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Tissue Culture in Vegetable Crops

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The increasing demand for high-quality vegetables, coupled with challenges such as climate change, emerging diseases, declining seed quality and shrinking agricultural resources, has necessitated the adoption of advanced propagation technologies. Among these innovations, plant tissue culture has emerged as one of the most powerful biotechnological tools for rapid multiplication, disease-free plant production, germplasm conservation and genetic improvement of vegetable crops. By exploiting the inherent ability of plant cells to regenerate into complete plants, tissue culture enables year-round production of uniform planting materials under controlled conditions. The technology has transformed the propagation of important vegetable crops such as potato, tomato, chilli, brinjal, okra and cucurbits. This article explores the principles, techniques, applications, advantages, challenges and future prospects of tissue culture in vegetable crops, highlighting its significance in sustainable horticultural production.

Introduction: A New Green Revolution in Vegetable Production

Vegetables are indispensable components of a balanced human diet, providing essential vitamins, minerals, dietary fibre, antioxidants and health-promoting phytochemicals. However, conventional methods of vegetable propagation often face limitations such as low multiplication rates, seed dormancy, disease transmission, poor germination and genetic variability. To overcome these constraints, plant scientists have increasingly turned towards tissue culture, a sophisticated laboratory technique that enables the growth and multiplication of plant cells, tissues, organs or entire plants under sterile and controlled environmental conditions.

The concept of tissue culture was first proposed by the German botanist **Gottlieb Haberlandt** in 1902, who envisioned that individual plant cells could be cultured independently and regenerated into complete plants. Today, his vision has evolved into a global industry contributing significantly to modern agriculture and horticulture. Tissue culture is widely used for commercial micropropagation, production of disease-free planting material, conservation of rare germplasm, genetic transformation, haploid production and crop improvement.

The Scientific Foundation: Totipotency

The success of tissue culture is based on the principle of **totipotency**, which refers to the remarkable ability of a single plant cell to regenerate into an entire plant under suitable environmental and nutritional conditions.

Every living plant cell contains the complete genetic information required to produce roots, shoots, leaves, flowers, fruits and seeds. When provided with the right combination of nutrients and plant growth regulators, these cells can re-enter active growth and develop into a complete plant. This unique property distinguishes plants from most animals and forms the biological basis of modern plant biotechnology.

How Tissue Culture Works

The tissue culture process involves several carefully controlled steps:

