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IN VITRO MICROPROPAGATION OF STRAWBERRY (*Fragaria ananassa*)

S.M.L. RAHMAN[1], M.M. HOSSAIN[2], M.M. RAHMAN[3], M.A.K. MIAN[4] AND T. HOSSAIN[5]

[1]Citrus Research Station, Bangladesh Agricultural Research Institute, Jaintapur, Sylhet; [2&3]Department of Horticulture; [4]Department of Genetics and Plant Breeding; [5]Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

Corresponding author & address: Shah Md. Luthfur Rahman, E-mail: luthfr_r@yahoo.com
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ABSTRACT

Rahman SML, Hossain MM, Rahman MM, Mian MAK, Hossain T (2014) *In vitro* micropropagation of strawberry (*Fragaria ananassa*). *Int. J. Expt. Agric.* 4(1), 11-16.

The experiment was conducted at the Tissue Culture Laboratory, Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during the period from February 2008 to December 2009 to investigate the effect of different concentrations of BAP on virus free plant regeneration, shoot multiplication and different concentrations of IBA on *in vitro* root formation of strawberry accession FA002. Number of shoots/explant was the highest of 29.0 for runner tip with 10.0 mg/l BAP and the controls were the least (3.0 for runner tip and 2.6 for shoot tip. The maximum leaves/culture (10.2) was produced for 10.0 mg/l BAP with runner tip while the minimum (3.6) for 0.0 mg/l BAP with shoot tip. The highest length (5.0 cm) was produced for 1.0 mg/l BAP with runner tip which was statistically identical to 0.5, 1.5, 2.0 and 2.5 mg/l BAP with runner tip (4.2 cm, 4.4 cm, 4.6 cm and 4.2 cm respectively) and also statistically identical to 1.0, 1.5 and 2.0 mg/l BAP with shoot tip (4.2, 4.04 and 4.24 cm, respectively). For root initiation half strength MS medium supplemented with different levels of IBA (0, 0.5, 1.0, 1.5 and 2.0 mg/l) were used. Root numbers varied with different concentrations of IBA and type of explants. Number of roots/explant was found maximum (19.0) at 1mg/l IBA with shoot tip which was statistically identical to 1.0 mg/l with runner tip explants.

Key words: *in vitro regeneration, pineapple strawberry*

INTRODUCTION

Strawberry (*Fragaria ananassa*) is a fruit of temperate regions of the world (Hossain 2009). It belongs to the family Rosaceae (Sing 2002). The garden strawberry (*Fragaria ananassa*) known as pineapple strawberry or ananas strawberry was first bred in Brittany, France, in the 1750s via a cross of *Fragaria virginiana* from eastern North America and *Fragaria chiloensis* from Chile (Hossain 2009). The strawberry is, in technical terms, an aggregate accessory fruit, meaning that the fleshy part is derived not from the plant's ovaries but from the receptacle that holds the ovaries. Each apparent "seed" (achene) on the outside of the fruit is actually one of the ovaries of the flower, with a seed inside it. In both culinary and botanical terms, the entire structure is considered as fruit. This fruit is widely appreciated for its characteristic aroma, bright red color, juicy texture, and sweetness. Strawberries are grown throughout Europe, in every state of the United States, as well as in Canada and South America. The wide variation in climates within these regions and the wide adaptation of the strawberry plant permit harvesting and marketing the fruit during greater part of the year. Strawberry has a tremendous scope for cultivation near towns and canning units where the produce can be utilized immediately after harvest. It is more profitable in the shortest possible time as compared to other fruits (Sing 2002). Strawberry is a delicious fruit taken fresh in several ways. It also makes excellent ice cream and jam on account of its pleasant aroma and delicate flavor. It is also nutritious and beneficial to anemic persons. One cup (236 g) of strawberries contains approximately 45 calories (188 kJ) and other nutrients such as water (132 g), protein (0.88 g), fat (0.53 g) carbohydrate (10.10 g), fiber (3.3 g), calcium (20.00 g), iron (0.55 g), vitamin C (82 µg), thiamin (0.03 µg), riboflavin (0.1 µg), vitamin (B-6 0.09 mg), folate (25 µg) and vitamin A (IU 39) (Strawberry Wikipedia 2009).

