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Optimizing In Vitro Runner Production and Survival of Strawberry (cv. Camarosa) Under Subtropical Conditions

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ABSTRACT

Strawberry (*Fragaria × ananassa* Duch.), a high-value horticultural crop, is valued for its rich nutritional profile and global consumption. However, its commercial cultivation faces significant challenges, including susceptibility to viral, fungal, and bacterial pathogens, particularly when propagated via conventional methods. This study developed an efficient micropropagation protocol for the strawberry cultivar Camarosa, addressing key factors influencing phenolic exudation, explants establishment, shoot proliferation, rooting and acclimatization under subtropical conditions. Explants sterilized with 0.1% HgCl₂ for 3 minutes showed the highest establishment rate (93.3%) and minimal contamination. Phenolic exudation was effectively mitigated using ascorbic acid (300 mg/l), ensuring higher explant viability. Optimal shoot proliferation was achieved with MS medium supplemented with BAP (3.0 mg/l) and NAA (0.5 mg/l), yielding an average of 5.4 shoots per explant with significant shoot elongation. Root induction was most successful on half-strength MS medium containing IAA (1.5 mg/l), which produced the highest root formation percentage (53.96%), maximum root length (4.80 cm), and a rapid rooting response. Acclimatization trials revealed cocopeat as the most effective potting medium, achieving a survival rate of 70% due to its superior water retention and aeration. These results underscore the effectiveness of micropropagation in generating disease-free, uniform planting material suitable for Bihar's subtropical climate. While significant advancements have been made, further research is recommended to refine the protocol for large-scale adoption and to explore genetic transformation techniques to enhance the crop's commercial viability. This study lays the foundation for expanding strawberry cultivation in Bihar, contributing to its agricultural diversification and economic growth.

Keywords: Strawberry, Growth Regulators, Shoot and root proliferation, Mass Multiplication, Acclimatization, Coco peat, vermiculite, perlite etc)

Introduction

The strawberry (*Fragaria × ananassa* Duch.), a member of the Rosaceae family, is a natural hybrid derived from *Fragaria virginiana* Duch. and *Fragaria chiloensis* Duch. Known for its characteristic aroma, vibrant red color, and juicy texture, strawberries are consumed globally in various forms, including fresh fruit, processed products like jams, juices, and ice creams, and even as dried fruit [29] & [1]. Originally a temperate crop, advancements in cultivation practices have enabled strawberry farming to thrive in diverse climates, including subtropical and tropical regions. In India, strawberries are now commercially grown in states like Jammu and Kashmir, Maharashtra, Karnataka, and Madhya Pradesh, with emerging markets in northern states such as Haryana, Punjab, and Himachal Pradesh [19].

Strawberries are widely known for their nutritional value, a rich source of essential nutrients like folate, vitamin C, fiber, potassium, flavonoids, anthocyanins, and various antioxidants

[13] & [12]. These bioactive compounds contribute to numerous health benefits, including reducing the risks of cardiovascular diseases, thrombosis, and certain cancers, while also aiding in the prevention of age-related cognitive decline [4] & [21] & [16]. Despite their high commercial and agricultural potential, strawberry cultivation faces challenges due to susceptibility to various diseases caused by viruses such as strawberry mottle virus (SMoV), *Fragaria chiloensis* cryptic virus (FCICV), strawberry necrotic shock virus (SNSV), and strawberry mild yellow edge virus (SMYEV), along with fungal pathogens like *Botrytis cinerea*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Colletotrichum* spp., *Verticillium dahliae*, and others. Additionally, bacterial infections, such as those caused by *Xanthomonas fragariae*, further hinder cultivation. These pathogens are often transmitted during propagation through stolons, presenting a significant challenge in disease management [7].

Strawberries are propagated vegetatively through runners to ensure plants remain true type. Successful strawberry cultivation relies on an ample supply of runners and high-quality planting material. Conventional propagation methods, primarily through runners, are limited by their slow pace, seasonal dependence, and vulnerability to diseases. In vitro propagation offers an efficient alternative, ensuring the rapid multiplication of disease-free and uniform plants, essential for

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preparing strawberry propagating material [24]. Tissue culture *i.e.* micropropagation, offers an effective alternative for producing healthy and genetically uniform planting material [17].

