The Pharma Innovation Journal 2023; 12(8): 899-902

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(8): 899-902

www.thepharmajournal.com
Received: 09-05-2023
Accepted: 13-06-2023

Sumit Singh
Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University Agriculture Technology and Sciences, Naini, Prayagraj, Uttar Pradesh, India

Vijay Bahadur
Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University Agriculture Technology and Sciences, Naini, Prayagraj, Uttar Pradesh, India

Gaurav Singh Vishen
Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University Agriculture Technology and Sciences, Naini, Prayagraj, Uttar Pradesh, India

Sudhir Kumar Mishra
Department of Horticulture, National Post Graduate College, Barhalganj, Gorakhpur, Uttar Pradesh, India

Corresponding Author:
Sudhir Kumar Mishra
Department of Horticulture, National Post Graduate College, Barhalganj, Gorakhpur, Uttar Pradesh, India

In vitro breeding techniques in vegetable crops and their achievements

Sumit Singh, Vijay Bahadur, Gaurav Singh Vishen and Sudhir Kumar Mishra

Abstract
In the present era, virtually all plants and their constituents have become valuable resources for generating cell cultures, which have gained recognition as crucial research instruments. Moreover, numerous *in vitro* techniques have become commonplace in the horticulture industry. Commercial plant growers are employing tissue culture techniques and principles to propagate a diverse array of plants clonally. Additionally, various *in vitro* methods are employed to produce pathogen-free stocks, effectively compensating for yield and quality losses incurred due to diseases. The combination of cryogenics and *in vitro* approaches shows promise in transporting and preserving germplasm. For the past five decades, embryo cultures have been the standard practice in plant breeding endeavors. However, there are other techniques that hold great potential for inclusion in the plant breeder's toolkit. These techniques include *in vitro* fertilization using ovary and ovule cultures, the production of haploid plants from anthers and microspores, mutagenesis using cell cultures, somatic hybridization, and gene transfer using protoplasts. Furthermore, there have been promising developments in harvesting cultured plant cells and organs for their medicinal and associated compounds. Nevertheless, refining this technique for practical applications remains an ongoing area of improvement.

Keywords: *In vitro* breeding techniques, vegetable crops, their achievements

Introduction
Over the last few decades, the field of plant tissue culture has made remarkable strides, leading to the widespread commercial adoption of methods for rapid multiplication and improved production processes of various crops worldwide. This progress in plant tissue culture traces back to the concept of cell totipotency proposed by Gottlieb Haberlandt in the 20th century (Vasil 2008) [36]. Subsequently, the discovery of cytokinins by Folke Skoog and others in the 1950s and auxins by Frits Warmolt Went in 1926 marked the initial application of *in vitro* techniques to plant tissues (Pennazio 2002) [24]. Since then, the technology has undergone significant advancements and has become indispensable for crop development and genetic engineering. Plant tissue culture offers a diverse range of strategies that complement traditional plant propagation and breeding methods. While *in vitro* techniques have been most commonly used for plant propagation, their most significant recent application lies in crop enhancement through gene technology (Khan 2009; Takeda and Matsuoka 2008; Thakur *et al*. 2012) [18, 33, 34]. These innovations have revolutionized the way crops are developed and improved, playing a pivotal role in modern agriculture and biotechnology.

Embryo Rescue
The technique aimed at fostering the growth of immature embryos into viable plants through *in vitro* methods is commonly known as "embryo rescue." This approach has found widespread application in preventing embryo abortion in hybridized regenerated plants. Embryo rescue has proven to be a vital strategy in plant breeding, enabling the successful development of numerous interspecific and intergeneric crop species. The process involves removing embryos and placing them on a sterile culture medium, also referred to as embryo culture, and was first invented by Tukey in 1933. The groundbreaking experiment involved growing a cherry embryo on a synthetic medium, and since then, this method has been employed to rescue embryos of various other crops, such as Malus (Dantas *et al.* 2006) [9] and Capsicum (Debbarama *et al*. 2013) [10]. Embryo culture has primarily been applied in cases of interspecies or intergeneric hybridization, where the endosperm's growth is hindered by hybridization barriers.

By removing dormancy in seeds, embryo culture also serves to expedite the breeding cycle. Seed dormancy is often induced by factors like endogenous inhibitors, light, temperatures, humidity, or embryo immaturity. By isolating the embryos from these influences, they can be stimulated to germinate and mature more rapidly, effectively shortening the breeding cycle.

