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A grape breeding programme using different *Vitis* species

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Abstract

Intensive breeding programmes have been carried out to obtain high-quality seedless grape cultivars. Seedless grape cultivars were used as pollinizer parents, but the number of hybrid seeds and the viability of the seed obtained could differ greatly. In this study, carried out over 3 years, the results obtained from crossing with some pollinizer cultivars belonging to different *Vitis* species were compared. Maternal cultivars that do not need emasculation (male sterile) and emasculated cultivars were also compared. Evaluations were based on the total number of hybrid seeds and the proportion of viable seeds. In addition, the effects of various treatments on the rates of germination of the hybrid seed were compared. Although the female parents (with female flower structure) produced more seed than did hermaphrodite mothers in 2019, different results were obtained in cross-breeding studies in 2018 and 2020. Cold stratification was very effective in increasing germination by breaking dormancy of the hybrid seed. In addition, after treatment of cold moist storage (cold stratification), keeping the seed in distilled water overnight before planting increased germination. Although large numbers of seeds were produced by the genotype 86/1 and the cultivar 'Beyaz Çavuş', both of which do not require emasculation, many of these seeds did not germinate. Crosses with the cultivar 'Red Globe' as the maternal parent produced large numbers of viable seeds and high germination rates.

KEYWORDS
cold stratification, crosses, GA_3, hybrid seeds, *Vitis* spp

1 | INTRODUCTION

The genus *Vitis* comprises about 60 species, plus some natural interspecific hybrids (Wen, 2007). Nearly all grapes cultivated for fruit production belong to the species *Vitis vinifera* or are hybrids that include *V. vinifera* in their parentage. Interspecific hybrid cultivars, which are selected from crosses of *V. vinifera* with other species, including *V. labrusca*, *V. riparia*, *V. rupestris*, *V. amurensis* and *V. aestivalis*, are important locally but are mostly minor components of world viticulture (Eibach & Töpfer, 2015). In different countries of the world, grapes are grown for different purposes, most commonly for production of table grapes, wine and raisins. Table grape cultivation is of great importance for Turkey and some other countries. There are different cultivars of seeded and seedless table grapes. The seedless *V. vinifera* table grape market has exploded over the past 50 years, and consumers are much more accepting new cultivars because they do not rely as much on name recognition as they do on visual and sensory appeal (Ibanez et al., 2015).

Controlled grape breeding is thought to have started almost 200 years ago. The birth of modern grape breeding is strongly connected to the arrival of North American diseases and insects in Europe (Paul, 1996). In successive waves in the mid-19th century phylloxera (*Daktulosphaira vitifoliae* Fitch), powdery mildew (*Uncinula necator* Burr), downy mildew (*Plasmopara viticola* Berl.) and black rot (*Guignardia bidwellii* Ellis) arrived in European vineyards where they caused substantial losses of the highly susceptible *V. vinifera* vines then planted. Although *V. vinifera* grapes constitute over 95% of the world market, they are, in general, highly susceptible to many diseases. It is expected that as interest in organic and sustainable viticulture increases, demand for resistant cultivars will increase (Reisch et al., 2012).

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The table grape market responds rapidly to changing consumer demands. Breeding programmes are carried out in different countries to meet these demands. Projects are carried out for different aims, especially development of seedless, larger berried, early/late harvest and disease resistant/tolerant cultivars. In recent years, with increasing consumer awareness, there has been increasing demand for more disease-resistant cultivars that are environmentally friendly and require less spraying (Atak & Kahraman, 2014; Burger et al., 2009; Perl et al., 2000). During the last 50 years, Japan and Korea have led the way in the development of large-berried, seeded and seedless table grapes. Most have large berries because they are tetraploid, leading to an increase in cell size and a resulting increase in berry size. Cultivars, such as 'Kyoho', 'Pione', 'Olympia' and 'Heukgoosul', are examples of seeded tetraploid cultivars grown in Asia. All originated from tetraploid crosses. There are also recent examples of triploid seedless cultivars, such as 'Honey Seedless', 'King Dela' and 'Mirei' (Morinaga, 2001).

Because pollination is required for seed development the development and viability rates of the hybrid seeds obtained may vary according to the pollination technique used, the quality of the pollen and the cultivar. Fruit set occurs at different rates depending on the pollen source used in pollination of hermaphrodite flowering vines where both foreign pollination and self-fertilization are possible (Cadot et al., 2006).

Pollinizer cultivars play an important role in grape yield and quality. The pollinizer (male) cultivar can significantly affect the characteristics of the berry and seed of the mother (female) cultivar. These issues must be considered when choosing hybrid combinations (Sabır, 2015).

Rates of the germination of the hybrid seeds developed from breeding programmes can vary. Although high rates of germination have been obtained in some studies (Uzun et al., 2019), very low rates were obtained in other studies (Conner, 2008; Sun, 2011). Among the reasons suggested for this variation, dormancy seems particularly common. Although environmental conditions such as temperature, humidity, oxygen and light can be suitable for the germination of the seed of many fruit species, they still do not germinate because of dormancy (Penfield, 2017). Dormancy can be broken by removing or softening the layers surrounding the embryo. Even so, optimum conditions are still required for good germination (Arabacı et al., 2014).

Although only water is required for the seeds of some plant species to germinate, environmental factors such as light, temperature, soil conditions can influence germination in other species (Humphries et al., 2018; Travlos et al., 2020). Plant growth regulators such as gibberellic acid (GA) and abscisic acid (ABA), especially in seeds, have been reported to have important effects on germination. Some treatments are very effective in overcoming dormancy. Such treatments include cold stratification (CS), viability testing, pre-sowing soaking, treatment with growth regulators, acid etching, soaking in vitamins, nutrients or osmotic solutions, sowing in gel after germination, coating and banding (Ercişli et al., 1999; Hartmann et al., 1990; Hilhorst & Karssen, 1992; Yamaguchi & Kamiya, 2002). CS, moist stratification, soaking and GA_3 treatments, in particular, can increase rates of germination.

