Chapter 1

Applications of Embryo Culture in Grapevine

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Abstract

Plant tissue culture is a method that enables the regeneration or propagation of plant parts or whole plants by culturing plant protoplasts, cells, tissues, or organs under controlled conditions in vitro. Among these techniques, anther culture, meristem culture, shoot tip culture, ovule culture, and embryo culture are widely used in vitro techniques.

Embryo culture, defined as the cultivation of embryos isolated from seeds or seed primordia of higher plants on sterile nutrient media, is utilized for purposes such as the rapid development of new varieties, the creation of varieties more resistant to diseases, pests, and abiotic stresses, and enabling hybridization efforts hindered by incompatibility between plant genera and species.

In grapevine, embryo culture is employed for objectives such as developing new grape varieties, propagating seedless grape cultivars, and creating varieties resistant to diseases, pests, and diverse ecological conditions. However, despite extensive research on embryo rescue in viticulture, the number of newly developed cultivars remains limited. Breeding new grape varieties through embryo rescue is still a challenging and long-term technique, requiring a patient and dedicated approach from grape breeders.

This compilation will provide current and comprehensive insights into the embryo rescue technique in grape breeding, discussing its applications and practices.

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1. Introduction

Plant tissue culture is defined as the cultivation of plant cells obtained from various parts of the plant (root, stem, leaf, apical and meristem) under aseptic conditions and in an synthetic nutrient medium (Güven and Gürsul, 2014). Compared to traditional propagation methods, tissue culture is a modern vegetative propagation technique that provides high efficiency and quality. It is particularly suitable for mass production, ensures genetic homogeneity, and significantly shortens the juvenile period (Driver and Kuniyuki, 1986; Scaltsoyiannes et al., 1998).

In vitro propagation of various plant species using tissue culture techniques employs several types of cultures. These include shoot tip culture, node culture, callus culture, embryo culture, cell, and protoplast culture (Guney et al., 2020; Ahuja, 1986; Pierik, 1986; Srivastava and Steinbaver, 1981).

One of the earliest and most important applications of in vitro culture is embryo culture, also known as embryo rescue. It is an in vitro technique used to rescue zygotic embryos extracted from seeds and ovules in a nutrient medium, by identifying the essential requirements for embryo morphogenesis, differentiation, and continued development under aseptic conditions (Sharman and Kaur, 1996; Raghavan, 2003). Numerous studies have been conducted on various plants and species concerning the embryo rescue technique (Chen et al., 2025; Doyğacı et al., 2024; Reddy et al., 2024; Yao et al., 2024; Yıldırım, 2012).

Grape is a fruit species of global significance. It has numerous uses, including as table grapes, raisin, and wine production. In grape cultivars, seedlessness is a highly valued trait for both table and raisin, ranking high among consumer preferences. Therefore, the breeding of seedless grape varieties has long maintained its importance and relevance (Alleweldt and Possingham, 1988; Wang et al., 2002; Ebadi et al., 2009).

Embryo culture plays a significant role in the breeding of seedless grapes. Many breeders have obtained new seedless grape varieties from stenospermocarpic varieties using as female parents by the embryo rescue technique (Spiegel-Roy et al., 1985; Gribaudo et al., 1993). Although seedless grape varieties are preferred by consumers, they tend to be more susceptible to diseases. Therefore, breeding seedless varieties with improved disease resistance has emerged as a separate and important objective (Nigar et al., 2024).

The embryo rescue technique has great potential in developing disease-resistant seedless grape varieties. Difficulties are encountered in traditional breeding methods due to the stenospermocarpic berry set in grapes. Stenospermocarpy refers to the degeneration of grape embryos before full development, which stems from abnormal development of the ovule and integuments. Thus, the embryo rescue technique offers a valuable and promising solution to overcome these limitations and develop desirable, disease-resistant, seedless grape varieties (Cui et al., 2017; Conner et al., 2018).

2.1. History of Embryo Culture

Embryo culture, which involves isolating embryos from seeds and seed primordia and cultivating them in specific nutrient media, is an important plant tissue culture technique. This technique is based on the principles of isolating the embryo without damage, establishing a suitable nutrient medium, and ensuring embryogenic development and transformation into a plant (Bridgen, 1994; Reed, 2005).

The embryo rescue technique is used to eliminate dormancy in seeds of certain plants, overcome incompatibility barriers in some hybrids, and initiate callus cultures.

Embryo rescue began to be used by plant breeders in the 18th century with Charles Bonnet's studies on *Phaseolus* and *Fagopyrum* (Schopfer, 1943; Sharma et al., 1996). The first successful experiment was conducted in the early 20th century by Hanning (1904), who used two radish species (*Raphanus* and *Cochlearia*). Mature embryos isolated from seeds were cultured in a nutrient medium containing macro and micro elements, and sugar, resulting in new plantlets (Narayanaswami and Norstog, 1964; Ramming, 1990). Later, in 1925, Laibach succeeded in cultivating hybrid embryos obtained from a cross between *Linum perenne* and *Linum austriacum*, which normally could not develop on the plant, thereby producing hybrid plants (Laibach, 1925).

