tips began to appear from the seeds, the viols were moved from the germination room to the greenhouse.

Weight of 100 seed (g) in each combination;

In 50 randomly selected seed, the proportion of seeds floating in water (viability rate %) was calculated.

Viability rate (%) = (100 x sunken seeds)/(floating seeds + sunken seeds)

The following characteristics will be examined in the seeds obtained as a result of crossbreeding during the germination phase.

- Seeded berry (number)
- Seedless berry (number)
- Seed weight (100 number)
- Seed width (mm)
- Seed length (mm)
- Germination rate of seeds (%)
- Seedling formation rate (%)
- Floating seed in water (%)
- Seed viability (%)
- Shoot length of the second year's plants (cm)

2.3. Greenhouse Cultivation Stages

During daily checks, the viols belonging to the combinations whose germination started and whose root tips were visible were transferred to the greenhouse. The germinated grape seeds were planted in the greenhouse in May. After two or three cotyledons had emerged, the young plants were transplanted into deep square pots with a diameter of 5 x 5 cm, allowing them to grow and develop in the greenhouse. The plants transferred to the viols were numbered and labeled individually according to each cross-breeding combination. In the second year, the plants were transferred 17 x 22 cm polyethylene plastic pots in open field condition. To ensure upright growth of the plants, a 100 cm long plant support rod was planted at the base of the plants and the plants were tied to these rods as they grew taller.

2.4. Statistical Analysis

In the research, % ratio was taken as basis in evaluating the results of hybrid combinations. However, analysis of variance was applied to determine the germination rate. Differences between combinations were made with Duncan's multiple range test. Differences were determined at the 5% confidence interval ($P < 0.05$). SPSS (v.16) statistical package program was used for statistical analysis. Correlations between the studied seed characteristics were evaluated with Pearson's coefficients (r).

3. RESULT AND DISCUSSION

In the research, the number of seeded and seedless berries per cluster (berry set) and the number of seeds per berry were evaluated after harvesting the clusters (Table 3). Significant differences were determined between the hybrid combinations in terms of the seed number per berry and berry set ($P<0.05$). The highest number of seeded berries was obtained in the 'Cayuga White' x 'Marechal Foch' combination (146.4 number). The lowest seeded berry numbers were determined in 'Ülkemiz' x 'Marechal Foch' (12.3 number), 'Red Globe' x 'Valiant' (25.5 number), and 'Cayuga White' x 'Valiant' (27.6 number) crossings. In this study, the seedless small berries in the cluster were identified in the harvested clusters (Table 3). Statistical differences were found between the hybrid combinations in terms of the number of seedless berry ($P<0.05$). According to the obtained results; the hybrid combinations with the highest seedless berries were determined as 'Çavuş' x 'Prima' (32.8 number per cluster) and 'Trakya İlkeren' x 'Valiant' (30.4 number per cluster) combinations. On the other hand, the hybrid combination with the least number of seedless berries in the cluster was determined as 'Cayuga White' x 'Marechal Foch' (0.5 number per cluster). In this study; it has been revealed that the cultivars/genotypes used in cross-breedings may have different effects on berry setting, and caution should be taken, especially in cross-breedings with the