Grape seeds are dormant and are therefore stratified when moist under controlled temperatures (usually around 4°C) for 3–4 months, followed by germination at around 25°C. Treatment with plant growth regulators is sometimes recommended to overcome dormancy. Spiegel-Roy et al. (1987) proposed cyanamide, whereas Manivel and Weaver (1974) suggested gibberellic acid (GA_3). Ellis et al. (1983) proposed the following procedure for grape seed germination: 24 h soaking in H_2O_2 (.5 M), a further 24 h soaking in 1,000 ppm GA_3, followed by a 21-day pre-chilling at 3–5°C with germination under a diurnal alternating temperature regime of 20/30°C (16/8 h).

In this study, the results obtained from the crossbreeding studies with grape cultivars belonging to different species are described. The effects of emasculation or crosses that do not involve emasculation (physiologically female) on rates of obtaining viable seed production were studied over 3 years. The effects of cold moist stratification and different doses of GA_3 treatments on germination of seeds of hybrid F_1 genotypes obtained from different parents were also investigated.

2 | MATERIAL AND METHODS

2.1 | Plant material

This study was carried out in the research vineyard, laboratories and germination greenhouse of the Yalova Atatürk Horticulture Central Research Institute. Twenty-two grape cultivars belonging to different species were used in the 3-year crossbreeding part of the study (Table 1). Of these, 8-grape cultivars were used as the maternal parent and 14 as the father (pollinizer) parent. Using these cultivars, 24 different hybridization combinations were created in the first year, 20 in the second year, and 25 in the third year. In the study, hybridization was undertaken using 15-year-old grapevines grafted on Kober 5 BB rootstock. Viability rates of seeds obtained from these breeding studies, from the 28 different combinations, as well as germination rates as a result of varying treatments, were studied.

2.2 | Emasculation

Flower clusters that were to be used were covered with a special bag to prevent fertilization by external pollen sources. Before grape flowers open, they are covered by a cap (calyptra). The cap and the anthers need to be removed in order to prevent self-pollination. Although the cap eventually dries and comes off on its own, it must be removed a day or two before the cross is to be made to prevent self-pollination in hermaphrodite cultivars. Before the flowers open, the corolla of the flower bud was removed together with the anthers taking care not to damage the female parts. Anthers were removed using forceps with very fine tips. Each flower cluster had a least 100 flowers, so during emasculation, any flower that had opened was removed. Four days after emasculation, the female parts of the flower

TABLE 1 Grape cultivars/genotypes used in crossbreeding studies and their characteristics

Cultivar/genotype	Species	Male/female parent	Seeded/seedless	Berry colour
Kishmish Vatkana	*Vitis vinifera*	Male (pollinizer)	Seedless	Black
Crimson seedless	*Vitis vinifera*	Male (pollinizer)	Seedless	Red
Superior seedless	*Vitis vinifera*	Male (pollinizer)	Seedless	Yellow
Autumn Royal	*Vitis vinifera*	Male (pollinizer)	Seedless	Black
Samancı seedless	*Vitis vinifera*	Male (pollinizer)	Seedless	Yellow
Fantasy seedless	*Vitis vinifera*	Male (pollinizer)	Seedless	Black
Yalova seedless	*Vitis vinifera*	Male (pollinizer)	Seedless	Yellow
Bronx seedless	Interspecific	Male (pollinizer)	Seedless	Black
Ergin seedless	*Vitis vinifera*	Male (pollinizer)	Seedless	Yellow
Ruby seedless	*Vitis vinifera*	Male (pollinizer)	Seedless	Red
Mars seedless	Interspecific	Male (pollinizer)	Seedless	Black
Exalta	*Vitis vinifera*	Male (pollinizer)	Seedless	Yellow
Glenora	Interspecific	Male (pollinizer)	Seedless	Red
Beauty seedless	*Vitis vinifera*	Male (pollinizer)	Seedless	Red
Beyaz Çavuş[a]	*Vitis vinifera*	Female	Seeded	Yellow
Köfteci	*Vitis labrusca*	Female	Seeded	Black
Red Globe	*Vitis vinifera*	Female	Seeded	Dark red
Atasarısı	*Vitis vinifera*	Female	Seeded	Yellow
Yalova Beyazı	*Vitis vinifera*	Female	Seeded	Yellow
Muscat Bailey	Interspecific	Female	Seeded	Black
86/1[a]	*Vitis vinifera*	Female	Seeded	Yellow
Kyoho	Interspecific	Female	Seeded	Black

[a]Imperfect (female) flower.

were mature and ready for pollination. Maturity is often indicated by the appearance of a bead of moisture on the stigma.

If the female cultivar produces female (imperfect) flowers, no emasculation is necessary. In our study, because both the cultivar 'Beyaz Çavuş' and the genotype 86/1 had female flowers, emasculation was not needed and their flowers were simply bagged until they were ready for pollination. To check that the pollen of 'Beyaz Çavuş' and the genotype 86/1 was indeed sterile, 10 flower clusters of each were bagged and then pollinated with their own pollen. No berry or seed formation resulted.

2.3 | Pollen collection

Pollen was collected each year because fresh pollen is always more effective in crossbreeding. Because some pollinizer cultivars flower late in the season in Yalova, flowers of these cultivars were instead obtained from Manisa Viticulture Research Institute in the south in the same year. Flower clusters of the pollinizer cultivars were collected at full bloom and held in plastic bags for immediate treatment. In the laboratory, pollen was separated from other components by passing through a sieve. After being kept at room temperature for overnight, during which it dried into powder, pollen was stored in jars with desiccant silica gel. Pollen was held at +4°C until used.

