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Embryo Rescue: Tool to Overcome Interspecific Barrier in *Solanaceae* Crops

Prashant Sharma[1*], A.R. Kurubar[1], B.V. Thembhurne[2] and Anusha[3]
[1]Department of Horticulture,
University of Agricultural Sciences, Raichur (Karnataka), India.
[2]Department of Genetics and Plant Breeding,
University of Agricultural Sciences, Raichur (Karnataka), India.
[3]Department of Fruit Science,
University of Horticultural Sciences, Bagalkot (Karnataka), India.

(Corresponding author: Prashant Sharma)*
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ABSTRACT: Embryo rescue, also known as embryo culture, is an in vitro technique frequently employed in horticulture crops to save hybrid results of fertilization that would otherwise deteriorate. In embryo culture operations, the artificial nutrient medium replaces the endosperm, allowing the embryo to continue developing. Laibach demonstrated the first successful embryo culture to produce an interspecific hybrid between *Linumperenne* and *L. austriacum*. Several improvements have been achieved since then in embryo culture/embryo rescue procedures, which have been a popular approach for generating hybrids from a variety of incompatible crossings. Currently, embryo rescue shows enormous promise not just for wide crossings, but also for producing plants from essentially poor embryos, producing haploid plants, and shortening the breeding cycle. This review presents application of embryo rescue that is being used in *Solanaceae* crops for overcoming different interspecific pre- and post-barrier which is important resistant to different abiotic and biotic stresses.

Keywords: Embryo rescue, Fertilization barrier, Hybrids, Interspecific barrier, *Solanaceae.*

INTRODUCTION

A plant embryo is a seed component that includes precursor tissues for the leaves, stem, and root, as well as one or more cotyledons. The young sporophyte of a seed plant, which typically consists of a rudimentary plant with a plumule, radical, and cotyledon. It is a multicellular structure with the ability to grow into a new plant. It marks the start of a new saprophytic generation. The embryo can develop from a specialised cell, such as a zygote, or from a somatic cell. The former is referred to as an azygotic embryo, whereas the latter is referred to as a somatic, adventive, or asexual embryo. An embryo is essentially a bipolar structure with primordial root and shoot structures. Normally, embryos, no matter how small they are, develop into whole organisms as small as foliage and as large as Sequoia species. An embryo's vast potential makes it a very unique structure. Although young embryos mature and unfold their potential under normal and favourable conditions, in some cases, their subsequent development is halted at an early stage. When the embryos have desirable genotypes, this becomes a factor to consider. In such cases, embryos (or proembryos, as the case may be) can be saved from abortion by culturing them under appropriate conditions, a process known as embryo rescue.

The culture of isolated mature or immature embryos is called embryo rescue. Zygotic or seed embryos are frequently used as explants in plant tissue culture, such as to start callus cultures. When nourishing tissue, such as endosperm, is present in the seed during development, the embryo develops properly. Crossing between the two distant species, however, resulted in endosperm tissue degeneration, which impedes embryo development and, as a result, no viable plant will develop (Bhatia *et al.,* 2015). Culturing of the embryo was developed to recover such a hybrid embryo. The culture of embryos (hybrids) in vitro is known as embryo rescue, and it is widely used for crop improvement. Hanning was the first to successfully culture an isolated mature embryo of a cross between Cochlearia × Raphanus in 1904. (Hannig, 1904). Laibach isolated zygotic embryos from nonviable *Linumperenne* × *L. austriacum* seeds in 1925 and 1929. (Laibach, 1925). These hybrids cannot form naturally. Van Overbeek (1941) introduced coconut milk for the first time in 1941 for the development of embryo and callus formation in Datura. In other research, embryo culture has resulted in the development of a new area of hybrid embryos, which has provided a solution to embryos that are typically destroyed (for a variety of reasons) at an early stage and cannot be raised naturally. This technique is used for incompletely developed embryo culture (orchids), to overcome seed dormancy, shorten the breeding cycle, determine seed viability, microcloning of the source material, rapid

multiplication, and conservation of many important medicinal plant and endangered plants (e.g., recently embryo culture of important medicinal endangered forest tree species *Strychnos potatorum* with 100% germination rate was conserved by using this technique) (Kagithoju *et al*., 2013). Furthermore, embryo culture is a rich source of therapeutic secondary metabolites; for example, an alkaloid (neferine) with the potential to inhibit human lung cancer has recently been investigated from lotus seed embryo (Poornima *et al*., 2014). The establishment of successful in vitro cultures is hampered by the difficulty of obtaining contamination-free seeds or embryos without damage or oversterilization. This paper reviews work done on embryo rescue in *solanaceae* crops.

