*Indian J. Plant Physiol.*, Vol. 11, No. 2, (N.S.) pp. 136-144 (April-June, 2006)

# ESTABLISHMENT OF CALLUS CULTURES AND PLANT REGENERATION IN STRAWBERRY (*FRAGARIA* x *ANANASSA* DUCH.) CV. CHANDLER

**KANIKA KAUSHAL, AMARJIT K. NATH* AND D.R. SHARMA**

Department of Biotechnology, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan-173230 (HP).

Received on 13 April 2006, Revised on 12 June, 2006

## SUMMARY

**Leaf, petiole and stipule explants of strawberry were treated for the establishment of callus cultures, but callus initiation could be obtained only in leaf and petiole explants. The maximum callus induction percentage ( 88.89%) was recorded in MS medium containing 0.5 mg/l BAP + 0.75 mg/ 1 NAA. Callus obtained from both leaf and petiole explants was light green, friable and compact. Regeneration was difficult to achieve. A total of 93 different combinations of growth regulators were tried. Callus differentiated into shoots after transfer to MS medium supplemented with 2 mg/1 BAP+ 0.25 mg/l NAA + 0.5 mg/l kinetin. The regeneration percentage was higher in petiolar callus (83.33%) as compared with the leaf-derived callus (81.67%). Well developed shoots in both leaf and petiolar calli obtained after 50-55 days were transferred to multiplication medium containing 0.5 mg/l BAP + 0.5 mg/l kinetin and 1.0 mg/l $GA_3$. Rooting was done in MS half strength + 1.0 mg/l IBA and 0.2 mg/l activated charcoal. Once the root system was fully developed, the plantlets after washing were kept in 0.5% bavistin for 15-20 minutes and were planted in pre-sterilised mixture of soil : FYM (1:1) filled to two third of the capacity and rest filled with sterilised sand. Plantlets were hardened and then transferred to field.**

***Key words:*** **Callus cultures, growth regulator, micropropagation, plant regeneration, strawberry.**

## INTRODUCTION

Strawberry (*Fragaria* x *ananassa* Duch), a fruit of family Rosaceae, occupies an important place among the small fruits. The cultivated strawberry of today is a natural interspecific hybrid between the two octoploid (2n=56) species, i.e. *F. virginiana* and *F. chiloensis*. *Fragaria* x *ananassa* is perhaps the only fruit in which all the cultivars have been evolved by man through hybridization. Compared to many cultivated crops, its origin is relatively recent dating to 18$^{th}$ century. Years for selection of adaptation to cultivated environment has reduced genetic diversity within the cultivated strawberry (Dale and Sjulin 1990, Luby *et al*. 1991). A narrow genetic base can be associated with inbreeding depression and vulnerability for diseases, pests and stresses (Luby and Stahler 1993). Therefore, the breeders are interested in expanding the narrow genetic base of this fruit crop.

In recent years, the developments for plant regeneration from tissue culture have given breeders new tools to increase the speed and efficiency of the breeding process. They also provide new methods to improve accessibility of the existing germplasm to create new variation for crop improvement. Somaclonal variation is often more frequent among the regenerants from callus and suspension culture as compared to meristem and shoot apex culture (Gould, 1986). Keeping in view the above fact, present studies were aimed to develop a

*Corresponding author, E.mail : amarjitnath@yahoo.com

reproducible protocol for plant regeneration in strawberry (cv. Chandler).

## MATERIALS AND METHODS

The plants of strawberry (*Fragaria* x *ananassa* Duch) cv. Chandler were obtained from the Department of Fruit Breeding and Genetic Resources, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP). In order to maintain supply of explants (leaf, petiole and stipules) for callus cultures, the vegetative buds were established *in vitro*. Shoot buds were excised with the help of sharp scalpel blade from thoroughly washed plants and collected in a beaker containing water. The axillary buds were washed 3-5 times under running tap water, treated with Tween-20 (2-3 drops/100 ml of $H_2O$) for 4-5 minutes and washed again with distilled water. Henceforth, the manipulation was carried out in the laminar flow chamber. The washed buds were treated with bavistin (0.5%) for 5-6 minutes, then rinsed with sterilized distilled water and treated with $HgCl_2$ (0.1%) for 2-3 minutes. After further washing with sterile distilled water the buds were inoculated on the establishment medium (MS + BAP 0.1 mg/l + kin 0.5 mg/l + $GA_3$ 2.0 mg/l). Leaf discs (0.3-0.5 cm) from young, fully expanded leaves, petiolar sections (0.75- 1.0 cm) and stipules were cut from actively growing *in vitro* shoots and were inoculated on the MS medium supplemented with various concentrations and combinations of BAP (0-3 mg/l), NAA (0-1.0 mg/l) and 2,4-D (0-1.10 mg/l). After inoculation of the explants, the cultures were incubated at 25± 2° C in dark for 20 days and were subsequently exposed to 16 hours photoperiod.