Tukey's embryo culture study on cherries in 1933 was an important step in the application of embryo culture in fruit breeding (Tukey, 1993). Since then, embryo rescue has been widely applied in various fruit species. For example, it has been used in apple (Dantas et al., 2006), banana (Uma et al., 2011), citrus (Viloria et al., 2005), mango (Krishna and Singh, 2007), melon (Nunez-Palenius et al., 2006), peach (Anderson et al., 2002), persimmon (Hu et al., 2013), and watermelon (Taşkın et al., 2013; Li et al., 2014). Embryo rescue is the most commonly used method in seedless grape

breeding and is also applied to develop early-ripening and triploid grape varieties (Li et al., 2014).

Ovule culture is defined as the in vitro cultivation of ovules and the subsequent isolation of embryos developing from these ovules. After the first in-ovulo culture in grapevine was performed in 1982, embryo culture became widely used (Emershad and Ramming, 1982). Factors influencing the success of culture include genotype (Liu et al., 2003), the age of the ovule at isolation (Ji et al., 2013b), and the composition of the nutrient medium (Amaral et al., 2001).

Embryo culture is also used to overcome pre-zygotic and post-zygotic incompatibilities, which limit the development of sexual hybrids and result in sterility under natural conditions (George and Eapen, 1993). Additionally, It is also a valuable technique in preventing losses in the germination of hybrid seeds obtained from breeding studies under field conditions. Zygotic embryos are known as the best explant source for in vitro cultures due to their juvenile characteristics and high regeneration potential (Burgos and Ledbetter, 1993).

2.2. Advantages of Embryo Culture

In a classical breeding study, the chance of obtaining a seedless individual in a seeded × seedless cross is up to 49%, while in seedless × seedless crosses with embryo culture, this rate increases to 92%.

Embryo rescue method is used because germination is generally poor in the embryos of early-ripening grape varieties. While in traditional breeding methods, early maturing grape varieties are used only as male parents, these varieties can also be used as female parents in embryo culture, and the chance of earliness in the resulting hybrids can be increased (Ramming et al., 1990).

In some cases, fertilization occurs after pollination, but divisions do not occur in the zygote or only a few-celled embryo is formed and development cannot continue. The endosperm may not support embryo growth, resulting in small, undeveloped embryos. In such situations, seeds containing embryos at a certain physiological maturity are sterilized, and embryos are isolated from surrounding tissues under sterile conditions. During isolation, small embryos should be isolated without damaging them, and the isolated embryos should be inoculated under appropriate conditions to ensure their transformation into full plants (Ergönül et al., 2021).

2.3. Disadvantages of Embryo Culture

Embryo culture, despite its many advantages, also has some disadvantages. These can be summarized as follows:

i. Low success rates: Success rates in embryo recovery vary depending on the plant species and even the variety.

ii. Genetic diversity: Genetic variations that affect plant traits may emerge during the culture process.

iii. Environmental factors: Plants grown under controlled conditions may have difficulty adapting to natural environments.

iv. High costs: Embryo culture is a costly technique that requires expert experience and a well-equipped laboratory infrastructure.

3. Applications of Embryo Culture in Viticulture

Due to its heterozygous genetic structure, significant genetic diversity is seen among grapevines adapted to various climatic conditions around the world. Therefore, seeds play an important role in grapevine breeding. In breeding programs, seeds with high germination potential are of particular significance (Elidemir et al., 1999).

Today, biotechnological approaches such as molecular techniques and tissue culture are integrated into traditional breeding efforts to enhance their effectiveness. Embryo rescue technique is a technique applied in cases where it is not possible to obtain hybrid plants with classical breeding methods. In recent years, embryo culture and embryo rescue techniques have significantly contributed to the improvement of seedlessness and polyploidy in grapevine breeding programs (Emershad and Ramming, 1982). This technique also provides significant benefits in the breeding of early-maturing grape varieties. In grapevine breeding where seedlessness is a priority, embryo culture is used to increase the likelihood of obtaining seedless individuals.

In addition to breeding for seedlessness and earliness, embryo culture is also integrated into programs aimed at producing haploid, triploid, and tetraploid plants. Moreover, it is utilized in interspecific hybridizations between *Vitis* species to combine seedlessness with other traits such as cold tolerance and resistance to fungal diseases (Ji and Wang, 2013; Li et al., 2015). Embryo and embryo rescue techniques are used in hybridization studies to develop varieties that are resistant to diseases and pests and produce high-quality grapes (Goldy et al., 1988).