'Trakya İlkeren' cv. Since the aim of breeding studies based on cross-breeding is to achieve the highest possible number of seeds, thus obtaining different individuals according to the principles of expansion, it becomes important to know the effects of pollination method and technique on seed development as well as fruit set (Marasalı and Ergül 1997). In addition, between grape varieties in terms of cross-breeding compatibility and potential are an important process for berry set (Sabır and Küçükbasmacı, 2020). In this study, the number of seeds per berry varied between 1.5 and 3.0 depending on the hybrid combination. The least seed number per berry was obtained at both 'Ülkemiz' x 'Marechal Foch' and 'Trakya İlkeren' x Valiant' (1.5 seed/berry) crossings. The highest seed number per berry was determined at 'Red Globe' x 'Valiant' combination. This result shows that parentage especially mother plant can be effective on seed numbers of the berries. In the study, the highest number of seeds was obtained from crosses when used 'Red Globe' as the mother (Table 4). In this way, Sabir (2011) implanted that the berry settings of the crosses were varied depending on the pollinators. Also, pollen source significantly affects cluster size (Sabır and Küçükbasmacı, 2020). Sabır et al., (2020) found that the berry set in 'Trakya İlkeren' cultivar ranging from 21.3% (self-pollination) to 23.7% (hand-pollination). Kowalczyk et al. (2022), determined that the number of germinating pollen grains on the stigma and the number of pollen tubes at the base of the pistil were statistically the highest in 'Solaris', 'Marechal Foch', 'Aurora', 'Bianca', and 'Muscat Odesskij' cultivars. Similar result reported by Dardeniz et al. (2011) that there were differences in the size and number of berries obtained as a result of pollination of the 'Çavuş' grape cultivar with different pollinators. Atak et al. (2019) found that in terms of the number of seeds per cluster, the combination using 'Çavuş' x 'Crimson Seedless' (148.7 seeds per cluster) combination gave the highest number of hybrid seeds. They reported that although a large number of seeds were obtained in combinations in which 'Çavuş' was used as the main, the viability rates remained lower than in combinations in which 'Red Globe' and 'Köfteci' grapes were used as the main. However, the researchers stated that the combinations in which the 'Red Globe', 'Köfteci' (*Vitis labrusca*) and 'Çavuş' cultivars were used as the main ones came to the fore, and that in almost every combination in which these varieties were crossed, they gave better results in terms of both seed number and viability, and that in the cross-breeding studies to be carried out in the following years. Our findings in 'Cayuga White' x 'Marechal Foch' and 'Çavuş' x 'Yalova Incisi' crossings gave the highest berry set compare to the other combinations (146.4 and 109.3 berry per cluster, respectively). On the other hand, the least seedless berry has been found in 'Cayuga White' x 'Marechal Foch' crossings. Similar findings determinated from Karataş et al. (2007) that among pollinated grape cultivars with 'Kalecik Karası', the highest berry set rate (38.0%) was obtained in 'Hamburg Misketi', while the lowest rate (18.0%) was found in 'Riesling' cultivar. Morever, in our study; the highest berry set was obtained when the 'Çavuş' cv. was pollinated with 'Yalova İncisi'. In the light with these results, 'Yalova İncisi' is recommended as a pollinator for 'Çavuş' cultivar.

Table 3. Effects of Cross-Breedings on berry set and seed number per berry

Crossings ID	Seeded Berry Number Per Cluster	Seedless Berry Number Per Cluster	Seed Number Per Berry
Çavuş x Yalova İncisi	109.3±17.2 ab*	14.8±5.0 bc	2.5±0.5 ab
Çavuş x Trakya İlkeren	47.5±22.5 cde	7.3±4.5 c	2.2±0.4 ab
Çavuş x Marechal Foch	76.3±23.8 bcd	19.7±9.7 abc	2.0±0.6 ab
Çavuş x Prima	87.7±24.2 bc	32.8±8.6 a	2.3±1.0 ab
Çavuş x 1103P	42.0±18.6 cde	5.0±6.2 c	2.3±1.0 ab
Red Globe x Ülkemiz	40.6±22.1 cde	4.0±5.7 c	2.5±0.7 ab
Red Globe x Valiant	25.0±2.8 de	5.3±8.4 c	3.0±0.0 a
Çeliksu x Marechal Foch	24.3±14.0 de	10.0±7.3 c	1.8±0.6 b
Çeliksu x Valiant	32.8±7.8 cde	3.0±0.5 c	2.0±0.0 ab
Ülkemiz x Marechal Foch	12.3±5.6 e	3.4±3.3 c	1.5±0.4 b
Cayuga White x Valiant	27.6±20.4 de	3.3±5.3 c	1.7±0.6 b
Cayuga White x Marechal Foch	146.4±33.7 a	0.5±0.6 c	1.8±0.8 b
Trakya İlkeren x Valiant	55.5±9.2 b-e	30.4±2.0 ab	1.5±0.5 b
Significiance	*P<0.05*	*P<0.05*	*P<0.05*
*SEM	4.924	1.623	0.091

*SEM: Standard errors of the means. Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