Department of Botany, Rajshahi University is the pioneer of introducing strawberry in Bangladesh. Under the leadership of Dr. Monjur Hossain, Professor of the Department, strawberry research and development has been initiated since 1998. They developed three varieties of strawberry, *viz.*, Rabi Strawberry-1, Rabi Strawberry-2 and Rabi Strawberry-3. Bangladesh Agricultural Research Institute also released one variety of strawberry, named BARI Strawberry-1. These varieties play a vital role in the quality strawberry production due their popularity and acceptability to marginal, commercial and urban strwberry growers. Strawberry is traditionally propagated vegetatively by rooted runners. To improve the strawberry varieties this method was not suitable due to incidence of many diseases infection and environmental hazards and resulting in the gradual degeneration of cultivars. The rate of strawberry propagation through conventional techniques is quite low and difficult to maintain planting materials during summer in Bangladesh (Rahman 2007). Moreover, the conventional way of saplings production is not adequate to meet the increasing commercial demand day by day (Biswas *et al*. 2008). Khanam *et al.* (1998) reported that the main hurdle in the successful strawberry cultivation was the occurrence of viral diseases which seriously affecting the yield of this crop. Belkengren and Miller (1963) were the first to recommed the use of meristem culture for the elimination of virus from Fragaria. Since then Boxus (1974) produced virus free strawberry using *in-vitro* callus culture. Adams (1972) was the first to report on the micropropagation of strawberry. According to him it would be possible to obtain an unlimited number of strawberry plantlets from a single meristem.

Therefore, to develop a mass propagation protocol for virus free strawberry plantlets production, an investigation has been undertaken:

1. To study the effect of BAP growth regulator in MS media on *in vitro* shoot proliferation of strawberry accession FA002;
2. To determine the effect of IBA growth regulator in half strength MS media for *in vitro* root development of strawberry; and
3. To develop protocol for in vitro rapid propagation of strawberry.

MATERIAL AND METHODS

The present study was carried out in the Tissue Culture Laboratory of the Department of Horticulture, Bangabandhu Sheikh Mujibur Brahman Agricultural University, Gazipur during the period from February, 2008 to December, 2009. The planting materials of strawberry accession FA002 was selected from a Ph. D. study experiment titled "Growth and yield performance of six strawberry genotypes in Bangladesh" (Rahman 2007). Runner tips and shoot tips were collected from the field experiment stated above and was brought to the preparation room. The two types of tips were washed throroughly under running tap water for about 30 minutes. The outer tissues of the tips were removed with the help of a sharp knife until the tips measured about 2-2.5 cm in length. Both the tips used for establishment of culture were prepared through dissection under the microscope. Then the initial explants were prepared under stereomicroscope by removal of outer tissues with the help of sterile scalpel, which was about 1-1.5 cm in length. Two treatments were conducted to assess the effect of different concentrations of BAP and IBA on shoot proliferation and subsequent rooting of the multiplied shoot. In the first experiment, runner tip and shoot tip of the strawberry accession FA002 were used as sources of ex plants to investigate the effect of BAP at different concentrations on shoot proliferation. Ten levels of BAP (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, and 10.0 mg/l) were used as treatments. In the second experiment, there were 5 levels of IBA (0.0, 0.5, 1.0, 1.5, and 2.0 mg/l) were used as treatments. The experiments were arranged in factorial design with 4 replications. Each treatment consisted of 10 culture tubes per replication. Data were recorded on the effect of different treatments on shoot proliferation and rooting. Murashige and Skoog (1962) medium supplemented with different phytohormones as per treatments were used as culture medium for shoot induction, shoot multiplication and maintenance and regeneration of roots from multiplied shoots. Hormones were added separately to different media according to the requirements. For the preparation of media, stock solutions were prepared at the beginning and stored at 9±1°C temperature. The respective medium was prepared from the stock solutions. The culture tubes with media were then autoclaved at 1.06 kg/cm^2 pressure at 121°C for 25 minutes. The medium was then cooled at room temperature before use.

Both the two ex plants (1-1.5 cm length) were taken separately in two beakers. After surface sterilization under laminar Airflow Cabinet with 70% ethyl alcohol, the explants were again surface sterilized with 0.1% mercuric chloride and a few drops of Tween 20 for 15 minutes. Finally, the explants were then rinsed three to four times with sterile distilled water.

