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RESEARCH ARTICLE

An integrate methods to improve the high efficiency of embryo rescue breeding in seedless grapes

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Highlights

- Optimization of culture media for embryo development and germination in seedless grapes demonstrated that supplementation with zeatin and auxin significantly enhances both embryo development and germination rates.
- Refinement of the conversion medium for abnormal plantlets in embryo rescue, combined with establishment of a regeneration system for inducing adventitious buds from these plantlets, enabled the recovery of 35% of otherwise abnormal plantlets, thereby improving seedling formation efficiency in embryo rescue.

Abstract

The embryo rescue technique plays an essential role in developing new seedless grape varieties. To enhance the efficiency of seedless grape embryo rescue breeding, this study evaluated 22 hybrid combinations and systematically investigated the effects of parental genotypes and plant hormones on embryo development and germination. Additionally, an in-depth analysis was conducted on the conversion of abnormal plantlets. Results indicate that 'Ruby Seedless', 'Delight', 'Huozhouheiyu', 'Zitian Seedless', and 'Zhengyan Seedless' are suitable as maternal parents, whereas 'Zitian Seedless', 'Shennongxiangfeng', 'Hongqitezao', and 'Guibao' perform optimally as paternal parents. Among these, the crosses 'Ruby Seedless×Shennongxiangfeng' and 'Ruby Seedless×Zitian Seedless' exhibited the highest embryo rescue efficiency, with embryo development rates of 55.05 and 59.76%, yielding 1,348 and 2,235 viable plantlets, respectively. When 1.0 mg L^{-1} zeatin (ZT) was added to the MM3 medium supplemented with 0.2 mg L^{-1} indole-3-acetic acid (IAA), the embryo development rate of 'Ruby Seedless×Zitian Seedless' increased by 64.73%. In the WPM germination medium, supplementation with 0.2 mg L^{-1} ZT and 0.2 mg L^{-1} IAA resulted in the highest germination rate of 85.71% for the hybrid combination 'Huozhouheiyu×Shine Muscat'. Furthermore, 3,365 abnormal plantlets were rescued *via* direct transformation and hypocotyl-induced adventitious bud regeneration, among which 1,234 were transformed into normal plantlets. Following hybridization, a total of 4,287 plants were successfully acclimatized and transplanted. This study provides theoretical insights to improve the efficiency of embryo rescue breeding in seedless grapes and offers valuable genetic resources for future breeding programs.

Keywords: seedless grape, embryo rescue, parental genotype, plant hormones, abnormal plantlets

1. Introduction

Seedless grapes are a vital component of both the fresh and dried grape markets, significantly contributing to the advancement of the grape industry. Based on their pollination and fruit-setting characteristics, seedless grapes are classified into two types: parthenocarpic and stenospermocarpic. The parthenocarpic type develops fruit from the ovary without pollination or fertilization. In contrast,

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the stenospermocarpic type undergoes normal pollination and fertilization, but the zygote fails to develop fully, resulting in the absence of mature seeds and leaving only seed remnants of varying sizes — a process commonly referred to as seed abortion (Stout 1936). Since parthenocarpic grapes do not form zygotic embryos, their genetic traits cannot be transmitted to subsequent generations, making them rarely used in seedless grape breeding. In contrast, the seedless trait in stenospermocarpic grapes is inheritable, rendering them the primary source of parental material for embryo rescue breeding in seedless grape programs.

Traditional hybridization in seedless grape breeding typically involves using seeded varieties as the maternal parent and seedless varieties as the paternal parent. However, using seedless grapes as the paternal parent presents significant challenges, often resulting in a low proportion of seedless offspring. The seedless rate in hybrid progeny from such crosses generally ranges from 10.0 to 40.0%, with only a few combinations reaching up to 55% (Loomis and Weinberger 1979). The introduction of embryo rescue techniques in 1982 revolutionized this process by enabling *in vitro* culture of ovules from seedless grape maternal parents, facilitating the germination of seedlings and the development of seedless hybrid offspring (Ramming and Emershad 1982). This technique made it feasible to use seedless grapes as maternal parents, thereby expanding the range of viable hybrid combinations and substantially increasing the proportion of seedless progeny. Moreover, it shortened the breeding cycle and has become a critical auxiliary method for generating novel seedless grape germplasm (Cain *et al.* 1983; Goldy and Amborn 1987; Ramming *et al.* 1990a, b; Tian and Wang 2008a; Li *et al.* 2020). Researchers have also applied embryo rescue in triploid grape breeding (Hiroyuki *et al.* 1995; Yamashita *et al.* 1998; Yang *et al.* 2007; Guo *et al.* 2011) and in distant hybridizations between different grape species (David *et al.* 2000). Over the past four decades, countries including the United States, France, Israel, Bulgaria, India, Japan, China, Mexico, Turkey, and Italy have adopted this technology, leading to the development of over 150 new seedless grape varieties (USPTO, United States Patent Public Trademark Office). Despite these advances, limited understanding of the genetic mechanisms underlying seedlessness continues to pose challenges, making the breeding of new seedless grape varieties through embryo rescue a complex and time-consuming endeavor.

Despite extensive research into the various factors influencing embryo rescue methodologies in seedless grapes, the average seedling survival rate of hybrids obtained via embryo rescue remains disappointingly low. Substantial opportunities exist for improving both ovule development and embryo germination rates (Emershad and Ramming 1984; Gray *et al.* 1987; Bruce and James 1994; Tian *et al.* 2008b; Li *et al.* 2020; Chiaromonte *et al.* 2023). Several key factors have been identified that influence the efficiency of embryo rescue, including parental genotype, timing of ovule sampling, and the composition of the culture medium (including additives) during ovule development and embryo germination stages.

The selection of parental genotypes is critically important for maximizing embryo rescue efficiency (Celso *et al.* 1995). Strong evidence indicates that the maternal genotype exerts a more pronounced influence on the seedless traits of progeny than the paternal genotype (Loomis and Weinberger 1979; Chiaromonte *et al.* 2023; Cui *et al.* 2024). The capacity for embryo formation varies widely among grape cultivars, directly affecting the number of embryos that can be successfully rescued (Ponce *et al.* 2000; Tian *et al.* 2008b; Li *et al.* 2013). For example, studies show that using 'Ruby Seedless' as the maternal parent yields hybrid fruits with superior embryo germination and seedling survival rates compared to 'Perlette', 'Beauty Seedless', and 'Crimson Seedless' (Liu *et al.* 2016). Although the paternal genotype also affects outcomes, its impact is generally less significant than that of the maternal genotype (Mazdak *et al.* 2013; Puglisi *et al.* 2022; Chu *et al.* 2023).

Sampling time is a crucial factor determining the success of embryo rescue. Identifying the optimal sampling period is essential, as it corresponds to a critical stage in embryo development in seedless grapes. Early studies suggested that fruit ripening or color change were suitable indicators for sampling; however, these phenological markers often led to suboptimal results (Gray *et al.* 1990). Because different grape varieties mature at different times, the timing of embryo development and degeneration varies accordingly, aligning with the maternal plant's maturation period. Later studies adopted days after flowering (DAF) or days after pollination (DAP) as more precise metrics for determining the optimal sampling window across genotypes, as they provide a more accurate reflection of embryonic development within ovules (Liu *et al.* 2003; Li *et al.* 2013; Luo *et al.* 2023). Cytological observation of embryo degeneration offers an advanced method to pinpoint the ideal sampling time from the maternal plant (Liu *et al.* 2003; Li *et al.* 2013; Luo *et al.* 2023). Ultimately, the optimal sampling time depends on the parental genotypes of the hybrid combination. While it may remain consistent for certain maternal backgrounds, it can vary even for the same hybrid combination across different years (Ji *et al.* 2013b; Giancaspro *et al.* 2022).

