Crop Improvement 37 (2) : 153-156 (2010)

MICROPROPAGATION OF STRAWBERRY *(FRAGARIA X ANANASSA)*

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ABSTRACT

For obtaining basic material for the establishment of large scale, stable and high yielding strawberry plantlets for cultivation requires mass production of uniform propagules. Micropropagation is one of the effective means to achieve this goal. Continuous planting of runners from old mother plants for five or more years that are prone to diseases & viruses and the lack of ideal planting materials are the main causes of low productivity of strawberry. The rate of strawberry propagation through conventional technique is quite low and it is difficult to maintain plant material during the summer months. Efficient method for shoot regeneration, proliferation and rooting from nodal segments of strawberry (*Fragaria x ananassa*) cultivars Chandler, Oso-Grande and Cama-Rosa was developed. After six weeks of incubation maximum regeneration of 90 per cent was recorded in cv. Chandler on MS medium with 2 mg l^{-1} BA + 0.5 mg l^{-1} NAA . In all cultivars maximum mean number of shoots per explant was observed in MS + 2.0 mg l^{-1} BA + 0.5 mg l^{-1} NAA. Maximum length (10.18 cm) and number of shoots (10.4) were observed in cv Chandler on MS Media supplemented with BA (2 mg l^{-1}) and NAA (0.5 mg l^{-1}). The regenerated shoots were rooted on MS basal medium with different concentrations IBA. The maximum frequency of rooting, highest number of roots (10) and maximum length of roots (7.5 cm) was observed in cv Chandler on MS medium containing 2 mg l^{-1} IBA and 2.3 mg l^{-1} IBA respectively. The plantlets, thus developed were hardened and successfully established in soil.

Key words: Strawberry, micropropagation, nodal segment, Chandler, Oso-Grande and Cama-Rosa

Strawberry (*Fragaria x ananassa* Duch.) is a perennial, stoloniferous herb belongs to the family Rosaceae. It is one of the most popular fruits growing in the Northern hemisphere in temperate and sub temperate environment (Biswas, Islam and Hossain, 2008).The fruits are rich in bioactive phytochemicals, especially phenolic compounds with high antioxidant capacity, and as a part of daily diet could be beneficial for human health Hannum (2004). Micro propagation of strawberry has been used in horticultural production since 30 years Boxus (1974). Although, some problems are still remaining such as multiple shoots regeneration ability, micropropagation media varied from strawberry cultivars to cultivars. Some workers reported high concentration of BAP is the best for strawberry micropropagation (Morozova, 2002) while other authors suggested IAA + BAP + GA3 (Boxus, 1999) and BA + IBA (Bozena, 2001) for strawberry micropropagation. Organogenesis of strawberry has been reported by several workers (Lal, Sharma and Hedge, 2003, Damiano *et al*., 2004, Biswas, *et al*., 2010). Efficient methods for shoot regeneration, proliferation and rooting from runner tip explants of strawberry (*Fragaria X ananassa*) cultivars Chandler, Oso-Grande and Ofra have been developed (Lal, Sharma and Hegde, 2003).

In view of the potential commercial value of this crop, it is highly desireable to develop an efficient tissue culture protocol for rapid and large scale multiplication of popular strawberry genotypes grown under Kashmir conditions, where temperature remains sub-zero during most of the winter season. This is the first report on *in-vitro* propagation of strawberry in Kashmir. In present study a simple protocol has been developed from nodal segments in order to ensure large scale production of quality planting material.

MATERIAL AND METHODS

Plant material

Three genotypes Chandler, Cama-Rosa and Oso-Grande from the *Fragaria* germplasm collection of the Central Institute of Temperate Horticulture, Srinagar, J & K, India were cultured *in vitro*. Nodal segments were collected from nursery grown stock plants of these three strawberry genotypes.

