The Pharma Innovation Journal 2022; 11(12): 287-293

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 287-293

www.thepharmajournal.com
Received: 16-09-2022
Accepted: 24-10-2022

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In vitro culture methods and molecular approaches for crop improvement in solanaceous and cucurbitaceous vegetables

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Abstract
In vitro culture methods play important role in crop improvement in agricultural as well as horticultural crops through different *In vitro* culture methods like micro propagation, Soma clonal variation, Anther culture, endosperm culture, zygotic embryo culture and protoplast fusion etc. Somatic hybridization in solanaceous vegetables like potato has provided few new opportunities for the introgression of novel sources of many diseases and pest resistance into the cultivated potato. Embryo culture has so many potential uses ranging from overcoming seed dormancy to the facilitation of inter-specific hybridization. Some molecular approaches like genetic engineering and genome editing tools improved the generation of tomato and overcome the restrictions in important traits. Molecular techniques have been heavily utilized in the genetic development of agronomics such as biotic and abiotic stress tolerance and fruit quality (self-life) in tomato due to changing global climate and market competitiveness. Triploid plants are unfavorable for plants whose seeds are used economically because they produce sterile seeds. However, triploid plants is extremely useful when seed lessness is used to enhance the quality of vegetables like cucumber and watermelon.

Keywords: *In vitro* Culture, solanaceous, cucurbitaceous, micro propagation, molecular, soma clonal variation, transgenic

Introduction
Plant biotechnology is a relatively new science, although it has developed quickly during the last 20 years. In order to modify and utilize biological processes for the benefit of humans, biotechnology relies on such research and encourages the development of such technologies. Strategies that result in a far greater knowledge of the basic functions of life. As a result, it has developed the fastest and most quickly in the history of technology. Biotechnology is described as a process that creates goods or services that are beneficial to people by using live microbes, animal or plant cells, or their subcellular components. Thus, biotechnology creates new goods, alters current products, enhances plant and animal genotypes, and genetically changes microbes to make them useful for certain purposes. Modern biotechnology has opened up new possibilities for developing vegetable crops. The word "biotechnology" refers to a range of technologies that utilize biological agents or processes to develop fresh and practical goods and methods (James *et al.*, 2011) [2]. The goal of creating new vegetable plant types with high and reliable yields, superior quality, and pest and stress tolerance is becoming increasingly important (Schleiden *et al*., 1838 & Schwann *et al*., 1839) [3, 4]. Molecular markers clearly show the DNA-level polymorphism and genetic variation used to create high-resolution genetic data genetic linkage between markers as shown on maps and significant crop characteristics have been utilized for enhancing vegetable breeding (Virchow *et al*., 1858 & Edwards *et al*., 1987) [5, 6]. The age of plant biotechnology, however, is to have started in the early 1980s with the mearstone recounts the creation of transgenic plants. *Agrobacterium tumefaciens*-using plants (Paterson *et al*., 1988) [7]. Nathan and Smith's discovery of restriction enzymes and Kary Mullis and his team's invention of the polymerase chain reaction (PCR) have made it possible to learn about the DNA makeup of organisms and acquire their so-called genetic fingerprints. Currently, these investigations are regularly carried out by separating DNA fragments (using gel electrophoresis) originating from selective digestion of DNA using restriction enzymes or from selective amplification of DNA using

PCR. Polymorphic markers are DNA fragments that cause distinct gel patterns in various samples or people. Isozyme markers are among the molecular markers that have previously demonstrated their use in genetic research and crop breeding programs. Additionally, they contributed the fundamental ideas that aided in the creation of DNA-based molecular markers. There are two common methods used by all DNA-based marker techniques: Southern hybridization and polymerase chain reaction (PCR). (Moose *et al.*, 2008) [8]

Methods of *in vitro culture*

1. Micro propagation

Tissue culture, another name for micro propagation, is a technique used to artificially propagate plants. It is the quickest artificial vegetative multiplication of plants in controlled environment condition. The of various types of vegetables can also be propagated using micro propagation. (Murashige 1978, Reynolds 1986, Seckinger 1991 & Krikorian 1994) [10, 11, 12, 13]. Virus-free micro-tubers and *in vitro* mini-tubers are the exceptions. Additionally, little planting material is produced on a large scale. For top vegetable crops, reform able techniques based on accidental budding, shoot tip culture, and or neither direct nor indirect somatic embryogenesis are available. These include Solanaceous crops, cucurbitaceous crops, Cole crops and leguminous crops. Since the majority of these veggies are grown as transplants, their cost has increased as a result. The micro propagation technology is not a part of the production cycle is astounding (Thorpe 1990) [14]. Traditional tropical root and tuber crops that are vegetatively propagated, such as cassava, aroids, sweet potatoes, and yams, aid in advancement since they are made with some pathogen-free material and help in producing larger yields. The development of synthetic seed technology and the progress of seed-based male sterile hybrid vegetative propagation will undoubtedly improve the production and use of micro propagated vegetative planting material.

