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Embryo recovery(rescue) studies in different *Vitis* species

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Abstract

Backround In recent years, with the increasing demand for seedless grape varieties that have lower production costs, are disease resistant/tolerant and require less chemical pesticides, the embryo recovery technique has begun to be used more in table grape breeding studies. However, the desired high success rate has not yet been achieved in these studies. Although there are different reasons for this, especially the grape varieties selected for cross-breeding and the timing of transferring the embryos to medium are among the most important reasons. In this study, focusing on these two important factors, the embryos obtained from different hybridization combinations were transferred to agar medium at different weeks for 4 years and the most successful combination and time were determined. In addition, seedless and large berry grape varieties and some seeded varieties that are resistant/tolerant to fungal diseases were selected as parents because they can provide resistance to disease infections in vitro and thus increase the success rate.

Results The results obtained from the study showed that the selected variety and combination significantly affected the success rate in embryo rescue. Especially in combinations with the 'Yalova Seedless' variety as the female parent, more successful results were obtained compared to combinations of other varieties. When 'Yalova Seedless' variety was pollinated with pollen of 'Red Globe', 'Muscat Bailey A' and 'Exalta' varieties, more seedlings were obtained with the help of embryo rescue. The results obtained over four years showed that the best sampling time after pollination was the eighth week and then the seventh week.

Conclusions According to the results obtained, it has been shown that the selected varieties and the sampling time significantly affect the success rate in embryo rescue studies. Therefore, higher success rates can be achieved in comprehensive breeding studies in which they will be included as pollinators, especially in different seeded varieties that are resistant to diseases and have larger berry size.

Keywords Live embryo, Cross-breeding, Weeks, Pollinator, Seedling, Disease resistance

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Introduction

Hundreds of different grape varieties are grown all over the world. However, the number of commercially valuable grape varieties is very limited. In addition, since consumer demands change very rapidly, breeding studies are carried out in different parts of the world to develop new grape varieties that meet these demands. One of the main purposes of these breeding studies is to develop new seedless grape varieties that meet consumer expectations [1]. Using stenospermocarpic grapes as female parents could improve the seedless ratio of offspring via embryo rescue techniques, so they are widely used for breeding seedless grapes [2].

In recent years, the inclusion of embryo rescue techniques in grape breeding studies has made significant contributions to obtaining more seedless and polyploid hybrid genotypes. The embryo recovery technique has previously been tried in different plant species and fruits [3]. In the eighties, this technique began to be used on grapes [4].

The embryo rescue technique is used to obtain seedlings from hybrid embryos of different plant species that have been aborted at certain stages of their development. Embryo rescue has been used successfully not only to facilitate successful crosses but also to obtain haploids, double haploids, and to manipulate ploidy levels for monosomic and disomic insertion as well as chromosome engineering. Additionally, embryo rescue can be used to shorten the breeding cycle and propagate rare plants. In addition, it has been used repeatedly to study stages of embryonic development, especially in embryo-lethal mutants. The most commonly used embryo rescue method is placing immature embryos directly in the culture medium. In some cases, in vitro culture of ovaries, ovaries, or placentas allows the successful development of young embryos from the zygote stage to maturity [5–7].

Embryo rescue is a technique used to turn hybrid embryos, which are intraspecific and interspecific hybrids that cannot survive under normal conditions, into a living plant with the help of in vivo plant growing environments. Particular attention should be paid to hygiene in this technique. The procedure involves removing mature/mature-lethal embryos and culturing them in vitro in a specific nutrient culture medium. Especially depending on the species, climate chambers with different food types and different climatic characteristics are required [8, 9].

The success of obtaining seedless grape embryo rescue depends on many factors. In embryo rescue studies, especially species, variety, polyploidy level, sapling time and environmental conditions can greatly affect the success rate [3]. Therefore, depending on the parents used in the crossbreeding, embryos that have partially completed their development 6–9 weeks after pollination must be placed in artificial nutrient media and continue their development under sterile conditions [10]. Seedless grape varieties developed in recent years with their large and juicy berries and wide adaptations to different ecological environments are widely grown in different countries in Europe, America, and Asia. However, due to their sensitivity to fungal diseases, they must be frequently sprayed. Therefore, in recent years, efforts have been made to develop new resistant and tolerant varieties by including disease-resistant parents in embryo rescue studies [2, 11].

Embryos also need to be cleaned of endosperm and other tissues, possibly to prevent infection. Otherwise, any pathogen source may infect other tissues, first stopping the development of embryos and even terminating their viability [12]. The embryo rescue technique is also used in studies on crossbreeding grapes with different chromosome numbers [13–15]. When diploid grape varieties are used as female parents, it is easier to obtain viable hybrid genotypes [16]. It is also reported that the ovule fertility of tetraploid varieties is lower than that of diploid varieties [14].

When the studies conducted for embryo rescue in grapes are examined, it is stated that the selected parents and the combinations formed can significantly affect the success rate of embryo rescue. In seedless grapes, the maternal (female parent) genotype has an essential effect on saving the embryo and obtaining a live grapevine plant, but It is reported that the pollinator (male parent) variety has a more limited impact on embryo recovery in seedless grapes [17–22]. Although different studies have shown that the sampling time is important for embryo recovery, it is also reported that the success rate varies in different weeks depending on the parent combination [23–27]. It is also reported that the medium used for embryo development, different culture conditions, and plant growth regulators can affect the success rate of embryo rescue [28–34].

While many factors are cited as effective in the success rate of the studies conducted, it is understood from the results that the most prominent of these are the selected species or varieties themselves and that they can be effective in the sampling time. In addition, it has been seen that including different *Vitis* species in these studies in recent years can effectively increase the success rate. Since the selected parents (even the selection of female or male) and the most appropriate sampling time are reported as the most critical factors in the success rate of embryo rescue, we planned a study considering that a comprehensive embryo rescue study on these factors would benefit breeders. In this comprehensive embryo rescue study that we planned, different parent combinations were created considering the conditions

