

# MICRO PROPAGATION ON STRAWBERRY: A REVIEW

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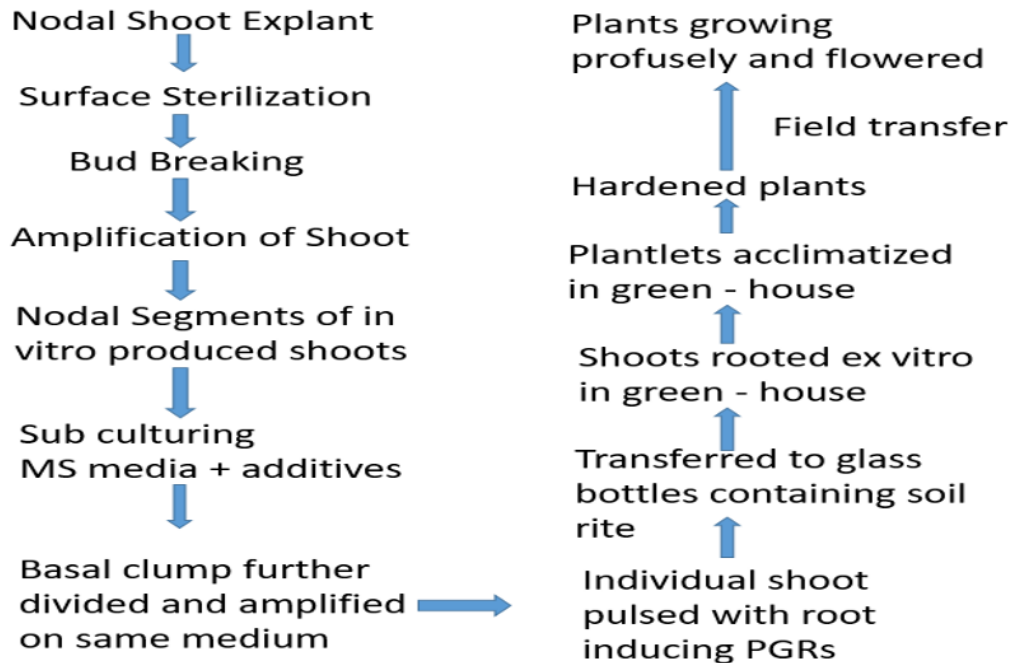
**Abstract.** The fruit crop known as strawberries (*Fragaria ananassa*) produces maximum revenues in the quickest period of time. It is the diet's richest source of the vitamins and minerals needed for human health. The major method of growing strawberries is by runners, which produce susceptible-to-disease plants. Plantlets produced using in vitro micropropagation are free of disease and can be used for further culture. Using MS media supplemented with 3-4% sugar, 0.75-1.0% agar, and an adequate combination of plant growth hormones, such as 6-benzyladenine, NAA, IBA, and kinetin, shoot cultures can be grown from shoot tips. Strawberry explants have been cleaned, multiplied into shoots, rooted, and ex vitro acclimated as part of a routine regeneration technique. The difficulties in getting better-quality plants and their higher endurance rate during ex vitro acclimatization can be greatly reduced by in vitro micro propagation. The Culture of Tissue Laboratory, a division of Ain Shams University's Faculty of Agriculture in Egypt, is where the study was conducted. This work's main goal was to determine whether using runners as meristem cultures to micropropagate a strawberry cultivars Festival and Marquez is a feasible approach. This was done while testing various gibberellic acid (GA3) concentrations (i.e., 0.1, 0.2, 0.3, 0.4, and 0.5 mg l<sup>-1</sup>) during the multiplication phase. It was discovered that 0.4 mg l<sup>-1</sup> of GA3 produced the greatest number of shoots each organ transplant and each shoot's leaves, whereas 0.5 mg l<sup>-1</sup> was the most effective focal point for growing buds.