Stages of the micro propagation are given below

Generally, micro propagation is completed with three stages (I, II, and III). Some authors include two additional stages (stages 0 and IV) for complete illustration.

Stage 0: This is the fundamental stage of plant micro propagation and involves selecting and growing stock plants for around three months under controlled circumstances.

Stage I: In this stage, a culture's conception and establishment in a workable medium are achieved. Selecting appropriate explants is important. The organs, shoot tips, and axillary buds are the explants that are most frequently used. Before use, the surface of the harvested explant is cleaned and disinfected.

Stage II: In a defined culture media, this stage is when considerable micro propagation movement occurs. The majority of Stage II consists of fast incipient organism organization from the explant or augmentation of shoots. A development chamber with a temperature range of 20 to 24 °C, a light intensity of 2000 to 4000 lux, and a lighting period of 16 hours or so is used.

Stage III: During this stage, shootings are switched to a mode that allows for quick advancement into shoots. The shoots are occasionally genuinely put in the ground to form roots. Establishing shoots *in vitro* while caring for numerous species is popular.

Stage IV: Plantlets are establishing themselves in the soil during this period. Moving the stage III plantlets from the research facility to the nursery's ground completes the process. Some plant species bypass stage III and instead grow unestablished stage II branches in containers or with the optimum fertilizer mix.

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 protocones 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) [15].

Application of soma clonal variations

- Soma clonal variation is helpful in increase production of secondary metabolites
- Soma clonal variation is useful in breeding of tree species.
- Soma clonal variation is useful in crop improvement which resistant to several disease and pest.
- Soma clonal variation is useful in creation of genetic variations.

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

Another culture, pollen culture and ovary culture, these all methods used in production of haploid plants in large numbers.

A. Another culture: This a method whereby the developing anthers are surgically removed from unopened flower buds at a precise and crucial stage and cultured on a nutrient medium where the microspores develop into callus tissue or embryoids that give rise to haploid plantlets either through organogenesis or embryogenesis.

B. Pollen culture: Pollen culture and microspore culture is an *in vitro* technique through which the pollen grains are squeezed out aseptically from the anther. After that pollen as well as microspores are cultured on nutrient medium.

C. Ovary culture: The *in-vitro* development of plants from unfertilized cells of female gametophyte ovaries. It was first reported in barley (Noeum *et al*., 1976) [16]. It is mostly used in early development of embryo with different aspects such as fruit physiology including

respiration and maturation.

D. Ovule culture: Is an excellent experimental approach in which ovules are aseptically separated from the ovary and cultivated under controlled circumstances on chemically specified feeding media.

5. Endosperm culture

A. Triploid plants are unfavorable 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)

- Somatic hybridization is the process of producing hybrid plants by fusing protoplasts from two different plant species as well as varieties. Such types of hybrids are referred to as somatic hybrids.
- Higher plant protoplasts have the capacity to absorb foreign DNA, cellular components, bacterial, or viral particles. Due to the distinctive characteristics of protoplasts and the totipotent nature of plant cells, a new field of basic and applied research in experimental biology and somatic cell genetics has been opened up.

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

Tomato is the most sensitive vegetable crop to biotechnology because it has comprehensive genetic and cytogenetic mapping, many genetic markers, facile transformation and regeneration of plants from explants from a variety of tissues, and easy inter and intra-species hybridization. Several studies on tomato have been conducted in order to increase tomato quality as well as resistance to illnesses and pests. Some notable breakthroughs include gene transfer via Agrobacterium, generate fusion to create somatic hybrids and embryo rescue to transmit desirable features from foreign species. There have been several types of research on worked on tomato micro propagation in order to increase the number of axillary shoots per nodal micro cutting. (Soressi *et al*., 2007) [17]. the improved *in vitro* micro propagation technique in tomato by the using of various media, with or without PGR and discovered that both IAA and Zeatin were able to gradually induce the proliferation of 2 extra axillary shoots. The findings present a critical step for improving micro propagation efficiency with a view to replacing or avoiding seed reproduction in the tomato. In their efforts to create a novel, effective and affordable procedure for *in-vitro* micro propagation of the tomato male sterile line (Shalimar FMS-1) (Hussain *et al*., 2021) [18]. When applied MS medium supplemented with calcium D pantothenate 2 mg l-1, calcium chloride 440 mg l-1, and gibberellic acid 0.4 mg l-1 and recorded maximum root and shoot regeneration.

