

EMBRYO ABORTION IN STONE FRUIT SPECIES

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Abstract

*Embryo abortion in stone fruit species (*Prunus* spp.) represents a major physiological and genetic constraint that creates significant post-zygotic barriers in breeding programs. This phenomenon is associated with developmental asynchrony between fruit and seed tissues, particularly the delayed development of embryos in early-ripening cultivars, nutrient deprivation (starvation) resulting from endosperm and chalazal haustorium dysfunction, and programmed cell death (PCD). Furthermore, self-incompatibility, genetic deficiencies, and interspecific incompatibility are among the principal factors triggering embryo abortion. The most effective strategy for overcoming this limitation is embryo culture (embryo rescue), in which embryos are excised before losing viability and subsequently cultured under in vitro conditions. The success of this technique largely depends on the optimal stage of embryo excision ($PF \approx 0.5$), the selection of an appropriate culture medium ($\frac{1}{2}$ MS or WPM), and a balanced plant growth regulator regime. Moreover, the integration of embryo rescue with somatic embryogenesis and synthetic seed technologies facilitates the propagation of rare hybrids and provides substantial support for modern fruit breeding programs. In conclusion, embryo abortion is a complex physiological and genetic challenge that constitutes a critical barrier to successful hybrid development. A comprehensive understanding of the underlying mechanisms and the effective application of biotechnological approaches for its management are essential for enhancing the efficiency and success of contemporary fruit breeding programs.*

Keywords: Embryo abortion, embryo culture, somatic embryogenesis, post-zygotic barriers, *Prunus*.

1. INTRODUCTION

Stone fruits, including peach, sweet cherry, sour cherry, apricot, plum, and almond (family Rosaceae, genus *Prunus*), constitute one of the most economically important groups of fruit crops worldwide (Özdemir Eroğlu et al., 2012). High fruit set and proper seed development are essential prerequisites for sustainable and profitable fruit production (Kalkışım, 2020). However, developmental abnormalities occurring between flowering and fruit maturation frequently result in embryo abortion (embryo death), leading to severe fruit drop (Martínez-García et al., 2012). This

phenomenon is particularly prevalent in early-ripening peach, nectarine, and sweet cherry cultivars, in which fruits enter the ripening phase before embryo development is complete, ultimately causing seed inviability through embryo abortion (Tukey, 1933). Although embryo abortion functions as an evolutionary adaptive mechanism in natural populations, it represents one of the most significant post-zygotic barriers limiting the transfer of desirable genes among *Prunus* species through interspecific hybridization in breeding programs (Ramming, 1990).

The asynchronous development of the fruit (pericarp) and the seed constitutes one of the primary physiological causes of embryo abortion in stone fruit species. In peach (*Prunus persica*), fruit development proceeds through three distinct stages. Stage I is characterized by rapid fruit growth immediately after flowering and exhibits a relatively similar duration across cultivars regardless of the ripening season. Stage II is marked by a slowdown in fruit growth accompanied by pit hardening; this stage is extremely short in early-ripening cultivars (e.g., approximately 5 days) but considerably longer in late-ripening cultivars (e.g., approximately 42 days). During Stage III, the fruit resumes rapid growth and reaches its final size. Throughout Stage I, embryo development remains extremely slow, with the embryo maintaining a microscopic size. However, the onset of Stage II initiates a period of rapid embryo growth, during which the embryo attempts to attain its maximum size. In very early-ripening cultivars, Stage III begins while the embryo is still undergoing this rapid developmental phase.

As a consequence, the embryo fails to achieve its maximum size, the nucellus and integuments collapse, and the characteristic shriveled, abortive seeds are formed. In contrast, Stage III in late-ripening cultivars does not commence until the embryo has nearly completed its development, allowing seeds to retain viability and germination capacity (Tukey & Lee, 1937).

Embryo abortion is associated not only with the early-ripening characteristic but also with competition for assimilates between maternal tissues and the developing embryo, as well as severe physiological disturbances. Anatomical investigations of fruit abscission have revealed typical necrotic symptoms in species such as peach and sour cherry (*Prunus cerasus*), including cytoplasmic shrinkage and nuclear collapse within both endosperm and embryonic cells.