The average 100 seed weights obtained from the harvested cluster before germination, and the weights of the hybrid combinations were determined (Table 4). Seed weights showed statistically significant differences (P<0.05), and seed weights varied between 2.7 and 6.0 g depending on the hybrid combinations. Among the combinations examined, the lowest average seed weight was determined to be at the 'Çavuş' x 'Trakya İlkeren' (2.7 g) and 'Trakya İlkeren' x 'Valiant' (2.9 g) combinations. Despite that; 'Çeliksu' x 'Marechal Foch' (6.0 g) and 'Çeliksu' x 'Valiant' (5.8 g) crossings were the heaviest seed combinations. In the study, it was observed that the seed weights, width and length obtained from cross-breedings with the cv. 'Çavuş' showed differences depending on the pollinator (father). It was determined that seed weights of the 'Çavuş' crossings varied between 2.7 g and 3.7 g, and that there were differences in seed weights according to pollinator. Accordingly, while the lowest seed weight was obtained by pollinating the 'Çavuş' with the 'Trakya İlkeren' (2.7 g), the seed weight obtained by hybridizing with 'Marechal Foch' increased and was determined to be 3.7 g.

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Table 4. Seed weight, seed width and seed length of the hybrid combinations.

Crossings ID	100 Seed Weight (g)	Seed Width (mm)	Seed Length (mm)
Çavuş x Yalova İncisi	3.4±0.2 g*	3.86±0.25 ef	6.38±0.35 e
Çavuş x Trakya İlkeren	2.7±0.1 h	3.82±0.21 ef	5.96±0.20 h
Çavuş x Marechal Foch	3.7±0.2 f	3.98±0.08 de	5.94±0.22 f
Çavuş x Prima	2.9±0.1 h	3.68±0.17 f	5.45±0.12 g
Çavuş x 1103P	3.5±0.3 g	4.10±0.15 d	6.47±0.20 de
Red Globe x Ülkemiz	3.9±0.1 e	3.77±0.17 f	7.50±0.36 b
Red Globe x Valiant	3.9±0.1 e	4.00±0.12 de	7.98±0.32 a
Çeliksu x Marechal Foch	6.0±0.2 a	4.85±0.10 a	7.04±0.20 c
Çeliksu x Valiant	5.8±0.3 a	4.70±0.21 ab	6.70±0.34 d
Ülkemiz x Marechal Foch	5.2±0.1 b	4.63±0.19 b	6.31±0.24 e
Cayuga White x Valiant	4.3±0.1 d	4.14±0.17 d	6.38±0.21 e
Cayuga White x Marechal Foch	4.6±0.1 c	4.37±0.17 c	6.53±0.18 de
Trakya İlkeren x Valiant	2.9±0.1 h	3.78±0.11 f	5.72±0.26 f
Significiance	*P<0.05*	*P<0.05*	*P<0.05*
*SEM	0.017	0.02	0.02

*SEM: Standard errors of the means. Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

When the seed width values of the grape seeds obtained in the research were examined, significant differences were observed between the combinations. In this topic; while Ledbetter and Shonnard (1991) considered grape varieties with a single seed weight of 20 mg or less as rudimentary. Ramming et al. (1990) accepted 25 mg as the threshold value for abortive seeds formed as a result of embryo and endosperm degeneration. Barış et al. (1991) examined the seeds and there was no germination was observed in seeds weighing less than 20 mg. On the other hand; Ergönül et al. (2023) in determining seedless individuals, taking into account the single seed dry weight data, individuals with a single seed dry weight below 20 mg were classified as seedless, and individuals with values above 20 mg were classified as having seeds. In this research, the lowest seed weight of 27 mg was obtained in the combination of 'Çavuş' x 'Trakya İlkeren'. This value was found to be above the minimum seed weight reported by the researchers. In this way; Ramming et al. (1990) stated that the germination rates of seeds of early maturing grape varieties are poor. In this conducted study, 'Trakya İlkeren' cv. is an early ripens and showed lower seed germination rates than the other combinations. Ergönül et al. (2021); stated that in many early ripen grape varieties, embryos degenerate at certain stages of seed development and they found the germination rate of the 'Trakya İlkeren' x 'Barış' seeds as 2.6%. In this study, on the contrary; germination rates of 'Trakya İlkeren' x 'Valiant' hybrid seeds were found to be 38.7%.

