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MICROPROPAGATION OF STRAWBERRY (*FRAGARIA X ANANASSA* DUCH.)

Murari Lal, Suneel Sharma and M. V. Hegde

Department of Horticulture,
CCS Haryana Agricultural University, Hisar - 125 004, India

ABSTRACT

Efficient methods for shoot regeneration, proliferation and rooting from runner tip explants of strawberry (*Fragaria X ananassa*) cultivars Chandler, Oso Grande and Ofra were developed. After seven weeks of incubation maximum regeneration of 100 per cent regeneration was recorded in cv. Ofra on MS medium with 4 mg l^{-1} BAP. On Knop's medium maximum regeneration (70.0 %) was observed on K_4 (Knop's + 4.0 mg l^{-1} IBA + 0.4 mg l^{-1} BAP + 0.4 mg l^{-1} GA_3) medium. In all cultivars maximum mean number of shoots per explant was observed in M_9 medium (MS + 4.0 mg l^{-1} BAP). Among cultivars maximum mean number of multiple shoots (13.8±3.6) and range of shoots per explant (4-36) was observed in Ofra cultivar. Maximum rooting (100 %) was observed on MS media (Full or half strength of MS basal salts) with 1.0 mg l^{-1} IBA. Whereas, in case of Knop's based media maximum rooting of 60.0 per cent was observed on K_4 medium.

INTRODUCTION

Commercial scale propagation by runners has been widely used in the strawberry industry. However, plants raised through runners suffer from viral diseases. Micropropagation is a tool in this direction to have true to type, disease free and quality plants throughout the year. The effectiveness of meristem culture in producing virus free material is reported to vary among the cultivars (Popova *et al.*, 1985). Plants have been regenerated from meristematic callus (Jones *et al.*, 1988), anthers (Laneri and Damiano, 1980) and immature embryos (Wang *et. al.*, 1984). Methods have also been developed for efficient shoot regeneration from leaf and runner tissues (Liu and Sanford, 1988). Presently, availability of good quality planting material of a required cultivar in a large quantity is a major limitation in expansion of strawberry cultivation in northern India and considering these problems the study was planned to develop an efficient protocol for plant regeneration in strawberry (*Fragaria X ananassa* Duch.).

MATERIAL AND METHODS

The present investigation was carried out in tissue culture laboratory of Department of Horticulture, CCS Haryana Agricultural University, Hisar, India. The runner tips (2-5 mm in length) of disease free plants of cvs. Chandler, Oso Grande and Ofra were excised during the month of October from the field grown plants. Explants were first washed under running tap water, and thereafter surface sterilized with 70 per cent aqueous ethanol (v/v) for 1-2 minute followed by 0.1 per cent aqueous solution of $HgCl_2$ (w/v) for 2-10 minutes. Finally explants were thoroughly washed in sterilized double distilled water and cultured aseptically on two basal media *viz.*, Murashige and Skoog (MS) media (1962) and Knop's media (1865) with some modifications used for plant regeneration and rooting. The cultures were incubated under fluorescent white light and dark cycle of 16hr/8hr at 25±2°C. The sub culturing was done at every 20-25 days interval to promote shoot proliferation. Visual observations were recorded after 7 weeks of culturing for shoot regeneration and after 2 weeks for rooting.

RESULTS AND DISCUSSION

Shoot regeneration : Results of shoot regeneration from runner tip explants observed after seven weeks in all the three cultivars tested are summarised in Table 1. Maximum

Table 1. Effect of media on shoot regeneration from runner tip explants in different cultivars of strawberry (after 7 weeks of incubation)

Media	Per cent regeneration		
	Chandler	Oso Grande	Ofra
M_5	66.6	70.0	70.0
M_6	70.0	75.0	75.0
M_7	75.0	77.0	80.0
M_8	80.0	83.0	90.0
M_9	91.0	91.7	100.0
K_1	40.0	30.0	30.0
K_2	60.0	40.0	40.0
K_3	60.0	60.0	50.0
K_4	70.0	60.0	50.0

M_5=MS + 0.4 mgl^{-1} BAP
M_6=MS + 1.0 mgl^{-1} BAP
M_7=MS + 2.0 mgl^{-1} BAP
M_8=MS + 3.0 mgl^{-1} BAP
M_9=MS + 4.0 mgl^{-1} BAP

K_1=KNOP + 1.0 mgl^{-1} IBA + 0.1 mgl^{-1} BAP + 0.1 mgl^{-1} GA_3
K_2=KNOP + 2.0 IBA + 0.2 BAP + 0.2 GA_3
K_3=KNOP + 3.0 IBA + 0.3 BAP + 0.3 GA_3
K_4=KNOP + 4.0 IBA + 0.4 BAP + 0.4 GA_3

regeneration frequency was observed on M_9 medium (100%) in cv. Ofra followed by Oso Grande (91.7 %) and Chandler (91.0 %). Minimum regeneration frequency (66.6 %) was observed in cultivar Chandler on M_5 medium (MS+0.4 mgl^{-1} BAP). In case of media based on Knop's basic media, the maximum shoot regeneration (70 %) was recorded on K_4 medium in cv. Chandler followed by K_3 medium with 60, 60, and 50 per cent shoot regeneration in the three cvs. Chandler, Oso Grande and Ofra, respectively and minimum number of shoot regeneration was recorded on K_1 medium (Knop's+1.0 mg l^{-1} IBA+0.1 mg l^{-1} GA_3) in cvs. Oso Grande and Ofra.