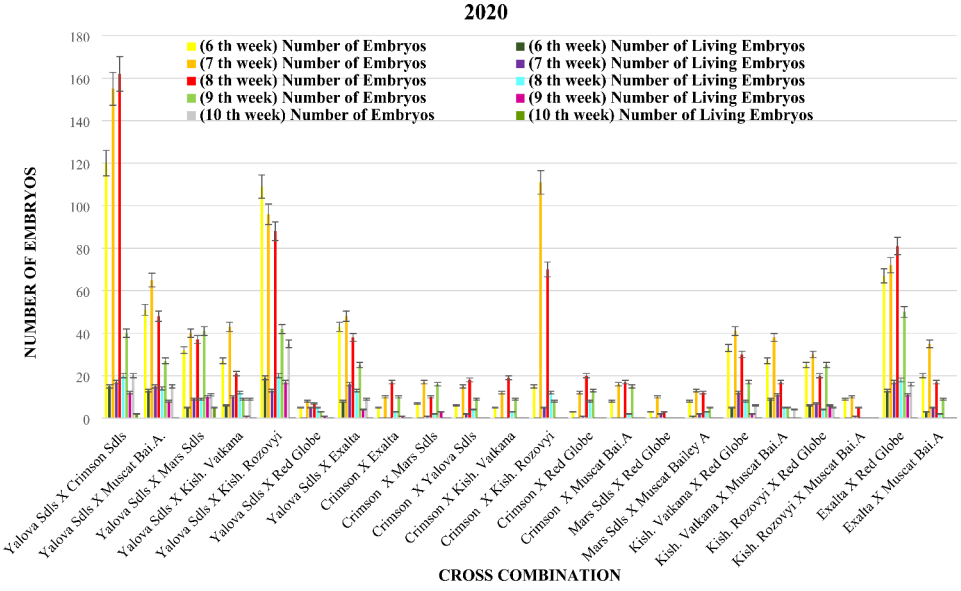

Fig. 1 Total number of planted and developing embryos in 2020 embryo rescue studies

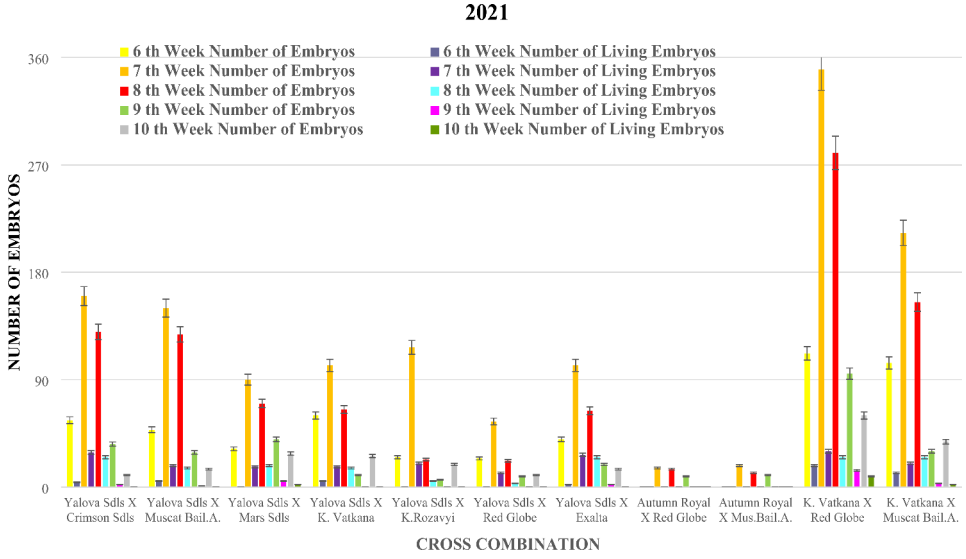

Fig. 2 Total number of planted and developing embryos in 2021 embryo rescue studies

recommended in the literature. Samples were taken for embryo rescue studies between the sixth and tenth weeks after pollination. The effects of different *Vitis* spp. combinations and sampling times on the live embryo rate and the number of grapevine plants have been evaluated in detail within the scope of this study for 4 years.

Results

In 2020, the first year of the study, embryo rescue studies were carried out in 22 different hybrid combinations by taking samples in the sixth and tenth weeks after pollination, and the same studies were repeated in 2021, 2022 and 2023, especially with those that gave the best results among the parents/combinations. Number of ovules planted on agar medium per combination and the number of embryo developed are given in Figs. 1, 2, 3 and 4. Percentage of embryo developed (%) and number of seedling of combinations on a weekly basis in embryo rescue studies are given in Tables 1, 2, 3 and 4.

In embryo rescue studies carried out with 22 different combinations in 2020 (when weeks are evaluated separately), the best results were obtained from embryos belonging to the 'Yalova Seedless X Red Globe' combination, which were planted in E20A medium at the 6th and 7th weeks after pollination. Then, it was obtained from 'Yalova Seedless X Mars Seedless' embryos, which were planted in 10 weeks, and 'Kishmish Vatkana X Muscat Bailey A' embryos, which were planted in 6 weeks,

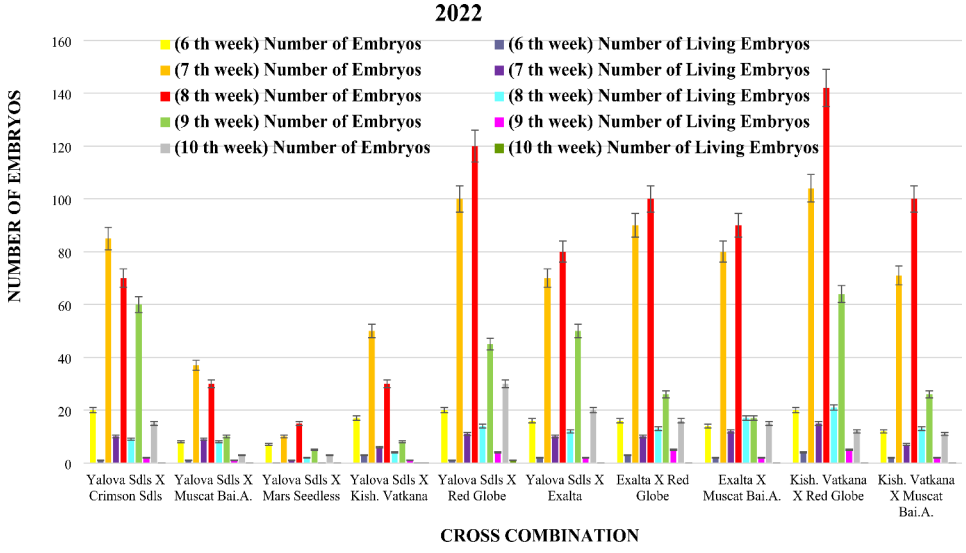

Fig. 3 Total number of planted and developing embryos in 2022 embryo rescue studies

