Bangladesh J. Nuclear Agric, 35: 93-104, 2021 *ISSN 0258-7130*

IN VITRO REGENERATION POTENTIALITY OF STRAWBERRY FROM DIFFERENT EXPLANT UNDER VARIOUS HORMONAL CONCENTRATIONS

K.F. Sukhi[1], M.M. Rahman[2], N. Mitu[1], M.M. Rahman[3] and M.N.H. Mehedi*[4]

Abstract

An experiment was carried out to examine the effects of explants and different combinations of plant growth regulators *in vitro* micro propagation of strawberry. The research was laid out in 2 factorial experiments in completely randomized design. For shoot induction, six concentrations viz. BAP (0.5, 1.0, 1.5), GA_3 (0.5) and KIN (0.1, 0.5) and for root induction four combinations viz. IBA (0.1, 0.5) and 2, 4-D (0.1, 0.5) were used. The interaction effects between explants and growth regulators showed significant differences for all the parameters used in the experiment. In case of shoot initiation, runner tip explants performed the best for days required to shoot initiation (8.00), number of shoot per plantlet (11.20), number of leaves per plantlet (12.80) and shoot length (4.70 cm) when 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L was used. In case of root initiation, runner tip explants exhibited the best results for days to root initiation (10.00) and number of roots per plantlets (12.00) when 1.0 mg IBA + 0.5 mg 2, 4-D/L was used. Maximum good results observed when 1.0 mg BAP was combined with different concentration of GA_3 and KIN. Low concentration of IBA and 2, 4-D produces significant results. The findings of the present study could be useful to develop protocol to identify the potentiality of exact concentration of different growth regulators.

Key words: Micro propagation, MS media, strawberry, explants, plant growth regulators

Introduction

The strawberry is an important and popular fruit produced in temperate and sub-tropical climates due to its fragrance, taste and nutritional properties. It is the most widely consumed berry fruits throughout the world (Sultana, 2011). It is cultivated in 73 countries world-wide on 2,00,000 hectares and produced 31 lac metric tons strawberry (FAO, 2016). The cultivated strawberry (*Fragariax ananassa* Duch) is a member of the Rosaceaealong with blackberries and raspberries (Sultana, 2011). It contains 90.6 g water, 0.89 g protein, 0.5 g fat, 7.6 g carbohydrates, 1.7 g fiber, 53.0 mg vitamin C and 30.0 g vitamin B per 100 g edible fruit (Rahman, 2011). Strawberry is also qualified as a very good source of iodine as well as a good source of potassium, folate, omega-3 fatty acids, vitamin K, magnesium and

[1]Department of Horticulture, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh
[2]Upazilla Agriculture Officer, Department of Agricultural Extension (DAE), Bangladesh
[3]Agriculture Extension Officer, Department of Agricultural Extension (DAE), Bangladesh
[4]Horticulture division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202
*Corresponding author: email: nazmul02348@gmail.com

copper (Sultana, 2011). It contains relatively high quantities of ellagic acid, which is thought to be an anti-carcinogenic (ICAR news, 2005) and a wide range of biological activity (Sakila *et al.*, 2007). Strawberries are growing in many areas of Bangladesh from last few years. In Bangladesh, commercial cultivation has been started with the released varieties of Rajshahi University. Bangladesh Agricultural Research Institute has also released two varieties of strawberry (Rahman, 2011). It has become very popular fruit to the people of the country. As a result, a number of farmers have now taken up their activity.

Tissue culture considered as an *in vitro* aseptic culture of cells, tissues, organs or whole plant below exact nutritional and ecological circumstances (Thorpe, 2007). Through using plant tissue culture methods plants can be attained from several explants over direct or indirect morphogenesis. Direct morphogenesis refers to the manufacture of shoots from explants deprived of passing over callus (unorganised tissue) stage while indirect morphogenesis relates to the generation of shoots over the callus stage (Kadhimi, 2014). The standardization of protocol and procedure of micropropagation of strawberry was successfully attempted by many researchers (Kaur *et al.*, 2005; Sakila *et al.*, 2007; Gantait *et al.*, 2010). Micropropagation of strawberry has been used in horticultural production since 30 years (Boxus, 1974). Some workers reported that high concentration of BAP is the best for strawberry micropropagation (Morozova, 2002) while other authors suggested 1.0 mg/L IAA + 1.0 mg/L BAP + 0.05 mg/L GA_3; 0.5 mg/L BAP + 0.1 mg/L GA_3 + 0.1 mg/L IBA (Boxus, 1999; Litwinczuk, 2004) and 0.5 mg/L BAP + 0.1 mg/L IBA (Bozena, 2001) for strawberry micropropagation.