There are two types of embryo culture: mature embryo culture and immature embryo culture (embryo rescue).

• **Mature embryo**: Ripe seeds are used to separate mature embryos for *in vitro* cultivation. Mature embryo cultures are used when the embryos remain latent for extended periods of time; embryos have a low rate of survival *in vivo*; to prevent seed germination inhibition; or to transform sterile seeds into live seedlings. In some plants, chemical inhibitors or mechanical resistance provided by the structures that surround the embryo may be the cause of seed dormancy. The embryos can be effectively cultured *in vitro* to get around seed dormancy. In order to create viable seedlings, embryos can be fed on a basic inorganic medium supplemented with an energy source (often sucrose). Since the adult embryos removed from the growing seeds are autotrophic in nature, so this is possible.

• **Immature embryo:** Immature embryos are cultured in order to save them from immature or hybrid seeds that do not germinate. This strategy helps to develop a healthy plant and prevent embryo abortion. Wild hybridization, which involves mating two distinct plant species from the same genus or from different genera, frequently fails. This is mostly because genetic obstacles prevent normal zygote and seed development. As a result, the hybrid embryo is aborted because the endosperm in the hybrid does not mature. It's also possible for the endosperm to release chemicals that finally harm the embryo. Under normal circumstances, endosperm initially develops and provides nutritional support for the development of the embryo. Thus, endosperm development failure is the primary cause of embryo abortions. By isolating and cultivating the hybrid embryos before abortion, abortion of embryos can be prevented. The creation of interspecific and intergeneric hybrids from wild plant species is the most significant use of embryo rescue.

According to cultural requirements, there are different stages of embryo development:

— Globular Stage
— Hearted shape Stage
— Torpedo Stage
— Cotyledonary Stage

The embryo is heterotrophic up until the globular stage because it receives some of its nutrients from endosperm. Once the embryo reaches beyond this stage, it turns autotrophic and is able to produce its own biochemical needs from basic nutrients like salt and sugar.

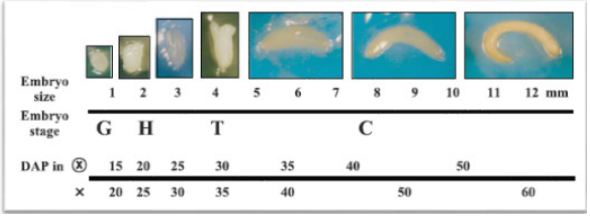

Fig. 1. Embryo stage in chilli are indicated as follows: G=globular; H=heart; T=torpedo; C=cotyledonary Symbols: ⊗ = Selfing; x=Intercrossing (Yoon *et al.,* 2006).

Nutritional needs for embryo cultures

1. If the embryo is heterotrophic, its primary sources of nutrition are the mother's tissues and endosperm.
2. If the embryo is autotrophic, it has the metabolic capacity to manufacture the materials it needs to grow and gradually become independent. At this stage, the nutrient supply is highly varied and mostly dependent on the type of plant. In general, a complicated medium is needed to cultivate immature embryos as opposed to adult embryos, which can grow on a straightforward inorganic medium. Furthermore, in order for embryos to fully develop, they frequently need to be transferred from one medium to another.

Culture Technique for Embryo Rescue: Isolating developing embryos can frequently be challenging. The aseptically separated embryos can be optimally developed in a suitable medium. In general, culture techniques involving embryo rescue calls for a complicated nutritional media. Embryo-endosperm transplantation is utilised to provide young embryos with enough nutritional support.