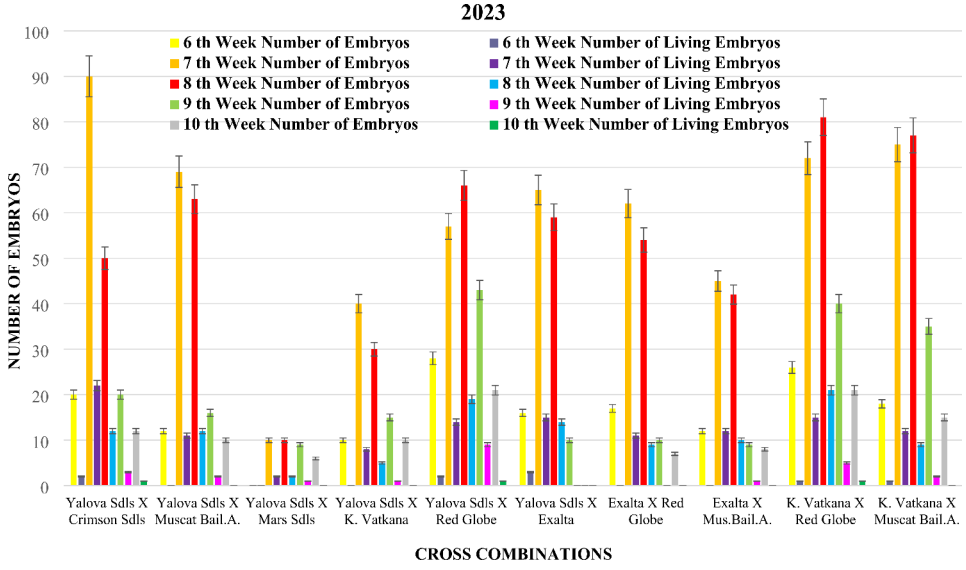

Fig. 4 Total number of planted and developing embryos in 2023 embryo rescue studies

respectively. Considering the average of the weeks, the most successful results were the eighth and seventh weeks, respectively. Especially the tenth week gave the worst results. Considering the average of the combinations, the combinations in which the 'Yalova Seedless' variety was used as the female parent gave better results than all other combinations, except for one exception. Especially the 'Yalova Seedless X Red Globe' combination was the best combination in terms of percentage of embryo developed (%) compared to all other combinations. When we look at the number of seedling results, we see that more seedlings were obtained from the combinations in which 'Yalova Seedless' was used as the female parent than the other combinations. On average, the highest number of seedlings was obtained in the eighth week, while no seedling was obtained, especially in the sixth week (Table 1; Fig. 1).

According to the results obtained in 2020, since a very low number of embryo developed, especially in combinations in which the 'Crimson Seedless' variety was used as female and parent, such unsuccessful combinations were removed from the embryo recovery program in the following years. Apart from these, changes were made in the following years in a few combinations where the desired number of embryo development could not be reached.

Embryo rescue studies were carried out in 11 different combinations in 2021. Two combinations using the 'Autumn Royal' variety as maternal parent were first tried this year, but no embryo developments were obtained from these combinations. When embryo development

Table 1 Percentage of weekly embryo development (%) and number of seedlings of combinations in embryo rescue studies in 2020*

Combinations (FemaleXMale)	Percentage of developed embryo (%)						Number of Seedling				
	6.th week**	7.th week	8.th week	9.th week	10. th week	Average	6.th week	7.th week	8.th week	9.th week	10.th week
Yalova Sdls X Crimson Sdls	12.5j	11.0n	12.3p	30.0c	10.0b	**15.2 J**	0	0c	10a	1b	0b
Yalova Sdls X Muscat Bail.A.	25.5b	23.1 g	29.2f	29.6d	0.0c	**21.5D**	0	0c	1d	2a	0b
Yalova Sdls X Mars Sdls	15.6 h	22.5 h	24.3i	24.4e	45.5a	**26.5B**	0	0c	1d	2a	2a
Yalova Sdls X Kishmish Vatkana	22.2d	23.3f	57.1b	11.1k	0.0c	**22.7 C**	0	0c	4b	0c	0b
Yalova Sdls X Kishmish Rozavyi	17.4 g	13.5 L	22.7j	40.5a	0.0c	**18.8 F**	0	1b	0e	0c	0b
Yalova Sdls X Red Globe	0.0k	62.5a	71.4a	33.3b	0.0c	**33.5 A**	0	1b	2c	0c	0b
Yalova Sdls X Exalta	18.6f	33.3b	34.2d	16.0i	0.0c	**20.4E**	0	0c	1d	0c	0b
Crimson Sdls X Exalta	0.0k	0.0s	17.6 m	10.0 L	0.0c	**5.5R**	0	0c	0e	0c	0b
Crimson Sdls X Mars Sdls	0.0k	5.9q	20.0 L	18.8 h	0.0c	**8.9 M**	0	0c	0e	0c	0b
Crimson Sdls X Yalova Sdls	0.0k	13.3 m	22.2k	0.0 m	0.0c	**7.1P**	0	0c	0e	0c	0b
Crimson Sdls X Kishmish Vatkana	0.0k	0.0s	15.8o	0.0 m	0.0c	**3.2U**	0	0c	0e	0c	0b
Crimson Sdls X Kishmish Rozavyi	0.0k	4.5r	17.1n	0.0 m	0.0c	**4.3 S**	0	0c	0e	0c	0b
Crimson Sdls X Red Globe	0.0k	8.3p	40.0c	0.0 m	0.0c	**9.7 L**	0	1b	0e	0c	0b
Crimson Sdls X Muscat Bail.A.	0.0k	0.0s	11.8q	0.0 m	0.0c	**2.4 V**	0	0c	0e	0c	0b
Mars Sdls X Red Globe	0.0k	20.0i	0.0r	0.0 m	0.0c	**4.0T**	0	0c	0e	0c	0b
Mars Sdls X Muscat Bail.A.	12.5j	15.4	25.0 h	0.0 m	0.0c	**10.6 K**	0	0c	0e	0c	0b
Kishmish Vatkana X Red Globe	15.2i	29.3c	26.7 g	11.8j	0.0c	**16.6I**	0	2a	1d	0c	0b
Kishmish Vatkana X Muscat Bail.A.	33.3a	28.9d	29.4e	0.0 m	0.0c	**18.3G**	0	0c	0e	0c	0b
Kishmish Rozavyi X Red Globe	24.0c	23.3f	20.0 L	24.0f	0.0c	**18.3G**	0	0c	0e	0c	0b
Kishmish Rozavyi X Muscat Bail.A.	0.0k	10.0o	0.0r	0.0 m	0.0c	**2.0Y**	0	0c	0e	0c	0b
Exalta X Red Globe	19.4e	23.6e	22.2k	22.0 g	0.0c	**17.4 H**	0	0c	0e	0c	0b
Exalta X Muscat Bail.A.	15.0i	14.3k	11.8q	0.0 m	0.0c	**8.2 N**	0	0c	0e	0c	0b
TOTAL	**-**	**-**	**-**	**-**	**-**	**-**	**0D**	**5B**	**20 A**	**5B**	**2 C**
AVERAGE	**10.5D**	**17.5B**	**24.1 A**	**12.3 C**	**2.5E**	-	-	-	-	-	-