During the critical stages of seed development, the chalazal haustorium plays a pivotal role in transporting water and nutrients to the developing embryo. Degeneration or collapse of this structure prevents the transfer of assimilated nutrients from the endosperm to the embryo, resulting in a nutritional bottleneck known as the starvation syndrome. This ultimately leads to embryo necrosis and physiological fruit abscission (Fukuda et al., 2006). Likewise, both abiotic and biotic stress factors may induce DNA fragmentation and programmed cell death (PCD) in maternal tissues, particularly the chalaza and integuments, thereby disrupting nutrient supply to the embryo and triggering embryo abortion (Li et al., 2021).

In addition to these physiological constraints, genetic and reproductive incompatibilities constitute another major cause of embryo abortion in *Prunus* species. Most commercially important species, including almond (*Prunus dulcis*), sweet cherry, apricot, and plum, exhibit genetically controlled gametophytic self-incompatibility mediated by the S-RNase system. Under self-incompatibility reactions, pollen tube growth is generally arrested within the style, thereby preventing fertilization (Kalkışım, 2020). Nevertheless, even when fertilization is successful, post-zygotic genetic constraints such as inbreeding depression may become evident. Embryological studies in almond have demonstrated that highly selfed genotypes exhibit not only pre-zygotic abnormalities but also post-zygotic defects, including failure of endosperm cellularization and necrosis in the chalazal

region, ultimately resulting in extensive embryo abortion and fruit drop (Martínez-García et al., 2012).

A similar genetic barrier is observed in distant interspecific hybridizations. Studies involving peach crossed with plum or apricot (*Prunus persica* × *P. salicina* or *P. armeniaca*) have shown that hybrid embryos undergo rapid abortion beginning approximately seven to eight weeks after fertilization owing to arrested or degenerated endosperm development (Liu et al., 2007).

From an evolutionary and ecological perspective, the production of numerous ovules followed by the abortion of a proportion of them is not a random event. Rather, it is considered a strategy of selective embryo abortion, enabling the maternal plant to allocate its limited resources preferentially to the most competitive and genetically superior offspring (Melser & Klinkhamer, 2001). This mechanism, often referred to as developmental selection or bet-hedging, maximizes reproductive success under unpredictable environmental conditions (Wiens et al., 1987). While this maternal strategy enhances offspring quality in natural ecosystems, it presents a major obstacle in modern fruit breeding programs, where the recovery of novel hybrid genotypes is a primary objective. Fruit breeders invest substantial resources in exploiting the genetic potential of early-ripening cultivars while simultaneously introgressing desirable traits, such as stress resistance and fruit quality, among different *Prunus* species. However, embryo abortion remains one of the principal causes of failure in these hybridization programs (Zhang et al., 2014).

To overcome this barrier, plant biotechnology and tissue culture techniques have been extensively employed, with embryo culture (embryo rescue) currently recognized as the most reliable approach (Pramanik et al., 2021). In early-ripening peach and nectarine cultivars, where the interval between full bloom and fruit maturity is typically shorter than 100–120 days, embryos are excised from developing fruits before natural abortion occurs and while they still retain viability (Özdemir Eroğlu et al., 2012). Under aseptic conditions, immature embryos are cultured on nutrient media such as Murashige and Skoog (MS) or Woody Plant Medium (WPM), supplemented with specific concentrations of plant growth regulators including 6-benzylaminopurine (BAP), gibberellic acid (GA₃), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA), thereby promoting successful *in vitro* germination (Şahin et al., 2016). Hybrid embryos derived from incompatible interspecific crosses, which would otherwise perish because of insufficient endosperm development, can likewise be rescued using this biotechnological approach and subsequently incorporated into breeding programs for horticultural and commercial cultivation (Pramanik et al., 2021).

