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Advances In Tissue Culture And Somatic Embryogenesis In Fruit Crops.

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Abstract: -

In modern era, traditional propagation techniques are overpowered by the advanced in vitro culture, that offers the highest commercial value of the plants, due to its rapid generation of clonal plants. Additionally, it is very helpful in supporting the preservation of germplasm, the creation and upkeep of disease-free planting material, and additions to standard plant advancement techniques such as plant genetic engineering. Micropropagation approaches are frequently based on tissue culture techniques. The main objectives of these techniques are to increase the effectiveness and efficiency of plant regeneration. The maximum and minimum use of a few fundamental concepts related to plant tissue culture *in vitro*, particularly micropropagation, have been discussed in this article. These concepts include types of tissue culture and recent advances for general studies of micropropagation, callus induction, shoot and root induction, and somatic embryogenesis (SE). Additionally, somatic embryogenesis is a significant breakthrough in plant vegetative propagation technology, with implications for high-value horticulture crops and tree breeding. Within a limited amount of time and resources, the technique depicts the mass proliferation of new and elite types. Conventional breeding techniques multiply slowly and rely on natural processes, but somatic embryogenesis may help better in terms of quick mass production of plantlets in a limited time. There are several practical and economic uses of the rapidly evolving somatic embryogenesis techniques, especially in the area of *in vitro* clonal micropropagation. The low frequency of somatic seedling recovery and the poor germination of its embryos have hampered the application of somatic embryogenesis in fruit crop improvement. In this review, the advances achieved till date, have been elaborated more efficiently, which can effectively utilized for achieving future perspectives.

Key words--- Somatic embryogenesis, Tissue culture,

A. Introduction: -

Micropropagation refers to the aseptic *in-vitro* cultivation of cells, tissues, organs, and their constituent parts under certain chemical and physical conditions (Gupta *et al.,* 2020). Considered to be the plant regeneration method with the least genetic instability and soma clonal variation is micropropagation through the growth of axillary buds (Encina *et al.*, 2023). Plant Tissue Culture is important for agriculture and plant breeding since it enhances crop production through micropropagation, hybridization, synthetic seed production, haploid culture, and pathogen

eradication (Gulzar *et al*., 2020). In order to enable micropropagation from altered passion fruit cells, regeneration systems have been constructed from the shoot apex, leaf discs, nodal, and hypocotyl segments, as well as the roots (da Silva *et al.,* 2021). For the study of the morphophysiological, biochemical, and molecular processes that take place during the development of higher plants, somatic embryogenesis makes it simple to manipulate and control the culture conditions (Gerdakaneh *et al*., 2021). Protoplasts are the parts of plant cells that are enclosed by their cell walls. By physically or by an enzymatic digesting process, the cell membrane can be removed, allowing for plasmolysis and separation. The preservation of this precious biological resource would greatly benefit from the development of a fruitful propagation mechanism. (Cobo *et al*., 2018). For the sustainable and ongoing synthesis of bioactive chemicals from this threatened species, callus culture is an option (Koufan *et al*., 2020). A thorough investigation of the genetic and epigenetic factors causing callus induction is warranted, as well as the general use and understanding of molecular mechanisms (Iqbal *et al*.,2019). Somatic hybridization is the *in vitro* fusing of plant protoplasts produced either from somatic cells of the same plant or from two genetically distinct plants (Chakraborty *et al*., 2016). Indirect and Direct SE relate, respectively, to the presence or absence of a callus formation phase (Joshi *et al*., 2013). Since the first reports on carrot in 1958, somatic embryogenesis (SE) has been reported in various plant species (Solís-Ramos *et al*., 2012). Somatic Embryogenesis (SE) can arise in vitro either directly from the explant without any intermediary callus stage (direct SE) or indirectly from the callus (indirect SE). In contrast to primary SE created from explant cells, somatic embryogenesis can also be directly triggered or by callus in the culture of somatic embryos. This process is referred to as secondary SE (Solís-Ramos *et al*., 2012). When mass producing synchronized developmental stages of somatic embryos, somatic embryogenesis (SE) is more advantageous than zygotic embryogenesis since it is simpler to monitor and manage the cultures (Botini *et al.,* 2021). One of the most important components in the induction of somatic embryogenesis in plants is growth regulator, which has been detected in around 80% of somatic embryogenesis (SE) induction techniques. Somatic embryogenesis is caused by variations in the concentration of known growth regulators, specifically auxins and cytokinin, in the plant tissue culture media (Gerdakaneh *et al*., 2021). Axillary shoot culture and plantlet recovery after shoot regeneration from somatic embryos are two other methods that have been used. After genetic transformation operations in this species, regeneration, and plant recovery frequently take place (Botini *et al*., 2021). The greatest potential for totipotent growth is seen in in vitro tissue culture, where a wider range of explants may be stimulated to undergo embryogenesis, including haploid cells of the male and female gametophyte (gametophytic embryogenesis) (Horstman *et al*., 2017). The ability to create plant material without regard to the time of year or the plant's growth cycle is made possible by tissue culture. Additionally, by altering the environment, the genes, and the chemicals in a tissue culture system, it is possible to increase the concentration and purity of therapeutic (Menbari *et al*., 2021). The development of embryogenic cell suspension cultures requires the selection of large quantities of high-quality embryogenic callus; embryogenic calli and transparent pro embryos should make up the initiating tissues, whereas compact calli, cotyledonary stage embryos, and meristematic globules should be avoided (Kong *et al*., 2020). Plant protoplasts are living, naked cells surrounded by a plasma membrane that, in the right culture conditions, can lead to the regeneration of plants (Xiangmin *et al.,* 2019). As a source of protoclonal variation, also known as soma clonal variation, protoplast culture could be of great benefit in enhancing the existing germplasm by producing variations that can be applied in breeding programs (Barceló *et al.,* 2019).For many crops, in vitro anther culture is the preferred method of regeneration because to the high responsiveness of explants, the ability to stimulate somatic embryogenesis, and the