Culture conditions and *in-vitro* multiplication

Basal media employed was MS at pH 5.8 and with 0.8% agar supplemented with different concentrations of BA, NAA and IBA in combinations (Table 1 & 2). After preparing the media, explants were cultured in glass tubes (90x 25mm) and flasks (150 ml). Cultures were maintained at 25±1 °C under 16/8 h (light/darkness) photoperiod with a light intensity of approximately 4000 lux. These cultures were sub-cultured every 3 weeks. Observations with respect to shoot and root development were taken after every four weeks. This study was carried out as a factorial experiment based on completely randomized design (CRD) with 4 treatments, in 4 replications.

Acclimatization and Field performance of micro propagated plantlets

Four week-old rooted shoots were removed from the culture tubes, thoroughly washed to remove agar traces and then transferred to plastic pot containing cocopeat:vermiculite:perlite (2:1:1). Potted plantlets were covered with transparent polythene bags to ensure high humidity and watered every 3-4 days with half strength MS salt solution for 2 weeks. Polythene bags were gradually perforated after 4 weeks and were removed after 8 weeks in order to acclimatize plants to field conditions. After 8 weeks, acclimatized plants were transferred to greenhouse, where ambient conditions of temperature and humidity were maintained. The hardening and acclimatization procedures were followed as described by Kumar *et al*., (2007).

Statistical analysis

Each treatment was replicated 4 times and observations in stages of development were recorded periodically. The data was analyzed by comparing means using one way ANOVA and the significance was determined by Duncan's Multiple Range Test using

Table 1. Effect of BA and NAA on shoot regeneration and multiplication of Strawberry

Treatment (mgl^{-1})		Shoot number			Shoot length (cm)		
BA	NAA	Chandler	Oso-Grande	Cama-Rosa	Chandler	Oso-Grande	Cama-Rosa
1.0	0.5	3.4^{ab}±0.51	2^{a}±0.32	1.8^{ab}±0.37	3.26^{b}±0.33	2.32^{b}±0.12	1.84^{b}±0.15
1.0	0.1	4.2^{b}±0.58	2.2^{ab}±0.37	2.25^{ab}±0.43	4.44^{c}±0.23	4.42^{d}±0.10	3.64^{d}±0.10
1.5	0.5	6.6^{cd}±0.51	4^{cd}±0.45	2.8^{bc}±0.37	5.6^{d}±0.30	4.92^{e}±0.09	3.9^{d}±0.19
1.5	0.1	7.4^{cd}±0.60	5.2^{d}±0.66	3.6^{c}±0.51	7.82^{f}±0.27	6.26^{f}±0.19	5.08^{e}±0.09
2.0	0.5	10.4^{e}±0.51	7.8^{e}±0.66	7.2^{d}±0.37	10.18^{g}±0.49	8.3^{g}±0.15	7.18^{g}±0.05
2.0	0.1	7.6^{d}±0.51	5.4^{d}±0.51	2.8^{bc}±0.37	6.96^{e}±0.23	4.98^{e}±0.21	5.8^{f}±0.31
2.5	0.5	6^{c}±0.32	3.6^{bc}±0.51	2.4^{b}±0.51	5.34^{d}±0.15	4.48^{d}±0.12	3.82^{d}±0.12
2.5	0.1	4.4^{b}±0.51	3.2^{abc}±0.37	2.4^{b}±0.24	4.06^{c}±0.16	3.38^{c}±0.16	2.58^{c}±0.10
3.0	0.5	3.8^{b}±0.37	2.2^{ab}±0.37	1.6^{ab}±0.24	2.96^{ab}±0.16	2.18^{b}±0.05	1.72^{b}±0.13
3.5	0.1	2.2^{a}±0.37	2^{a}±0.32	1.2^{a}±0.20	2.38^{a}±0.13	1.48^{a}±0.20	1.22^{a}±0.09

Means followed by the same letter within the columns are not significantly different (P= 0.05) using Duncan's multiple range test.