In summary, embryo abortion in stone fruit species (*Prunus* spp.) is a multifaceted physiological and genetic disorder involving developmental asynchrony during fruit growth, structural abnormalities of the endosperm and chalazal haustorium, programmed cell death (PCD), genetic incompatibility, and inbreeding depression. This review aims to comprehensively examine the pre-zygotic and post-zygotic factors responsible for embryo abortion, elucidate its underlying genetic and cellular mechanisms in light of current scientific evidence, and critically evaluate the role and significance of *in vitro* embryo culture (embryo rescue) strategies in overcoming hybridization barriers and improving the efficiency of modern fruit breeding programs.

2. ARREST OF EMBRYONIC DEVELOPMENT: PHYSIOLOGICAL AND MORPHOLOGICAL CAUSES

In fruit crops, particularly stone fruit species of the genus *Prunus*, embryo abortion is the cumulative outcome of cellular, anatomical, and physiological disruptions occurring during both pre-zygotic and post-zygotic stages. These mechanisms, which ultimately arrest embryo

development and lead to loss of viability, are primarily driven by developmental asynchrony between tissues, competition for assimilates, and the activation of programmed cell death pathways at the cellular level (Bradbury, 1929).

2.1. Asynchronous Tissue Growth and Developmental Incompatibility

From a morphological perspective, embryo abortion primarily results from developmental asynchrony between the maternal tissues forming the fruit (pericarp) and the zygotic tissues constituting the seed (embryo and endosperm). Fruit development in stone fruit species generally consists of three distinct stages (Stages I, II, and III). Stage I is characterized by rapid fruit enlargement immediately after flowering and exhibits a relatively similar duration among cultivars with different ripening periods. However, Stage II, during which fruit growth slows and pit hardening occurs, represents the critical developmental threshold determining the fate of the embryo. In late-ripening cultivars, this stage extends for several weeks (e.g., approximately 42 days), providing sufficient time for the embryo to complete its development. In contrast, Stage II lasts only a few days (e.g., approximately 5 days) in early-ripening cultivars. Consequently, these cultivars enter Stage III—the period of rapid fruit enlargement and ripening—while the embryo is still at a microscopic developmental stage (Tukey & Lee, 1937).

This developmental asynchrony induces dramatic structural collapse within the ovule. As the pericarp rapidly expands by accumulating water and assimilates, embryonic development is abruptly arrested. Cellular investigations have demonstrated that, immediately before embryo growth ceases, the nucellus, which functions as a nutritive tissue, together with the integuments that give rise to the seed coat, loses turgor pressure and undergoes rapid collapse. This structural degeneration physically compresses the embryo and obstructs nutrient transport pathways, ultimately resulting in the formation of the characteristic flattened and abortive seeds (Tukey, 1933).

2.2. Biochemical Deficiencies, Starvation, and Resource Competition

The physiological basis of embryo abortion is closely associated with severe nutrient deprivation and intense competition for assimilates. Analyses of the chemical composition of aborted embryos have revealed substantially lower accumulations of lipids, nitrogen, and sucrose compared with embryos from normally developing or late-ripening cultivars. Conversely, aborted embryos retain abnormally high water contents, reaching approximately 93%. Under normal developmental conditions, embryos accumulate storage proteins and lipids following the attainment of maximum size while simultaneously undergoing dehydration. In contrast, aborted embryos remain flaccid, fail to synthesize these storage reserves, and eventually lose viability (Tukey & Lee, 1937).

The interruption of carbohydrate and mineral transport from maternal tissues to the embryo is also closely associated with the maternal plant's strategy for allocating limited resources. In species producing multiple ovules, such as *Cercis canadensis*, intense sibling competition occurs among developing zygotes, and the maternal plant preferentially allocates assimilates to embryos possessing the greatest sink strength (Arathi, 2011). Embryos that fail to compete successfully, or that lose vascular continuity due to the cessation of transport through the funiculus, experience severe nutritional restriction and ultimately undergo starvation-induced abortion (Du et al., 2024).