Several factors influence the success of embryo rescue in grapes, with genotype being the most critical. Various researchers have reported that genotype plays a vital role in embryo development (Ji et al.,2013a; Razi et al., 2013). These studies have shown that genotype significantly affects the success of embryo rescue methods and that better results can be obtained from certain grape varieties. Therefore, optimizing genotype differences is of great importance for embryo rescue succes.

Parthenocarpic seedless grapes (e.g., Black Pearl and White Corinth) are not suitable as female parents for breeding via embryo rescue. However, this technique can be applied using them as female parents in stenospermocarpic grapes (Singh et al., 2011).

Another factor affecting the success of embryo rescue is the time at which the embryo is isolated. While the early berry development stages of stenospermocarpic grapes similar to seeded varieties, they later undergo degeneration, limiting the transition of embryos into mature stages. Therefore, identifying the optimal period for embryo isolation plays a key role in improving success rates. Numerous studies have emphasized the importance of this period (Ponce et al., 2000; Roichev et al., 2007; Li et al., 2013). For instance, Gray et al. (1990) cultured ovules from Orlando Seedless grapes at 10, 20, 40, and 60 days after flowering. They reported that more embryos and plants were obtained from ovules cultured 40–60 days after flowering and the highest embryo recovery was from ovules cultured at 60–70 days. Other studies have also shown that genotype affects the sampling time of ovules (Ji et al., 2013b; Yang et al., 2007; Xu et al., 2005).

Xu et al. (2005) determined that the optimum sampling time in crosses between diploid and tetraploid cultivars depends on the maturation time of the female parent. This period was 6–9 weeks after pollination in early-ripening varieties, 7–10 weeks in mid-ripening varieties, and 9–12 weeks in late-ripening varieties.

The nutrient medium, which is part of the embryo rescue technique, is crucial for success. Since embryo culture involves multiple stages, different basal media and media solidity (liquid, solid, liquid-solid) are used. Examples include White (White, 1954), MS (Murashige and Skoog, 1962), B5 (Gamborg et al., 1968), NN (Nitsch and Nitsch, 1969), Smith (Smith et al., 1969), SH (Stewart and Hsu, 1977), Cain (Cain et al., 1983), ER (Emershad and Ramming, 1982), BD (Bouquet and Davis, 1989), and WPM (Woody Plant Basal Medium, Lloyd and McCown, 1980). These media contain macro and micro elements, sugars, and growth-promoting

substances. Liu et al. (2008) reported that increasing $CaCl_2$ concentration in the medium enhanced success in embryo rescue (Liu et al., 2008).

In grape embryo culture, sucrose is the most commonly used sugar. While the most commonly used concentration in tissue culture studies is 20-30 g/L, this level is as high as 60 g/L for the culture of immature grape embryos. Sugar supports normal growth by balancing osmotic pressure and preventing premature germination of young embryos (Tian et al., 2008; Ji et al., 2013b). Many additives are also included in the culture medium, such as coconut water, casein hydrolysate, and malt extract—substances derived from endosperm tissues (Sharma et al., 1996).

Another factor affecting the success of the grape embryo culture technique is the type and concentration of plant growth regulators (PGRs). Success rates increase when plant growth regulators are added to the culture medium. Application before or during flowering can also be effective (Razi et al., 2013). Common PGRs in the culture medium include antigibberellins (e.g., chlormequat and uniconazole, which promote embryo germination when applied before flowering), cytokinins, and putrescine (Ledbetter and Shonnard, 1990; Tang et al., 2009).

4. Studies on Applications of Embryo Culture in Viticulture

Grape (*Vitis vinifera* L.) is one of the most widely consumed fruits globally, available in many forms. Among consumer preferences, seedlessness has always been a prominent research area in grape breeding. Therefore, extensive breeding programs have been conducted to develop new seedless cultivars. Embryo rescue has been effectively applied by plant breeders to overcome the biological barriers encountered in crosses between seedless cultivars, aiming to recover immature embryos. For over thirty years, embryo rescue has been used in grape breeding. The most critical factors affecting embryo rescue success are genotype, the timing of embryo isolation, and the composition of the nutrient medium. Additionally, other factors such as culture techniques and the use of plant growth regulators also play significant roles. To date, this technique has been employed in wide hybridizations among various *Vitis* species to rescue weak embryos and to produce seedless and triploid lines. However, the development of new grape varieties through embryo rescue remains a challenging and long-term task, requiring consistent effort from breeders (Li et al., 2015).

