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Effective decontamination and regeneration protocol for *in vitro* culture of strawberry cv. Chandler

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ABSTRACT

Nodal segments of strawberry cv. Chandler were collected from the field and surface sterilization of the explants was carried out using various levels of decontaminants as different treatments viz. sodium hypochlorite (NaClO) 0.5% for 7, 5 and 3 min and mercuric chloride ($HgCl_2$) 0.1% for 5 min, 3 min and 3 min 10 sec. The explants were excised and cultured for 3 weeks on the initiation medium supplemented with various levels of BAP (0.5, 1, 1.5 and 2mg/l) and its combination with GA (0.5 and 1mg/l). Sub culturing was done and finally rooting was initiated on the medium supplemented with various levels of IBA (0.5, 1, 1.5 and 2mg/l) for 4 weeks. The results obtained indicated that, among the decontaminants, treatment of explants with $HgCl_2$ 0.1% for 3 min 10 sec resulted in the minimum contamination and browning of explants. Further, maximum shoot proliferation percentage, shoots per explant and minimum number of days to shoot initiation was observed when explants were cultured on MS medium supplemented with 1.5 mg/l BAP over other concentrations of either BAP or in combination with GA. However, the maximum length of shoots was obtained in medium supplemented with 1.5 mg/l BAP + 0.5 mg/l GA. While, medium supplemented with 0.5 mg/l IBA supported highest percentage of rooting, the highest number of roots, maximum root length of micro-cuttings and minimum number of days to root initiation were observed in medium supplemented with 1mg/l of IBA.

Key words: Decontaminants, growth regulators, *in vitro*, nodal segments, strawberry

INTRODUCTION

Strawberry (*Fragaria ananassa* Duch) is propagated conventionally through runners but it is not feasible for mass multiplication. Alternative methods of propagation like micro-propagation have tremendous potential and attempts have been made for commercially exploitation (Boxus, 1974; Boxus *et al*, 1977). In the process of mass multiplication, the initial step of surface sterilization of explants is one of the most crucial factors in plant tissue culture. Contamination in plant cultures generally originates from explants, operators, laboratory environments or ineffective sterilization techniques. Microbes existed even among the healthy plants and their presence is often missed under field conditions, but the deleterious effects get expressed during *in vitro* conditions. Further, enzymatic browning at the initial establishment of *in vitro* culture is another primary cause failure of the cultures, leading to the death explants (Pirttilla *et al*, 2008; Zaid, 1984). Thus, establishment of a simple and effective disinfection protocol for escalating the survival of explants is imperative both for research and commercial production. The growth and development of explants *in vitro* is very much influenced by the addition of growth regulators to the basal medium for culture (Skoog and Miller, 1957). Morozova (2002) reported that high concentration of cytokinins (BA) required for strawberry micro-propagation, while Boxus (1999) suggested lower concentrations (0.5-1mg/l) of BA. Therefore, the present study was undertaken to develop an effective disinfection protocol for nodal segments of strawberry cv. Chandler obtained from field grown plants and assess the efficacies of various growth regulators at different levels for efficient *in vitro* culture of strawberry cv. Chandler.

MATERIAL AND METHODS

Explant selection and sterilization

Field grown runners (7-10cm size) obtained from open field (Block-I) of the ICAR-Indian Institute of Horticultural Research, Hesarghatta, Bengaluru, Karnataka,

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India, were used in the present studies. The leaves and root initials were trimmed off and nodal segments (1-2cm) were pretreated with a mixture of Bavistin (1.0%), CTAB (Cetyl Trimethyl Ammonium Bromide- 0.5%) and antibiotic formulation (streptomycin + tetracycline) 0.1% each for 90min. The explants were then washed off with all the residues using sterile distilled water three times. Thereafter, the explants were treated for 1 minute using 75% ethanol containing 2 drops of tween-20. Subsequently, the explants were treated with various surface sterilants at different concentrations and timings as per the treatments. The surface sterilants used were sodium hypochlorite (Na ClO) 0.5% for 7, 5 and 3 min and mercuric chloride ($HgCl_2$) 0.1% for 5 min, 3 min and 3 min 10 sec. After subsequent washing for six times, exposed ends were trimmed off, and the excised nodal explants of 1-1.5 cm size were cultured *in vitro* (Fig. 1).