potential to regenerate haploid plants from microspores. Many variables, including the anthers physiological state, stage of differentiation, endogenous and external hormonal signalling, and culture medium composition, affect the anthers reactivity in *in vitro* situations (dos Santos *et al.,* 2021). Meristem culture is a popular technique for removing viruses from horticultural plants. Meristems are thought to be virus-free because of the uneven distribution of viruses in plants. Determining out the meristem tip's size might be helpful in improving the effectiveness of viral eradication. (Vivek *et al*., 2018).

Different types of Plant Tissue Culture

Tissue culture can be divided into various types as described in Table no 1 and it depends upon the plant part which is used explant for preparing the culture.

Table 1. Types of Tissue Culture

Sr.no	**Types of tissue culture based on the part of plant used as explant**	**Reference**	**Sr No**	**Types of tissue culture based on the part of plant used as explant**	**Reference**
1	Apical meristem culture	(Gaikwad *et al.,* 2020)	7	Root & Shoot tip culture	(Gupta *et al.,* 2020)
2	Axillary bud culture	(Gaikwad *et al.,* 2020)	8	Flower organ culture	(Gupta *et al.,* 2020)
3	Callus culture	(Gaikwad *et al.,* 2020)	9	Fruit organ culture	(Gupta *et al.,* 2020)
4	Cell Suspension culture	(Gaikwad *et al.,* 2020)	10	Anther culture	(Gupta *et al.,* 2020)
5	Protoplast culture	(Gaikwad *et al.,* 2020)	11	Somatic Embryogenesis	(Singh *et al.,* 2019)
6	Embryo culture	(Gaikwad *et al.,* 2020)			

1.Apical meristem culture

The most promising method for removing viruses from plant tissue culture as well as for replicating a chosen plant material true to its type and exhibiting the same agronomic properties is plant regeneration through the apical meristem. Shoot tip explants with a single apical meristem responded to regeneration less well than those with two axillary meristems (Sahraroo *et al*., 2019). The shoot apical meristem (SAM) and root apical meristem (RAM) are formed at the highest point of embryogenesis, while the lowest point is situated at either the shoot top or the root bottom (Xue *et al*., 2020). Larger meristems could not completely eliminate the viruses under study (Vivek *et al*., 2018). The details of *in- vitro* studies of different fruit crop by using apical meristem are given in table no 2.

Table 2. *In-vitro* studies of different fruit crop by using apical meristem

Species cultivar	**Explant used**	**Culture medium, PGR,**	**Culture condition**	**Result**	**References**
Purple passion fruit	Apical meristem	MS medium, BA, NAA, sucrose,IBA		Root formation was satisfactory with IBA @ 0.4 and 0.6 mg/l	Prammanee *et al*., 2011
Apple	Apical meristem	MS medium supplemented with BA (1.0 mg/l), IBA (0.05 mg/ l) and GA3 (0.1 mg/l)	Temp 25 ± 2°C. It was a kept at 16-hour light and 8-hour dark photoperiod.	Mostly explants are established, and more number of meristems proliferated with BA (0.5 mg/l), IBA (0.08 mg/l), and GA3 concentration	Vivek *et al*., 2018

Fig	Apical meristem	MS medium, BA NAA, and IBA was used for shoot regenerating.	pH 5.8 culture was kept at 25 ± 2 °C under 16 h photoperiod,	More numbers of rooted micro-shoots were observed with 1.5mg/l IBA	Sahraroo *et al.*, 2019
Wild Guava	Apical meristem	JADS culture medium ,myo-inositol, PVP ,sucrose, agar	pH 5.7, autoclaving at 121 °C for 20 min	The culture media containing 2.22 μM BAP exhibited the highest responsiveness and the maximum number of shoots per explant.	dos Santos *et al.*,2021
Strawberry	Apical meristem	MS basal medium enhanced with 0.2 mg/l IAA and 1 mg/l BA	Incubated under 16/8 h light/dark cycle at 25 ± 2°C by cool white fluorescent lights	The medium added with 500 mg/l polyvinylpyrrolidone (PVP) produced the maximum results.	Hemmati Asl, S., & Dorani, E. (2023)

2.Axillary bud culture.