Tissue culture techniques, including meristematic tissue culture, enable the rapid multiplication of disease-free plants while ensuring uniformity and high phytosanitary quality. This method is important for overcoming the limitations of conventional propagation techniques, which are labor-intensive and less efficient. Successful *in vitro* propagation requires meticulous regulation of contamination, optimization of growth regulators, and careful management of the *ex vitro* acclimatization process to achieve high survival rates and robust plant development [5].

Conventional strawberry propagation through runners is slow and inefficient, often yielding limited planting material and requiring intensive labor. Furthermore, vegetative propagation makes plants susceptible to biotic stresses, highlighting the need for advanced propagation techniques. Micropropagation addresses these challenges by enabling the rapid multiplication of high-quality, disease-free plants. *In vitro* propagation methods for strawberries have been widely adopted for their efficiency in generating uniform plants with superior growth, yield, and resistance to pests and diseases. However, challenges such as contamination, phenolic exudation, and poor acclimatization of *in vitro* plants persist, necessitating further refinement of tissue culture protocols [17]. The critical issue of low survival rate of plantlets during acclimatization to greenhouse or field conditions [24] and bacterial and fungal contamination during the initiation and multiplication phases of tissue culture, leading to 3–15% plant loss per subculture due to commercial viability [20]. Additionally, the presence of phenolic compounds, often released due to tissue injury during culture, can lead to tissue browning, growth inhibition, or explant death. This condition particularly prevails in woody perennials, where phenolic oxidation occurs due to polyphenol oxidases and the influence of light, media composition, and oxidative conditions [8] [25] [26]. In Bihar, the expansion of commercial strawberry cultivation is hindered by the unavailability of quality planting material. Conventional runner propagation is inefficient and unsuitable for the region's hot climate during April–June. Planting time significantly affects flowering, fruiting, and runner production, making it essential to optimize environmental and cultural practices for profitable production [17] [22]. Tissue culture offers a solution by producing uniform, disease-free plants with higher flowering and fruiting potential. This technique also facilitates rapid propagation and genetic improvement, complementing conventional breeding methods [6]. To address the demand for high-quality planting material in Bihar, it is necessary to develop efficient tissue culture protocols for rapid and large-scale multiplication of regionally adapted strawberry cv. Camarosa to ensure the availability of superior planting material and support the region's strawberry industry.

2. Materials and Methods

"*In vitro* runner production of strawberry cv. Camarosa and its survivability under subtropical conditions," was conducted in the Plant Tissue Culture Laboratory at Bihar Agricultural College, Sabour, Bhagalpur. The experimental design followed a Completely Randomized Design (CRD), and runners of strawberry cv. Camarosa, free from pests and diseases, were used as explants.

High-purity chemicals and reagents were procured and used. Growth regulators such as gibberellic acid (GA3), 1-naphthaleneacetic acid (NAA), and other components like vitamins, myo-inositol, and chelating agents (EDTA sodium salt) were sourced from Sigma (USA) and Hi-Media (India). Additional chemicals like sucrose and agar were obtained from Sisco Research Laboratories (SRL), BDH, and Hi-Media. Borosilicate glassware was used for culture work, while culturing was performed in sterilized conical flasks and jam bottles. Glassware was initially washed with a detergent solution (teepol) under running tap water and subsequently rinsed with double-distilled water. After drying, they were sterilized at 180°C in a hot air oven for 2 hours. Plasticware, including beakers and measuring cylinders, were wrapped in aluminum foil and autoclaved at 121°C (15 psi) for 20 minutes. These were performed under aseptic conditions in a laminar airflow chamber.