Micropropagation of strawberry has been reported a large number of disease free plants (Boxus, 1974). Micropropagated strawberry plant has been introduced to prevent most of the plant and soil transmissible diseases (Biswas *et al.*, 2008). In addition, the storage of tissue cultured propagules requires less space than traditional propagated plant and the *in vitro* storage can be initiated at any time during the production cycle (Litwinczuk, 2004). Virus free shoot tips, runner tips, leaves and nodal segments are commonly used as a source of plant material for regeneration and transformation. Prior experiences with strawberry micropropagation indicate that *in vitro* plants are more uniform, produce higher number of runners and have better survival in the field and the fruit yield increases 24% than plants propagated by the traditional method. Each square meter of growing area of strawberry can produce 40000 plantlets per year (Boxus, 1999), these plants were vigorous and after transplanting in the soil some produced up to 500 new runner plants. Micropropagated strawberry plants were comparatively better in different characters (canopy size, number of runners, flowering time and yield of berries) than conventionally propagated runner plants, which is very labour intensive (Sakila *et al.*, 2007), time consuming and results in the transmission of viral diseases. Moreover, the conventional way of production is not adequate to meet the commercial demand. So, there is an urgent need to develop an efficient protocol for *in vitro* regeneration of strawberry plantlet for mass production of planting materials. Therefore, the present experiment has been planned to identify the suitable explants of strawberry for large scale production and determine the effective concentration and combination of BAP, GA_3, KIN, IBA and 2, 4-D on callus induction and plantlet regeneration of strawberry.

Materials and Methods

The experiment was conducted in the Plant Biotechnology Laboratory, Patuakhali Science and Technology University, Bangladesh during the period from July to October, 2015. The mother parts of the BARI strawberry-1wascollected from Regional Horticultural Research Station, Lebukhali, Patuakhali used as explants such as Runner tip, Shoot tip and Nodal segments.

Healthy, disease free and young runner tips, shoot tip and nodes were cut from the field grown plants of strawberry and were collected in a conical flask. The runner tips, shoot tip and nodes were washed thoroughly under running tap water for 20-25 minutes to remove the dust and then transferred in another conical flask containing distilled water adding with 2-3 drops of tween-80 and a few drops of savlon with constant shaking, then kept them 3-5 minutes and were washed 4-5 minutes with distilled water to remove sterilizing agents. The materials were separated into different segments. Inside the laminar air flow cabinet surface disinfection was done with 0.1% $HgCl_2$ solution by gently shaking for 3-8 minutes. After exposure to the sterilant, the materials were then washed in several times with double distilled water to remove all traces of $HgCl_2$ and the material was ready for inoculation on appropriate nutrient medium.

MS (Murashige & koog, 1962) basal media supplemented with different concentrations and combinations of growth regulators viz. 6-benzyladenine (BA), gibberellic acid (GA_3) and 6-ferfuryl amino purine (KIN) for shoot proliferation andindole butyric acid (IBA) and 2, 4-Dichlorophenoxy Acetic acid (2, 4-D) for root proliferation.

After mixing all stock solutions and growth regulators at appropriate volume, 3% sucrose was added. The pH of the medium was adjusted at 5.8 and then agar (0.7%) was added and dissolved. The media weredispensed in a 20-25 ml glass bottles. The media were sterilized by autoclaving at 121^0C for 15 minutes. Sterilized explants materials were dissected and cultured on MS medium supplemented with BAP (0.5, 1.0 and 1.5 mg/L), GA_3 (0.5 mg/L) and KIN (0.1 and 0.5 mg/L). After 30-35 days of culture inoculated shoots were produces multiple shoots.

When the regenerated shoot apices were reached 3-5 cm in length with 4-5 well developed leaves, they were rescued aseptically from the culture vessel and were separated from each other. Micro-cutting were prepared from these shoots by snapping off the basal leaves and cultured them individually in tubes containing 20 ml of rooting medium with different combinations of auxins (IBA and 2, 4-D). The pH of the medium was adjusted at 5.8 and then agar (0.7%) was added and dissolved. The media were sterilized by autoclaving at 121^0C for 15 minutes.