Interspecific and Intergeneric Hybridization

Hybridization between diploids and tetraploids, interspecies, or intergeneric crosses often results in nonviable embryos due to slow or no development of the endosperm. Nonetheless, these embryos may have the potential for growth and future development. Overcoming this hybridization barrier can be achieved by aseptically isolating and cultivating them in a nutrient-rich medium. Stebbins (1950) categorized two types of hybridization barriers: pre-fertilization and post-fertilization barriers. Pre-fertilization barriers include factors like geographical distance, apomixis, and pollen-pistil incompatibilities. On the other hand, post-fertilization barriers encompass mechanisms that prevent successful fertilization and may arise from factors like ploidy variations, chromosomal elimination, and seed dormancy. The post-fertilization barrier, which has been a significant challenge in plant breeding, has been effectively surmounted through the application of the embryo rescue procedure. This technique has successfully rescued young embryos resulting from interspecific crosses, such as *Lycopersicon esculentum* × L. peruvianum (Thomas and Pratt 1981) [35]. By employing embryo rescue, these hybrid embryos that would have otherwise been nonviable can now be nurtured and developed, opening up new possibilities for crop improvement and interspecific breeding endeavors.

Table 1: Achievements in vegetable crops through Embryo Rescue.

Species	Use of embryo culture	References
Allium cepa × *A. roylei*	Introgression desirable traits of *Allium roylei* into the *A. cepa* Genome	Chuda and Adamus (2012) [8]
Capsicum annuum, *C. chinense*, and *C. frutescens*	Interspecifi c hybridization for crop improvement	Debbarama *et al.* (2013) [10]
Solanum pinnatisectum × *S. tuberosum*	Introgression of resistance to late blight from *Solanum pinnatisectum* into *S. tuberosum* genome	Ramon and Hanneman Jr. (2002) [27]

The embryo rescue technique is not only applicable to interspecific hybrids but also proves valuable in saving young embryos from intraspecific hybrids, which typically yield nonviable seeds. One example is the production of seedless triploid embryos through crosses between diploid and tetraploid members of the same species. These seedless embryos can successfully develop into fully grown plants when nurtured through *in vitro* cultivation on a sterile and nutrient-rich medium. By overcoming postzygotic barriers, such as endosperm failure, this approach enables the successful growth and maturation of these previously nonviable embryos (Razdan 1996; Hu and Wang 1986) [4, 16]. This advancement in plant biotechnology opens up new opportunities for crop improvement and the development of seedless varieties with enhanced characteristics.

Shortening Breeding Cycle by Overcoming Seed Dormancy

The embryo rescue approach has proven effective in reducing the breeding cycle for various fruit crops by overcoming seed dormancy. In some species, seedlings cannot be immediately grown after fruit ripening, as these species require sufficient time for embryo maturation. Seed dormancy in certain plants may even necessitate an extended period before germination occurs. For instance, seeds from iris, apple, brussels sprouts, and roses do not germinate right after fruit maturation.

By culturing immature embryos on appropriate growth media, the embryo rescue technique enables rapid germination, thus shortening the breeding cycle. Seed dormancy can be triggered by various environmental factors like light, temperature, and humidity, as well as internal factors such as endogenous inhibitors and embryo immaturity. Debbarama *et al.* (2013) [10] highlighted that seed dormancy might be limited to either the endosperm, the seed coat, or both. Removing these inhibitory influences allows the embryos to germinate, expand, and develop more effectively and expediently.

Researchers like Bridgen (1994) [5], Chuanen and Guangmin (2005) [7], and more recently Fathi and Jahani have explored the application of diverse embryo rescue techniques across a wide range of plant studies, further validating its significance in enhancing plant breeding and propagation.

Somatic Embryogenesis

Somatic embryogenesis is the process of generating embryos from somatic cells, bypassing the conventional fertilization procedure. These embryos are clones of the parent tissue, as they are genetically identical due to the absence of natural fertilization.

The discovery of somatic embryogenesis can be attributed to significant research milestones. Steward *et al.* (1958) [32] first described the phenomenon in Daucus carota using suspension culture, and Reinert (1959) [29] observed it in callus culture of the same species. Additionally, Harry Waris' pioneering work on somatic embryogenesis in Oenanthe aquatica (Umbelliferae) was highlighted by Krikorian and Simola (1999) [19], underscoring Waris' contributions to observing and identifying somatic embryo formation in aseptic culture (Simola 2000) [30].