2.4 | Pollination

The clusters that were bagged after emasculation were frequently checked and pollinated with pollen collected from the seedless pollinizer cultivars when sugary liquid was seen on the stigma. This was the optimal time for pollination. Because the sugary liquid disappears during the very sunny, hot hours of the day, pollination was undertaken early in the morning. Pollen of the paternal cultivar were first brushed on the stigma of the maternal cultivar, and then the nylon bag containing the pollen was closed around the cluster and shaken to provide pollen. This process was repeated several times during the flowering period. Brushes and hands were sterilized with 70% ethanol between pollinations. After pollination, the clusters were again bagged and labelled with the details of the maternal and paternal cultivars and pollination dates. After 2–3 weeks, depending on the combination, the pollination bags were removed and were replaced by net bags to protect the developing berries, especially against bird damage. Vines were subjected to regular cultural practices and sprays.

2.5 | Harvest

Seeds were harvested when the grapes were ripe. Harvesting could have been earlier because the embryos of the seeds are fully developed early in veraison. However, harvesting when the grapes are fully

ripe is more convenient, especially for the separation of the seed from the fruit flesh. Moreover, seed germination rates increase, starting from veraison, and reach a maximum when the grapes are physiologically ripe (Eibach & Töpfer, 2015). Depending on the cross, grapes were harvested between the third week of August and the last week of September. Fruit harvested had to be at a minimum of 20°Brix with the colour fully developed. The clusters of harvested grapes were brought to the laboratory, the berries were separated from the cluster skeleton, and the seeds were removed by sieving. The seeds were washed with distilled water to separate them from other fruit components and then left to dry on blotting paper for several days.

2.6 | Viability test

To separate non-viable seed from those that are viable, the seeds were placed in a small beaker of distilled water. Non-viable seeds tend to float; those that sink are considered viable. Percentage viability rates were calculated:

$$\%\text{Viability rate} = (100 \times \text{viable seed})/(\text{viable seed} + \text{non-viable seed})$$

Viable seeds were cleaned of any remaining pulp, were dried and stored at +4°C.

2.7 | Cold stratification

The viable seeds, along with a dilute solution of fungicide (MAXIM), were placed in a zip plastic bag containing moist perlite and held in a refrigerator (+4°C) for 100 days.

2.8 | Gibberellic acid (GA_3) treatment

The following treatments were imposed to determine the effects of GA_3 on seed germination rates:

- Control (100-day CS);
- CS + 1 night soaking in distilled water containing no GA_3;
- CS + 1 night soaking in distilled water containing 1,000-ppm GA_3;
- CS + 1 night soaking in distilled water containing 3,000-ppm GA_3.

There were three replicates for each treatment and 40 seeds for each replicate.

2.9 | Germination studies

Seeds were sown in a plastic vial (top diameter 6 cm, bottom diameter 5 cm, depth 7 cm) filled with sterile peat to the top. Seeds were planted at a depth of about 1–2 cm. After planting, the vials were watered, and the excess water was allowed to drain away. Because the number of viable seeds obtained from the combinations was limited, 40 seeds were planted in each vial. Each vial was treated as a replicate, so germination evaluation was made on a total of 120 viable seeds for each application. Vials were then placed in plastic containers (50 × 60 × 30 cm). The plastic containers were closed and placed in a germination chamber at a temperature of 25–27°C and relative humidity of 50%–60%. Weekly fungicide treatments were applied. The temperature and humidity of the environment in the glass house were regularly monitored. After the first seed had germinated, germinated seeds were counted every day. Seeds appearing on the surface of the hypocotyl fold were considered to have germinated.

2.10 | Statistical analysis

In order to test the results of the research, in determining the viability rates depending on the combination in the crossbreeding studies, % viability rate was taken as a basis. However, variance analysis was applied for the determination of germination rate. Two factorial and one-way analysis of variance (ANOVA) was performed to determine differences among means of treatments and combinations. After factorial ANOVA, Tukey multiple comparison test was carried out to determine different treatments. The differences determined at a 5% confidence interval ($P < .05$). For statistical analysis, JMP 13 (SAS Institute, 2020) statistical package program was used.

3 | RESULTS

In 2018, 24 different crosses were made (Table 2). In addition to the cultivar 'Beyaz Çavuş', which does not require emasculation because its pollen is sterile, 68 clusters were emasculated. Crossbreeding studies were carried out on a total of 99 clusters, of which 13 clusters from different crosses failed to develop fruit and seed. In total, 4,492 hybrid seeds were obtained from the remaining clusters, of which 1,508 were determined to be viable and 2,984 to be non-viable. The average percentage viability was 33.6%, the average number of seed per bunch was 44.4, and the average numbers of viable seed per bunch and of non-viable seed per bunch were 17.6 and 27.5, respectively.

More hybrid seeds were obtained from crosses where emasculation was not required and the cultivar 'Beyaz Çavuş' was used as the maternal parent. However, seed viability varied between 5.5% and 53.8%. In crosses in which the cultivars 'Red Globe' and 'Atasarısı' were the maternal parents, more than 80% of the seeds were viable despite fewer seeds being obtained. Seed viability was very low in combinations in which the interspecific hybrid 'Kyoho' (tetraploid, 4*n*) was the maternal parent. In crosses with the cultivar 'Köfteci' (*V. labrusca*), seed viability varied between 9.6% and 85.3% depending on the pollinizer cultivar (Table 2).