In embryo culture studies, the choice of culture medium is paramount, with media typically categorized as liquid, semi-solid, or solid. There is no universal consensus on the optimal medium type, leading to variable outcomes across studies (Cain *et al.* 1983; Emershad and Ramming 1984; Tian and Wang 2008a; Zhu *et al.* 2022a). The success of ovule culture largely depends on the selected medium, which significantly influences embryo survival and continued development. Numerous basic media have been employed, including Cain medium (Emershad and Ramming 1984), Nitsch and Nitsch medium (Gray *et al.* 1990; Benke *et al.* 2021), White (or modified) medium (Cain *et al.* 1983; Ramming *et al.* 1990a), MS (modified) medium (Bouquet *et al.* 1989; Hiroyuki *et al.* 1995), 1/2 MS medium (Yamashita *et al.* 1998; Celik and Ilbay 2003), ER (modified) medium (Tian and Wang 2008a; Li *et al.* 2013), and MM3 medium (Zhu and Zhang 2022b). Currently, MM3 medium is the most widely used in China. During later stages of embryo development, WPM medium is frequently used due to its ability to meet the nutritional requirements for both embryo germination and

seedling growth, thus supporting subsequent subculturing (Giancaspro *et al.* 2022; Chu *et al.* 2023).

Additives in the culture medium play a significant role in enhancing embryo rescue efficiency. Many researchers agree that selecting appropriate plant hormones throughout the process is critical for improving culture outcomes. Commonly used hormones include indole-3-acetic acid (IAA), naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), 6-benzylaminopurine (6-BA), zeatin (ZT), and gibberellic acid 3 (GA_3) (Emershad and Ramming 1984; Ding and Qi 2001; Tian *et al.* 2008b). During ovule development culture, both the type and concentration of hormones significantly affect outcomes. Liu *et al.* (2016) investigated the effects of varying IAA concentrations in MM3 medium on hybrid ovule development, finding that 1.75 mg L^{-1} IAA improved embryo rescue efficiency in the crosses 'Ruby Seedless×00-1-5' and 'Beauty Seedless×Beichun'. During embryo germination, hormonal requirements are simpler, and seedlings can develop without exogenous hormones. Nevertheless, research shows that applying suitable hormone concentrations can further promote germination and enhance seedling survival (Liu *et al.* 2003; Zhu *et al.* 2020). Additional components such as antioxidants, amino acids, polyamines, and banana puree, when added to the ovule development medium alongside plant hormones, have been shown to support ovule growth, improve germination, and reduce the incidence of abnormal plantlets (Tian and Wang 2008a; Ji *et al.* 2013b; Li *et al.* 2014; Jiao *et al.* 2018).

Besides the aforementioned factors, during the *in vitro* germination phase of seedless grape embryos in embryo rescue breeding, approximately 40% of plantlets exhibit abnormalities. This high rate significantly undermines the effectiveness of embryo rescue in seedless grape breeding. Factors such as parental genotypes, the ovule development medium, the embryo germination medium, and the morphological characteristics of developing embryos all contribute to the emergence of abnormal plantlets. Current strategies for rescuing abnormal plantlets involve modifying the culture medium to encourage their transition into normal seedlings, although rescue efficiency varies depending on the type of abnormality (Hiroyuki *et al.* 1995; Ji *et al.* 2013a; Li *et al.* 2020).

This study focuses on optimizing embryo rescue technology by selecting grape varieties with superior horticultural traits and rose fragrance as parental materials. It examines the effects of various hybrid combinations (seedless×seeded, seedless×seedless, diploid×tetraploid, and tetraploid×diploid) on embryo rescue efficiency. The research evaluates how parental genotypes and plant hormones influence ovule development and embryo germination media. Additionally, it emphasizes the recovery of abnormal plantlets to improve overall success rates. The ultimate goal is to refine embryo rescue techniques for seedless grape breeding, thereby establishing a robust foundation for the development of new seedless grape varieties.

2. Materials and methods

2.1. Plant materials

This experiment was conducted from May 2023 to June 2024 at the State Key Laboratory of Crop Stress Resistance and High-Efficiency Production, Northwest A&F University, China, and at the Xinjiang Uygur Autonomous Region Research and Development Center for Facility Agriculture and Specialty Agriculture, China. A total of 22 hybrid combinations were evaluated. 'Shennongxiangfeng' is a tetraploid seeded grape variety, whereas the remaining varieties are all diploid seedless (or seeded) grape varieties. These hybrid combinations resulted from crosses between specific paternal and maternal parents, whose characteristics are detailed in Table 1.

2.2. Hybridization

When collecting pollen from the paternal parent, it is ideal to

Table 1 The characteristics of paternal and maternal parents

Cross-combinations	Maternal characteristics	Paternal characteristics
Zhengyan Seedless×Italy	Stenospermic; Muscat	Seeded; Muscat
Zhengyan Seedless×Guibao	Stenospermic; Muscat	Seeded; Muscat
Huozhouheiyu×Sweet Scarlet	Stenospermic	Stenospermic; Muscat
Huozhouheiyu×Shine Muscat	Stenospermic	Seeded; Muscat
Delight×Hongqitezao	Stenospermic	Seeded; Muscat
Delight×Guibao	Stenospermic	Seeded; Muscat
Melissa Seedless×Heihali	Stenospermic; Muscat	Seeded; high fruit quality
Melissa Seedless×Lüzhoubaoshi	Stenospermic; Muscat	Seeded; high fruit quality
Flame Seedless×Canadice Seedless	Stenospermic; high fruit quality	Stenospermic; Muscat
Flame Seedless×Hongqitezao	Stenospermic; high fruit quality	Seeded; Muscat
Flame Seedless×Italy	Stenospermic; high fruit quality	Seeded; Muscat
Zitian Seedless×Melissa Seedless	Stenospermic; high fruit quality	Stenospermic; Muscat
Zitian Seedless×Heihali	Stenospermic; high fruit quality	Seeded; high fruit quality
Ruby Seedless×Zitian Seedless	Stenospermic; high fruit quality	Stenospermic; high fruit quality
Shennongxiangfeng×Sweet Scarlet	Seeded; high fruit quality; Diploid	Stenospermic; Muscat
Zitian Seedless×Shennongxiangfeng	Stenospermic; high fruit quality	Seeded;high fruitquality; Diploid
Jingzaojing×Muscat Hamburg	Stenospermic; Muscat	Seeded; Muscat
Jingzaojing×Shine Muscat	Stenospermic; Muscat	Seeded; Muscat
Jingzaojing×Sweet Scarlet	Stenospermic; Muscat	Stenospermic; Muscat
Crimson Seedless×Muscat Hamburg	Stenospermic; high fruit quality	Seeded; Muscat
Crimson Seedless×Sweet Scarlet	Stenospermic; high fruit quality	Stenospermic; Muscat

select inflorescences from vigorous, fruit-bearing branches. Pollen viability peaks when approximately 5% of the flowers in an inflorescence are open (Fig. 1-A). After harvesting, grind the inflorescences into powder and store the pollen at 4°C for future use. For the maternal grape variety, select healthy plants with well-developed inflorescences suitable for emasculation, based on their phenological stage in the local area. During emasculation, care must be taken not to damage the stigma. Following emasculation, double-check for any missed flowers, spray water to remove residual pollen, and maintain the stigma in a moist condition (Fig. 1-B). Then, bag the inflorescence, attach a label, and record the maternal variety and emasculation date. Two to three days after emasculation, when the stigma secretes a large amount of clear mucilage (Fig. 1-C), use clean tweezers to pick up a cotton ball dipped in pollen. Gently tap the cotton ball to evenly distribute the pollen onto the stigma (Fig. 1-D). Immediately cover the inflorescence with a paper bag (Fig. 1-E) and continue pollinating for 2 to 3 d, recording the paternal parent information and pollination date. Then, at 7 DAP, observe the inflorescence's growth (Fig. 1-F) and confirm that the hybridization bag remains securely in place.



