JUST AGRICULTURE
multidisciplinary e-Newsletter

Plant Tissue Culture Technique for Production of Strawberry (*Fragaria Ananassa*)

Subaran Singh[1]* and Bijender Singh[2]
[1]Deptt. of Biotechnology, College of Horticulture, Maharana Pratap Horticultural University, Karnal- 132001 (Haryana)
[2]Deptt. of Fruit Science, College of Horticulture, Maharana Pratap Horticultural University, Karnal- 132001 (Haryana)

ARTICLE ID: 65

Abstract

Strawberry (*Fragaria ananassa* Duch.) is a highly profit-generating fruit crop that produces maximum returns in a shorter period of time. It is the richest source of vitamins and minerals required for human health in a balanced diet. Strawberry is mainly grown through runners which give rise to plants which are prone to diseases. *In vitro* micro propagated plantlets are disease-free and can be utilized for further cultivation. Shoot cultures can be produced from shoot tips using Murashige and Skoog (MS) medium supplemented with 3-4% sugar, 0.75–1.0% agar and an appropriate combination of plant growth hormones, such as 6-benzyladenine, NAA, IBA, and Kinetin. A standard regeneration protocol has been carried out with explants disinfection, shoot multiplication, rooting, and *ex vitro* acclimatization of strawberries. *In vitro* micro propagation is significantly helpful to overcome the challenges faced in achieving better quality plants and their higher endurance rate in the *ex vitro* acclimatization.

Key words: Strawberry, Explant, Micro propagation, Plant growth regulators, Growth conditions, Regeneration

Introduction

Strawberry which features a fragrantly sweet flavor is most widely consumed fruit belonging to Rosaceae family. Cultivated strawberry (*Fragaria* × *ananassa* Duch.) which is an octaploid (2n=8x=56) is a hybrid of (*Fragaria virginiana* × *Fragaria chiloensis*). It is largely known for its distinctive aroma, juicy texture and bright red colour and mainly consumed as either fresh or in prepared foods like ice creams, milk shakes and preserved fruit juices. Artificial strawberry aroma is also widely used in many industrialized food products. It is one of the most captivating fruits worldwide and is a good source of vitamin C, vitamin

JUST AGRICULTURE
multidisciplinary e-Newsletter

B1 & B2, protein, potassium, copper, calcium and other nutritious components necessary for human health. It is very costly to import mother plants and there are no available healthy stocks for conventional techniques of propagation (Nehra et al., 1994). Propagation of strawberry is achieved either by runner/nodal segments (Anuradha et al., 2016; Jhajhra et al., 2018) or by runner tips *in vitro* micro propagation (Ashrafuzzaman et al., 2013) with using different combination of plant growth regulators. The micro propagation of strawberry plants using nodal cuttings by *in vitro* technique has proved to be successful (Karhu and Hakala, 2002). Plant growth regulators widely effect the *in vitro* rooting of micro shoots of strawberry *cv.* Sweet Charlie (Madumali et al., 2021). Different explants like leaves, sepals, petioles and nodes have been widely used for proficient strawberry production through plant tissue culture technique (Debnath, 2005, 2006, 2008; Bhatt, 2000). But, meristem culture using runner tips is most useful to regenerate virus free plants of strawberry under *in vitro* conditions (Mercado et al., 2007). Plant growth regulators such as cytokinins and auxins are added to the culture media in order to control the morphogenesis and organogenesis of *in vitro* cultured explants. The advantages of *in vitro* propagation are to produce large number of plantlets with high multiplication rate (Chawla, 2002). *In vitro* techniques are important for germplasm improvement, clonal multiplication and for gene conservation of fruit quality and nutrition value. A generalized standard *in vitro* micro propagation protocol for Strawberry has been described in this article.

Micro propagation Steps

Explant

Runner/nodal segments and runner tips are mostly used as explants. Only the tender and vigorously growing runner tips and runner segments having 1-1.25 cm length should be prepared to use as explants in the media.