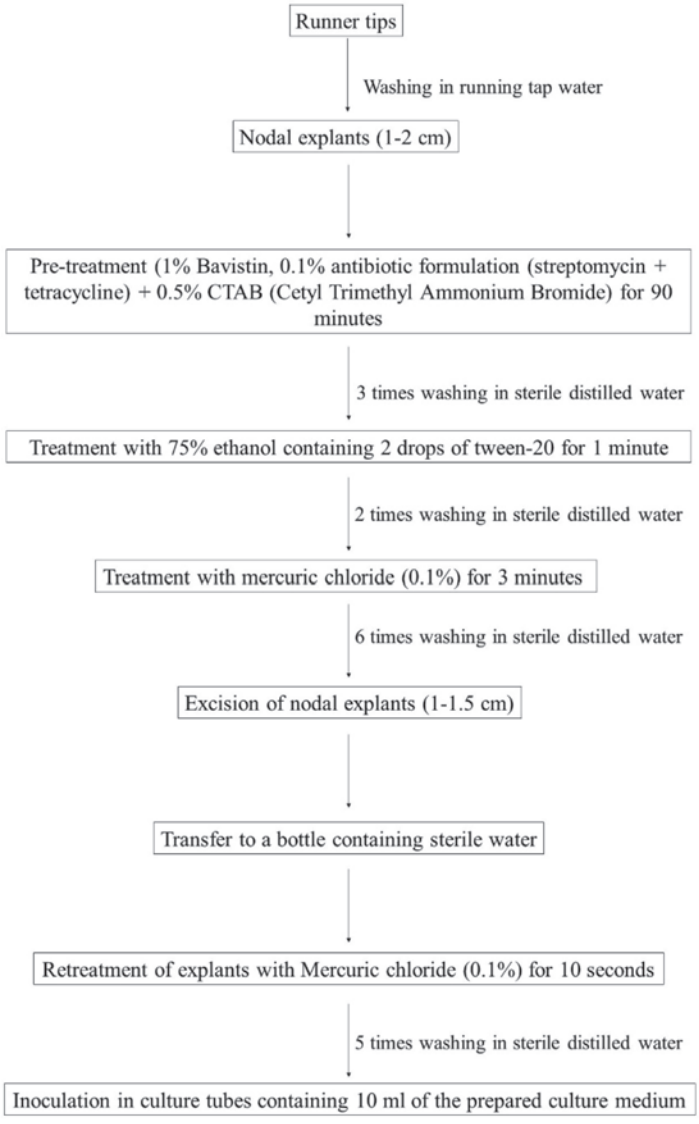

Fig. 1. Decontamination protocol of strawberry nodal explants *in vitro* culture.

Shoot proliferation and root initiation

Surface sterilized nodal segments were cultured on MS medium containing 0.5, 1.0, 1.5 or 2.0 mg/l of BAP. Another set of treatments consisting of 0.5 or 1 mg/l of GA in combination of the four concentrations of BAP mentioned above with were also tested. The cultures were examined regularly and the data on shoot proliferation was recorded after 3 weeks of culture. These shoots were used as micro-cuttings and sub-cultured on fresh medium for further shoot proliferation and root initiation. Growth characteristics such as percentage of explants showing shoot proliferation, days to shoot initiation, number of shoots per explant and average length of shoot were recorded. Further, the micro-cuttings obtained from the shoot induction treatments were transferred to MS media containing 0.5, 1.0, 1.5 and 2.0 mg/l of IBA and the percentage of rooting, number of days to root initiation, number of roots per micro-cutting and average root length were recorded in each treatment after 4 weeks of sub-culturing and the data were subjected to appropriate statistically analysis.

Hardening

Hardening of the rooted plantlets was carried out on the medium consisting of a mixture of sterilized sand, soil and cocopeat (1:1:2). One third of the plastic cups punched with drainage holes were filled with hardening media. After

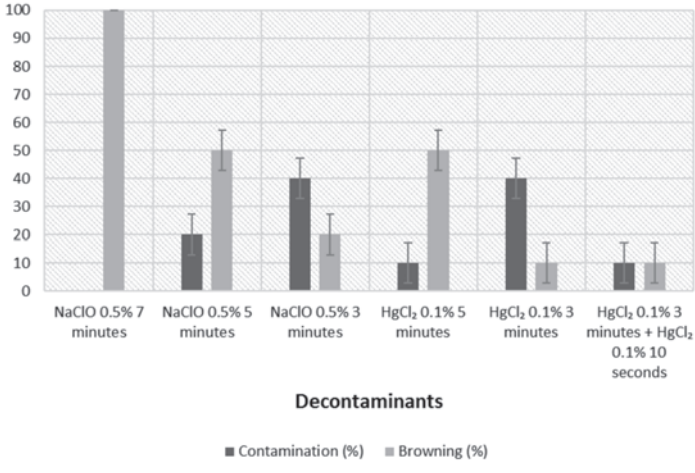

Fig. 2. Efficacy of decontaminants in reducing the contamination and browning of explants

