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Micropropagation of Strawberry Crop (*Fragaria ananassa*): A Review

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ABSTRACT

The objective of this review paper is to summarize on the history, advantages, disadvantages of *in vitro* micropropagation of strawberry crop. Since the traditional method of propagation, that is, the use of runners as propagating material, is not that efficient because it leads to the spread of viral diseases. We have to go for *in vitro* micropropagation, the crops also yield more when compared to traditional methods of propagation. The paper also reviews a study done to develop a protocol for large-scale propagation of strawberry cv. 'Sweet Charlie' and 'Winter Dawn' using the micropropagation technique. Shoot cultures were obtained from shoot tips on Murashige and Skoog (MS) medium with 4% table sugar, 0.75% agar, 5 mg L^{-1} 6-benzyladenine and 0.01 mg L^{-1} kinetin. These shoots were multiplied and maintained on MS medium with 1 mg L^{-1} 6-benzyladenine and 0.1 mg L^{-1} kinetin. Rooting of *in vitro* raised shoots was successfully conducted by pulse treatment with 500 mg L^{-1} indole-3-butyric acid for 30 s and subsequent culturing in MS medium with 1 mg L^{-1} indole-3-butyric acid, 0.1 g L^{-1} activated charcoal and 6% table sugar. Plants were successfully acclimatized and survived in field conditions.

Key words: Fragaria, Micropropagation, Plant propagation, Strawberry.

Strawberry (*Fragaria ananassa* Duch.), belonging to the family Rosaceae, is one of the important small fruits. It is produced over an area of 1800600 hectares spread over 71 countries. The annual world production of 9,125,913 tonnes (FAOSTAT, 2017). All the strawberry cultivars present today all octoploid (2n=56) and well adapted to a wide range of climatic conditions. Strawberry is commercially grown in Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Maharashtra, West Bengal, the Nilgiri Hills and sections of Delhi, Haryana and Punjab in India. Strawberries have always been a popular delicacy due to its flavour, taste, freshness, freezing and processing capabilities. It contains relatively high quantities of ellagic acid, which has a wide range of biological activity. Strawberries contain many important dietary components including vitamins, minerals, folate and fibre and are a rich source of phytochemical compounds mostly represented by polyphenols (Giampieri *et al*., 2012).

Runners are used to propagate the strawberry crop and viral infections are frequently transmitted through runners. As a result, *in-vitro* micropropagation techniques have been proved to be a viable alternative to traditional runner production for mass propagation (Singh, 2002) of virus free planting material (Lal *et al*., 2003; Kanwar *et al*., 2013). It's also a known fact that tissue cultured strawberry plants have higher yield as compared to conventionally grown plants (Cameron and Hancock, 1986). Despite the benefits of micropropagation, the expensive cost of plant production has prevented this approach from being used in the past. Certain elements, however, can be tweaked to make micropropagation more cost-effective.

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Micropropagation in strawberry

Micropropagation or tissue culture is the process of rapidly multiplying plant stock material to produce many progeny plants, using plant tissue culture methods.

The first report of *in vitro* strawberry propagation by (Boxus, 1974), there have been many reports on types of medium, plant growth regulators, genotypes and types of explants used in strawberry regeneration. Nevertheless, there are still problems associated with the regeneration of strawberry explant, *i.e*., meristem; for example, the highest percentage of explant producing shoots for cv. *Elsanta* was only 4% (Debnath, 2006), which seems to be insufficient for commercialization (Boxus, 1974).

Young strawberry plantlets, obtained from meristems, are initially maintained in a medium containing undiluted Knop solution, the micro-elements used by (Murashige and Skoog, 1962), nicotinic acid 0.5 mg/L, pyridoxine HCl 0.5 mg/L, glycine 2.0 mg/L, thiamine HCl 0.1 mg/L, meso-inositol 100.0 mg/L, indolybutyric acid 1.0 mg/L, glucose 40.0 g/L,

agar 8.0 g/L, adjusted pH 5.6. This medium is based on the mixture of two media recommended by (Vine, 1968) for strawberry meristem culture (Boxus, 1974).

Somatic embryogenesis and bioreactor systems could be techniques that replace conventional micropropagation using agar sucrose-based media (Cardosa *et al.*, 2018).

Methods of micropropagation

- **Meristem culture**

Subtending leaf primordial and a meristem is placed into their respective growing media culture and allowed to grow.

- **Callus culture**

Selected plant tissue is placed in an artificial growing medium culture until the callus is formed.

