MASS PRODUCTION OF STRAWBERRY PLANTS THROUGH MICROPORPAGATION

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Abstract

An investigation was made during 2008-2009 to standardize a protocol for mass propagation of strawberry. The MS medium supplemented with BAP (0.5 mg/l) and Kinetin (0.5 mg/l) was found the best for culture survival (90%), early shoot emergence (13.33 days), shoot elongation (17.67 days) and number of shoots per explant (11.44). Different combinations of BAP, Kinetin, GA_3 and NAA were tried for further shoot multiplication. In case of shoot proliferation, MS medium in combination with 1.0 mg/l of BAP and 0.5 mg/l of Kinetin resulted maximum shoot length (7.50 cm) as well as maximum number of leaves (23.11), shoots (12.33) and fragments (4.56) per explant. For root induction, the best results were found using ½ MS medium supplemented with 0.5 mg/l of IBA. Here, 80% microcuttings were rooted, root induction took the least time (8.86 days) and the highest number of roots per microcutting (23.43) and the longest root (4.01 cm) were observed.

Keywords: Strawberry, Micropropagation, BAP, Kinetin.

Introduction

Fragaria X ananassa Duch., the cultivated strawberry is a perennial, stoloniferous herb. Strawberry is one of the most popular fruits growing in the northern hemisphere in temperate and sub-temperate environment and the most widely consumed fruits throughout the world (Biswas *et al.*, 2008). It is an exotic fruits in Bangladesh. Strawberry is the most favourite fruit for its yogurt flavour in many countries. Fruits are eaten raw or used in making juice, desserts, jam, syrup, ice-cream and wine. According to FAO, 2004, United States is the highest strawberry producing country in the world. About 75 % of the strawberries grown in the USA are sold fresh, and 25 % processed (mostly frozen). Strawberries are higher in vit-C than many citrus fruits. It is also enriched in protein (14%), calories (37%), water (9%), carbohydrate (8%), vitamins A (1.2%), B1, B2, calcium, phosphorus, iron, sodium, potassium, etc. (FAO, 2004)

Majority of strawberry cultivars are generally vegetatively propagated by runners. But the viral diseases can frequently be transmitted through runners during the process of vegetative propagation and the rate of multiplication through this conventional means is too slow (Negi *et al.*, 2008). As in the case with all vegetatively propagated plants, strawberry is often infected with virus and mycoplasma diseases (Boxus, 1974). To improve the strawberry varieties, this conventional method is not suitable and resulting in the gradual degeneration of cultivars performance (Biswas *et al.*, 2008). Thus tissue culture methods in strawberry have been developed with the objectives of rapid clonal multiplication of disease free plant materials and preservation of germplasm (Boxus, 1974). The rate of strawberry propagation through conventional techniques is difficult to maintain plant materials during summer in Bangladesh. Moreover, the conventional way of propagation is not adequate to meet the commercial demand. Several improvements of the tissue culture technology have been proposed by authors working with strawberry (Damiano, 1980; Drew *et al.*, 1986; Swartz *et al.*, 1987) but the highest genotypic, physiological and morphological quality of micropropagated plants is produced by the method described by Boxus and co-workers (Boxas, 1974; Jemmali *et al.*, 1995). Micropropagated strawberry plant has been introduced to prevent most of the plant and soil transmissible diseases. So, the present study was undertaken to develop a method for the rapid clonal multiplication of disease free strawberry plant in order to ensure abundant supply for commercial cultivation.

Materials and Methods

Runner tips collected from nursery grown stock plants of Rabi-3 variety, were washed with tween-20 for 30 minutes followed by a thorough wash in running tap water to remove traces of the detergent. These were then surface sterilized with 0.1 percent $HgCl_2$ solution for 4-5 minutes inside laminar flow cabinet. Then the treated explants were washed with sterile distilled water. Afterwards, sterile runner tips having terminal buds (3-4 mm) were dissected and cultured in MS medium (Murashige and Skoog, 1962) alone and in combination with growth regulators, BAP (0.5mg/l) and Kinetin (0.5mg/l). The pH of the medium was adjusted to 5.8 with 0.1 N NaOH before adding agar and autoclaving at 1.06 kg/cm^2 and 121°C for 20 min. The cultures were incubated in a growth chamber under 16/8 hour light/dark cycle at 25±2°C. Shoot initiated within 16 days after inoculation. The survival percent, days taken to shoot emergence and shoot

elongation, number of shoots per explant were recorded to establish media for shoot induction.

The aseptic shoots obtained after 16 days of culture were used as source of explants to establish media for shoot proliferation. The aseptic shoots were subcultured on MS media supplemented with BAP and Kinetin/GA_3/NAA to find out the appropriate concentration and combination of growth regulators in media. In this case, shoot length, number of leaves and number of fragments per explant were recorded after 21 days of subculturing.

Subcultures were done every 21 days interval. Nodal segments from the proliferated shoots were subcultured in ½ MS medium containing different concentrations of NAA and IBA for root induction. After 5 weeks data, such as percentage of microcuttings rooted, days taken to root initiation, number of roots per microcutting and root length were recorded to observe the rooting frequency.

