Original Research Article

IN VITRO PROPAGATION OF STRAWBERRY (*Fragaria* X *Ananassa* DUCH.) PLANTLETS THROUGH RUNNER TIPS EXPLANTS

Abstract

Runner tips explants of strawberry give rise to multiple shoots when cultured on MS medium supplemented with different concentrations and combinations of BAP with KIN or NAA or GA_3.The highest response of shoot multiplication was obtained on MS containing 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kin + 0.5 mgl^{-1} GA_3. The maximum frequency of rooting (83%) and highest number of roots (3.49) was produced in medium containing 1.0 mgl^{-1} IBA. The well grown rooted plantlets were acclimatized and successfully established in autoclaved vermiculate soil and as well as natural condition. Using our established protocol, it is also possible to provide large numbers of micropropagated plantlets of this cultivars to produce high quality strawberry fruit for commercial cultivation practices.

Key words: *Strawberry, in vitro, regeneration, multiple shoots and roots,* acclimatization

1. Introduction

The cultivated strawberry (*Fragaria* X *ananassa* Duch.) is a natural hybrid between the Scarlet or Virginia strawberry (*F. virginiana* Duch.) and pistillatet South American (*F. chiloensis* L. Duch), is an octoploid (2n=8x=56), a member of the Rosaceae family, and the most important soft fruit worldwide (Jiajun et al., 2005; Debnath et al., 2007). It is a perennial, stoloniferous herb. Strawberries have traditionally been a popular delicious fruit for its flavor and taste. It can be consumed fresh, frozen or processed. It contains relatively high quantities of allagic acid, which has a wide range of biological activity. It is produced in 71 countries worldwide on 506000 acres (Sakila et al., 2007).

Strawberry is widely appreciated, mainly for its characteristic aroma, bright red colour, and juicy texture. It is consumed in large quantities, either fresh or in prepared foods, such as preserved fruit juice, pies, ice creams, and milk shakes. Artificial strawberry aroma is also widely used in many industrialized food products. Strawberry is one of the most fascinating fruits of the world, which is a rich source of vitamins and minerals and has fabulous and tantalizing aroma. It contains numerous important dietary components and is rich source of vitamin C. It also contains significant levels of ellagic acid, which is thought to be an anti-carcinogenic (ICAR News, 2005).

The strawberry fruits are rich of vitamin C, B1, B2, protein, calcium, potassium, copper and iron, most of the nutritious elements essential for human being (Nehra et al., 1994). It contains relatively high quantities of ellagic acid having a range of biological activity

and especially the fruit contains higher vitamin C concentration than orange or lemon. It is produced in 73 countries worldwide on 200,000 hectors and produced 31 lac metric tons strawberry (FAO, 2008).

Most of the strawberry cultivars are grown in the temperate parts of the world, although a few day-neutral cultivars can be grown in the sub-tropical regions. However, commercial cultivation is not popular in Bangladesh due to lack of proper cultivars (Biswas et al., 2009). For better strawberry production photoperiod 10 to 20h, day temperature 12 to 30°C and number of short days 12 to 24 are essential (Michel et al, 2006). Bangladesh is a sub-tropical country and here in winter average day temperature is 15 to 25°C, photoperiod 12 to 16 h and short days about 30 to 50 days (Biswas et al., 2008). Therefore, in winter season, strawberry can be grown and now a day it becomes very popular due to attractiveness of fruits, fragrance and nutritional quality. Since last few years, strawberries are cultivated but the main constrain of its cultivation is to maintain plant materials due to hot summer in Bangladesh (Ara et al., 2013).

Strawberries can be propagated *in vitro* condition by tissue culture methods whereas micro-propagation is a very useful technique of improvement of plant. Micro-propagated strawberry plants were comparatively better in different characters (crown size, number of runners, flowering time and yield of berries) than conventionally propagated runner plants (Karhu and Hakala, 2002; 2007). 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 (Dijkstra, 1993).