To develop seedless and disease-resistant cultivars, Li et al. (2014) crossed stenospermocarpic *Vitis vinifera* cultivars with Asian grape species. They studied seven hybrid combinations in different media containing

growth regulators and evaluated embryo formation, germination, and plant development. The highest embryo germination and plant development rates were achieved using a medium composed of Woody Plant Medium (WPM) + 5.7 μM indole-3-acetic acid (IAA) + 4.4 μM 6-benzylaminopurine (BAP) + 1.4 μM gibberellic acid (GA) + 2% sucrose + 0.05% casein hydrolysate + 0.3% activated charcoal + 0.7% agar. Adding proline increased embryo formation by 36.1%, germination by 64.6%, and plant development by 90.5%. The study established a high-efficiency protocol for hybridizing seedless *Vitis vinifera* with Asian species, significantly improving breeding efficiency for disease-resistant seedless grapes.

In breeding studies, it is of great importance to develop varieties that will meet consumer demands. De Menezes et al. (2014) emphasized the importance of developing new genetic materials for export-oriented fruit production in irrigated regions of Brazil. They highlighted that embryo rescue is effective in semi-arid regions to obtain such materials. They evaluated the in vitro development of intraspecific grape hybrids obtained by rescuing immature embryos from crosses between Brazilian clones "Superior Seedless" and "Thompson Seedless." The culture medium contained 30 g/L sucrose, 0.1 g/L myo-inositol, 0.002 g/L glycine, 0.1 mg/L IAA, and 6.5 g/L agar, adjusted to pH 5.7. Parameters such as node number, leaf number, plant height, root number, and root length were evaluated over 90 days. Ovules cultured at 60 days showed the highest values, with approximately 50% embryo formation and a germination rate of 47.3%.

Embryo rescue is the most important in vitro method used in breeding seedless grapes. Breeding programs incorporating seedlessness along with other traits have also been studied. Moreira and Clark (2021) investigated the impact of embryo isolation timing and nutrient media on embryo rescue efficiency in cold-hardy hybrid grapes. Ovules were collected 5 to 9 weeks after flowering and cultured in four different media. The highest germination and plant development occurred from ovules collected eight weeks post-flowering. Lloyd & McCown WPM medium was found to support the best results. Although different growth regulators did not significantly differ in performance, the WPM plus medium containing BAP, IAA, GA, and casein provided the highest seedling yields. The authors concluded that ovule collection at eight weeks and culture in WPM plus is optimal for breeding programs.

Similarly, Doygaci et al. (2024) emphasized that genotype and embryo isolation timing are the most critical factors in achieving high success rates in embryo rescue. After four years of study, they identified the eighth week after

pollination as the optimal isolation time. They found that the combination using 'Yalova Seedless' as the female parent resulted in more plant recovery, especially when pollinated with Red Globe, Muscat Bailey A, or Exalta.

Giancaspro et al. (2022) noted that early-ripening grapes often experience embryo abortion, limiting traditional breeding. They applied a three-stage in vitro culture protocol—embryo development, germination, and rooting—to obtain viable plants from immature ovules of stenospermocarpic table grape hybrids. Factors such as parent genotype, ovule sampling time (30, 40, 50 days post-pollination), and duration of embryo germination induction (4, 6, 8 weeks) were evaluated. The optimized protocol involved isolating immature ovules 40 days after pollination and inducing germination for eight weeks. The best results came from hybrids of Thompson, Superior, and Regal, while the highest plant survival was from Luisa × Thompson crosses sampled at 50 days.

Chiaromonte et al. (2023), within the Italian Variety Club's 2017–2021 breeding program, validated an optimized embryo culture protocol across 39 cultivars and 41 hybrid combinations. They reported that sampling time (43–62 days after pollination) did not significantly affect success, but genotype did.

Chu et al. (2023) used embryo rescue to develop cold-resistant grape varieties. Using stenospermocarpic female parents and seeded, cold-hardy male parents, they evaluated genotype, sampling time, and culture conditions across 14 hybrid combinations. The highest embryo development (39.9%) and plant formation (21.5%) were observed in the cross of 'Ruby Seedless' × 'Beibinghong.' Optimal embryo isolation days for 'Yuehong Wuhe,' 'Ruby Seedless,' and 'Melissa Seedless' were 37, 55, and 52 days post-flowering, respectively. A sucrose concentration of 1.0–1.5% yielded the best results. They identified 91 seedless and 18 cold-tolerant hybrids using molecular markers.

In another study, different sampling times (20, 30, 40, and 50 days after pollination) were tested using NN medium with varying concentrations of BAP (0.1, 0.2, and 0.5 mg/L) and activated charcoal (1.5, 2, and 2.5 g/L). The best results across all parameters were achieved from ovules sampled at 40 days and cultured in NN medium supplemented with 0.5 mg/L BAP and 2 g/L activated charcoal. The study emphasized the importance of correct sampling timing and protocol standardization for efficient embryo rescue and seedless hybrid development (Nigar et al., 2024).