In the study, seed width and length showed differences according to crossing combinations. The highest seed width was obtained from 'Çeliksu' and 'Ülkemiz' crossings. On the other hand, the longest seeds were determined in 'Red Globe' crosses (Table 4). Among the hybrid combinations examined, the highest seed width was determined as 'Çeliksu' x 'Marechal Foch' (4.85 mm) and 'Çeliksu' x 'Valiant' (4.70 mm). The combinations with the lowest seed width are; it was determined as 'Çavuş' x 'Prima' (3.68 mm), 'Red Globe' x 'Ülkemiz' (3.77 mm) and 'Trakya İlkeren' x 'Valiant' (3.78 mm). In the study; 'Çeliksu' (*V. labrusca*) cultivar gave the highest seed width and seed weight. Differences were determined between hybrid combinations in terms of seed length ($p<0.05$). Seed lengths of the hybrid combinations examined are given in Table 4. Among the hybrid combinations, the combination with the highest seed length was determined as 'Red Globe' x 'Valiant' (7.98 mm) and 'Red Globe' x 'Ülkemiz' (7.50 mm) crossings. On the other hand, the shortest seed length was determined as 'Çavuş' x 'Prima' (5.45 mm), 'Çavuş' x 'Marechal Foch' (5.94), and 'Trakya İlkeren' x 'Valiant' (5.72 mm) crossings. As a matter of fact, Odabaşıoğlu and Gürsöz (2020) determined the seed width and length as 3.62-5.82 mm in the 'Trakya İlkeren' and 4.21-7.78 mm in the 'Red Globe' cultivars. In this subject, Martín-Gómez et al. (2020) stated that *V. labrusca* seeds larger than *V. vinifera* seeds. The findings of the researchers are similar to our obtained results. The seeds of the 'Red Globe' gave the longer seeds and the seeds of *V. labrusca* grapes produced larger seeds than the other cultivars.

In floating tests performed to determine seed viability before germination, and showed significant differences in seed viability were obtained between hybrid combinations (Table 5). The highest seed viability rate was determined as the combinations with the lowest floating seed rate, while the lowest viability rate was evaluated as the combination with the highest floating seed rate. According to the obtained results; the highest floating seed rate rates were actually obtained in the combination of 'Trakya İlkeren' x 'Valiant' (50.4%) and 'Çavuş' x 'Prima' (45.32%). The highest seed viability rates are; the combinations with the lowest floating seed rates were 'Red Globe' x 'Ülkemiz' (5.64%), 'Çeliksu' x 'Marechal Foch' (3.78%), 'Çeliksu' x 'Valiant' (7.63%), 'Ülkemiz' x 'Marechal Foch' (4.92%), and 'Cayuga' x 'Valiant' (6.52%) crossings. These viability rates obtained in hybrid combinations varied depending on the parents. In the research, the lowest seed viability rate was obtained from the combination of 'Trakya İlkeren' x 'Valiant' crossings. This result reveals that caution should be taken when using the 'Trakya İlkeren' cultivar in cross-breeding and that cross-breeding should be done in more bunches to obtain a large number of seeds. As a matter of fact, on this subject, Uzun et al. (1995) reported that the 'Trakya İlkeren' grape cultivar had soft seeds and a rudimentary structure. Ledbetter and Shonnard (1991) considered *Vitis vinifera* grape varieties with a seed size of 2 mm or less or a seed weight of 20 mg or less as rudimentary. In the results of this study shows that the pollinator had a significant effect on the viability of the seed in the cross-breedings made with the 'Çavuş' grape. In the cross-breedings; when we made with the 'Çavuş' cultivar, it was determined that the combination of 'Çavuş' x 'Marechal Foch' crossing had the lowest floating seed rate and therefore gave the highest seed viability rate (Table 5). In order to determine the viability of seeds, Karataş et al. (2007) determined that 1350 of 1537 seeds were alive as a result of the floating test. Many researchers also reported that the 'Çavuş' grape cultivar has empty seeds, and 99.5% of the seeds in this cultivar are empty (Atak and Şen, 2021; Ergönül et al., 2021; Fidan and Cemali, 1974). In our study; for 'Çavuş' cultivar, the empty seed ratio varied between 11.7% and 45.3% depending on the pollinators. This result was found to be lower than the findings obtained by different researchers. Morover, before germinating the seeds, performing a water floating test to determine the viable seeds and separating the non-viable seeds will increase the germination rate.

