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Tissue Culture: Aeon of Micro Propagation in Vegetable Crops

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

Tissue culture in vegetable crops is a technique that has revolutionized the way we produce and propagate plants. It involves the growth of plant cells, tissues or organs in an artificial nutrient medium under sterile conditions. This method offers numerous advantages, including the production of disease-free plants, rapid multiplication and the ability to grow plants year-round. One of the key benefits of tissue culture in vegetable crops is the production of disease-free plants. By starting with a small piece of healthy tissue such as a leaf or stem, it is possible to grow an entire plant that is free from any pathogens. This is particularly important in vegetable crops, as diseases can significantly reduce yields and quality. By using tissue culture, farmers can ensure that the plants they grow are healthy and resistant to common diseases. Another advantage of tissue culture is the rapid multiplication of plants. Through a process called micropropagation, a single piece of tissue can be used to produce hundreds or even thousands of identical plants within a short period. This is particularly useful for vegetable crops that have a high demand or are difficult to propagate through traditional methods. By using tissue culture, farmers can quickly and efficiently produce large quantities of plants to meet market demands. Furthermore, tissue culture allows for year-round plant production. Unlike traditional methods that are limited by seasonal variations, tissue culture can be done in controlled environments such as laboratories or

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greenhouses. This means that farmers can grow vegetable crops regardless of the weather conditions outside. This is particularly advantageous for regions with harsh climates or limited growing seasons.

Keywords: Tissue culture; micro propagation; growing media; explants; vegetable crops.

1. INTRODUCTION

The term “plant tissue culture” broadly refers to the *in vitro* cultivation of plants, seeds, plants parts (tissue, organs, embryos, single cells, protoplasts, *etc*.) on nutrient media under aseptic conditions [1]. Tissue culture had its origins at the beginning of the 20th century with the work of Gottlieb Haberlandt and known as father of tissue culture [2]. In 1902 G. Haberlandt proposed that single plant cell could be cultured *in vitro* in nutrient culture media [3]. The most important growth hormone for tissue culture IAA was discovered in 1930 by F. Kogle et al. [4]. Professor Philip White in 1934 got success in regeneration of tomato plant from using root as explant and it was first tissue culture vegetable crop [5]. In 1962, T. Murashige and Skoog published the composition of a plant tissue culture medium known as MS medium [6]. “Commercial tissue culture was born in India when A.V. Thomas and Company (AVT) established their first production unit in 1987 at Cochin for clonal propagation of superior genotypes of selected cardamom plants. A second company Indo-American Hybrid Seeds established in 1988 at Bangalore, Karnataka, who were in the nursery business in hybrid flowers and vegetables, imported a tissue culture laboratory and green houses with a capacity of 10 million plants/ annum” [7].

2. PRINCIPLES OF PLANT TISSUE CULTURE

“There are three principles of plant tissue culture *viz.,* totipotency, dedifferentiation and competency” [8].

1. Totipotency

“The potential or inherent capacity of a plant cell to develop into an entire plant if suitably stimulated is called totipotency. It implies that all the information necessary for growth and reproduction of the organism is contained in the cell” [9].

2. Dedifferentiation

“Dedifferentiation refers as capacity of mature cells to return to meristematic condition and development of a new growing point, follow by redifferentiation which is the ability to reorganize into new organ” [10].

3. Competency

“Competency defined as the endogenous potential of a given cells or tissue to develop in a particular way” [11].

3. METHODS OF PLANT TISSUE CULTURE

1. Meristem Tip Culture

“In this method, the meristem tip consisting of one or two pairs of leaf primordial are cultured in a cultured medium” [12]. Rapid multiplication of the plants, which not easily propagated by vegetative means, is also possible through meristem tip culture. Plants produced are free from pathogens and can be stored for longer period in a smaller space [13]. Different stages of meristems tip culture is given in Fig. 1.

2. Anther or Pollen Culture

Regeneration of whole plant from anther or pollen in the culture medium is called anther culture [14]. Haploid plants can be developed by Anther or Pollen culture. Production of haploid plant through Anther culture is known as *Androgenesis*. Plant developed through anther culture are haploid, in general the haploid plants are highly sterile in nature. Therefore, chromosome numbers of all haploids are doubled by treating with Colchicine to produce double haploid which have normal somatic chromosome (2n) of the species and are fully fertile [15]. Different stages involve in Anther or Pollen culture is given Fig. 2.

3. Embryo Culture

“Isolation and growth of an immature or mature embryo *in vitro*, with the goal of obtaining a viable plant. The first attempt to grow the embryos of angiosperms was made by Hanning in 1904 from two Crucifers *Cochleria* and *Raphanus”* [16]. Different stages in embryo culture are noted in below Fig. 3.