Li et al. (2024) aimed to explain the relationship between embryo rescue and hormonal changes in seedless grape breeding. They used four Eurasian seedless varieties: 'Thompson Seedless,' 'Flame Seedless,' 'Heshi Seedless,' and 'Ruby Seedless.' Endogenous hormone levels (3-IAA, GA, ABA) were measured at optimal embryo rescue times in both fruit and in vitro ovules. Exogenous application of these hormones during embryo rescue revealed significant variations in internal hormone levels among ovules from the same cultivar. This indicated a hormonal influence on ovule abortion and embryo development. Effective BDM concentrations were also determined:

For ovule development in fruit: 30 mg/L IAA + 30 mg/L ABA

For in vitro ovule development: 1.0 mg/L IAA + 2.0 mg/L 6-BA + 1.0 mg/L GA + 1.0 mg/L ABA

For embryo germination and seedling formation: 1.0 mg/L IAA + 2.0 mg/L 6-BA + 1.0 mg/L GA

The study concluded that hormonal changes significantly influence ovule and embryo development, closely linking embryo rescue success with hormone regulation. This research deepens the understanding of hormonal interactions in embryo rescue and supports the development of new seedless grape cultivars through advanced breeding technologies.

Embryo rescue, a fundamental technique in seedless grape production, has limited success due to the inadequacy of existing protocols. In one study, the 00-1-5 ('Muscat Hamburg' × 'Vitis amurensis'), a cold-tolerant seeded hybrid, was crossed with four stenospermocarpic seedless grape cultivars ('Flame Seedless', 'Qinhong No. 2', 'Qinhong No. 10', 'Ruby Seedless'). Application of 30–50 mg L^{-1} IBA 14 days before flowering was found to significantly increase embryo development, germination, and seedling emergence. Embryo development was found to be higher on solid MM3 medium than on solid-liquid dual-phase medium. 'Ruby Seedless', 'Qinhong No. 2', and 'Qinhong No. 10' were reported to be the most suitable genotypes for embryo rescue as maternal parents. The most effective medium for ensuring normal development of deformed seedlings was 2×MS medium containing 0.2 mg L^{-1} 6-BA, 0.1 mg L^{-1} IAA, and 1.6 mg L^{-1} $ZnSO_4$. As a result of the study, a total of 311 embryos were successfully rescued, and these embryos were evaluated as cold-tolerant seedless hybrid candidates (Zhu et al., 2024).

The success of embryo rescue studies in seedless grapes depends on understanding the mechanism of egg cell abort. In a study conducted for this purpose, changes in 21 free amino acids and 9 mineral elements in egg

cells of four grape varieties ("Flame Seedless," "Ruby Seedless," "Muscat Hamburg," and "Pinot Noir") were examined across six developmental stages over two years. Metabolomic analyses revealed significant differences between seeded and seedless varieties. Multivariate statistical analyses revealed that 11 amino acids (e.g., glutamine, arginine, alanine, GABA) and 5 mineral elements (N, P, K, Ca, Mg) were associated with egg cell degeneration. Based on these data, 12 different culture media were designed for embryo development, and AM3 medium was found to increase embryo development rates by 8% and plantlet formation rates by 5%. The study provided important findings regarding the improvement of embryo rescue efficiency in seedless grape breeding (Wang et al., 2025).

In addition, the success rate of embryo rescue technique, widely used in seedless grape breeding, depends on many factors. In a study, the effect of 0.5 mg L^{-1} thidiazuron application 10 days before flowering was investigated on two seedless cultivars of *Vitis vinifera* ('Qinhong No. 2' and 'Qinhong No. 10'). Ovules obtained from open and self-pollinations were cultured in solid-liquid two-phase MM3 medium to evaluate the effect of thidiazuron on embryo development. The results showed that thidiazuron application significantly promoted ovule development and increased the success of embryo rescue in vitro. After the application, embryo development, germination, and seedling emergence rates ranged from 30.77%–42.62%, 29.36%–41.64%, and 22.02%–40.33%, respectively. Ovules obtained from open-pollinations showed higher success than those from self-pollinations. Additionally, MM3 medium was found to be more suitable for embryo development compared to NN and ER media. The germination rate of 'Qinhong No. 10' embryos obtained from open pollination on solid WPM medium was also higher than that of embryos from unpeeled ovaries on NN medium (Zhu and Zhang, 2025).

In a similar study, 22 hybrid combinations were investigated to increase embryo rescue efficiency in seedless grape breeding, and the effects of different parental genotypes and plant hormones on embryo development and germination were evaluated. Studies on the conversion of abnormal plantlets to normal were also conducted. Based on the results, 'Ruby Seedless', 'Delight', 'Huozhouheiyu', 'Zitian Seedless', and 'Zhengyan Seedless' were identified as the maternal parents, while 'Zitian Seedless', 'Shennongxiangfeng', 'Hongqitezao', and 'Guibao' were identified as the paternal parents. The highest embryo rescue rates were obtained in the combinations 'Ruby Seedless × Shennongxiangfeng' (55.05%) and 'Ruby Seedless × Zitian Seedless' (59.76%). The addition of 1.0 mg·L^{-1} zeatin to MM3 medium increased the development rate of 'Ruby Seedless × Zitian

Seedless' embryos to 64.73%, while the addition of 0.2 mg·L^{-1} ZT + 0.2 mg·L^{-1} IAA to WPM medium achieved the highest germination rate of 85.71% in 'Huozhouheiyu × Shine Muscat.' Furthermore, 3,365 abnormal plants were successfully rescued, 1,234 normal plants were regenerated, and a total of 4,287 seedlings were transplanted by direct transformation and cotyledon induction methods (Chen et al., 2025).