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 poly bags. After 7 days the hardened plantlets were planted in potted soil.

Results and discussion

The MS medium supplemented with BAP (0.5 mg/l) and Kinetin (0.5 mg/l) (M_3 treatment) was found the best for culture survival (90%) and the earliest to shoot emergence (13.33±0.17 days). The lowest culture survival (70%) and maximum days to shoot emergence (15.57±0.20 days) were observed on MS medium (M_1 treatment) (Table 1). Similar trend was reported by Pathania *et al.* (2001) in gladiolus. In MS medium with Kinetin (0.2 ppm) and BAP (0.5 ppm), buds turned green and opened early (Barve *et al.*, 1984). Earlier shoot elongation (17.67±0.29 days) and maximum number of shoots per explant (11.44±0.24) were observed for MS medium supplemented with BAP (0.5 mg/l) and Kinetin (0.5mg/l). However, minimum number of shoots per explant (2.43±0.20) took maximum time (21.43±0.20 days) when explants were inoculated in MS medium. Hussain (1995) and Hussain *et al.* (1996) observed higher shoot proliferation rate of gladiolus in MS basal medium supplemented with BAP and NAA. Indra and Uppeandra (2000) reported that multiple shoot regeneration from Indian wild strawberry using MS medium supplemented with 4.0 mg/l IBA and 0.1 mg/l NAA.

It was observed that S_4 medium, having 1.0 mg/l of BAP and 0.5 mg/l of Kinetin, had maximum shoot length (7.50±0.07), the highest number of leaves (23.11±0.45), shoots (12.33±0.33) and fragments per explants (4.56±0.18) (Table 2, Plate 1A).

To study the effect of GA_3 in shoot proliferation as an alternative of Kinetin, S_8 and S_9 treatments were used having 0.5 mg/l and 1.0 mg/l of GA_3 respectively (Table 2). The effect of GA_3 was studied because Negi *et al.* (2008) found maximum number of leaves when used MS medium supplemented with 0.5 mg/l BAP and 1.0 mg/l of GA_3. But in this study no satisfactory results were observed when GA_3 was used instead of Kinetin. The effect of NAA was also investigated as a substitute of Kinetin (S_{10} and S_{11} treatments) and here also no satisfactory results were observed.

Indra and Uppeandra (2000) reported multiple shoot regeneration from Indian wild strawberry using MS medium supplemented with 4.0 mg/l BA and 0.1mg/l NAA. Some workers also reported shoot regeneration in strawberry using MS medium containing BA also of in combination with Kinetin (Lis, 1990; Boxus, 1999; Neeru *et al.*, 2000; Mereti *et al.*, 2003). However, the results of the present investigation slightly differed with that of the previous works. Our results indicated that, 0.5 mg/l of BAP and 0.5 mg/l of Kinetin were suitable for shoot initiation and 1.0 mg/l of BAP and 0.5 mg/l of Kinetin were suitable for further shoot multiplication of Rabi-3 variety of strawberry. This difference might be attributed by the difference of genotype and physiological condition of the explants.

Then to establish the rooting medium for proliferated shoots, different concentrations of IBA and NAA were used with ½ MS media. Out of different concentrations of IBA (0.2, 0.5 & 1.0 mg/l) and NAA (0.2, 0.5 & 1.0 mg/l) tested, 0.5 mg/l IBA (R_6 treatment) was proved to be the most suitable for root induction with 23.43±0.28 roots per microcutting and the average root length being 4.01±0.09 cm (Table 3, Plate 1C). Similar effects of IBA were also observed in *Calotropis gigantea* (Roy and De, 1986), *Capsicum annum* (Agarwas *et al.*, 1989) and *Prunus* sp. (Mante *et al.*, 1989).

Table 1. Effects of shoot induction media on explants of strawberry

Treatments	Composition	Survival (%)	Days taken to shoot emergence	Days taken to shoot elongation	Number of shoots per explant
M_1	MS	70	15.57±0.20	21.43±0.20	2.43±0.20
M_2	MS + BAP (0.5 mg/l)	80	14.13±0.30	19.00±0.33	4.38±0.18
M_3	MS + BAP (0.5 mg/l) + Kinetin (0.5 mg/l)	90	13.33±0.17	17.67±0.29	11.44±0.24