## Introduction

Small plant tissue samples, or explants, are cultivated in a controlled laboratory setting as part of the plant propagation process known as micropropagation, sometimes called tissue culture or in vitro propagation. This method enables the rapid multiplication of plants, generation of virus-free and genetically uniform specimens, and the productivity of large numbers of offspring with desirable traits. Micropropagation is commonly used in agriculture, horticulture, and the cultivation of valuable or rare plant species. Originally thriving in subtropical areas, the strawberry, or *Fragaria x ananassa* Duch, has recently spread to tropical nations like Indonesia. The *Fragaria* genus in the Rosaceae family includes this strawberry, which is a highly prized fruit crop and a perennial stoloniferous herb. Developed almost 300 years ago in Europe by crossing *Fragaria virginiana* with *Fragaria chiloensis*, the octaploid hybrid *Fragaria X ananassa* is largely responsible for the world's most popular berry crop, strawberries. Strawberries are known for their vivid red colour, unique scent, and distinctive liquid consistency. Strawberries are usually eaten fresh or processed in meals like ice cream, milk shakes, and preserved fruit juice. They are also used as dried fruit in various parts of the world (1). Artificial strawberry fragrance is also widely used in many manufactured food products. Strawberries contain high concentrations of most essential nutrients for human health, such as protein, calcium, potassium, iron, copper, and vitamin

C (35). Since strawberry genetic engineering has already been reported, they are essential for genetic engineering and transformation genes (24, 36). The colour, flavour, size, shape, level of fertility, fruit shelf life, ripening season, resistance to disease, and nutritional content of strawberry cultivars vary (44). Additionally, it contains large levels of ellagic acid, which may have anti-carcinogenic effects (23). It produced on 506000 acres in 71 countries worldwide. Roughly twenty strawberry species have been identified in five chromosome groupings ( $x=7$ ): ten diploids, four tetraploids, one pentaploid, one hexaploid, and four octaploids (42, 26). Its remarkable environmental adaptation is demonstrated by its ability to withstand circumstances seen in temperate, Mediterranean, subtropical, and taiga zones (21). Runners are commonly used in vegetative reproduction of strawberries to produce true-to-type plants. A significant number of runners and suitable planting stock are necessary for the successful planting of strawberries. There are numerous combinations of plant growth regulators that can be used to propagate strawberries, either by runner/nodal segments (3, 25) or by runner tips in vitro micro propagation (5). It has been demonstrated that the in vitro method of micropropagating strawberry plants from nodal cuttings is successful (28). (10) spoke about the many advantages of micropropagation, a technique that makes it possible to produce vast quantities of plants quickly and efficiently from a single human in a constrained amount of time and area. Tissue culture permits bulk multiplication in a short length of time by direct or indirect regeneration, with plants created in vitro having greater yield, vigour, pest resistance, and numbers of runners and leaves per plant compared to conventionally propagated plants (39). In 1974, Boxus released the first study on the in vitro production of strawberries. Numerous media types, explants, genotypes, and plant growth regulators have been employed since then (20). Hormones, vitamins, amino acids, and the physical state of the medium were all found to play a role in strawberry micropropagation over time (27). Importing mother plants comes at a steep expense. There are no healthy stocks available for use in conventional propagation methods. It was thought that several plants may be produced from a small number of mother plants in less than a year by employing the tissue culture approach (9, 35). In the case that new cultivars are introduced, this approach is useful. Strawberries can be multiplied using runners or in vitro micropropagation. Strawberry branches and runners are not always the best for this type of culture because disease agents are emotionally sensitive (16, 43, 35). Strawberry micropropagation has been shown to successfully generate a sizable number of disease-free plants, beginning with runners (8). Tissue-grown propagules also require less storage space than standard runner plants, and in vitro storage can begin at any stage of the production cycle (43). The introduction of micropropagated strawberry plants has prevented the bulk of diseases that are transmissible through plants and soil. Nodal cuttings cultivated in vitro have been used to successfully micropropagate strawberry plants (28). The extensive rooting mechanism of strawberry cv. micro shoots in vitro is influenced by plant growth regulators. Charlie (age 31) Sweet. Using a range of explants, such as leaves, sepals, petioles, and nodes, plant tissue culture technique has been extensively used to successfully grow strawberries (11, 12, 13, 6). However, in vitro meristem culture with runner tips is the most effective method for cultivating virus-free strawberry plants once more (32). Auxins and cytokinins are two examples of plant growth regulators that are introduced to the culture media to regulate the morphogenesis and organogenesis of explants produced in vitro. One advantage of propagation in vitro is the capacity to generate an enormous amount of plants with an elevated rate of replication (10). In vitro techniques enable improved germplasm, clonal multiplication, and gene conservation of fruit quality and nutritional content. In vitro microreproduction, cytokinins abolish apical dominance and promote the initiation of axillary buds and shoots. The hormonal regulation of plant growth

and development depends on cytokinins. Plants have an efficient system in place to maintain the natural level of physiologically active cytokinin forms. When exogenous cytokinins are given to nutritional media, the apical meristem and the group of micro-shoots simultaneously form (2, 38).