Embryo-endosperm transplant: In order to cultivate immature embryos, the endosperm transfer procedure is performed as follows:

Excision is used to remove the hybrid embryo from the ovule inside which endosperm development had failed. Another ovule with endosperm encapsulating an embryo and normal development is chosen. The normal embryo is forced out of this ovule after it has been dissected. A typical endosperm with an open hole is the result. Through the open hole, the hybrid embryo can now be inserted into the healthy endosperm. An appropriate medium can be used to cultivate the embryo-endosperm transplant that arises from this. Many interspecific and intergeneric plants, such as hybrid legume plants, have been developed utilising embryo-endosperm transfer.

Two environmental elements that are crucial for embryo culture are light and temperature. In order to promote chlorophyll development, embryos might occasionally grow best when kept in the dark for the first one to two weeks of culture. Compared to whole seeds, isolated embryos usually germinate over a larger temperature range. Although the ideal temperature depends on the type of plant, a high range of 25 to 30°C is typically employed (Narayanaswami and Norstog 1964). To disrupt dormancy, certain embryos from

species like Lilium required a cold treatment of 4 °C, while others need a lower temperature, 17°C (Pierik, 1987). In embryo culture, the mother plant's growing circumstances are also taken into account. If the mother plant is grown under carefully controlled conditions, the endosperm and cotyledon development will increase, promoting embryo growth.

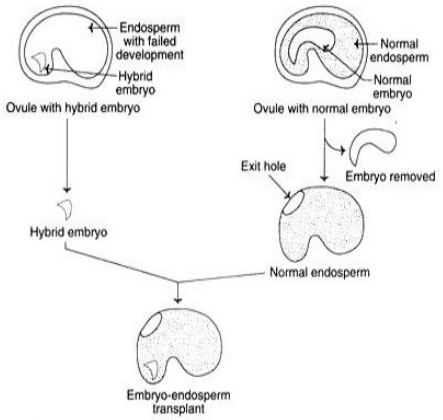

Fig. 2. Embryo-endosperm transplant technique used in embryo rescue (Chadipiralla *et al.*, 2020).

Media for embryo rescue. Dieterich showed that mature embryos could grow normally on a semisolid medium containing only Knop's mineral salts and 2.5% to 5% sucrose (Dietrich, 1924). However, many scientists believe that the most important aspect of embryo culture is medium selection. Several formulations of mineral salts have been used for embryo culture without much critical evaluation of the role of individual elements (Bhojwani and Razdan 1983). Murashige and Skoog (1962) and Gamborg'sB5 medium (Gamborg *et al.,* 1968), with certain degrees of modification, are the most widely used basal media in embryo culture. In general the older the embryo the simpler its nutritional needs.

Ammonium nitrate and potassium nitrate are the most frequently used sources of inorganic nitrogen in embryo culture. Ammonium in the medium is essential or preferential for proper growth and differentiation of immature embryos (Matsubara, 1964; Umbeck and Norstog 1979). Ammonium usually is combined with an organic acid, particularly with malate or citrate anions. Among various amino acids, glutamine and asparagine are the most effective (Dietrich, 1924).

It is common to add vitamins, including biotin, thiamine, pantothenic acid, nicotinic acid, ascorbic acid, inositol, and pyroxidine. These vitamins have not been proven to be essential. Adding amino acids to the culture medium may stimulate embryo growth (Bhojwani and Razdan 1983). Glutamine is the most effective amino acid for cultured embryo growth (Monnier, 1995). Asparagine may also enhance embryo growth (Hannig, 1904), but it can be inhibitory (Matsubara, 1964).

In embryo culture media, casein hydrolysate is used to stimulate growth because it is a complex mixture of amino acids. It contains18 amino acids that has been widely used as an additive to embryo culture media. When added alone to a medium, none of the amino acids match the beneficial effect of casein hydrolysate (Sanders and Burkholder 1948). Proline, serine, and glutamine, which can be substituted for casein hydrolysate, have been demonstrated to be compatible with the induction and maturation of somatic embryos.