Somatic embryogenesis finds numerous applications in various fields. It is utilized for *in vitro* selection techniques to improve resistance to biotic and abiotic stresses. Moreover, it allows for the large-scale clonal propagation of elite cultivars, leading to the production of synthetic seeds (Pintos *et al.* 2008) [25]. Gene transfer for genetic improvement and its use as potential models for studying molecular, regulatory, and morphogenetic events during plant embryogenesis are among other valuable applications (Kamle *et al.* 2011; Ravi and Anand 2012) [17, 28].

Somatic embryos can be formed directly from organized tissue without passing through a callus phase or indirectly by dedifferentiating the organized tissue into a callus mass before embryo development (Slater *et al.* 2003) [31]. These somatic embryos exhibit morphological and physiological characteristics similar to zygotic embryos resulting from

conventional fertilization (Dobrowolska *et al.* 2012; Mathew and Philip 2003; Palada-Nicolaua and Hausman 2001) [12, 22, 23]. While up to the octant stage, embryogenesis in dicots and monocots follows similar patterns, they diverge into distinct routes afterward (Raghavan 1986) [26]. In monocots, the stages of embryogenesis include globular, elongated, scutellar, and coleoptile stages, while in dicots, the stages encompass globular, heart, torpedo, and cotyledon or plantlet stages (Godbole *et al*. 2002; Mandal and Gupta 2002) [15, 21].

Conclusion

In response to the increasing demand for superior quality crops, traditional plant breeding methods are proving insufficient, leading to a greater reliance on biotechnology approaches. Initially, biotechnological technologies complemented traditional breeding through *in vitro* culture techniques, such as micropropagation, which facilitated rapid multiplication and enhanced uniformity. As a result of their practicality and ability to expedite the breeding cycle, *in vitro* techniques have now become widely adopted in breeding projects. However, the lack of proven *in vitro* methods can sometimes hinder their practical application. The complete process of *in vitro* screening and breeding involves steps like variant or mutant induction, selection, plant regeneration, acclimation, and evaluation of *in vivo* plants. To ensure the success of plant screening and breeding programs, thorough research is necessary to design effective *in vitro* procedures for each technique. Climate change on a global scale has significantly impacted plant growth and crop yields. Growers may now seek new crop varieties that can thrive in changing environmental conditions, such as extreme heat, drought, or chemically contaminated soil. To address these challenges, the development of crops better suited to the evolving environmental circumstances becomes crucial. Biotechnology, in conjunction with *in vitro* methods, holds the potential to play a vital role in breeding resilient and adaptive crops capable of meeting the demands of modern agriculture.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Ahlawat A, Pahuja SK, Dhingra HR. Studies on interspecifi c hybridization in Cyamopsis; c2013.
2. Ahmad I, Johansson S, Andrade G, *et al*. Somatic embryogenesis for mass propagation of Am J Bot. 2011;45:693-713.
3. Ammirato PV. (eds) Handbook of plant cell culture, Macmillan, New York, and *Lycopersicon peruvianum* via embryo callus. Theor Appl Genet. p. 43-96; 59:215-219.
4. Bhojwani SS, Razdan MK. Plant tissue culture: theory and practice (rev ed). Studies in plant science, 5. Elsevier Science, Amsterdam; c1996.
5. Bridgen MP. A review of plant embryo culture. Hortscience. 1994;29(11):1243-1246.
6. Cambridge University Press, Cambridge. *Cepa* × *Allium roylei* plants. Acta Biol Cracov Bot. 54(2), 25-31.
7. Chuanen Z, Guangmin X. Embryo rescue technology of distant hybrids in wheat. J Triticeae Crops. 2005;25(3):88-92.
8. Chuda A, Adamus A. Hybridization and molecular characterization of F1 Allium; c2012.
9. Dantas ACM, Boneti JI, Nodari RO, Guerra MP. Embryo rescue from interspecific crosses in apple rootstocks. Pesq Agrop Bras. 2006;41(6):969-973.
10. Debbarama C, Khanna VK, Tyagi W, *et al*. Wide hybridization and embryo-rescue for crop Improvement in *Capsicum*. Agrotechnology. 2013;S11:003. DOI: 10.4172/2168–9881S11–003.
11. Dhiman M, Sharma V, Moitra S. Somatic embryogenesis and plant regeneration in *Ephedra foliata* (Boiss.): a non coniferous Gymnosperm. Plant Tiss Cult Biotech. 2010;20(2):133-143.
12. Dobrowolska I, Majchrzak O, Baldwin TC, Kurczynska EU. Differences in protodermal cell wall structure in zygotic and somatic embryos of *Daucus carota* (L.) cultured on solid and in Dordrecht; c2012. p. 1-16.
13. Elite Spruce families: effect of storage time on somatic embryogenesis initiation. BMC Proc embryos of rose via *Agrobacterium tumefaciens*. Plant Physiol Biochem; 40:453-459.
14. Fathi H, Jahani. Review of embryo culture in fruit trees. Ann Biol Res. 2012;3(9):4276-4281.
15. Godbole S, Sood A, Thakur R, *et al*. Somatic embryogenesis and it's conversion into plantlets in a multipurpose bamboo, *Dendrocalamus hamiltonii* Nees *et* Arn. Ex Munro. Curr Sci; c2002.
16. Hu C, Wang P. Embryo culture: technique and applications. In: Evans DA, Sharp WR; c1986.
17. Kamle M, Bajpai A, Chandra R, *et al*. Somatic embryogenesis for crop improvement. GERF Bull Biosci. 2011;2(1):54-59.
18. Khan KH. Gene transfer technologies in plants: Roles in improving crops. Recent Res Sci; c2009.
19. Krikorian AD, Simola LK. Totipotency, somatic embryogenesis, and Harry Waris; c1999, 1893.
20. Li X, Krasnyanski SF, Korban SS. Optimization of the *uidA* gene transfer into somatic liquid media. Protoplas. 2002;249(1):117-129.
21. Mandal AKA, Gupta SD. Direct somatic embryogenesis of safflower: A scanning electron microscopic study. Curr Sci. 2002;83:1138-1140.
22. Mathew MM, Philip VJ. Somatic embryogenesis versus zygotic embryogenesis in *Ensete* Newton RJ (eds) Somatic Embryogenesis in Woody Plants, vol 6. Kluwer Academic Publishers; c2003.
23. Palada-Nicolaua M, Hausman JF. Comparison between somatic and zygotic embryo development in *Quercus robur* L. Plant Biosyst. 2001;135(1):47-55.
24. Pennazio S. The discovery of the chemical nature of the plant hormone auxin. Riv Biol; c2002.
25. Pintos B, Bueno MA, Cuenca B, Manzanera JA. Synthetic seed production from encapsulated somatic embryos of cork oak (*Quercus suber* L.) and automated growth monitoring. Plant Cell Tissue Org Cult. 2008;95(2):217-225.
26. Raghavan V. Embryogenesis in angiosperms: A developmental and experimental study; c1986.
27. Ramon M, Hanneman RE Jr Introgression of resistance to late blight (*Phytophthora infestense*) from *Solanum pinnatisectum* into *S. tuberosum* using embryo rescue and double pollination. Euphytica. 2002;127:421-435.
28. Ravi D, Anand P. Production and applications of artifi