Fig. 1 Process of field hybridization. A, collection of pollen from the paternal parent. B, emasculation of the maternal inflorescence. C, secretion of clear mucilage from the stigma. D, pollination. E, bagging and recording hybridization information. F, observation of hybrid young fruit growth status seven DAP.

2.3. Sampling

The genotype of the maternal parent and the number of DAP determine the sampling time for hybrid ovules. Based on previous studies by the research group and annual climatic conditions, the specific sampling times for different maternal parents are as follows: 'Zhengyan Seedless': 41 DAP; 'Huozhouheiyu' and 'Jingzaojing': 40 DAP; 'Melissa Seedless' and 'Delight': 41 DAP; 'Flame Seedless': 41 DAP; 'Crimson Seedless': 43–45 DAP; 'Zitian Seedless': 47 DAP; and 'Ruby Seedless': 55 DAP.

2.4. Process of *in vitro* embryo rescue

Following the optimal sampling time, hybrid young fruits from various combinations are collected and transported to the laboratory (Fig. 2-A). Subsequently, they are stored at 4°C for further use. First, remove the fruit peduncles and place the fruits in a beaker, submerging them in diluted dishwashing liquid for 15–20 min, followed by rinsing under running water for 2–3 h. The cleaned fruits are then transferred to a sterile workspace and placed in sterilized glass bottles. Sterilization involves two rinses with sterile water, immersion in 75% ethanol for 30 s, 2–3 additional rinses with sterile water, soaking in 2% NaClO solution for 20 min, and a final five rinses with sterile water. After sterilization, the fruits are transferred to petri dishes (Fig. 2-B) and dissected longitudinally to extract the ovules, which are then inoculated into an ovule development medium (Fig. 2-C). The petri dishes are sealed, and the number and date of inoculated ovules, along with the hybrid combination

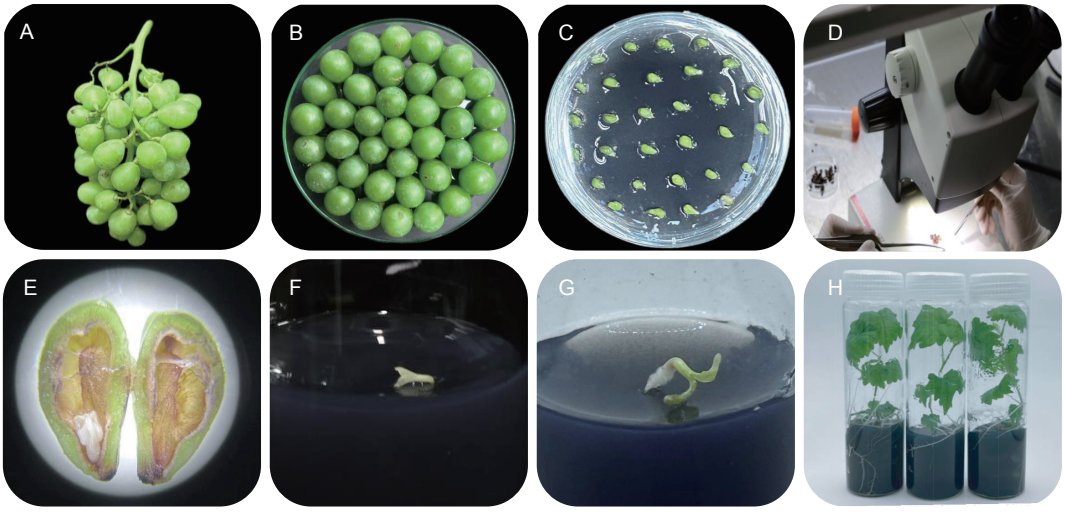


Fig. 2 Process of *in vitro* embryo rescue. A, collected hybrid young fruits. B, sterilized hybrid young fruits. C, ovule culture. D, extraction of embryos under a stereomicroscope. E, embryos after dissecting the ovules. F, inoculation of embryos into the embryo germination medium. G, emergence of cotyledons and embryonic roots. H, development of seedlings from the embryos.

name, are recorded. The dishes are incubated in a constant temperature chamber at (25±1)°C under dark conditions for 8–10 weeks. In a sterile working area, embryos within the ovules are extracted under a stereomicroscope (Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland) (Fig. 2-D and E) and transferred to an embryo germination medium (Fig. 2-F). After recording the inoculation details, the dishes are placed in a constant temperature incubation room at 25±1°C with a 16-h light/8-h dark photoperiod. Over 30–40 d, hybrid embryos develop into seedlings (Fig. 2-G and H).

2.5. Effect of different ZT concentrations on embryo development

Hybrid ovules from 'Flame Seedless×Hongqitezao', 'Ruby Seedless×Zitian Seedless', 'Zitian Seedless×Heihali', and 'Delight×Guibao' were used as experimental materials. These ovules were excised under *in vitro* conditions and inoculated onto different treatment media. The base medium consisted of MM3, 0.1 g L^{-1} myo-inositol, 3 g L^{-1} activated charcoal, 7 g L^{-1} agar, 60 g L^{-1} sucrose, and 0.5 g L^{-1} hydrolyzed casein, supplemented with varying concentrations of ZT (0 mg L^{-1}, 0.5 mg L^{-1}, 1.0 mg L^{-1}, and 1.5 mg L^{-1}) and a constant concentration of IAA (0.2 mg L^{-1}). Treatments were designated as CK, T1, T2, T3, and T4, respectively. After 8 weeks of dark incubation, the embryo development rate was recorded.

2.6. Effect of different plant hormones on embryo germination

Hybrid embryos from 'Flame Seedless×Hongqitezao', 'Huizhouheiyu×Shine Muscat', and 'Zitian Seedless×Heihali' were used as experimental materials and inoculated onto five different germination media. The basic medium was WPM+7 g L^{-1} agar+20 g L^{-1} sucrose+1.5 g L^{-1} activated charcoal+0.1 g L^{-1} myo-inositol, with the following treatments: 0.2 mg L^{-1} 6-BA; 0.2 mg L^{-1} 6-BA+0.2 mg L^{-1} IAA; 0.2 mg L^{-1} ZT; 0.2 mg L^{-1} ZT+0.2 mg L^{-1} IAA; and 0.5 mg L^{-1} ZT+0.2 mg L^{-1} IAA. Treatments were labeled C1, C2, C3, C4, and C5, respectively. After 40 d, the germination rate was recorded.

2.7. Effect of transformation culture screening on the transformation rate of abnormal plantlets

Three transformation media for abnormal plantlets were established: Medium A (MS+2 mg L^{-1} 6-BA+0.5 mg L^{-1} IBA); Medium B (WPM+2 mg L^{-1} 6-BA+2.0 mg L^{-1} IAA); and Medium C (WPM+2 mg L^{-1} 6-BA+2.0 mg L^{-1} IBA). After 30 d, the medium exhibiting the highest transformation rate was selected.

