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Yield potential study of three genotypes (RABI-3, JP- 3 and Camarosa) of strawberry (*fragaria* X *ananassa* Duch.) in Bangladesh

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Abstract

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the important and popular fruits in the temperate countries of the world due to its fragrance, taste and nutritional properties. Due to its popularity and increasing demand in Bangladesh, an experiment was conducted to establish a rapid *in vitro* clonal propagation of three strawberry genotypes and yield potential study of these three strawberry genotypes. Runner tips of three strawberry genotypes viz. RABI-3, JP-3 and Camarosa were cultured *in vitro* for multiple shoot proliferation and root induction. Proliferation of runner tips was obtained on MS basal medium containing three different concentrations (0.5, 1.0 and 1.5 mg/l) of BA singly and with 0.5 mg/l KIN or GA_3. The best shoot proliferation was obtained from cultures grown on medium supplemented with 1.5 mg/l BA with 0.5 mg/l KIN. Microcuttings were rooted on half strength MS medium with 0.5-1.5 mg/l IBA or IAA. Maximum rooting (87-100%) with 9-12 roots/ cultures was achieved at 1.0 mg/l IBA. The plantlets, thus developed were hardened and successfully stablished in soil. RABI-3 showed best performance in all morphological and yield contributing characrers followed by JP-3 and Camarosa. This result indicated that Bangladeshi strawberry variety was more responsive under *in vitro* and *ex vitro* condition than Japanese and American variety.

Key words: Yield potential, Strawberry, Runner tips, Nodal segments, Bangladesh.

Introduction

The cultivated strawberry (*Fragaria* x *ananassa* Duch.), a member of Rosaceae, is the most important soft fruit worldwide. The cultivated strawberry (*Fragaria* x *ananassa* Duch.), a hybrid between the scarlet or Virginia strawberry (*F. virginiana* Duch.) and the pistillate South American *F. chiloensis* (L.) Duch., is a dicotyledonous, perennial low-growing herb grown in most arable region of the world.

The cultivated strawberry is an octaploid (2n=2X=56) stoloniferous perennial herb (Debnath and Teixeira da silva, 2007). It has a wide range of climatic adaptation which includes Mediterranean, temperate, subtropical and taiga zones (Hancock *et al.,* 1991). Conventionally, strawberry is propagated by runners (sakila *et al.,* 2007), which is very labour intensive, time consuming and results in the transmission at viral diseases (Gautam *et al.,* 2001). In contrast of these, mass multiplication *in vitro* through tissue culture results high yield in disease free plant material (Mohan *et al.,* 2005) and proved to be the best alternative approach to conventional propagation method (Mahajan *et al.,* 2001). The standardization of protocol and procedure of micropropagation of strawberry was successfully attempted by many (Kaur *et al.,* 2005; Sakila *et al.,* 2007; Gantait *et al.,* 2010; Hasan *et al.,* 2005; Biswas *et al*., 2008) scientists. But complete field performance of micropropagated plants was not studied enough where extensive field evaluation is necessary for commercial utilization of tissue culture (Smith and Hamill, 1996).

For better strawberry production photoperiod 10-20h, day temperature 12-30 ^{o}C and number of short days 12-24 are essential (Michel *et al.,* 2006). Bangladesh day temperature is 15-25^{o}C, photoperiod 12-16h and short days about 30-50 days (Biswas *et al.,* 2008). Therefore in winter season Bangladeshi strawberry variety RABI-3 can be grown.

In addition now strawberry genotype RABI-3 is successfully cultivated in different regions of Bangladesh for commercial purpose (Ara, 2009). The present investigation was curried out to evaluate the field performance of micropropagated Japanese and American varieties in comparision to *ex vitro* propagated commercially cultivated RABI-3 variety.