Another culture has been used to produce haploid tomato plants. Protoplast culture has also been used in different studies to improve tomato crops. From the cotyledons of UC82, a strain sensitive to *Fusarium oxysporum* race 2 and cultivated on a medium containing the non-specific toxin fusaric acid extracted protoplast (Shahin *et al*., 1986) [19]. In case of tomato embryo culture has also been performed when embryo rescue is used to establish backcross progenies from two hexaploid somatic hybrids with diploid tomato varieties like Moneymaker and Pusa Ruby (Patil *et al*., 1993) [20].

Molecular techniques including metabolic genetic engineering and genome editing tools have been capable of overcoming the limits and have produced the development of tomatoes with enhanced, economically significant features. The molecular techniques have been heavily utilized in the genetic development of agronomic (such as biotic and abiotic stress tolerance) and fruit quality (such as antioxidant enrichment and prolonging of shelf-life) features in tomato due to ongoing global climate change and market competitiveness. The number of accessible molecular markers for tomatoes is relatively abundant. The majority of the more than 1000 RFLP markers now available have been located on the 12th chromosomes on tomato. Other molecular marker types, such as SSRs, CAPS, RAPDs, SCARs, RGAs, and AFLPs, have been generated and mapped in tomato in addition to RFLPs and ESTs. The high-density tomato genetic map has been mapped with a minimum of 148 SSR markers and 77 CAPS. Much earlier than in many other crop species, the tagging and mapping of single-gene features in tomatoes, including several morphological, physiological, and disease resistance traits, began in the 1930s. Beginning in the 1970s, single-gene features were marked using molecular and biochemical markers (Foolad *et al*., 2007) [21]. Recent developments in the crop improvement of tomatoes include research on the mapping of genes for resistance against biotic and abiotic stress. MAS and salt tolerance of beneficial traits in tomato crop improvement.

B. Pepper

It is a major vegetable as well as spice crop. It is a member of the Solanaceae family and a plant that is significant commercially. There are several species of chilli, but only

five are domesticated. A crucial technique in biology and agriculture, the assessment of the degree of genetic variation within a species is now required to maintain, manage and develop the many species of chilli. Morphological and molecular markers are two of the primary criteria employed for that aim. Many of the works that have been done on chilli *in vitro* culture have been done on another culture. In *Capsicum annum,* Anther culture is most useful for producing fully homozygous plants and viable haploids. These are also employed in the development of double haploid lines that exhibit pest and disease resistance. With the use of molecular biology tools, modern plant breeding selects or, in the case of genetic modification, introduces desired features into plants. It has been found that the chilli plant exhibits a saturated (RFLP) map. The map has a total coverage of 720 cm and 192 chilli genomic DNA clones with 19 linkage groupings. *Agrobacterium tumefactions* has generally been utilized as a genetic transformation vector. The sole vector for chilli transformation to yet is Agrobacterium (Madala *et al*., 2020) [22].

C. Brinjal

Several Asian and African countries produce the brinjal, which is significant commercially grown vegetable. Brinjal is susceptible to a variety of biotic and abiotic stresses, making the insertion of resistance genes for improved production and fruit quality one of the key issues with traditional breeding and biotechnology. Numerous studies have discovered that somatic embryogenesis and vitro organogenesis may swiftly rejuvenate plants in Aubergines. Successful regeneration of haploid plants from cultured eggplant microspores has also been accomplished. *In vitro* androgenesis is being used more often by eggplant breeders to swiftly create fixed lines from heterozygous material and create marketable F1 hybrids. Somatic hybridization experiments have been carried out through protoplast fusion to encourage the integration of agronomic ally significant traits from wild relatives into the cultivated eggplant. This is done in order to get around potential sexual barriers or increase the fertility of hybrids typically obtained through traditional breeding techniques. The majority of the illnesses and pathogens to which they were resistant included bacterial and fungal wilts, worms, mites and fruit borers. By analyzing the chloroplast DNA of eggplants, phylogenetic maps and DNA markers were created. The classification of eggplants and their wild cousins also included the use of RAPDs, AFLP studies, isoenzymes, and seed storage protein. Genome engineering uses a range of methods to change, remove, swap out, or insert special genomic sequences into a living organism's genome at a specific location. For effective built-in fruit and shoot borer control, Bt brinjal is a transgenic brinjal that has had the cry1Ac gene introgressed under the direction of a high-level expression amplified CaMV 35S promoter. The npt II (neomycin phosphotransferase) gene, derived from the prokaryotic transposon Tn5, and the aad gene, isolated from the transposon Tn7 gene, are the selectable marker genes for the insecticidal protein derived from the soil bacterium *Bacillus thuringiensis*, derived by enhanced CaMV 35S (promoter).