The actively growing callus from the initial explants was separated and sub cultured onto fresh medium. Visual observations with respect to growth, type and colour of callus were recorded. On the basis of these observations, the callus was again subcultured onto the medium giving best results for further multiplication. The callus was allowed to grow for 60 days then half of it was further subcultured on to the fresh callus multiplication medium and rest was transferred to the shoot regeneration medium.

In order to optimize the medium for shoot regeneration, the calli were divided into small pieces (1.0–1.5 cm diameter) and cultured on MS basal and MS modified medium containing various combinations and concentrations of growth regulators, i.e. BAP (0- 0.5 mg/l), NAA (0-0.5 mg/l), Kin (0-0.5 mg/l), 2,4-D (0-1.0 mg/l), IBA (0- 1.0 mg/l) and IAA (0-0.5 mg/l). The regenerated shoots were then multiplied on standardized MS medium containing BAP (0.5 mg/l), kin (0.5 mg/l) and $GA_3$ (1.0 mg/l).

The multiplied shoots of size 2.5– 3.0 cm were transferred singly to culture tube each containing about 15 ml of rooting medium. The standardized rooting medium (Kaushal 2002) used was half strength MS basal medium supplemented with IBA (0.5 mg/l) and charcoal (0.2 g/l). Once the root system was fully developed (after 5 weeks), the plantlets were taken out of the culture tube, washed thoroughly and kept in 0.5% bavistin for 15-20 minutes. They were then transferred to the plastic pots containing pre sterilized mixture of soil: FYM 1:1 filled to two third of the capacity and rest filled with sand. The plantlets were watered and covered with jam bottles to maintain high humidity. Water was gently sprayed regularly over the plants and per cent survival was recorded.

## RESULTS

Initially, the explants inoculated on to various callus induction media exhibited browning, which was controlled to a great extent in leaf and petiole explants by frequent transfer to the fresh medium. The stipule explants turned brown and died after 2-3 days of inoculation. Therefore, in the present investigations, only leaf and petiole explants were used for the induction of callus. Time taken for the initiation of callus in both cultures was about 20-25 days. Effects of various concentrations and combinations and of growth regulators on the percentage callus induction, are presented in Table 1 (Fig. 1.1b, 1.2 b). The maximum callus induction was obtained in MS medium + BAP 0.5 mg/l + NAA 0.75 mg/l, i.e. 88.89% followed by 68.89% in MS medium + BAP 1.0 mg/l + IAA 0.9 mg/l. The per cent response of leaf explants (48.38%) was significantly higher than the petiole explants (29.63%) for the induction of callus.

**Table 1.** Effect of different concentrations and combinations of growth regulators on callus induction from the leaf and petiole explants of strawberry cv. Chandler.

| Medium | Medium composition MS supplemented with | | | Callus induction (%) | | Mean |
|---|---|---|---|---|---|---|
| | BAP (mg $l^{-1}$) | NAA (mg $l^{-1}$) | 2,4-D (mg $l^{-1}$) | Leaf explant | Petiole explant | |
| CIM1 | - | - | - | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| CIM2 | 0.25 | 0.25 | - | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| CIM3 | 0.25 | 0.50 | - | 51.11 (45.64) | 37.78 (37.87) | 44.44 (41.76) |
| CIM4 | 0.25 | 0.75 | - | 57.78 (49.64) | 42.22 (40.52) | 50.00 (45.08) |
| CIM5 | 0.25 | 1.00 | - | 44.44 (41.79) | 24.45 (29.58) | 34.44 (35.69) |
| CIM6 | 0.25 | 1.50 | - | 62.22 (52.09) | 26.67 (30.97) | 44.44 (41.53) |
| CIM7 | 0.50 | 0.25 | - | 53.33 (46.92) | 0.00 (0.00) | 26.67 (23.46) |
| CIM8 | 0.50 | 0.50 | - | 62.22 (52.09) | 0.00 (0.00) | 31.11 (26.05) |
| CIM9 | 0.50 | 0.75 | - | 95.55 (80.02) | 82.22 (65.36) | 88.89 (72.69) |
| CIM10 | 0.50 | 1.00 | - | 66.67 (54.81) | 0.00 (0.00) | 33.34 (27.41) |
| CIM11 | 0.75 | 0.25 | - | 53.32 (46.91) | 0.00 (0.00) | 26.66 (23.46) |
| CIM12 | 1.00 | 0.90 | - | 86.67 (69.02) | 51.11 (45.69) | 68.89 (57.35) |
| CIM13 | 2.00 | 1.00 | - | 66.67 (54.81) | 48.89 (44.36) | 57.78 (49.59) |
| CIM14 | 3.00 | 1.00 | - | 53.33 (46.92) | 0.00 (0.00) | 26.67 (23.46) |
| CIM15 | 0.50 | - | 0.50 | 20.00 (26.36) | 0.00 (0.00) | 10.00 (13.18) |
| CIM16 | 0.75 | - | 0.75 | 53.12 (46.84) | 84.43 (67.29) | 68.78 (57.07) |
| CIM17 | 1.00 | - | 1.00 | 44.65 (41.81) | 64.44 (53.48) | 54.44 (47.67) |
| CIM18 | 1.10 | - | 1.10 | 0.00 (0.00) | 71.22 (57.53) | 35.56 (28.76) |
| Mean | | | | 48.38 (0.00) | 29.63 (26.26) | |
| | | | | Medium | Explant | Medium x Explant |
| SE± | | | | 2.38 | 0.79 | 3.37 |
| CD at 5% | | | | 4.75 | 1.58 | 6.77 |