One of the various methods for cultivating plants in vitro is xylem bud culture. One of the main issues with in vitro culture systems is the occurrence of somaclonal variation among subclones of a single parental line. Numerous characteristics can be associated with this variant, such as transposon and retrotransposon activation, sequence modifications, cytological abnormalities, frequent qualitative and quantitative phenotypic alterations, and gene silencing and activation. Therefore, somaclonal variation is taken into account at both the genetic and epigenetic levels. The axillary bud culture approach is commonly thought to present the least danger of genetic instability because of meristems' greater resistance to genetic alterations compared to disordered tissues (Ngezahayo F., & Liu, B. 2014). These techniques still have the potential to produce more plant material faster, cheaper, and use less labour. Over four weeks, these *in vitro* propagation techniques produce, on average, four to six shoots from a single source shoot. Considered to be the *in vitro* plant regeneration method with the least genetic instability and soma clonal variation is micropropagation through the growth of axillary buds. Papaya species have benefited greatly from the widespread use of tissue culture-based micropropagation, which provides advantages over traditional propagation techniques. Various strategies have been used, including axillary shoot cultivation. Considered to be the *in vitro* plant regeneration method with the least genetic instability and soma clonal variation is micropropagation through the growth of axillary buds (Encina *et al.*, 2023). The details of *in-vitro* studies of different fruit crops using axillary bud are given in table no 3.

Table 3. *In-vitro* studies of different fruit crop by using Axillary bud to develop culture

Species, Cultivar	Explant used	Culture medium, PGR,	Culture condition	Result	References
Apple	Axillary bud	MS medium , BA and Kn, sucrose, agar, vitamins, NAA	Minimum and maximum temperatures from 6°C to 12°C and 17°C to 25°C	Maximum calli formed at medium supplemented with 2g/l NAA in combination with 2 mg/l cytokinin and higher root length were recorded at 0.25 mg-L of IBA.	Itana *et al.*, 2022
Papaya cv. Solo	Axillary bud	MS medium supplemented with NAA, IBA, and adenine hemisulphate	Cultures were incubated at 25 ± 1 ◦C under a 16 h & pH 5.7	The rooting medium and the substrate to MS liquid plus 1 mg L−1 IBA in vermiculite achieved best acclimatization.	Encina *et al.*, 2023

Caucasian apple	Axillary bud	MS medium, glycin, pyridoxin·HCL, thyamine·HCL, nicotinic acid, BA, GA_3, IAA, sucrose, plant agar	16-hour photoperiod, 45% relative moisture, and 25 ± 1°C temperature.	The results showed that a rooting percentage of 77.8% was obtained on half strength, LS medium in combination with 0.9 mg L−1 IBA	Amirchakhmaghi *et al.*,2019
Blackberry	Axillary bud	BA , NAA, GA_3, Agar	pH 5.8 25⁰C under 16 h light and 8 h dark cycle.	The cultivar "Chester Thornless" had the maximum rate of proliferation, yielding 9.66 shoots on a medium containing a mixture of 2 mg/l BA and 0.2 mg/l IBA.	KEFAYATI *et al.*,2019

3. Callus Culture

In order to undergo genetic change, callus induction and subsequent plantlet regeneration are necessary (Ikeuchi *et al.*, 2019). The change of cell fate from the somatic state to pluripotency is necessary for callus induction (Ikeuchi *et al.*, 2016). Numerous explant types, including leaves, mature embryos, stems, and immature embryos, can be used to generate callus; nevertheless, the embryogenic potential of various calli varies greatly (Wu *et al.*, 2020). Cotyledon explants have demonstrated high frequency regeneration as callus induction materials because of their increased meristematic tissue composition, less contamination, and enhanced browning. All things considered, explants of leaves and cotyledons are the main sources of callus induction and ensuing plantlet regeneration (Gao *et al.*, 2023). To study plant genetics and improve plant varieties, callus is a desirable target explant (Cimen *et al.*, 2020). The detailed i*n- vitro* studies of different fruit crop by using apical meristem are given in table no 4