In conventional cultivation, the strawberry (*Fragaria* X *ananassa* Duch.) is a species vegetatively propagated via runner seedlings. Nevertheless, such seedlings due to their

weakness and susceptibility to pathological agents are not always suitable for this cultivation. Nowadays, the advantageous alternative to this conventional method seems to be the use of micro-propagated plants for cultivation (Jadwiga et al., 2015). Micro-propagation of strawberry has been applied on a large scale in commercial production since mid1970s. First applied to strawberry meristems *in vitro* techniques have been amplified to an efficient method of mass propagation (Jadwiga et al., 2015; Boxus, 1992].

However, strawberry was introduced in Bangladesh two decades ago and in becoming popular in recent years. Bangladesh Agricultural Research Institute (BARI) recently released a strawberry variety known as BARI Strawberry-1 (Ashrafuzzaman et al., 2013). Conventional propagation methods are slow, laborious, and expensive with many limitations and may not be recommended for effective and commercial multiplication (Dhar, 1998). Commercial multiplication of the released variety in this country is essential for rapid extension. The advantages of *in vitro* propagation is that it offers fast multiplication rates (Mott, 1981). The significant advantages of micro-propagation by which a large number of plants can be produced from a single individual in a relatively short span of time and space (Chawla, 2002).

Moreover, the conventional way of production is not adequate to meet the commercial demand. Micropropagated strawberry plant has been introduced to prevent most of the plant and soil transmissible diseases (Biswas et al., 2008). Large-scale commercial propagation by *in vitro* techniques has been used widely in the strawberry industry (Abdullah et al., 2013).

Therefore, in our investigation to considering the view of the potential commercial value and market demand we established an efficient and cost-effective method of *in*

vitro propagation of strawberry to ensure availability of planting materials for commercial cultivation practice.

2. Materials and Methods

2.1 Collection of explants and surface sterilization

Juvenile runner tips from strawberry mature and healthy plants (Fig. 1A) were collected from the strawberry garden of Jessore University of Science and Technology, Jessore 7408, Bangladesh. Collected explants were washed in a running tap water for 30 min and then thoroughly washed again by adding a few drops of Savlon (ACI Ltd. Bangladesh) and Tween-20 (UNI Chem, China) for 5 minutes followed by rinsing with three times autoclaved distilled water. Again, surface sterilized in a 0.1% mercuric chloride (MERCK, India) for 5 min followed by rinsing them five times with autoclaved dH_2O inside the Laminar Air Flow Cabinet.

2.2 Inoculation

Small runner tips (0.5-1.0 cm) were cultured on MS medium (Murashige, and Skoog, 1962) supplemented with different concentration of growth regulators (BA, NAA, KIN and GA_3) singly or in combination adding 30 gl^{-1} sugar and the medium was solidified with 0.6% agar. The pH of the medium was adjusted to 5.7 with 1M KOH before autoclaving at 0.15 MPa and 121°C for 20 min.

2.3 Incubation

The cultures were maintained in a growth chamber at 25 ± 2°C under photoperiod of 16 h/day and a light intensity of 2000-3000 lux provided by white fluorescence tube (Philips) with 55-60% relative humidity.

2.4 Sub-culture and multiple shoot regeneration

Subcultures were done every 2-3 weeks interval depending on the explants growth conditions. Nodal segments from the proliferated shoots were sub-cultured again for further multiple shoots regeneration.

2.5 Rooting and acclimatization

Regenerated multiple shoots were cut (approximately 2-3 cm long) and individual shoots were placed on MS solidified medium containing different single concentrations of IBA and IAA for root induction. Well grown *in vitro* rooted plantlets were transferred to autoclaved vermiculate soil in green house conditions.

2.6 Data analysis

Surface sterilization, callus induction, shoot regeneration, multiple shooting and rooting data were recorded during the experiments. Only some advantageous effect showed data were included in the tables and 10 explants were used per treatment and repeated three times.

3. Results and Discussion

Runner tips explants of strawberry cultured on MS medium with different concentration of BAP (0.5-2.5 mgl^{-1}), KIN (1.0-2.0 mgl^{-1}), and GA_3 (0.1-1.0 mgl^{-1}) (Table 1). Within 4-5 weeks of culture multiple shoots were directly initiated from the explants. The maximum number of shoots (7) in the medium with BAP (2.0 mgl^{-1}) were greater than those observed in the medium supplemented with KIN and GA_3 singly, where 82% explants showed shoot proliferation (Table 1 and Fig. 1, B). But when BAP concentration was decreased or increased, the shoot proliferation rate was decreased.