D. Potato

The majority of people's staple meal is the potato, making it one of the most significant vegetables in the world. Applications of biotechnology for agricultural enhancement have a long history. Tissue culture has been used to rescue embryos that are about to be aborted in potatoes (Watanabe *et al.,* 1995). Additionally, useful for avoiding various kinds of interspecific incompatibility is embryo culture. For instance, the introduction of potato leaf roll virus resistance into *Solanum tuberosum* by the use of embryo culture (Chavez *et al.,* 1988) [24]. The successful hybridization of the disomic hexaploid *Solanum nigrum* (black nightshade) as a female parent with tetraploid potato serves as an extreme example of how embryo culture is used to help recover wide hybrids (Eijlander *et al*., 1994) [25].Protoplast fusion has opened up a few new doors for the introduction of fresh sources of pest and disease resistance into cultivated potatoes from germplasm of taxa possessing sexual reproductive barriers with potato. Through somatic fusion of potato protoplasts with those of wild relatives like *Solanum palustre* (formerly *S. brevidens*) and *Solanum bulbocastanum*, resistance to diseases brought on by the leaf roll virus, potato virus Y, early and late blight, soft rot, Columbia root-knot nematode and Colorado potato beetle has been introduced (Bradshaw *et al*., 2006) [26]. Although these hybrids are excellent sources of resistance to abiotic stress and infections, they frequently yield undersized, malformed tubers that are not appropriate for agricultural production. For these plants to be useful in agriculture, several rounds of backcrossing are necessary (Tek *et al*., 2004) [27]. Somatic cell selection has been used to improvement in potatoes, mostly through selecting for disease resistance in the crop. This process required exposing vast numbers of grown potato cells to path toxins or pathogen culture filtrates, then picking out the unusual survivors (Barrell *et al*., 2013) [28]. The creation of potato clones which resistant to the scab disease is one example of how somatic cell selection is used to enhance potatoes (Wilson *et al.,* 2010) [29]. A successful transgenic potato production employing vir gene-mediated gene transfer from Rhizobium species and *Ensifer adhaerens* has recently been described (Wendt *et al*., 2011) [30]. Potato transformation has also been accomplished through direct DNA absorption (Valkov *et al*., 2011) [31]. The preferred method, however, is Agrobacterium-mediated gene transfer, which is regularly carried out in labs all over the world (Wendt *et al*., 2012) [32]. The transformation of potatoes using a variety of Bt-based transgenes has shown to be a very effective method for reducing potato insect pests. Using 1 bulked segregant analysis and (RAPD) markers, DNA sequences associated to important gene features of interest in potatoes have been found (Jacobs *et al*., 1996). However, more trusted markers like SSR have replaced RAPD markers because of their limited repeatability (Simple Sequence Repeats or microsatellites). The researchers have used them to locate SSR markers on linkage groups using segregating mapping populations as well as to identify and fingerprint potato germplasm accessions and cultivars (Reid *et al*., 2011) [34]. Only recently have studies utilizing Diversity Array Technology markers been reported in the potato (Śliwka *et al*., 2012) [35] employed DArT markers to identify the late blight resistance gene Rpi-mch1.

E. Cucurbits

The Cucurbitaceae family plays a significant role in the preparation as a source of vegetables and medicinal plant. Ayurvedic medicine and the processing industry both heavily rely on the qualitative and quantitative improvement of this

family, which includes hundreds of edible species (AYUSH). Since a few decades ago, family Cucurbitaceae have been propagated utilizing a variety of explants and approaches using plant tissue culture techniques. Over the past 20 years, *Cucumis species'* zygotic embryogenesis *in vitro* has been researched and employed for *in vivo* intraspecific and interspecific hybridization (Skálová *et al*., 2004) [36]. For experiments, cucumber (*C. sativus*), muskmelon *(C. melo*) and a few wild species of Cucumis (*C. metuliferus*, *C. zeyheri*, and *C. anguria*) have been employed. *In vivo* pollination and fertilization with subsequent *in vitro* embryogenesis can take the place of *in vivo* pollination and *in vitro* embryo rescue. On specific media, mature cucumber ovules and pollen grains from the cucumber and melon have been separated and grown together. Numerous factors affect how well protoplasts may be isolated and grown, including genotype, explant type, protoplast density, viability, cultivation technique and medium composition (Gajdová *et al*., 2004) [37]. Mesophyll protoplast cultures from cucumbers are used to examine how UV-C radiation affects protoplast physiology. Asymmetric hybridization can use this technique (Navrátilová *et al*., 2008) [38]. Somatic hybridization through protoplast fusion between *Cucumis sativus*, *C. metuliferus*, *C. melo*, *C. anguria*, *C. melo*, *a*nd *C. myriocarpus* is documented.