Crossbreeding studies continued with 20 different crosses in 2019 (Table 3). A total of 191 clusters was used of which 18 failed for various reasons and 173 were harvested. Except for 'Beyaz Çavuş'

TABLE 2 Hybrid combinations and viable seed rates in 2018

Female parent	Male parent	Bunch number		Seed number		% Viability	Total number of seed per bunch	Number of viable seed per bunch	Number of non-viable per bunch
		After pollination	Before harvest	Viable	Non-viable				
Köfteci	Kishmish Vatkana	5	5	34	69	33.0	20.6	6.8	13.8
Köfteci	Crimson seedless	3	3	161	180	47.2	113.7	53.7	60.0
Köfteci	Superior seedless	3	3	80	37	68.4	39.0	26.7	12.3
Köfteci	Autumn Royal	4	4	27	254	9.6	70.3	6.8	63.5
Köfteci	Samancı seedless	2	2	107	24	81.7	65.5	53.5	12.0
Köfteci	Fantasy seedless	2	2	20	8	71.4	14.0	10.0	4.0
Köfteci	Yalova seedless	2	2	65	130	33.3	97.5	32.5	65.0
Köfteci	Bronx seedless	1	1	40	63	38.8	103.0	40.0	63.0
Beyaz Çavuş[a]	Kishmish Vatkana	5	5	134	115	53.8	49.8	26.8	23.0
Beyaz Çavuş[a]	Crimson seedless	6	6	190	702	21.3	148.7	31.7	117.0
Beyaz Çavuş[a]	Ergin seedless	6	6	164	412	28.5	96.0	27.3	68.7
Beyaz Çavuş[a]	Autumn Royal	4	4	9	154	5.5	40.8	2.3	38.5
Beyaz Çavuş[a]	Yalova seedless	10	10	237	685	25.7	92.2	23.7	68.5
Red Globe	Kishmish Vatkana	5	4	99	17	85.3	29.0	24.8	4.3
Red Globe	Crimson seedless	5	5	88	17	83.8	21.0	17.6	3.4
Kyoho	Kishmish Vatkana	5	4	4	20	16.7	6.0	1.0	5.0
Kyoho	Crimson seedless	5	2	5	45	10.0	25.0	2.5	22.5
Kyoho	Superior seedless	6	4	6	33	15.4	9.8	1.5	8.3
Atasarısı	Autumn Royal	3	2	–	1	0.0	0.0	–	0.5
Atasarısı	Yalova seedless	4	3	6	8	42.9	4.7	2.0	2.7
Atasarısı	Crimson seedless	4	3	23	4	85.2	9.0	7.7	1.3
Atasarısı	Kishmish Vatkana	4	1	4	1	80.0	5.0	4.0	1.0
Yalova Beyazı	Samancı seedless	3	3	2	2	50.0	1.3	0.7	0.7
Yalova Beyazı	Fantasy seedless	2	2	3	3	50.0	3.0	1.5	1.5
Total		99	86	1,508	2,984	–	–	–	–
Average		4.1	3.6	62.8	1,24.3	33.6	44.4	17.6	27.5

[a]Imperfect (female) flower.

TABLE 3 Hybrid combinations and viable seed rates in 2019

Female parent	Male parent	Bunch number		Seed number		% Viability	Total number of seed per bunch	Number of viable seed per bunch	Number of non-viable per bunch
		After pollination	Before harvest	Viable	Non-viable				
Köfteci	Crimson seedless	10	10	499	400	55.5	89.9	49.9	40.0
Köfteci	Superior seedless	5	5	169	301	36.0	94.0	33.8	60.2
Köfteci	Bronx seedless	10	10	430	428	50.1	85.8	43.0	42.8
Köfteci	Ruby seedless	10	8	95	194	32.9	36.1	11.9	24.3
Köfteci	Beauty seedless	10	10	795	514	60.7	130.9	79.5	51.4
Köfteci	Yalova seedless	6	5	155	329	32.0	96.8	31.0	65.8
Beyaz Çavuş[a]	Bronx seedless	11	11	2,304	179	92.8	225.7	209.5	16.3
Beyaz Çavuş[a]	Crimson seedless	10	10	2,557	142	94.7	269.9	255.7	14.2
Beyaz Çavuş[a]	Mars seedless	11	11	1,952	152	92.8	191.3	177.5	13.8
Beyaz Çavuş[a]	Glenora	12	12	2,095	388	84.4	206.9	174.6	32.3
Beyaz Çavuş[a]	Yalova seedless	15	15	2,314	407	85.0	181.4	154.3	27.1
Red Globe	Ruby seedless	7	5	73	13	84.9	17.2	14.6	2.6
Red Globe	Yalova seedless	10	9	808	38	95.5	94.0	89.8	4.2
Red Globe	Crimson seedless	8	8	403	36	91.8	54.9	50.4	4.5
Muscat Bailey	Yalova seedless	5	5	181	83	68.6	52.8	36.2	16.6
Kyoho	Superior seedless	11	6	9	10	47.4	3.2	1.5	1.7
Kyoho	Yalova seedless	10	3	3	48	5.9	17.0	1.0	16.0
86/1a	Ruby seedless	10	10	1,839	29	98.4	186.8	183.9	2.9
86/1a	Beauty seedless	10	10	1,856	52	97.3	190.8	185.6	5.2
86/1a	Bronx seedless	10	10	2,108	82	96.3	219.0	210.8	8.2
Total		191	173	20,645	3,825	–	–	–	–
Average		9.6	8.7	1,032.3	1,91.3	84.4	117.1	93.9	23.3

[a]Imperfect (female) flower.

and the genotype 86/1, both of which do not need emasculation, 82 clusters were emasculated. In total, 24.470 hybrid seeds were obtained from the remaining clusters, of which 20,645 hybrid seeds were determined as being viable and 3,825 hybrid seeds as non-viable. The average seed viability was 84.4%, the average number of seed per bunch was 117.1, and the average numbers of viable seeds per bunch and of non-viable seeds per bunch were 93.9 and 23.3, respectively.

More viable hybrid seed was obtained in 2019. This was also the most successful year in terms of the overall average number of viable seed from the crosses. The highest seed viability came from crosses in which genotype 86/1, 'Red Globe' and 'Beyaz Çavuş' were used as maternal parents. Seed viability rates between 32% and 6.7% were obtained in crosses with 'Köfteci' (*V. labrusca*), depending on the pollinizer cultivar. In 2019, both the number of seeds obtained and the number of viable seeds remained very low in crosses in which 'Kyoho' was the maternal parent (Table 3).