**Fig 1: Flowchart of micro propagation process**

Item	Choices accessible	Utilized in this project	Price
<b>Light Source</b>	White light-colored bulbs / halogen bulb	Low power use LEDs are employed.	Compared to halogen lamps, LED lamps consume 25–30% less energy and have an 8–25 times longer lifespan (Energy.gov, 2021).
<b>Energy-dense source</b>	Sugar	Tabletop of sugar from the nearby store	Typical sugar cubes cost one US dollar per kilogramme, while Labogen AR Quality sucrose costs 15.16 US dollars per kilogramme of weight.
<b>Agent that adheres</b>	Phytigel, gelrite or gel	Type II Agar-Agar (minimum price)	50% low cost
<b>Halite</b>	Water that was purified	Filtered water	In comparison to RO systems, purified water technologies are more costly to maintain and need more time for purifying water (Applequist 2018).

<b>Grade of chemicals</b>	Analytical	Laboratory grade	Laboratory grade chemicals have low cost
<b>Acclimatization matrix</b>	Soilrite / rockwool / perlite cocopeat / moss	Only cocopeat	In comparison to perlite as a cocopeat that is less costly.

**Table 1: Cost effective micro propagation on strawberry varieties “Sweet Charlie” and “Winter Dawn” (14)**

<i>Sl No</i>	<b>Approach</b>	<b>Motive</b>
1.	Just robust crops cultivated in greenhouses were chosen to be donor plants.	Issue with the subculture, the stock plant, or the disinfection process.
2	One explant per culture vessel should be used for the first subculture, and diseased plants should be discarded at that point.	Beneficial for microbial contamination early detection.
3	From third subculture onwards: • ¾ MS medium, • Benzyl adenine The amount present is now only 0.5 mg L <sup>-1</sup> . • The amount of agar has been raised to 0.85%.	In order to avoid shoot tip necrosis and hyperhydricity while in subculture.
4	Activated carbon is added to the rooting media along with a 500 mg L <sup>-1</sup> indole-3-butyric ester pulsed stimulation.	In both cultivars, pulse treatment enhances rooting, while activated carbon creates a dark environment that enhances rooting.

**Table 2: Techniques for establishing "Sweet Charlie" and "Winter Dawn" strawberry cultivars for micro propagation (16)**

<i>Hormone</i>	<b>Function in Micropropagation</b>
<i>Cytokinin (e.g., BA)</i>	Promotes shoot initiation and multiplication. Helps control the rate of shoot growth.
<i>Auxin (e.g., IBA)</i>	Stimulates root development in micropropagated plantlets.
<i>Gibberellic Acid (GA)</i>	Influences stem elongation and internodal spacing in micropropagated plants.
<i>Abscisic Acid (ABA)</i>	Used to induce dormancy in certain stages of micropropagation, depending on the specific protocol.

