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Advances in Embryo Rescue as an In-Vitro Crop Improvement Strategy

Padminee Das[1], Mukesh Kumar Singh[2], Mouli Paul[3], Th. Nengparmoi[4] and Jatoth Uday Bhargav[5]

Abstract:

Important challenges in horticultural crop breeding include long juvenility, seedlessness, embryo abortion, seed dormancy, low seed viability, cross and self-incompatibility, etc. One of these, post-zygotic embryo abortion in remote hybridization, has become a significant biological constraint in the traditional method to crop development. Breeders and cytogenecists use embryo rescue, a non-GM biotechnological in vitro technique, to create hybrids in interspecific and intergeneric hybridization. Post-crosses frequently result in embryo abortion, degeneration, and self and cross-incompatibilty, which drastically reduces the ability to transfer desirable genes from wild species to elite cultivars. There is very little chance of variety being created and subsequent development of favorable features in crop plants if seedless parents do not generate fertile seeds. Adopting the embryo rescue and culturing techniques will help horticultural crops overcome key constraints such excessive fruit drop, seed dormancy, etc. However, the success rate of embryo rescue techniques is always determined by the following factors: seedling growth and development, standardizing a workable nutritional media, commencing and continuing embryonic development, and embryo recovery without injury. Additionally, embryo cultivation is a vital method for developing monoploid, triploid, synthetic seeds, testing the viability of seeds, and conserving germplasm under biotic and abiotic stressors, types that are resistant or tolerant.

Padminee Das[1], Mukesh Kumar Singh[2], Mouli Paul[3], Th. Nengparmoi[4] and Jatoth Uday Bhargav[5]

[1]Scientist, Department of Plant Breeding and Genetics, AAU- Zonal Research Station, Assam Agricultural University, Shillongani, Nagaon- 782002, Assam

[2]Assistant Professor-cum-Junior Scientist, Department of Genetics & Plant Breeding, Tilka Manjhi Agriculture College, Godda (Birsa Agricultural University, Ranchi) Jharkhand

[3]M.Sc Scholar, Department of Genetics and Plant Breeding, Institute Ramakrishna Mission Vivekananda Educational and Research Institute, Kolkata

[4]Ph.D. Research Scholar, Department of Agronomy, School of Agricultural Sciences, Nagaland University, Medziphema, Nagaland

[5]Assistant professor, Department of Agriculture, Loyola academy, Osmania University, Hyderabad, Telangana

Introduction:

The process of cultivating removed embryos in a sterile culture media is known as embryo rescue procedure or embryo culture technique. This method was initially applied by Tukey in Cherry in 1933. By preventing embryo abortion in regenerated plants via varied crossings for crop improvement, "embryo rescue" refers to in vitro technology that develops immature embryos into whole plants. In embryo rescue procedures, artificial nutritional medium serves as a stand-in for endosperm, promoting ongoing embryonic growth. The embryo rescue technique has been a key tool for scientists to achieve considerable success in plant breeding during interspecific and intergeneric crop species hybridization. There have been successful applications of the embryo rescue procedure in tomatoes, capsicum, chillies, okra, radish, papaya, grapes, mangos, and olives. Physiological requirements for embryogenesis, overcoming seed dormancy, shortening the breeding period, testing seed viability, micro-propagation, and isolating degenerating embryos from distant crosses are some of the areas of research conducted under embryo rescue techniques since the early 1940s. One of the most promising methods for producing viable seeds from seedless parents, rescuing and cultivating naturally weak, immature, and/or abortive embryos in artificial nutrient media, developing triploids, and overcoming cross and self-incompatibilities in distant hybridization is embryo culture.

Through a successful ontogeny process, the embryo culture and rescue method recovers immature or mature zygotic embryos that typically abort and cultivates them on artificial nutritional media in an aseptic environment to produce a robust and healthy plant. It is difficult to standardize a nutrient media, isolate an embryo without harm, and promote ongoing embryogenesis and seedling development. To reduce the risk of incompatibility and embryo abortion, young embryos are saved from successful distant crosses of Medicago sativa × M. rubestris and Lycopersicon esculentum × L. peruvianum and raised in artificial medium. One of the main causes of incompatibility in interspecific crosses, which leads to the generation of small, shrunken seeds and disrupts seed development, is embryo abortion. Embryo rescue methods have been used to various genetic resources in order to get over the restriction of remote hybridization. Furthermore, haploids and triploids have been created via chromosome removal following remote hybridization, which has saved the embryo. For instance, crossing diploid and tetraploid species and then cultivating the resulting embryo to preserve the dying embryo has produced triploids. A highly helpful method for creating offspring from intraspecific hybridizations in which the maternal parents possess the seedlessness characteristic is embryo rescue. Sometimes there is no issue with embryo abortion, but breeders have difficulties because of things like large fruit drop, low seed viability, and seed dormancy. In these situations, embryo rescue methods not only get over these obstacles but also provide up opportunities for research on seed germination and development.

General Embryo Rescue Procedures

There are three methods if culturing embryos.