On the other hand, runner tips explants were cultured on MS medium supplemented with different concentration and combination of BAP (1.0-2.5 mgl^{-1}) with KIN (0.1-0.5 mgl^{-1}) or BAP (1.0-2.5 mgl^{-1}) with GA_3 (0.05-0.5 mgl^{-1}) or BAP (1.0-2.0 mgl^{-1}) with NAA (0.1-0.5 mgl^{-1}). And also cultured on MS medium with BAP (1.0-2.5 mgl^{-1}) with KIN (0.5-1.0 mgl^{-1}) and GA_3 0.5 mgl^{-1}. The numbers of shoots in medium with BAP+KIN+GA_3 combinations were greater than those observed in the medium supplemented with BAP + KIN or BAP + GA_3 or BAP + NAA treatments. The highest rate of response was obtained at 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} KIN combination (Table 2) where 92% explants showed shoot proliferation and 7 ± 0.23 shoots were developed. When BA concentration was decreased below 2.0 mgl^{-1}, the rate of shoot multiplication reduced. In our present investigation, maximum number of shoots per explant (20 ± 0.23) and highest average length (3.5 ± 0.26) were recorded at BAP 2.5 mgl^{-1} + KIN 0.5 mgl^{-1} + GA_3 0.5 mgl^{-1}, which was found to be the best combination for high frequency of multiple shoot induction of strawberry (Table 2, Fig. 1, C). When BAP was supplemented with KIN or GA_3 or NAA the rate of shoot proliferation and the shoot length were increased but number of shoots were decreased. The high concentration of cytokinin reduced the number of micropropagated shoots (Hu and Wang, 1983). Similar results have already been reported in *Fragaria indica* Andr. (Bhatt and Dhar, 2000). Also this result is in consistent with the findings of in papaya (Cononer and Litz, 1978) as well as in *Eucalyptus grandis* (Teixetra, and Silva, 1990). The shoot organogenesis varied from strawberry genotypes to genotypes (Singh and Pandey, 2004). Some workers reported high concentration of BAP is the best for strawberry micro propagation (Morozova, 2002) while other authors suggested 1.0 mg/l IAA + 1.0 mg/l

BAP + 0.05 mg/l GA3; 0.5 mg/l BA + 0.1 mg/l GA3 + 0.1 mg/l IBA (Boxus, 1999; Litwińczuk, 2004) and 0.5 mg/l BA + 0.1 mg/l IBA for strawberry micropropagation.

The developing shoots were elongated by sub-culturing on the same combinations of growth regulators. Later, elongated shoots were excised and used for root induction. Well grown shooted plantlets were cultured on different concentrations of IBA (0.5-2.0 mgl^{-1}) and IAA (0.5-2.0 mgl^{-1}) singly on ½MS solidified medium. Among the different treatments 1.0 mgl^{-1} IBA proved to be the most suitable for root induction with 83% of rooting and 3.49 ± 0.05 roots per explant and the average root length being 3.18 cm (Table 3; Fig. 1, F). Also, were observed similar effects of IBA in case of rooting of strawberry plantlets (Sakila et al., 2007). Well grown and 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 planted to sterilized vermiculate soil in small pot and after acclimatization grown in a natural condition.

Table 1: Effects of different concentration of BAP, KIN and GA_3 on multiple shoot regeneration from runner tips explants of strawberry.