The isolated and surfaced sterilized explants collected carefully under the stereomicroscope through maintaining aseptic condition inside the laminar air flow cabinet to use those as ex plants. The individual explants were directly inoculated to each of the culture tube containing 20 ml of MS medium supplemented with different concentrations of hormones as per treatment covered with aluminium foil.

The culture tubes were transferred to growth room and allowed to grow in controlled environment. The temperature of the growth room was maintained with 25±10°C by an air conditioner. A 16 -hour light period was maintained with light intensity of 2000 lux for the growth and development of culture.

Some explants become black in colour within 5-6 days after inoculation. To control blackening after one week, the blackish tissues on the ex plants were removed and the meristematic tissues were transferred to fresh medium. It was repeated 10 days interval for about one month to minimize further blackening of the tissues.

Initial subculturing was done when the ex plants produced some shoots. For sub-culturing, the entire samples of in vitro shoot were cut into small pieces so that each piece would contain about one shoot. Each piece was inoculated into a similar fresh medium. It was practiced at the interval of every one month.

When the shoots grew about 3-5 cm in length with 3-6 well developed leaves, they were rescued aseptically from the culture tubes and were separated from each other and again cultured on freshly prepared medium containing different combinations of hormonal supplements for root induction.

Potting mixture contain grerden soil and cowdung in the ratio of 1:1 was mixed thoroughly and were placed into a 10 x 15 cm polyethylene bag for growing in vitro grown plantlets under ex vitro condition.

RESULTS AND DISCUSSION

Regeneration of shoots from shoot tips and runner tip ex plants

Regeneration of strawberry plant lets through shoot tip and runner tip culture offers a unique scope of developing disease free planting materials. Results of multiple shoot development due to runner tip and shoot tip

were presented in Table 1. Runner tip developed significantly higher number (17.36) of shoots per culture than that of shoot tip explants (14.40). Runner tip is comparatively more easy to excise, clean and sterilize than shoot tip. Shoot tip is complex structure compared to runner tip which facilitate more pathogen contamination. It might be the cause of higher number of shoots under runner tip explants which is in agreement with the findings of Biswas *et al.* (2008). They found best response from runner tip explants with 0.5 mg/l BAP.

Runner tip also developed significantly higher number (6.98) of leaves per culture compared to shoot tip. It is due to the higher number of shoots developed from runner tip. With that period, under runner tip and shoot tip explants, length of shoots was found 3.69 cm and 3.21 cm respectively. Higher number of shoots containing higher number of leaves competed themselves for space, light and nutrition which might be the probable cause for maximum length of shoots under runner tip explants.

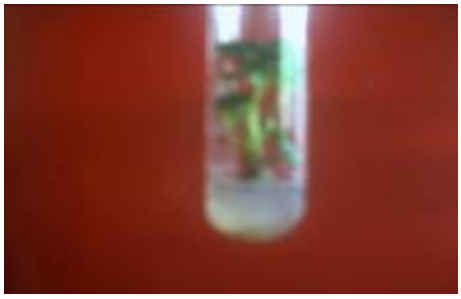

Fig. 1. Shoot regeneration of strawberry from runner tip explant

Fig. 2. Shoot regeneration of strawberry from shoot tip explant

Table 1. Effect of explants on different growth parameters at 30 days after inoculation

Explants	No. of shoots	No. of leaves culture	Length of shoots (cm)
Runner tip (Rt)	17.36	6.98	3.69
Shoot tip (St)	14.40	6.14	3.21
Significance level	**	**	**

Effect of different concentrations of BAP on multiple shoot proliferation from runner tip and shoot tip derived explants

The results obtained from this experiment have been presented in Table 2-3 and discussed under the following heading:

Number of shoots per explant

Variable number of shoots were produced per explant in MS media supplemented with different concentrations of BAP. Data were recorded at 30 days after inoculation (DAI) and results have been presented in Table 2. The effect of different concentrations of BAP on shoot regeneration and proliferation were statistically significant. Among the different concentrations, 10 mg/l BAP showed highest shoot proliferation of 27.7 shoots per explant at 30 days after inoculation whereas from the control treatments (0.00 mg/l) lowest shoot proliferation of 2.8 shoots per explant were recorded. The number of shoots per explant was gradually increased with the increment of BAP levels and the number of leaves followed the same trend (Table 2). In this Table, it has been shown that the length of shoots was inversely related to the number of shoots per explant. Bhuiyan (2005) also reported similar result in his experiment of different BAP levels on *Colocasia esculenta* var. Globulifera for multiple shoot production with BAP levels.