(i) Embryo Culture

This popular technique involves removing, saving, and cultivating embryos in culture media. Another name for it is ovary-embryo or ovule-embryo culture. Ovum or ovarian culture can occasionally come before embryo culture. By delaying excision until the embryo reaches a sufficient size, this procedure causes little to no harm. The presence of the integument throughout the ovule or ovary culture stage reduces precocious germination. The excised embryo and the medium come into direct contact. Before embryo abortion, desired plants that are cultivated in a field or greenhouse with regulated pollination conditions first have their fruits harvested. Following the recovery of ovules from the ovary under aseptic conditions, disinfection is carried out. The embryo is carefully removed using a laminar flow hood and stereomicroscope, which improves the success of embryo culture. It is important to know the incision site beforehand, as it varies depending on the species. The ovule's micropylar end is severed, and the opposite end is gently pushed to extract the embryo via the aperture. Certain plant species have employed this method. The saved embryo is immediately put into culture medium to prevent drying. Hu and Wang have noticed that plant species that have heart-shaped embryos use the suspension intact approach. Nurse cultures as a means of preserving embryos. After an embryo from an incompatible cross is saved and placed into endosperm derived from a similar compatible cross, the two are cultivated together.

(ii) Ovule Culture

The use of the embryo culture method is challenging for plant species with extremely small seeds because it can harm the embryo during the rescue procedure. This process, known as ovule culture or ovolo embryo cultivation method, involves cultivating the entire ovule without removing the embryo. Before an embryo is aborted, the ovaries containing the embryo are first extracted from the surface-sterilized ovary under aseptic conditions under a stereomicroscope. The entire ovule is then placed in the culture media. Additionally, this method saves time when rescuing embryos and improves success in large-seeded species. This technique has shown remarkable success in cultivating plant species that include placental tissue. The ovule culture technique regimen for peaches (Prunus persica L. Batsch). Ovule perforation is a recently developed procedure that involves making tiny holes in each ovule right before medium culture. Care is used during perforation to prevent harm to the embryo, and the perforated ovule effectively absorbs nutrients and water. In addition, two kinds of ovule support systems—filter paper support systems and vermiculite support systems—have been used. Under a filter paper support system, perforated ovules are cultivated on top of filter paper that is set over a liquid medium. On the other hand, the micropylar side of the ovules is positioned beneath the vermiculite support system in a mixture of liquid substrates or sterilized vermiculite. However, the vermiculite support systems and ovule piercing technique might not be practical for small seeded crop species.

(iii) Ovary Culture

Another name for this method is pod cultivation. This method involves separating the entire ovary from the fruit and cultivating it in a medium. Disinfestation procedures are used to eliminate surface contaminants, and caution should be exercised to prevent ovarian damage. Finally, the seed and the cultured ovary are separated. In order to prevent embryo abortion by saving embryos from interspecific hybridization in Tulipa, employed a modified ovary culture technique called as ovary-slice culture. The transversely cut tulipa ovaries are divided into pieces, and the basal cut end of each segment is put in culture media. Compared to basic ovary culture, this method allows for early embryo growth because of its effective water and nutrient absorption. Both, however, yield the same germination rate percentage.

Applications of Embryo Rescue Technique

i) Overcoming embryo abortion, Self- and Cross-Incompatibility

In hermaphrodite flowers, self-incompatibility occurs when functional male and female reproductive cells cannot produce seeds through self-pollination, which naturally prevents inbreeding and promotes outcrossing. Conversely, when reproductive cells from different parent plants are unable to successfully cross-pollinate, this creates cross-incompatibility and results in inbreeding. These reproductive barriers can be overcome using artificial pollination, fertilization, and in vitro embryo culture. The embryo rescue technique has been successfully used to create hybrids between different plant genera, including crosses between radish and rapeseed, different kiwi species, and various Carica and Citrus plants. Research by North and Wills showed that viable lily seeds could be produced from Lilium lankongens crosses using embryo rescue, even without endosperm. This technique has proven effective in overcoming both self- and cross-incompatibility barriers after fertilization. Scientists have achieved various successful applications of embryo rescue. Bentvelsen and colleagues created Pelargonium hybrids, while others produced banana hybrids between different Musa species. The technique also enabled the transfer of genetic material between different tulip species, overcoming post-fertilization barriers. The process involves removing the entire embryo during development to prevent it from being aborted. However, this is technically challenging due to the extremely small size of embryos after fertilization. As a solution, scientists often culture the entire ovary or fertilized ovules instead. The technique has broad applications in plant breeding. It allows the recovery of fertile embryos from crosses between plants with different chromosome numbers (diploids and tetraploids). Kato and colleagues used it to create inter-section hybrids in primulas, while others developed rhododendron hybrids. Skálová's team used ovule culture to overcome incompatibility between cucumber and other Cucumis species that had different chromosome numbers. The method has also proven successful with vine cacti like Hylocereus and Selenicereus, as reported by Cisneros and Tel-Zur.