Materials and Methods

Runners with tips of three strawberry varieties viz. RABI-3, JP-3 and Camarosa were collected from strawberry germplasm stocks maintained in Akafuji Agrotechnologies, Rajshahi, Bangladesh. RABI-3 is a Bangladeshi strawberry variety, Camarosa is a North American variety and JP-3 is a Japanese variety. Fresh runner tips from 2 mounth old mature strawberry plants were collected during the first week of November 2007. Runner tips of three strawberry varieties were washed first under running tap water for 30 minutes and treated with 1% Tween 80 for ten minutes followed by repeated rinsing with sterile distilled water. Further sterilization was done under aseptic condition in laminar air flow cabinet. Explants were surface sterilized with 50% (v/v) ethyl alcohol (1 min) followed by 0.1 % (w/v) $HgCl_2$ (4 min). Finally, the explants were washed thoroughly (five times) with sterilized distilled water and cut into appropriate size (1.5 cm) and cultured on MS basal medium supplemented with specific concentration of growth regulators (BA, KIN and GA_3) adding 30 g/l sugar (market sugar) and 0.8% agar (BDH, England). The pH of the medium was adjusted to 5.7 before autoclaving at 1.06 kg/cm^2 and 121°C for 20 min. The cultures were incubated in growth chamber 16/8 light/dark cycle at 25±2°C. Proliferated multiple shoots after elongation were cut and individual shoots were placed in half strength MS medium containing different concentration of IBA or IAA for root induction. Data on shooting and rooting efficiency were recorded after 5 weeks of culture initiation. For each treatment 10-12 explants were used and the experiments were repeated three times. Three-week-old rooted shoots were taken out from the culture tubes, thoroughly washed in water to remove agar gel and then transferred to plastic pot containing garden soil and compost (3:1 v/v) and were kept under transparent plastic shed to control the moisture condition. After one week plants were taken out from the shed and successfully survived plants were transferred to the field. Plantlets of three strawberry genotypes (RABI-3, Camarosa and JP-3) were planted at the research field of Department of Botany, Rajshahi University, Rajshahi, Bangladesh in a Randomized Block Design with three replications during winter season of 2011 and 10 plants per replication. RABI-3 is a Bangladeshi strawberry variety, Camarosa is a American variety and JP-3 is a Japanese variety maintained in strawberry germplasm bank of plant breeding and gene engineering lab, Department of Botany, Rajshahi University, Bangladesh.

The fertilizers (Nitrogen 55 Kg/hec, P 70 Kg/hec, K 65 Kg/hec.) and 10 plants of each variety were used to ensure optimum plant population (Hossain, 2007). Irrigation and intercultural operations were done for raising good crops.

The time of harvest is very important in strawberry. After plantation of strawberry plantlets fruits were collected from field within 80-100 days. Berries were harvested every day to maintain the quality of the berries. When 2/3 of the berries became red color then they were was ready to harvest (Ara, 2009). Other standard agronomic practices were followed.

Data were collected from 10 randomly selected plants on different morphological and agronomical characters such as plant height, no. of leaves/plant, petiole length, no. of stolon/plant, no. of nodes/stolon, stolon length, no. of crowns/plant, canopy size (cm^2), days to flowering, no. of flower cluster/plant, no. of flower/plant, no. of fruits/plant, no. of fruits/cluster, days to fruit harvest, average fruit weight (g) and fruit weight/plant (g). The Results of these data are summarized in Table (2). Data on eight morphological and eight yield contributing characters were recorded from different selected somaclones and their donor parents. Out of eight morphological characters three characters were recorded after 60 days of plantation in the field and other five characters were recorded 70 days after plantation in the field. Data were analyzed following biometrical techniques developed by Mather 1949, Hayman (1958), De-wey and LU (1959), Allard (1960).