Table 4. *In-vitro* studies of different fruit crop by using callus to develop culture

Species, Cultivar	Explant used	Culture medium, PGR	Culture condition	Result	References
Citrus	Ovules extracted from the immature fruits	MS, vitamins, malt extract, sucrose, and supplemented with 2,4-D, BA, and Kn.	pH 5.8, autoclaving at 121°C for 15 min.	The highest callus initiation using EME (MT basal medium + 0.5 gL-1 malt extract) + Kn (1.0 mg L-1) were observed	Cimen *et al.*, (2020).
Argan	Leaf and mature fruit samples of argan	Gallic acid and (DPPH), (BF3), 2,4-D, NAA,(KOH), (NaOH), hexane, and ethanol Sucrose, (IAA) ,(BA) and Agar	pH 5.7 autoclave at 121 °C for 20 min. cultures kept at 25 °C	Combination of 1 mgl−1 NAA and 1 mgl−1 2,4-D resulted in the highest callus formation rate	Koufan *et al.*, 2020
Apple	Calli obtained from fruit	MS salt, thiamine, Myoinositol, Glycine Nicotinic acid Pyridoxine,	25 ± 1∘C, and a photoperiod of 16 h light: 8h darkne	It was also discovered that MS medium containing 2,4-D + BA produced the finest callus quality, which was friable and white-yellowish.	MenBAri *et al.*, 2021

			ss and light		
Quince	Fruit pulp was used establish callus	MS media, GB_5, sucrose, BA, NAA,	pH 5.8 Incubated @ 25 ± 2 ∘C in the dark	The ideal growth, maintenance, and induction conditions for quince callus biomass generation were determined to be B5 medium plus 1.77 µM BA and 5.40 µM NAA.	De Bellis *et al.*, 2022
Apple	Fruit pulp was used establish callus	MS culture, B_5 medium, sucrose, BA, NAA, 2,4-D	pH 5.8, Incubated at 25 ± 2 °C in dark	When combined with 2.0 mg/L BA and 2.0 mg/L NAA, MS produced the maximum amount of biomass from GD pulp explants, whereas B5 combined with 2.0 mg/L BA and 0.2 mg/L 2,4-D produced the highest amount of biomass from MRM pulp explants.	Verardo *et al.*,2016
Feijoa	Fruit pulp	Cultures were grown on MS; and B5 media, sucrose, BA, NAA	pH 5.8, Incubated in the dark at 25 ± 2 °C.	The combination of B5 medium containing 0.89 µM BA and 10.7 µM NAA produced the maximum biomass formation of calluses.	Verardo *et al.*,2019

4. Protoplast culture

Plant protoplasts were isolated for the first time in 1960 by treating cells with enzymes like cellulase, pectolyase, and hemicelluloses that would dissolve cell walls (Sharma *et al.*, 2014). Single, wall-free cells known as protoplasts can divide, multiply, and differentiate by developing calluses before giving rise to the full plant. A flexible system for conducting functional genetic studies and researching a variety of biological functions, including cell shape, membrane function, and hormone signalling, is the protoplast. Protoplasts can also be a valuable biotechnological tool in breeding operations, particularly for vegetatively propagated species like strawberries that have significant levels of polyploidy and heterozygosity. Leaf mesophyll, embryogenic calli, embryogenic suspension cultures, and nonembryonic calli are only a few examples of diverse tissues from which protoplasts can be recovered. From friable embryogenic ovule-derived calli maintained on a growth-regulator-free medium, the protoplasts can be separated (Cimen *et al.*, 2020). By using protoplasts, it is possible to generate combinations of core genes and/or unique cytoplasmic organelles, with the resulting hybrid cells and asymmetric hybrid cells being one in the main applications of cytoplasmic fusion. The protoplast culture can be very valuable since the variance gained in plants regenerated from protoplasts is far more than the variation obtained in plants from structured growth cultures. As a source of proline variation, also known as soma clonal variation, by enlarging the genetic material already present and producing variants that may be employed in breeding initiatives (Barceló *et al.*, 2019). Each protoplast has the potential to regenerate a new wall and perform recurrent mitosis division to produce daughter cells, which may then be used to regenerate viable plants through tissue culture. This process is dependent on the protoplasts receiving the appropriate chemical and physical stimuli. Many species have protoplast-to-plant systems accessible, and their use has been well documented in the literature. Remarkably, not much has changed in terms of the fundamental protocols for protoplast isolation since they were initially documented. Nonetheless, a significant advancement has been achieved in the quantity of species for which protoplast-to-plant systems are available (Davey *et al.*, 2005).

