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Invitro Techniques in Major Vegetable Crops

Rashmi H, Gandikota Brahmani and Yoshita Sri Krishna Shastri
Department of Vegetable Science, University of Horticultural Sciences Bagalkot, 587 103 Karnataka, India.
*Corresponding Author: rashmihadapad75@gmail.com

Abstract

In vitro propagation techniques have become indispensable tools in modern agriculture, offering unprecedented advantages for the improvement of vegetable crops. This abstract provides a comprehensive overview of the current state of in vitro propagation methods and their applications in enhancing the genetic diversity, selection and multiplication of vegetable crops. Various techniques, including embryo rescue, anther and ovary culture, protoplast fusion, and the transgenic approach are explored in the context of their contributions to breeding resilient and high-yielding vegetable varieties. The efficiency of in vitro culture suspensions, regenerative callus cultures and diverse forms of embryos, such as zygotic and somatic embryos are highlighted, particularly for plants propagated from vegetative parts. Additionally, the potential for induced variation through irradiation, mutagenesis of inserted genes in transgenic plant and the elimination of unwanted genes in cybrids are discussed as promising strategies to expand the genetic repertoire of vegetable crops. This abstract sets the stage for a detailed exploration of the diverse applications and future prospects of in vitro propagation techniques in the dynamic field of vegetable crop production and breeding.

Introduction

In the past decade, plant tissue and cell culture in-vitro techniques have advanced conventional plant breeding. These techniques, such as embryo rescue, anther and ovary culture, and protoplast fusion, enhance efficiency in obtaining, selecting, and multiplying desired genotypes, especially for plants propagated from vegetative parts. In-vitro culture methods, including regenerative callus cultures, zygotic and somatic embryos, fused protoplasts, and cybrids, can be irradiated to induce variation. This complements soma clonal and proto clonal variation. Mutations in inserted genes in transgenic plants may be achievable, leading to new alleles transferable through gene insertion or conventional back-crossing. Eliminating linkage drag in cybrids with unwanted genes is possible through irradiation of protoplasts or fusion products. For seed-propagated crops like pepper, haploid production from anther culture, followed by doubled haploid production, is feasible (Reynolds et al., 1996). Irradiation of parental plants or microspores at anthesis induces variation.

Various classes of vegetables based on planting material and sowing method (Source: Thamburaj and Singh, 2000)

Crop Propagation method

- Planting bulbs and rhizomes: Onion, garlic, ginger and turmeric
- Planting tubers and corms: Potato, yam, taro
- Planting vines and cuttings: Pointed gourd, sweet potato and cassava
- Divisions of crown/suckers: Asparagus, rhubarb and globe artichoke

Methods of *in vitro culture*

1. Micro propagation

Tissue culture, or micropropagation, rapidly multiplies plants in controlled environments and is widely used for various vegetables (Murashige 1978, Reynolds 1986, Seckinger 1991, & Krikorian 1994). However, it's not commonly integrated into the production cycle for top vegetable crops (Thorpe 1990), excluding some exceptions like virus-free micro-tubers. Traditional tropical root and tuber crops, such as cassava and sweet potatoes, play a vital role in advancing agriculture with their pathogen-free material and increased yields.

2. Soma clonal variation

Soma clonal variants relate to all of the genetic variation seen in *in vitro* grown cells. Variations could be cytogenetic as well as genetic. Soma clones are the plants created from these types of cells. Some authors referred to cultures made from callus and protoplasts

as proto clones and calli clones, respectively. In soma clonal variation" to describe variances carried on by cell cultures or variability produced by a tissue culture (Larkin *et al*., 1981).

3. Production of disease-free plants

In many agricultural plants, the apical dome shape with the first and second primordial leaves (sized between 100 and 500 m) is the best planting material (tissue) for growing plants free of viruses. The reasons for being virus free are:

- Movement of virus takes place plant body through the vascular system which is totally absent in meristematic cells. And one other method that is cell-to-cell movement of the virus by plasmodesmata
- Virus multiplication does not allow due to High metabolic activity through actively dividing meristem cells.
- Virus multiplication is inhibited due to high endogenous auxin level in shoots

4. Production of haploids

Three methods, anther culture, pollen culture, and ovary culture, are employed for mass production of haploid plants.

A. Anther Culture: Developing anthers are surgically removed, cultured on a nutrient medium, and produce haploid plantlets through organogenesis or embryogenesis.

B. Pollen Culture: In vitro technique involving aseptic removal of pollen grains from anthers, followed by culture on a nutrient medium.

C. Ovary Culture: In-vitro development of plants from unfertilized cells of female gametophyte ovaries, primarily used for early embryo development and studying fruit physiology.

D. Ovule Culture: Experimental approach where ovules are aseptically separated from the ovary and cultivated on chemically specified feeding media under controlled conditions.

5. Endosperm culture

A. Triploid plants are un favourable for plants whose seeds are used economically because they produce sterile seeds. However, of triploid plants is extremely useful when seed lessness is used to enhance the quality of vegetables like cucumber and watermelon.