Disinfection of explants

An efficient method of sterilization is very helpful for survival of explants (Jan et al., 2013). The explants must be properly washed by running tap water and should be sterilized using autoclaved distilled water containing a drop of tween 20 followed by 1% sodium hypochlorite (NaOCl) for 5-10 mins. Then, the sterilized runners were washed with autoclaved distilled water three times. Inoculations and other operations should be carried out in a laminar airflow cabinet to maintain aseptic conditions. During the culture, 70%

JUST AGRICULTURE
multidisciplinary e-Newsletter

ethanol should be frequently applied to the hands and cabinet base to maintain aseptic conditions. A fine, sterile forceps and scalpel should be used to prepare the shoot tips carefully inside the laminar airflow cabinet.

Culture media

Murashige and Skoog (MS) media is used for culturing the explants, supplemented with specific concentration of growth regulators *viz.*, indole acetic acid (IAA), napthelene acetic acid (NAA), benzylaminopurine (BAP) and Kinetin in combinations.

Conditions for culturing

A temperature of 25 ± 1^{0}C and a light intensity of 3000-4000 lux should be maintained as the ideal physical conditions for the growth and development of cultures. The photoperiod is maintained at 16 hours light and 8 hours dark with a relative humidity of 60-70%.

Shooting and multiplication

Shoot formation is initiated with emergence of small green fresh leaves and it is the first sign of regeneration. Once fully formed, these tiny leaves are transplanted into fresh medium having same hormonal composition to promote further growth and development of shoots. After initiation of the shoot formation, the first and second subcultures should be conducted at three and five weeks, respectively. The regenerated multiple shoots should be placed carefully on a sterile hard paper. The highest average number, length of shoots and leaves were recorded at 0.5 mg/L BAP concentration (Ashrafuzzaman et al., 2013). Additionally, explants generated from cultures on media with 0.5 mg/L Kinetin showed more surviving shoots and better plant development (Naing et al., 2019).

Rooting

The newly grown shoots can be rooted in MS medium at half strength with IBA/NAA. Maximum root length and rooting percentage can be obtained in medium supplemented with 1.0 mg/L IBA, however more number of roots can be obtained in MS media at half strength (1.0 mg/L NAA) (Anuradha et al., 2016). On the other hand, (Ashrafuzzaman et al., 2013) suggested that 0.5 mg/L IBA enriched media shows the best response in terms of root development.

Acclimatization

JUST AGRICULTURE
multidisciplinary e-Newsletter

The strongest and most uniformly hardened seedlings can be removed and transplanted into a nursery by shifting them into nursery bags with a 3:1:1 mixture of coco peat, perlite and vermiculite under 70% shade. During the period of acclimatization, optimum temperature should be 16.4 ^{0}C with a relative humidity of 78 to 80% (Neri et al., 2022).

Conclusion

Plant tissue culture can be very useful to develop disease resistance, salinity resistance and stress tolerance among high yielding varieties. The quantity of propagules that can be produced through traditional runner segment propagation is limited, and the propagules that are produced are vulnerable to plant diseases like those brought on by fungi (Dijkstra, 1993). A protocol has been developed for large-scale propagation of strawberry cv. 'Winter Dawn' and 'Sweet Charlie' using *in vitro* propagation technique (Dhukate, 2021; Valliath and Mondal, 2023). It is challenging to obtain genetically similar plants for continuous production of better quality fruits as strawberries are prone to somaclonal variation when regenerated through *in vitro* tissue culture techniques. For cultivation to be successful, high-quality planting material is required. The absence of runners throughout the cropping season is a significant disadvantage in strawberry cultivation. Different types of medium, explants and combinations of growth regulators should be used to successfully propagate strawberries under *in vitro* conditions for mass multiplication of disease free plants in short time period.

References

Anuradha, Sehrawat, S. K., Poonia, A K., Kajla, S. and Bhat, S. (2016). Production of strawberry plant by in vitro propagation. *Research on Crops*. 17 (3) : 545-549.