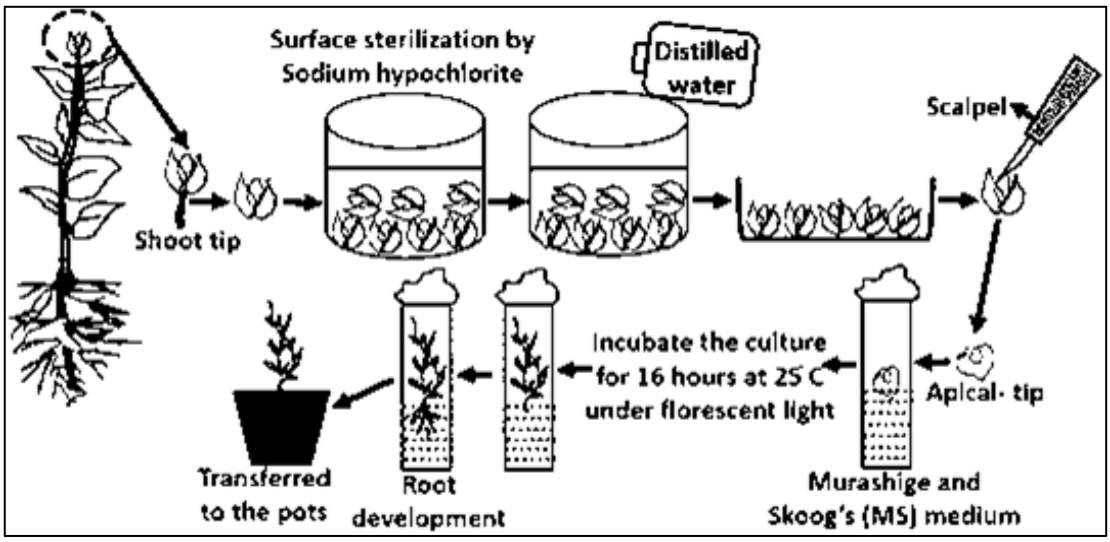

Fig. 1. Different stages of meristem culture

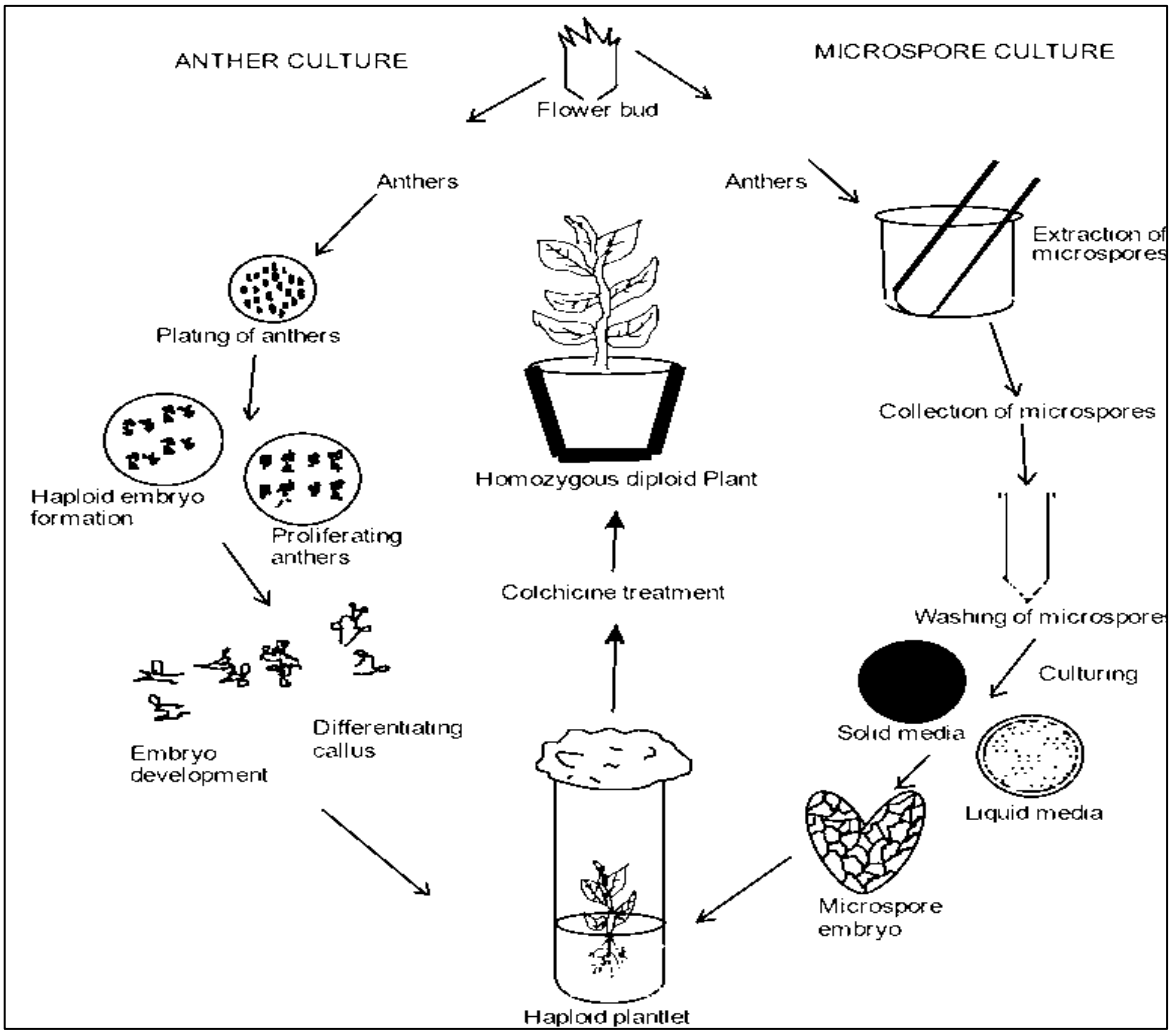

Fig. 2. Different stages involve in anther or pollen culture

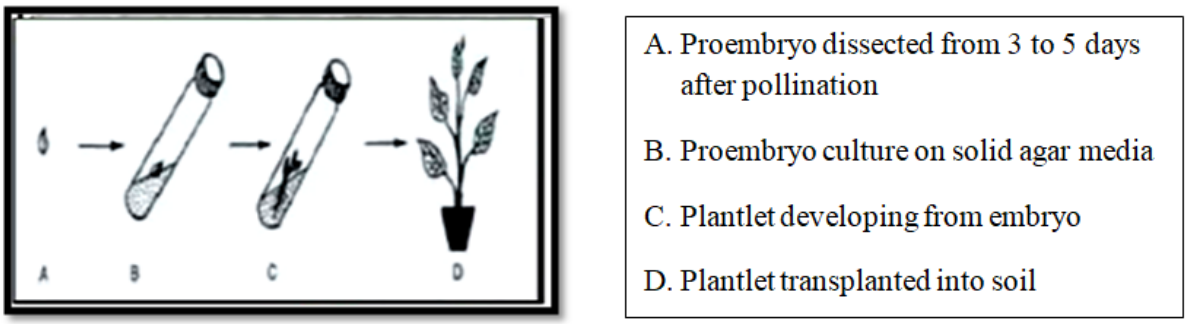

Fig. 3. Different stages involve in embryo culture

4. Cell Culture

"Cell suspension culture is obtained by homogenizing a piece of callus into liquid medium by shaking with shaker until medium becomes cloudy with suspended cells. This technique is useful to plant breeders because now it is possible to induce desirable variability in an individual cell, which can be regenerated into a full plant" [17].

5. Protoplast Culture

"In protoplast culture, the rigid cell walls are first removed either mechanically or enzymatically to expose the protoplast. The exposed protoplast is then cultured onto a suitable culture medium" [18].

Advantages of protoplast culture are:

- A large homogeneous population of plants can be obtained from a small sample.
- Fusion of two protoplast of two different plant species is possible, which may bring a greater variability in plants.

6. Ovule Culture

"In this technique, unfertilized ovules are excised and cultured into medium. This technique has potential application to produce hybrid seeds in wide genetic crosses, which is rather difficult through conventional means. It is also efficient method to obtain plants in self incompatible species" [19]. Steps involve in ovule culture is given in below Fig. 4.