**Table 3: Different hormones are used in micro propagation in strawberry**

### 2.1. In-Vitro regeneration:

Strawberry transplants were treated with sodium hypochlorite at concentrations of 0.5% for 7, 5, and 3 minutes, and mercuric chloride (HgCl<sub>2</sub>) at quantities of 0.1% for 5 minutes, 3 minutes, and 10 seconds. After three days of HgCl<sub>2</sub> 0.1% counselling on explants, the least amount of damage and browning was observed. Kichaoui (2014) investigated ways of avoiding In vitro explant contamination and oxidative browning. Sodium hypochlorite surface sterilization of runner tips concentrations of 0.5, 1, 1.5, 2.0, and 2.5% were used respectively. The preparation with 1, 1.5, and 2% Polyvinylpyrrolidone (PVPP) was investigated for its effects on evidence discoloration and mortality rate. Minimise the number of explants and maximise aseptic cultures 1.5% sodium was added, and turning brown ensued. 1% of hypochlorite with mercuric chloride. The highest percentage of explants in previous therapy was 2% PVPP (15). An effective method for regenerating plants in three cultivars of strawberries, Ofra, Chandler, and Oso Grande, employs modified forms of Knop's Media along with Murashige and Skoog (MS). It was observed that the Ofra and Oso Grande Chandler types' regeneration rates peaked when MS A supplement of 4 mg/liter of 6-(BAP) Benzylaminopurine was added to the medium. With Knop's medium-sized, which included 0.4 mg/l of BAP, 0.4 mg/l of Gibberellic acid (GA<sub>3</sub>), and 4.0 mg/l of the acid Indole-3-butyric acid (IBA), maximum regeneration was attained (30). On MS media supplemented with kienetin (KIN) (0.1 and 0.5 mg/l) or GA<sub>3</sub> (0.1 and 0.5 mg/l) and benzyl adenine (BA) (0.5-2.0 mg/l), strawberry nodule segments were grown. 0.5 mg/l of BA plus 1.5 mg/l Kinetin demonstrated the greatest reaction in terms of development and advancement, despite the fact that BA + KIN produced more shoots than BA + GA<sub>3</sub>. The highest multiple shoot induction was observed in the media treated with 1.5 mg/l BA + 0.1–0.5 mg/l KIN (40). n MS media enriched with varying doses of 1-Naphthalene acetic acid (NAA), BA, and kinetin, either singly or in combination, runner tips were employed. Although the growing medium supplied with low BA produced the largest variety of launches, harvestable shoots per culture, and repeated regrowth of shot proliferation, the MS medium added with 0.5 mg/l BA created the best percentage of culture response. It has been demonstrated that the types and concentrations of cytokines used had an impact on how quickly buds from running tips proliferated. When BA dosages were reduced from 4.0 to 0.1 mg/l, the percentage of explants exhibiting proliferation and the number of shoots per culture gradually rose. Strawberry runners of the 'Camarosa' variety were used by Haddadi et al. (2010) to generate branches on MS medium augmented with 0, 2, 4, and 8 M Thidiazuron (TDZ) and 0, 4, 9, 18, and 27 M BAP. The MS medium combined with 2M TDZ and M BAP showed the highest proportion of seedlings per explant (7).

## **2.2. Plant material:**

The seeds were given a five to ten minute soak in tap water to get rid of any surface contaminants. After giving them a good shake and submerging them in 70% ethanol for a minute, they were sterilised for 20 minutes in 4% sodium hypochlorite with a drop of Tween 20. The seeds were then meticulously rinsed three times with sterile, deionized water in a cabinet with laminar flow in order to get rid of any remaining disinfectant solution residues (33).

## **2.3. Culture media and conditions:**

To aid in germination, the seeds were grown in mugs on 30 millilitres of the typical Murashige and Skoog (1962) medium, which included 0.6% (m/v) agar and 3% (m/v) sucrose. The cultures were kept in a growth chamber with a 16-hour photoperiod, 2000 lux of illumination from white fluorescent bulbs, and a temperature of 24°C (33). The tissue samples are cultivated in a Murashige and Skoog (MS) medium supplemented at a predetermined concentration with kinetin,

benzylaminopurine (BAP), naphthalene acetic acid (NAA), and indole acetic acid (IAA). The optimal environmental conditions for the formation and development of cultures are  $25 \pm C$  and  $3000 \pm 4000$  lux of bright light. A humidity level of 60–70% is maintained during the photoperiod, which consists of 16 hours of daylight and eight hours of sleep (41). Murashige and Skoog's (1962; MS) nutritional medium was also used, along with 4% local sugar from sugar cane, 0.7 g L<sup>-1</sup> ascorbic acid (AA), 10 mg L<sup>-1</sup> adenine sulphate (ADS), and 0.75% tissue culture quality agar (Titan Biotech, Delhi, India) as culture media. After adjusting the pH of the culture medium to 5.8, 50 mL was added, and the jars were autoclaved for 15 minutes at 121 °C. These bottles of glass with semi-transparent polypropylene lids with screws (300 mL volume; made by Hindustan National Glass & Sectors, Nasik, India) were then added. The MS medium was made using only ingredients of laboratory quality that were purchased from SRL supplies in Mumbai, India. Every 35 days, sprout clusters were to be subculturing according to the design. For the purposes of the cultures, a light/dark daylight hours of 16:8 hours and 2500 lux of light strength were supplied by Wipro 4-Foot 20 W LED Canopy Chill Day Light, just Pune, India (14).