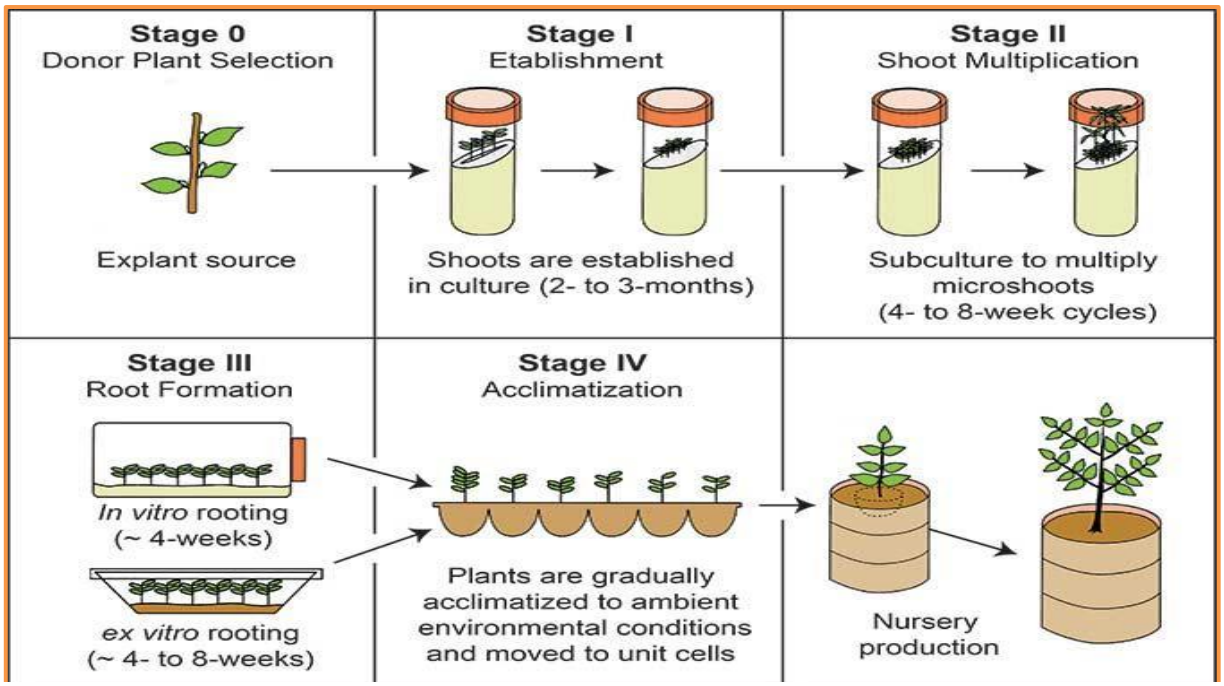

Fig 1. General workflow of tissue culture from explant to field establishment

1. Selection of Explant: An explant is a small piece of plant tissue selected from a healthy mother plant. Common explants include: Shoot tips, Meristems, Leaves, Cotyledons, Hypocotyls, Nodal segments, Embryos and Anthers.

2. Surface Sterilization: A typical sterilization procedure involves washing explants under running tap water, treating them with a fungicide solution, followed by 70% ethanol for 30–60 seconds and sodium hypochlorite or mercuric chloride for a few minutes. Finally, explants are rinsed three to five times with sterile distilled water to remove chemical residues before inoculation.

3. Culture Establishment: After sterilization, explants are inoculated onto a sterile nutrient medium that supplies all essential elements required for growth and development. The most widely used medium for vegetable crops is the **Murashige and Skoog (MS) medium**, developed in 1962. The nutrient medium contains the following components:

- Macronutrients
- Micronutrients
- Vitamins – B_1, B_2, B_6, Myo-inositol, glycine
- Carbon source (sucrose) - 20–30 g L^{-1} (2–3%)
- Plant growth regulators
- Gelling agent: Agar, Phytagel/Gellan gum

4. Multiplication Phase: Cells divide rapidly and produce either:

- Direct shoots
- Callus tissue followed by shoot regeneration

5. Root Induction: Regenerated shoots are transferred to rooting media containing auxins.

6. Hardening and Acclimatization: Plantlets are gradually adapted to greenhouse and field conditions before transplanting.

Major Tissue Culture Techniques Used in Vegetable Crops

Meristem Culture

Meristem culture is one of the most widely adopted tissue culture techniques in vegetable crop improvement. The meristem is the actively dividing region located at the shoot apex and is generally free from systemic pathogens, particularly viruses. In this technique, a small meristematic dome along with one or two leaf primordia is carefully excised under sterile

conditions and cultured on a suitable nutrient medium. The cultured meristem gradually develops into a complete plantlet that is genetically identical to the parent plant. The major advantage of meristem culture is the production of disease-free planting material, which is particularly important in vegetatively propagated crops where pathogens accumulate over successive generations. This technique has been extensively used for eliminating viral diseases in potato, garlic, sweet potato and several other vegetable crops. Besides disease eradication, meristem culture also facilitates rapid multiplication of elite genotypes and long-term conservation of valuable germplasm resources.

Anther Culture and Doubled Haploid Production

Anther culture is a specialized tissue culture technique in which immature anthers containing pollen grains are cultured on artificial nutrient media under controlled environmental conditions. Instead of developing into male gametes, the pollen grains are induced to form embryos and eventually regenerate into haploid plants possessing only a single set of chromosomes. Since haploid plants are usually sterile, their chromosome number is artificially doubled using chemicals such as colchicine to produce doubled haploid plants. These doubled haploids are completely homozygous and genetically stable, making them extremely valuable in plant breeding programmes. The use of doubled haploid technology significantly reduces the time required to develop pure lines, which would otherwise take several generations through conventional selfing. In vegetable crops such as tomato, brinjal, pepper, cucumber, radish and various cole crops, anther culture has become an important tool for accelerating breeding programmes, studying genetic inheritance and developing improved cultivars with desirable agronomic traits.