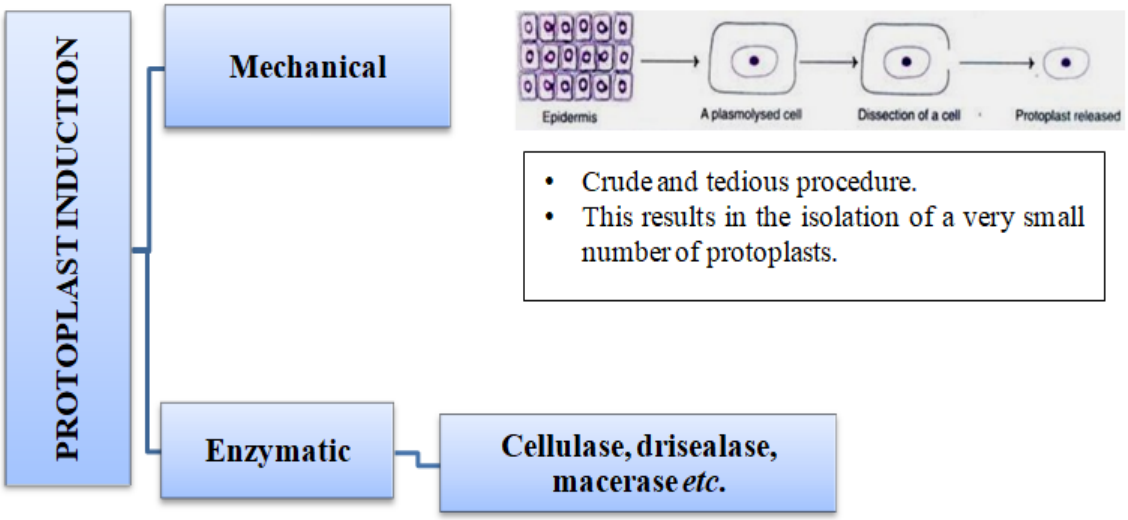

Fig. 4. Steps of protoplast culture

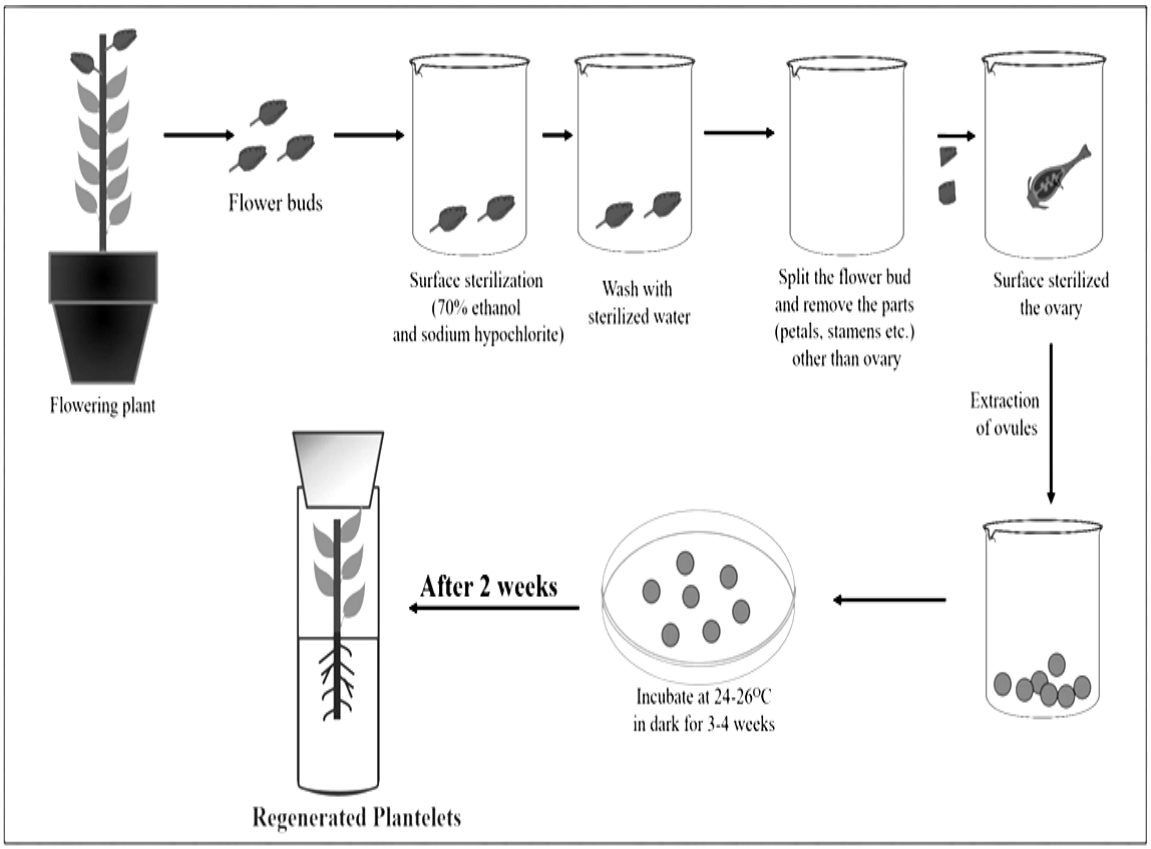

Fig. 5. Different steps involve in ovule culture

7. Callus Culture

A callus is an unorganized and undifferentiated mass cell. In callus culture, the stimulation of cell division and multiplication is required and thus culture medium is accordingly supplemented with necessary regulators [20]. In callus culture, organogenesis occurs in two stages. In first stage, formation of meristems takes place and in second stage, active growth of stem buds and roots takes place [21].

4. PROCEDURE OF PLANT TISSUE CULTURE

The process of plant tissue culture begins with the selection of a suitable plant material, which can be any part of the plant such as leaves, stems, or roots. The chosen plant material is then surface sterilized to remove any contaminants that may interfere with the growth of the cultured cells. This is usually done by washing the plant material with a disinfectant solution, followed by rinsing with sterile water. Once the plant material is sterilized, it is cut into small pieces called explants. These explants are then placed onto a nutrient-rich medium, which contains a combination of macronutrients, micronutrients, vitamins, and growth regulators. The growth regulators, such as auxins and cytokinins, play a crucial role in stimulating the growth and development of the cultured cells. The explants are incubated in a growth chamber under controlled conditions of temperature, light, and humidity. This ensures optimal conditions for the growth of the cultured cells. The growth chamber is equipped with artificial lighting to provide the necessary light intensity for photosynthesis. The temperature and humidity are regulated to promote the growth of the cells and prevent contamination. As the explants grow, they form a mass of undifferentiated cells called a callus. The callus can be subcultured onto fresh media to promote further growth and multiplication. By manipulating the composition of the growth medium, plant tissue culture techniques can be used to induce the differentiation of the callus into specific plant organs, such as roots, shoots, or embryos. Once the desired plant organs have been formed, they can be transferred to a rooting medium to induce the development of roots. This is an important step in the tissue culture process, as it allows the

plants to establish a root system and become self-sustaining. After rooting, the plants can be transferred to soil or a suitable growing medium for further growth and development. Now tissue culture plant ready for primary hardening which done in green house conditions. After primary hardening, secondary hardening was done in field condition. After that plant is ready for main field planting [22]. Different steps in tissue culture process are showed in below Fig. 5.

5. FACTORS AFFECTING PLANT TISSUE CULTURE

1. Explants

Cell, tissue or any organ of a plant that is used to start *in-vitro* culture is called explants. Explants are isolated from parent plants and grown in a nutritional medium and maintained in controlled environment for prolonged period of time under aseptic condition [23].

2. Modulation and components in cultural media

Optimal growth and morphogenesis of tissues may vary for different plants according to their nutritional requirements. Tissues from different parts of plants may also have different requirements for satisfactory growth. Cultural media and its combination and composition play vital role in tissue culture. For the establishment of a new protocol for a specific purpose in tissue culture, a suitable medium is better formulated by testing the individual addition of a series of concentrations of a given compound to a universal basal medium such as MS, LS or B5 [32].