The data collected on different parameters were statistically analyzed to ascertain the significance of the experimental results. The analyses of variances were performed and the means were compared by DMRT for interpretation of results (Gomez and Gomez, 1984). The significance of the difference between the pair of means was evaluated using the MSTAT-C computer package program.

Results and Discussion

Effect of different explants and growth regulators (BAP, GA_3 and KIN) on in vitro shoot regeneration

Significant variation was observed among the different explants and combination of plant growth regulators regarding the shoot regeneration characteristics.

Among the explants, runner tip took the lowest number of days (13.17) and produced significantly the highest percentage (90.83) of shoot while nodal segment took the highest number of days (17.17) for shoot initiation and lowest percentage (64.67) of shoots (Fig. 1 and 2). This means specific explants took higher or lower percentage to initiate shoot with certain concentrations of BAP, GA_3 and KIN. MS Medium that containing 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T2) took the least number of days (10.67) for shoot initiation and produced the highest percentage (90.67%) of shoots (Fig. 3 and 4). In contrast, concentration 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T3) took the highest number of days (19.67) for shoot initiation and produced the lowest percentage of shoot (70.67%). Negi *et al.* (2008) observed that MS medium supplemented with BAP (0.5 mg/L) and KIN (0.5 mg/L) took less time to shoot emergence (9.81 days).

In case of interaction effect, runner tips with 1.0 mg BAP+0.5 mg GA_3+0.5 mg KIN/L (T2) required the lowest number of days (8.00) for shoot initiation and highest percentage of shoot initiation (97.00). In contrast, the lowest (70.00%) percentage and highest number of days required (21.00) for shoot initiation were obtained from nodal segments with the supplement of 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T3) (Table 1). Murari *et al.* (2003) also found that runner tip with BAP at 4.0 mg/L produced the maximum shoot regeneration (100%) after 7 weeks of incubation.

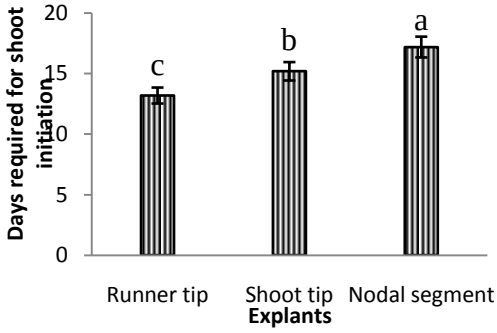

Fig. 1. Effects of different explants on days required for shoot initiation

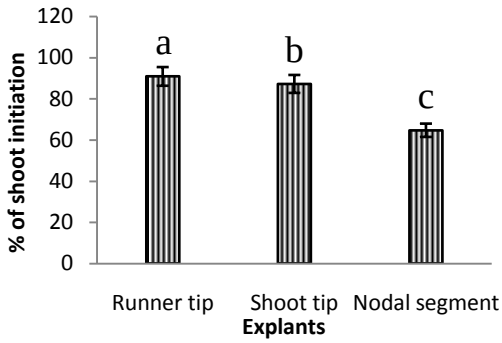

Fig. 2. Effects of different explants on percentage of shoot initiation

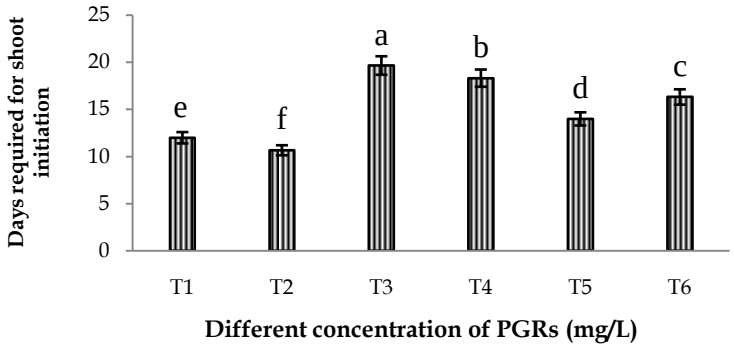

Fig 3. Effects of different concentrations of BAP, GA_3 and KIN on days required for shoot initiation