The chamber's working surface was cleaned with 70% alcohol, followed by UV light exposure for 20–30 minutes before use. Instruments such as forceps and scalpels were sterilized by dipping in 70% alcohol and flame-sterilized during operations. Hands were swabbed with alcohol before handling materials. Murashige and Skoog's (1962) medium served as the base for all experiments. Stock solutions of macronutrients, micronutrients, vitamins, and growth regulators were prepared at 10x or 100x concentrations using double-distilled water and stored at $4 \pm 1^\circ\text{C}$. Auxins and cytokinins were dissolved in small volumes of NaOH or HCl, respectively, before dilution. These solutions were stored in amber-colored bottles to prevent light degradation and consumed within a month of preparation. Specified amounts of stock solutions were mixed in a beaker, followed by the addition of sucrose and myo-inositol. The pH of the medium was adjusted to 5.8 using 0.1N NaOH or 0.1N HCl. Agar (0.8%) was added to the boiling medium for homogenization. The prepared medium was dispensed into sterilized flasks (40–50 ml per flask) and culture tubes (10–15 ml per tube) and autoclaved at 121°C (15 psi) for 20 minutes. The media were stored at $25 \pm 2^\circ\text{C}$ and used within 10 days. All inoculations and subcultures were carried out in a sterile environment within the laminar airflow hood. Surface sterilization of explants was done before inoculation. After inoculation, cultures were incubated at $25 \pm 2^\circ\text{C}$ in an air-conditioned culture room with a light intensity of 2000–3000 lux, provided by cool white fluorescent lights. A photoperiod of 16 hours light and 8 hours dark was maintained. This protocol ensured a controlled environment for the successful *in vitro* propagation of strawberry cv. Camarosa.

3. Results and Discussion

Effect of Sterilizing Agents on Contamination and Establishment (Table 4.1)

The sterilization process plays a pivotal role in tissue culture, particularly in preventing microbial contamination and ensuring explant survival. The efficiency of mercury chloride (HgCl_2) and sodium hypochlorite (NaOCl) as sterilizing agents for runner explants of strawberry cv. Camarosa. Treatment of HgCl_2 (0.1%) for 3 minutes emerged as the most effective treatment, yielding 75% contamination-free explants and achieving a high plant establishment rate of 93.3%, with an overall establishment rate of 70% which showed its disinfectant properties, to mitigate microbial contamination without adversely affecting the viability of the explants. It has validated the efficacy of HgCl_2 in plant tissue culture, to control microbial

contamination at low concentrations for short period [25]. NaOCl (1%) treatments were comparatively less effective, with T8 (3 minutes exposure) achieving 70% contamination-free explants and a lower overall establishment rate of 65%. Although NaOCl is widely used as a sterilizing agent due to its affordability and accessibility, its lower efficacy compared to HgCl_2 might be attributed to its relatively weaker oxidizing ability and reduced penetration capacity in explant tissues [27]. This suggests that optimizing NaOCl concentrations and exposure times could improve its sterilization performance. The control treatment resulted in 100% contamination, emphasizing the indispensable role of sterilization in vitro propagation. Sterilization is critical to eliminate microbial contaminants that hinder explant establishment in tissue culture [28]. This finding can still be utilized as an alternative sterilant with further optimization and need for tailored sterilization protocols to achieve successful in vitro propagation of specific plant species.

Impact on Phenolic Exudation and Mortality (Table 4.2)

Phenolic exudation poses a major obstacle in the successful establishment of in vitro cultures of strawberry (*Fragaria × ananassa*), as the oxidation of phenolic compounds creates a toxic environment that inhibits explant growth and viability [33]. The effectiveness of various antioxidants in mitigating phenolic exudation and improving explant survival and proliferation rates. Among all the treatments, ascorbic acid at a concentration of 300 mg/l showed the highest efficacy, resulting in 85% contamination-free explants and 70% shoot proliferation. This significant improvement can be attributed to the role of ascorbic acid as a potent antioxidant, which actively scavenges reactive oxygen species and prevents phenolic oxidation, thereby creating a more favorable environment for explant growth [30]. These findings are consistent with the work of micropropagation who highlighted the ability of ascorbic acid to enhance tissue culture outcomes by reducing oxidative stress in strawberries and other fruit crops [2]. The difference in efficacy between ascorbic and citric acid may stem from the lower antioxidant potential of citric acid under oxidative stress conditions prevalent in strawberry explants. Polyvinylpyrrolidone (PVP), a phenolic scavenger, showed limited success, with higher levels of phenolic exudation (+++) and establishment rates ranging from 40–60%. PVP primarily acts by binding free phenolics, which may not effectively prevent the oxidative chain reactions that generate toxic compounds [31] [18]. While PVP remains a widely used agent, its limited impact compared to ascorbic acid underlines the importance of selecting antioxidants with active phenolic oxidation prevention mechanisms.