The detailed *in- vitro* studies of protoplast culture in different fruit crop is given in table no 5

Table 5. In-vitro studies of protoplast culture of different fruit crop

Species, Cultivar or Rootstock	Explant used Size and sourced	Culture medium, PGR, and Additives	Culture condition	Result	References
Strawberry	Shoots	MS medium supplemented with 10 g l−1 sucrose, 2 mg l−1 BA and 0.3% Gelrite for 3-4 subcultures	16 h photoperiod, 25 ± 2 °C	Higher shoot regeneration rates in protoplasts cultured in a medium supplemented with TDZ in comparison with BA,17% of callus regenerating shoot	BArceló *et al.*, 2019
Apple	Leaf mesophyll	MS medium, NAA, BA, mannitol, pectinase, sucrose, KH_2PO_4, KNO_3, $CaCl_2.2H_2O$: $MgSO_4.7H_2O$, KI, $CuSO_4.5H2O$	pH: 5.8, Incubation periods for 16 h	In the CPW medium, a 20-hour incubation time with a pore size of 25 µm resulted in a maximum yield of protoplast production.	El-Gioushy *et al.*, 2019
Papaya	Shoots	MS medium, Agar, Sugar, Vitamins, BA, NAA, GA_3	The cultures were kept at 25±1°C Incubation period 16-hour photoperiod.	2.0 mg/L of IBA was found to have the maximum root induction response (86%) and roots suited for acclimatization.	Waidyaratne *et al.*,2023

5. Anther Culture.

Both internal and exterior cues have an impact on the embryogenic response of anthers in culture. The genotype, physiological state and growth circumstances of donor plants, gamete developmental stage, pre-treatment of flower buds, media, and incubation conditions, as well as the interactions among these parameters, all have a substantial impact on the anther response to in vitro culture. It is likely that anthers from different species, as well as different cultivars within a species, may require quite diverse circumstances in order to go through an embryogenic pathway and mature. There is no one fixed setting or technique for creating plants from anther cultures. Unlike gynogenesis, which uses female gametes like ovules, androgenogenesis, which uses male gametes such anthers, microspores, or pollen to generate haploids, is androgenesis. The basic concept is to prevent an immature gametic cell from maturing into a gamete and instead induce it to grow into a haploid plant (Das *et al.,* 2018).Haploid embryos were created using parthenogenesis, pseudogamy, and broad hybridization under *in vivo* conditions. The haploid embryo must be saved using embryo rescue techniques, cultured further to make haploid, and then chromosome doubling must be carried out to produce doubled haploids. For the creation of haploid cells, androgenesis (anther and microspore culture) and gynogenesis (ovary and ovule) were employed, with androgenesis being the preferred method (Das *et al.,* 2018). Another triploid papaya that was derived was divided into tall, semi-tall, and dwarf trees. The triploid strains and the diploid control did not significantly differ in all morphological characteristics. The fruit-bearing triploid strains parthenocarpically. Each variety of dwarf and semi-dwarf had large yields and good bearing. combined with low stature and the triploid strains have a high parthenocarpy fruit output could be used to produce fruit commercially (Rimberia, *et al.,*2007). In androgenesis via anther culture, male gametophyte cells or their progenitors diverge towards a sporophytic phase, resulting in the formation of haploid embryos and plants (Vasanth *et al.*, 2017). When transplanted into a hormone-free media, embryos taken from another culture can grow roots and transform into plantlets. The detailed studies of anther culture in different fruit crops are given

below in Table 6-

Table 6. *In-vitro* studies of anther culture of different fruit crop

Species, Cultivar or Rootstock	**Explant used Size and sourced**	**Culture medium, PGR, and Additives**	**Culture condition**	**Result**	**References**
Wild passion fruit	Anthers	MS medium, B5 vitamins 0.01% (w/v) myo-inositol, 3% (w/v) sucrose, and 0.28% (w/v) h 4.5 µM BA and different conc 2,4-D	Temp 27±2 °C Incubated at 16-h photoperiod, pH 5.7±0.1,	More numerous only when anthers at the DS2 stage were cultivated with 18.1 µM 2,4-D and 4.5 µM BA.	da Silva *et al.*, 2021
Apple	Anther	10 µM BAP, 0.5 µM naphthaleneacetic acid (NAA), and 5.0% sucrose	Temp 25°C, Incubation at 16 h a day and 8 hours at night	The rate of shoot formation from anthers showed a high-low order of 'Senshu', 'SD', 'ASP', and 'Tsugaru'	Zhang *et al.*,2017