5. Conclusion

Embryo culture is a tissue culture technique that can be successfully used for purposes such as developing new cultivars, using seedless grape varieties in breeding, rescuing degenerate embryos, and producing haploid plants. It can also be used to breed cultivars resistant to diseases, pests, and various stresses. In situations where conventional breeding methods are inadequate, embryo culture is an effective alternative, enabling rapid results. Integrating conventional breeding methods with embryo culture is crucial in this regard. Furthermore, factors such as genotype, the physiological developmental stage of the isolated explant, nutrient medium, and culture conditions influence the success of this technique. Embryo culture also requires significant technical experience and laboratory equipment.

References

Ahuja, M. R. (1986). Application of biotechnology to forest tree species and problems involved. *Holzwirtschaft, 154,* 187–199.

Bajaj, Y. P. S. (1990). In vitro production of haploids and their use in cell genetics and plant breeding. In *Haploids in crop improvement I* (pp. 3-44). Berlin, Heidelberg: Springer Berlin Heidelberg.

Bouquet, A., Davis, H.P. (1989). In vitro ovule and embryo culture for breeding seedless table grapes (*Vitis vinifera* L.). Agronomie 9:565–574.

Bridgen, M. P. (1994). A review of plant embryo culture. *HortScience, 29*(11), 1243–1246.

Burgos, L., & Ledbetter, C. A. (1993). Improved efficiency in apricot breeding: Effects of embryo development and nutrient media on in vitro germination and seedling establishment. *Plant Cell, Tissue and Organ Culture*, *35*, 217-222.

Cain, D.W., Emershad, R.L., Tarailo, R.E. (1983). In-ovulo embryo culture and seedling development of seeded and seedless grapes (*Vitis vinifera* L.). *Vitis* 22:9–14.

Chen, X., Ayesha, K., Wen, X., Zhang, Y., Dou, M., Jia, K., ... & Xu, Y. (2025). An integrate methods to improve the high efficiency of embryo rescue breeding in seedless grapes. *Journal of Integrative Agriculture*.

Chiaromonte, E., Bottalico, G., Lanotte, P., Campanale, A., Montilon, V., Morano, M., ... & Savino, V. N. (2023). A Large-Scale Validation of an Improved Embryo-Rescue Protocol for the Obtainment of New Table-Grape Seedless Genotypes. *Plants*, *12*(19), 3469.

Chu, Y., Li, M., Li, R., Zhang, K., Qiu, P., Yuan, X., ... & Liu, G. (2023). Embryo Rescue Breeding of New Cold-Resistant, Seedless Grapes. *Horticulturae*, *9*(9), 992.

de Menezes, E. F., Silva, E. M., Melo, A., Leão, P. D. S., & de Melo, N. F. (2014). Immature embryo rescue and in vitro development evaluation of intraspecific hybrids from Brazilian seedless grapevine Superior × Thompson clones. *American Journal of Plant Sciences*, 5(13), 1956-1960.

Doyğacı, Y., Boztepe, Ö., Kandilli, G. G., & Atak, A. (2024). Embryo recovery (rescue) studies in different *Vitis* species. *BMC Plant Biology*, *24*(1), 822.

Drew, R. A. (1997). The application of biotechnology to the conservation and improvement of tropical and subtropical fruit species. Seed and Plant Genetic Resources Service, Food and Agriculture Organization of the United Nations, Rome. Retrieved from http://www.fao.org/ag/agp/agps/pgr/drew1.htm#Sub41.4

Driver, J. A., & Kuniyuki, A. H. (1984). In vitro propagation of Paradox walnut rootstock. *HortScience*, *19*(4), 507-509.

Elidemir, A. Y., & Uzun, H. İ. (1999). Erkenci ve çekirdeksiz üzümlerin (*Vitis vinifera* L.) embriyo kültürü yoluyla çoğaltılması üzerine araştırmalar. *Akdeniz University Journal of the Faculty of Agriculture, 12*(1), 93–106.

Emershad, R.L., Ramming, D.W. (1982). In-ovulo embryo culture of *Vitis vinifera* L. cv. 'Thompson Seedless'. *HortScience,* 17:576.