Effect of Growth Regulators on Shoot Proliferation (Table 4.3)

The application of growth regulators has proven to be an essential aspect that significantly influences shoot proliferation in strawberries. The results demonstrated that specific combinations of BAP and NAA with MS media yield better outcomes, supporting its critical role of growth regulators in tissue culture. The combination of (BAP @ 3.0 mg/l + NAA @ 0.5 mg/l) emerged as the most effective treatment. It resulted in the fastest shoot proliferation, with shoots emerging in just 21.67 days. Additionally, MS7 recorded the highest number of shoots per explant, averaging 5.40 shoots, and the maximum shoot length at 5.20 cm.

It indicates synergistic interaction between BAP and NAA at these concentrations, providing an optimal hormonal environment for shoot initiation and elongation. Comparable results were obtained with MS8 (BAP @ 4.5 mg/l + NAA @ 0.5 mg/l), which yielded 5.13 shoots per explant and displayed robust leaf development. However, the slightly higher BAP concentration may have triggered secondary metabolic pathways, resulting in slightly longer leaves but a marginal reduction in shoot numbers compared to MS7. The control (MS0) demonstrated the least response, with only a single shoot per explant and limited growth which showed that growth regulators, are useful in overcoming the endogenous hormonal deficiencies in explants under in vitro conditions.

The shortest time for shoot proliferation was significantly faster compared to the control due to the synergistic effect of an optimized cytokinin-auxin ratio in accelerating shoot initiation. The effective role of BAP at higher concentrations with NAA in promoting shoot multiplication. The balanced hormonal ratios in MS7 and MS8 provided the optimal conditions for shoot initiation, proliferation, and vegetative growth, confirming the synergistic effects of BAP and NAA. The combination of BAP with NAA enhances shoot initiation by balancing auxin-cytokinin interactions, which are critical for organogenesis. Previous studies have corroborated that cytokinins, especially BAP, effectively promote shoot multiplication in strawberry tissue culture [15]. The ability to achieve rapid shoot proliferation, high shoot numbers, and robust growth has shown their potential for commercial propagation and can meet the growing demand for high-quality, virus-free strawberry planting materials.

Rooting Response to Growth Regulators in Half MS Medium (Table 4.4)

The impact of various concentrations of Indole-3-acetic acid (IAA) and Naphthaleneacetic acid (NAA) on rooting in strawberry plantlets using half-strength MS medium. The findings revealed that IAA at 1.5 mg/l (HMS2) provided the best rooting outcomes, achieving the highest root formation percentage (53.96%), maximum root length (4.80 cm), and the greatest number of roots per shoot (6.80), while also initiating rooting in the shortest time (18.40 days). Similarly, IAA at 3.0 mg/l (HMS3) demonstrated comparable results, with a slightly lower rooting percentage (50.87%) and a minor increase in the time required for root initiation (18.80 days). NAA treatments, while effective, were slightly less potent than IAA. Among them, NAA at 1.5 mg/l (HMS5) exhibited the highest root formation percentage (45.44%) and good root elongation (4.20 cm) but required slightly more time for root initiation (19.50 days). Control treatments (HMS0) showed the least efficiency across all parameters, with a rooting percentage of just 14.62%, limited root length (1.35 cm), and minimal root regeneration (2.90 roots per shoot), highlighting the critical role of growth regulators in promoting rooting. Overall, IAA, particularly at 1.5 mg/l, emerged as the most effective auxin for rooting in strawberries, promoting better root initiation and growth compared to NAA. These findings emphasize the importance of optimizing growth regulator concentrations to improve in vitro propagation efficiency in strawberries. The above finding is in agreement with [9] on an efficient micropropagation protocol for nodal segments of strawberry cultivar festival having maximum shoot length (3.50 ± 0.07) was found with TDZ @ 1mg/l.

Effect of Potting Mixtures on Hardening (Table 4.5)

The results indicate that the potting mixture significantly influences the survival and establishment of plantlets. This treatment showed the highest survival rate at 70%, attributed to its excellent water-holding capacity and aeration, which supports root development and reduces stress during acclimatization. These mixtures resulted in a survival rate of 50%, likely due to suboptimal moisture retention or drainage properties, which could either limit water availability or lead to over-drainage. While these combinations offer balanced aeration and moisture retention, they may not be as efficient in maintaining optimal conditions for micropropagated plantlets. For effective acclimatization, like pure coco peat simpler mixtures emphasizing high moisture retention and aeration. Similar findings were reported in bananas using peat moss and soil.