B. The endosperm is the primary nutritive tissue for the embryo in angiosperms. The endosperm is the result of double fertilization, in which one male gamete fertilizes the egg to form a zygote and the other fuses with secondary nuclei to form triploid endosperm.

C. Production of triploid plants through endosperm (matured and Immature) is used for initiation of culture.

D. Triploid plants grow faster than diploid plants in terms of vegetative growth. As a result, triploids can be used in plants where the vegetative parts are economically valuable.

6. *In vitro* fertilization

A. *In vitro* fertilization/*in vitro* pollination occurs when pollen is directly applied to ovules cultured with or without placental tissues, or to the stigma of *in vitro* cultured ovaries.

B. Ovaries from emasculated flowers are removed and cultured either intact or with the ovarian wall removed to reveal the placenta, 1-2 days after anthesis. You might even cultivate the entire placenta or parts of it that contain ovules.

7. Protoplast fusion (Somatic hybridization)

The technique of somatic hybridization involves four stages

1. Selection of somatic hybrid cells
2. Culture of the hybrid cells and regeneration of hybrid plants from them.
3. Fusion of the protoplast of desired species or varieties
4. Isolation of protoplast

Application of *in vitro* culture methods in solanaceous and cucurbitaceous vegetable crops

A) Tomato

Researchers improved in vitro tomato micropropagation by enhancing axillary shoot proliferation in nodal microcuttings (Soressi et al., 2007). They induced critical proliferation of additional

axillary shoots using various media with or without plant growth regulators like indole-3-acetic acid (IAA) and zeatin, potentially replacing seed reproduction. A cost-effective micropropagation procedure for the tomato male sterile line (Shalimar FMS-1) was established using a Murashige and Skoog (MS) medium supplemented with calcium D-pantothenate, calcium chloride, and gibberellic acid (Hussain et al., 2021). Protoplast culture contributed to tomato crop improvement by extracting protoplasts from Fusarium oxysporum race 2-sensitive UC82 cotyledons (Shahin et al., 1986).

B) Pepper

In chilli cultivation, another culture in Capsicum annum is crucial for genetic variation assessment, generating fully homozygous plants and viable haploids, notably in developing double haploid lines with enhanced resistance to pests and diseases. These in vitro techniques are essential for advancing the understanding and cultivation of chilli varieties in both biology and agriculture.

C) Brinjal

In the realm of in vitro propagation for brinjal cultivation, somatic embryogenesis and vitro organogenesis prove effective for rapid plant rejuvenation, with studies showcasing successful regeneration of haploid plants from eggplant microspores. Utilizing in vitro androgenesis, eggplant breeders swiftly generate fixed lines and develop marketable F1 hybrids. Protoplast fusion in somatic hybridization integrates agronomically significant traits from wild relatives, enhancing hybrid fertility and overcoming sexual barriers, aiming to expedite the creation of improved eggplant varieties resistant to various illnesses and pathogens for enhanced resilience and productivity.

D) Potato

Tissue culture preserves endangered potato embryos, overcomes interspecific incompatibility, and introduces resistance to potato leaf roll virus. Successful hybridization demonstrates how embryo culture aids in recovering wide hybrids. Protoplast fusion introduces pest and disease resistance but often yields undersized tubers, requiring multiple backcrossing rounds for agricultural suitability. Somatic cell selection plays a crucial role in potato improvement, leading to the creation of disease-resistant clones, showcasing diverse applications of in vitro techniques (Watanabe et al., 1995; Chavez et al., 1988; Eijlander et al., 1994; Bradshaw et al., 2006; Tek et al., 2004; Barrell et al., 2013; Wilson et al., 2010).

E) Cucurbits

Plant tissue culture techniques are crucial for propagating the Cucurbitaceae family for Ayurvedic medicine and the processing industry, with studies exploring zygotic embryogenesis in Cucumis species (Skálová et al., 2004). In vivo and in vitro methods provide alternatives for growing mature cucumber ovules and pollen grains (Gajdová et al., 2004). Protoplast isolation and growth, influenced by various factors, have been extensively studied, including mesophyll protoplast cultures used for UV-C radiation effects and asymmetric hybridization (Navrátilová et al., 2008). Somatic hybridization through protoplast fusion between various Cucumis species contributes to the development of disease-resistant rootstocks, significantly advancing Cucurbitaceae cultivation and research (AYUSH).

Conclusion

In vitro culture methods are crucial for enhancing vegetable crops by producing competitive and marketable varieties. They facilitate the development of nutritious vegetables essential for maintaining good health, providing vital vitamins, minerals, antioxidants, and dietary fibers. These techniques enable precise control over cultivation, resulting in the rapid production of disease-resistant, high-yielding, and genetically stable plants. Their efficiency has been demonstrated in inducing genetic variations, creating disease-resistant lines, and accelerating breeding programs. In summary, the strategic utilization of in vitro propagation is essential for advancing vegetable crop research, ensuring sustainable agriculture, and meeting the diverse nutritional needs of a growing global population.