#### **2.4. Shoot multiplication:**

Three dosages of KIN (0, 0.2, and 0.5 mg/l) or IBA (0, 0.2, and 0.5 mg/l) were added to MS media supplemented with BAP at four doses (0.1, 0.5, 1, and 1.5 mg/l) for nodal segments (0.5–1 cm in length). Autoclaving was used to achieve sterilisation; it took 20 minutes at 121 °C. Before adding 0.4% (w/v) activated charcoal (AC) and 0.6% (w/v) agar, the pH was brought to 5.8. To establish an aseptic culture, five explants, or nodal categories were placed in a vessel with 50 cc of the inducement medium. Before the ceramic cups were exposed to the previously specified circumstances, they were sealed and covered with regular household plastic foil for a period of five weeks (33). The emergence of little, tender green leaves marks the beginning of branch development and renewal. When these tiny leaves are fully formed, they are placed into a new medium that has the same testosterone content to support the shoots' continuing growth and development. Three and five weeks following the beginning of the shoot development, both the first and second cultural groups should be initiated, correspondingly. Place each regenerated shoot on a piece of solid, sterile paper with care. The highest mean number, length of sentence, and number of sprouts and leaves were seen at a BAP dosage of 0.5 mg/L (5). Furthermore, explants from conditions that received 0.5 mg/L kenetinden showed greater numbers of surviving shoots and better plant development (34) (41). To avoid culture loss from bacterial contamination, just one tip of the shoot or a bunch of branches was infected per bottle between the start of the culture through the first growth (up to the second subculture). To avoid cultures from being lost due to endophytic contamination throughout shoot multiplication, MS media comprising 1 mg L<sup>-1</sup> BA and 0.01 mg L<sup>-1</sup> KN were used for the first two subcultures (each shoot cluster consisted of five bud shoots that were at least 1 cm in diameter). Three branch aggregates from the third subculture were maintained on 3/4 concentration MS medium including 0.5 mg L<sup>-1</sup> BA and 0.1 mg L<sup>-1</sup> KN (each bottle encompassed three shoot clusters with 5 to 6 shoots with > 1 cm shoot length) to avoid vitrification and stunted growth. After the ninth subculture, cultures were restarted using seedlings that had been micropropagated and grown in the greenhouse (14). Whether applied alone or in conjunction with BAP, the study's results confirmed the importance of TDZ as a cytokinin for encouraging a high quantity and proportion of sprout production in strawberries. It demonstrated how shoot branching brought an end to apical dominance when TDZ and BAP were present. The chemical TDZ, which is a substituted phenylurea, is extensively employed as a cytokinin in the micropropagation of diverse woody plants. It possesses the remarkable ability to promote the

growth of axillary shoots. Studies on the effects of this cytokinin on various cultivars of *Fragaria ananassa* have also been conducted (12). The sprouts in culture multiplied after a week. The amount of shoots per explant rose throughout each four-week subculture and peaked at 12 weeks on MS media with 2 mM TDZ and 4 mMBAP. With an average of fifteenth shoots per explant, this medium produced a substantial number of shoots compared to earlier treatments. It was also the only therapy where every transplant produced new shoots. Merely 77% of the tissue samples exhibited development in the medium lacking TDZ or BAP, with each explant displaying a median number of two different branches (19).

### **2.5. Bud elongation:**

After three weeks, the transplanted tissue regeneration was transferred to an elongation medium and BAP (0.1 mg/l) and IBA (0.2 mg/l) were added. The established plantlets were given five weeks to acclimatise before being put in pots with a disinfected peat material to vermiculite ratio of 3:1. The planters in a phytotron were covered with a clear flask with a few holes in it and preserved at an elevated humidity level for 10 days. This was accomplished by frequent watering. Reinforced plantlets were placed in an enclosed conservatory with the following parameters: 21°C during the day, 19°C at night, 85% relative humidity, a 16-hour daylight hours, and 3000 lumens of light from white fluorescent lights. The plantlets were given daily irrigations after planting in order to maintain a sufficiently wet soil (33).