Embryo Culture (Embryo Rescue)

Embryo culture, commonly referred to as embryo rescue, is employed when embryos fail to develop naturally within seeds due to genetic incompatibility between parents or unfavourable developmental conditions. In this technique, immature or mature embryos are excised from developing seeds and cultured aseptically on nutrient media where they continue their growth independently. The technique is particularly useful in overcoming post-fertilization barriers encountered during wide hybridization programmes. Plant breeders frequently use embryo rescue to recover interspecific and intergeneric hybrids that would otherwise abort before maturity. Apart from hybrid development, embryo culture is useful in breaking seed dormancy, shortening breeding cycles and facilitating the transfer of useful genes from wild relatives into cultivated vegetable crops. This approach has contributed significantly to the development of improved varieties possessing resistance to pests, diseases and environmental stresses.

Callus Culture

Callus culture involves the induction and maintenance of an unorganized mass of rapidly dividing cells known as callus. When explants such as leaves, cotyledons, hypocotyls, stem segments or roots are placed on culture media containing appropriate concentrations of auxins and cytokinins, the differentiated cells lose their specialized functions and revert to a meristematic state, resulting in callus formation. The callus serves as a versatile biological material that can be manipulated for various biotechnological applications. Under suitable conditions, callus tissues can regenerate into complete plants through organogenesis or somatic embryogenesis. Callus culture forms the foundation of genetic transformation studies, mutation breeding, somaclonal variation and secondary metabolite production. In vegetable crops such as tomato, chilli, brinjal, cucumber and potato, callus culture has played a crucial role in developing regeneration protocols and supporting crop improvement programmes.

Cell Suspension Culture

Cell suspension culture is an advanced form of callus culture in which small pieces of friable callus are transferred into liquid nutrient media and maintained under continuous agitation. The shaking action disperses individual cells and small cell aggregates throughout the medium, creating a uniform suspension. These actively growing cells can be multiplied

rapidly and harvested in large quantities. Cell suspension cultures are particularly valuable for physiological, biochemical and molecular studies because they provide a homogeneous population of cells. In addition, they are widely used for the production of secondary metabolites, pharmaceuticals, pigments, flavour compounds and other commercially important bioactive substances. The technique also serves as the basis for large-scale bioreactor systems, enabling industrial production of valuable plant-derived compounds. Although less common in commercial vegetable propagation, cell suspension cultures are increasingly important in vegetable biotechnology research.

Protoplast Culture

Protoplast culture represents one of the most sophisticated techniques in plant biotechnology. Protoplasts are plant cells from which the cell wall has been completely removed using enzymes such as cellulase and pectinase. The resulting naked cells are capable of regenerating new cell walls, dividing and ultimately developing into complete plants under suitable culture conditions. One of the most significant applications of protoplast culture is somatic hybridization, where protoplasts from different species or genera are fused to create novel hybrids that may not be obtainable through conventional sexual hybridization. This technology has opened new avenues for transferring desirable traits such as disease resistance, stress tolerance and improved quality characteristics between distantly related plant species. In vegetable crops, protoplast culture has been used extensively in members of the Solanaceae and Brassicaceae families for genetic improvement and breeding purposes.

Ovule Culture

Ovule culture involves the aseptic isolation and cultivation of unfertilized or fertilized ovules on nutrient media. This technique is particularly useful when normal seed development is hindered due to incompatibility barriers, embryo abortion or poor endosperm development. Cultured ovules can continue their growth in vitro and eventually develop into complete plants. Ovule culture has found important applications in wide hybridization programmes, especially in species where conventional crossing methods are unsuccessful. It is also useful for obtaining haploid plants, studying reproductive biology and overcoming self-incompatibility barriers in certain vegetable crops. By enabling the recovery of otherwise unattainable hybrids, ovule culture contributes significantly to the creation of novel genetic variability and the development of improved vegetable cultivars.

Growth Media: The Lifeline of Tissue Culture

The success of tissue culture depends heavily on nutrient media.