Number of leaves per explant

The effect of different concentrations of BAP on number of leaves per explant has been presented in Table 2. The results showed that the maximum number of leaves of 8.6 per explant at 30 DAI was produced on the medium supplemented with 10 mg/l BAP. The second highest number of leaves (8.20 leaves/explant) was

produced on the medium supplemented with 6.00 mg/l of BAP. The lowest number of leaves per explant of 4.2 was obtained from control treatment (Table 2). The highest level of BAP (10.0 mg/l) produced the maximum shoots per explant (27.7) and the control treatment produced the The length of shoots was statistically higher with 0.0 mg/l BAP (4.6 cm) which was statistically identical with 0.5 and 1.0 mg/l BAP. The treatments which produced more number of shoots gave the lowest length. It might be due to competition among the shoots for limited space and nutrition.

Table 2. Main effect of BAP on different growth parameters at 30 days after inoculation

BAP (mg/l)	No. of shoots/explant	No. of leaves/explant	Length of shoots (cm)
T_1-0.0	2.8i	4.20d	4.60a
T_2-0.5	8.6h	5.90c	4.42ab
T_3-1.0	10.0g	5.97c	4.22ab
T_4-1.5	12.2f	6.10c	3.85bc
T_5-2.0	14.8e	6.30c	3.84bc
T_6-2.5	18.1d	6.40c	3.20cd
T_7-3.0	20.0c	6.50c	3.05de
T_8-4.0	20.8c	7.5b	2.80de
T_9-6.0	24.5b	8.20ab	2.5ef
T_{10}-10.0	27.7a	8.60a	2.0f
Mean	15.95	6.57	3.45

In a column, means followed by common letters are not significantly different from each other at 1% level of probability by DMRT

In case of combined effect, the number of shoots/explant was the highest of 29.0 for runner tip with 10.0 mg/l BAP and the controls were the least (3.0 for runner tip and 2.6 for shoot tip (Table 3). The maximum leaves/culture (10.2) was produced for 10.0 mg/l BAP with runner tip while the minimum (3.6) for 0.0 mg/l BAP with shoot tip. The highest length (5.0 cm) was produced for 1.0 mg/l BAP with runner tip which was statistically identical to 0.5, 1.5, 2.0 and 2.5 mg/l BAP with runner tip (4.2, 4.4, 4.6 and 4.2 cm, respectively) and also statistically identical to 1.0, 1.5 and 2.0 mg/l BAP with shoot tip (4.2, 4.04 and 4.24 cm, respectively).

Table 3. Combined effect of explants and BAP on different growth parameters at 30 days after inoculation

Treatments		No. of shoots/explant	No. of leaves/explant	Length of shoots(cm)
Explants	BAP (mg/l)			
Runner tip	0.0	3.0m	4.8hi	3.4b-d
Shoot tip	0.5	10.0j	7.2b-e	4.2ab
	1.0	11.0i	7.6bc	5.0a
	1.5	13.8h	5.4gh	4.4ab
	2.0	16.0g	6.0d-h	4.6a
	2.5	20.0de	7.0c-f	4.2ab
	3.0	21.0d	6.4c-g	3.4b-d
	4.0	22.0bc	8.6b	2.6c-f
	6.0	25.0b	6.6c-g	3.0c-e
	10.0	29.0a	10.2a	2.1ef
	0.0	2.6m	3.6i	2.7c-f
	0.5	7.2l	5.6f-h	3.48bc
	1.0	8.4k	7.4b-d	4.2ab
	1.5	10.6ij	6.4c-g	4.04ab
	2.0	13.6h	5.8e-h	4.24ab
	2.5	16.2g	5.6f-h	3.5bc
	3.0	19.2e	6.6c-g	3.0c-e
	4.0	17.4f	7.8bc	2.4d-f
	6.0	23.2c	5.6f-h	2.6c-f
	10	25.6b	7.0c-f	1.9f