2.8. Effect of 9 types of abnormal plantlets on the transformation of normal plantlets

Abnormal plantlets from 18 hybrid combinations were classified into 13 types (Fig. 3), with the 9 most prevalent abnormalities selected for analysis. These included monocotyledon abnormalities, leafless and rootless abnormalities, cotyledon twisting and wrinkling, cotyledon fusion, root-bearing but cotyledon-less abnormalities, cotyledon-bearing but rootless abnormalities, halted development, multiple cotyledons, and tubular cotyledon abnormalities. A total of 3,365 plantlets were inoculated onto the transformation medium for cultivation. After 30 d, the number of transformed normal plantlets derived from each of the 9 abnormal types across different hybrid combinations was recorded.

2.9. Effect of different induction media on the regeneration of adventitious buds

For abnormal plantlets that could not be directly transformed, explants were divided into cotyledons, hypocotyls, and roots, and inoculated onto four induction media: T1 (MS+2 mg L^{-1} 6-BA+1.0 mg L^{-1} 2,4-D+60 g L^{-1} sucrose+3 g L^{-1} phytagel); T2 (MS+2 mg L^{-1} 6-BA+1.0 mg L^{-1} IAA+60 g L^{-1} sucrose+3 g L^{-1} phytagel); T3 (MS+0.2 mg L^{-1} 6-BA+2.0 mg L^{-1} IBA+20 g L^{-1} sucrose+7 g L^{-1} agar); and T4 (MS+2 mg L^{-1} ZT+0.2 mg L^{-1} 2,4-D+20 g L^{-1} sucrose+7 g L^{-1} agar), to induce adventitious bud regeneration. For each treatment, a number of 20 abnormal plantlets were selected, with 3 replicates conducted. After 30 d of differentiation, regenerating tissues were transferred to the seedling culture medium: 1/2 MS+0.3 mg L^{-1} IBA+30 g L^{-1} sucrose+3 g L^{-1} phytagel+1.5 g

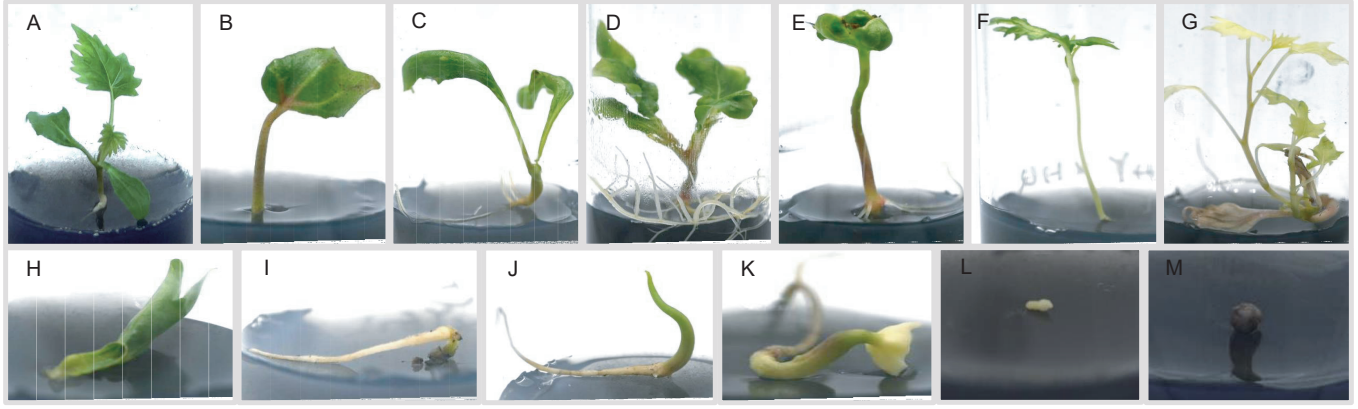


Fig. 3 Thirteen types of plantlets that appeared during the rescue process of embryos. A, normal plantlets. B, monocotyledon abnormalities. C, cotyledon twisting and wrinkling abnormalities. D, multiple cotyledon abnormalities. E, tubular cotyledon abnormalities. F, elongated plantlets. G, albino plantlets. H, cotyledon-bearing but rootless abnormalities. I, root-bearing but cotyledon-less abnormalities. J, developmentally halted plantlets. K, cotyledon fusion abnormalities. L, not germinated. M, leafless and rootless abnormalities.

L^{-1} activated charcoal for further development. After 30 d, the number of developed plantlets was recorded.

2.10. Hardening and transplanting hybrid plantlets

In March, select vigorous, well-rooted tissue-cultured plantlets and transfer them from the tissue culture room to a climate-controlled chamber. Gradually loosen the bottle caps over 3–4 d to acclimatize the plantlets (Fig. 4-A). Transplant the plantlets into sterile matrix and apply a 1/8 MS nutrient solution. Cover the plantlets with a transparent seedling lid (Fig. 4-B), gradually removing the cover over the next 1–2 weeks (Fig. 4-C). After one month, select robust hybrid plantlets with healthy, well-proportioned leaves for field planting. Ensure proper labeling and proceed with standard field management (Fig. 4-D and E). Three months after transplantation, evaluate the survival rate of the transplanted plantlets (Fig. 4-F).

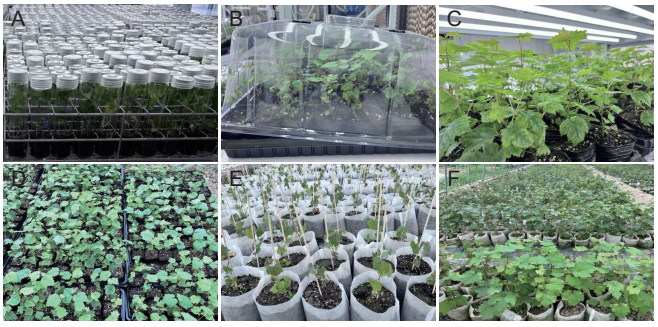


Fig. 4 Transplanting process of hybrid plants. A, opening the lid of the tissue culture plantlets. B, covering the plantlets with a transparent seedling lid. C, opening the lid completely. D and E, transfering to an outdoor greenhouse for transplantation. F, growth performance after 3 months of transplanting.

2.11. Data analysis

Data processing was conducted using Microsoft Excel 2020, and statistical analyses were performed using SPSS 26.0. The embryo development rate, plantlet formation rate, abnormal plantlet transformation rate, and transplant survival rate were calculated as follows: Embryo development rate (%)=(Number of developed embryos/Number of ovules cultured)×100; Plantlet formation rate (%)=(Number of plantlets formed/Number of ovules cultured)×100; Abnormal plantlet transformation rate (%)=(Number of normal plantlet transformations/Number of abnormal plantlets cultured)×100; Transplant survival rate (%)=(Number of surviving transplants/Number of transplants)×100.

3. Results

3.1. Effect of different parental genotypes on embryo rescue efficiency

In this study, 22 hybrid combinations were evaluated, yielding a total of 20,055 hybrid berries, 25,969 excised ovules, and 9,207 developed embryos, resulting in an average embryo development rate of 35.45%. A total of 6,091 plantlets were obtained, corresponding to an average plantlet formation rate of 23.45% (Table 2). The maternal genotype significantly influenced embryo rescue efficiency, with embryo development rates ranging from 3.80 to 55.50% and plantlet formation rates varying between 0.63 and 40.77% across combinations. Notably, the cross using ‘Shennongxiangfeng’ as the paternal parent and ‘Ruby Seedless’ as the maternal