*Letters show significant difference at $p \leq 0.05$ according to Duncan's multiple range test

** It refers to how many weeks after pollination the sample is taken

Table 2 Percentage of weekly embryo development (%) and number of seedlings of combinations in embryo rescue studies in 2021*

Combinations (FemaleXMale)	Percentage of developed embryo (%)						Number of Seedling				
	6.th week**	7.th week	8.th week	9.th week	10. th week	Average	6.th week	7.th week	8.th week	9.th week	10. th week
Yalova Sdls X Crimson Sdls	7.14d	18.13d	19.23e	5.56e	0.00d	**10.01 C**	0	1c	2c	0c	0
Yalova Sdls X Muscat Bail.A.	10.42c	12.00 g	12.50 h	3.45f	0.00d	**7.67 F**	0	3a	8a	0c	0
Yalova Sdls X Mars Sdls	0.00 g	18.89c	25.71b	12.50b	7.14b	**12.85B**	0	2b	3b	2a	0
Yalova Sdls X K. Vatkana	8.33e	16.67f	24.62c	0.00 g	0.00d	**9.92D**	0	1c	1d	0c	0
Yalova Sdls X K.Rozavyi	0.00 g	17.09e	21.74d	0.00 g	0.00d	**7.77E**	0	0d	1d	0c	0
Yalova Sdls X Red Globe	0.00 g	21.82b	13.64 g	0.00 g	0.00d	**7.09G**	0	2b	3b	0c	0
Yalova Sdls X Exalta	5.00 g	26.47a	39.06a	10.53c	0.00d	**16.21 A**	0	0d	2c	0c	0
Autumn Royal X Red Globe	0.00 g	0.00j	0.00j	0.00 g	0.00d	**0.00 H**	0	0d	0e	0c	0
Autumn Royal X Mus.Bail.A.	0.00 g	0.00j	0.00j	0.00 g	0.00d	**0.00 H**	0	0d	0e	0c	0
K. Vatkana X Red Globe	16.07a	8.57i	8.93i	14.74a	15.00a	**10.01 C**	0	2b	2c	1b	0
K. Vatkana X Muscat Bail.A.	11.54b	9.39 h	16.13f	10.00d	5.26c	**7.67 F**	0	0d	2c	0c	0
TOTAL	-	-	-	-	-	**-**	**0D**	**11B**	**24 A**	**3 C**	**0D**
AVERAGE	**5.32 C**	**13.55B**	**16.51 A**	**5.16D**	**2.49E**	-	-	-	-	-	-

*Letters show significant difference at $p \leq 0.05$ according to Duncan's multiple range test

** It refers to how many weeks after pollination the sample is taken

rates were examined on a weekly basis, the most successful results were obtained from the 'Kishmish Vatkana X Red Globe' combination in the sixth, ninth and tenth weeks after pollination, and from the 'Yalova Seedless X Exalta' combination in the seventh and eighth weeks. When the average of weeks was evaluated, it was stated that the results were similar to 2020. The most successful week was the eighth week, followed by the seventh week.

Table 3 Percentage of weekly embryo development (%) and number of seedlings of combinations in embryo rescue studies in 2022*

Combinations (FemaleXMale)	Percentage of developed embryo (%)						Number of Seedling				
	6.th week**	7.th week	8.th week	9.th week	10. th week	Average	6.th week	7.th week	8.th week	9.th week	10. th week
Yalova SdIs X Crimson SdIs	5.00 g	11.76f	12.86 g	3.33 h	0.00b	**6.59I**	0	0d	2b	0	0
Yalova SdIs X Muscat Bail.A.	12.50f	24.32a	26.67a	10.00d	0.00b	**14.70 A**	0	2b	3a	0	0
Yalova SdIs X Mars SdIs	0.00 h	10.00i	13.33e	0.00i	0.00b	**4.67 J**	0	1c	2b	0	0
Yalova SdIs X K. Vatkana	17.65c	12.00e	13.33e	12.50b	0.00b	**11.10E**	0	1c	1c	0	0
Yalova SdIs X Red Globe	5.00 g	11.00 h	11.67 h	8.89e	3.33a	**7.98 H**	0	3a	3a	0	0
Yalova SdIs X Exalta	12.50f	14.29d	15.00c	4.00 g	0.00b	**9.16G**	0	0d	2b	0	0
Exalta X Red Globe	18.75b	11.11 g	13.00f	19.23a	0.00b	**12.42B**	0	1c	1c	0	0
Exalta X Muscat Bail.A.	14.29e	15.00b	18.89b	11.76c	0.00b	**11.99 C**	0	1c	2b	0	0
K. Vatkana X Red Globe	20.00a	14.42c	14.79d	7.81f	0.00b	**11.40D**	0	2b	3a	0	0
K. Vatkana X Muscat Bail.A.	16.67d	9.86j	13.00f	7.69f	0.00b	**9.44 F**	0	0d	2b	0	0
TOTAL	-	-	-	-	-	**-**	**0 C**	**11B**	**21 A**	**0 C**	**0 C**
AVERAGE	**12.23 C**	**13.38B**	**15.25 A**	**8.52D**	**0.33E**	-	-	-	-	-	-

*Letters show significant difference at $p \leq 0.05$ according to Duncan's multiple range test.

** It refers to how many weeks after pollination the sample is taken.

Table 4 Percentage of weekly embryo development (%) and number of seedlings of combinations in embryo rescue studies in 2023*

Combinations (FemaleXMale)	Percentage of developed embryo (%)					
	6.th week**	7.th week	8.th week	9.th week	10.th week	Average
Yalova Seedless X Crimson Seedless	10.00b	24.44c	24.00c	15.00b	8.33a	**16.36B**
Yalova Seedless X Muscat Bailey A	0.00f	15.94i	19.05 g	12.50c	0.00c	**9.50G**
Yalova Seedless X Mars Seedless	0.00f	20.00f	20.00f	11.11d	0.00c	**10.22 F**
Yalova Seedless X Kishmish Vatkana	0.00f	20.00f	16.67 g	6.67e	0.00c	**8.67 H**
Yalova Seedless X Red Globe	7.14c	24.56b	28.79a	20.93a	4.76b	**17.24 A**
Yalova Seedless X Exalta	18.75a	23.08d	23.73e	0.00 g	0.00c	**13.11D**
Exalta X Red Globe	0.00f	17.74 g	16.67 g	0.00 g	0.00c	**6.88 J**
Exalta X Muscat Bailey A	0.00f	26.67a	23.81d	11.11d	0.00c	**12.32E**
Kishmish Vatkana X Red Globe	3.85e	20.83e	25.93b	12.50c	4.76b	**13.57 C**
Kishmish Vatkana X Muscat Bailey A	5.56d	16.00 h	11.69 h	5.71f	0.00c	**7.79I**
AVERAGE	**4.53D**	**20.93B**	**21.03 A**	**9.55 C**	**1.79E**	**-**

*Letters show significant difference at $p \leq 0.05$ according to Duncan's multiple range test.