Acclimatization

It was recorded that a maximum number of plants has survived when coco peat was used as hardening media (70%) (Table 4.5). However, coco peat: vermiculite (2:1 v/v), coco peat: vermiculite (3:1 v/v), coco peat: vermiculite: soil (1:1:1 v/v/v), coco peat: perlite (2:1 v/v), coco peat: perlite (3:1 v/v) and coco peat: perlite (3:1 v/v) also gave promising results as hardening media with (60 %) plant survival rate in each mixture with a minimum survival rate (40 %) was recorded when plantlets were hardened in combined mixture of coco peat: vermiculite: perlite (1:1:1 v/v/v). A

similar finding was also recorded in hydroponic strawberry cultivation with 38.5 to 49.5 in the different substrate (50% coco peat + 50% perlite) [10] and perlite, vermiculite, and cocopeat (2:1:2 v/v/v) [27]. The mortality was observed due to decaying at collar region and subsequent wilting of shoots. The leaves of in vitro-raised plants are generally photosynthetically not competent because they were growing in sucrose rich medium but are active in transpiration and respiration, which may be the reason for low establishment of plantlets during hardening. The findings are somewhat similar with those of [8]. The plantlets thus developed were hardened and successfully established in the field. The results are in agreement with those of Sharma [30] reported that the rooted plants transferred in cocopeat + perlite + vermicompost (3:1:1) showed maximum transplanting success (94.33%). Hence, suitable mixtures are useful for good plant development, i.e. easily accessible and low-cost [32] [14].

Conclusion

It can be concluded that a successful micropropagation protocol for strawberry (*Fragaria X ananassa* Duch.) cv. camarosa has been developed. Our results described various factors that influence the phenolic exudation, establishment, shoot proliferation, shoot multiplication, root induction and acclimatization of strawberries. Strawberry is still a new crop for Bihar, therefore, further research needs to be conducted to study all these factors in-depth to make it commercially viable and to optimize protocols for use in genetic transformation.

Table No. 4.1: Effect of different treatments duration of sterilizing agent on runner explants of strawberry cv. Camarosa

Treatments	Treatment duration	No. of explants	3 days	6 days	9 days	12 days	15 days	18 days	21 days	Contamination free plants	Percentage contamination free explants	Percent establishment of contamination free plants	Shoot establishment/proliferation after 4 weeks	Overall Percent establishment
T1	Control	20	12	18	20					0				
T2	HgCl ₂ (0.1 %) for 1 minutes.	20	6	10	12	13	13	13	13	7	35	100	7	35
T3	HgCl ₂ (0.1 %) for 2 minutes.	20	3	5	6	7	7	7	7	13	65	92.3	12	60
T4	HgCl ₂ (0.1 %) for 3 minutes.	20	2	2	3	4	5	5	5	15	75	93.3	14	70
T5	HgCl ₂ (0.1 %) for 4 minutes.	20	2	3	3	4	4	4	4	16	80	62.5	10	50
T6	NaOCl (1%) for 1 minutes.	20	7	10	13	14	15	16	16	4	20	100	4	20
T7	NaOCl (1%) for 2 minutes.	20	5	8	10	12	13	14	14	6	30	100	6	30
T8	NaOCl (1%) for 3 minutes.	20	4	4	5	5	5	6	6	14	70	92.85	13	65
T9	NaOCl (1%) for 4 minutes.	20	3	4	4	4	5	5	5	15	75	73.3	11	55

Table 4.2: Effect of various treatments on in vitro phenolic exudation from runner of strawberry cv. camarosa

Treatments	Number of explants	Concentration of treatments	Degree of phenolic exudation	Mortality	Healthy plant	Percentage	Shoot proliferation	Percent Establishment
Control	20		++++	19	1	5	1	5
Ascorbic acid	20	150 mg/l	++	4	16	80	14	70
	20	300 mg/l	+	3	17	85	12	60
Citric acid	20	150 mg/l	++	9	11	55	10	50
	20	300 mg/l	++	6	14	70	9	45
PVP	20	150 mg/l	+++	10	10	50	8	40
	20	300 mg/l	++	8	12	60	10	50

Table 4.3: MS basal medium treatment with different concentration of growth regulators