Results and Discussion

Sterilized runner tips were cultured in MS medium without any growth regulators. After two weeks aseptic runner tips were transferred to

multiple shoot induction media. To evaluate the best media formulations for mass propagation of three strawberry genotypes were tasted on MS medium was tested and fortified with different concentration of BA (0.5, 1.0 and 1.5 mg/l) singly and with KIN (0.5 mg/l) or GA_3 (0.5 mg/l). Within 8-14 days of culture multiple shoots emerged directly from the explants. RABI-3 showed the maximum frequency (88%) of shoot formation followed by JP-3 (86%) and Camarosa (80%) (Figure 1. C-D). When BA concentration was increased from 1.0 mg/l to 1.5 mg/l the number of shoots/explant increased but further increase of BA number of shoots decreased. Hu and Wang (1983) reported that high concentration of cytokinin reduced the number of micropropagated shoots. Similar results have already been reported in *Fragaria indica* Andr. (Bhatt and Dhar, 2000). Also this result is in consistent with the findings in papaya (Corner and Litz, 1978) as well as in *Eucalyptus grandis* (Teixetra and Silva, 1990). 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. Indra and Uppeandra (2000) reported multiple shoot regeneration from Indian wild strawberry using MS supplemented with 4.0 mg/l BA and 0.1 mg/l NAA.

Some workers also reported shoot regeneration in strawberry using MS medium containing BA in combination with KIN (Lee and de Fossard, 1977; Sobczykiewicz, 1980; Lis, 1990; Boxus, 1999; Neeru, *et al*., 2000; Mereti, *et al*., 2003). Our results indicated that, low concentration of BA alone or with KIN were found suitable for shoot initiation and further multiplication. This difference may be attributed by the difference of genotype and physiological condition of the explants. The daughter shoots (3-4 cm length) were excised and transferred to root induction media. Both IBA and IAA were found to be effective for adventitious root induction and frequency of root induction ranged from 87-100%. Out of two concentrations of IBA or IAA 1.0 mg/l was proved to be superior where the shoots produced roots early and between the two auxins IBA showed better performance for root induction than IAA (Figure 1. F). RABI-3 produced highest number of roots per shoot with highest frequency (100%) of root induction in medium fortified with 1.0 mg/l IBA.

No difference was observed in root length in this experiment. Similar effects of IBA were also observed in *Calotropis gigentea* (Roy and De, 1986), *Capsicum annum* (Agarwal, Chandra and Kothari, 1989) and *Prunus* sp. (Mante, Scorza and Cordts, 1989).

Table (1): Effects of different concentrations and combinations of growth regulators on multiple shoot proliferation and root induction of three strawberry genotypes.

Concentrations of growth regulators (mg/l)	Parameters	Genotypes		
		RABI-3	JP-3	Camarosa
MS+ 0.5 mg/l BA	Explants induced multiple shoots (%)	50	45	40
1.0+ 0.5 (BA+KIN)		55	48	45
1.5+0.5 (BA+KIN)		88	86	80
1.5+ 0.5 (BA+GA_3)		56	51	51
MS+0.5 mg/l BA	No. of shoots/culture	3.33	3.00	2.67
1.0+0.5 (BA+KIN)		2.67	2.33	2.00
1.5+0.5 (BA+KIN)		9.00	8.33	8.33
1.5+ 0.5 (BA+GA_3)		7.33	5.67	5.33
MS + 0.5 mg/l IBA	Root formation (%)	88	87	87
MS + 1.0 mg/l IBA		100	95	95
MS + 0.5 mg/l IAA		89	88	88
MS + 0.5 mg/l IBA	No. of roots/culture	7.33	6.33	6.33
MS + 1.0 mg/l IBA		12.00	10.67	10.00
MS + 0.5 mg/l IAA		6.67	3.67	3.00

Rooted plantlets ware 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 plastic pot (Figure 1. G). After 7 days, the hardened planlets were planted in soil. After acclimatization of *in vitro* regenerated plantlets, the successfully survived plants were then transferred to evaluate their field performance. Data were collected from 10 randomly selected plants on different morphological and agronomical characters such as plant height, no. of leaves/plant, petiole length, no. of stolons/plant, no. of nodes/stolon, stolon length, no. of crowns/plant, canopy size (cm^2), days to flowering, no. of flowers/cluster, no. of flowers/plant, no. of fruits/plant, no. of fruits/cluster, days to fruit harvest, average fruit weight (g) and fruit weight/plant (g). In the present study three strawberry genotypes were evaluated for sixteen characters. Collected data were analyzed in order to estimate mean with standard error, analysis of variance (ANOVA), least significant difference (LSD) and coefficient of variability. The Results of these data are summarized in Table (2).