** It refers to how many weeks after pollination the sample is taken.

When the embryo development rates were compared on the basis of combination averages, the most successful combinations were 'Yalova Seedless X Exalta' and 'Yalova Seedless X Mars Seedless', respectively. No embryo development and seedling were obtained from the combinations of 'Autumn Royal X Red Globe' and 'Autumn Royal X Muscat Bailey A'. In terms of seedling number, the most successful week was the eighth week, while the most successful combination was 'Yalova Seedless X Muscat Bailey A' combination. Although there are fewer combinations in 2021 compared to 2020, more seedlings were obtained from embryo rescue studies (Table 2; Fig. 2).

In 2022, especially considering the results of the first two years of the trial, embryo rescue studies were carried out with combinations using 'Yalova Seedless', 'Exalta' and 'Kishmish Vatkana' varieties as female(maternal) parents. According to the results obtained on the basis of sample weeks after pollination, the highest embryo development rate in the sixth week was obtained from the 'Kishmish Vatkana X Red Globe' combination, similar to 2021. In the seventh and eighth weeks, the combination of 'Yalova Seedless X Muscat Bailey A' gave the most successful results. The most successful result in the ninth week was obtained from the 'Exalta X Red Globe' combination. In the tenth week, live embryos could only be obtained from the 'Yalova Seedless X Red Globe' combination. Considering the average percentage of embryo developed of the weeks, the most successful results were obtained in the eighth and seventh weeks, respectively, similar to the results obtained in previous years. The tenth week attracted attention as the week that yielded the lowest number of embryo development during the four years of the study.

According to the results obtained in terms of number of seedlings in 2022, the eighth and seventh weeks were the weeks with the highest number of seedlings, similar to other years. No seedlings plants were obtained in the sixth, tenth and tenth weeks (Table 3; Fig. 3).

In 2023, the last year of the study, embryo rescue studies were carried out using the same combinations as in 2022. In the evaluation made on a weekly basis in terms of live embryo rates, the 'Yalova Seedless X Exalta' combination gave the most successful results in the sixth week, while the in the studies conducted this year, embryo development at varying rates was obtained from different combinations on a weekly basis, but unfortunately no seedlings were obtained from any week or combination. In the eighth and ninth weeks, the highest embryo development was obtained from the 'Yalova Seedless X Red Globe' combination. In the tenth week, the combination 'Yalova Seedless X Crimson Seedless' had the highest embryo development rate.

When we look at the averages of the weeks, it was determined that the most successful weeks were the eighth and seventh weeks, respectively, similar to the results obtained in other years. When we look at the combination averages, the most successful results were obtained from the combinations of 'Yalova Seedless X Exalta' and 'Yalova Seedless X Crimson Seedless', respectively (Table 4; Fig. 4).

Considering the results obtained from all years, it has been shown that combinations with the 'Yalova Seedless' variety as the female parent give better results than other varieties in terms of both percentage of embryo developed (%) and number of seedling. In studies conducted to determine the sampling time according to the number of weeks after pollination, the eighth and seventh weeks gave much better results than the other weeks in all years.

Discussion

The success rate in embryo rescue studies is quite low because it is affected by many factors. Among these factors, selected parents and sampling time are among the factors that most affect the success rate. In terms of parent combinations, especially the combinations in which 'Yalova Seedless' variety was used as the female parent gave better results than other combinations. More live embryos and live grapevine plants were obtained from hybrid embryos obtained by pollinating the 'Yalova Seedless' variety with 'Red Globe', 'Muscat Bailey A' and 'Exalta' grape varieties, compared to other combinations from different years. In addition, during embryo rescue studies, the embryos must be cleaned of all other tissues and the risk of infection, especially fungal diseases, must be minimized. At this stage, there is a lower risk of infection, especially if varieties that are resistant/tolerant to fungal diseases are used. In this study, a more live embryo rate and live grapevine plants were obtained in combinations in which the interspecific hybrid varieties 'Muscat Bailey A' and 'Mars Seedless', which are more resistant to fungal diseases, and the 'Red Globe' variety, which is more tolerant in terms of diseases, were used as pollinators.

Luo et al., [22] studied the effects of parental genotypes on embryo rescue, they conducted 11 different combinations using nine stenospermocarpic seedless grapes as female parents and three seedless or seeded grapes as male parents. When 'FZ42' was the female parent, 'Su-67' was better than 'Jupiter' as the male parent; when 'Perlette' was the female parent, 'Jupiter' and 'Su-67' as male parents had no significant difference, indicating that paternal genotype has a certain effect on embryo rescue. These results are similar to our study; the success rate may vary depending on the combinations of parents and even whether the parent is used as female or male (pollinator).

In another embryo rescue study conducted by Cui et al. [34], similar results were obtained with our embryo rescue study. In their studies, two varieties, one with seeds ('Beichun') and one without seeds ('Venus Seedless'), were used as male parents and 5 different stenospermocarpic seedless grape varieties were used as female parents. As a result of their study, they reported that similar to our scientific study, particularly selected combinations were very effective in success. They reported that the effect of the parent selected as female on the success rate of embryo rescue was greater than that of male (pollinator) varieties. However, unlike our study, the 10th week gave better results than the other weeks in terms of the combination and embryo recovery technique they used in their study. The different results obtained in some embryo recovery studies may be due to different sampling periods of the researchers, as well as differences in the growth and development stages of the ovaries. Additionally, in embryo rescue studies, it is reported that the paternal (male) genotype is as effective as the female parent in embryo development.

It is reported that the success rate in embryo rescue studies is closely related to the maturity of the female parents. The embryos of early maturing varieties abort earlier and cannot show the desired development, and as a result, the desired success rate in embryo rescue studies cannot be achieved. It has been determined that late-maturing varieties have a higher success rate in embryo rescue studies than early-maturing varieties [34, 35]. In addition to these findings, in our study, the high success rate was obtained from male (pollinator) varieties such as 'Red Globe' and 'Muscat Bailey A', which mature in the late period.