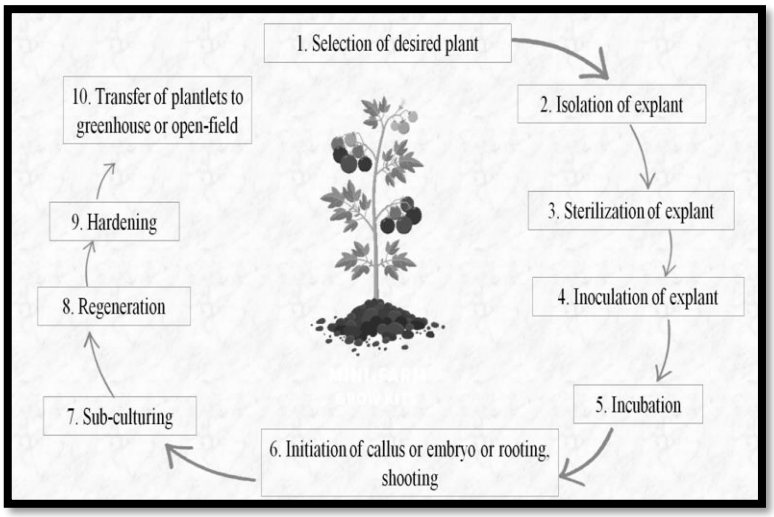

Fig. 6. Procedure of tissue culture

Table 1. Different explants use for regeneration of Vegetables

Vegetable crops	Explants	Sources
Potato	Leaf, Stem, Internodal segments and Tuber	Kumlay and Sezai [24].
Tomato	Hypocotyls, Leaf discs, Root, Shoot tips, Cotyledons and Anther	Papry et al. [25]
Brinjal	Cotyledons, Shoot tips, Hypocotyls and Root	Kaur et al. [26].
Chilli	Hypocotyls, Cotyledons and Shoot tips	Ashrafuzzaman et al. [27]
Okra	Cotyledonary node and Hypocotyls	Rizwan et al. [28]
Cucumber	Hypocotyls, Leaf discs and Cotyledons	H. Sultana et al. [29]
Little gourd	Nodal segments	Amin et al. [30]
Pointed gourd	Nodal segments and Shoot tips	Malek et al. [31]

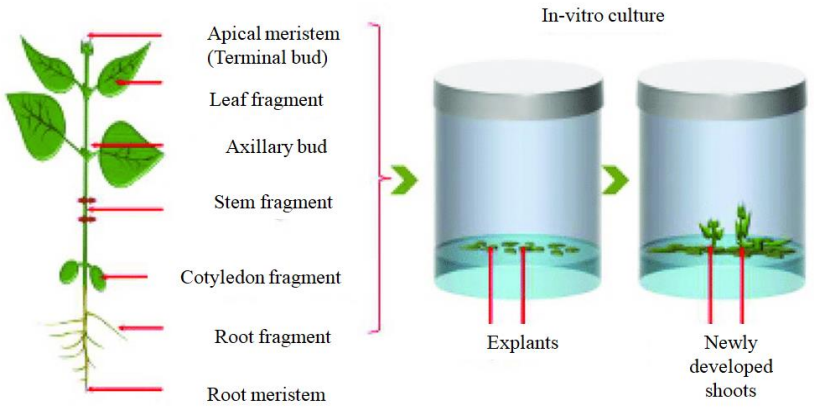

Fig. 7. Explants of trees

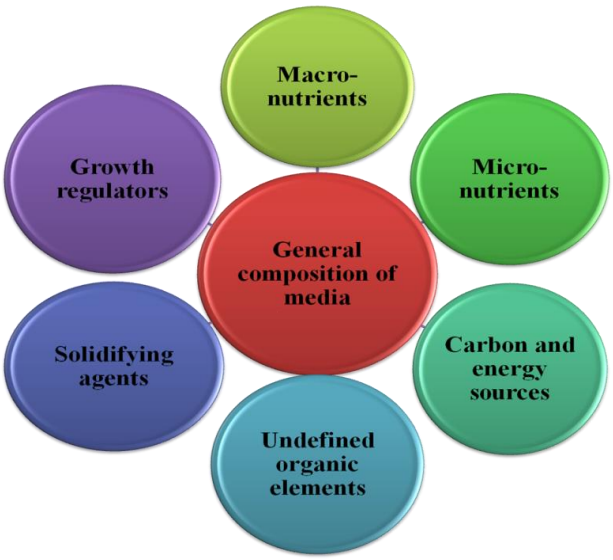

Fig. 8. Modulation and components in cultural media

3. Cultural conditions

The temperature should be normally 25ºC ± 2 (maintained with the help of sensor based air conditioner and heater). Initially callus is kept in dark condition and after formation of shoot/root light intensity is being increased gradually. During shoot/root subculturing 16 hours light is provided [33].

6. ADVANTAGES OF TISSUE CULTURE

Following are the advantages of tissue culture [34]:

- The plantlets are obtain in very short time with small amount of plant cell or tissue
- The new plants produced are disease free
- The plants can be grown throughout the year, irrespective of the season
- The production of exact copies of mother plant
- Reduce stock plant space
- Long term germplasm storage
- Production of difficult to propagate species

7. DISADVANTAGES OF TISSUE CULTURE

With advantages there are some disadvantages of tissue culture which given given below [34]:

- Specialized equipments are required
- More technique expertise required
- Protocol is not optimized for all species
- Relatively expensive to set up