Ashrafuzzaman, M., Faisal, S. M., Yadav, D., Khanam, D. and Raihan, F. (2013). Micropropagation of Strawberry (*Fragaria ananassa*) through Runner Culture. *Bangladesh Journal of Agricultural. Research.* 38(3): 467-472.

Bhatt, I.D. and Dhar, U. (2000). Micropropagation of Indian wild strawberry. *Plant Cell Tissue Organ Culture.* 60:83–88.

Chawla, H. S. (2002). Introduction to Plant Biotechnology. Oxford & IBH publishing Co. Pvt. Ltd., 66 Janapath, New Delhi 110001, India. P. 39

Debnath, S.C. (2005). Strawberry sepal: another explant for thidiazuron-induced adventitious shoot regeneration. *In Vitro Cellular & Developmental Biology - Plant*. 41:671–676.

JUST AGRICULTURE
multidisciplinary e-Newsletter

Debnath, S.C. (2006). Zeatin overcomes thidiazuron-induced inhibition of shoot elongation and promotes rooting in strawberry culture in vitro. *Journal of Horticultural Science & Biotechnology*. 81:349–354.

Debnath, S.C. (2008). Developing a scale-up system for the in vitro multiplication of thidiazuron-induced strawberry shoots using a bioreactor. *Canadian Journal of Plant Science*. 88:737–746.

Dhukate, M. R., Kher, M.M., Vadawale, A.V., and Giri, P. (2021). Protocol for micropropagation of strawberry (*Fragaria* × *ananassa*) cv. 'Sweet Charlie' and 'Winter Dawn'. *Environmental and Experimental Biology*. 19: 1–6

Dijkstra, J. (1993). Development of alternative methods for healthy propagation of strawberry plants using cuttings. *Acta Hortic*. 348:234–236.

Jan, A., Bhat, K.M., Bhat, S.J.A., Mir, M.A., Bhat, M.A., Imtiyaz, A., and Rather, J.A. (2013). Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for in vitro culture. *African Journal of Biotechnology*. 12(39): 5749-5753.

Jhajhra, S., Dashora, L.K., Singh, J., Bhatnagar, P., Kumar, A. and Arya, C.K. (2018). In-vitro Propagation of Strawberry (*Fragaria* × *ananassa* Duch.) *International Journal of Current Microbiology and Applied Sciences*. 7(10): 3030-3035.

Karhu, S. and Hakala, K. (2002). Micropropagated strawberries on the field. *ISHS Acta Horticulture*. 2: 182.

Madumali, H.K.C., Abeythilakarathna, P.D. and Seran, T.H. (2021). Rooting performance of in vitro microshoots of strawberry (*Fragaria x ananassa* Duch.) as influenced by plant growth regulators. *Agrieast.*15 (2): 69-73.

Mercado, J. A., Pliego Alfaro, F. and Quesada, M.A. (2007). Strawberry. Edited by T. Nagata (Managing Editor) H. Lörz, 309.

Naing, A. H., Kim, S. H., Chung, M. Y., Park, S.K. and Kim, C.K. (2019). In vitro propagation method for production of morphologically and genetically stable plants of different strawberry cultivars. *Plant Methods*. 15:36.

Nehra, N.S., Kartha, K.K., Stushnoff and Giles, K.L. (1994). Effect of in-vitro propagation methods on field performance of strawberry cultivars. *Euphytica*. 76: 107-115.

JUST AGRICULTURE
multidisciplinary e-Newsletter

Neri, J.C., Meléndez Mori, J.B., Tejada Alvarado, J.J.,Vilca Valqui, N.C., Huaman Huaman, E., Oliva, M. and Goñas, M. (2022). An Optimized Protocol for Micropropagation and Acclimatization of Strawberry (*Fragaria* × *ananassa* Duch.) Variety'Aroma'. *Agronomy* . 12: 968.

Valliath, A.S. and Mondal, R. (2023). Micropropagation of Strawberry Crop (*Fragaria ananassa*): A Review. *Bhartiya Krishi Anusandhan Patrika*. doi: 10.18805/BKAP529.