Crossbreeding studies continued with 25 different crosses in 2020 (Table 4). A total of 174 clusters were used, of which 13 failed for various reasons and 161 were harvested. The flowers of 'Köfteci', 'Red Globe' and 'Muscat Bailey' were emasculated, but those of 'Beyaz Çavuş' and genotype 86/1 were not. In total, 9,904 hybrid seeds were obtained in 2020, of which 5,756 were determined to be viable and 4,148 to be non-viable. The average percentage seed viability was 58.1%. The average number of seeds per bunch, the average of number of viable seeds per bunch and the number of non-viable seeds per bunch were 61.3, 34.9 and 26.3, respectively.

The results of 2020 were similar to those of 2019, especially in the successful crosses with high rates of seed viability. The cultivars 'Red Globe' and 'Beyaz Çavuş' gave the highest rates of seed viability. The crosses with 'Köfteci' (*V. labrusca*) gave seed viability rates between 19.3% and 64.5% depending on the pollinizer cultivar. In 2020, seed viability rates varied in 'Muscat Bailey' and 86/1 depending on the pollinizer cultivar (Table 4).

Over the 3 years of study, the highest number of hybrid seeds (24,470) and the highest number of viable seeds (20,645) were obtained in 2019. In terms of the average number of viable seeds obtained from all crosses, the highest success (84.4%) was also achieved in 2019. The year with the lowest number of hybrid seeds (4,492) was 2018, which was also the year with the lowest number of viable seeds (1,508) and the lowest average seed viability (33.6%). The cross with the highest percentage of viable seed (98.4%) was 86/1 X 'Ruby Seedless' in 2019. The cross with the largest number (2,557) of viable hybrid seeds was 'Beyaz Çavuş' X 'Crimson Seedless' in 2019.

Over the 3 years of study, the most successful maternal parent, in terms of the seed viability of the F_1 hybrids, was 'Red Globe' (85.8%) (Table 5), followed by 'Beyaz Çavuş' and the genotype 86/1. 'Beyaz Çavuş' and 86/1 do not need to be emasculated because their pollen is sterile. The lowest seed viability was obtained when the interspecific hybrid 'Kyoho', which is tetraploid, was the maternal parent. When diploid and tetraploid grape cultivars are crossed, the hybrids produced may be triploid or contain unbalanced numbers of chromosomes, resulting in high proportions of non-viable seed. Embryo rescue techniques may prevent the early abortion of young triploid embryos, allowing triploid plants to be produced. However, the results of this tetraploid by diploid cross varied from year to year, possibly because of differing climatic conditions each year, pollen source or success in emasculation.

Germination studies were carried out for 2 years with viable hybrid seed from different crosses (Tables 6 and 7). The effects of CS, soaking and GA_3 treatments on germination in hybrid grape seed were investigated. In the 2018 studies, germination rates were compared over 10 different crosses and four different treatments. In the 2019 germination studies, the most successful crosses were those in which 'Köfteci' was the maternal parent and later crosses in which 'Red Globe' was the maternal parent. Seed from crosses of 'Köfteci' (*V. labrusca*) and 'Crimson Seedless' gave the highest germination rates. Seed from other crosses with 'Köfteci' gave particularly high rates of germination. Crosses with 'Beyaz Çavuş' as the maternal parent resulted in the lowest germination rates, with one exception. The highest germination rates were obtained from hybrid seed subjected to CS and soaking in distilled water. On average, about half (54%) of all hybrid seeds planted in 2018 germinated (Table 6).

Germination studies were carried out with hybrid genotypes obtained from 18 different crosses carried out in 2019. Very high rates of germination were obtained for seed produced in all crosses with 'Köfteci' as the maternal parent, except for the cross 'Köfteci' X 'Ruby Seedless'. In the only cross using 'Muscat Bailey', the seed produced showed very high germination rates in all except the control treatment. In addition, seed from crosses in which 'Red Globe' was the maternal parent gave high rates of germination. Germination rates remained much lower in crosses with the genotype 86/1. As in the hybrids produced in 2018, seed obtained in 2019 from the cross with 'B. Çavuş' germinated very poorly.

The seed produced by crosses in 2018 and 2019 responded similarly to germination treatments. Germination was best in hybrid seed that had been subjected to CS and soaking in distilled water. CS + soaking in 1,000-ppm GA_3 treatment was second-best followed by CS alone (only CS). On average, less than half (42%) the sown hybrid seed germinated (Table 7).

4 | DISCUSSION

This study contains data over 3 years from a breeding programme with the aim of developing new seedless grape cultivars that are increasingly popular all over the world. Parents were chosen in order to obtain cultivars that have particularly large fruit and are tolerant of the main fungal diseases. Breeding programmes with grapes and some other plant species show that the results may differ according to year, species/cultivar genotype, ploidy and emasculation technique (Perl et al., 2000; Sun et al., 2011; Tounekti et al., 2018).