Growth regulators conc.(mgl^{-1})	No. of explants inoculated	% of explants responded	Days to shoot formation	No. of shoots per culture (M±S.E.)	Highest length of shoots in cm (M±S.E.)
BAP					
0.5	15	20	18-22	3±0.57	1.90±0.05
1.0	15	40	15-20	5±0.58	2.10±0.19
1.5	15	70	12-15	5±0.45	2.51±0.57
2.0	15	82	8-15	7±0.73	2.10±0.15
2.5	15	71	12-15	5±0.66	2.03±0.03
KIN					
1.0	15	40	10-15	2±0.43	2.17±0.08
1.5	15	65	10-16	4±0.45	2.06±0.03
2.0	15	73	8-15	5±0.21	2.20±0.06
GA_3					
0.1	15	25	10-15	3±0.57	2.04±0.04
0.5	15	45	8-15	4±0.22	2.25±0.02
1.0	15	33	10-15	5±0.65	2.03±0.10

Note: M = Mean, S.E. =Standard Error

Table 2: Effects of different concentration and combination of auxin with cytokinin and GA_3 in MS medium on multiple shoot regeneration from runner tips explants of strawberry.

Growth regulators (mgl^{-1})	No. of explants inoculated	% of explants responded	Days to shoot formation	No. of shoots per culture (M±S.E.)	Highest length of shoots in cm (M±S.E.)
BAP+KIN					
1.5+0.5	15	65	10-15	3±0.58	2.20±.11
2.0+0.1	15	75	9-12	3±0.40	3.0±0.16
2.0+0.5	15	85	8-12	5±0.34	3.4±0.21
2.5+0.5	15	92	8-12	7±0.23	3.5±0.66
BAP+GA_3					
1.0+0.05	15	28	12-16	2±0.21	3.2±0.11
1.5+0.1	15	60	10-15	3±0.76	2.9±0.42
1.5+0.5	15	87	12-16	5±0.24	3.06±0.05
2.0+0.5	15	90	12-16	8±0.58	3.3±0.24
2.5+0.5	15	82	12-16	7±0.33	3.47±0.63
BAP+NAA					
1.0+0.5	15	28	12-20	2±0.55	2.0±0.26
2.0+0.1	15	57	12-20	4±0.32	2.3±0.27
2.0+0.5	15	60	10-15	5±0.35	2.56±0.38
BAP+KIN+GA_3					
1.0+0.5+0.5	15	80	7-12	12±.33	3.2±0.51
1.5+0.5+0.5	15	85	7-14	12±.66	2.5±0.88
1.5+1.0+0.5	15	72	6-15	14±.57	2.5±0.58
2.0+0.5+0.5	15	90	6-12	17±.39	3.4±0.32
2.5+0.5+0.5	15	98	5-10	20±.23	3.5±0.26

Note: M = Mean, S.E. =Standard Error

Table 3: Effects of different concentration of auxins (IBA, IAA) in MS medium on *in vitro*

root induction from regenerated shoots of strawberry after 7-8 weeks of culture.

Growth regulators conc. (mgl^{-1})	No. of shoot sub-cultured	Shoot derived from the explants of mature plants			
		% of rooting	Days to root generation	Average no. of roots	Average root length (cm)
IBA					
0.5	15	26	16-22	2.26±0.23	2.86±0.09
1.0	15	83	12-20	3.49±0.05	3.18±0.04
1.5	15	64	14-20	2.50±0.04	2.90±0.65
2.0	15	71	16-22	3.36±0.03	3.18±0.56
IAA					
0.5	15	39	18-26	2.04±0.35	3.18±0.30
1.0	15	56	16-22	3.45±0.33	3.50±0.50
1.5	15	64	16-26	3. 20±0.20	3.22±0.39
2.0	15	53	21-26	2.92±0.32	3.08±0.52