### **2.6. Experimental design and statistical analysis:**

A completely randomly design multivariate experimentation was used to carry out the inquiry. The data that was gathered was statistically evaluated using SAS software (version 8). When the results of the F- test of the Analyses revealed considerable treatment effects (5 or 1%), the treatments were evaluated for statistical differences using the Duncan's Multiple Range Test for statistical significance (P0.05) (33). Based on monthly visual inspections of the cultures, the average rate at which individual plantlets, subdivisions, and roots developed and multiplied was recorded. Information on growth and anchoring per culture were examined using Duncan's novel multiple range tests (18).

### **2.7. Histological studies:**

Using histological staining, buds on shoots along with additional explant ontogeny were confirmed. The transplanted cells were preserved in FAA (formalin, acetic acid, pure alcohol: 10, 5, 85; v/v) for a full day after regeneration on days three, eight, and twelve. Textures were divided at an angle of 30 metres using a microtome. After that, they were evaporated in methanol (70, 95, and 100%), fixed in paraffin, finally coloured with metylen blue. The sections were ready for examination using light microscopy in a lam (33).

### **2.8. Rooting and acclimatization:**

In the expansion of media that contained 0.1 mg/l BAP after adding 0.2 mg/l IBA, more than half of the shoots were also rooted. Before being out-planted, 4 week-old established plantlets were typically placed in containers with sterilised clay mass and vermiculite (3:1). For twenty-five days, the pots in a phytotron were wrapped with a clear glassware with a few holes in it and continuously watered in order to preserve a high humidity level. strengthened plantlets were out-planted in a greenhouse with a daily room temperature of 21°C, an overnight temperature of 19°C, a relative humidity of 85%, and a 12-hour day. After planting, the plantlets received daily irrigations to keep

the soil adequately moist. Upon acclimatisation and hardening, the duplicated plants exhibited an impeccable survival rate (33). MS media can be used to root freshly formed stems when IBA/NAA is half strength. The longest roots and the highest rooting rates may be obtained with 1.0 mg/L IBA supplementation, but 1.0 mg/L NAA in MS media may generate more roots (3). However, (5) discovered that the best performance was in the area of growth of roots for 0.5 mg/L IBA added to the media. The toughest and most evenly hardened seedlings can be selected, and they can be planted in plantation bags with a 3:1:1 mixture of coco peat, perlite, and vermiculite under 70% shade. During the acclimatisation period, 16.4 °C and 78 to 80% relative humidity are the optimal values (37, 41). Nursery trays were kept at a high relative humidity (95%) and shielded from water and light stress for 10 days by being covered with transparent polythene (100 micron) in a low tunnel. Sardar WSF-19-19-19TM (GSFC, Vadodara, India) fertilizer solution containing 0.2% (w/v) liquid nitrogen, phosphorous, and potassium was used to irrigate these plants at regular intervals of three days. Plants were raised in an organically rich, well-drained medium loam soil. Soil had a pH that varied from 5.7 to 6.5. It was discovered that in the field, the production of secondary roots and runners was suppressed at higher pH levels. In beds measuring 4 by 4 or 4 by 3, plants were raised. The space between each plant was 45 cm, while the distance between each row was 60 to 75 cm. At intervals of 25 to 28 days, these plants received nighttime irrigation (14). On media containing NAA, collected samples on hormone-free MS media also started to grow roots after 10 days, but they lacked callus tissue. On conditions containing 1 mM NAA or 2 mM IBA, which produced an average of six or five roots per explant, respectively, the majority of the explants formed roots (70% in both treatments). Without hormones, explants typically produced three roots, with 68% of them developing roots; nevertheless, the roots were generally longer than in any hormone treatments (19). Five weeks later, the elongated shoots were moved to the rooting media. Among the concentrations, IBA at mg/l performed best overall based on all metrics (41). After five weeks, the longer shoots were placed in the rooting medium. Among the concentrations, IBA at 0.5 mg/l performed the best across all criteria. The IBA concentrations of 0.5 and 1.0 mg/l needed the least amount of time (8–10 days) for root induction. The biggest roots (3.05 cm) and the greatest amount of roots/culture (number: 6) were also obtained from the same concentration. Conversely, the roots produced by alternative therapies were limited. There were no roots produced in the control condition (5).