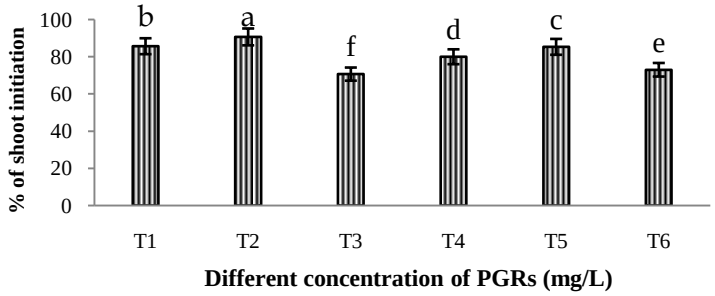

Fig 4. Effects of different concentrations of BAP, GA_3 and KIN on percentage of shoot initiation

Here,

T1= 0.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T2= 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T3=1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T4= 0.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T5= 1.0 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T6= 1.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L

Among the explants, runner tip took the highest number of shoot (7.98), number of leaves (9.31) and longest shoot (3.15 cm) per plantlet (Fig. 5 and 6). In contrast, nodal segment took the lowest number of shoot (4.30), lowest number of leaves (7.28) and shortest shoot (2.65 cm). Rahman *et al.* (2011), Karim *et al.* (2011), Biswas *et al.* (2008), Sakila *et al.* (2007) also observed that the different number of leaves produced from different explants.

Medium that containing 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T2) took the highest (9.40) number of shoots, maximum number of leaves (11.60) per plantlet and highest shoot length (4.23 cm) while the concentration 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T3) took the lowest number of shoot per plantlet (3.60), minimum number of leaves (4.93) per plantlet and smallest shoot length (2.06 cm). This observation agreed with the findings of Negi *et al.* (2008), they also observed that MS medium supplemented with BAP (0.5 mg/L) and kinetin (0.5 mg/L) was found best for shoot elongation.

The highest number of shoot per plantlet (11.20), number of leaves per plantlet (12.80) and longest shoot (4.70 cm) were recorded from the combination of runner tip with 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T2) concentration (Table 2). In contrast, the lowest number of shoot per plantlet (2.00), lowest number of leaves per plantlet (4.20) and smallest shoot (2.00 cm) were recorded from the combination of nodal segment with 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T3) concentration. Biswas*et al.* (2008) also reported the highest number of shoots per plantlet from runner tip explants of strawberry having 0.5 mg/L BAP.

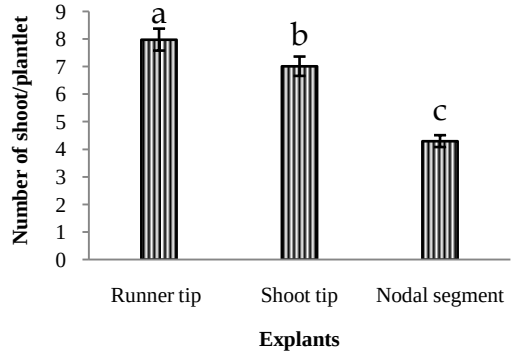

Fig. 5. Effects of different explants on number of shoots per plantlet for shoot initiation

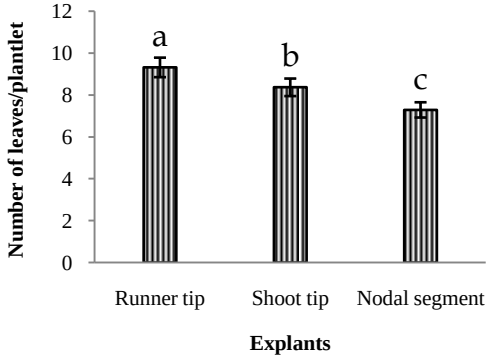

Fig. 6. Effects of different explants on number of leaves for shoot initiation

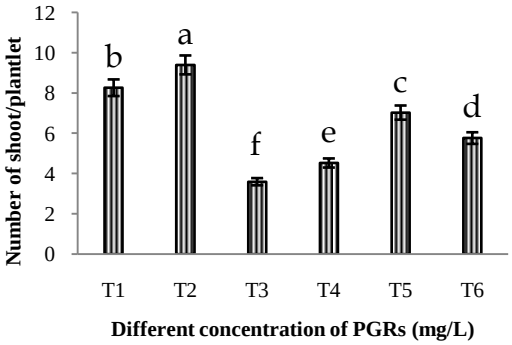

Fig. 7. Effect of different concentrations of BAP, GA_3 and KIN on number of shoot per plantlet of strawberry