6. Somatic Embryogenesis

Somatic Embryogenesis is defined as process in which embryo-like structures are formed from somatic tissue and develop into whole plant (Savita *et al.*, 2015). The survival and development of regenerated plants depend on the cultivation environment and are genotype dependent during the complex process of somatic embryogenesis (Morais-Lino *et al.*, 2016). To regenerate clonal plants and create biotechnological breeding tools like transgenic utilized embryogenic cell suspension cultures, somatic embryogenesis is an alternate technique (Savita *et al.*, 2015). This process yields disease-free plantlets which can be applied for breeding, conservation, sanitation, and micropropagation (Meziane *et al.*, 2017). Organogenesis and somatic embryogenesis have generally been employed for transgenic plant regeneration. While organogenesis is the process by which plants regenerate by producing new organs (shoots and roots) on explants, somatic embryogenesis is the process by which bipolar embryos grow from somatic cells. Somatic embryogenesis seems to have several benefits over organogenesis, including the potential for high rates of multiplication, the capacity to scale up using bioreactors, and the ability to distribute using synthetic seeds. As such, somatic embryogenesis has been emphasized as a promising target for gene transfer (Hota *et al.*, 2019). Micropropagation by somatic embryogenesis acts as a powerful tool for genetic improvement of any plant species because of its single cell origin (Jha *et al.*, 2007). Synchronized generation, maturation, and conversion of embryos into plants are essential for propagation, particularly through somatic embryogenesis. Dedifferentiation, chromatin re-modelling, and gene expression programming in somatic cells are all necessary for the phenomenon of somatic embryogenesis to occur (Ali *et al.*, 2017). A typical somatic embryogenesis technique includes many stages such as callus induction, embryogenic callus multiplication, somatic embryos development, and germination. It seems to be influenced by the interaction of the genotype, explant source, media make-up, plant growth regulators, and explant stage at the start of culture. Nevertheless, because a variety of factors affect how an embryogenic callus differentiates, success with the same cultivar might still vary across years (Vasanth *et al.*, 2017). Citrus plants that were recovered *in vitro* via somatic embryogenesis (SE) from stigmas and styles were true to type, had a brief juvenile phase, and were free of the primary infectious agents (Meziane *et al.*, 2017). To create somatic embryos from embryogenic calli, solid culture media are typically utilized. These media are also useful for observing the many developmental stages of

somatic embryogenesis in date palm. Solid medium, however, cannot be employed to guarantee widespread dispersion. Due to this, every effort has been made to develop embryogenic cell suspension cultures that have successful plant regeneration and a high morphogenetic potential (Abohatem *et al.,* 2017). Plant growth regulators and carbohydrate sources can be changed in a culture medium to boost the rate of cell proliferation, greatly enhancing the pace of regeneration. Although cytokinin like 6-benzyladenine (BA) and kinetin are thought to be necessary for micropropagation processes like embryogenesis, what is necessary for the best regeneration depends on the genotype (Agisimanto *et al., 2019*). Modern breeding techniques for bananas must take into account somatic embryogenesis. However, somatic embryogenesis is labor-intensive and extremely genotype-dependent. Only a small number of banana cultivars, including the majority of EAHBs, have so far reacted to somatic embryogenesis. Notably, only a small number of cultivars that are receptive to the process have modest frequencies of somatic embryogenesis. To enhance somatic embryogenesis success rates in cultivars that exhibit poor sensitivity to the process, it is necessary to investigate novel methods for inducing somatic embryogenesis in resistant elite cultivars. There is mounting evidence that certain genes, such as transcription factors, control somatic embryogenesis in bananas. These genes are triggered by specific PGRs and environmental stimuli. Somatic embryogenesis and embryogenic cell suspension cultures developed rapidly, and breakthroughs in 1991 completely transformed the landscape. (Adero *et at.,* 2023*).* The exudation of phenols at higher levels from plant tissues will remove the darkness and resistant character of plant tissues, which will help with tissue regeneration from in vivo sources, which has been hindered by increasing microbial contamination. Citric acid, ascorbic acid, and polyvinylpyrrolidone (PVP) solution soaking and agitation of plant tissue in nutritive medium significantly reduced the phenolic exudation in the medium and helped to form plant tissue from explants from in vivo sources. Due to the lower production of phenolic compounds in seedlings, tall explant seedlings or greenhouse-grown plants (low light and low temperatures) produced less contamination and phenol (Singh *et al.,* 2019)

Type of Somatic Embryogenesis-- Direct somatic embryogenesis

In direct somatic embryogenesis, embryos are formed directly from the cell without the callus formation (Bhatia *et al.*,2015).