2.3. Chalazal Haustorium and Endosperm Dysfunction

The transport of nutrients between the maternal plant and the developing endosperm, which serves as the principal nutritional source for the embryo, is mediated by specialized structures known as the chalazal haustorium. Anatomical studies of physiological fruit abscission have demonstrated that the chalazal haustorium loses its functionality at an early developmental stage. Under normal conditions, this structure extends toward the chalaza and facilitates nutrient translocation. However,

in ovules destined for abortion, the chalazal haustorium ceases development, loses cellular integrity, undergoes collapse, and prevents the transfer of assimilated nutrients from the nucellus to the embryo sac. The interruption of nutrient flow subsequently initiates rapid degeneration of the endosperm (Fukuda et al., 2006).

Extensive histological investigations have further demonstrated that, in aborting ovules, the primary endosperm nucleus either fails to divide or, when division occurs, is unable to progress from the free-nuclear stage to cellularization (Geerts et al., 2002).

As a consequence, the cytoplasm of the embryo sac contracts, the free endosperm nuclei degenerate, and the embryo sac ultimately collapses completely. The observation that endosperm degeneration consistently precedes embryonic necrosis provides compelling evidence that embryo death is primarily attributable to nutritional deficiency resulting from endosperm dysfunction (Chen et al., 2022).

Furthermore, during interspecific hybridization, excessive proliferation of maternal endothelial cells (inner integument) may occupy the embryonic cavity, physically compressing the developing embryo and thereby constituting an additional anatomical barrier leading to embryo abortion (Geerts et al., 2002).

2.4. Cellular Degeneration: Programmed Cell Death (PCD) and Embryo Isolation

Once triggered by physiological disturbances and nutritional limitations, embryo abortion progresses irreversibly through the activation of programmed cell death (PCD) pathways. TUNEL assays performed on aborting ovules have confirmed extensive DNA fragmentation within maternal tissues, the nucellus, and nuclei of cells within the embryo sac. Ultrastructural analyses have further revealed irregular thickening of cell walls, disruption of plasma membrane integrity, aggregation and collapse of mitochondria, and vacuole-mediated autophagic degradation (autolysis) of cellular contents (Du et al., 2024).

At the molecular level, aborted embryos exhibit profound alterations in gene expression associated with cell survival and death. Genes involved in energy metabolism, including ATP synthase, are markedly downregulated, whereas caspase-like proteases responsible for regulating plant cell death cascades—particularly VEIDase and LEHDase activities—are strongly upregulated. Together with oxidative stress caused by reactive oxygen species (ROS) and cellular senescence, these molecular events ultimately drive the embryo toward irreversible cell death (Zhang et al., 2014).

One of the most striking physiological defense responses observed during this process is the massive deposition of callose. In stressed or aborting ovules, substantial callose accumulation occurs within endothelial cells and along the walls of the embryo sac. Under normal conditions, these regions facilitate symplastic transport between maternal and filial tissues. However, callose deposition forms a physical and physiological barrier that effectively isolates the degenerating embryo from the maternal plant. By functioning as a molecular filter that blocks the movement of sugars and other essential nutrients into the embryo sac, callose deposition irreversibly reinforces starvation and ultimately leads to complete embryonic desiccation and mummification (Sun et al., 2004).

3. GENETIC FACTORS, STERILITY, AND SEXUAL INCOMPATIBILITY

In stone fruit species, particularly within the genus *Prunus*, embryo abortion is not merely a consequence of physiological or morphological nutrient competition, but rather a multifaceted crisis rooted in complex genetic barriers, incompatibility mechanisms, and sterility. These factors, which

lead to hybridization failure in breeding programs, manifest across a broad spectrum ranging from pre-zygotic barriers to post-zygotic cellular collapse (Kalkışım, 2020).

3.1. Gametophytic Self-Incompatibility and the S-RNase Mechanism

A large proportion of cherry, almond, plum, and apricot cultivars exhibit gametophytic self-incompatibility (GSI), an evolutionary adaptation that prevents fertilization by self-pollen or genetically closely related pollen. This system is regulated by specific S-RNase enzymes encoded by multiple allelic genes (S-alleles) located at the S-locus and expressed in the style tissue of the pistil (Čolić et al., 2010).