In both years, crosses with the cultivar 'Kyoho', a hybrid that is tetraploid, yielded only low numbers of seeds and low viability rates, even though different pollinizers were tested. Similar results have

TABLE 4 Hybrid combinations and viable seed rates in 2020

Female parent	Male parent	Bunch number		Seed number		% Viability	Total number of seed per bunch	Number of viable seed per bunch	Number of non-viable per bunch
		After pollination	Before harvest	Viable	Non-viable				
Köfteci	Yalova seedless	6	6	244	1021	19.3	210.8	40.7	170.2
Köfteci	Ruby seedless	6	6	276	780	26.1	176.0	46.0	130.0
Köfteci	Bronx seedless	8	8	411	689	37.4	137.5	51.4	86.1
Köfteci	Exalta	6	6	321	286	52.9	101.2	53.5	47.7
Köfteci	Beauty seedless	6	6	423	233	64.5	109.3	70.5	38.8
Beyaz Çavuş[a]	Yalova seedless	12	11	852	268	76.1	101.8	77.5	24.4
Beyaz Çavuş[a]	Ruby seedless	10	9	492	110	81.7	66.9	54.7	12.2
Beyaz Çavuş[a]	Exalta	10	2	30	22	57.7	26.0	15.0	11.0
Beyaz Çavuş[a]	Beauty seedless	10	9	270	62	81.3	36.9	30.0	6.9
Beyaz Çavuş[a]	Bronx seedless	9	9	701	57	92.5	84.2	77.9	6.3
Red Globe	Bronx seedless	4	4	518	82	86.3	150.0	129.5	20.5
Red Globe	Kishmish Vatkana	5	4	7	6	53.8	3.3	1.8	1.5
Red Globe	Yalova seedless	5	5	127	10	92.7	27.4	25.4	2.0
Red Globe	Mars seedless	5	5	171	17	91.0	37.6	34.2	3.4
Red Globe	Crimson seedless	4	4	268	44	85.9	78.0	67.0	11.0
Muscat Bailey	Yalova seedless	4	4	43	17	71.7	15.0	10.8	4.3
Muscat Bailey	Bronx seedless	4	4	127	26	83.0	38.3	31.8	6.5
Muscat Bailey	Kishmish Vatkana	4	4	2	148	1.3	37.5	0.5	37.0
Muscat Bailey	Ruby seedless	4	4	31	35	47.0	16.5	7.8	8.8
Muscat Bailey	Beauty seedless	3	3	13	25	34.2	12.7	4.3	8.3
86/1[a]	Ruby seedless	10	10	97	100	49.2	19.7	9.7	10.0
86/1[a]	Bronx seedless	10	10	179	14	92.7	19.3	17.9	1.4
86/1[a]	Yalova seedless	10	10	146	36	80.2	18.2	14.6	3.6
86/1[a]	Beauty seedless	9	9	6	30	16.7	4.0	0.7	3.3
86/1[a]	Exalta	10	9	1	30	3.2	3.4	0.1	3.3
Total		174	161	5,756	4,148	–	–	–	–
Average		7.0	6.4	2,30.2	1,65.9	58.1	61.3	34.9	26.3

[a]Imperfect (female) flower.

TABLE 5 Three-year viable seed rates of the maternal parents

Female parent	Year	Bunch number		Seed number		% Viability	Total number of seed per bunch	Number of viable seed per bunch	Number of non-viable per bunch
		After pollination	Before harvest	Viable	Non-viable				
Köfteci	2018	2.8	2.8	66.8	95.6	47.9	65.4	28.7	36.7
	2019	8.5	8.0	357.2	361.0	44.5	88.9	41.5	47.4
	2020	6.4	6.4	335.0	601.8	40.0	147.0	52.4	94.6
	Average	5.9	5.7	253.0	352.8	44.2	100.4	40.9	59.6
Beyaz Çavuş[a]	2018	6.2	6.2	146.8	413.6	27.0	85.5	22.4	63.1
	2019	11.8	11.8	2244.4	253.6	89.9	215.0	194.3	20.8
	2020	10.2	8.0	469.0	103.8	77.9	63.2	51.0	12.2
	Average	9.4	8.7	953.4	257.0	64.9	121.2	89.2	32.0
Red Globe	2018	5.0	4.5	93.5	17.0	84.6	25.0	21.2	3.8
	2019	8.3	7.3	428.0	29.0	90.7	55.4	51.6	3.8
	2020	4.6	4.4	218.2	31.8	81.9	59.3	51.6	7.7
	Average	6.0	5.4	246.6	25.9	85.8	46.5	41.4	5.1
Kyoho	2018	5.3	3.3	5.0	32.7	14.0	13.6	1.7	11.9
	2019	10.5	4.5	6.0	29.0	26.6	10.1	1.3	8.8
	2020	NC	NC	NC	NC	NC	NC	NC	NC
	Average	7.9	3.9	5.5	30.8	20.3	11.8	1.5	10.4
86/1[a]	2018	NC	NC	NC	NC	NC	NC	NC	NC
	2019	10.0	10.0	1934.3	54.3	97.3	198.9	193.4	5.4
	2020	9.8	9.6	85.8	42.0	48.4	12.9	8.6	4.3
	Average	9.9	9.8	1010.1	48.2	72.9	105.9	101.0	4.9
Muscat Bailey	2018	NC	NC	NC	NC	NC	NC	NC	NC
	2019	5.0	5.0	181.0	83.0	68.6	52.8	36.2	16.6
	2020	3.8	3.8	43.2	50.2	47.4	24.0	11.0	13.0
	Average	4.4	4.4	112.1	66.6	58.0	38.4	23.6	14.8
Atasarısı	2018	3.8	2.3	11.0	3.5	52.0	4.7	4.6	1.4
	2019	NC	NC	NC	NC	NC	NC	NC	NC
	2020	NC	NC	NC	NC	NC	NC	NC	NC
	Average	3.8	2.3	11.0	3.5	52.0	4.7	4.6	1.4
Yalova Beyazı	2018	2.5	2.5	2.5	2.5	50	2.2	1.1	1.1
	2019	NC	NC	NC	NC	NC	NC	NC	NC
	2020	NC	NC	NC	NC	NC	NC	NC	NC
	Average	2.5	2.5	2.5	2.5	50.0	2.2	1.1	1.1

Abbreviation: NC, no cross (no crossbreeding was done with this cultivar).
[a]Imperfect (female) flower.