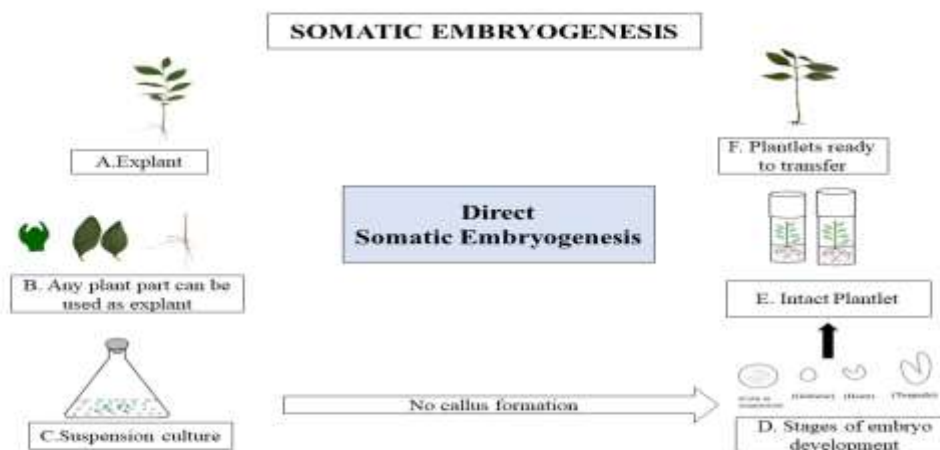

Fig 1. Steps of Direct Somatic Embryogenesis

Indirect somatic embryogenesis

In indirect somatic embryogenesis the callus is formed from the explant and then from the callus tissue embryo is formed (Bhatia *et al.*,2015)

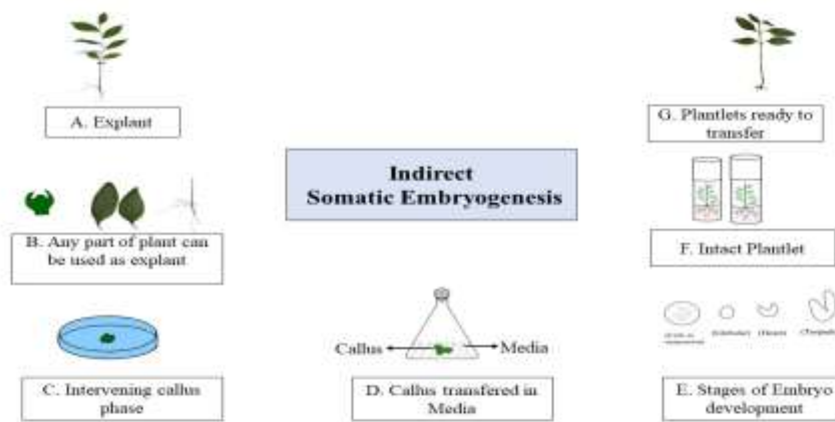

Fig 2. Steps of Indirect Somatic Embryogenesis

Table 6. *In-vitro* studies of somatic embryogenesis of different fruit crops

Crop name	Explant used	Culture medium, PGR	Culture Condition	Result	References
Pineapple	Crown leaf and Plantlet leaf	BAP + copper + Picloram on MS medium supplemented with vitamins B_5	Cultures were kept in the dark at 27±1°C 80% humidity for 4 to 6 weeks.	Medium MS with NAA (0.5 mg/l) + BAP (1mg/l) was highly influenced with 8.8 mature somatic embryos per explant.	Cacai *et al.*,2021
Strawberry	Leaf blade	MS medium containing 3 mg/L TDZ with 0.25 mg/L 2,4-D	Explants were cultured under a 16/8 h light/dark cycle at 25±2°c.	The maximum number of globular embryos per explant obtained for the cv. of Strawberry.	Gerdakaneh *et al.*, 2021
Passion fruit	Anther	2,4-D,4.5 µM BA, vitamins B_5, myo-inositol, sucrose, and Phytagel	Cultures grew for 60 days at 27±2°C, with 16-hour light, 8 hours dark, and 36 µmol m^{-2} s^{-1} irradiance.	Embryogenic calluses and embryos were more only when anthers were cultivated in 18.1 µM 2,4-D and 4.5 µM BA.	da Silva *et al.*, 2021
Kinnow mandarin	Nucellus tissues	MS medium , auxins, cytokinin's and malt extract, BAP	Cultures were kept at 25˚C with a photoperiod of 16 hours of light. Light intensity was maintained up to 2500 lux.	**Highest Somatic Embryo Induction:** Medium: MS, BAP:1 mg/l **Highest Somatic Embryo Maturation:** Medium:MS,2,4-D:5mg/l BAP: 1 mg/l	Hussain *et al.*, 2016
Guava	Immature ZE	MS medium enhanced with 500 mgL-1 malt extract, 6% sucrose, 400 mgL-1 L-glutamine, and 2.0 mgL-1 2, 4-D	Incubate at 25 ± 2 °C in dark for 4-6 weeks for induction. Somatic embryos were exposed to a 16/8 hr. photoperiod for plantlet conversion.	SE induction was achieved using explant on MS medium, 2, 4-D and L-glutamine and sucrose. Maximum no of cotyledonary embryos and matured embryos was achieved using MS medium supplemented with polyethylene glycol and sucrose	Bajpai *et al.*, 2016
Guava	endosperm and immature	Basal salts and vitamins supplemented with sucrose, casein	Cultures were maintained in darkness at 23 ±	Ghosi had the maximum rates of embryogenic callus as well	Ameri *et al.*, 2018