Note: M = Mean, S.E. =Standard Error

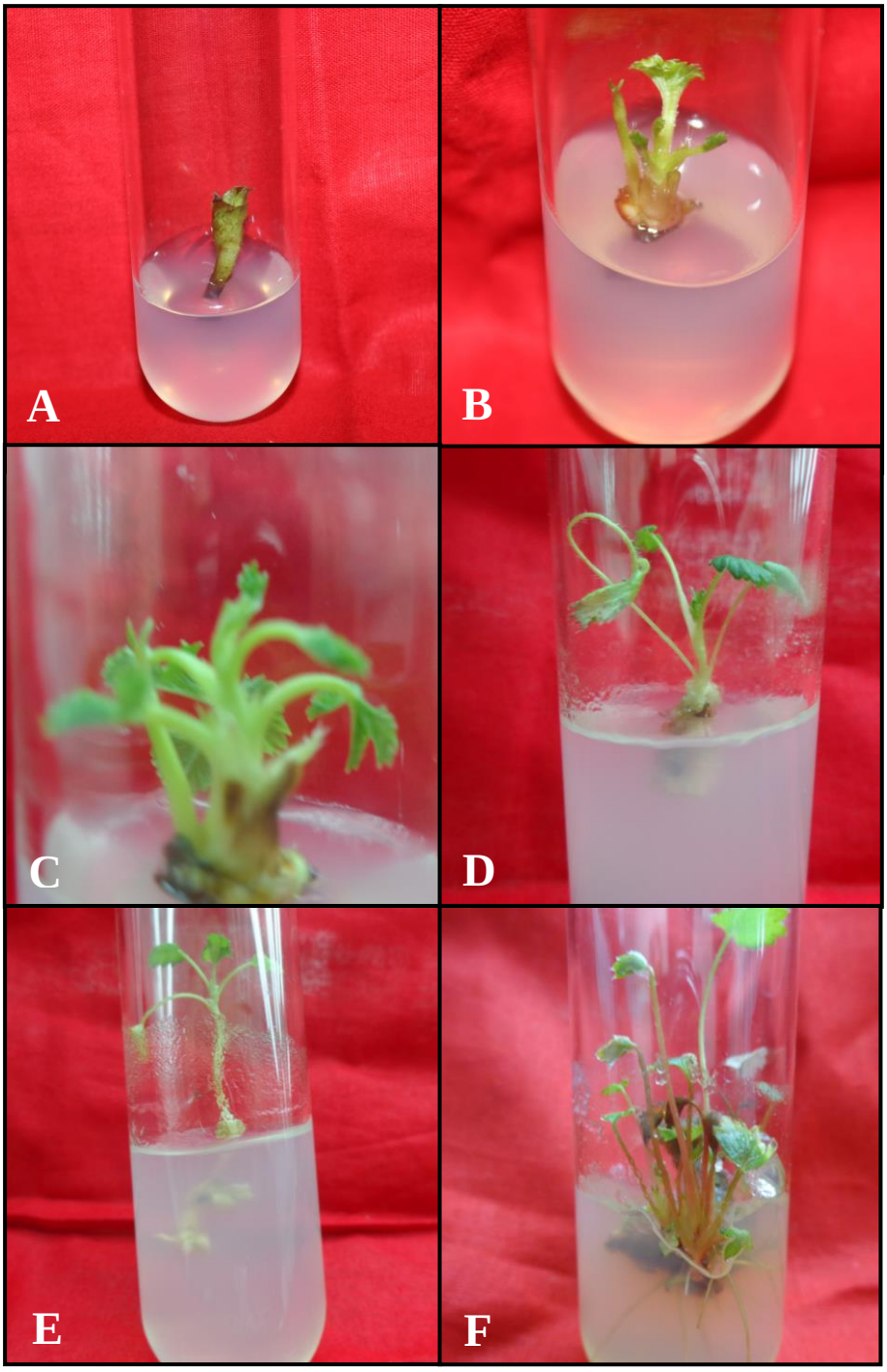

Figure 1: *In vitro* propagation of Strawberry plantlets from runner tips explants; A. Runner tips used as explants. B. Shoot proliferation on MS supplemented with 2.0 mgl^{-1} BAP after 3 weeks of culture. C. Proliferated of multiple shoots on MS+2.0 mgl^{-1} BAP+0.5 mgl^{-1} KIN+0.5 mgl^{-1} GA_3 after 4 weeks of culture. D and E. Establishment of shoot after 7 weeks of culture. E. Rooted shoots on 1.0 mg/l IBA after 7-8 weeks of culture.

4. Conclusion

From our investigation, it can be concluded that many strawberry plantlets even an off season can be raised from an explant within a short period of time by using micropropagation technique following our established protocol. Also, our investigation, provides a reliable and economical method of maintaining pathogen free plants. Few years ago, strawberry plants introduced in Bangladesh and within a short time this fruit become very popular to us. So, applying the above standard protocol we can easily fulfill our demand of strawberry plantlets for the growing of the commercial cultivation purposes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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