- **Suspension culture**

Cells or groups of cells are dispersed and allowed to grow in an aerated and sterile liquid culture medium.

- **Embryo culture**

The embryo is extracted and placed into a culture medium with proper nutrient in aseptic condition.

- **Protoplast culture**

The plant cell is isolated and cultured in an appropriate medium to reform the cell wall and callus. (https://en.wikipedia.org/wiki/Micropropagation#References).

When TIBs system was used to carry out tissue culture and rapid propagation and study the effects of different generations, inoculation densities, intermittent immersion frequencies and immersion time on the proliferation of strawberry tissue culture seedlings. The results showed that better culture effect could be obtained by selecting the 5th generation of sub-cultured plantlets, adopting the inoculating density of 2 plants/bottle and intermittent immersion frequency of 10 min/L h and selecting the hormone combination of 3.0 mg/L 6 - BA + 0.01 mg/L NAA (Mengxing *et al.*, 2020).

The main advantages of micropropagation over the conventional methods of clonal propagation are

a) From a single individual, a great number of plants can be generated in a short amount of time and area.
b) Very small pieces of plant tissues are required to initiate aseptic cultures.
c) The rate of multiplication *in vitro* is often much faster than the *in vivo* methods of vegetative propagation (Mir *et al.*, 2010) because in cultures it is possible to manipulate the nutrient and growth regulator levels, temperature and light more effectively.
d) It can be used to propagate many genotypes that are difficult or impossible to propagate *in vivo*.
e) It goes on round the year.
f) The plants are protected from re-infection if they are derived from virus-free stock and the micropropagated plants can be exported with no quarantine hassle.
g) The plants are relatively free of microorganism infestation.
h) *In vitro* production can be better planned by storing cultures at a low temperature during low-demand seasons.
i) Plants grown by micropropagation may develop new desirable characteristics, such as a bushy habit of ornamental plants and a higher number of runners in strawberries (Bhojwani *et al.*, 2013).

Disadvantages of micropropagation

Labour may make up 50%-69% of operating costs

- A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.
- An infected plant sample can produce infected progeny. This is uncommon as the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.
- Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant.
- Sometimes plants or cultivars do not come true to type after being tissue cultured. This is often dependent on the type of explant material utilized during the initiation phase or the result of the age of the cell or propagule line.
- Some plants are very difficult to disinfect of fungal organisms. (https://en.wikipedia.org/wiki/Micropropagation# References).

Process of micropropagation in strawberry

To develop a cost-effective protocol, table sugar cubes from the local market, LED lights to reduce cost of electricity, low-cost agar, reverse osmosis (RO) water and laboratory grade chemicals were used. (Dhukate *et al.*, 2021).

Disinfection of shoot tip

Aseptic cultures were initiated from 3 to 4 cm long runner tips of two-month-old healthy plants of strawberry cultivars 'Sweet Charlie' and 'Winter Dawn'. Shoot-tips were washed with running tap water for 10 min to remove adhering dust. Afterwards, these shoot tips were soaked in 3% Teepol™ (liquid soap solution) for 5 min and washed in running tap water for 10 min. Shoot tips were disinfected in aseptic condition with 1% Bavistin (a fungicide solution consisting of carbendazin 12% + mancozeb 63%) for 10 min. Later, these shoot-tips were treated using 0.5% sodium hypochloride solution for 7 to 8 min, followed by immersion in 0.05% mercuric chloride for 1 min and finally the shoot tips were washed three times with sterilized RO water. Shoot tips were trimmed (0.4 to 0.5 cm) at the cut end prior to inoculation onto culture initiation medium (Dhukate *et al.*, 2021).

Culture medium

Culture medium consisted of Murashige and Skoog (1962; MS) nutrient medium containing 0.75% tissue culture grade agar, 0.7 g L^{-1} ascorbic acid (AA), 10 mg L^{-1} adenine sulphate (ADS) and 4% table sugar. The pH of the culture media was adjusted to 5.8 and about 50 mL medium was poured in glass bottles with semi-transparent polypropylene screw-

caps and autoclaved for 15 min at 121°C. Shoot clumps were subcultured at regular interval of 35 days (Dhukate *et al*., 2021).

Sugarcane bagasse can be used as a substitute for agar in the rooting medium of shoots in plant micropropagation process. Sugarcane bagasse were better than the commercial medium (agar). In the acclimatization step 100% of survival were obtained, which could manipulate the costs of the total micropropagation process (Mohan *et al*., 2005).