the medium was saturated with water to field capacity, the plantlets were transplanted in to plastic cups after thorough washing and finally the cups were covered with an inverted punched transparent cups to maintain humidity and placed under cool fluorescent light in the culture room. After one week, the covered plastic cups covers were removed to permit further hardening, and after one month, hardened plantlets were relocated to glasshouse conditions. Such hardened plantlets of two months old were ready for transfer to the field.

Statistical analysis

Analysis of variance was carried out using Completely Randomized Design (CRD) using SPSS statistics version 17.0. For each of the treatment combinations, ten replications were maintained and the test for significance (*p* d"0.05) was conducted among different surface sterilants and plant growth regulators treatments.

RESULTS AND DISCUSSION

The efficacy of various decontaminants in reducing the contamination of explants of strawberry cv. Chandler for *in vitro* culture was evaluated. The data on percentage of contamination showed significant differences among the surface sterilants, and it was observed that explants treated with Na ClO 0.5% for 7 min resulted in zero contamination, but the browning percentage was highest. Treatment of explants with $HgCl_2$ 0.1% 3 min 10 sec gave the minimum percentage of contamination and browning compared to other treatment combinations. The best decontamination protocol suggested by Ying Ko *et al* (2009) using sodium hypochlorite (0.5%) for 7 min, Biswas *et al* (2007) with 0.1% $HgCl_2$ for 5 min and Sharma *et al* (2009) with 0.1% $HgCl_2$ for 3 min were also tried in the present study but of little help in overcoming contamination. Since, the decontamination treatment identified in the present study worked efficiently with the explants collected from the open field, it saves the cost for installation of protected structure for raising plants as source of explants, thereby increasing the economic return.

Significant differences in shoot proliferation of strawberry cv. Chandler to *in vitro* culture were observed under the influence of growth regulator treatments (Table 1). It was observed that medium supplemented with 1.5mg/l BAP resulted in the highest percentage of shoot proliferation and minimum number of days to shoot initiation. Maximum number of shoots per explant was obtained on medium supplemented with 1.5mg/l BAP and was significantly higher than the other concentrations of BAP alone or in combinations with GA. The maximum number of days taken for shoot initiation and minimum number of

Table 1. Influence of plant growth regulators on the number of days to shoot initiation, number of shoots per explant and shoot length of nodal segments of strawberry cv. Chandler

Plant growth regulators (mg/l)	Days to shoot initiation	Number of shoots per explant	Shoot length (cm)
Control	10.50±0.45	2.30±0.21	1.06±0.09
BAP 0.5	9.20±0.47	3.60±0.16	1.63±0.10
BAP1	8.90±0.43	5.00±0.26	2.42±0.14
BAP 1.5	8.00±0.26	6.30±0.15	3.47±0.10
BAP 2	8.90±0.41	5.00±0.21	2.72±0.06
BAP 0.5 + GA 0.5	9.50±0.22	3.50±0.17	3.10±0.07
BAP 0.5 + GA 1	9.40±0.43	3.70±0.15	3.64±0.07
BAP1 + GA 0.5	9.10±0.41	4.10±0.23	4.00±0.06
BAP 1 + GA 1	8.50±0.45	4.50±0.17	4.43±0.07
BAP 1.5 + GA 0.5	8.00±0.26	5.50±0.17	5.20±0.05
BAP 1.5 + GA 1	8.40±0.48	4.80±0.20	4.77±0.07
BAP 2 + GA 0.5	9.20±0.36	4.20±0.20	4.10±0.04
BAP 2 + GA 1	9.90±0.35	3.60±0.16	3.66±0.07
CD *(P=0.05)*	1.10	0.54	0.22

Table 2. Rooting responses of micro-cuttings of strawberry cv. Chandler under the influence of various concentrations of IBA