## **2.9. Disinfection of explants:**

The survival of explants is greatly aided by an effective sterilization technique. The explants must be thoroughly cleaned with running tap water before being sterilised for 5–10 minutes with autoclaved distilled water laced with a drop of Tween 20. The sterilised runners were after that rinsed three times in autoclaved distilled water. To preserve aseptic conditions, vaccinations and other procedures should be performed in a laminar airflow cabinet. To maintain aseptic conditions during the culture, 70% ethanol should be frequently administered to the hands and cabinet base. Inside the laminar airflow cabinet, the shoot tips should be meticulously prepared using a pair of fine, sterile forceps and a scalpel (41). These shoot tips were then immersed in 3% Teepol™ (liquid soap solution) for 5 minutes, followed by 10 minutes of washing under running water. Shoot tips were cleaned for 10 minutes in an aseptic environment using (a fungicide solution containing carbendazin 12% + mancozeb 63%). These shoot-tips were then treated for 7 to 8 minutes with a 0.5% sodium hypochloride solution, submerged for 1 minute in a 0.05% mercuric chloride solution, and then rinsed three times in sterilised RO water. Prior to inoculation onto culture initiation medium were clipped (0.4 to 0.5 cm) near the cutoff (14).

### **2.10. Shoot Proliferation:**

After seven weeks, there was evidence of shoot proliferation from runner tip explants on all medium examined. On MS-based media, both solitary and multiple shoots were seen. On various mixes of Knop's material, however, we only saw a lone shoot per explant. In all cvs, the Mg medium (MS+4.0 mg 1-1 BAP) had the highest mean number of shoots per explant and the Ms Medium (MS+0.4 mg 1-1 BAP) had the lowest. On Mg medium, cv. Ofra showed the highest mean number (13.83.6) and range of shoots per explant (4-36) across cultivars (30). After four weeks, the cultivated explants were subcultured. Runner sections were cultured in Knop media with MS microelements with varied concentrations of BAP and Kinetin, i.e. 0.0 mg/l (control), 0.5 mg/l, 1.5 mg/l, and 2 mg/l (41). In MS media supplemented with varied BAP concentrations, such as 0.0 mg/l (control), 0.5 mg/l, 1.0 mg/l, 1.5 mg/l, and 2 mg/l, runner segments and tips were cultivated. The cultured explants were subcultured after three weeks. At a concentration of 0.5 mg/l BAP, the maximum average number of shoots (7), length of shoots (3.34 cm), and number of leaves (5) were noted. It displayed the strawberry's numerous shoots. The greatest BAP concentration (2.0 mg/l) did not generate any shoots. The remaining treatments yielded numerous weak shoots. In BAP-free media, the average shoot length (0.57 cm) and average number of leaves (2) were both lowest (5).