Table 2 Effect of different parental genotypes on embryo rescue efficiency

Cross-combinations	No. of berries	No. of ovules cultured	Embryos developed		Plantlet formation	
			No.	%	No.	%
Zhengyan Seedless×Italy	1,030	720	168	23.33	108	15.00
Zhengyan Seedless×Guibao	747	392	74	18.88	49	12.50
Huozhouheiyu×Sweet Scarlet	753	960	254	26.46	191	19.90
Huozhouheiyu×Shine Muscat	1,803	2,630	820	31.18	395	15.02
Melissa Seedless×Heihali	367	332	31	9.34	24	7.23
Melissa Seedless×Lüzhoubaoshi	334	312	84	26.92	51	16.35
Flame Seedless×Canadice Seedless	554	500	30	6.00	14	2.80
Flame Seedless×Hongqitezao	1,085	1,408	333	23.65	169	12.00
Flame Seedless×Italy	739	600	28	4.67	12	2.00
Delight×Hongqitezao	801	404	132	32.67	93	23.02
Delight×Guibao	808	746	245	32.84	163	21.85
Jingzaojing×Muscat Hamburg	364	158	6	3.80	1	0.63
Jingzaojing×Sweet Scarlet	35	43	7	16.28	2	4.65
Jingzaojing×Shine Muscat	3	3	3	100.00	3	100.00
Crimson Seedless×Muscat Hamburg	226	136	6	4.41	6	4.41
CrimsonSeedless×Sweet Scarlet	814	455	38	8.35	14	3.08
Zitian Seedless×Heihali	868	1,580	426	26.96	289	18.29
Zitian Seedless×Melissa Seedless	1,547	3,659	1,018	27.82	685	18.72
Zitian Seedless×Shennongxiangfeng	1,119	1,800	352	19.56	226	12.56
Ruby Seedless×Zitian Seedless	3,253	5,505	3,290	59.76	2,235	40.60
Ruby Seedless×Shennongxiangfeng	2,289	3,306	1,820	55.05	1,348	40.77
Shennongxiangfeng×Sweet Scarlet	516	320	42	13.13	13	4.06
∑	20,055	25,969	9,207	35.45	6,091	23.45

parent achieved the highest efficiency, with an embryo development rate of 55.50% and a plantlet formation rate of 40.77%, outperforming combinations involving 'Zitian Seedless'. When 'Sweet Scarlet' was used as the paternal parent in crosses with 'Huozhouheiyu', 'Jingzaojing', 'Crimson Seedless', or 'Shennongxiangfeng' as maternal parents, 'Huozhouheiyu' exhibited superior performance, achieving a plantlet formation rate of 19.90%; in comparison, the other maternal genotypes yielded rates of 4.65, 3.08, and 4.06%, respectively. Furthermore, paternal genotypes including 'Italy', 'Guibao', 'Hongqitezao', and 'Heihali' enhanced embryo rescue outcomes in maternal lines such as 'Zhengyan Seedless', 'Italy', and 'Zitian Seedless', whereas 'Flame Seedless' and 'Melissa Seedless' resulted in comparatively lower efficiencies. In contrast, when 'Muscat Hamburg' served as the paternal parent, both 'Crimson Seedless' and 'Jingzaojing' showed poor embryo rescue success.

The influence of paternal genotypes on embryo rescue efficiency was relatively limited. However, when 'Ruby Seedless' was used as the maternal parent, crosses with 'Zitian Seedless' and 'Shennongxiangfeng' as paternal parents demonstrated superior embryo development and plantlet regeneration. Specifically, embryo development rates reached 59.76 and 55.05%, while plantlet formation rates were 40.60 and 40.77%, respectively. Similarly, maternal genotypes 'Delight', 'Italy', 'Huozhouheiyu', and 'Zitian Seedless' produced favorable results, with embryo development rates ranging from 18.88 to 32.84% and plantlet formation rates from 12.50 to 23.02%. Conversely, when 'Melissa Seedless' was the maternal parent, pairing with 'Lüzhoubaoshi' as the paternal parent resulted in higher embryo development (26.92%) and plantlet formation (16.35%) compared to 'Heihali' (9.34 and 7.23%). For 'Flame Seedless' as the maternal parent, the combination with 'Hongqitezao' as the paternal parent yielded significantly improved embryo development (23.65%) and plantlet formation (12.00%), surpassing those obtained with 'Canadice Seedless' (2.00 and 2.80%) and 'Italy' (4.67 and 2.00%).

3.2. Effect of ZT concentration in embryo development culture medium on embryo rescue efficiency

After eight weeks of dark culture, ovules were examined under a stereomicroscope for various developmental stages of embryos, including the occurrence of multiple and malformed embryos (Fig. 5). Using MM3 supplemented with 0.2 mg L^{-1} IAA as the basal medium and varying concentrations of ZT, embryo rescue efficiency differed significantly across hybrid combinations (Table 3). For the crosses 'Flame Seedless×Hongqitezao' and 'Ruby Seedless×Zitian Seedless', T2 yielded embryo development rates of (29.33%, 64.73%) and plantlet formation rates of (14.46%, 31.76%), respectively, both significantly higher than those of the CK, T1, and T3. For 'Zitian Seedless×Heihali', T1 achieved notably higher embryo development (35.61%) and plantlet formation (21.94%) rates than CK, T2, or T3. In the 'Delight×Guibao' cross, T2 resulted in an embryo development rate of 41.25%, markedly higher than CK, T1, and T3; meanwhile, plantlet formation rates in CK (17.22%) and T2 (15.63%) were considerably greater than those in T1 and T4, with no significant difference between the CK and T2.

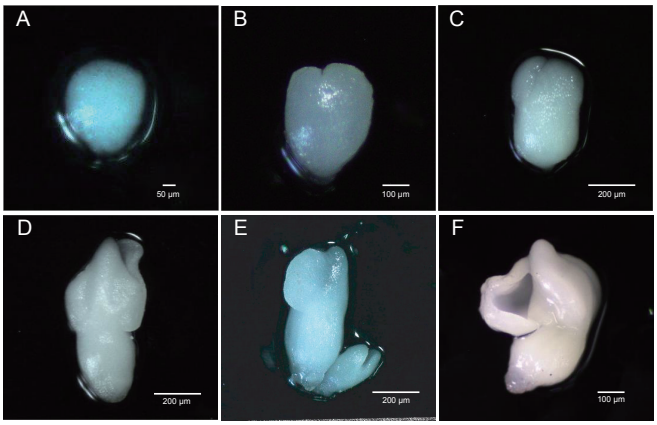


Fig. 5 Developmental appearance of embryos in ovules after 8 weeks of dark culture. A, spherical embryo. B, heart-shaped embryo. C, torpedo-shaped embryo. D, cotyledon embryo. E, multiple embryos. F, malformed embryo.

3.3. Effect of different plant hormones on embryo rescue efficiency in embryo germination culture medium

The addition of diverse growth regulators to the embryo germination culture medium significantly influenced embryo rescue efficiency (Table 4). In the cross 'Flame Seedless×Hongqitezao', treatments incorporating two growth regulators yielded better outcomes than those with a single regulator. Specifically, embryo germination and plantlet formation rates in C2, C4, and C5 exceeded those in C1 and C3. Notably, C4 achieved the highest performance, with an embryo germination rate of 85.71% and a plantlet formation rate of 57.14%. For the cross 'Huozhouheiyu×Muscat Hamburg', C3, C4, and C5, which containing ZT, exhibited higher embryo germination and plantlet formation rates compared to C1 and C2, which included 6-BA. Again, C4 demonstrated the highest values, achieving 85.71% embryo germination and 66.67% plantlet formation. In the cross 'Zitian Seedless×Heihali', C1 showed the highest embryo germination rate at 91.30%. However, when 0.2 mg L^{-1} IAA was added in C2, both embryo germination and plantlet formation rates decreased. Although C4 had a lower embryo germination rate than C1 and C3, it attained a higher plantlet formation rate (56.52%) relative to other groups.