When the S-allele carried by the haploid pollen nucleus matches one of the alleles present in the style (e.g., S¹ pollen on an S¹S² style), a molecular recognition event is triggered, initiating the incompatibility response (Kalkışım, 2020).

At the cellular level, this response is highly destructive. Incompatible pollen tubes attempting to grow through the style are typically arrested within the upper third of the stylar tissue. Fluorescence microscopy studies have revealed characteristic abnormalities in incompatible pollen tubes, including tip swelling, tube coiling and looping, and eventual rupture and disintegration of the pollen tube apex (Čolić et al., 2010).

This physical and biochemical blockage of pollen tube progression toward the ovule and egg cell constitutes a primary pre-zygotic barrier, preventing fertilization and leading to rapid flower abscission. Nevertheless, in cases where the pollen tube bypasses this barrier and fertilization occurs, genetic incompatibility effects may persist as post-zygotic embryo abortion (Kalkışım, 2020).

3.2. Inbreeding Depression and Post-Zygotic Developmental Collapse

In breeding programs, or even in naturally self-compatible species, successful fertilization does not necessarily guarantee viable seed development. In-depth histological studies on almond (*Prunus dulcis*) selections carrying the Sf (self-compatibility) allele have demonstrated that inbreeding depression can directly induce embryo abortion (Martínez-García et al., 2012).

In highly inbred genotypes, cryptic genetic incompatibilities or genetic load may activate a cascade of developmental abnormalities (Alonso & Socias i Company, 2005).

At the pre-zygotic level, selfed genotypes often exhibit reduced pollen germination rates and slower pollen tube growth within the style. However, the most severe effects occur post-zygotically during embryo sac development. In ovules affected by inbreeding depression, endosperm development becomes asynchronous despite successful fertilization. The failure of endosperm cellularization after the free-nuclear stage results in severe nutrient deprivation of the embryo (starvation). Simultaneously, extensive necrosis develops in the chalazal region (haustorium), which plays a crucial role in nutrient transport to the embryo.

This genetically programmed degenerative collapse within the female gametophyte ultimately arrests fruit development and leads to extensive fruit drop (Martínez-García et al., 2012).

3.3. Interspecific Hybridization Barriers and Endosperm Degeneration

Interspecific hybridization within the genus *Prunus*, aimed at combining desirable traits such as disease resistance, earliness, and environmental adaptability, often encounters severe post-zygotic barriers such as hybrid sterility and embryo abortion (Liu et al., 2007).

Even when fertilization is successful, the combination of divergent genomes leads to genetic incompatibilities either between parental genomes or between the embryo and maternal tissues, resulting in maternal recognition and elimination of the developing embryo (Mwangangi et al., 2019).

This phenomenon is particularly evident in crosses such as peach $\times$ plum (*Prunus persica* $\times$ *P. salicina*) and Myrobalan plum $\times$ apricot (*P. cerasifera* $\times$ *P. armeniaca*). In such crosses, hybrid embryo development is typically arrested at very early stages within the ovule (Arbeloa et al., 2009). Anatomical studies have shown that developmental coordination between the embryo and the endosperm is disrupted (endosperm failure/degeneration), accompanied by browning of the seed coat (integuments) and collapse of nucellar tissues. In peach $\times$ plum hybrids, endosperm degeneration typically begins around 7–8 weeks after pollination, leading to embryo abortion and subsequent fruit drop (post-pollination incompatibility). Because this genetic incompatibility prevents completion of embryonic development, breeders are often compelled to excise these aborting embryos and culture them under laboratory conditions using embryo culture (embryo rescue) techniques (Liu et al., 2007).

3.4. Cytological and Morphological Sterility

Another fundamental genetic cause of embryo abortion and poor fruit set is hereditary sterility at the cellular level. In fruit trees, sterility is generally classified as cytological or morphological/genetic sterility.