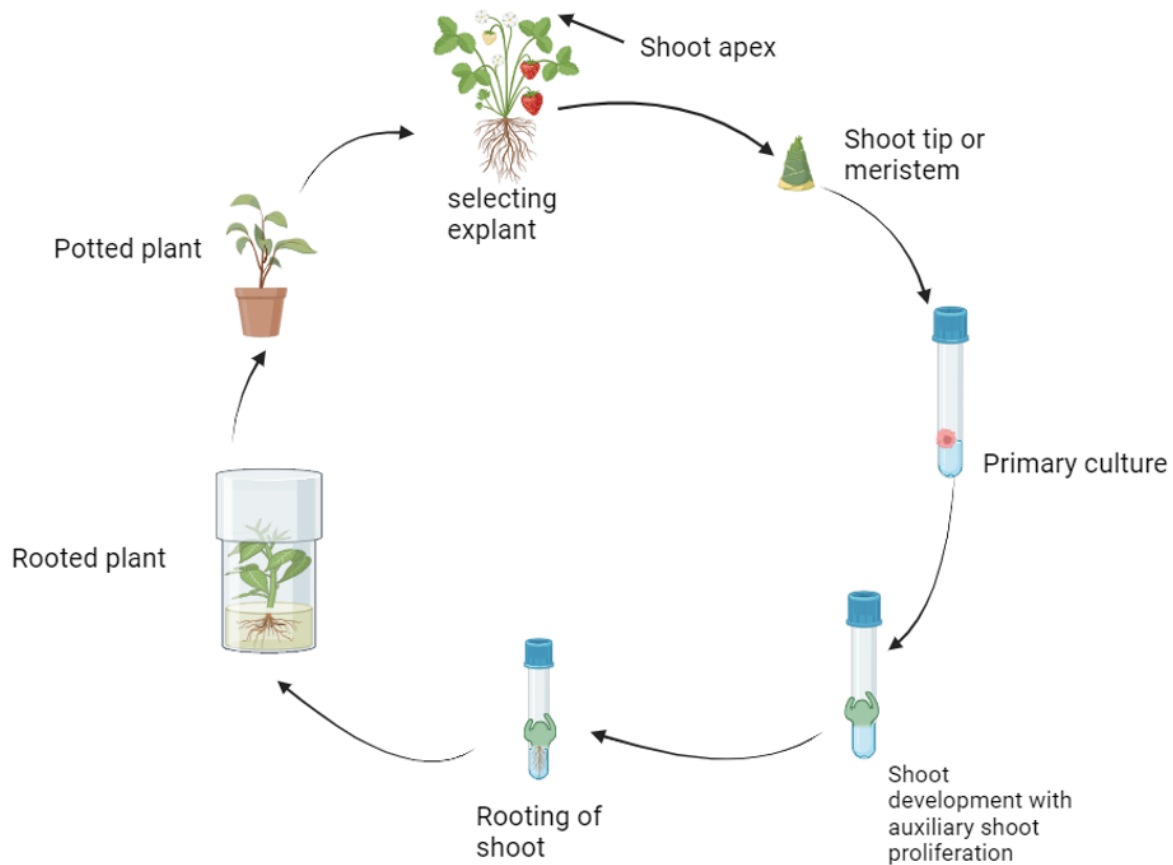
### **2.11. Transfer to the soil:**

After being taken out of the culture tubes, the medium used for cultivation from the rooted plantlets' roots was thoroughly cleaned with tap water. Washing plantlets were treated with fungicide prior to planting them in polybags with sterilised ordinary soil. After seven days, the hard plantlets were immersed in soil (22).

## **3. Conclusion:**

The utilization of high-yielding cultivars in agriculture can experience significant advantages when we focus on enhancing their resistance to diseases, tolerance to salinity, and capacity to withstand various environmental stresses through the application of plant tissue culture techniques. Traditional methods like runner segment propagation are constrained in their ability to produce plant propagules efficiently, and the resulting propagules are often vulnerable to fungal infections, as documented (16). To address these limitations, researchers have developed an innovative approach involving in vitro propagation, which has yielded promising results. Notably, protocols have been established for the large-scale propagation of two specific strawberry cultivars, "Winter Dawn" and "Sweet Charlie," as highlighted in the studies conducted (14, 45). However, it's important to acknowledge that strawberries, as a species, are prone to somaclonal variation when they are regenerated using in vitro tissue culture methods. This variation can make it challenging to obtain genetically consistent plants that consistently produce high-quality fruits. Consequently, having reliable planting materials is critical for the success of strawberry farming endeavors. Moreover, an inherent challenge in strawberry farming is the absence of runners throughout the entire cropping season, which can hinder the traditional propagation process. To successfully leverage in vitro techniques for the rapid and mass production of disease-free strawberry plants, a multifaceted approach is required. This includes utilizing various growth media specific. Such an approach is essential to ensure the efficient and sustainable propagation of high-quality strawberry cultivars and to meet the demands of modern agriculture. The future of strawberry micro propagation holds promise in developing disease-resistant and climate-adaptive cultivars,

increasing year-round production, and ensuring genetic stability. These advancements can enhance commercial viability and meet the rising demand for high-quality strawberries.



**Fig 2: Graphically represented the Micro-propagation of Strawberry**

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