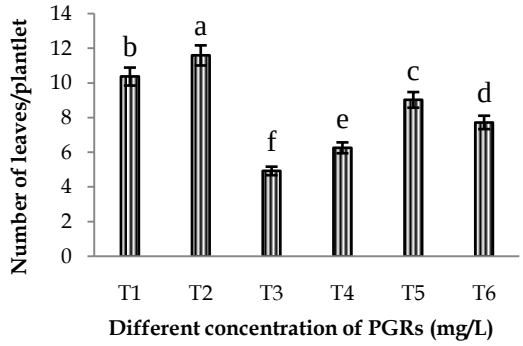

Fig. 8. Effects of different concentrations of BAP, GA_3 and KIN on number of leaves per plantlet of strawberry

Here,

T1= 0.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T2= 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T3= 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T4= 0.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T5= 1.0 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T6= 1.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L

Table 1. Interaction effects between explants and different concentrations of BAP, GA_3 and KIN on days required for shoot initiation, % of shoot initiation, number of shoot per plant, number of leaves per shoot and shoot length

Treatment combination	Days to shoot initiation	% of shoot initiation	No. of shoot per plant	No. of leaves per shoot	Shoot length (cm)
T1P1	9.00k	95.00b	9.80b	11.50b	3.60b
T1P2	12.00i	92.00c	9.00c	10.50c	3.50bc
T1P3	15.00f	70.00l	6.00g	9.13d	3.00d
T2P1	8.00l	97.00a	11.20a	12.80a	4.70a
T2P2	11.00j	95.00b	10.00b	11.60b	4.50a
T2P3	13.00h	80.00j	7.00f	10.40c	3.50bc
T3P1	18.00c	84.00h	5.00h	5.70j	2.10g
T3P2	20.00b	80.00j	3.80i	4.90k	2.10g
T3P3	21.00a	48.00o	2.00k	4.20l	2.00g
T4P1	17.00d	87.00f	6.10g	7.20g	2.40fg
T4P2	18.00c	82.00i	5.00h	6.20i	2.50efg
T4P3	20.00b	50.00n	2.50j	5.40j	2.20g
T5P1	12.00i	92.00c	8.50d	10.10c	3.10cd
T5P2	14.00g	89.00e	7.80e	9.20d	3.10cd
T5P3	16.00e	75.00k	4.80h	7.80f	2.80def
T6P1	15.00f	90.00d	7.30f	8.60e	3.00cd
T6P2	16.00e	85.00g	6.50g	7.80f	2.90df
T6P3	18.00c	65.00m	3.50i	6.80h	2.40fg
$LSD_{0.05}$	0.496	0.331	0.496	0.384	0.447
CV (%)	1.98	0.25	4.90	2.78	9.13

In a column values having different letter (s) differ significantly at 5% level of probability

Here,

T1 = 0.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T2 = 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T3 = 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T4 = 0.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T5 = 1.0 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T6 = 1.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L and
P1 = Runner tip; P2 = Shoot tip and P3 = Nodal segment