Embryo culture typically uses sucrose as its carbon energy source. In addition to being an energy source, sucrose keeps nutrient media at a suitable osmotic potential. An immature embryo typically grows on media with 8% to 12% sucrose, mimicking the high osmotic potential within an immature embryo sac. Mature embryos grow on media with 2% to 3% sucrose. Generally, the younger the excised embryo, the higher the medium osmolarity required. Raghavan and Barlow (1986) believes that this high osmolarity prevents precocious germination and keeps cells that are in a state of division from going into a state of elongation. Agar is the most commonly used agent to solidify culture media. Concentrations of 0.5% to 1.5% are generally used for embryo culture (Hu and Wang 1986). In the presence of high concentrations of agar, growth can be inhibited by reduced water availability, agar's quality, or contaminants. Plant growth regulators generally play a small role in embryo culture. Exogenous auxins do not seem to be required for plant embryo growth in vitro (Norstog, 1979). Exogenous auxin in high concentration in media inhibit somatic embryo induction whereas when in low concentration it stimulate growth. When utilised as the single hormone, cytokinins either have no effect or have very little effect on the growth of immature embryos. Mathew *et al.* (2021) showed groundnut leaves when cultured in MS medium supplemented with 5.0 mg l^{-1} 2, 4-Dcallus induction frequency of 97.22% was observed. So, when paired with particular auxins, they encourage the growth and differentiation of embryos (Veen, 1963). Hormones shouldn't be added to the media used for embryo cultivation, according to (Monnier, 1995), because they can result in structural defects. If callus induction is required, auxins and cytokinins are not often employed for embryo cultivation. Gibberellins are occasionally employed to induce early germination or to break dormancy.

Steps involved in rescuing embryos: According to research by Raghavan and Barlow (1986), inviable hybrid embryos had the capacity to start developing but were prevented from growing to adult size through normal differentiation. The aberrant embryo eventually becomes starved as a result of endosperm failure. Prior to abortion, hybrid embryos are isolated and cultured in order to get beyond these robust post-zygotic hurdles. The most striking and obvious use of the embryo rescue and culture approach, specifically for eventual beneficial gene transfer from wild species, is the production of interspecific and intergeneric hybrids.

The selection of the medium required to support ongoing embryo growth is the most crucial step in the culture of embryos. The requirements are more demanding the younger the embryo. Although early embryos also need a variety of vitamins, amino acids, growth regulators, and, in certain circumstances, natural

endosperm extracts, adult embryos can be cultured on basal salt media with a carbon-energy source like sucrose. Early embryos should be transplanted to a medium with a high concentration of sucrose (8–12 percent), which mimic the high osmotic potential of the young embryo-intracellular sac's environment, and a combination of hormones that encourage the growth of heart-stage embryos (i.e., a moderate level of auxin and a low level of cytokinin). Asparagine, glutamine, or casein hydrolysate are all forms of reduced organic nitrogen that are always advantageous. Adding malic acid to the culture medium is common practise. The embryo must be moved to a second medium with a normal sucrose concentration, low auxin levels, and a moderate level of cytokinin after 1-2 weeks (when it stops growing), as this allows for restarted embryo growth, often with direct shoot growth. When an embryo does not immediately develop a shoot, it might be placed in a medium for callus induction before a shoot forms. When the embryos have developed into plantlets in a lab setting, they are often transplanted to sterile soil and brought to maturity there.

Embryo culture in *Solanaceae*: Cap *et al*. (1991) conducted a study in which genotypes of *Lycopersicum peruvianum* (L.) Mill. and *L. peruvianum* var. *gladulosum* (Rick) selected from accessions with resistance to *Meloidogyne incognitia* at high soil temperature (30°C) were used as male parents in crosses with *L. esculentum* (Mill.) susceptible cultivars UC82, Lukullus, Tropic, the embryo callus and embryo cloning techniques overcame the incongruity barrier between the two plant species. F_1 plants were extremely resistant to *M. incognitia* in soil at 25 and 30°C in greenhouse inoculation studies. These findings supported the successful transmission and expression of heat-stable resistance to *M. incognitia* from *L. peruvianum* to *L. esculentum* hybrids.

To create inter-EBN hybrids, Singsit and Hanneman (1991) used a combination of mentor pollination and embryo rescue. In potato, this approach proved successful in producing 4x (2EBN) 4x (4EBN) and 2x (1EBN) 2x (2EBN) hybrids.