Components of Culture Media

Component	Function
Macronutrients	Cell growth and metabolism
Micronutrients	Enzyme activation
Vitamins	Cellular processes
Sucrose	Energy source
Auxins	Root formation and callus induction
Cytokinins	Shoot multiplication
Organic additives	Enhanced growth

Commonly Used Media

Medium	Developed By	Application
MS Medium	Murashige and Skoog (1962)	Most vegetable crops
White Medium	White	Root cultures
Gamborg B5	Gamborg	Cell suspension cultures
Chu N6	Chu	Cereal cultures

Factors Influencing Tissue Culture Success

Several factors determine the efficiency of tissue culture:

1. **Explant Factors –** Age, Physiological condition, Genotype and Size
2. **Environmental Factors -** Temperature (25 ± 2°C), Light intensity, Photoperiod and Humidity
3. **Media Factors -** Hormone concentration, Nutrient balance, pH and Carbon source

Applications of Tissue Culture in Major Vegetable Crops:

Potato: The Flagship Crop of Tissue Culture Technology

Potato (*Solanum tuberosum* L.) is one of the most successful examples of commercial tissue culture application in vegetable crops. Conventionally, potato is propagated through seed tubers, which possess a very low multiplication rate and often serve as carriers of viral, bacterial and fungal pathogens. Consequently, the availability of quality seed tubers becomes a major constraint in potato cultivation. Tissue culture has emerged as a revolutionary technology for overcoming these limitations by enabling the rapid production of disease-free planting material. Through meristem culture and micropropagation techniques, thousands of genetically identical plantlets can be produced from a single healthy explant within a relatively short period. The technology has significantly contributed to the production of virus-free seed potatoes and mini-tubers used in seed multiplication programmes worldwide.

Fig.2. In vitro collection of transgenic potato plant and original varieties

Several studies have demonstrated the effectiveness of tissue culture in potato improvement. Walia *et al*. (2021) reported maximum shoot multiplication and shoot length in the late blight-resistant variety Kufri Girdhari when cultured on MS medium supplemented with appropriate concentrations of BAP and kinetin. Similarly, Srivastava *et al*. (2012) observed superior microtuber production and plant vigour in cultivar Kufri Giriraj under in vitro conditions. The integration of tissue culture with aeroponics and protected cultivation has further enhanced the efficiency of seed potato production systems, making potato a model crop for successful commercialization of plant tissue culture technology.

Tomato: A Platform for Rapid Multiplication and Genetic Improvement

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops in the world and an important source of vitamins, minerals, antioxidants and lycopene. However, its production is severely affected by numerous diseases, insect pests and environmental stresses. Conventional breeding methods often require several years to develop improved varieties, creating a need for faster and more efficient propagation techniques. Plant tissue culture has become an indispensable tool in tomato improvement programmes. Through in vitro regeneration, large numbers of uniform and disease-free seedlings can be produced throughout the year. Tissue culture also provides the foundation for genetic transformation, somaclonal variation, embryo rescue and synthetic seed production. Researchers have successfully developed regeneration protocols using cotyledons, hypocotyls, leaf discs, shoot tips and nodal segments as explants. Jawad *et al*. (2020) reported 100 percent rooting in regenerated shoots using a combination of IBA and IAA, while Sharma *et al*. (2021) achieved high shoot regeneration efficiency using BAP and IAA. These developments have facilitated the production of elite planting material and accelerated breeding programmes aimed at developing disease-resistant and climate-resilient tomato cultivars.

Chilli: Enhancing Propagation Efficiency and Crop Productivity

Chilli (*Capsicum annuum* L.) is valued both as a vegetable and as a spice crop because of its pungency, flavour and nutritional properties. The crop is often affected by viral diseases, bacterial wilt and fungal pathogens that reduce yield and fruit quality. Since chilli lacks an efficient natural vegetative propagation system, tissue culture offers an attractive alternative for rapid multiplication of superior genotypes. Micropropagation techniques have enabled the large-scale production of uniform and disease-free chilli plants. Hypocotyls, cotyledons, shoot tips and leaf segments have been widely utilized as explants for callus induction and plant regeneration. Studies have shown that the addition of auxins and cytokinins in suitable concentrations significantly improves regeneration efficiency. Fatima *et al*. (2014) observed high callus induction and shoot regeneration from cotyledon explants, while Kaaby *et al*. (2015) reported enhanced callus growth in hypocotyl-derived cultures. Tissue culture has also provided opportunities for genetic transformation and the development of improved cultivars possessing resistance to biotic and abiotic stresses. Consequently, it has become an important component of modern chilli breeding programmes.