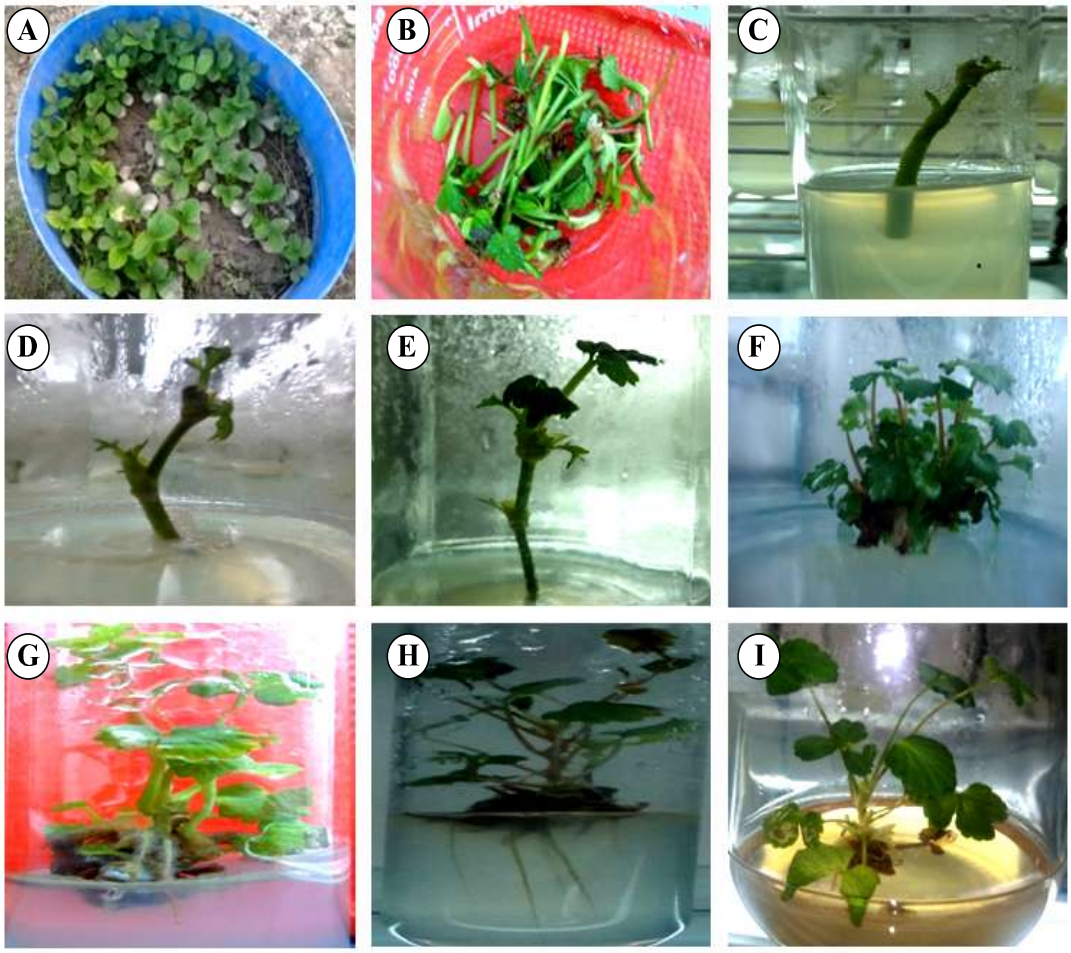

Plate 1. Source of explants for micropropagation (A); washing of explants (B); runner tips proliferation on MS + 1.0 mg BAP + 0.5 mg GA3 + 0.5 mg KIN/L (C); after 7 days (D); after 15 days (E); After 35 days (F) of culture medium. Rooted shoots on 1.0 mg IBA + 0.5 mg 2, 4-D/L after 2 weeks (G); after 4 weeks (H) of culture medium Regenerated plantlets with roots (I).

Effect of IBA and 2, 4-D on in vitro root regeneration

Distinct variations were found in respects of rooting characteristics due to the effect of different explants and different concentrations of growth regulators.

Among the explants, runner tip took the shortest number of days (13.50) for root initiation, highest percentage (93.00) of roots, highest number (8.60) of roots and the longest (2.40 cm) root. In contrast, nodal segment had required the highest days (19.00) for root initiation, lowest (73.50) percentage of roots, lowest (5.34) number of roots and shortest root (2.07 cm) (Table 2). Sakila *et al.* (2007) observed the different number of roots produced from different explants.

Table 2. Effects of different explants on the days to root initiation, percentage of root initiation no. of roots/plantlet and root length (cm)

Explants	Days to root initiation	Percentage of root initiation	No. of root per plantlet	Root length (cm)
Runner tip	13.50c	93.00a	8.60a	2.40a
Shoot tip	16.56b	82.50b	6.78b	2.18b
Nodal segment	19.00a	73.50c	5.34c	2.07c
$LSD_{0.05}$	0.571	0.805	0.629	0.323
CV (%)	2.10	4.50	1.70	2.55

In a column values having different letter (s) differ significantly at 5% level of probability.