Runner tip explants exhibited variable response for shoot formation in various media tested. Runner tip explants after 7 weeks of culture revealed maximum regeneration potential on M_9 medium (MS+4.0 mg l^{-1} BAP). In media based on Knop's basal medium plant regeneration increased with the increased concentrations of IBA, BAP and GA_3. Media formulations based on MS or Knop's medium are most oftenly used for culturing of meristem explants. However, best results with MS based media formulations have been reported by several workers (Anderson and Hanner, 1978; Boxus *et al.*, 1977 and Damiano, 1980)

Cultivar differences can be critical in the success of micropropagation (Mullin *et al.*, 1974 and Boxus, 1981). However, in present study on shoot regeneration, no differences were observed among the cvs. Chandler, Oso Grande and Ofra when cultured on MS based formulations whereas slight cultivar differences in shoot regeneration on the Knop's based media formulations were observed.

Shoot proliferation: Shoot proliferation from runner tip explants was observed after seven week on all media tested (Table 2). Solitary as well as multiple number of shoots were observed on MS based media. However, only solitary shoots per explant were observed on various combinations of Knop's media. In all cvs. maximum mean number of shoots per explant was observed in M_9 medium (MS+4.0 mg l^{-1} BAP) and minimum was in M_5 medium (MS+0.4 mg l^{-1} BAP). Among cultivars maximum mean number (13.8$\pm$3.6) and range of shoots per explant (4-36) was observed in cv. Ofra on M_9 medium.

In the present investigation, with the increase in concentration of BAP in MS based media, there was linear increase in number of

Table 2. Effect of media on shoot proliferation of regenerated plantlets from runnertip explant in different cultivars of strawberry (after 7 days of incubation)

Media	Shoots per explants					
	Chandler		Oso Grande		Ofra	
	Range	Mean	Range	Mean	Range	Mean
M_5	1-2	1.2±0.2	1-2	1.3±0.2	1-2	1.3±0.2
M_6	1-2	1.3±0.2	1-2	1.4±0.2	1-2	1.5±0.2
M_7	4-12	7.1±1.2	4-16	8.9±1.6	4-16	9.4±1.2
M_8	4-16	9.1±1.5	4-20	10.4±2.2	4-25	11.6±2.4
M_9	4-20	10.1±1.9	4-30	12.9±3.2	4-36	13.8±3.6

Table 3. Effect of media on rooting of regenerated plantlets derived from runnertip explant in strawberry cultivars (after 2 weeks of incubation)

Media	Per cent shoots rooted	Rooting intensity
R_1	100	+
R_2	100	+++
K_1	33.3	+
K_2	46.7	++
K_3	56.7	++
K_4	60.0	+++

+ = Low ; ++ = Moderate ; +++ = High

K_1=KNOP + 1.0 mgl^{-1} IBA + 0.1 mgl^{-1} + BAP + 0.1 mgl^{-1} GA_3
K_2=KNOP + 2.0 IBA + 0.2 BAP + 0.2 GA_3
K_3=KNOP + 3.0 IBA + 0.3 BAP + 0.3 GA_3
K_4=KNOP + 4.0 IBA + 0.4 BAP + 0.4 GA_3
R_1=MS + 1.0 mgl^{-1} IBA
R_2=1/2 MS + 1.0 mg l^{-1} IBA

shoots developed per explant (Table 2). With BAP at 4 mg l^{-1} concentration the maximum number of shoots i.e., 10.1, 12.9 and 13.8 in the cvs. Chandler, Oso Grande and Ofra, respectively was observed. It was contrary with the findings of James and Newton (1977) and Hunter *et al.* (1984). James and Newton (1977) showed that the best BAP concentration for axillary branching in strawberry ranged between 0.05 to 0.5 mg l^{-1} whereas Hunter *et al.* (1984) reported the concentration of 0.1 mg l^{-1} to be the optimum for multiple shoot development in strawberry. The higher requirement of BAP above 0.4 mg l^{-1} observed in the present study may probably be due to the genotypic variation and lower levels of endogenous cytokinins in the source plant material.

Rooting : Hundred per cent rooting was observed on R_1 and R_2 media in all the cultivars (Table 3). In case of Knop's based media maximum rooting of 60.0 per cent was observed in K_4 medium followed by K_3 (56.7 %) and K_2 (46.7 %) media. Low, and high intensity of root growth was observed on R_1 and R_2 media, respectively. The intensity of rooting in Knop's media varied from high in K_4 medium to moderate in K_2, K_3 and low in K_1 media. Hundred per cent shoot stimulated roots in R_1 and R_2 media. Whereas high percentage rooting was observed in half strength MS medium (R_2) indicating that 50 per cent reduction of major salts helps in promoting the number and length of roots thereby increasing the root intensity. Hunter *et al.* (1984) suggested that for root formation the optimum concentration being in the range of 0.75 to 1.0 mg l^{-1}.

Hundred per cent survival of plantlets

in the pots containing soil medium consisting of 1:1 field soil and river sand was observed in the plantlets raised from irrespective of media used.

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