Out of eight morphological characters three characters were recorded after 60 days of plantation in the field and other five characters were recorded 70 days after plantation in the field. On the other hand out of eight characters three characters were recorded after 70 days, three characters were recorded after 80 days and other two characters (average fruit weight and fruit weight/plant) were recorded at harvesting.

In the analysis of variance the main item genotype was highly significant for all characters at 5% level of significance. These results indicate that genotypes were genotypically different from each other and justify their inclusion in the present investigation as materials. The replication items were non significant for all characters. Haque and Hoque (1997) obtained similar results in chickpea. RABI-3 showed best performance in all morphological and yield contributing characters followed by JP-3 and Camarosa (Table 2). These results indicated that Bangladeshi strawberry variety was more responsive under *in vitro* and *ex vitro* condition than Japanese and American variety.

The protocol reported here is reproducible; it has a potential for allowing a large scale multiplication of this important and new fruit plant in Bangladesh.

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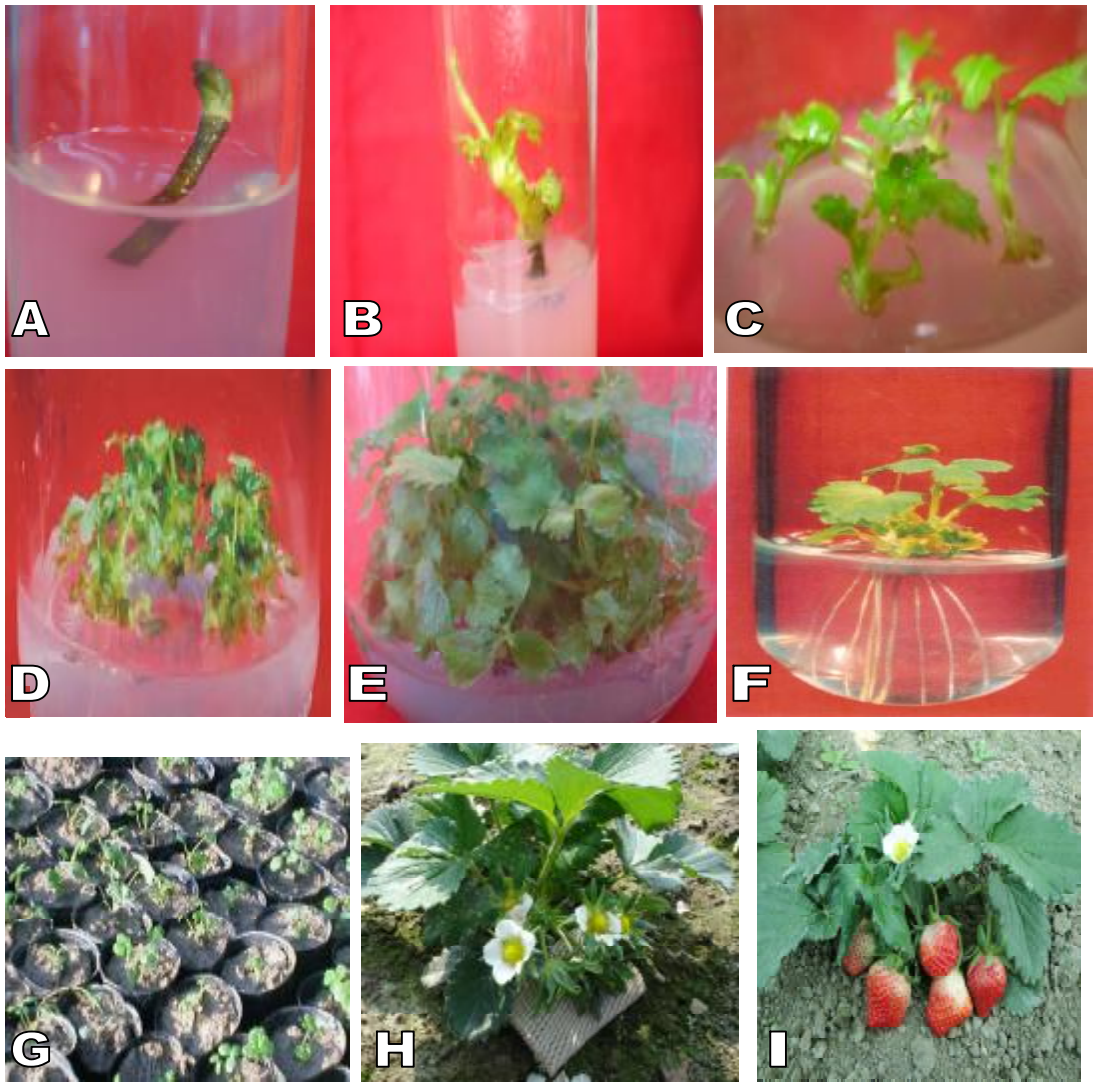