Table 5. Floating seeds, germinating, seedling formation rates (%) and shoot length of the seedlings of the hybrid seeds

Crossings ID	Seed Floating Rates (%)	Seed Germination Rates (%)	Seedling Survival Rates (%)	2nd Year's Shoot Length (cm)
Çavuş x Yalova İncisi	16.3±0.7 abc	65.3±5.7 ab	52.67±6.2 c	82.6±4.9 ab
Çavuş x Trakya İlkeren	26.0±1.1 abc	17.7±5.7 d	17.00±2.1 e	75.2±9.2 a-d
Çavuş x Marechal Foch	11.7±0.5 bc	40.7±5.7 c	35.33±6.5 d	78.0±2.4 a-d
Çavuş x Prima	45.3±3.4 ab	39.3±5.7 c	33.33±2.4 d	65.1±7.9 de
Çavuş x 1103P	27.3±1.1 abc	43.0±5.7 c	40.67±2.7 d	86.9±2.5 a
Red Globe x Ülkemiz	5.6±0.2 c	84.0±8.1 a	72.67±6.4 b	79.9±1.5 a-c
Red Globe x Valiant	16.2±1.8 abc	38.3±5.7 c	71.33±5.0 b	76.7±1.5 a-d
Çeliksu x Marechal Foch	3.8±0.3 c	78.7±8.1 a	77.33±5.0 ab	68.6±3.7 c-e
Çeliksu x Valiant	7.6±0.7 c	72.3±5.7 a	69.67±1.5 b	74.1±3.6 a-d
Ülkemiz x Marechal Foch	4.9±0.6 c	51.7±5.7 bc	49.33±8.2 c	59.7±1.6 e
Cayuga White x Valiant	6.5±0.5 c	84.3±5.7 a	78.00±9.5 ab	86.6±2.2 a
Cayuga White x Marechal Foch	29.8±4.4 abc	81.7±5.7 a	81.67±7.5 a	90.9±2.0 a
Trakya İlkeren x Valiant	50.4±3.4 a	38.7±8.1 c	37.33±5.0 d	72.4±3.6 b-e
Significiance	*P<0.05*	*P<0.05*	*P<0.05*	*P<0.05*
*SEM	4.244	1.765	0.757	1.192

*SEM: Standard errors of the means. Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