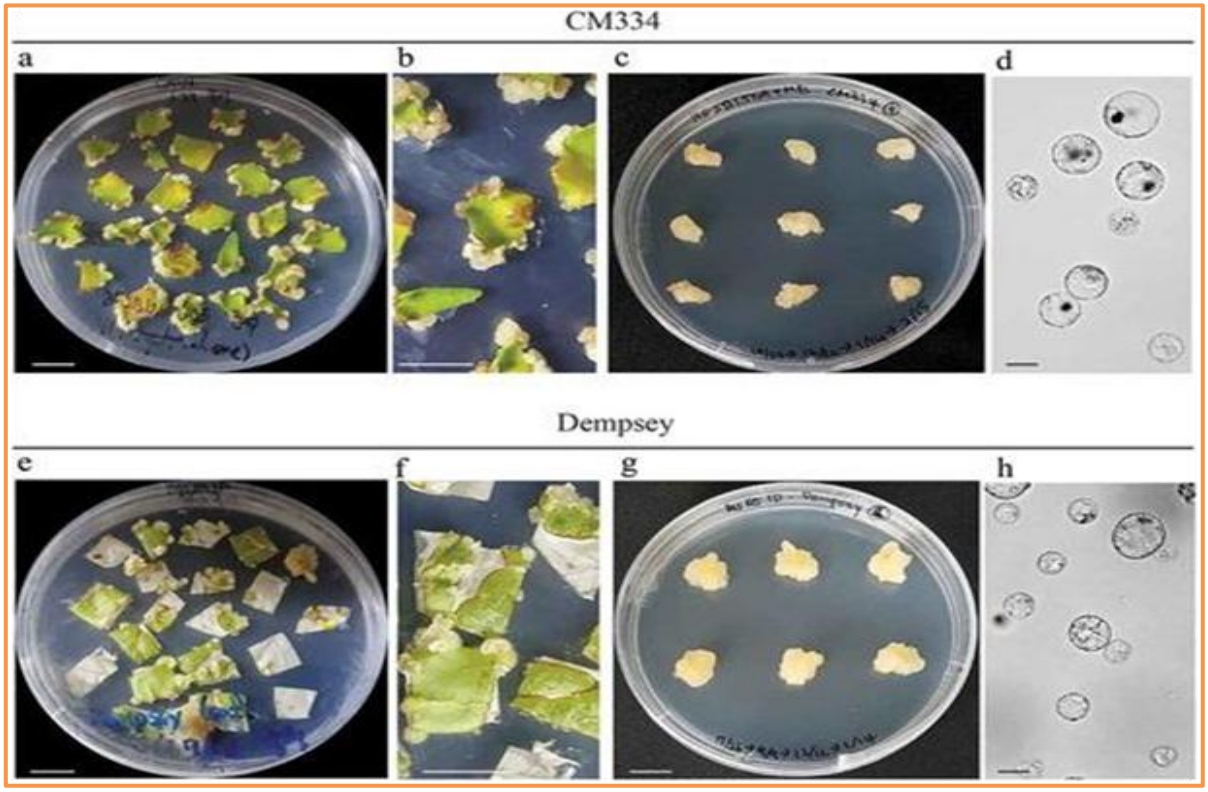

Fig.3. Leaf-induced callus formation of *Capsicum annum* 'CM334' and 'Dempsey'. (a-c) Leaf segment-derived calli of 'CM334'. (a) 4-week-old leaf segments placed in Gamborg's B5 medium supplemented with 2 mg/L 6-benzylaminopurine (2BAP), and 1 mg/L α-naphthalene acetic acid (1NAA) medium under long-day conditions (16 h light/8 h dark) at 25°C in a growth chamber. (b) Enlarged image of (a). (c) Propagated calli in B5 medium containing 2BAP, 1.5 mg/L NAA, and 2-morpholinoethanesulphonic acid (MES) under dark conditions at 25°C. (d) Protoplasts isolated from the propagated 'CM334' calli. (e-g) Leaf segment-derived calli of 'Dempsey'. (e) 4.5-week-old leaf segments placed in Murashige and Skoog (MS) medium supplemented with B5 vitamins and 1 mg/L 2,4-dichlorophenoxyacetic acid (1 2,4D) under long-day conditions (16 h light/8 h dark) at 25°C in a growth chamber. (f) Enlarged image of (e). (g) Calli propagated in MSB5 (MS basal salt mixture and B5 vitamins) medium containing 1 2,4D under dark conditions at 25°C. (h) Protoplasts isolated from the propagated 'Dempsey' calli. White scale bars = 1 cm. Black scale bars = 10 µm.