Figure (1): Micropropagation of strawberry from runner tips. Runner tips were used as explants (A-B); shoot proliferation on MS + 1.5 mg/l + 0.5 mg/l KIN after 7 days (C), after 20 days (D), after and 35 days (E) of culture. Rooted shoots on 1.0 mg/l IBA after 3 weeks (F) and after 4 weeks (I) of culture. Regenerated plantlets in thump pots after 20 days of transplantation (G). Plants with flowers (H) and fruits (I) of strawberry.

Table 2. Morphological and yield contributing characters of three strawberry varieties.

Characters	Varieties			F value (at 5% level of significance)	LSD value (at 5% level of significance)
	RABI-3	JP-3	Camarosa		
Plant height (cm) (Mean±SE)	25.03±0.26	22.00±0.23	16.13±0.18	6502.71*	0.3536
No. of leaves/plant (Mean±SE)	19.60±0.20	18.60±0.21	15.47±0.20	107.78*	1.3090
Petiole length (cm) (Mean±SE)	14.23±0.15	13.80±0.15	13.27±0.15	10.55*	0.9394
No. of stolons/Plant (Mean±SE)	6.27±0.15	6.10±0.10	4.40±0.05	77.86*	0.7377
No. of nodes/Stolon (Mean±SE)	4.23±0.12	3.13±0.09	2.33±0.09	819*	0.2101
Stolon length (cm) (Mean±SE)	150.13±0.23	149.73±0.39	100.70±0.44	9166.54*	1.8710
No. of crowns/Plant (Mean±SE)	6.63±0.09	5.40±0.06	3.03±0.26	129.1*	1.0147
Canopy size (cm^2) (Mean±SE)	462.37±0.30	451.23±0.16	383.40±3.31	533.04*	11.6663
Days to Flowering (Mean±SE)	61.67±0.33	61.67±0.33	70.00±0.33	250*	1.9176
No. of flower clusters/plant (Mean±SE)	7.67±0.33	6.67±0.33	6.00±0.33	19*	1.2128
No. of flowers/Plant (Mean±SE)	18.67±0.33	17.67±0.33	14.33±0.33	34.75*	2.4255
No. of fruits/ Cluster (Mean±SE)	6.00±0.57	2.67±0.33	2.00±0.33	22.54*	2.8442
No. of fruits/plant (Mean±SE)	6.67±0.33	14.33±0.33	14.33±0.33	529*	1.2128
Days to fruit Harvest (Mean±SE)	92.67±0.33	84.67±0.33	83.67±1.20	73*	3.6383
Average fruit wt. (g) (Mean±SE)	32.43±0.23	31.17±0.65	22.27±0.15	398.46*	1.7491
Fruit wt./plant (Mean±SE)	154.57±0.43	497.03±2.57	482.27±3.05	7379.32*	14.2024

*= significant at 5% level of probability

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