8. ROLE OF TISSUE CULTURE IN VEGETABLE CROPS

8.1 Potato

Potato (*Solanum tuberosum* L.) is one of the most important crops in the world, it is grown in 100 countries worldwide. Conventionally, potato is propagated through tubers. This propagation is characterized by low ratio of multiplication that ranges from 1:4 to 1:15. Through tissue culture we can create a large number of clones from a single seed or explants, select desirable traits, decrease the amount of space required for field trials and to eliminate plant diseases through careful selection and sterile techniques. The system is characterized by very flexible rapid multiplication giving a high rate of multiplication in a very short period [35]. Walia et al. [36] recorded that applications of 0.75 mg L^{-1} BAP + 0.25 mg L^{-1} in culture media gave maximum number of shoots per explant and shoot length in grown shoot explant of potato. Molla et al. [37] noted that higher shoot length, number of leaf per shoot and stem diameters with lower days require for shoot appearance were observed in application of 5.0 mg L^{-1} Zeatin in potato which regenerated through internode explant. Applications of 3.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA reported highest callus induction, diameter of callus and weight of callus with lowest days for callus induction when potato plant regenerated through stem node explant which studied by Kumlay and Sezai [24]. Another experiment in potato is carried out by Srivastava et al. [38] and they recorded that K. Giriraj cultivar have higher micro tube potential as compare to other cultivars when it regenerated through nodal cutting explant.

8.2 Tomato

Tomato is a highly popular crop for its rich nutritional values and constitutes an integral part of daily diets for substantial portion of human population of the world. Tomato production is declining day-by-day due to the attack of several insect-pests, fungal, bacterial, viral diseases, and nematodes. Because of limitations in conventional breeding methods and increasing demand of this important crop for fast growing population, large scale propagation of tomato through plant tissue culture techniques becomes highly significant. Hence, the development of germplasm, which may be resistant to these pests, is a need of the hour, which in a shortest period can be possible through tissue culture technique [39]. Alicja [40] reviled that all growth and biochemical parameters were recorded maximum in applications of 50 mg L^{-1} Silver nanoparticles in modified MS mdium. Jawad et al. [41] studied that application of 0.5 IBA + 0.5 IAA recorded higher root formation percent in node and ST explant. Sharma et al. [42] also experimented that applications of 2.0 mg L^{-1} BAP + 0.5 mg L^{-1} IAA concentration in culture media were found superior for number of shoot per explant and shoot regeneration percent in cotyledon explant. Addition of 3.0 mg L^{-1} BAP + 0.25 mg L^{-1} NAA in modified MS medium give utmost dry weight and fresh weight of callus at 15,30 and 45 days after culture which observed by Papry et al. [25].

8.3 Chilli

“Chillies are cultivated for vegetables as well as condiments and also used around the world as sweet peppers, pungent chilli peppers, or as a source of dried powders of various colors” [43]. To meet the increasing demand for the crops, faster propagation techniques for mass multiplication have become imperative. Tissue culture methods provide a way for the asexual multiplication of chilli plants as the plants lack natural vegetative propagation. Therefore, *in vitro* culture followed by gene transfer could be an easy, efficient and economic means for obtaining large number of disease-free, consistently uniform and true-to-type plants within a short span of time to improvement of yield and quality of chilli [27]. Dong Phuong [44] recorded that the shape of anther was too much change and too many anther was started to appear as small white protuberances during ebryogenesis with applications of 2.0 mg L^{-1} Kinetin in culture medium. In vitro regenerated plants Jalapeno cultivar of chilli perform superior in field conditions as compare to those plant which developed through seed which recorded by Bustos et al. [45]. Sanatombi and Sharma [46] reviled that maximum shoot length, number of roots and root length recorded with 0.5 IAA concentrations in medium.

8.4 Brinjal

Brinjal (*Solanum melongena* L.) is economically important vegetable crop grown in the sub-tropics and tropics. Brinjal is infected by many insects and diseases, but among them fruit and shoot borer (FSB) is major, causing yield losses of 60-70% even after repeated insecticidal sprays. The seed-borne pathogens of previous

year can be perpetuated over the generations with symptoms expressed. To overcome this situation, plant tissue culture offers an efficient method for pathogen free materials and germplasm preservation of plants [47]. Minimum day to callus initiation and maximum callus initiation percentage were recorded in brijal plant regenerated through Hypocotyl explant with applications of 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} BAP in culture medium which studied by Kaur et al. [26]. Puja et al. [48] reviled that applications of 1 mg L^{-1} IBA in MS medium give maximum number of roots and root regeneration percentage.

8.5 Okra

Okra (*Abelmoschus esculentus* L. Moench) is belongs to the family Malvaceae. Conventional propagation of okra in a large scale is limited due to bacterial, fungal, viral disease and seed dormancy. Hence, micro propagation offers great potential to increasing the commercial availability of okra. Rizwana et al. (2018) observed that higher shoot proliferation percent and number of shoot per explant were recorded with applications of 2.0 mg L^{-1} BAP in medium in cotyledonary node explant. Maximum shoot regeneration percent and length of shoot recorded by application of 1.0 mg L^{-1} IBA in apical shoot explant which studied by Dhande et al. [49].

8.6 Cucumber

Sultana et al. [29] studied that leaf disc explant of cucumber recorded maximum callus induction percentage and dry matter accumulation as well as minimum days to callus initiation with application of 2 mg L^{-1} 2,4 - D in culture medium.

8.7 Ivy Gourd

Amin et al. [30] reviled that the *In vitro* regeneration of ivy gourd through nodal segment explant noted maximum number of bud sprouted, shoot bud induction, number of shoot per explant and culture establishment index in media supplemented with 3.0 mg L^{-1} BAP and 0.1 mg L^{-1} NAA.

