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Micropropagation of Strawberry (*Fragaria* X *ananassa* Duch.) A Newly Introduced Crop in Bangladesh

[1]S. Sakila, [1]M.B. Ahmed, [1]U.K. Roy, [2]M.K. Biswas, [1]R. Karim, [1]M.A. Razvy, [1]M. Hossain, [1]R. Islam and [1]A. Hoque

[1]Plant Breeding and Gene Engineering Lab.,
Dept. of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh
[2]Department of Pomology, College of Horticultre and Forestry, Huazhong Agricultural University, China

Abstract: Nodal segments of strawberry gave rise to multiple shoots when cultured on MS medium supplemented with different concentration of BA with KIN or GA_3. The highest response of shoot multiplication was obtained in MS containing 1.5 mg l^{-1} BA + 0.5-0.1 mg l^{-1} KIN. The regenerated shootlets were rooted on MS basal medium with different concentrations IBA and IAA. The maximum frequency of rooting and highest number of roots was produced on medium containing 1.0 mg l^{-1} IBA. The plantlets, thus developed were hardened and successfully established in soil. The plants raised through tissue culture exhibited normal growth, flowering and fruit setting.

Key words: Regeneration · Node · Strawberry · Fragaria X ananassa

INTRODUCTION

Strawberry (*Fragaria* x *ananassa* Duch.) is a natural hybrid of *Fragaria chiloensis* L. P. Mill. and *Fragaria virginiana* Duch. It is a perinnial, 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. Strawberries are now getting popularity in Bangladesh. Karhu and Hakala [1] observed that 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 [2].

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. through tissue culture.

This is the first time to report on *in vitro* propagation of strawberry in Bangladesh. 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 mature plants (Fig. 1A) were collected during the first week of November 2006 from the Akafugi Agrotechnologies, Padma Abashik, Vadra, Rajshahi. 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_3) singly or in combination adding 30 g l^{-1} 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^2 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

Corresponding Author: Md. Bulbul Ahmed, c/o: Professor M. Monzur Hossain, Dept. of Botany, Rajshahi University, Rajshahi-6205, Bangladesh

