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Plant tissue culture techniques for vegetable crop improvement

Sheetal[1*], Manisha[2]

[1]Ph.D. Scholar, Department of Vegetable Science and Floriculture, CSK HPKV, Palampur, HP, India-176062
[2]Ph.D. Scholar, Department of Vegetable Science and Floriculture, Palampur, HP, India-176062

*Corresponding Author
Sheetal[*]

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INTRODUCTION

Plant tissue culture is a technique of growing cells, tissues, organs or whole organism *in-vitro* (in glass) on an artificial culture medium under aseptic and controlled environment condition. It has emerged as a revolutionary biotechnological tool with a significant impact on agriculture, by providing plants necessaryto meet the growing global demand. It offers a controlled environment for rapid multiplication, disease-free plant production and genetic improvement, making it an essential tool in modern agriculture.The basic principle of plant tissue culture is totipotency i.e., the capacity of a plant cell to regenerate into a whole plant (Bhojwani and Razdan 1996).Techniques like meristem culture, embryo culture, anther or pollen culture, protoplast culture and ovary culture can be utilized for vegetable crop improvement.

Factors impacting plant tissue culture

- **The genetic makeup of the plant:** The plant's genetic background plays a crucial role in its response to tissue culture. Some species or varieties are naturally more responsive to *in-vitro* culture, while others may be recalcitrant (difficult to culture). This response can affect callus formation, organogenesis, somatic embryogenesis and regeneration ability.
- **Source of explants:** The type, age and physiological state of the plant tissue used as an explant greatly influence the success of culture. Younger, actively dividing tissues give better results. Explants from different plant parts (leaf, stem, root and bud) may show different abilities to regenerate.
- **Growth medium:** The composition of the culture medium is a major determinant of success in tissue culture. It typically includes: macronutrients, micronutrients, vitamins, carbonsource and plant growth regulators**.**
- **Environmental factors:** The physical and atmospheric conditions like temperature, light, humidity and sterility plays a vital role in determining the success of *in-vitro* growth and development. These factors directly influence cell metabolism, organ development and prevention of contamination.

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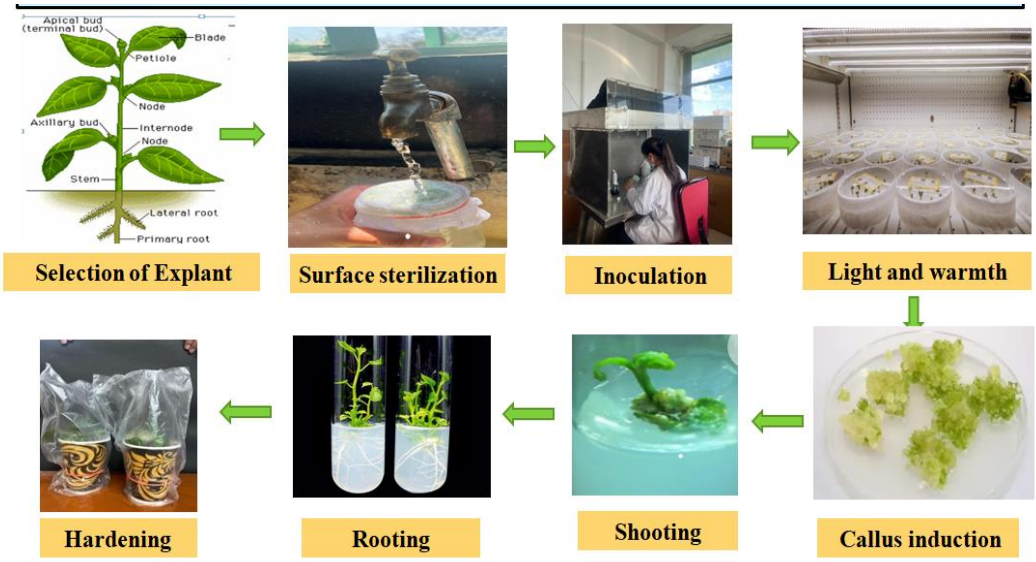
Basic process of plant tissue culture



Techniques of plant tissue culture

1. Meristem culture: Meristem is a region of actively dividing, undifferentiated plant cells that are responsible for plant growth and development. In this method, the meristem tip, consisting of one or two pairs of leaf primordial is cultured in a cultured medium. Smaller meristem tips (0.2-0.4 mm) have a higher chance of being virus-free compared to larger ones. Virus-free potato and sweet potato plants are commonly produced through this method.