MS Medium that containing 1.0 mg IBA + 0.5 mg 2, 4-D/L (T4) took the least number of days (10.00) for root initiation, produced the highest percentage (91.33) of roots, maximum number of roots per plantlet (10.07) and recorded highest roots length (3.33 cm)while required the highest number of days (12.00) for root initiation, lowest percentage of root (80.67), minimum (4.50) number of roots per plantlet and shortest (1.23 cm) roots length were recorded from the concentration 1.0 mg IBA + 0.1 mg 2, 4-D/L (T2) (Table 3). Rahman (2011) found that runner tip explants showed better compared to shoot tip with 1 mg/L BAP whereas for root development, earlier roots could be found from both the explants with 0.5 mg/L IBA. Sakila *et al.* (2007) observed that the maximum frequency of rooting and highest number of roots was produced on medium containing 1.0 mg/L IBA.

Table 3. Effects of different concentrations of IBA and 2, 4-Don the days to root initiation, Percentage of root initiation, no. of roots/plantlet and root length (cm)

Concentrations and combinations of PGRs (mg/L)	Days to root initiation	Percentage of root initiation	No. of root per plantlet	Root length (cm)
T_1 (0.5 mg IBA + 0.1 mg 2, 4-D/L)	10.98b	89.33ab	8.03b	2.65b
T_2 (1.0 mg IBA + 0.1 mg 2, 4-D/L)	10.00c	80.67c	4.50d	1.23c
T_3 (0.5 mg IBA + 0.5 mg 2, 4-D/L)	11.87a	85.67b	6.78c	1.87bc
T_4 (1.0 mg IBA + 0.5 mg 2, 4-D/L)	12.00a	91.33a	10.07a	3.33a
$LSD_{0.01}$ value	0.742	0.531	0.814	0.420

In a column values having different letter(s) differ significantly at and 5% level of probability.

In interaction effect, the lowest number of days (10.00) for root initiation, highest percentage of root initiation (98.00), highest number of root (12.00) and the longest root (3.70 cm) were found from the explant runner tip with 1.0 mg IBA + 0.5 mg 2, 4-D/L (T4). In contrast, highest number of days required (23.00) for root initiation, lowest (68.00) percentage of root initiation and lowest (3.10) root number were recorded from nodal segments with (T2) 1.0 mg IBA + 0.1 mg 2, 4-D/L (Table 4). It observed that rest of the combination showed intermediate results compared to the highest and lowest value of root

length. This result corroborates with that of Biswas *et al*. (2007), Karim *et al.* (2011), they also observed significant interaction effects between explants and concentrations of IBA.

From the above discussion, it is revealed that, different explants and growth regulators with their different concentration and combination provided the best results in *in vitro* plant regeneration of strawberry. Runner tip explants showed the best performance than other explants when MS media supplemented with (1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L) for shoot initiation and MS media supplemented with (1.0 mg IBA + 0.5 mg 2, 4-D/L) for root initiation.

Table 4. Interaction effects between explants and different concentrations of IBA and 2, 4-D on days required for root initiation, % of root initiation, number of roots per plantlet and root length

Treatment Combination	Days to root initiation	% of root initiation	No. of roots per plantlet	Root length (cm)
T1P1	15.00f	91.00e	7.50e	1.90de
T1P2	17.00e	89.00f	6.16f	1.40ef
T1P3	20.00b	71.00k	4.16i	2.30d
T2P1	17.00e	88.00g	5.60g	1.50ef
T2P2	19.00c	86.00h	4.80h	1.20f
T2P3	23.00a	68.00l	3.10j	1.00f
T3P1	12.00h	95.00c	9.30c	2.50cd
T3P2	14.00g	93.00d	8.10d	2.40cd
T3P3	18.00d	75.00j	6.10f	2.00de
T4P1	10.00j	98.00a	12.00a	3.70a
T4P2	11.00i	96.00b	10.20b	3.30ab
T4P3	15.00f	80.00i	8.00d	3.00bc
$LSD_{0.05}$	0.3370	0.3370	0.4522	0.6005
CV (%)	1.26	0.23	3.79	16.30

In a column values having different letter (s) differ significantly at 5% level of probability

Here,

T1 = 0.5 mg IBA + 0.1 mg 2, 4-D/L T3 = 0.5 mg IBA + 0.5 mg 2, 4-D/L
T2 = 1.0 mg IBA + 0.1 mg 2, 4-D/L T4 = 1.0 mg IBA + 0.5 mg 2, 4-D/L
and P1= Runner tip, P2= Shoot tip, P3 = Nodal segment

The findings of the present study could be useful to develop protocol to identify the potentiality of exact concentration of different growth regulators. Furthermore, the results could be used to produce large scale production of healthy and disease free planting materials commercially.