cial seeds: a review. Int Res J Biol Sci. 2012;1(5):74-78.
29. Reinert J. Ueber die kontrolle der morphogenese und die induktion von adventive embryon en an gewebekulturen aus karotten. Planta. 1959;53:318-333.
30. Simola L. Harry Waris, A Pioneer in Somatic Embryogenesis. In: Jain SM, Gupta PK; c2000.
31. Slater A, Scott NW, Fowler MR. Plant biotechnology. Oxford University Press, Oxford. species. Afr J Agric. Res. 2003;8(27):3590-3597.
32. Steward FC, Mapes MO, Mears K. Growth and organized development of cultured cells. Superbum. Plant Cell Tiss Org Cult. 1958;72(3):267-275.
33. Takeda, Matsuoka. Somatic (asexual) procedures (haploids, protoplasts, cell selection) and their applications. In: Altman A, Hasegawa PM (eds) Plant biotechnology and agriculture: Prospects for the 21st century. Academic, Oxford; Technol. 2008;1(3):116-123, 139-162.
34. Thakur AK, Chauhan DK, Parmar N, Verma V. Role of genetic engineering in horticultural crop improvement – a review. Agr Rev. 2012;33(3):248-255.
35. Thomas BR, Pratt D. Effi cient hybridization between *Lycopersicon esculentum* tomatoes; c1981.
36. Vasil IK. A history of plant biotechnology: From the cell theory of Schleiden and Schwann to biotech crops. Plant Cell Rep. 2008;27:1423-1440.