Segeren (1993) sought to generate hybrid tomato plants through interspecific crosses using embryo rescue. Culture mediums containing varying quantities of auxins and cytokinins, as well as complicated additions, were evaluated. The addition of 10 M 6-BA and 2.5 M IAA was the most effective therapy, causing callus development in around 20% of the immature embryos. Four callus lines with normal shoot development were isolated. A total of 128 hybrid plants were transplanted to a field nursery after rooting plantlets were gradually adapted to greenhouse conditions.

Watanabe *et al*. (1995) used mentor pollination and embryo rescue to produce the first sexual hybrids between non-tuber-bearing and cultivated Solanum species. The cultured female parents were interspecific hybrids consisting primarily of cultivated material. They were pollinated by three non-tuber-bearing species, *S. brevidens*, *S. etuberosum*, and *S. fernandezianum* and then mentor pollinated by a Phureja Group pollinator. While sexual hybrids were formed, the fraction of actual hybrids was low, and confirming putative hybrids required substantial effort.

Janssen *et al*. (1997) crossed the 4x (2EBN) species *S. stoloniferum* and *S. fendleri* with the 4x (4EBN) and 2x (2EBN) Tuberosum Group, as well as the 6x (4EBN) species *S. hougasii*. 20-day-old seeds were extracted from berries and cultivated in Petri dishes for various cross combinations. This method resulted in hybrid plants from both inter- and intra-EBN crossings.

Through mentor pollination and embryo rescue, Ramon and Hanneman (2002) aimed to expand the range of Mexican 2x (1EBN) species accessible. However, just one hybrid [2x (2EBN) Tuberosum Group] x 2x (1EBN) *S. pinnatisectum* with different morphological features was observed after 6365 pollinations. The scientists speculate that the *S. pinnatisectum* parent may have carried the anthocyanin-producing allele but did not express it.

Hossain *et al*. (2003) investigated the effects of medium composition and embryo age on plant recovery for successful rescue of hybrid embryos from *Capsicum annuum* and *C. frutescens*. Only embryos that were used to create hybrid plants were from 28 to 33 days following pollination. Because of the presence of several post-zygotic incompatible obstacles, the plant recovery rate from hybrid embryos was low and less than the embryo germination rate. The recovered plants hybridity was established using random amplified polymorphic DNA (RAPD) markers and morphological traits acquired from the pollen parent.

According to Chen *et al*., 2004, most cross combinations of 2X (1EBN) *S. pinnatisectum* and 2X (1EBN) *S. cardiophyllum* failed unless embryos rescue protocol was followed. The embryos that were saved grew into hybrid plants that could be backcrossed to *S. cardiophyllum* for further breeding program.

Due to embryo abortion, which has been identified as a post fertilization genetic barrier in conventional interspecific hybridization between the *Capsicum annuum* and *Capsicum baccatum* species, hybrids could not be created. Using a wide number of *C. annuum* accessions as pistillate parents, some somewhat suitable cross combinations were identified through studies of embryo development after pollination. Yoon *et al*. (2006) effectively used embryo rescue procedure to develop hybrids in these partially suitable crossings. Immature seeds containing torpedo or early cotyledonary embryos were excised 35-40 days after pollination and the embryos cultivated on MS medium containing sucrose and plant growth regulators. The observation of corolla yellow spot as a major species characteristic trait of *C. baccatum* verified hybridity. Hybridity was established utilising random amplified polymorphic DNA (RAPD) marker analysis and observation of corolla yellow spot as a prominent species characteristic attribute of *C. baccatum*. All of the hybrid plants grew rapidly but had full pollen sterility. Using *C. annuum* as the pollen parent, vigorous backcrossing was used to overcome hybrid sterility.