Cytological sterility arises from abnormalities in chromosome number. For instance, in some triploid (3n) cultivars of apple and pear, irregular chromosome segregation during meiosis results in the formation of non-viable pollen grains with abnormal chromosome numbers. Although less frequent in stone fruits, such cytological abnormalities directly lead to gamete failure and consequently prevent embryo formation at the earliest stage (Kalkışım, 2020).

Morphological and male sterility refers to the abnormal or incomplete development of floral organs due to genetic mutations. Defects in pistil or anther development—such as the formation of non-functional anthers incapable of producing viable pollen—constitute major barriers in hybridization programs. These structural abnormalities often result in complete absence of seeds and, consequently, embryos (Kalkışım, 2020).

In conclusion, genetic factors in stone fruit species act as the principal and most irreversible determinants of embryo abortion. These mechanisms operate across all developmental stages, from S-allele mediated stylar rejection in gametophytic self-incompatibility, to endosperm cellularization failure and chalazal necrosis associated with inbreeding depression, and finally to endosperm/embryo incompatibilities in interspecific hybrids and cytological sterility. Collectively, these pre-zygotic and post-zygotic genetic constraints represent the most critical barriers limiting successful fertilization, embryo development, and seed formation in *Prunus* breeding systems.

4. BIOTECHNOLOGICAL APPROACHES TO OVERCOMING EMBRYO ABORTION

Fruit breeders, particularly for obtaining progeny from early-ripening cultivars and interspecific hybrids, increasingly rely on tissue culture techniques that bypass embryo abortion. Embryo culture (embryo rescue) refers to the excision of a developing embryo from the seed before degeneration occurs, followed by its germination under *in vitro* conditions on artificial nutrient media. This technique is based on replacing the function of the arrested or degenerated endosperm with a controlled artificial culture environment and is regarded as one of the most reliable biotechnological tools for overcoming post-zygotic barriers (Noga et al., 2016).

Historically, the first successful *in vitro* culture of mature embryos was achieved by E. Hannig in 1904, while its application for rescuing hybrid embryos was initiated by Friedrich Laibach in 1925 (Uysal et al., 2007). In fruit trees, particularly stone fruits (*Prunus* spp.), the pioneering work was conducted by H.B. Tukey, who successfully cultured cherry embryos in 1933 (Ramming, 1990).

4.1. Isolation Timing, Embryo Size, and Culture Systems

In early-ripening peach, nectarine, cherry, plum, and apricot hybridizations, embryos abort within a relatively short developmental window (less than 100–120 days) due to nutrient limitation caused by rapid pericarp growth. Therefore, fruits must often be harvested before full maturity, and embryos must be transferred under sterile laboratory conditions. At this stage, both the timing of embryo isolation and embryo size are critical determinants of culture success (Özdemir Eroğlu et al., 2012).

Embryo development is generally divided into two phases: a heterotrophic phase from the zygote to the globular stage, and an autotrophic phase beginning with cotyledon development. Embryos in the heterotrophic phase are extremely small and fully dependent on specific nutrients and hormonal signals supplied by maternal tissues. Therefore, in highly incompatible interspecific crosses where abortion occurs very early, direct isolation of the naked embryo is technically challenging, increases the risk of mechanical damage, and reduces survival rates. In such cases, culturing the entire ovule or even the entire ovary is often more effective (Rogo et al., 2023).

Ovule or ovary culture allows maternal tissues (e.g., integuments) to continue protecting the embryo for a limited period, preventing premature germination and enabling further accumulation of dry matter and growth within the ovule (Pramanik et al., 2021).

When direct embryo isolation is possible (embryo culture), the goal is to recover the embryo at the latest viable developmental stage before abortion begins. In *Prunus* species, anatomical studies have shown that the most suitable stage for embryo rescue corresponds to a PF (proportion factor) value of approximately 0.5, meaning the embryo has reached about 50% of the seed length (Liu et al., 2007).

For example, in studies on *Prunus cerasifera* (Myrobalan plum), embryos reaching 50% of seed size (approximately 4.5–6 mm) exhibited germination rates of 70–90%, whereas embryos smaller than 30% of seed size showed drastically reduced success rates (Arbeloa et al., 2009).