Researchers have reported that the large berry late-maturing 'Crimson Seedless' grape variety has a very low viability rate in embryo rescue studies due to smaller seed ovules [21, 34–39]. Similarly, in our study in 2020, much lower embryo viability was detected in combinations in

which the 'Crimson Seedless' grape variety was used as the female parent, compared to other combinations.

In their embryo rescue study, Chiaromonte et al. [10] examined the embryo development rates from ovules extracted with the embryo rescue technique of crosses between different combinations of seedless parents for 4 years. In their studies, they achieved success rates ranging from 3.5 to 35.5%, depending on the combination, similar to our results. They reported that there were no significant differences between different samples taken between 43 and 62 days (6–9 weeks) after pollination (DAP). They also reported that grapevine varieties and genotypes significantly affect the success rate of embryo rescue, as in our study.

Li et al., [2] used stenospermocarpic grapes as female parents in order to obtain new seedless grapes. They reported that the seed coat of abnormal ovules turned brown from 38 to 42 days after flowering. At the hormone level, two ratios of endogenous hormones' content [gibberellic acid+indole-3-acetic acid] / abscisic acid and zeatin riboside / abscisic acid had decreased by 36 DAF. In the paraffin sections of the embryo sac, they observed few or no embryos in ovules between 37 and 42 DAF. In addition, depending on the variety, the addition of 5 mg/L exogenous brassinolide and 1 µM 6-BA to the medium has been shown to have a positive effect on embryo recovery, embryo germination, and polyembryony formation. In line with our results, the researchers observed that embryo size depends on the selected parent and reported that their viability varied.

Using the modified embryo rescue technique Giancaspro et al., [40] planted the immature ovules on agar nutrient medium 40 days after pollination and allowed embryo development for 8 weeks. As a result of their studies, they obtained the highest embryo development from hybrids of 'Thompson', 'Superior' and 'Regal' grape varieties. The highest percentage of embryo development was obtained from the ovaries taken 50 days after pollination from the 'Luisa x Thompson' combination. Especially the results based on variety and cultivation week were found to be compatible with our study.

Tian et al., [11] used *V. vinifera* as female parent and disease-resistant wild Chinese *Vitis* spp. as male parent in embryo rescue studies, similar to our study. After 7 weeks of pollination, embryos were developed in modified nutrient medium and embryo formation, embryo germination and plant development rates were obtained as 34.0%, 91.2% and 77.4%, respectively. When they applied their protocol to other hybrid combinations; They reported that genotype significantly affects embryo formation, embryo germination and plant development, and hybrid plants were successfully obtained at different frequencies in all crosses. This result shows that when the embryo rescue method is selected in the breeding studies of new seedless varieties resistant to diseases, the nutrient medium used as well as the combinations can be effective in increasing the success rate.

Li et al., [41] conducted embryo rescue studies with 4 naturally pollinated Eurasian seedless grape varieties. They have been reported that there are some endogenous compounds among the physiological reasons affecting embryo development and abortion of seedless grapes. When the change in polyamine content among these is examined, it has been reported that especially high amounts of endogenous polyamine content support the growth and development of embryos. In this case, it has been reported that when different seedless grapes are used as female parents, different amounts of exogenous polyamines may be synthesized and different embryo rescue efficiency may be achieved. The different embryo recovery success rates obtained from different grape varieties used as females in our study may be related to this situation.

When we evaluate the performance of grape varieties over the 4 years, the result we see is that the 'Yalova seedless' variety and the 'Exalta' variety, which were selected as the female parent, gave more successful results, especially in terms of the percentage of development embryo (%). However, 'Kismish Vatkana' variety also showed good results from time to time, depending on the sampling time. 'Red Globe,' 'Mars,' 'Muscat Bailey A,' and 'Crimson Seedless' varieties selected as pollinator varieties were determined to be the grape varieties that gave the best results in terms of percentage of the developed embryos (%) and a number of seedlings. It is also important that 'Mars' and Muscat Bailey A' varieties are interspecific hybrid varieties among these varieties. This situation also shows that it is possible to obtain more successful results by using more interspecific hybrids in embryo rescue studies in the future.

Conclusion

Embryo rescue studies are important method that helps breeding, and they have become more preferred in recent years, especially with the increasing consumer demand for seedless grapes. In these studies, the success rate may vary considerably depending on the combination chosen and the timing of placing the immature ovules into the ready-made nutrient medium. This study's results confirmed the importance of the conditions associated with this success rate. In future studies, it has been revealed that grape breeders prefer combinations with higher success rates and place the ovules in a ready-made nutrient medium between 50 and 60 days after pollination. These results may be helpful, especially in studies on polyploidy breeding and other combinations where healthy plants cannot be obtained with traditional methods. Although it was observed that a seedless variety, in particular, stood

out in our study and increased the success rate, it should be taken into account that there are many factors affecting the success rate in embryo rescue studies and that the success rate will increase by taking all factors into account and providing the best possible conditions in vitro and afterward.

Materials and methods

Plant material

In this study, varieties belonging to different *Vitis* species in the vineyard collection parcel located within the Yalova Atatürk Horticulture Central Research Institute were used as plant material. While 'Crimson Seedless', 'Autumn Royal', 'Mars Seedless', 'Yalova Seedless', 'Kishmish Vatkana', 'Kishmish Rozovyi', 'Exalta' are used as seedless varieties; 'Red Globe' and 'Muscat Bailey A' were used as seeded varieties. While seeded varieties were used only as pollinators, seedless varieties were used both as female parent and pollinator (male parent) within the scope of the study.

In this study, 'Crimson Seedless', 'Autumn Royal', 'Mars Seedless' and 'Yalova Seedless' were selected as parents because they have a larger berry size. 'Kishmish Vatkana' and 'Kishmish Rozovyi 'were selected because they are resistant/tolerant to diseases and the 'Exalta' variety was selected because of its muscat aroma. 'Red Globe' was selected because of its very large berry size and 'Muscat Bailey A' were selected for highly resistant to fungal diseases.

The grape varieties used in cross-breeding studies and some of their basic characteristics are given in Table 5. Additionally, photographs of the grapes used as parents are given in Fig. 5. All grape varieties used in this study consist of grape varieties previously collected within the scope of different projects, and all legal permissions for their use have been obtained.

Cross-breeding

As a result of phenological observations, hybridization studies were carried out in late May and early June for 3 years in 2020, 2021 and 2022 (Fig. 6a). Before cross-breeding, emasculation processes were carried out before the flowers opened (Fig. 6b). The emasculated bunches were placed in pouches (special bags) designed to prevent foreign pollination. Emasculation processes were completed according to the flowering status of the varieties. 5 bunches from each combination were emasculated. Pollen taken from the variety determined as pollinator was stored in a dry and cool environment in the laboratory until pollination. Pollination was carried out by taking the emasculated bunch into the polyethylene bag containing the pollen and shaking the bag. This procedure was performed early in the morning, which is the most suitable time for pollination, and was applied 3 times with 2 days' intervals. The labels with the names of the parents and the pollination date were left in the tied bunches.