3.4. Effect of different transformation media on the rescue efficiency of abnormal plantlets

The efficiency of transforming abnormal plantlets into normal ones varied significantly depending on the transformation medium used (Table 5; Fig. 6). Among the three media tested, Medium C produced the highest number of normal plantlets, yielding 178 individuals. The transformation rate with Medium C was 54.95%, which was significantly higher than that of Medium A (10.88%) and Medium B (30.61%).

3.5. Effect of nine abnormal plantlet types on transformed normal plantlets

The analysis of transformation efficiency across nine types of abnormal plantlets from 18 hybrid combinations (Table 6), indicates that no normal plantlets were obtained from the

Table 3 Effect of ZT concentration in ovule development medium on embryo rescue efficiency

Cross combination	Treatment	Exogenous hormone[1)]		No. of berries	No. of ovules cultured	Embryos developed		Plantlet formation	
		ZT (mg L^{-1})	IAA (mg L^{-1})			No.	%	No.	%
Flame Seedless× Hongqitezao	CK	0	0.2	335	480	112	23.33 b	51	11.04 b
	T1	0.5	0.2	146	168	23	13.69 c	11	6.55 c
	T2	1.0	0.2	392	470	138	29.33 a	68	14.46 a
	T3	1.5	0.2	212	300	60	20.00 b	19	7.00 c
Ruby Seedless× Zitian Seedless	CK	0	0.2	354	698	391	56.02 c	206	29.51 c
	T1	0.5	0.2	448	790	475	60.13 b	227	28.73 d
	T2	1.0	0.2	635	979	634	64.76 a	311	31.76 a
	T3	1.5	0.2	369	620	346	55.81 c	190	30.64 b
	T4	2.0	0.2	243	360	180	50.00 d	96	26.67 e
Zitian Seedless×Heihali	CK	0	0.2	145	333	94	28.23 c	45	13.51 c
	T1	0.5	0.2	165	278	99	35.61 a	61	21.94 a
	T2	1.0	0.2	167	300	94	31.33 b	53	17.67 b
	T3	1.5	0.2	148	300	78	26.00 d	37	12.33 c
Delight×Guibao	CK	0	0.2	181	175	64	36.56 b	31	17.22 a
	T1	0.5	0.2	154	160	53	33.13 b	19	11.88 b
	T2	1.0	0.2	190	160	66	41.25 a	25	15.63 a
	T3	1.5	0.2	151	160	40	25.00 c	17	10.63 b

[1)] ZT, zeatin; IAA, indole-3-acetic acid.
Data are shown as mean (n=3). Different lowercase letters indicate significant differences among groups as determined by Tukey's multiple comparison test (P<0.05).

Table 4 Effect of different plant hormones in embryo germination medium on embryo rescue efficiency

Cross combination	Treatment	Exogenous hormone[1)]			No. of embryos developed	Per. of embryos germinated (%)	Per. of plantlet formation (%)
		6-BA (mg L^{-1})	ZT (mg L^{-1})	IAA (mg L^{-1})			
FlameSeedless× Hongqitezao	C1	0.2			23	78.26	34.78
	C2	0.2		0.2	20	85.00	55.00
	C3		0.2		22	68.18	45.45
	C4		0.2	0.2	21	85.71	57.14
	C5		0.5	0.2	23	82.61	47.83
Huozhouheiyu× Muscat Hamburg	C1	0.2			22	63.64	36.36
	C2	0.2		0.2	22	59.09	18.18
	C3		0.2		19	78.95	36.84
	C4		0.2	0.2	21	85.71	66.67
	C5		0.5	0.2	21	66.67	38.10
Zitian Seedless× Heihali	C1	0.2			23	91.30	52.17
	C2	0.2		0.2	27	77.78	51.85
	C3		0.2		23	86.96	52.17
	C4		0.2	0.2	23	82.61	56.52
	C5		0.5	0.2	27	66.67	29.63

[1)] 6-BA, 6-benzylaminopurine; ZT, zeatin; IAA, indole-3-acetic acid.

leafless and rootless abnormalities. Among the remaining eight types, transformation rates decreased in the following order: multiple cotyledons>developmentally halted>cotyledon twisting and wrinkling>monocotyledon>cotyledon fusion>tubular cotyledons>root-bearing but cotyledon-less>cotyledon-bearing but rootless, with corresponding rates of 59.13, 51.28, 41.67, 32.47, 23.07, 14.29, and 9.18%, respectively. Across all 18 hybrid combinations, a total of 3,365 abnormal plantlets were obtained, from which 1,201 normal plantlets were successfully transformed, yielding an overall transformation rate of 35.69%.

3.6. Effect of abnormal plantlets-induced adventitious bud regeneration on the transformation of normal plantlets

Different culture media exert distinct effects on adventitious bud regeneration from abnormal plantlets (Table 7, Fig. 7). Among the four media tested, callus tissue differentiation was observed in both T1 and T4 media (Fig. 7-A and C). In contrast, only the hypocotyl explants cultured in T2 and T3 media exhibited adventitious bud formation, accompanied by aerial root development (Fig. 7-B and D). Notably, T3

Table 5 Effect of different transformation media on the rescue efficiency of abnormal plantlets

Medium type	No. of abnormal plantlets	No. of normal plantlets transformation	Per. of normal plantlet transformation (%)
Medium A	247	27	10.88 c
Medium B	307	94	30.61 b
Medium C	324	178	54.95 a

Data are shown as mean (n=3). Different lowercase letters indicate significant differences among groups as determined by Tukey's multiple comparison test (P<0.05).

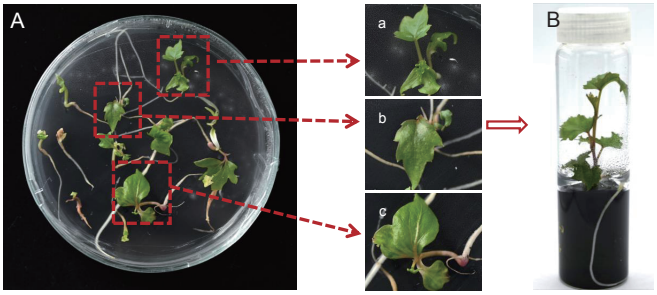


Fig. 6 The process of transforming abnormal plantlets into normal plantlets through embryo rescue. A, abnormal plantlets are inoculated and cultured on the transformation medium. a–c, abnormal plantlets develop true leaves. B, abnormal plantlets grow into normal plantlets.

Table 6 Effect of nine abnormal plantlet types on transformed normal plantlets

Abnormal plantlets type	No. of abnormal plantlets	No. of normal plantlets transformation	Per. of normal plantlet transformation (%)
Monocotyledon	555	215	38.74 c
Cotyledon twisting and wrinkling	336	140	41.66 c
Cotyledon fusion	539	175	32.48 d
Root-bearing but cotyledon-less	189	27	14.30 f
Cotyledon-bearing but rootless	294	27	9.02 g
Developmentally halted	392	201	51.27 b
Multiple cotyledons	668	395	59.13 a
Tubular cotyledons	91	21	23.18 e
Leafless and rootless	301	0	0.00 h
∑	3,365	1,201	35.69

Data are shown as mean (n=3). Different lowercase letters indicate significant differences among groups as determined by Tukey's multiple comparison test (P<0.05).

medium yielded the highest adventitious bud regeneration efficiency at 33.33%, resulting in the recovery of 20 normal plantlets (Fig. 7-E).