Treatments	PGR (Mg/l).	No. of days taken for shoot proliferation	Number of shoots generated per explant	Shoot length (cm.)	Number of leaves per regenerated shoots
MS0	Control	28.67 ± 0.882a	1.35 ± 0.06e	1.74±0.08f	2.42 ±0.11g
MS1	BAP @ 0.5 mg/l	27.33 ± 0.333ab	2.75 ± 0.12d	3.15±0.14e	3.34 ±0.15f
MS2	BAP @ 1.5 mg/l	26.00 ± 1.155ab	3.40 ± 0.08d	4.60±0.10bc	4.20 ±0.10de
MS3	BAP @ 3.0 mg/l	25.67 ± 0.882abc	4.60 ± 0.03bc	5.80±0.04a	5.10 ±0.03bc
MS4	BAP @ 4.5 mg/l	25.00 ± 0.577abc	4.64 ± 0.58abc	5.41± 0.67ab	4.83 ±0.60cd
MS5	BAP @ 0.5 mg/l + NAA @ 0.5 mg/l	25.00 ± 0.577abc	3.00 ± 0.26d	3.40±0.29de	3.90 ±0.34ef
MS6	BAP @ 1.5 mg/l + NAA @ 0.5 mg/l	24.00 ± 3.055bc	4.20 ± 0.12c	4.20±0.12cd	4.80 ±0.14cd
MS7	BAP @ 3.0mg/l + NAA @ 0.5mg/l	21.67 ± 0.882c	5.40 ± 0.28a	5.20±0.27ab	5.80 ±0.30ab
MS8	BAP @ 4.5mg/l + NAA @ 0.5 mg/l	24.00 ± 0.577bc	5.13 ± 0.18ab	5.03±0.18abc	6.04 ±0.21a
C.D		3.749	0.743	0.833	0.82
C.V		8.586	11.223	11.261	10.554

Table 4.4: Half MS basal medium treatments with different concentration of growth regulators

Treatments	PGR (Mg/l)	Percent root formation	Root length (cm.)	Number of roots regenerated per shoots	No. of days taken for root initiation
HMS ₀	Control	14.62 ± 0.34d	1.35 ± 0.06d	2.90 ± 0.13d	25.81±1.18a
HMS ₁	IAA @ 0.5 mg/l	41.79 ± 1.15b	3.15 ± 0.14c	4.62 ±0.21bc	20.16±0.91bc
HMS ₂	IAA @ 1.5 mg/l	53.96 ± 0.91a	4.80 ± 0.11a	6.80 ±0.16a	18.40±0.43c
HMS ₃	IAA @ 3.0 mg/l	50.87 ±0.20a	4.40 ± 0.02ab	6.60 ±0.04a	18.80±0.11bc
HMS ₄	NAA @ 0.5 mg/l	36.25 ±0.73c	3.00 ± 0.10c	4.20 ±0.14c	22.50±0.78ab
HMS ₅	NAA @ 1.5 mg/l	45.44 ±0.50b	4.20 ± 0.08ab	5.40 ±0.09b	19.50±0.34bc
HMS ₆	NAA @ 3.0 mg/l	41.66 ±3.18b	3.96 ± 0.49b	5.03 ±0.63bc	19.43±2.42bc
C.D		4.205	0.63	0.824	3.47
C.V		5.85	10.02	9.18	9.49

Table 4.5: Effect of different potting mixtures for hardening of strawberry micropropagated plantlets

S. No.	Treatments	Total number of plants	Plant survival	Percent survival
1.	Coco peat	20	14	70 %
2.	Coco peat: Vermiculite(1:1 v/v)	20	10	50 %
3.	Coco peat: Vermiculite(2:1 v/v)	20	12	60 %
4.	Coco peat: Vermiculite(3:1 v/v)	20	12	60 %
5.	Coco peat: Vermiculite : Soil (1:1:1 v/v/v)	20	12	60 %
6.	Coco peat: perlite (1:1 v/v)	20	10	50 %
7.	Coco peat: perlite (2:1 v/v)	20	12	60 %
8.	Coco peat: perlite (3:1 v/v)	20	12	60%
9.	Coco peat: perlite: Soil (1:1:1 v/v)	20	12	60%
10.	Coco peat: vermiculite: perlite (1:1:1 v/v/v)	20	8	40%