Table 5 Important characteristics of grape varieties used as parents in embryo rescue studies

Cultivar/Variety	Species	Berry colour	Special aroma	Flesh status
Yalova Seedless	*V.vinifera*	White	No	Soft
Crimson Seedless	*V.vinifera*	Red/Pink	No	Crunchy
Mars Seedless	Interspecies	Black	Yes	Soft
Kishmish Vatkana	*V.vinifera*	Black	No	Soft
Kishmish Rozovyi	*V.vinifera*	Red/Pink	No	Crunchy
Exalta	*V.vinifera*	White	Yes	Soft
Autumn Royal	*V.vinifera*	Black	No	Crunchy
Muscat Bailey A	Interspecies	Black	Yes	Soft
Red Globe	*V.vinifera*	Red/Pink	No	Soft

Embryo rescue

Although the harvest of clusters after hybridization varies depending on the variety, it generally started on the 42nd day (6 weeks) from the last pollination date and continued to be sampled until the 70th day (10 weeks). The harvested bunches were brought to the tissue culture laboratory and subjected to surface sterilization. After harvesting, the berries separated from the clusters were kept under running water for approximately 20 min. Then, 3 min in 70% ethyl alcohol and 10 min in 20% Sodium Hypochlorite (NaOCl or NaClO) solution into which 1–2 drops of 0.1% tween 20 were added. Surface sterilization was applied. After the sterilization process, the ovules were isolated and planted in E20A nutrient medium (Table 6; Fig. 6c). Ovules and embryos were cultured a climate chamber with a temperature controlled at 25±1 °C, in a photoperiod of 8 h of darkness and 16 h of light, under lamps with an illumination intensity of 4000 lm m^{-2} (or 371.61 foot-candle) (Fig. 6d and e).

Plant development

Embryos planted in the agar medium were left to grow for approximately 3 months in the climate chambers, depending on the combination, and were sub-cultured according to their developmental status (Fig. 6f). Grapevine plantlets, with adequate roots and upper parts formed from embryos developing in the climate chamber, were planted in pots containing sterile peat-perlite mixture (Fig. 6g and h). The pots were kept in the climate chamber for a while after being covered with a plastic tent to reduce moisture loss and prevent disease infection. Then, they were gradually uncovered and transferred to the greenhouse. The grapevine plants remained in the greenhouse for 3–6 months, depending on their development, and were then taken to the Institute

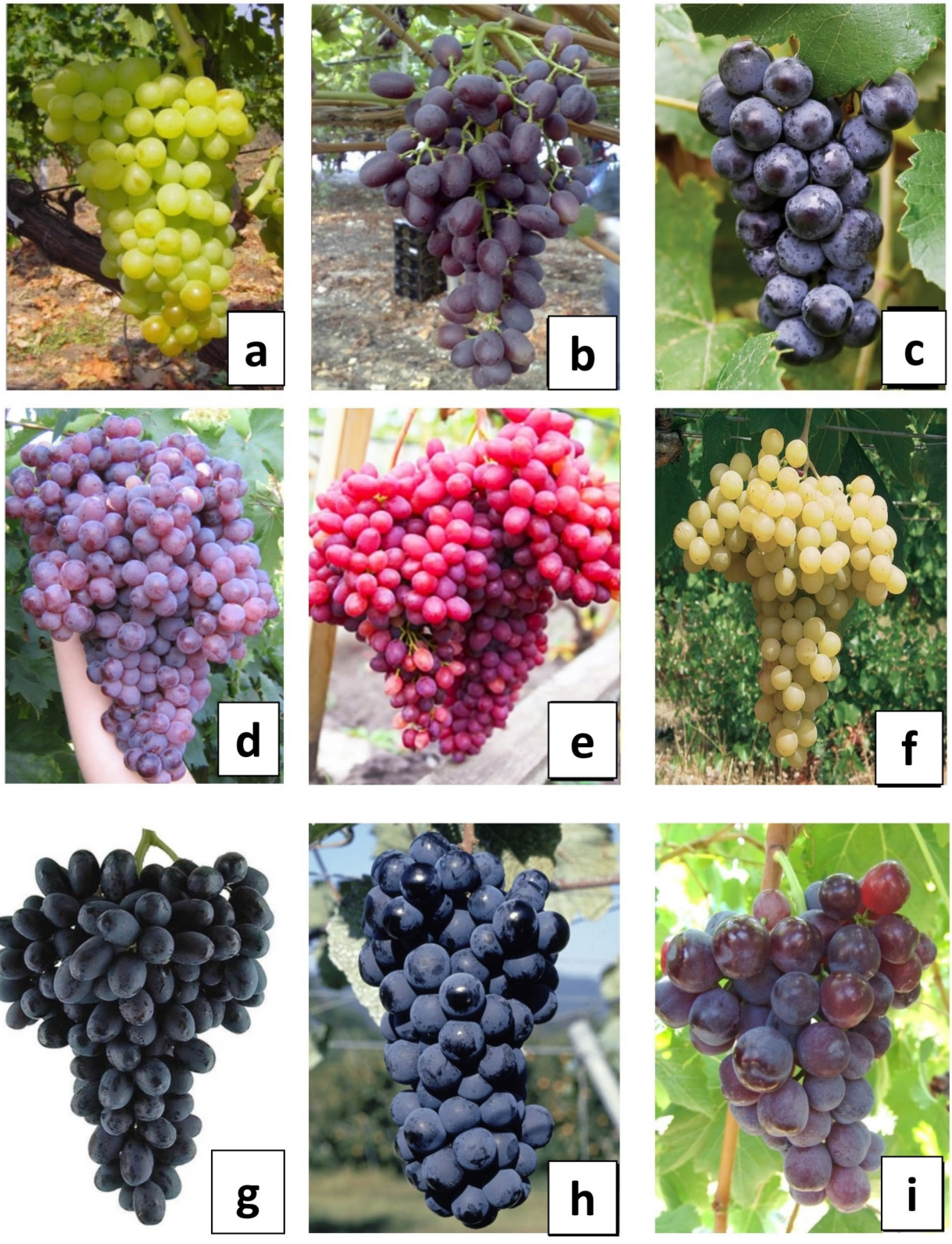

Fig. 5 Photos of grape varieties used as parents in embryo rescue studies. Yalova Seedless (**a**), Crimson Seedless (**b**), Mars Seedless (**c**), Kishmish Vatkana (**d**), Kishmish Rozovyi (**e**), Exalta (**f**), Autumn Royal (**g**), Muscat Bailey A (**h**) and Red Globe (**i**)