2.Embryo culture: Embryo is the young, developing plant enclosed within a seed, formed after fertilization. The term embryo culture means excision of embryos and growing them under artificial environmental condition.

Two types of embryo culture:

- **Mature embryo culture:** *In-vitro* technique where fully developed embryos (from mature seeds) are excised and grown on nutrient media to develop into whole plants. It is used to overcome long dormancy period.
- **Immature embryo culture/embryo rescue:** The isolation and *in-vitro* cultivation of embryos that are not yet fully developed - typically harvested days after pollination, before seeds are fully mature. Used in wide hybridization where the embryo would normally abort inside the seed. Successfully used in crops like tomato, brinjal and onion.

3. Anther/pollen culture: Anther or pollen culture is a plant tissue culture technique used to produce haploid plants (plants with only one set of chromosomes, n) from immature pollen grains (microspores) or whole anthers in a nutrient medium under sterile condition. This process is called androgenesis. *Brassica* sp. was identified as one of the best-responding species for androgenesis. Production of haploids and double haploids through anther/pollen culture from F_1 plants is a very useful technique for compressing the breeding cycle.

Achievements of anther/pollen culture:

Crop	Achievements	Country	References
Broccoli	Double Haploid inbred lines USLV048 and USLV131 of heading broccoli	USA	Farnham 2013
White cabbage	Developed good anti-oxidative properties in DH lines 4013, 4014 and 4024 with high phenol content	Poland	Leja et al. 2006
Cauliflower	Inbred line development	India	Bhattacharya et al. 2017
Cucurbits	High yield and disease resistance	India	Rakha et al. 2012

4.Protoplast fusion/Somatic hybridization: Development of hybrid plants through the fusion of somatic protoplast from two different plant species varieties is called somatic hybridization. Many sources of useful genes cannot be included in crop improvement programme because of sexual incompatibilities. Genetic transformation is expensive approach that requires isolation, identification and cloning, while it can be achieved through somatic hybridization. Pomato (Potato + Tomato) is one of the first successful somatic hybrid developed through protoplast fusion.

5. Ovary culture: Thetechnique of ovary culture was developed by Nitsch in 1951.It is used for the propagation of plants, especially in cases where it is difficult to regenerate plants from other parts like leaves or stems. It involves the *in-vitro* culture of ovaries excised from the parent plant under sterile conditions to induce embryo development and plant regeneration. Ovary culture is applied in vegetables like tomato, brinjal, cucumber and carrot etc.

Achievements of ovary culture:

Crop	Achievements	Country	References
Cucumber	Developed cucumber lines CSL 0011 and CSL 0006 resistant to CMV	Thailand	Plapung et al. 2018
Suger beet	Developed high sugar yield crosses of monogerm line DH 150-4 and multigerm pollinator DH 58 and TH 55	Bulgaria	Kikindonov et al. 2020
Onion	Variation in gynogenic potential for haploid induction in Indian short day onion	India	Anandan et al. 2016

Advantages of plant tissue culture:

1. **Mass propagation:** Tissue culture allows rapid production of large numbers of genetically identical plants in a short time, ensuring uniformity and disease-free plants. It also enables year-round production and the propagation of hard-to-grow species, making it highly efficient for commercial and conservation purposes.
2. **Disease-free plants:** Since the process begins with small, sterile explants, it ensures that the resulting plants are free from soil-borne pathogens, viruses and fungi. This is especially valuable for crops that are susceptible to diseases, as it helps maintain plant health and increases productivity.
3. **True-to-type:** The new plants are genetically identical to the parent, which is crucial for maintaining desirable traits in crops.
4. **Space efficient:** Tissue culture is space efficient because it allows for the production ofa large number of plants in a small and controlled environment.

CONCLUSION

Plant tissue culture offers a range of precise and innovative techniques such as meristem culture, embryo rescue, anther culture, protoplast fusion and ovary culture, that are instrumental in the genetic improvement and propagation of vegetable crops. Each method plays a distinct role in achieving goals like disease elimination, haploid production, hybrid development and regeneration of difficult-to-propagate species.

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