4.2. Culture Media Modification and Plant Growth Regulators (PGRs)

Following isolation, embryos are typically cultured on nutrient media such as Murashige and Skoog (MS), half-strength MS ($\frac{1}{2}$ MS), or Woody Plant Medium (WPM). Since *Prunus* embryos are sensitive to high salt and nitrogen concentrations, reduced-strength ($\frac{1}{2}$ MS) or woody plant-specific media (WPM) generally provide higher success rates. Supplementation with plant growth regulators such as BAP (6-benzylaminopurine), GA₃ (gibberellic acid), IAA (indole-3-acetic acid), and IBA (indole-3-butyric acid) significantly enhances germination, shoot and root elongation, and plantlet conversion efficiency (Özdemir Eroğlu et al., 2012).

The physiological roles of these hormones are as follows:

Cytokinins (BAP, kinetin): Promote cell division and morphogenesis in embryos. In aborted seeds, high levels of abscisic acid (ABA) inhibit germination; thus, the addition of BAP helps overcome dormancy and counteracts ABA's inhibitory effects (Özdemir Eroğlu et al., 2012).

In peach $\times$ plum (*P. persica* $\times$ *P. salicina*) hybrids, supplementation of half-strength MS medium with high concentrations of 6-BA (4 mg/L) and IBA (0.5 mg/L) increased germination rates up to 90%. However, excessive BAP levels may cause abnormal morphology such as shortened internodes, thickened leaves, rosette formation, and vitrification (hyperhydricity), thereby limiting normal plant development (Liu et al., 2007).

Gibberellins (GA₃): Strongly promote germination, break dormancy, and enhance cell elongation. In Japanese plum (*P. salicina*) and nectarine, combinations of 1.5 mg/L BAP and 0.1 mg/L GA₃ have resulted in up to 100% embryo germination (Şahin et al., 2016).

Auxins (IAA, IBA, NAA): Essential for root meristem induction and proper root system development. Low concentrations of auxins are often added to balance the negative morphological effects of cytokinins such as rosetting, thereby improving overall plant quality (Özdemir Eroğlu et al., 2012).

4.3. Somatic Embryogenesis and Synthetic Seed Technology

In addition to conventional embryo culture, induction of somatic (secondary) embryogenesis under *in vitro* conditions is a key strategy for rescuing abortive embryos and increasing plant regeneration efficiency in hybrid breeding programs (Wakana et al., 2003).

Somatic embryogenesis is the process by which any somatic (non-reproductive) cell of the plant—such as leaf, stem, or cotyledon tissue—develops into a bipolar embryo (with both shoot and root poles) under *in vitro* conditions without fertilization (Sülüsoğlu Durul & Tekin, 2022).

This process may occur via direct somatic embryogenesis or indirectly through an embryogenic callus phase. Immature zygotic embryos that cannot be germinated through conventional methods, or their cotyledons, are isolated and cultured under high-auxin media conditions (e.g., 2,4-D, NAA, or picloram). These strong auxins reprogram differentiated cells into totipotent embryogenic cells, leading to the formation of embryogenic callus tissue (Sülüsoğlu Durul & Tekin, 2022).

Somatic embryogenesis provides several revolutionary advantages in fruit breeding:

Secondary embryogenesis: Once a single abortive zygotic embryo is rescued, it can be used to generate hundreds or even thousands of genetically identical (clonal) somatic embryos. This enables unlimited propagation of rare hybrid genotypes from a single rescued embryo (Wakana et al., 2003).

Synthetic seed production: Embryo abortion in stone fruits prevents seed propagation of valuable genotypes such as early-ripening or interspecific hybrids. Synthetic seed technology provides a biotechnological solution by encapsulating or converting *in vitro*-derived embryos into artificial seeds that can be directly sown. This approach has high potential for clonal propagation in species such as sour cherry, sweet cherry, peach, grapevine, and citrus (Sülüsoğlu Durul & Tekin, 2022).