8.8 Pointed Gourd

Application of 2.0 mg L^{-1} BAP in culture medium recorded maximum shoot induction percent, number of shhot per explant and shoot length in both nodal explants (shoot tip and nodal segments) which experimented by Malek et al. [31]. Uddin et al. [50] observed that the maximum multiple shoot regeneration were recorded in media containing 2.0 mg L^{-1} BAP and 0.1 mg L^{-1} NAA.

9. CONCLUSIONS

Tissue culture has undoubtedly revolutionized vegetable crop production by offering numerous advantages such as rapid multiplication, disease-free plants, genetic uniformity, conservation of rare species, and season-independent production. As the demand for vegetables continues to rise, the adoption of tissue culture techniques is expected to increase further. However, it is crucial to strike a balance between tissue culture and traditional propagation methods to maintain genetic diversity and preserve the natural variability of vegetable crops. With ongoing research and advancements in tissue culture technology, the future of vegetable crop production looks promising, ensuring food security and sustainability for generations to come.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Hussain A, Ahmed I, Nazir H, Ullah I, Plant tissue culture: Current status and opportunities. Rece. Adv. in Pl. *In vitro* Culture. 2012;6(10):1-28.
2. Dangla HR. Plant tissue culture: Historical developments and applied aspects. Resonance. 2012;17(8):759-767.
3. Asmita VG, Singh SK, Ritu G. Plant tissue culture: A review. J. Pharma. Res. and Edu. 2017;2(1):217-220.
4. Thorpe T. History of plant tissue culture. Pl. Cell Culture Protocols. 2012;9-27.
5. James DC, Gray DJ, Robert NT. History of plant tissue culture and cell culture. Pl. Tissue Culture Concepts and Laboratory Exercises. 2018;11-17.
6. Thorpe TA. History of plant tissue culture. Molecular Biotechnol. 2007;37: 169-180.
7. Arie A. Plant tissue culture and biotechnology: Perspectives in the history and prospect of the international association of plant biotechnology. *In vitro* Culture and Devlopmental Biol. Pl. 2019;55(5): 590-594.
8. Shahzad A, Sharma S, Parveen S, Saeed T, Shaheem A. Historical perspective and

basic principles of plant tissue culture. Pl. Biotechnol. 2017;1-36.
9. Haberlandt G. Culture experiment with isolated plant cells. Math Naturwiss. 1902;**111**: 69-92.
10. Attila F. Callus, dedifferentiation, totipotency, somatic embryogenesis: What these term means in the era of molecular plant biology. Frontiers in Pl. Sci. 2019;10: 536.
11. Akira I, Mita K, Nonaka S, Momoko I, Chie K, Hirosi O. WIND 1 based acquisition of regeneration competency in Arabidopsis and rapeseed. J. Pl. Res. 2015;128: 389-397.
12. Pramesh D, Baranwal VK. Productions of virus free garlic through meristems tip culture after solar or hot air treatment of cloves. The J. Hortic. Sci. and Biotechnol. 2015;90(2):180-186.
13. Verma N, Ram R, Hallan V, Kumar K, Zaidi AA. Production of cucumber mosaic virus free chrysanthemums by meristems tip culture. Crop Protection. 2004;23(5): 469-473.
14. Teodora I, Grozeva S, Rodeva V. Anther culture in pepper in vitro. Acta Physiol. Pl. 2011;33: 1559-1570.
15. Maria AG. Anther culture for haploid and double haploid productions. Pl. Cell Tissue and Organ Culture. 2011;104: 283-300.
16. Fathi H, Jahani U. Review of embryo culture in fruit trees. Annals of Bolo. Res. 2012;3(9): 4276-4281.
17. Filova A. Production of secondary metabolities in plant tissue culture. Res. J. Agril. Sci. 2014;46(1): 2-8.
18. Navratilova B. Protoplast culture and protoplast fusion focused on Brassicaceae: a review. Hortic. Sci. 2004;31(4):140-157.
19. Williams EG, Maheshwaran G, Hutchinson JF. Embryo and ovule culture in crop improvement. Pl. Breeding Rev. 1987;5:181-236.
20. Thomas E. Biotechnology applications of plant callus culture. Engineering. 2019;5(1):50-59.
21. Emmanuel DB, Gali AI, Andrew F, Samuel A. Callus culture for production of therapeutic compounds. American J. Pl. Biol. 2019;4(4):76-84.
22. Ahloowalia BS, Prakash J, Savangikar VA, Savangikar C. Plant tissue culture. Low Cost Options for Tissue Culture Technol. In Devloping Countries. 2004;1:3-10.
23. Singh V, Tyagi A, Chauhan P, Kumari P, Seema K. Identification and prevention of bacterial contamination on explant used in plant tissue culture labs. Alcohol. 2011;3 (1):1-722.
24. Kumlay AM, Sezai E. Effect of different plant growth regulators on callus induction from stem node explant of potato. J. Biotech. and Biotech. Equipment. 2015;29(6):1075-1084.
25. Papry M, Ahsan SM, Sayeed S. Effect of different concentrations of BAP and NAA on stem explant for callus proliferation of tomato at different days after culture. Asian J. Med. and Biol. Res. 2015;1(3):589-599.
26. Kaur G, Rattan P, Pathania A. Effect of different concentrations of NAA and BAP in MS media on in vitro regeneration of callus of brinjal cv. Navkiran**.** J. Krishi Vigyan. 2020;8(2):166-173.
27. Ashrafuzzaman, M, Hossain MM, Razi M, Haque MS, Shahidhullah SM, Uz-zaman S. Regeneration potential of seedling explants of chilli. African J. Biotechnol. 2009;8(4):1-10.
28. Rizwan HM, Irshad M, He BZ, Liu S, Debnath B, Mitra S, Li M, Sun YT, Qui DL. Silver nitrate boosted high frequency multiple shoot regeneration from cotyledonary node explants of okra. App. Ecol. and Environ. Res. 2018;16(3):4-12.
29. Sultana H, Nahar L, Hossain M, Ghos K, Biswas S. Days to callus initiation and callus induction percentage in various explants of cucumber on MS medium supplemented with various concentration of 2,4-D. European J. Biol. and Biotech. 2021;2(5):2684-5199.
30. Amin M, Rao D, Kumar R, Salomi S. Percent shoot bud induction and culture establishment index as influenced by different growth regulator concentrations for *In vitro* culture establishment of ivy gourd. Int. J. Curr. Microbiol. and App. Sci. 2020;9(7): 2273-2286.
31. Malek MA, Miah B, Amin M, Khanam D, Khatun M. Effect of different concentrations of BAP on shoot induction and proliferation from nodal segment and shoot tip explants of pointed gourd. Bangladesh J. Agril. Res. 2007;32(3):461-471.
32. Abobkar IS, Elshahed AM. Plant tissue culture media. Rec. Adv. in Pl. *In vitro* Culture. 2012;1: 30-40.
33. Barbara R, Pistelli L, Bertoli A, Pistelli L. Plant cell culture: Bioreactors for industrial production. Biofarms for Nutraceuticals. 2010;1:203-221.