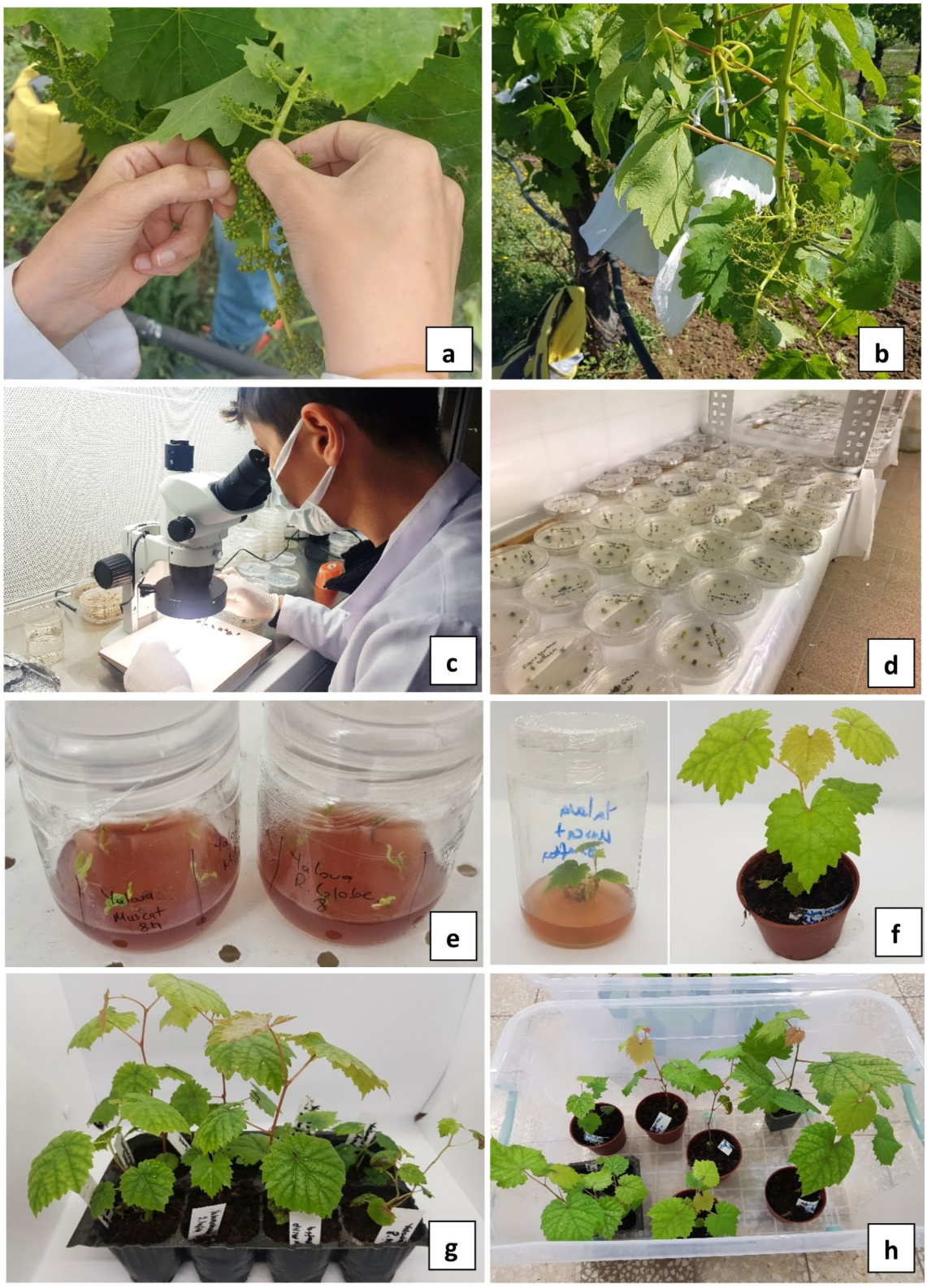

Fig. 6 Photographs of different stages of embryo recovery. Emasculation (**a**), hybridization/pouching (**b**), separation of embryos (**c**), planting in ready-made nutrient medium (**d**), placing live embryos in jars (**e**), placing hybrid plantlets in pots (**f**, **g**, **h**)

Table 6 Composition and amounts of E20A nutrient medium used in embryo recovery studies

Amount of Macro Elements (mg l^{-1})		Amount of Microelements (mg l^{-1})		Amount of Vitamins and Aminoacids (mg l^{-1})		Amount of Plant Growth Regulators (mg l^{-1})	
KNO_3	1075	$MnSO_4.H_2O$	11.065	Myo-Inositol	50.3	IAA	0.01
NH_4NO_3	619	$ZnSO_4.7H_2O$	1.812	Pyrodoxine-HCl	5.5		
$MgSO_4.7H_2O$	206	H_3BO_3	1.575	Nicotinic acid	0.7		
$CaCl_2.2H_2O$	156.5	KI	0.345	Thiamine-HCl	0.6		
KH_2PO_4	71	$Na_2MoO_4.2H_2O$	0.094	Calcium Pantothenate	0.5		
$Ca(NO_3)_2.4H_2O$	25	$CuSO_4.5H_2O$	0.008	Biotine	0.005		
$NaH_2PO_4.4H_2O$	19	$CoCl_2.6H_2O$	0.008	Glycine	0.1		
$(NH_4)_2SO_4$	17			Na_2EDTA	37.3		
KCl	3.5			$FeSO_4.7H_2O$	27.8		

nursery. Regular irrigation, fertilization, spraying and weed control were carried out in the nursery.

Statistical analyses

In the embryo recovery studies carried out in 2020, 2021, 2022 and 2023, the differences in the number of live embryos and plants obtained weekly on a combination basis were statistically analysed using a package program. Statistical analyses were performed using one-way analysis of variance, to determine the significance of differences between combination and weeks at $p<0.05$ using SAS statistical software of JPM-17 package program [42]. The differences between the combination and weeks were statistically evaluated using the LSD test at a significance level of 0.05. The differences were determined at a 5% confidence interval ($P \leq 0.05$).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05539-x.

Supplementary Material 1

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Author contributions
A.A. planned, designed, supervised and wrote the research. Y.D., Ö.B., and G.G.K. performed and analysed the study also did field, lab and greenhouse practices.All authors reviewed the manuscript.

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

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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