The results obtained by germinating seeds of hybrid combinations are given in Table 5. Among the combinations examined, the lowest seed germination rates were found in the seeds obtained from crosses with the 'Çavuş' cultivar, compared to other combinations with lower germination rates. In the combinations in which the 'Çavuş' cultivar was used, the highest germination rate was obtained from the cross-breeding of 'Çavuş' x 'Yalova İncisi'. And also; the germination rate of seeds belonging to the cross-breeding of 'Çavuş' x 'Trakya İlkeren' and 'Trakya İlkeren' x 'Valiant' was also determined to be quite low. According to the results of the research, it was seen that the 'Yalova İncisi' grape cultivar would be a good pollinator for the 'Çavuş'. In the research; in the crosses made with the 'Red Globe', high germination rates were obtained at the rates of 'Red Globe' x 'Ülkemiz' (84.0%) and 'Red Globe' x Valiant' (76.7%). Additionally, 'Cayuga White' x 'Valiant' (84.3%) and 'Cayuga White' x 'Marechal Foch' (72.3%) followed by hybrids. According to the research results; selection of pollinator (father) is very important for high germination rate in cross-breedings with 'Çavuş', which has a female flower structure, and the best germination rate among the pollinator used in the research was obtained from the cross-breedings with 'Yalova İncisi'. In addition, it would also be useful to use 'Red Globe' as a parent to achieve high germination. In this research; in terms of germination rate, 'Cayuga White', 'Çeliksu' and 'Red Globe' stood out as the main candidates that can be preferred in cross-breedings. In the research, the shoot lengths of the hybrids transferred to large polyethylene bags in the second year were measured in the second week of August, according to the combinations, and the average shoot lengths were determined (Table 5). In the research, survival rates were determined after the germinating plants were transferred to black polyethylene pots. For this purpose, the germinated plants were transferred to pots and calculated of the seedlings survival rates in the following year. Seedling death/drying occurred in some germinated hybrid combinations. The highest seedling survival rates obtained from 'Cayuga White' x 'Marechal Foch' and 'Çeliksu' x 'Marechal Foch' combinations as a parallel germination rate. Between germinated plant's shoot lengths showed varied depending on the crossing combinations. According to the combinations, the longest shoot length was obtained from the combinations 'Ülkemiz' x 'Marechal Foch' (90.9 cm), 'Çavuş' x '1103 Paulsen' (86.9 cm) and 'Çeliksu' x 'Marechal Foch' (86.6 cm) combinations. In the hybrids examined, the lowest shoot growth was measured in the 'Çeliksu' x 'Valiant' combination (59.7 cm). It was observed that the shoot length of the plants obtained by crossing the strong rootstock 1103 Paulsen and 'Çavuş' increased significantly. It was also determined that the shoot length increased when 'Marechal Foch' used in crossed (Table 5). It is known that the pollen of pollinator grape cultivars/genotypes in grapevines affects the berry and seed characteristics of the parent plant (Sabır, 2015). It is reported that 'Kuntra' (Karasakız, Mavrupalya, Makbule), 'Vasilâki' (Anatolia Yapıncağı, Altıntaş) and 'Cardinal' cultivars are planted as pollinators for 'Bozcaada Çavuşu' vineyards (Dardeniz et al., 2007; Dardeniz et al., 2011). Atak and Şen (2021), stated that when 'White Çavuş' was used as the maternal parent, seed viability varied between 5.5% and 53.8%. Atak et al. (2019) reported that the combinations in which 'Red Globe', 'Köfteci' (*Vitis labrusca*), and 'White Çavuş' cultivars were used as the main ones stood out compared to the others. Researchers reported that the germination rate of the 'Çavuş' grape cultivar was between 2% and 54%, depending on the pollinators. Also, Atak et al. (2019) stated that in cross-breedings in which the 'Red Globe' grape cultivar was used as the maternal, germination rates in the seeds were around 85% and that the use of the 'Red Globe' in cross-breedings would be appropriate in terms of producing seeds with a high germination rate. Sabır (2011) reported that the pollen source is very important, when Rup du Lot and 1103 Paulsen were used as pollen sources in the 'Italia' grape cultivar, the germination rates in 'Italia' hybrid seeds were 56.7 and 60.6%, respectively, and the germination rate in self-pollinated 'Italia seeds was 37.4%. The germination rates of seeds were significantly higher when 1103 P and Rupestris du Lot rootstocks (60.6 and 56.7%, respectively) were used as pollinator for 'İtalia' grape cultivar (Sabır, 2011). Atak and Şen (2021) stated that seed viability rates occurred between 6.7% and 32% in crosses with 'Köfteci' grape (*V. labrusca*), depending on the pollinizer cultivar. According to Akkurt et al. (2013), the highest germination ratio in 'Kalecik Karası' seeds were found in 0.5-1 g/L BAP + 2-3 g/L GA_3 +90-day treatments. In this research, we used 1000 ppm GA_3 +90 day and obtained between 17.7% and 84.0% according to crossbreeding combinations. In this conducted research; 'Red Globe' x 'Ülkemiz' gave the highest germination ratio (84.0%). Marasalı (1992) investigated that the effect of different pollinators on the germination ability of the seeds of 'Çavuş' grape cultivar had no effect as pollinators. In contrast, we found that when the pollinator changed, the seed germination rate has been showed differences. The results of this study obtained result has been changed according to crossings. For instance, when the 'Çavuş' cultivar pollinated different pollinators, the seed germination rates ranging from 17.7% (Trakya İlkeren) and 65.3% (Yalova İncisi).