Transgenic and mutation breeding: Because somatic embryos originate from a single cell, they provide an ideal target for genetic transformation techniques such as *Agrobacterium*-mediated gene transfer, CRISPR/Cas9 genome editing, and protoplast fusion. Somatic embryogenesis in suspension cultures of abortive hybrids enables rapid mass propagation of disease-resistant or genetically improved elite rootstocks (Sülüsoğlu Durul & Tekin, 2022).

In conclusion, in *Prunus* species where seeds lose viability due to early ripening or genetic incompatibility, the integration of embryo culture (embryo rescue) and somatic embryogenesis represents the most strategic biotechnological approach for overcoming natural reproductive barriers and ensuring the success of modern fruit breeding programs.

5. CONCLUSION AND RECOMMENDATIONS

In stone fruit species (*Prunus* spp.), embryo abortion is a multifactorial physiological and genetic crisis arising from the combined effects of asynchronous growth between fruit and seed tissues, nutrient deficiency (starvation) associated with chalazal haustorium and endosperm dysfunction, programmed cell death (PCD), and genetic incompatibilities including self-incompatibility, inbreeding depression, and interspecific hybridization barriers. While embryo abortion functions as an evolutionary adaptation in natural populations by enabling the maternal plant to allocate limited resources to the most competitive offspring, it constitutes an insurmountable post-zygotic barrier in modern fruit breeding programs aimed at exploiting the genetic potential of early-ripening cultivars and developing novel interspecific hybrids within the *Prunus* genus.

To overcome these barriers, in vitro biotechnological approaches such as embryo culture (embryo rescue), which revives embryos that have ceased development or are at risk of degeneration due to nutrient deficiency, and somatic embryogenesis, which exploits the totipotent capacity of plant cells, represent the most strategic and reliable solutions for improving the success of modern hybridization and breeding programs.

• Optimization of Isolation Timing

A critical determinant of success in embryo rescue studies is the timing of embryo excision before loss of viability. For optimal results, the isolation stage should correspond to a PF (proportion factor) value of approximately 0.5, meaning that the embryo has reached about 50% of the seed length.

• Selection of Culture Type According to Developmental Stage

In highly incompatible interspecific crosses where embryos abort at very early heterotrophic stages, direct embryo excision carries a high risk of mechanical damage and reduced survival. In such cases, ovule or ovary culture is preferred over direct embryo isolation in order to protect the embryo within maternal tissues and enhance survival rates.

• Optimization of Culture Media and Hormonal Balance

Considering the sensitivity of *Prunus* embryos to high salt and nitrogen concentrations, half-strength Murashige and Skoog ($\frac{1}{2}$ MS) or Woody Plant Medium (WPM) should be preferred over full-strength MS media. Cytokinins (particularly BAP) should be used to break dormancy and promote germination, gibberellins (GA_3) should be applied to enhance shoot elongation, and auxins (IAA, IBA) should be incorporated at optimized concentrations to stimulate root development and counteract morphological abnormalities such as rosetting induced by excessive cytokinin activity.

• Integration with Somatic Embryogenesis and Synthetic Seed Production

Following the rescue of rare and valuable hybrid embryos, secondary somatic embryogenesis should be induced to enable large-scale clonal propagation. Encapsulation of somatic embryos using protective agents such as sodium alginate to produce “synthetic seeds” is recommended, allowing direct sowing into soil and facilitating commercial-scale clonal propagation in agriculture.

• Platform for Advanced Molecular Breeding Applications

If the objective is rapid production of disease-resistant or elite transgenic rootstocks, somatic embryos originating from a single cell (single-cell origin) should be utilized as primary target material for advanced molecular breeding techniques such as CRISPR/Cas9 genome editing and *Agrobacterium*-mediated gene transfer.

In conclusion, the integration of embryo rescue and somatic embryogenesis provides a powerful and comprehensive biotechnological framework that effectively overcomes natural reproductive barriers in *Prunus* species, thereby ensuring the efficiency and success of contemporary fruit breeding programs.

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