ii) Shortening of breeding cycles

Endosperm or seed coat, or both, may be linked to seed dormancy determinants. By carefully separating the embryos from the seed and cultivating them in artificial medium, embryo culture has been employed in this instance to shorten breeding cycles by minimizing the effects of variables seen in the endosperm and/or seed coat. To break dormancy and shorten breeding cycles, apples employ the embryo rescue procedure. In order to improve the creation of theoretical models for inbreeding depression or genetic burden, heterosis, F1 hybrid development, and selfincompatibility genetics in chrysanthemums, By accelerating seedling growth, in vitro rescued and cultured embryos aid in reducing the breeding cycle. Fathi et al. looked into how the vernalization treatment of the removed embryos lengthens the breeding cycle and increases the percentage of germination in the sweet cherry cultivars "Silej-Delamarka." According to Cravero and Crointy, embryo rescue is used to reduce the time between seeds and shorten the breeding cycle. It also helps to manage infections that are spread by seeds. Using in vitro embryo rescue and culture techniques can help cassava overcome its lengthy seed-seed cycle, poor seed germination, and slow rate of multiplication by stem cuttings. In papaya, embryo culture combined with ethrel therapy can shorten the breeding cycle by about three months, compared to the typical six to nine months from pollination to seedling establishment. The immature heart stage of an embryo can be carried to the mature stage by Hollies (Ilex) embryo culture. Normally, it takes three years for full embryogenesis and the start of seed germination in mature berries. By using the culturing process and embryo rescue, hybrid plants can be produced in two to three weeks. In Rosa, which takes a year to flower, two generations can be produced annually with the aid of the embryo rescue procedure.

iii) Production of haploids

Gynogenesis is the process of cultivating egg cells or other haploid cells from the embryo sac to produce haploid plants. In 1976, San Noem was the first to report on haploid production in barley. Since then, it has been effectively documented in a number of crops, including tobacco, sugar beet, wheat, rice, maize, rubber, and so forth. Allium tuberosum uses in-vitro apogamy to create haploid plants from antipodal cells. One essential biotechnology technique for introducing better characteristics into plants is the induction and regeneration of haploids or monoploids. Interspecific crossings between Hordeum bulbosum as the pollen parent and H. vulgare as the female parent result in the chromosomal deletion of the pollen parents and the generation of monoploids in the female parents, which is how barley produces monopliods. Pseudo-fertilized ovule culture in carnation (Dianthus caryophyllus L.) produces doubled haploid. The embryo rescue strategy is used in lettuce to produce haploid plants.

iv) Resistance to biotic and abiotic stresses

Although interspecific and intergeneric hybridization are useful breeding techniques for introducing desired genes to give plants resilience to biotic and abiotic stress, these varied crossings typically result in embryo degeneration and unsuccessful embryonic development. Researchers are using the embryo cultivation method in a variety of horticultural crop development projects in order to get beyond these obstacles. By transferring genes directly from wild species, distant hybridization combined with back crossing, embryo rescue, and culture techniques saw the resynthesis of some hybrids and prevented embryo degeneration in plants with differing genetic compositions and cytoplasmic male sterility. The seedless hybrid created by hybridization followed by embryo rescue technology and screening showed superior field establishment against downy mildew, according to research by Murthy et al. Cheng et al. investigated how Chrysanthemum × grandiflorum and C. makinoi and Nakai crossed through interspecific hybridization and the embryo rescue method produced the MR1, MR2, and BC1F1 hybrids, which exhibit aphid and heat tolerance. According to Sohrab et al., interspecific crossings between Lycopersicon esculentum and L. peruvianum, followed by embryo culture, yield hybrids that are resistant to the peanut bud necrosis virus (PBNV) and have large, red, well-shaped fruits.

v) Conservation and exchange of germplasm

The process of in vitro embryo rescue and culturing is particularly helpful in reducing the bulkiness of seeds or plantlets for transportation or conservation. Embryos are a great source for in vitro preservation and propagation. Embryo culture is a common method of conserving Gramineae crops and conifers. Due to the high cost and cargo space requirements for shipping coconut germplasm, IPGRI, Rome, has mandated that coconut embryo culture be used for international transportation.

Conclusions

Many breeders and cytogeneticists prefer the embryo rescue procedure as a non-GM biotechnological tool for transferring desired genes from wild species into elite produced varieties. It is the most sophisticated agricultural advancement of human kind. Embryo rescue and culture techniques could effectively reduce breeding obstacles including protracted juvenility, seedlessness, embryo abortion, seed dormancy, low seed viability, cross and self incompatibility, etc. The most valuable advancements in embryo rescue techniques include the creation of monoploids, triploids, remote hybrids, qualitative trait hybrids, synthetic seeds, and biotic and abiotic resistant/tolerated types, among others. Because prebreeding and postbreeding obstacles are prevalent in horticultural crops, this strategy is widely accepted and used in crop improvement. Therefore, it is appropriate to conclude that this paper is not a comprehensive analysis of the research conducted and would serve as a resource for upcoming geneticists.

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