Significance of Pearson's coefficients(r) with respect to the correlations between all seed characters (Fig. 1). Significant relationships were obtained between the seed parameters examined in the study. Positive correlations were found between seed weight and seed width (**r** = 0.82**), seed length (**r** = 0.55**) and germination ratio (**r** = 0.552**). In the study, it was determined that there was a positive correlation between seed weight and germination rates. On the other hand, negative correlations were found among to floating seed ratio and seed weight (**r** = -0.492**), seed width (**r** = -0.320**), seed length (**r** = -0.454**) and germination ratio (**r** = 0.591**)

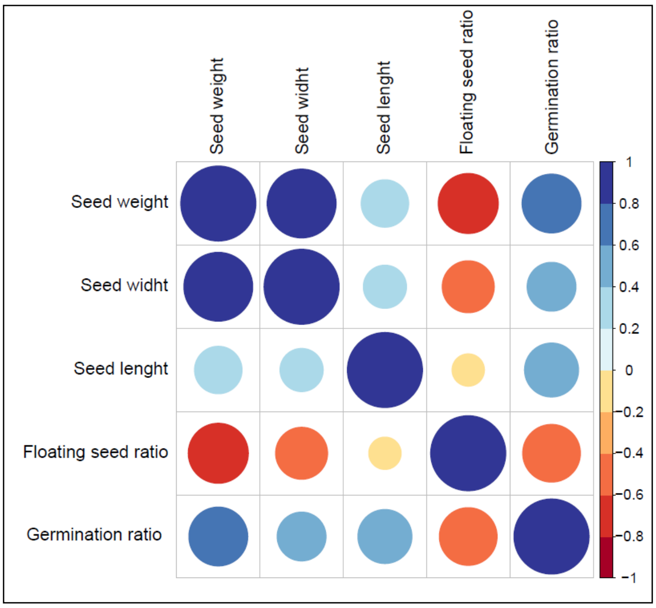

Figure 1. Pearson's correlation coefficient between hybrid seed characteristics is significant at the 0.01 level (1-tailed). Seed characteristics correlation among five seed specification seed weight (g); seed width (mm); seed length (mm); floating seed ratio (%), germination rates (%).

4. CONCLUSION

The use of American *Vitis* species in cross breeding studies with *V. vinifera* cultivars is very important in terms of resistance to phylloxera (*Daktulospharia vitifoliae* Fitch.), powdery mildew (*Uncinula necator*) and downy mildew (*Plasmopara viticola*). Before germinating of the seeds, performing a water floating test to determine the viable seeds and separating the non-viable seeds will increase the germination rate. It was seen that combinations with high germination rates could be obtained when 'Çeliksu', 'Red Globe' and 'Cayuga White' cultivars were used as a main parent. It seems likely that successful results will be obtained with different pollinators by using these cultivars as mother plants in future breeding studies. On the other hand, 'Yalova İncisi' can be considered as a suitable pollinator for 'Çavuş' grape cultivar in the vineyards. In the breeding studies, using female flower type grape cultivars that do not require emasculation are increased the berry set and number of seeds. On the other hand; even though some researchers found the germination rate of 'Çavuş' grape seeds to be low, the results we obtained in our research are considered satisfactory. Using of grape cultivars with morphological hermaphrodites and physiological female flower types in cross-breeding studies has been found important in hybridization studies in terms of both reducing the labor such as emasculation and providing convenience. For this purpose, it should be developed more female flower type grapes in cross-breeding studies to be increasing the seed numbers and hybrid plant populations. The 'Red Globe' cultivar is recommended as a mother plant for the development of new table grapes due to the large berry size and high seed germination ratio. On the other hand, since 'Çeliksu' and 'Cayuga White' is highly resistant to fungal diseases, it can be used as the mother plant in the development of fungal disease grapes in the future studies.

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Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on live animals or humans.

Author Contribution Rates

Design of the Study: BK (50%), YU(30%), FT(20%)

Data Acquisition: BK(20%), YU(30%), KB (30%), FT(20%)

Data Analysis: BK(60%), YU(40%)

Writing of the Article: BK(70%), YU(30%)

Submission and Revision: BK(70%),YU(30%)

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