	cotyledon.	hydrolysate CH, PGR's like NAA and BA	1 °C.	as the highest rates of embryo maturation and germination, at 77.06% and 37.66%, respectively.	
Papaya	Immature seeds	MS supplemented with 30 g L− 1 sucrose 20 μM ,2,4-D; and 2.0 g L− 1 Phytagel	pH 5.8. dark at 25 ± 1 ◦C.	The number of somatic embryos at each developmental stage were evaluated during the maturation phase and each developmental stage	Botini *et al.*, 2021
Kinnow Mandarin	Leaves and Nucellar embryo	MT Basal medium with 0.5mg/l BAP and 0.5mg/l Kinetin	pH-5.7 cultures were kept at 25°C under 16/8 hr light/dark with white fluorescent lights.	Protoplast yield: 5.4 x 10^4/g FW from nucellar embryos, 17.3 x 10^4/g FW non-viable protoplasts from leaves.	Kazmi *et al.*, 2022
Pomegranate	Axillary shoot	MS and WPM, $CoCl_2$, Casein hydrolysate, NAA and IBA	Kept at 25 ± 2 °C, 16-hour photoperiod with white fluorescent tubes.	Maximum roots (5.60 ± 0.74) and longest (6.78 ± 0.52 cm) on Woody plant medium + 2000 mg−1 AC.	Desai *et al.*, 2018
Grape	Nodal segment and leaf disc	MS+2.0mgL-12,4-D+0.5mgL-1Kn+30.0gL-1 sucrose	Darkness at 25±2°C, 16-hour light/8-hour dark, 2000-lux.	Improved root growth with (MS+2.0mgL-1 2,4-D+0.5mgL-1 BA+30.0gL-1 sucrose). Longer roots with (MS+0.5mgL-1 IBA+0.5mgL-1 Kn+15.0gL-1 sucrose+7.5gL-1 agar)	Sharma *et al.*, 2018
Grape	axillary bud	½ MS medium containing 2.5 % sucrose, PGR's	16-h light at 25/20°C, 60% RH, or dark at 25°C	No of embryogenic calli is more when there is higher conc of PGR's and 2,4-D/BAP (2:1)	Maillot *et al.*, 2016
Pineapple	Leaflets from crowns	MS medium, basal salts supplemented with of sucrose, vitaminsB5, glycine, glutamine, casein hydrolysate and MgCl2, BAP	Cultures were kept in the dark at 27±1°C humidity of 80%.	55.25% shoot regeneration from somatic embryos using BAP (3 mg/l) + GA3 (2 mg/l)	Cacaï *et al.*, 2021

Conclusion: -

In the recent period of time, tissue culture techniques herald a revolutionary period in horticulture. The development of these methods has significantly increased plant multiplication's effectiveness and scalability, allowing for the rapid mass production of high-quality planting material. Clonal propagation and genetic modification have emerged as critical areas of focus, protecting premium cultivars and guaranteeing the survival of desirable fruit crop traits. Notably, significant advancements in disease control have been made, with tissue culture providing a dependable way to generate pathogen-free plantlets, to combat various biotic stress. Tissue culture has several applications, as demonstrated by its integration with biotechnological technologies, that have ability to respond against abiotic stresses, and significant in the conservation of uncommon and endangered species. For several fruit crops, inefficient seedling regeneration from in vitro culture method remains an important barrier. Future advancements in micropropagation need careful thought, followed by the use of methods that have been designed to attain extremely effective in vitro regeneration. The early procedures employed stress or growth factor treatments for SE

induction and the process was examined at the histology level. SE can currently be induced by embryo- and meristem-expressed tissue factor (TF), and the other factors implicated in wound healing. Making a thoughtful decision on the utilization of designed methods that are extremely effective in vitro regeneration is crucial. The literature suggests that, it might provide incredibly effective suspension cultures of embryogenic cells from selected calluses lines to go over the present obstacles and produce a speedy technique of clonal propagation. Through the callus phase, somatic embryos can develop either directly or indirectly. genetic metamorphosis through the anther, leaf, shoots, cotyledon, and protoplast. Although transformation has been shown several times, more studies using indirect somatic embryo regeneration are still needed. Future research should focus on enhancing *in vitro* culture through the use of cell suspension culture technologies and medium additions to increase the induction of somatic embryos. Tissue culture has the potential to improve fruit crops' nutritional characteristics in the future, which would increase global food security.

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