Advances in Agrobacterium-mediated transformation have allowed for the introduction of foreign genes into watermelons, enhancing their quality and yield beyond what is feasible through conventional breeding (Maligeppagol *et al*., 2013) [39]. Watermelons are resistant to pathogens and stress may be created quickly via genetic engineering. Furthermore, by introducing enzymes that control the amount of carbohydrates in the watermelon genome, the sweetness of the fruit may be controlled (Choi *et al*., 1994) [40]. Water melon undergoes genetic modification in order to produce transgenic plants by Agrobacterium-mediated gene transfer methodology (Ellul *et al*., 2003) [41]. The transformation process was used to create genetically modified watermelon plants that expressed the salt tolerance-related *Saccharomyces cerevisiae* HAL1 gene. Grafting is typically used to create disease-resistant rootstocks for Cucurbitaceae plants like cucumber, watermelon and melons. The transgenic watermelon plants exhibit greater viral tolerance by over expressing the genes for the virus coat protein. The majority of viral infections that affect cucurbits are poty viruses such as the Zuchine Yellow Mosaic Virus (ZYMV), Watermelon Mosaic Virus II (WMVII) and Papaya Ring spot Virus (PRSV) (Ling 1991). Viral resistance has been developed using genetically modified coat proteins, although the resistance generally only affects homologous or closely similar viruses.

The two genes such as UGT and ACB, were utilized in cucumber to increase yield. The UGT gene, which was isolated from *Zea mays*, encodes an IAA glucose synthase called UDPG transferase. When corn endosperm is maturing, this gene is expressed which leads to an increase in the stored form of IAA accumulation in the kernels and an increase in yield. Cucumbers were used to produce the HBsAg gene for hepatitis B, is one of the most dangerous viral infections. It is economically feasible to produce oral vaccination from transgenic plants and the vaccine may be administered orally. Today, a variety of surface antigens, such as foreign proteins serum albumin, human a-interferon, human erythropoietin and murine IgG and IgA immunoglobulins have been produced in plants (Choi *et al*., 1994 & Birch 1997) [40, 43]

Conclusion

Wide application of *in vitro culture* methods is significant role in crop improvement, to developed very competitive and marketable varieties and hybrids of vegetable crops. Vegetable's important role in making good health due occurs ample number of vitamins, minerals, antioxidants and dietary fibers. Biotechnological tools provide new opportunities for development of different varieties which resistant to diseases and insect- pest. Embryo culture has so many potential uses ranging from overcoming seed dormancy to facilitation of inter-specific hybridization. Molecular markers are RFLP, RAPD and AFLP used for characterization and isolation of pest and pathogen resistance markers and genes that are useful for MAS and other gene transfers. The genetic research on in solanaceous and cucurbitaceous crop has gained traction over the past few years due to usages of PCR based markers.

Future scope of *in vitro culture*

In vitro culture techniques are part of a large group of strategies and technologies, ranging through molecular genetics, genome characterization, recombinant DNA studies, gene-transfer techniques, aseptic growth of cells, tissues, organs and *in vitro* regeneration of plants that are considered to be plant biotechnologies. The use of the term biotechnology has become widespread recently but, in its most restricted sense, it refers to the molecular techniques used to modify the genetic composition of a host plant, *i.e*., genetic engineering. The applications of various tissue-culture approaches to crop improvement, through breeding, wide hybridization, haploidy, Soma clonal variation and micro propagation. Through *In vitro culture* methods, now possible to regenerate species of any plant in the laboratory. To achieve the target of creating a new plant or a plant with desired characteristics, tissue culture is often coupled with recombinant DNA technology.

Acknowledgment

We are thankful to the authors whomever we have mentioned in this review paper as their precise content made the whole process easier to collect specific information about an *In vitro* culture methods and molecular approaches for crop improvement in solanaceous and cucurbitaceous vegetables.

Conflict of Interest

None

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