TABLE 6 Germination rate of 2018 hybrids with different treatments

Female parent	Male parent	Control (only CS)		CS + soaking (0-ppm GA_3)		CS + soaking (1,000-ppm GA_3)
		Hybrids number (after germination)	Germination (%)	Hybrids number (after germination)	Germination (%)	Hybrids number (after germination)
Köfteci	Crimson S.	36 a	90.0	36 a	90.0	32 b
Köfteci	Superior S.	16 f	40.0	32 b	80.0	35 a
Köfteci	Samancı S.	24 c	60.0	35 a	87.5	21 e
Köfteci	Yalova S.	32 b	80.0	36 a	90.0	18 f
B. Çavuş[a]	Kishmish V	8 g	20.0	24 d	60.0	12 g
B. Çavuş[a]	Crimson S.	12 f	30.0	12 g	30.0	10 h
B. Çavuş[a]	Ergin S.	16 e	40.0	21 e	52.5	24 d
B. Çavuş[a]	Yalova S.	16 e	40.0	19 f	47.5	9 hi
R. Globe	Kishmish V	19 d	47.5	27 c	67.5	8 i
R. Globe	Crimson S.	20 d	50.0	21 e	52.5	28 c
Average (%)			49.8		66	
Total		199 c		263 a		197 b

Note: Variance analysis was applied for the germination rate and the differences determined at a 5% confidence interval ($P < .05$). Letters after the numerals within the columns show significant difference at $\alpha = 0.05$.
[a]Imperfect (female) flower.

TABLE 6 (Continued)

Female parent	CS + soaking (1,000-ppm GA_3)	CS + soaking (3000 ppm GA_3)			
	Germination (%)	Hybrids number (after germination)	Germination (%)	Average (%)	Total
Köfteci	80.0	27 b	67.5	81.9	131 a
Köfteci	87.5	28 b	70.0	69.4	111 b
Köfteci	52.5	30 a	75.0	68.8	110 b
Köfteci	45.0	24 c	60.0	68.8	110 b
B. Çavuş[a]	30.0	12 g	30.0	35.0	56 g
B. Çavuş[a]	25.0	4 h	10.0	23.8	38 h
B. Çavuş[a]	60.0	20 e	50.0	50.6	81 d
B. Çavuş[a]	22.5	16 f	40.0	37.5	60 f
R. Globe	20.0	22 d	55.0	47.5	76 e
R. Globe	70.0	24 c	60.0	58.1	93 c
Average (%)	49.3		51.8	54	
Total		207 b			866

Note: Variance analysis was applied for the germination rate and the differences determined at a 5% confidence interval ($P < .05$). Letters after the numerals within the columns show significant difference at $\alpha = 0.05$.
[a]Imperfect (female) flower.

TABLE 7 Germination rate of 2019 hybrids with different treatments

Female parent	Male parent	Control (only CS)		CS + soaking (0 ppm GA_3)		CS + soaking (1000 ppm GA_3)		CS + soaking (3000 ppm GA_3)		Average (%)	Total
		Hybrids number (after germination)	Germination (%)	Hybrids number (after germination)	Germination (%)	Hybrids number (after germination)	Germination (%)	Hybrids number (after germination)	Germination (%)		
Köfteci	Crimson S.	18 f	45.0	18 h	45.0	17 h	42.5	14 i	35.0	41.9	67 g
Köfteci	Bronx S.	33 b	82.5	33 c	82.5	38 b	95.0	21 g	52.5	78.1	125 b
Köfteci	Ruby S.	2 l	5.0	6 j	15.0	6 l	15.0	3 m	7.5	10.6	17 m
Köfteci	Crimson S.	36 a	90.0	36 b	90.0	32 d	80.0	28 e	70.0	82.5	132 a
Köfteci	Superior S.	16 h	40.0	32 d	80.0	36 c	90.0	32 c	80.0	72.5	116 d
Köfteci	Yalova S.	2 l	5.0	12 i	30.0	21 g	52.5	21 g	52.5	35.0	56 i
B. Çavuş[a]	Bronx S.	0 n	0.0	1 k	2.5	0 m	0.0	0 o	0.0	0.6	1 o
B. Çavuş[a]	Crimson S.	13 i	32.5	18 h	45.0	9 j	22.5	5 l	12.5	28.1	45 k
B. Çavuş[a]	Mars S.	0 n	0.0	6 j	15.0	8 k	20.0	2 n	5.0	10.0	16 n
B. Çavuş[a]	Glenora	0 n	0.0	1 k	2.5	0 m	0.0	0 o	0.0	0.6	1 o
B. Çavuş[a]	Yalova S.	10 k	25.0	12 i	30.0	6 l	15.0	8 k	20.0	22.5	36 l
R. Globe	Yalova S.	26 d	65.0	25 g	62.5	26 e	65.0	30 d	75.0	66.9	107 e
R. Globe	Crimson S.	31 c	77.5	29 e	72.5	24 f	60.0	35 b	87.5	74.4	119 c
R. Globe	Kishmish V.	20 e	50.0	28 f	70.0	8 k	20.0	24 f	60.0	50.0	80 f
86/1[a]	Beauty S.	13 i	32.5	12 i	30.0	17 h	42.5	14 i	35.0	35.0	56 i
86/1[a]	Bronx S.	11 j	27.5	18 h	45.0	12 i	30.0	10 j	25.0	31.9	51 j
86/1[a]	Ruby S.	17 g	42.5	18 h	45.0	9 j	22.5	15 h	37.5	36.9	59 d
M. Bailey	Yalova S.	1 m	2.5	40 a	100.0	40 a	100.0	38 a	95.0	74.4	119 c
Average (%)			34.6		48		42.9		41.7	42	
Total		249 d		345 a		309 b		300 c			1,203

Note: Variance analysis was applied for the germination rate and the differences determined at a 5% confidence interval ($P < .05$). Letters after the numerals within the columns show significant difference at $\alpha = 0.05$.

Abbreviation: CS, cold stratification.