Rattan *et al*. (2015) experimented with the goal of standardising protocol and obtaining hybrids of

Solanum melongena and *Solanum khasianum* by embryo rescue technology. Cross pollination occurred in a reciprocal manner between indigenous collections of *S. melongena* and locally obtained *S. khasianum*. When *S. khasianum* was used as the male, IC 203585, IC 261767, IC 261797, IC 305129, IC 354611, IC 099736, and IC 261888 showed good fruit setting. When inoculated 25 days after pollination, the hybrid combinations IC 2035859 × *S. khasianum*, IC 2617679 × *S. khasianum*, IC 2617979 × *S. khasianum*, and IC 3051299 × *S. khasianum* produced the greatest results.

Sohrab *et al.* (2015) successfully developed interspecific hybrids F1 tomato by embryo rescue, which was not possible through conventional breeding, and were able to introgress the resistant genes into cultivated tomato and developed interspecific tomato plant by embryo rescue showing good resistance against PBNV and bearing large, red, and desired shape tomato fruits.

Because *Solanum sisymbriifolium* is resistant to various diseases and resistant interspecific hybrids have been developed, it has been deemed of potential importance for *S. melongena* L. breeding. Piosik *et al*. (2018) discovered that in vivo crossing between *S. lycopersicum* and *S. sisymbriifolium* diploid embryos were generated inside fruits, however their development was blocked at the globular stage. The embryorescue procedure was used to create F_1 hybrids. The results showed that *S. lycopersicum* can be interspecifically hybridised with *S. sisymbriifolium* to introduce unique features for application in tomato breeding programmes.

Walter *et al.* (2018) and his co-worker investigated the effect of genotype (*Capsicum baccatum* and *C. frutescens*), culture medium composition (MS and 1/2 MS medium with different concentrations of sucrose, IAA, and GA_3), and developmental stage (globular, cordiform, torpedo, and cotyledonary) on immature embryo in vitro culture. Regardless of species, the best culture medium for globular and cordiform embryo germination was 12 MS with 0.05 mg L^{-1} GA_3 and IAA and 40 g L^{-1} sucrose. Germination of torpedo and cotyledonary embryos is recommended in 12 MS culture medium with 20 g L^{-1} sucrose and no phytoregulators.

According to Afful *et al*. (2020), the success rate of eggplant hybridization between species is typically very low. This stifles the development of improved hybrids. Extracted embryos from two crosses (*Solanum melongena* × *S. torvum* × *S. anguivi*) were inoculated on Murashige and Skoog (MS) basal medium supplemented with different amounts of 3-indoleacetic acid (IAA) and 6-benzylaminopurine. After early acclimatisation, regenerated plantlets were successfully transported to the field. The high levels of heterosis observed for number of seeds/fruit and fruit breadth revealed a possibility for producing F_1 hybrids with favourable fruit characteristics for increased fruit output.

Rakha *et al.* (2020) aimed to create interspecific hybrids as prospective rootstocks from the eggplant (*Solanum melongena*) and the bacterial wilt resistant line EG203, as well as four wild accessions (*S. incanum* UPV1, *S. insanum* UPV2, *S. anguivi* UPV3, and *S. sisymbriifolium* UPV4). Embryo rescue was used to create viable interspecific hybrids between EG203 and UPV1.

CONCLUSIONS

In this study, we survey the literature regarding methods used to prevent embryo degeneration and to obtain viable interspecific plants from embryos. In spite of the fact that embryo rescue has not been used in many field trials, and how much plant material of agricultural and horticultural value has actually been generated. There is a large potential for rescuing desired embryos that would otherwise degenerate with the embryo rescue technique, as it is simple to use and possesses a wide range of potential applications. A plant tissue culture laboratory usually has the necessary facilities for this technique. Nevertheless, certain constraints are relevant under suboptimal conditions. On other hand, perennial plants are easier to handle as they can be kept in laboratories, transplanted at any time of the year, and acclimated until they become hardy enough to survive in nature or domestication.

FUTURE SCOPE

Breeders have only touched a small portion of these potential genetic resources due to fertilization barrier in interspecific crosses. Breeders should be optimistic about the prospects for genetic progress in *solanaceae* crops. Genetic variation abounds in germplasm resources, and the toolkit for utilising those resources is stocked with efficient strategies for capturing that diversity. Embryo rescue could be one of these toolkit which can be used for genetic improvement of chilli.

Conflict of Interest. None.

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