Table 2. Effects of shoot proliferation media on shoot tips of strawberry

Treatments	Composition	Shoot length (cm)	Number of leaves per explant	Number of shoots per explant	Number of fragments per explant
S_1	MS	2.74±0.07	6.75±0.31	2.88±0.30	0.00
S_2	MS + BAP (0.5 mg/l) + Kinetin (0.5 mg/l)	4.72±0.10	15.22±0.22	7.89±0.26	2.56±0.18
S_3	MS + BAP (1.0 mg/l) + Kinetin (1.0 mg/l)	5.14±0.06	16.22±0.28	8.44±0.34	2.44±0.18
S_4	MS + BAP (1.0 mg/l) + Kinetin (0.5 mg/l)	7.50±0.07	23.11±0.45	12.33±0.33	4.56±0.18
S_5	MS + BAP (0.5 mg/l) + Kinetin (1.0 mg/l)	4.77±0.06	15.78±0.32	8.78±0.32	2.67±0.17
S_6	MS + BAP (1.5 mg/l) + Kinetin (0.1 mg/l)	5.10±0.07	16.22±0.40	8.89±0.26	2.78±0.15
S_7	MS + BAP (1.5 mg/l) + Kinetin (0.5 mg/l)	5.61±0.11	18.89±0.31	9.67±0.29	3.56±0.18
S_8	MS + BAP (1.0 mg/l) + GA_3 (0.5 mg/l)	4.60±0.10	14.11±0.35	6.89±0.26	2.22±0.15
S_9	MS + BAP (1.0 mg/l) + GA_3 (1.0 mg/l)	4.80±0.08	15.30±0.33	7.10±0.31	2.80±0.15
S_{10}	MS + BAP (1.0 mg/l) + NAA (0.5 mg/l)	4.60±0.07	14.30±0.37	6.80±0.15	2.40±0.18
S_{11}	MS + BAP (1.0 mg/l) + NAA (1.0 mg/l)	4.70±0.08	14.60±0.47	8.90±0.20	2.70±0.17

Table 3. Effect of root induction media on shoots of strawberry

Treatments	Composition	% of microcuttings rooted	Days taken to root initiation	Number of roots per microcutting	Root length (cm)
R_1	½ MS	50	13.20±0.37	8.60±0.40	1.98±0.04
R_2	½ MS + NAA (0.2 mg/l)	60	11.33±0.33	10.67±0.33	2.32±0.07
R_3	½ MS + NAA (0.5 mg/l)	70	11.57±0.30	12.86±0.34	3.24±0.06
R_4	½ MS + NAA (1.0 mg/l)	60	11.50±0.34	12.50±0.34	2.87±0.07
R_5	½ MS + IBA (0.2 mg/l)	70	10.71±0.29	15.14±0.26	3.39±0.06
R_6	½ MS + IBA (0.5 mg/l)	80	8.86±0.29	23.43±0.28	4.01±0.09
R_7	½ MS + IBA (1.0 mg/l)	70	10.00±0.37	13.00±0.22	3.04±0.05

The protocol for the propagation strawberry, reported here is reproducible; it has a potential for allowing a large scale micropropagation of this important and new plant in Bangladesh.

Conclusion

An attempt may be made to evaluate the field performance of the Rabi-3 strawberry plants in Bangladeshi environment generated by the tissue culture techniques described here. This field performance results may be used further for improvement of this strawberry variety.

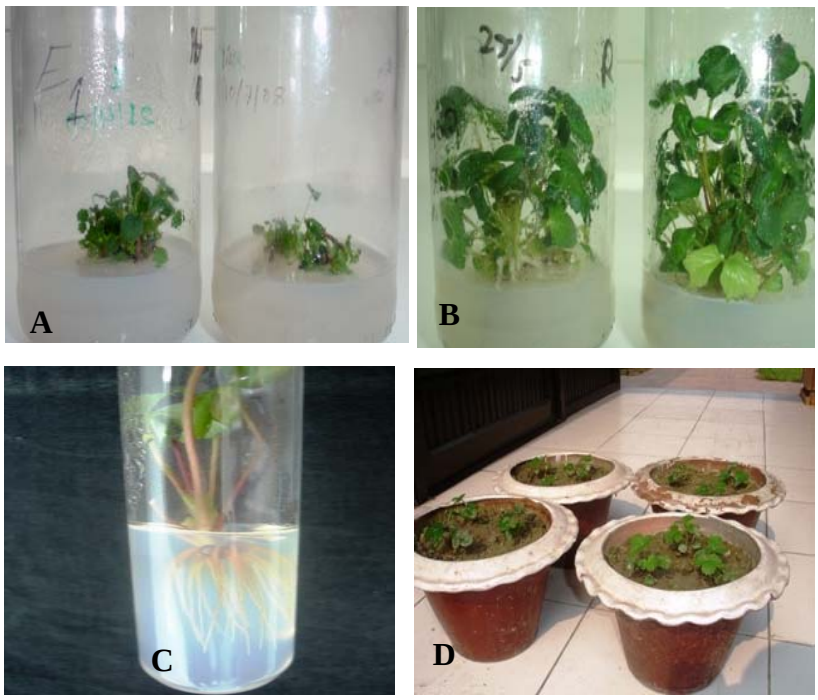

Plate 1. Micropropagation of strawberry from runner shoots tips. A. Shoot proliferation on MS + 1.5 mg/l BAP + 0.5 mg/l Kinetin after 21 days of culture. B. Shoot proliferation on MS + 1.0 mg/l BAP + 0.5 mg/l Kinetin after 42 days of subculture. C. Rooted shoots on ½ MS + 0.5 mg/l IBA + 0.5 mg/l Kinetin after 21 days of culture. D. Hardened plantlets of strawberry in potted soil after 20 days of transplantation.

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