34. Maharaj KR. An introduction to plant tissue culture. Oxford and IBH Publishing. 2002;10-15.
35. Mohapatra PP, Batra, VK. Tissue culture of potato: A trview. Int. J. Curr. Microbiol. And App. Sci. 2017;6(4):489-495.
36. Walia A, Anil B, Samnotra RK, Gaur A, Pankaj P. In vitro propagation studies for late blight resistant potato variety Kufri Girdhari. Biol. Forum An Int. J. 2021;13(3): 375-380.
37. Molla MH, Nasiruddin KM, Haque MS, Khanam D. Effect of different concentrations of Zeatin on in vitro regeneration of potato from internode explant at 28 days. Bangladesh J. Pl. Breed. and Genet. 2017;24(1):07-14.
38. Srivastava AK, Diengdoh LC, Rai R, Bag TK, Singh BP. In vitro micro propagation and micro tuberization potential of selected potato varieties. Indi. J. Hill Farming. 2012;25(2):14-17.
39. Oceania C, Doni T, Tikendra L., Nongdam P. Establishment of efficient In vitro culture and planted generation of tomato and development of synthetic seeds. J. Pl. Sci. 2015;10(1):15.
40. Alicja T. Effect of Silver Nanoparticles application on the growth and biochemical parameters of tomato seedlings after three weeks of in vitro culture on the modified MS medium. Materials. 2021;14(1):5340-5355.
41. Jawad AJ, Musa T, Fethi AO. Effect of different plant growth regulators on *In vitro* propagations of endangered plant yellow tomato. Int. J. Agric. Forest. and Life Sci. 2020;4 (1):92-98.
42. Sharma SK, Rajyaguru R, Gajera HP, Tomar RS, Kheni J, Bhalani H, Golakiya BA. Optimization of In vitro regeneration protocol for tomato cv. Junagadh Tomato-3. J. Cell and Tissue culture Res. 2021; 21(2): 7081-7090.
43. Ravishankar GA, Suresh B, Giridhar P, Rao SR, Johnson T. Biotechnological studies on capsicum for metabolite production and plant improvement. Hardwood Acadamic Publishers, U. K. 2003;98-128.
44. Dong Phuong NT. Effect different concentrations of kinetin on direct embryogenesis from anther culture of chilli at 14 DAC. J. Sci. 2021;11(1): 3-10.
45. Bustos GV, Aguado GA, Castaneda GC, Rincon HA. Agronomic performance of *In vitro* regenerated and seed derived plant of different cultivars of chilli under greenhouse condition. In Vitro Cell Dev. Biol. Pl. 2009;45:650-658.
46. Sanatombi K, Sharma GJ. Effect of auxins on rooting and elongation of *In vitro* induced shoot buds from shoot tip explant of chilli cv. Morok Amuba. J. Food Agric. and Environ. 2007;4(1): 205-208.
47. Mir KA, Dhatt AS, Sandhu JS, Sidhu AS. Effect of genotype, explant and culture medium on organogenesis in brinjal. Ind. J. Hort. 2011;68(3):332-335.
48. Puja R, Kumar S, Salgotra RK, Samnotra RK, Sharma F. Devlopment of interspacific hybrid in eggplant through embryo rescue technique. Pl. Cell Tissue and Organ Culture. 2015;120:379-386.
49. Dhande GA, Vijay MP, Raut RV, Rajput JC, Ingle AG. Regeneration of okra via apical shoot culture system. Africal J. of Biotechnol. 2012;11(86):15226-15230.
50. Uddin S, Huq S, Islam S, Khan S. Effect of different concentrations of BAP on shoot induction and proliferation from nodal segment and shoot tip explants of pointed gourd Bangladesh J. Agril. Res. 2004; 47(2):217-222.

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