MS media with Ascorbic acid 150 mg/l



MS media with Ascorbic acid 300 mg/l



MS media with citric acid 150 mg/l



MS media with citric acid 300 mg/l

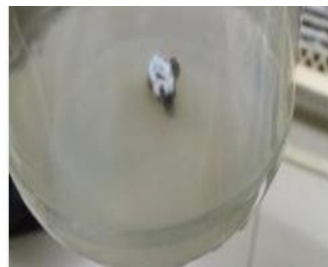


MS media with PVP 150 mg/l



MS media with PVP 300 mg/l

Micropropagation of Strawberry cv. Camarosa



Culture showing contamination in strawberry cv. Camarosa



Phenolic exudation in strawberry cv. Camarosa

Reference

1. Afrin S, Gasparrini M, Forbes-Hernandez TY, Reboredo-Rodriguez P, Mezzetti B, Varela LA, Giampieri F, Battino M. 2016. Promising health benefits of the strawberry: A focus on clinical studies. *Journal of Agricultural and Food Chemistry*. 64: 4435–4449.
2. Ali M, Ali Q, Athar HUR, and Ashraf M. 2018. Ameliorative role of ascorbic acid in plant tissue culture under stress conditions: A review. *Plant Stress Physiology*. 10 (3): 123-136.
3. Ali Q, Hafeez A, Sohu AA, and Noor MA. 2018. The role of ascorbic acid in enhancing in vitro tissue culture response and mitigating oxidative stress in strawberries and other fruit crops. *Acta Physiologiae Plantarum*. 40 (5): 105.
4. Beattie, J., Crozier, A., Duthie, G.G. 2005 Potential Health Benefits of Berries. *Curr. Nutr. Food Sci*, 1, 71–86.
5. Biswas, M.K., Islam, R., and Hossain, M., 2008. "Micro propagation and field evaluation of strawberry in Bangladesh." *J of Agri Tech*, 4(1), 167-182.
6. Cao, J., and Hammerschlag, F. A., 2000. Improved shoot regeneration from leaves of highbush blueberry. *HortScience*, 35(5), 945–947.
7. Clavero, R., Beltrán, A., Llauger, R., Rodríguez, A., Farrés, E., García, M.E., Placeres, J., Betancourt, M., Avalos, Y. Rodríguez, M., 2010. Apuntes sobre el cultivo de la fresa (*Fragaria* × *ananassa* Duch.). *Rev. CitriFrut*, 27, 67–71.
8. Compton, M.E., and Preece, J.E., 1986. Exudation and explant establishment. *International Association for Plant Tissue Culture. Newsletter*, 50, 9-18.
9. Diengngan, S., and Murthy, B.N.S., 2014. Influence of plant growth promoting substances in micropropagation of strawberry cv. Festival. *The Bioscan*, 9(4), 1491-1493.
10. Ebrahimi, R., Ebrahimi, F., Ahmadizadeh, M., 2012. Effect of Different Substrates on Herbaceous Pigments and Chlorophyll Amount of Strawberry in Hydroponic Cultivation System. *Am. Eurasian J. Agric. Environ. Sci*, 12, 154–158.
11. Emara, M. M. (2008). Photosynthetic competence and establishment challenges of in vitro raised plants during hardening. *Plant Tissue Cul & Biotech*, 18(2), 147-154.
12. Etienne, H., and Berthouly, M., 2002. Temporary immersion systems in plant micropropagation. *PCTOC*, 69, 215–231.
13. Giampieri, F., Alvarez-Suarez, J.M., Mazzoni, L., Forbes-Hernandez, T.Y., Gasparrini, M., González-Paramàs, A.M., Santos-Buelga, C., Quiles, J.L., Bompadre, S., Mezzetti, B., 2014. Polyphenol-rich strawberry extract protects human dermal fibroblasts against hydrogen peroxide oxidative damage and improves mitochondrial functionality. *Molecules*, 19, 7798–7816.
14. Grigatti, M., Giorgioni, M.E., Ciavatta, C., 2007. Compost-based growing media: Influence on growth and nutrient use of bedding plants. *Bioresour. Technol.*, 98, 3526–3534.
15. Haddadi, F., Aziz, M.A., Saleh, G., Rashid, A.A., Kamaladini, H., 2010. Micropropagation of Strawberry cv. Camarosa: Prolific Shoot Regeneration from In Vitro Shoot Tips Using Thidiazuron with N6-benzylamino-purine. *Hort Science*. 45(3): 453–456.
16. Hannum, S.M. 2004. Potential impact of strawberries on human health: A review of the science. *Crit. Rev. Food Sci. Nutr*, 44, 1–17.
17. Hasan, M.N., Nigar, S., Rabbi, M.A.K., Mizan, S.B., Rahman, M.S., 2010 "Micropropagation of strawberry (*Fragaria* × *ananassa* Duch.)." *Int. J. Sustain. Crop Prod*, 5(4): 36-41. ISSN-1991-3036.
18. Kumar, P., Bhatia, R., and Gupta, A. K. (2020). Effect of antioxidants on phenolic exudation and growth performance in in vitro propagated plants. *Journal of Horticultural Science and Biotechnology*, 95(1), 87-93.
19. Kumar, R., and Thakur, K.S., 2017. "Advances in strawberry production under changing climate conditions." *Ind J of Hort*, 74(1), 85-92.