IBA concentrations (mg/L)	Days to root formation	Number of roots per micro-cutting	Root length (cm)
IBA 0	15.40±0.34	1.80±0.13	1.07±0.09
IBA 0.5	12.00±0.47	3.60±0.27	2.20±0.08
IBA 1	9.30±0.21	5.50±0.22	3.47±0.11
IBA 1.5	9.40±0.22	4.40±0.22	2.97±0.08
IBA 2	10.70±0.33	3.60±0.27	2.18±0.07
CD *(P=0.05)*	0.94	0.65	0.25

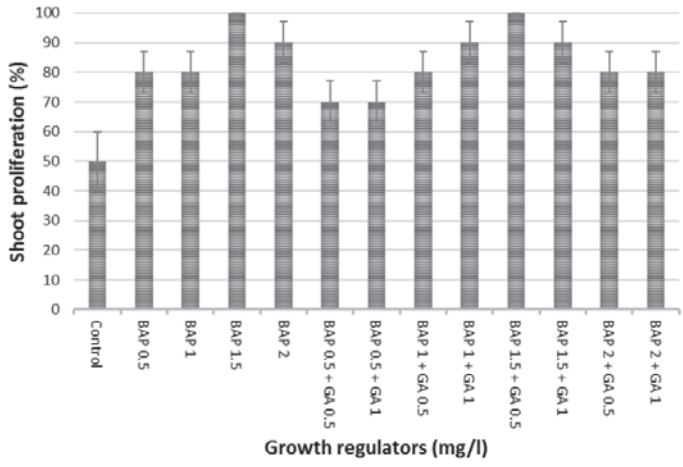

Fig. 3. Effect of plant growth regulators on shoot proliferation percentage of explants of strawberry cv. Chandler

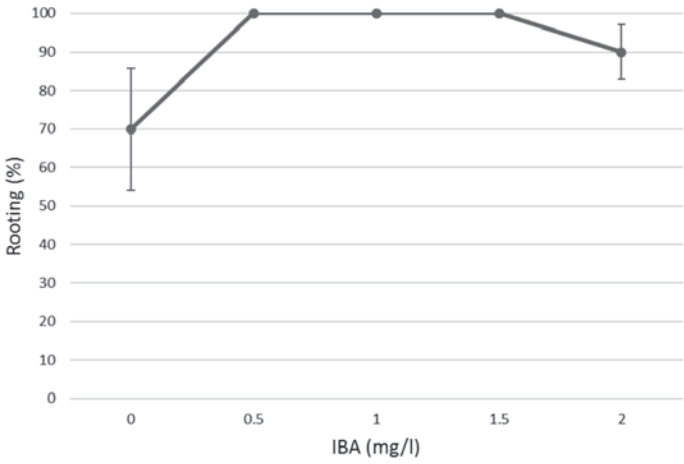

Fig. 4. Influence of IBA on rooting percentage in strawberry cv. Chandler

shoots per explant were recorded in basal MS medium. Biswas *et al* (2008) however reported that 0.5 mg/l BAP in MS medium gave the best response for multiple shoot induction. The higher requirement of concentration for shoot proliferation in the present study could be attributed to genotypic differences as genotypes responds differently *in vitro* (Passey *et al*, 2003). Supplementation of GA to the BAP containing medium did not improve the rate of shoot proliferation and is in accordance with the reports by Sakila *et al* (2007). However, the medium supplemented with 1.5mg/l BAP + 0.5mg/l GA exhibited the highest shoot length in explants in the present investigation and the minimum length of proliferated shoots were observed in basal MS medium thus GA contributed to elongation of proliferated shoots and again similar observation was reported by Sakila *et al* (2007).

On perusal of data, it was found that 0.5mg/l IBA when supplied to the medium resulted in the highest response to *in vitro* rooting reflected in highest percentage of rooting. The lowest percentage of rooting in micro-cuttings was observed on basal MS medium (Fig. 4). However, the minimum number of days taken to root formation was observed in medium supplemented with 1 mg/l IBA and this treatment also resulted in highest number of roots per micro-cutting. Maximum number of days taken for rooting and least number of roots per explant were observed in basal MS medium and interestingly, the average root length of micro-cuttings also recorded maximum in 1mg/l IBA, which was significantly superior to other IBA concentrations. Overall, results indicated that supplementation of 1mg/l to the media resulted in the highest rooting responses in micro-cuttings of strawberry cv. Chandler. Similar results have been obtained by Hemant *et al* (2001), Ritu *et al* (2001), Sakila *et al* (2007) on other cultivars of strawberry and Mante *et al*, 1989 in *Prunus* sp.

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