Ergönül, O., Özer, C., Orhan, Z., Uysal, T., Korkutal, İ. (2021). Studies on Embryo Culture and Embryo Viability in Early Ripening Cultivars (Vitis vinifera L.). *Journal of Agriculture and Nature*, 24 (1): 65-71.

Gamborg, O.L., Miller, R.A., Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research,* 50:151–158.

George, L., & Eapen, S. (1993). Influence of genotype and explant source on somatic embryogenesis in peanut.

Giancaspro, A., Mazzeo, A., Carlomagno, A., Gadaleta, A., Somma, S., & Ferrara, G. (2022). Optimization of an in vitro embryo rescue protocol for breeding seedless table grapes (*Vitis vinifera L.*) in Italy. *Horticulturae, 8,* 121.

Goldy, R. G., Emershad, R., Ramming, D., & Chaparro, J. X. (1988). Embryo culture as a means of introgressing seedlessness from *Vitis vinifera* to *V. rotundifolia*. *HortScience, 23*(5), 886–889.

Gray, D.J., Mortensen, J.A., Benton, C.M. (1990). Ovule culture to obtain progeny from hybrid seedless bunch grapes. *Journal of American Society of Horticultural Science,* 115:1019–1024.

Guney, M., Zarifikhosrohahi, M., Comlekcioglu, S., Keles, H., Gundesli, M. A., Kafkas, E., & Ercisli, S. (2020). Efficiency of various plant growth regulators on micropropagation of hawthorn (*Crataegus* spp.). *Comptes Rendus de l'Academie Bulgare des Sciences*, 73 (1), 65.

Güven, A. ve Gürsul I. (2014). Secondary metabolite synthesis in plant tissue cultures.

Ji W, Li Z, Yao W, Gong P, Wang Y (2013a). Abnormal seedlings emerged during embryo rescue and its remedy for seedless grape breeding. Korean Journal of Horticultural Science and Technology, 31:483–489.

Harbage, J. F., & Stimart, D. P. (1987). Adventitious shoot regeneration from in vitro subcultured callus of *Rhododendron exbury* hybrids. *HortScience, 22*(6), 1324–1325.

Ji, W., Li, Z.Q., Zhou, Q., Yao, W.K., Wang, Y.J. (2013b). Breeding new seedless grape by means of in vitro embryo rescue. *Genetics and Molecular Research (GMR)*, 12:859–869.

Kurt, O. (2001). Bitki Islahı. Ondokuz Mayıs Üniversitesi Ziraat Fakültesi, Ders Kitabı Yayın No: 43.

Laibach, F. (1925). das Taubwerden von Bastardsamen und die kunstliche Aufzucht fruh absterbender Bastardembryonen. Z Bot 17:417–459.

Li, G.R., Wang, G., Wang, Y.J. (2013). Embryo rescue to obtain progeny from seedless grapes (*Vitis vinifera* L.) 9 wild Chinese *Vitis* species. *Journal of Food, Agriculture and Environment,* 11:1058–1062.

Li, G., Li, K., Lu, Y., Fan, X., & Wang, L. (2024). The correlation between embryo rescue and hormonal changes in seedless grapes. *Frontiers in Plant Science, 15,* 1460886.

Li, J., Wang, X., Wang, X., & Wang, Y. (2015). Embryo rescue technique and its applications for seedless breeding in grape. *Plant Cell, Tissue and Organ Culture (PCTOC), 120,* 861–880.

Liu, S.M., Sykes, S.R., Clingeleffer, P.R. (2003). Improved in ovulo embryo culture for stenospermocarpic grapes (*Vitis vinifera* L.). *Australian Journal of Agricultural Research*, 54:869–876.

Lloyd, G., McCown, B. (1980). Commercially-feasible micropropagation of mountain laural, *Kalmia latifolia*, by use of shoot-tip culture. *Proceedings of the International Plant Propagator's Society*, 30:421–427.

Moreira, L. S., & Clark, M. D. (2021). Embryo rescue of cold-hardy table grapes. *HortScience*, *56*(9), 1059-1065.

Murashige, T., Skoog, L. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15:473–497.

Narayanaswami, S., Norstog, K. (1964). Plant embryo culture. *The Botanical Review,* 30:587–628.

Nigar, Q., ul Hassan, M., Rana, R. M., Ali, I., Almoallim, H. S., & Ansari, M. J. (2024). Optimization of embryo rescue technique for development of hybrid plants in stenospermic grapes. *Emirates Journal of Food and Agriculture, 36,* 1–11.

Nitsch, J.P., Nitsch, C. (1969). Haploid plants from pollen grains. *Science,* 163:85–87.

Pierik, R. L. M. (1986). *In vitro culture of higher plants.* The Netherlands.