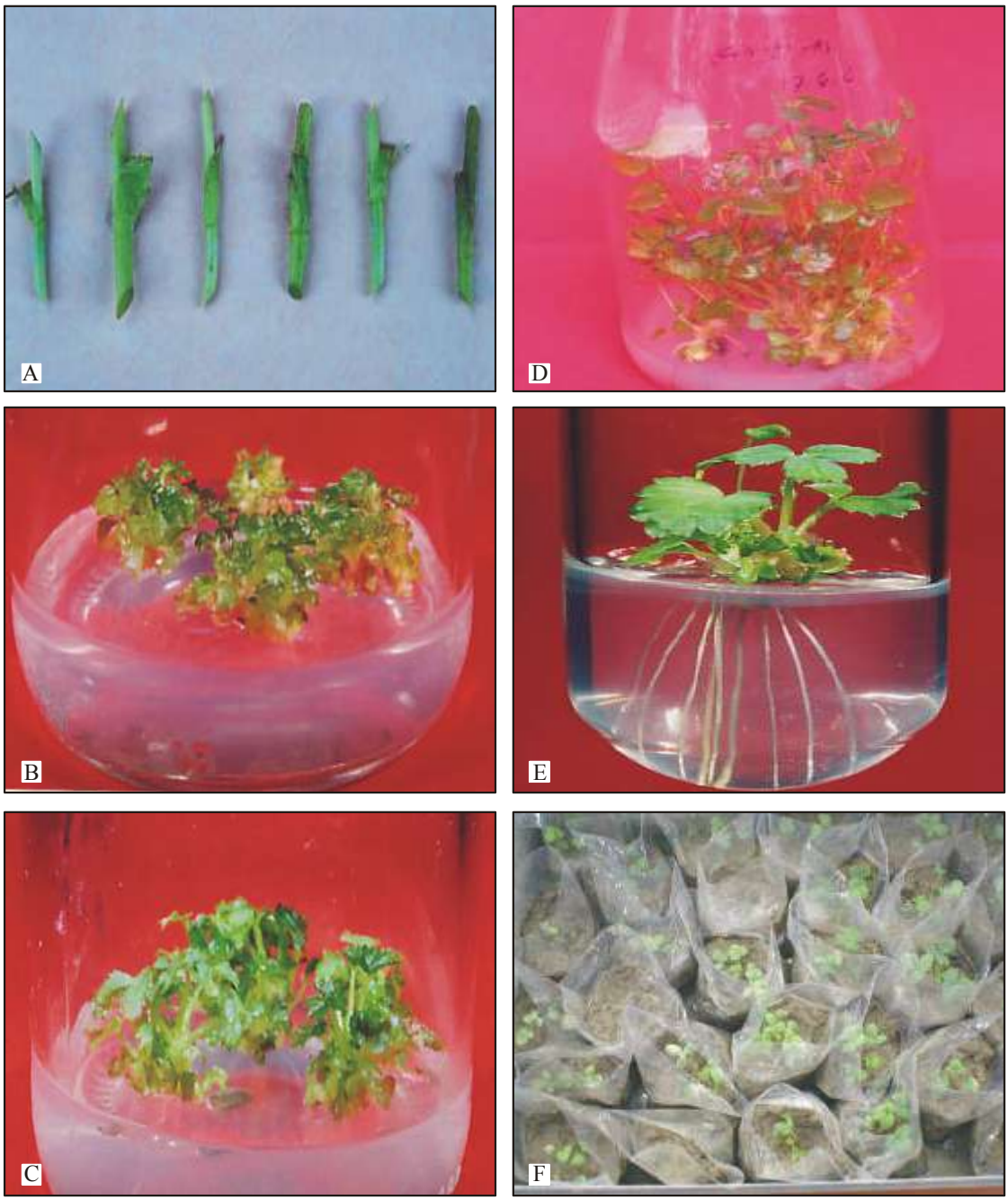

Fig. 1: Micropropagation of Strawberry from nodal segments. A. Nodal segments used as explants. B. Shoot proliferation on MS supplemented with 1.5 mg/l BA+0.1 mg/l KIN after 7 days of culture. C. Shoot proliferation on MS+1.5 mg/l BA+0.5 mh/l KIN after 20 days of culture. D. Shoot proliferation on MS+1.5 mg/l BA+0.5 mg/l KIN after 35 days. E. Rooted shoots on 1.0 mg/l IBA after 3 weeks of culture. D. Regenerated plantlets of strawberry in polybags after 20 days of transplantation

Table 1: Effects of different concentrations of BA with GA_3 or KIN on multiple shoot induction from nodal explants of Strawberry

Growth regulators conc. (mg l^{-1})	% of explants showing shoot proliferation	Days to shoot formation	No. of shoots/explant (mean ± SD)	Average length of shoot (cm)
BA+KIN				
0.5+0.1	50	10-12	6.2±0.44	1.1
0.5+0.5	61	10-12	6.5±0.15	1.5
1.0+0.1	65	10-12	5.8±0.80	1.9
1.0+0.5	70	10-12	7.0±0.23	2.3
1.5+0.1	79	9-12	10.1±0.33	3.4
1.5+0.5	88	8-10	9.3±0.58	3.0
2.0+0.1	64	10-12	6.6±0.22	2.4
2.0+0.5	53	10-12	7.5±0.48	1.1
BA+GA_3				
0.5+0.1	62	10-12	5.0±0.56	4.2
0.5+0.5	60	10-112	4.0±0.58	4.5
1.5+0.1	73	8-10	6.3±0.18	5.5
1.5+0.5	72	10-12	4.5±0.49	5.1
2.0+0.1	65	10-12	5.0±0.12	4.0
2.0+0.5	50	10-12	4.0±0.24	5.0

Table 2: Effects of different concentrations of IBA and IAA on *in vitro* rooting of shoots of strawberry after 5 weeks of culture

Growth regulators conc. (mg l^{-1})	% of microcutting rooted	Days to root formation	No. of roots/ microcutting	Average root length (cm)
IBA				
0.1	78	12-14	4.0±0.29	1.90
0.2	72	10-14	3.0±0.48	1.90
0.5	82	10-14	3.0±0.58	2.10
1.0	90	8-10	5.0±0.23	3.68
1.5	75	8-10	2.0±0.56	2.00
IAA				
0.1	77	10-14	2.0±0.44	1.90
0.2	80	12-14	3.0±0.58	2.20
0.5	75	12-14	3.0±0.23	2.10
1.0	75	12-14	2.0±0.48	2.10
1.5	65	12-14	1.5±0.22	1.75

multiple shoots were cut and individual shoots were placed in MS medium containing different concentrations of IBA and IAA 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

Nodal explants were inoculated on MS medium fortified with different concentration of BA (0.5-2.0 mg l^{-1}) w ith KIN (0.1 and 0.5 mg l^{-1}) or GA_3 (0.1and 0.5 mg l^{-1}). 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_3. The highest rate of response was obtained at 1.5 mg l^{-1} BA+0.5 mg l^{-1} 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^{-1}, 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^{-1} BA+0.1 mg l^{-1} KIN. When BA was supplemented with GA_3 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^{-1} BA + 0.1-0.5 mg l^{-1} KIN was found to be the ideal combination for high frequency multiple shoot induction. Results are shown in figure 1 (B, C and D). Hu and Wang [3] reported that high concentration of cytokinin reduced the number of micropropagated shoots. Similar results have already been reported in *Fragaria indica* Andr. [4]. Also this result is in consistent with the findings of in papaya [5] as well as in *Eucalyptus grandis* [6]. 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^{-1}) and IAA (0.1–1.5 mg l^{-1}) tested 1.0 mg l^{-1} IBA proved to be the most suitable for root induction with 5.0±0.23 roots per explant and the average root length being 3.68 cm (Table 2; Fig. 1, E). Similar effects of IBA were also observed in *Calotropis gigantea* [7], *Capsicum annum* [8] and *Prunus* sp. [9].

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 (Fig. 1, F). After 7 days the hardened plantlets were planted in soil. The protocol reported here is reproducible; it has a potential for allowing a large scale micropropagation of this important and new plant in Bangladesh.

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