References

Biswas M.K., Islam, R. and Hossain, M. 2008. Micropropagation and field evaluation of strawberry in Bangladesh. *J. Agric. Tech.* 4: 167-182.

Biswas, M.K., Hossain, M., Ahmed, M.B., Roy, U.K., Karim, R., Razvy, M.A., Salahin, M. and Islam, R. 2007. Multiple shoots regeneration of strawberry under various color illuminations. *American-Eurasin J. Sci. Res.* 2: 133-135.

Boxus, P. and Hall, R.D. (ed.) 1999. Micropropagation of strawberry via axillary shoots proliferation. In: plant cell culture protocols. Methods in molecular Biology. Plant propagation *In Vitro*.Humana Press Inc. pp. 103-114.

Boxus, P. H. 1974. The production of strawberry plants by *in vitro*micropropagation. *J. Hortic. Sci.* 49: 209-210.

Bozena. 2001. Morphological and physiological characteristics of micropropagated strawberry plants rooted *in vitro* or *ex vitro*. *Scientia Hort.* 89. 195-206.

FAO. 2016. Food and Agricultural Organization of the United Nations, Report on World-wide Crop Production, Rome, Italy.

Gantait, S., Mandal, N., Bhattacharyya, S. and Das, P.K. 2010. Field performance and molecular evaluation of micropropagated strawberry. *Res. Sci. Tech.* 2: 12-16.

Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agricultural Research (2^{nd} Edn.). John Willey and Sons, New York. pp. 207-215.

ICAR news, October-December 2005. Indian council of agricultural research, vol. 11, no. 4, New Delhi, India.

Kadhimi, A.A., Alhasnawi, A.N., Mohamad, A., Yusoff, W.M.W. and Zain, M. 2014. Tissure culture and some of the factors affecting them and the micropropagationof strawberry. *Life Sci. J.* 11: 484-493.

Karim, M.R., Aziz, M.A., Roy, U.K., Hoque, M.A. and Hossain, M.M. 2011. *In vitro* response of strawberry (*Fragariaxananassa* Dutch.) for callus induction and shoot regeneration.*Int. J. Agron. Agril. Res.* 1: 29-36.

Kaur, S. and Chopra, H.R. 2004. Studies on vegetative growth of runner plants obtained from micro propagation plants of strawberry (*Fragariaxananassa*Duch.). *J.-Res., Punjab-Agricultural-University*. 41: 336-340.

Morozova, T. 2002. Genetic stability of pure lines of *Fragariavesca* L. in micropropagation and long-term storage *in vitro*. *Acta Hort.* 567: 85-87.

Murari, L., Sharma, S. and Hegde, M.V. 2003. Micro propagation of strawberry (*Fragaria X ananassa* Duch.). *Indian J. Agril. Res.* 37: 231-234.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15(3): 473-497.

Negi, M., Singh, C.P. and Srivastava, R.K. 2008. *In vitro* multiplication of strawberry cv. Chandler. *Pantagar J. Res. 6:* 247-250.

Rahman, L. 2011. Production Technology of Strawberry in Bangladesh. PhD thesis, Department of Horticulture. BSMRAU. Salna, Gazipur. pp. 12-14.

Sakila, S., Ahmed, M.B., Roy, K., Biswas, K., Karim, R., Razvy, M.A., Hossain, M., Islam, R. and Hoque, A. 2007. Micro propagation of Strawberry (*Fragaria x ananassa Duch.*): A newly introduced crop in Bangladesh. *American-Eurasian J. Scientific Res.* 2: 151-154.

Sultana, H. 2011. In Vitro Propagation of Strawberry (*Fragaria x ananassa* Duch.) and It's Field Performance. MS Thesis. Dept. of Horticulture. BSMRAU. Salna, Gazipur. pp. 19-21.

Thorpe, T. 2007. History of plant tissue culture. *J. Mol. Microbial Biotechnol.* 37: 169-180.