3.7. Statistics of hardening and transplantation of embryo-rescue hybrid plantlets

The survival rate of hybrid plantlets was evaluated three months after field transplantation (Table 8). A total of 5,209 plantlets from 18 hybrid combinations were subjected to hardening, of which 4,728 survived, resulting in an overall survival rate of 85.92%. Plantlets that were not hardened prior to transplantation failed to meet the required acclimatization criteria and are currently maintained in the tissue culture room for future use. Survival rates among the hybrid progenies varied significantly, ranging from 33.33 to 98.99%. Notably, the following combinations — 'Huozhouheiyu×Sweet Scarlet', 'Huozhouheiyu×Muscat Hamburg', 'Flame Seedless×Hongqitezao', 'Delight×Hongqitezao', 'Delight×Guibao', 'Crimson Seedless×Muscat Hamburg', 'Zitian Seedless×Melissa Seedless', 'Zitian Seedless×Shennongxiangfeng', and 'Ruby Seedless× Shennongxiangfeng' — exhibited strong post-transplantation performance, with survival rates exceeding 90%.

4. Discussion

Numerous researchers have reported that maternal varieties such as 'Ruby Seedless', 'Delight', 'Flame Seedless', and 'Perlette' yield superior embryo rescue outcomes compared to other seedless grape cultivars (Ji and Wang 2013; Jiao *et al.* 2018; Li *et al.* 2020). The present study corroborates these findings, with the highest embryo rescue efficiency observed when 'Ruby Seedless' and 'Delight' were used as maternal parents. Notably, the cross 'Ruby Seedless×Shennongxiangfeng' exhibited exceptional performance, achieving an ovule development rate of 55.05% and a plantlet formation rate of 40.77%, ultimately generating 2,235 hybrid offspring. Among other maternal genotypes evaluated, 'Zitian Seedless', 'Huozhouheiyu', and 'Zhengyan Seedless' also demonstrated favorable *in vitro* characteristics, yielding plantlet formation rates ranging from 12.50 to 19.48%. In contrast, 'Jingzaojing', 'Crimson Seedless', and 'Melissa Seedless' performed poorly, with plantlet formation rates between 0.63 and 5.42%. Interestingly, Chu *et al.* (2023) reported a similarly low embryo rescue efficiency for 'Jingzaojing' (0.8% plantlet formation), yet observed higher efficiency with 'Melissa Seedless', contradicting the results of this study. This discrepancy may arise from differences in parental genotype combinations. Similarly, inconsistent results have been reported for 'Crimson Seedless': while Li *et al.* (2020) obtained findings consistent with this study, Liu *et al.* (2016) concluded that 'Crimson Seedless' is suitable for embryo rescue, highlighting the influence of genetic background on culture response.

Many studies on embryo rescue in seedless grapes emphasize that optimizing the type, concentration, and ratio of plant growth regulators during ovule culture and embryo germination significantly enhances culture efficacy. For instance, supplementing the ovule development medium with 0.2 mg L^{-1} GA_3, 1.5 mg L^{-1} IAA, and 1.0 mg L^{-1} ZT for the hybrid 'Perlette×Flame Seedless' resulted in a 70.7% ovule development rate and a 24.8% plantlet formation rate (Ding and Qi 2001), demonstrating the positive effects of ZT, IAA, and GA_3 on embryonic development. In this study, the impact of ZT and IAA on ovule development was specifically examined. The addition of 1.0–1.5 mg L^{-1} ZT and 0.2 mg L^{-1} IAA to MM3 medium markedly accelerated ovule

Table 7 Effect of different media on regeneration of adventitious shoots induced by abnormal plantlets

Medium types	No. of abnormal plantlets	No. of indefinite bud differentiation	Per. of indefinite bud differentiation	No. of normal plantlets transformation	Per. of normal plantlet transformation (%)
T1	60	0	0.00 c	0	0.00 c
T2	60	23	38.33 b	13	21.67 b
T3	60	27	45.00 a	20	33.33 a
T4	60	0	0.00 c	0	0.00 c

Data are shown as mean (n=3). Different lowercase letters indicate significant differences among groups as determined by Tukey's multiple comparison test (P<0.05).

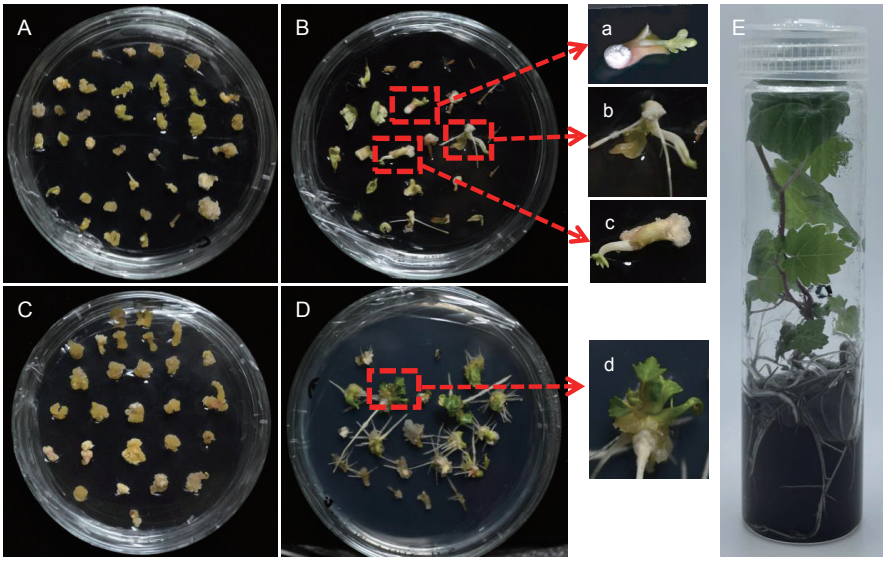


Fig. 7 Induction effects of adventitious buds in abnormal plantlets on four culture media. A, callus tissue differentiated in T1 medium. B, differentiation of adventitious buds in T2 medium with a few aerial roots. C, callus tissue differentiated in T4 medium. D, differentiation of adventitious buds in T3 medium with a larger number of aerial roots. a–d, all represent the induction of adventitious bud differentiation from the hypocotyl. E, normal plantlets.

Table 8 Statistics of the results from hardening and transplantation of embryo-rescue hybrid plantlets

Cross combination	No. of plantlets transplanted	No. of surviving plantlets	Per. of plantlet survival
Zhengyan Seedless×Italy	56	40	71.43
Zhengyan Seedless×Guibao	36	21	58.33
Huozhouheiyu×Sweet Scarlet	186	169	90.86
Huozhouheiyu×Muscat Hamburg	404	393	97.28
Melissa Seedless×Heihali	23	15	65.22
Melissa Seedless×Lüzhoubaoshi	58	35	60.34
Flame Seedless×Hongqitezao	121	115	95.41
Delight×Hongqitezao	99	98	98.99
Delight×Guibao	153	147	96.08
Zitian Seedless×Melissa Seedless	586	556	94.88
Jingzaojing×Shine Muscat	3	1	33.33
Crimson Seedless×Muscat Hamburg	15	14	93.33
Crimson Seedless×Sweet Scarlet	7	6	85.71
Zitian Seedless×Heihali	241	209	86.72
Ruby Seedless×Zitian Seedless	1,943	1,481	76.22
Shennongxiangfeng×Sweet Scarlet	14	6	42.86
Zitian Seedless×Shennongxiangfeng	145	134	92.41
RubySeedless×Shennongxiangfeng	1,395	1,288	92.33
∑	5,209	4,728	85.92

development. The cross 'Ruby Seedless×Zitian Seedless' achieved an ovule development rate of 64.76% and a plantlet formation rate of 31.76%. Embryo germinability during the embryo germination stage is a critical determinant of overall embryo rescue efficiency (Liu *et al.* 2003). Most research indicates that appropriate plant hormone supplementation at this stage improves culture success. For example, young embryos cultured on WPM supplemented with 1.0 μmol L^{-1} 6-BA showed increased potential to develop into normal plantlets (Tian *et al.* 2008; Li *et al.* 2020). Other studies suggest that either 1.0 mg L^{-1} 6-BA+1.0 mg L^{-1} IAA or 0.2 mg L^{-1} 6-BA+0.1 mg L^{-1} IAA can enhance both embryo germination and plantlet formation rates (Li *et al.* 2013; Zhu *et al.* 2020). Consistent with these reports, this study found that combined application of 6-BA+IAA or ZT+IAA yielded superior outcomes compared to single-hormone treatments. The highest embryo germination and plantlet formation rates were achieved in three hybrid combinations treated with 0.2 mg L^{-1} ZT+0.2 mg L^{-1} IAA, which also produced the lowest proportion of abnormal plantlets.