The number of developed buds per explant, the height of micro-shoots, the general appearance and development of microplants were taken into account. It was found that exclusion of ammonium nitrate (NH_4NO_3) and replacement of calcium chloride ($CaCl_2$) with calcium nitrate [Ca $(NO_3)_2$] in the Boxus nutrient medium in strawberry provided obtaining optimally developed plants (Tashmatova *et al*., 2021).

In an *in vitro* propagation method for five strawberry cultivars by culturing the meristem in MS medium containing different concentrations of Kn. The concentration of 0.5 mg L^{-1} Kn produced the most optimal shoot induction and plant growth parameters. The resulting meristem-derived plants were genetically stable in comparison with conventionally propagated plants and the vegetative growth and fruit quality attributes of the meristem-derived and conventionally propagated plants were similar when cultivated in a greenhouse for three continuous growing seasons (Naing *et al*., 2019).

Culture conditions

Cultures were kept at 16:8 h light/dark photoperiod with light intensity of 2500 lux and 25±2°C temperature (Dhukate *et al*., 2021).

Culture initiation

Culture initiation medium consisted of MS medium with additives as mentioned in the culture medium section with 5 mg L^{-1} benzyladenine (BA), 0.1 mg L^{-1} kinetin (KN), 0.7 g L^{-1} AA, 10 mg L^{-1} ADS and 4% table sugar, which produced around 4 to 5 shoots within one month (Dhukate *et al*., 2021).

Shoot multiplication

MS medium with 1 mg L^{-1} BA and 0.01 mg L^{-1} KN was used for first two subcultures to avoid loss of cultures due to endophytic contamination. From the third subculture, three shoot clumps were maintained on 3/4 strength MS medium with 0.5 mg L^{-1} BA and 0.1 mg L^{-1} KN to avoid vitrification and stunted growth. After the ninth subculture, cultures were again initiated from micropropagated plants maintained in the greenhouse (Dhukate *et al*., 2021).

In vitro rooting

Prior to *in vitro* rooting, the cut ends of in vitro raised shoots were treated with 500 mg L^{-1} indole-3-butyric acid (IBA) solution for 30 s and then were cultured on MS medium with 1 mg L^{-1} IBA, 0.1% activated charcoal and 6% table sugar (Dhukate *et al*., 2021).

Acclimatization of plantlets

For primary hardening, *in vitro* rooted shoots were taken out from the culture medium and gently washed with tap water to eliminate any traces of adhering medium and were transferred to nursery trays containing sterilized cocopeat in a polyhouse. Nursery trays were covered with transparent polythene in a low tunnel for 10 days to maintain high relative humidity (95%) and protect them from water and light stress. These plants were irrigated with 0.2% (w/v) liquid nitrogen, phosphorous and potassium fertilizer solution Sardar WSF-19-19-19TM at regular intervals of three days.

Secondary hardening and field transfer was performed after four weeks. The plantlets were transferred to the net-house onto plastic bags containing garden soil for acclimatization and hardening and then were transferred outdoors into fields (Dhukate *et al*., 2021).

CONCLUSION

To conclude we can agree that as strawberry is a very important fruit in the small fruits category, there is a need to improve the cultivation process for a more mass production standard. Strawberry propagation by runners, which is the most common, simple and natural way, is ineffective and viral infections are frequently transmitted through runners. As a result, *in vitro* micropropagation techniques have been proved to be a viable alternative to traditional runner production for mass propagation. It's also well-known fact that tissue cultured strawberry plants produce more fruit than conventionally grown plants. In this article, we also go over the basics of *in vitro* micropropagation in strawberries, including its history, advantages and disadvantages. For the commercial-scale production of strawberry plantlets, the micropropagation technique is most popular method (Boxus 1989; Capocasa *et al*., 2019). Apical meristem culture is the only method that ensures genetic uniformity of micropropagated plants (D'Amato, 1977). Towards the end, we also discuss on a method which was developed for rapid shoot multiplication of two commercially important strawberry cultivars, 'Sweet Charlie' and 'Winter Dawn', using shoot tip explants. Using this technique, a large number of high-quality planting material can be obtained in a very short period. With the help of this protocol, more than ten thousand plants can be produced from single shoot tip within one year. Thus, we can strongly say that the future of strawberry production in *in vitro* micropropagation.

Conflict of interest: None.

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