In a column, means followed by common letters are not significantly different from each other at 1 % level of probability by DMRT

Effect of IBA on root proliferation of strawberry

Results of root development due to runner tip and shoot tip were presented in Table 4. Number of roots/explant and length of roots (16.32 and 6.56 cm respectively) were highest in runner tip explants compared to shoot tip (16.20 and 6.44 cm) though these treatments showed statistically non-significant results. These results agrees the findings of Khanam *et al.* (1998). They found that shoot tip explants to initiated roots within 9 days after

culture. They also reported that the number of roots/culture and length of roots were 18.0 and 6.0 cm, respectively.

Table 4. Effect of explants on number of roots/explant and length of roots at 30 DAI

Explants	No. of roots/explant	Length of roots(cm)
Runner tip (Rt)	16.32	6.56
Shoot tip (St)	16.20	6.44
Significance level	NS	NS

Number of roots/explant and length of roots

The highest number of roots were produced (Table 5) by 1.0 mg/l IBA (10.9) which also produced shortest roots (4.4 cm) and 2.0 mg/l had the longest roots (6.9 cm). Auxin up to certain level (1.0 mg/l) helps to develop more, which enhanced by higher auxin level. Khanam *et al.* (1998) found 18.0 roots for 1.0 mg/l IBA which produced 6.8 cm long roots.

Table 5. Effect of different concentrations of IBA on number of roots/explant and length of roots at 30 DAI

IBA (mg/l)	No. of roots/explant	Length of roots (cm)
T_1-0.0	5.8cd	6.6a
T_2-0.5	8.9b	4.9b
T_3-1.0	10.9a	4.4b
T_4-1.5	6.3c	4.7b
T_5-2.0	5.4d	6.9a
Mean	7.46	5.5

In a column, means followed by common letters are not significantly different from each other at 1 % level of probability by DMRT

Combined effect of explants and different concentrations of IBA on root proliferation

Number of roots/explant was found maximum (19.0) at 1mg/l IBA with shoot tip explant which was statistically identical (18.8) to 1.0 mg/l with runner tip explants. These treatments also produced shortest roots 5.2 cm and 5.4 cm respectively. It was might be due to the competition among large number of roots for limited space and nutrition. On the other hand, the minimum number (14.4) of roots/explant at 30 DAI was recorded from the treatment of 2.0 mg/l of IBA with runner tip explant and same concentration with shoot tip explant. The highest length of roots (7.6, 8.0, 7.6 and 7.8) was produced from the treatments of 1.5 and 2.0 mg/l with runner tip and 0 and 2.0 mg/l with shoot tip explant at 30 DAI.

Table 6. Combined effect of explants and IBA on different growth parameters

Treatments		No. of roots/explant	Length of roots (cm)
Explants	IBA (mg/l)		
Runner tip	0.0	14.8de	5.7b
	0.5	18.0bc	5.7b
	1.0	18.8ab	5.4b
	1.5	15.6d	7.6a
	2.0	14.4e	8.0a
Shoot tip	0.0	14.8de	7.6a
	0.5	17.8c	5.6b
	1.0	19.0a	5.2b
	1.5	15.0de	5.8b
	2.0	14.4e	7.8a

In a column, means followed by common letters are not significantly different from each other at 1 % level of probability by DMRT

Plantlet formation and their establishment in the soil

The regenerated healthy rooted plantlets (Fig. 1 & Fig. 2) were replaced from culture room and kept in room temperature (20-25^{0}C). Plantlets were carefully removed from the culture vessels. After thouroughly washing the roots in tap water to remove the traces of nutrients, the rooted plantlets were placed in plastic pots (6 x 10 cm size) containing unsterilized garden soil and cow manure (1:1) for growing *ex vitro* condition.

CONCLUSION

For *in-vitro* shoot multiplication of strawberry, runner tip explants was found 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. For maximum roots/culture, 1.00 mg/l IBA with both runner tip and shoot tip was found better. Furthermore, the results could be used to produce large scale production of healthy and disease free planting materials commercially.

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