During embryo excision, various developmental stages and morphologies can be observed, including globular, heart-shaped, torpedo-shaped, and cotyledonary embryos. Additionally, multiple or malformed embryos may occur. These embryos subsequently germinate into both normal and abnormal plantlets on embryo germination medium. A substantial proportion of abnormal plantlets typically arises during embryo rescue, significantly reducing breeding efficiency. In this study, 22 hybrid combinations generated 3,577 abnormal plantlets out of 8,335 germinated embryos, resulting in an abnormality rate of 40%. Previous studies have reported similar rates, indicating that abnormal plantlet formation is an inherent challenge in embryo rescue protocols. Contributing factors may include insufficient dormancy breakage in immature embryos, leading to incomplete germination, or genetic incompatibilities between parental genotypes that result in asynchronous embryo development (Yamashita *et al.* 1995; Liu *et al.* 2003; Ji *et al.* 2013a).

This study classified abnormal plantlets (Fig. 3) following established criteria, aiming to assess their potential for recovery and reuse. Twelve distinct types were identified, and the nine most prevalent types — totaling 3,365 plantlets — were transferred to a transformation medium (WPM+2.0 mg L^{-1} 6-BA+2.0 mg L^{-1} IBA) to evaluate morphological correction. This treatment obtained 1,201 normal plantlets, achieving a transformation rate of 35.69%. Leafless or rootless plantlets could not be restored to normal morphology. However, several other types were successfully converted: monocotyledon abnormalities, cotyledon twisting and wrinkling, cotyledon fusion, root-bearing but cotyledon-less, cotyledon-bearing but rootless, developmentally halted, multiple cotyledons, and tubular cotyledons. Ji *et al.* (2013a) demonstrated that single cotyledon and twisted/wrinkled cotyledon abnormalities could be corrected using a medium supplemented with $ZnSO_4$ and banana puree (2MS+0.4 mg L^{-1} 6-BA+0.1 mg L^{-1} IBA). Another study by Xu *et al.* (2022) reported a 40.09% conversion rate when culturing abnormal plantlets from 'Kunxiang Seedless×Beibinghong' on WPM+0.2 mg L^{-1} 6-BA+0.5 mg L^{-1} IBA. The results of this study indicate that the transformation medium employed is broadly effective for rescuing diverse abnormal plantlets derived from multiple hybrid combinations. New shoot emergence occurred between 3 and 35 d post-inoculation. To further improve the rescue efficiency of abnormal plantlets, tissues of abnormally developed plantlets that failed to undergo successful transformation (including cotyledons, hypocotyls, and radicles) were transferred to shoot induction media for culture. Only the embryonic axis produced adventitious buds, yielding 33 normal plantlets. This approach presents a novel strategy for salvaging otherwise nonviable material during embryo rescue. Furthermore, the study confirms that utilizing multiple embryos per ovule can reduce the incidence of abnormal plantlets, aligning with previous findings by Ji *et al.* (2013a) and Valdez (2005).

5. Conclusion

This study investigates the factors influencing embryo rescue in seedless grapes and preliminarily establishes a comprehensive method to enhance breeding efficiency through this technique. Suitable maternal parents were identified as 'Ruby Seedless', 'Delight', 'Huozhouheiyu', 'Zitian Seedless', and 'Zhengyan Seedless', while appropriate paternal parents include 'Zitian Seedless', 'Shennongxiangfeng', 'Hongqitezao', and 'Guibao'. Supplementation of the embryo rescue medium with an optimal concentration of ZT significantly promotes both embryo development and germination. Regarding the rescue of abnormal plantlets, those exhibiting multiple cotyledons occurred most frequently and also demonstrated the highest conversion efficiency into normal plantlets; in contrast, leafless and rootless types could not be converted into viable normal plantlets. Additionally, adventitious bud regeneration induced from the embryo axis enables the recovery of certain abnormal plantlets. Employing these two approaches for the transformation of abnormal plantlets increases the seedling establishment rate by 5% (Fig. 8). Future efforts should focus on further optimizing medium composition to effectively reduce the proportion of abnormal embryos and plantlets during embryogenesis and germination stages. With continued refinement and application of this methodology, it is anticipated that more high-quality germplasm can be generated for the seedless grape industry, accelerating the development of new cultivars.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT 4.0 to assist in improving the clarity and readability of the language. All content generated was carefully reviewed

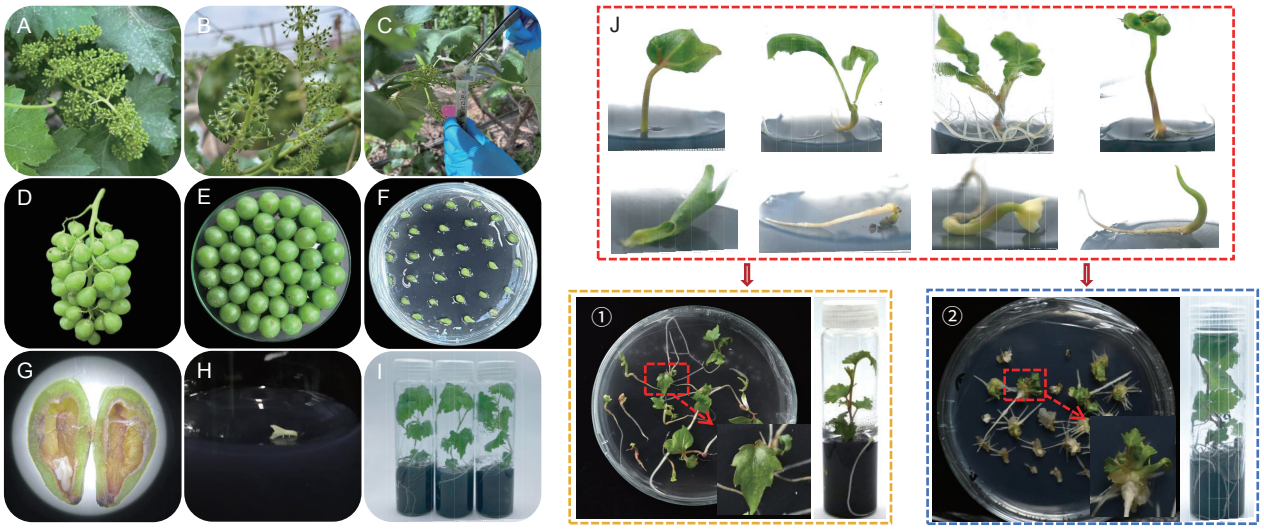


Fig. 8 Embryo rescue and *in vitro* culture process for seedless grapes and the transformation and reuse of abnormal plantlets. A–I, *in vitro* culture process for embryo rescue of seedless grapes. J, different types of abnormal plantlets produced from embryo rescue of seedless grapes. ① and ②, represent two pathways for transforming abnormal plantlets into normal ones.

and revised by the authors as necessary, and the authors take full responsibility for the final content of the article.

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