



## Original Research Article

### Micropagation of Strawberry (*Fragaria X ananassa* Duch.)

Shaila Harugade\*, R.H.Tabe and Sushama Chaphalkar

Vidya Pratishthan's School of Biotechnology, Vidyanagari, Baramati, India

\*Corresponding author

#### A B S T R A C T

##### Keywords

*Fragaria x ananassa* Duch;  
Strawberry;  
Shoot  
multiplication.

Nodal segments of Strawberry Variety Sweet charlie give rise to multiple shoots when cultured on MS medium supplemented with different concentrations of BA with Kin or GA3. The highest response of shoot multiplication was obtained on MS containing 1.5 mg/l BA +0.5 mg/l Kin. The maximum frequency of rooting and highest number of roots was produced in medium containing 1.0 mg/l IBA. The plantlets thus developed were hardened and successfully established in soil.

#### Introduction

Strawberry (*Fragaria x ananassa* Duch.) is a natural hybrid of *Fragaria chiloensis* L. P. Mill. and *Fragaria virginiana* Duch. It is a perennial, stoloniferous herb belongs to the Rosaceae family. Strawberries have traditionally been a popular delicious fruit for its flavour, taste, fresh use, freezing and processing. It contains relatively high quantities of ellagic acid, which has a wide range of biological activity. It is produced in 71 countries worldwide on 506000 acres. micropropagated strawberry plants were comparatively better in different characters (crown size, number of runners, flowering time and yield of berries) than conventionally propagated runner plants. Although production of propagules through runner has been reported to contribute 90% of total Dutch strawberry production, the product in Elsanta cultivars was found to be susceptible to

several fungal diseases. In the view of the potential commercial value, it is highly desirable to develop methods of rapid, efficient and large scale multiplication of *Fragaria X ananassa* Duch.variety Sweet charley through tissue culture. In the present study a simple protocol has been developed to propagate strawberry through tissue culture methods from nodal segments in order to ensure abundant supply of this plant material for commercial cultivation.

#### Materials and Methods

Fresh nodes from strawberry variety Sweet charley mature plants (Fig. 1A) were collected during the first week of November 2008 Explants were washed in running tap water and then washed again thoroughly by adding a few drops of

Tween-20. They were then surface sterilized in a 0.1% mercuric chloride for 5 min followed by rinsing them four times with double distilled water inside the Laminar Air flow chamber. Small nodal segments (0.5–1.0 cm) were cultured on MS supplemented with specific concentration of growth regulators (BA, KIN and GA ) singly or in combination adding 30 g l<sup>-1</sup> sugar (market sugar) and 0.7% agar. The pH of the medium was adjusted to 5.7 with 0.1 NaOH before autoclaving at 1.06 kg/cm and 121°C for 20 min. The cultures were incubated at 20 ± 2°C with 16 h photoperiod.

Subcultures were done every 21 days interval. Nodal segments from the proliferated shoots were subcultured again for further multiple shoot induction. Regenerated multiple shoots were cut and individual shoots were placed in MS medium containing different concentrations of IBA for root induction.

Data were recorded after 5 weeks for recording multiple shoot induction and rooting frequency. Only data which showed some advantageous effect were included in the tables and 25 explants were used per treatment and repeated three times.

## Results and Discussion

11) with KIN (0.1 and 0.5 mg l<sup>-1</sup>) or GA (0.1and 0.5 mg l<sup>-1</sup>). Within five weeks of culture multiple shoots emerged directly from the explants. The numbers of shoots in medium with BA + KIN were greater than those observed in the medium supplemented with BA + GA. The highest rate of response was obtained at 1.5 mg l<sup>-1</sup> BA+0.5 mg l<sup>-1</sup> KIN combination (Table 1) where 88% explants showed shoot proliferation and 9.3±058 shoots

developed. When BA concentration was increased above 1.5 mg l<sup>-1</sup>, the rate of shoot multiplication reduced. However, the maximum number of shoots per explant and highest average length were recorded at 1.5 mg l<sup>-1</sup> BA+0.1 mg l<sup>-1</sup>KIN. When BA was supplemented with GA instead of KIN the rate of shoot proliferation and number of shoot did not improve but the shoot length increased. In the present study under the moderate combination of 1.5 mg l<sup>-1</sup> BA + 0.1-0.5 mg l<sup>-1</sup> KIN was found to be the ideal combination for high frequency multiple shoot induction. It is observed that high concentration of cytokinin reduced the number of micropropagated shoots. The developing shoots were elongated by subculturing on the same combinations of growth regulators. Later on elongated shoots were excised and used for root induction.

Out of different concentrations of IBA (0.1-1.5 mg l<sup>-1</sup>) tested 1.0 mg l<sup>-1</sup> IBA proved to be the most suitable for root induction with 5 roots per explant and the average root length being 3.68 cm (Table1; fig 2). Rooted plantlets were taken out from culture tubes and washed thoroughly with tap water to remove the culture medium from the roots. Washed plantlets were sprayed with fungicide and planted to normal and sterilized soil in polybags. After 7 days the hardened plantlets were planted (Fig 3) in soil. The protocol reported here is reproducible; it has a potential for allowing a large scale micropropagation of this important plant.

In hardening soil and cocopeat(3:1) giving poor results 10% as compare to cocopeat and perlite(3:1) giving 80% results. We carry out Primary hardening in Laboratory giving 80% survival as compare to field conditions. Secondary hardening giving 50% survival for first time.(2)

**Table.1** Effects of different concentrations of IBA on *in vitrorooting* of shoots of strawberry variety Sweet charley after 5 weeks of culture

Growth regulators IBA Conce. (mg/l)	% of microcutting rooted	Days to root formation	No. of roots/ microcutting	Average root length( cm)
0.1	78	12-14	4	1.5
0.2	72	10-14	3	1.7
0.5	82	10-14	3	2.1
1.0	90	8-10	5	4
1.5	75	8-10	2	2

**Fig.1** Subcultured Strawberry



**Fig.2** Rooting



**Fig.3** Transplantation



**Fig.4** Hardened Strawberry



## References

- Sakila,S., M.B. Ahmed, U.K. Roy, M.K. Biswas,R. Karim,M.A. Razvy, M. Hossain, R. Islam and A. Hoque , Micropropagation of Strawberry (*Fragaria X ananassa* Duch.), A Newly Introduced Crop in Bangladesh. American-Eurasian Journal of Scientific Research 2 (2): 151-154, 2007.
- Hazarika, B.N., Acclimatization of tissue-cultured plants, Current science, Vol. 85, No. 12, 25 December 2003.
- Jofre-Garfias,A.E., M.N. Vazquez-Sanchez, A.R. Hernandez-Razo, P.A. Davalos-Gonzalez, Production and Acclimatization of in vitro produced Strawberry plants, ISHS Acta Horticulturae 727: X International Symposium on Plant Bioregulators in Fruit Production.
- Reinert , J., and Y.P. S. Bajaj. Applied and fundamental aspects of plant cell,tissue and organ culture, Narosa publishing house.