Ponce, M.T., Agu ero, C.B., Gregori, M.T., Tizio, R. (2000). Factors affecting the development of stenospermic grape (*Vitis vinifera*) embryos cultured in vitro. *Acta Horticulturae*, 528:667-671.

Raghavan, V. (2003). One hundred years of zygotic embryo culture investigations. *In Vitro Cellular and Developmental Biology-Plant, 39,* 437–442.

Ramming, D.W. (1990). The use of embryo culture in fruit breeding. *HortScience,* 25:393–398.

Ramming, D. W., Emershad, R. L., Spiegel-Roy, P., Sahar, N., & Baron, I. (1990). Embryo culture of early ripening seeded grape (*Vitis vinifera*) genotypes.

Razi, M., Marandi, R.J., Baneh, H.D., Hosseini, B., Darvishzadeh, R. (2013). Effect of paternal genotypes sprays with BA and IAA concen tration on embryo rescue of F1 progenies from 'Askari' (*Vitis vinifera* L.) cultivar. *Journal of Agricultural Science and Technology,* 15:1023–1032.

Reddy, C. S., Ramireddy, S., & Reddy, U. K. (2024). Widening genetic diversity using embryo rescue in cucurbit crops: a review. *Plants*, 13(10), 1320.

Reed, S. M. (2005). Embryo rescue. *Plant Development and Biotechnology,* 18, 235–239.

Roichev, V., Yancheva, S.D., Petkova, S. (2007). Embryogenesis in seedles grapes and hybrid combinations of (*Vitis vinifera* L.) somatic embryogenesis in liquid culture part I. *Biotechnology & Biotechnological Equipment,* 21(1):43–48.

Scaltsoyiannes, A., Tsoulpha, P., Panetsos, K. P., & Moulalis, D. (1998). Effect of genotype on micropropagation of walnut trees (*Juglans regia*). *Silvae Genetica*, *46*, 326-331.

Sharma, D.R., Kaur, R., Kumar, K. (1996). Embryo rescue in plants: a review. *Euphytica,* 89:32337.

Smith, C.A., Bailey, C.H., Hough, L.F. (1969). Methods for germinating seeds of some fruit species with special reference to growing seedlings from immature embryos. N J Agric Exp Stn Rutgers Univ Bul 823:1–29.

Srivastava, P. S., & Steinbaver, A. (1981). Regeneration of birch plants from catkin tissue cultures. *Plant Science Letters, 22.*

Stewart, J.M., Hsu, C.L. (1977). In-ovulo embryo culture and seedling development of cotton (*Gossypium hirsutum* L.). *Planta*, 137:113–117.

Tian, L.L., Wang, Y.J., Niu, L., Tang, D.M. (2008). Breeding of disease- resistant seedless grapes using *Chinese* wild *Vitis* spp. I. In vitro embryo rescue and plant development. Scientia Horticulturae, 117:136–141.

Tukey, H.B., (1933). Artificial culture of sweet cherry embryos. *Journal of Heredity*, 24: 7-12.

Vidalie, H. (1986). In vitro culture and its applications in horticulture. *Economic Botany, 51*(1), 92–93.

Wang, F., Bian, L., Xu, G., Cheng, M., Xu, T., Liu, G., & Xu, Y. (2025). Scientific optimization and application of embryo development medium for seedless grape embryo rescue. *Scientia Horticulturae*, 346, 114176.

White, P.R. (1954). The cultivation of animal and plant cells. *Ronald Press*, New York

Xu, H.Y., Yan, A.L., Zhang, G.J. (2005). Determination of the proper sampling period for embryo rescue from crosses between diploid and tetraploid grape cultivars. *Scientia Agricultura Sinica,* 38:629–633.

Yang D, Li W, Li S, Yang X, Wu J, Cao Z (2007) In vitro embryo rescue culture of F1 progenies from crosses between diploid and tetraploid grape varieties. *Plant Growth Regul*ation, 51:63–71.

Yao, H., Lelong, Y., Yanyan, C., Zhihu, M., Yongping, Z., & Chuntao, Q. (2024). Improvement of embryo rescue efficiency in haploid melon (Cucumis melo L.) via irradiated pollen pollination. Plant Cell, Tissue and Organ Culture (PCTOC), 159(2), 36.

Yıldırım, H. (2012). Effects of Media and Genotype on the in vitro Embryo Cultures from National Varieties of Apricot. *Journal of the Institute of Science and Technology*, *2*(2), 9-16.

Zhu, P., & Zhang, J. (2025). Effects of pre-bloom spraying thidiazuron and different embryo development media on seedless grape embryo rescue. New Zealand Journal of Crop and *Horticultural Science*, 53(1), 113-140.

Zhu, P., Tian, Y., Liu, Q., Ge, Q., & Zhang, J. (2024). Optimisation of embryo rescue for cold-resistant seedless grapevine. *New Zealand Journal of Crop and Horticultural Science*, 52(1), 38-51.