Table 2. Effect of different IBA concentrations on root proliferation in Strawberry

Treatment (mgl^{-1})	Root number			Root length (cm)		
IBA	Chandler	Oso-Grande	Cama-Rosa	Chandler	Oso-Grande	Cama-Rosa
0.3	1.6^{a}±0.24	1.8^{a}±0.37	1.2^{a}±0.20	1.7^{ab}±0.12	1.68^{b}±0.06	1.42^{ab}±0.07
0.6	1.6^{e}±0.24	1.4^{a}±0.24	1.2^{a}±0.20	1.52^{a}±0.16	1.24^{a}±0.10	1.2^{a}±0.08
0.9	2.8^{c}±0.37	1.6^{a}±0.24	2.2^{bc}±0.20	3.36^{d}±0.36	2.28^{c}±0.09	1.66^{bc}±0.15
1.2	3.4^{a}±0.50	2.2^{ab}±0.37	1.6^{ab}±0.24	2.56^{c}±0.13	2.7^{de}±0.14	2.04^{c}±0.10
1.5	5.4^{c}±0.50	5.2^{de}±0.37	2.6^{c}±0.40	4.78^{e}±0.25	3.34^{e}±0.13	2.82^{d}±0.09
1.8	7.4^{b}±0.39	3.6^{c}±0.50	2.4^{bc}±0.24	5.76^{f}±0.26	5.16^{g}±0.24	4.64^{g}±0.14
2.0	10^{c}±0.70	6.4^{e}±0.67	5.2^{d}±0.37	6.38^{f}±0.18	4.02^{f}±0.15	4.16^{f}±0.25
2.3	5.8^{ab}±0.37	4.2^{cd}±0.52	1.8^{abc}±0.37	7.58^{g}±0.26	7.08^{h}±0.21	5.6^{h}±0.20
2.6	5.2^{ab}±0.37	3.4^{bc}±0.50	1.8^{abc}±0.37	4.86^{e}±0.35	4.4^{f}±0.11	3.52^{e}±0.16
2.9	2.8^{d}±0.73	2^{a}±0.31	1.6^{ab}±0.24	2.26^{bc}±0.10	1.94^{bc}±0.09	1.58^{ab}±0.14

Means followed by the same letter within the columns are not significantly different (P= 0.05) using Duncan's multiple range test.

SPSS for windows (v. 15. SPSS Inc USA).

RESULTS AND DISCUSSION

Shoot multiplication and elongation

Among the various combinations of plant growth regulators supplemented, the best response towards multiple shoot production in cv Chandler (10.4) followed by cv Oso-Grande (7.8) was observed on MS medium supplemented with 2 mgl^{-1} BA + 0.5 mgl^{-1} NAA. The highest shoot length (10.1 cm) was also recorded in cv Chandler on the same media. Analysis of variance (Table 1) revealed that treatments were highly significant for both number and length of shoots. Indra and Uppeandra (2000) reported multiple shoot regeneration from Indian wild strawberry using MS supplemented with 4.0 mgl^{-1} BA and 0.1 mgl^{-1} NAA. Some workers also reported shoot regeneration in strawberry using MS medium containing BA alone or in combination with Kin or NAA (Lis, 1990; Boxus, 1999; Neeru *et al.*, 2000; Mereti *et al.*, 2003, Biswas *et al.*, 2008, Biswas *et al.*, 2010). Singh and Pandey (2004) reported that shoot organogenesis varied from strawberry genotypes to genotypes. In present study however, low concentrations of BA alone or with NAA were found suitable for shoot multiplication and elongation.

Root multiplication and elongation

Maximum number of roots were observed in cv Chandler (10) followed by Oso-Grande (6.4) on MS medium supplemented with 2 mgl^{-1} IBA (Table 2). Also the length of roots was higher in cv Chandler (7.58 cm) followed by Oso-Grande (7.08 cm) on MS medium supplemented with 2.3 mgl^{-1} IBA (Table 2, Fig 1). Similar effects of IBA on rooting in strawberry were observed by Sakila *et al.,* 2007 and Bozena, 2001.