Brinjal: Conserving Germplasm and Developing Improved Cultivars

Brinjal or eggplant (*Solanum melongena* L.) is an economically important vegetable crop grown extensively in tropical and subtropical regions. Despite its significance, brinjal

production suffers from severe infestations of fruit and shoot borer, bacterial wilt and other diseases. Conventional breeding efforts are often hampered by sexual incompatibility and difficulties in obtaining fertile progenies from interspecific crosses. Tissue culture has played a crucial role in overcoming these barriers by facilitating rapid clonal multiplication, germplasm conservation and genetic improvement. Various explants such as cotyledons, hypocotyls, roots and shoot tips have been successfully used for plant regeneration. Mir *et al*. (2011) reported excellent callus induction from cotyledon and hypocotyl explants, while Kaur *et al*. (2020) observed maximum callus formation in media supplemented with suitable combinations of NAA and BAP. In vitro regeneration systems have further enabled the development of transgenic brinjal lines and provided an effective platform for crop improvement through genetic engineering and mutation breeding.

Okra: Overcoming Seed Dormancy and Disease Constraints

Okra (*Abelmoschus esculentus* L.) is a nutritious vegetable widely consumed in tropical and subtropical countries. Traditional propagation through seeds often encounters problems such as poor germination, seed dormancy and transmission of seed-borne diseases. These limitations have encouraged the adoption of tissue culture methods for large-scale multiplication. Micropropagation of okra through meristem culture, shoot tip culture, and cotyledonary node culture has shown considerable success. Tissue culture allows the production of healthy and uniform planting material with improved field performance. Studies have demonstrated that the use of cytokinins such as BAP promotes multiple shoot formation, whereas auxins facilitate rooting and acclimatization. The technology has immense potential for rapid multiplication of elite genotypes and conservation of valuable germplasm resources.

Cole Crops: Accelerating Breeding and Hybrid Development

Cole crops such as cabbage, cauliflower, broccoli, kale and Brussels sprouts belong to the species *Brassica oleracea* and are among the most economically important vegetable groups worldwide. Tissue culture techniques have become integral components of Brassica improvement programmes because they facilitate rapid multiplication, doubled haploid production and genetic transformation. The production of haploids and doubled haploids through anther culture has significantly shortened breeding cycles and enabled the rapid development of homozygous lines. In vitro regeneration protocols have also been used for introducing disease resistance, improving nutritional quality and enhancing stress tolerance. Research findings indicate that explant type and growth regulator combinations greatly influence regeneration efficiency in cole crops. Consequently, tissue culture serves as a valuable tool for accelerating Brassica breeding programmes and improving crop productivity.

Cucurbits: Addressing Challenges in Clonal Propagation

The cucurbit family includes several economically important vegetable crops such as cucumber, watermelon, muskmelon, bottle gourd, ridge gourd, sponge gourd, pointed gourd, ivy gourd and spine gourd. Many of these crops exhibit poor seed viability, dioecious flowering habits and difficulties in maintaining desirable sex expression, making conventional propagation challenging. Tissue culture has emerged as an effective strategy for overcoming these constraints. Through micropropagation, large numbers of genetically uniform plants can be produced from selected elite genotypes. In cucumber, leaf disc explants have shown excellent callus induction and regeneration responses. Similarly, successful protocols have been developed for pointed gourd, ivy gourd, spine gourd and watermelon. The technology is particularly valuable for maintaining superior female lines, conserving germplasm and supporting hybrid seed production programmes. Furthermore, tissue culture provides opportunities for genetic transformation and the development of improved cucurbit varieties with enhanced resistance to diseases and environmental stresses.