Future scope of *in vitro culture*

In vitro culture techniques revolutionize agriculture by enabling rapid clonal propagation and genetic modification of vegetable crops for desirable traits like disease resistance and increased yield. These

methods also aid in conserving plant germplasm, especially for rare species, thereby contributing to biodiversity preservation. Moreover, they are crucial for producing high-quality hybrid seeds and developing stress-tolerant varieties necessary for sustainable crop production in the face of climate change. Integrating in vitro culture into precision agriculture systems promises to customize cultivation practices, optimizing resource use for a resilient and productive agricultural future.

References

Barrell, P. J., Meiyalaghan, S., Jacobs, J. M., & Conner, A. J. (2013). Applications of biotechnology and genomics in potato improvement. *Plant Biotechnology Journal*, *11*(8), 907-920.

Bradshaw, J. E., Bryan, G. J., & Ramsay, G. (2006). Genetic resources (including wild and cultivated Solanum species) and progress in their utilisation in potato breeding. *Potato Research*, *49*, 49-65.

Chavez, R., Brown, C. R., & Iwanaga, M. (1988). Application of interspecific sesquiploidy to introgression of PLRV resistance from non-tuber-bearing Solanum tuberosum.

Eijlander, R., & Stiekema, W. J. (1994). Biological containment of potato (Solanum tuberosum): outcrossing to the related wild species black nightshade (Solanum nigrum) and bittersweet (Solanum dulcamara). *Sexual Plant Reproduction*, *7*, 29-40.

Gajdová, J., Lebeda, A., & Navrátilová, B. (2004, July). Protoplast cultures of Cucumis and Cucurbita spp. In *Progress in cucurbit genetics and breeding research. Proceedings of Cucurbitaceae* (pp. 441-454).

Hussain, S. M., Hussain, K., Malik, A. A., Hussaini, A. M., Farwah, S., Rashid, M., & Ahmad, R. (2021). Development of a novel in-vitro protocol for micro propagation of tomato male sterile line (Shalimar FMS-1) of Kashmir Valley India. *Acta Scientific Agriculture*, *5*(4).

Larkin, P. J., & Scowcroft, W. R. (1981). Somaclonal variation—a novel source of variability from cell cultures for plant improvement. *Theoretical and applied genetics*, *60*, 197-214.

Murashige, T. (1987). The impact of plant tissue culture on agriculture. *Frontiers of plant tissue culture*, 15-26.

Navrátilová, A., Koblížková, A., & Macas, J. (2008). Survey of extrachromosomal circular DNA derived from plant satellite repeats. *BMC plant biology*, *8*, 1-13.

Reynolds, J. F. (1986). Regeneration in vegetable species. *Cell culture and somatic cell genetics of plants*, *3*, 151-178.

San Noeum, L. H. (1976). Haploides d, Hordeum vulgare L. par culture in vitro non fecondes. *Ann. Amelior Plantes*, *26*, 751-754.

Seckinger, G. R. (1991). Micropropagation of vegetable crop species. In *Micropropagation: Technology and Application* (pp. 265-284). Dordrecht: Springer Netherlands.

Shahin, E. A., & Spivey, R. (1986). A single dominant gene for Fusarium wilt resistance in protoplast-derived tomato plants. *Theoretical and Applied Genetics*, *73*, 164-169.

Skálová, D., Lebeda, A., & Navrátilová, B. (2004, July). Embryo and ovule cultures in Cucumis species and their utilization in interspecific hybridization. In *Progress in cucurbit genetics and breeding research. Proceedings of Cucurbitaceae* (pp. 415-430).

Soressi, G. P., Cammareri, G., & Picarella, M. E. (2007, September). Improvement of in vitro vegetative propagation technique in tomato (Solanum lycopersicum). In *III International Symposium on Acclimatization and Establishment of Micropropagated Plants 812* (pp. 283-288).

Tek, A. L., Stevenson, W. R., Helgeson, J. P., & Jiang, J. (2004). Transfer of tuber soft rot and early blight resistances from Solanum brevidens into cultivated potato. Theoretical and Applied Genetics, 109, 249-254.

Thamburaj, P & Singh, A. (2001). A Textbook of Vegetables, Tuber Crops and Spices. Indian agriculture research institute

Thorpe, T. A. (1990). The current status of plant tissue culture. In Developments in crop science (Vol. 19, pp. 1-33). Elsevier.

Valkov, V. T., Gargano, D., Manna, C., Formisano, G., Dix, P. J., Gray, J. C., ... & Cardi, T. (2011). High efficiency plastid transformation in potato and regulation of transgene expression in leaves and tubers by alternative 5′ and 3′ regulatory sequences. *Transgenic research*, *20*, 137-151.

Watanabe, K. N., Orrillo, M., Vega, S., Valkonen, J. P. T., Pehu, E., Hurtado, A., & Tanksley, S. D. (1995). Overcoming crossing barriers between nontuber-bearing and tuber-bearing Solanum species: towards potato germplasm enhancement with a broad spectrum of solanaceous genetic resources. *Genome*, *38*(1), 27-35.

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