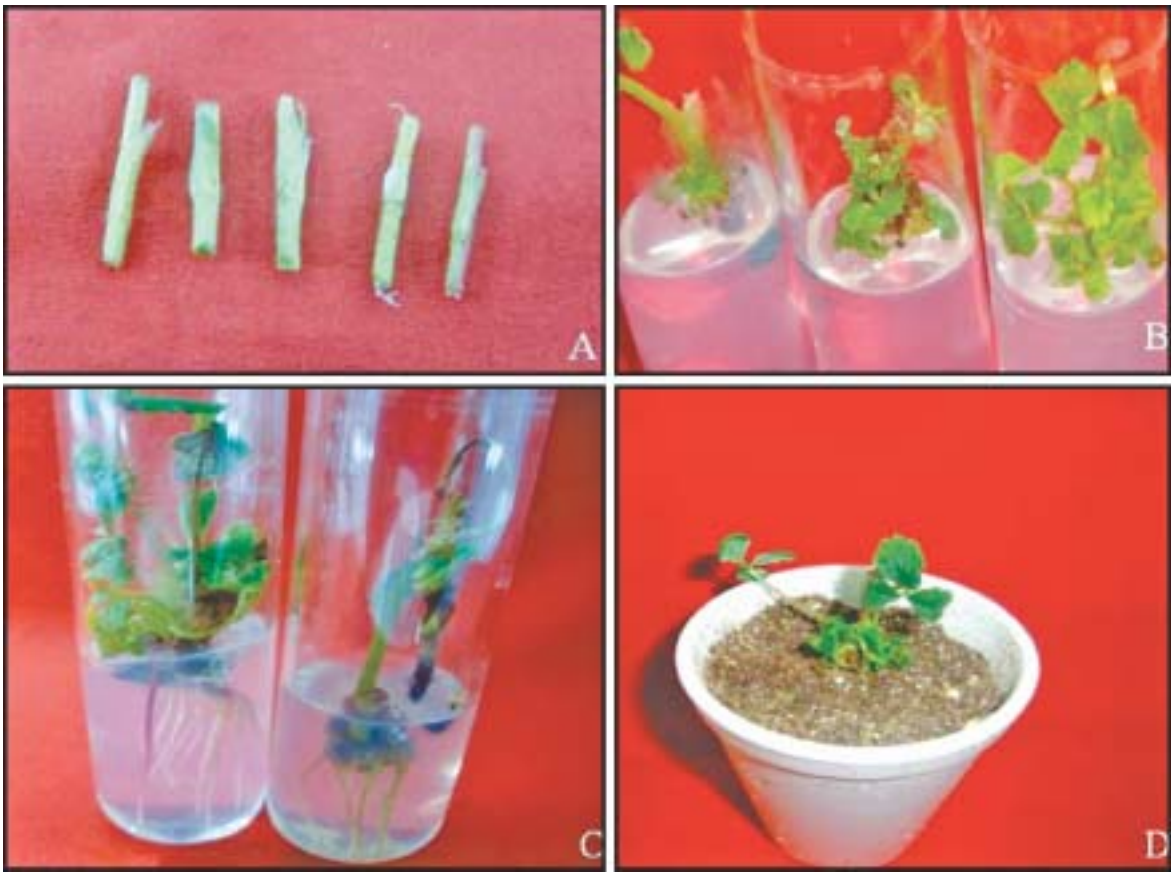

Fig 1: *In-vitro* propagation of strawberry (*Fragaria x ananassa Duch*)
A. Nodal segment as an explant
B. Shoot multiplication
C. Rooting
D. Hardening

Acclimatization of micro-propagated strawberry plants

After achieving the *in vitro* multiple plantlet regeneration, acclimatization of those plantlets is of utmost importance. Tissue culture derived plants can be directly transferred to small pots and allowed to raise on self system with manual water supply. Though it takes much more time to keep them in rooting medium, but the survival percentage reached up to 95-100% during the months of April-June (Koga *et al.*, 1999). Following the ideal hardening procedure, micropropagated plantlets were hardened in pots containing cocopeat: vermiculite: perlite (2:1:1). Hardening in February gives best result but planting in early April results in even more than a 95% survival rate (Kaur and Chopra 2004).

This study on *in vitro* mass multiplication of strawberry revealed that large number of high quality plant material can be achieved in a very short period of time. The planting material derived through tissue culture techniques using nodal segments as explant, show uniformity with respect to different quality parameters of the plant. Also the planting material will have the least chances of viral and other disease infestation.

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