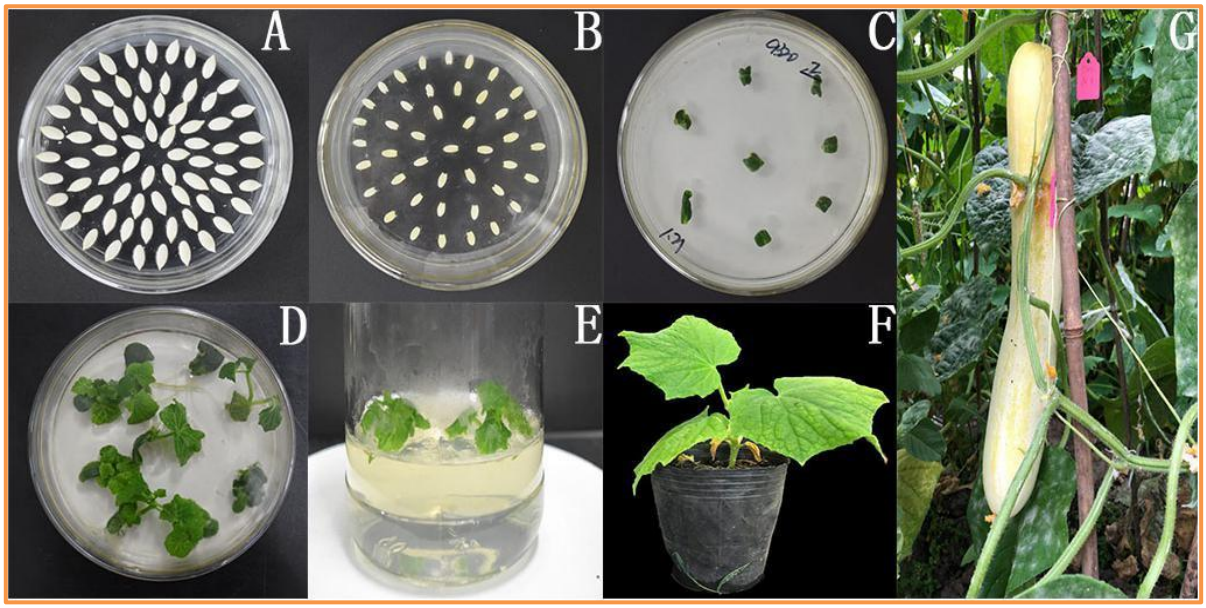

Fig.4. pCXSN-CsMCF genetic transformation of cucumber. **(A)** Cucumber seed. **(B)** Co-culture. **(C)** Screening culture. **(D)** Plant regeneration. **(E)** Rooting culture of resistant seedlings. **(F)** Regeneration of resistant seedlings. **(G)** Seed of transgenic plants.

Advantages of Tissue Culture

Advantage	Benefit
Rapid multiplication	Millions of plants from a single explant
Disease-free material	Improved crop health
Uniformity	True-to-type plants
Year-round production	Independent of seasons
Germplasm conservation	Preservation of elite genotypes
Space efficiency	Reduced nursery area
Breeding support	Faster crop improvement

Challenges and Limitations

Despite its advantages, tissue culture faces several constraints.

1. **Technical Challenges:** Requirement for aseptic conditions, Skilled manpower and Complex protocols
2. **Economic Constraints:** High initial investment, Costly infrastructure and Expensive consumables
3. **Biological Limitations:** Somaclonal variation, Genotype dependency and Acclimatization losses

Future Prospects

The future of tissue culture in vegetable crops is closely linked with advances in molecular biology, automation and artificial intelligence. Future developments may include:

- Automated micropropagation systems
- Robotic culture handling
- Genome-edited vegetables
- Synthetic seed commercialization
- Climate-resilient vegetable varieties
- Precision tissue culture using AI-based monitoring

As global food demand rises, tissue culture will continue to play a central role in ensuring sustainable vegetable production and nutritional security.

Conclusion

Plant tissue culture has evolved from a laboratory curiosity into a cornerstone of modern vegetable biotechnology. By enabling rapid multiplication, disease-free plant production,

germplasm conservation and crop improvement, it has transformed the way vegetable crops are propagated and managed. From potato and tomato to chilli, brinjal, okra and cucurbits, tissue culture offers practical solutions to many of the challenges facing contemporary agriculture. As biotechnology continues to advance, tissue culture will remain an indispensable tool for developing resilient, productive and high-quality vegetable crops capable of meeting future global food demands.

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