20. Leifert, C., Morris, C.E., Waites, W.M., 1994. Ecology of microbial saprophytes and pathogens in tissue culture and field grown plants: reasons for contamination problem in vitro. *Crit. Rev. Plant Sci.* 13: 139-183.
21. Meyers, K.J., Watkins, C.B., Pritts, M.P., and Liu, R.H.,2003. Antioxidant and antiproliferative activities of strawberries. *J. Agric. Food Chem*, 51, 6887–6892.
22. Mirza, F. G., Kadir, R A., Breymann, C., Fraser, I. S. & Taher, A.,2018. Impact and management of iron deficiency and iron deficiency anemia in women's health. [Expert Review of Hematology.9 \(11\): 727-736.](#)
23. Murashige T.,and Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473–497.
24. Pospisilova J., Ticha I., Kadlec P., Haisel D., and Plzakova S., 1999. Acclimatization of micropropagated plants to ex vitro conditions. *Biologia Plantarum* 42 (4): 481-497.
25. Rahman, M. M., Islam, T., Haque, M., and Hossain, M. A.,2020. Advances in tissue culture techniques for crop improvement. *J of Plant Res*, 133(3), 541-550.
26. Sakila, S., M.B. Ahmed, U.K. Roy, M.K. Biswas, R. Karim, M.A. Razvy, M. Hossain, R. Islam, and Hoque, A., 2007. Micropropagation of strawberry (*Fragaria ananassa* Duch.). A newly introduced crop in Bangladesh. *American-Eurasian Journal of Scientific Research* 2:151–154.
27. Scalbert, A., Monties, B., and Janin, G.,1988. Tannins in wood: Comparison of different estimation methods. *J of Agri and Food Che*,36(5),1324–1329. <https://doi.org/10.1021/jf00084a031>.
28. Seneviratne P,Wijesekara GAS(1996). The problem of phenolic exudates in *in vitro* cultures of mature *Hevea brasiliensis*. *Journal of Plantation Crops*,24: 54-62.
29. Sharma, R., Gupta, S., and Verma, P.,2019. Comparative analysis of sterilization methods in plant tissue culture. *Biotech Adv*, 37(2), 678-685. <https://doi.org/10.1016/j.biotechadv.2018.11.002>
30. Sharma, R., Singh, R., and Sood, N. (2019). Role of antioxidants in improving in vitro propagation and reducing phenolic browning in tissue culture. *Indian Journal of Horticulture*, 76(4), 405-412.
31. Sharma, R.R., and Goyal, R.K.,2002. "Strawberry cultivation in India: Progress and prospects." *Indian Horticulture Journal*, 56(2), 25-31.
32. Singh, D., Kumar, S., and Rana, R. K. (2021). Phenolic exudation and its management in in vitro cultures: A critical review. *Journal of Plant Biotechnology*, 23(2), 150-165.
33. Singh, V., Patel, S., and Kumar, R.,2021. Challenges and solutions in strawberry tissue culture under controlled conditions. *Horticultural Science*, 56(4), 310-318. <https://doi.org/10.17221/123/2020-HORTSCI>.
34. Tembe, K.O., Chemining'wa, G.N., Ambuko, J., Owino, W., 2017.Effect of water stress on yield and physiological traits among selected African tomato (*Solanum lycopersicum*) land races. *Int. J. Agron. Agric. Res.*, 10, 78–85. 45.