[a]Imperfect (female) flower.

been reported with grape cultivars that are not diploid (Bessho et al., 2000; Guo et al., 2011; Sattler et al., 2016). Furthermore, crosses between different *Vitis* species and *V. vinifera* can result in low numbers of seeds and low viability rates (Doucleff et al., 2004; Lu et al., 2000; Wang et al., 2016).

'Beyaz Çavuş' is known not only for having male-sterile flowers, but also for producing a high proportion of empty seed. Most of the seeds considered to be viable in the viability tests, performed with distilled water, do not germinate. Empty seeds of 'Beyaz Çavuş' are thought to result from zygote or embryo degeneration. The viability tests used separate most of the empty seeds, but not all degenerated hybrid seeds (Çelik & İlbay Karlı, 2003; Ebadi et al., 1996). In addition, as in our study, this situation may vary slightly depending on the pollinizer. Although seed viability appeared to be high in crosses with 'Beyaz Çavuş', germination rates remained very low. Generoso et al. (2019) reported that dormancy in grape seeds slows the progress of many breeding programmes of the crop, culminating in low uniformity and low germination percentages.

Sabır (2011) studied the effects of different male rootstocks as pollinizers of 'Italia' on fruit set, number of seed per fruit, seed viability, seed size, germination rate and germination period. The pollinizer used significantly affected the viability and the germination rate of the hybrid seed. In our study, depending on both the cultivars used as a pollinizer and the female cultivar, the number of seed formed, the viability and the rates of germination varied.

Grape seeds often germinate poorly. Research on germination and seedling growth has been largely the by-product of breeding programmes, rather than systematic physiological studies. *Vitis* seeds have a hard seed coat of variable thickness. Stratification at 0–10°C under moist conditions for about 3 months is essential for quick, uniform and high rates of germination.

Growth regulators affect seed germination positively, but overdoses have detrimental effects on seeds. Researchers (Akkurt et al., 2013; Terzi & Kocaçalışkan, 2010; Uzun et al., 2019) reported that H_2O_2 and GA_3 have positive effects on seed germination of grapes but higher doses than 1,000-ppm GA_3 can cause death of some seeds because of some adverse effects on germination (Conner, 2008). Soaking and soaking + GA_3 treatment have been reported to increase the germination of *Vitis* seed. Soaking and soaking + 1000-ppm GA_3 treatments after approximately 3 months of stratification at 4°C gave the best results (Conner, 2008; Rives, 1965; Seget et al., 2020).

Germination rate is determined genetically to a considerable extent. At the species level in general, most wild species exhibit a higher germination rate than cultivars belonging to *V. vinifera*. In our study, a higher rate was obtained in the germination of hybrids obtained from crosses with 'Köfteci' (*V. labrusca*) than other *V. vinifera* crosses. Germination rates of *V. vinifera* crosses showed differences in both years. Consistent with these results, Eibach and Töpfer (2015) reported that within *V. vinifera*, there is a huge variation. Also, it has been reported in a study with *V. riparia* seed that the amount of phenolic components in the seeds of different grape species changes during dormancy and affects germination (Wrobel et al., 2005).

V. labrusca cultivars, such as 'Köfteci' used in this study, are widely used in breeding programmes to their resistance to fungal diseases and their intense aroma (Reisch et al., 2012). However, there are differences in the germination of the seed of these cultivars. Similar to the results in our study, it is reported that GA_3 treatments were not very effective in promoting the germination of seed of cultivars of *V. labrusca*; rather germination media such as peat moss, perlite or silt and CS are more critical (Bordelon & Moore, 1994; Çelik, 2001; Toledo-Gaytan et al., 1992).

The female parents with female flower structure produced many more seeds than did hermaphrodite mothers in 2019. Differences were also observed in 2018 and 2020 in the crosses made with 'Beyaz Çavuş' and genotype 86/1, both of which have female flowers. In 2018, 734 viable and 2,068 non-viable seeds were obtained from 31 clusters that did not require emasculation, while 774 viable and 916 non-viable seeds were obtained from 55 clusters that were emasculated. In 2019, although the number of clusters with and without emasculation was very similar, the number of viable seeds obtained was 4.7 times higher in those that were not emasculated. In 2020, 2,774 viable and 729 non-viable seeds were obtained from 88 clusters that did not require emasculation, while 2,982 viable and 3,419 non-viable seeds were obtained from 73 clusters that were emasculated. The number of seeds and the seed viability rates vary according to both the cross and the year. Although a high number of seeds were obtained in some crosses, this number remained quite low in other crosses. Initially, this was thought to be related to the viability of the pollen, but it can be seen that the same pollen was effective in producing seed in other cultivars. Therefore, it seems likely that the problem may be due to some other factors that are important after pollination, rather than pollen viability (Sabır, 2015).

5 | CONCLUSION

Grape breeding studies in recent years have focused on developing larger fruited, seedless and disease-resistant cultivars. In the breeding study started for these purposes, crosses of grape cultivars belonging to different species were carried out for 3 years. In addition, the germination ratios of these hybrid seeds according to crosses were compared by adding some treatments. In these studies, different seedless pollinizer cultivars were used when comparing the female cultivars that require emasculation or not. The success of the obtained results was evaluated according to the total number of hybrid seeds and the number of viable seeds. Although a high number of hybrid seeds of genotype 86/1 and 'Beyaz Çavuş' that do not require emasculation were obtained, many of these seed failed to germinate. When 'Red Globe' was the maternal parent, both the number of viable seeds and germination rates were quite high. In other combinations, variable results were obtained depending on the maternal or paternal parent. In general, the emasculation application reduced the total number of seed, but not the number of viable seeds obtained. In addition, it was concluded that CS treatment of grape seed was very effective in promoting germination by breaking dormancy. The results of this study

will help in conducting appropriate crosses and germination studies by guiding future breeding studies.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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