Figures in parentheses are arc sine transformed values

To obtain shoot regeneration the callus pieces were cut to a size of 1.0 – 1.5 cm and transferred to various combinations and concentrations of growth regulators added to MS basal and modified MS medium. The regeneration was very difficult to achieve as the calli turned brown and died when transferred to regeneration media. A total of 93 different concentrations and combinations of growth regulators added to MS basal and MS modified medium (containing $B_5$ vitamins) were tried for shoot regeneration medium (Table 2). About 18 different combinations of BAP and NAA were used. None of the combinations resulted in shoot regeneration. The callus kept multiplying initially in all the concentrations, but at the higher concentration of BAP

**Table 2.** Different concentration and combinations of growth regulators used for shoot regeneration in strawberry cv. Chandler.

| Medium | Medium composition (MS supplemented with) | | | | | |
|---|---|---|---|---|---|---|
| | BAP ($mgl^{-1}$) | NAA ($mgl^{-1}$) | Kinetin ($mgl^{-1}$) | IBA ($mgl^{-1}$) | IAA ($mgl^{-1}$) | 2,4-D ($mgl^{-1}$) |
| SRM1 | 0 | 0 | - | - | - | - |
| SRM2 | 0.75 | 0.25 | - | - | - | - |
| SRM3 | 2.25 | 0.18 | - | - | - | - |
| SRM4 | 1 | 0 | - | - | - | - |
| SRM5 | 2 | 0 | - | - | - | - |
| SRM6 | 3 | 0 | - | - | - | - |
| SRM7 | 4 | 0 | - | - | - | - |
| SRM8 | 5 | 0 | - | - | - | - |
| SRM9 | 1 | 0.1 | - | - | - | - |
| SRM10 | 2 | 0.1 | - | - | - | - |
| SRM11 | 3 | 0.1 | - | - | - | - |
| SRM12 | 4 | 0.1 | - | - | - | - |
| SRM13 | 5 | 0.1 | - | - | - | - |
| SRM14 | 1 | 0.5 | - | - | - | - |
| SRM15 | 2 | 0.5 | - | - | - | - |
| SRM16 | 3 | 0.5 | - | - | - | - |
| SRM17 | 4 | 0.5 | - | - | - | - |
| SRM18 | 5 | 0.5 | - | - | - | - |
| SRM19 | 1 | 0.25 | 0.5 | - | - | - |
| SRM20 | 2 | 0.25 | 0.5 | - | - | - |
| SRM21 | 1 | 0.5 | 0.5 | - | - | - |
| SRM22 | 2 | 0.5 | 0.5 | - | - | - |
| SRM23 | 1 | - | 0.1 | - | - | - |
| SRM24 | 2 | - | 0.1 | - | - | - |
| SRM25 | 3 | - | 0.1 | - | - | - |
| SRM26 | 2.5 | - | - | - | - | 0.1 |
| SRM27 | 5 | - | - | - | - | 1 |
| SRM28 | 2.5 | - | - | 0.5 | - | - |
| SRM29 | 1 | - | - | 0.5 | - | - |
| SRM30 | 1.5 | - | - | 0.5 | - | - |
| SRM31 | 2 | - | - | 0.5 | - | - |
| SRM32 | 2.5 | - | - | 0.5 | - | - |
| SRM33 | 3 | - | - | 0.5 | - | - |
| SRM34 | 3.5 | - | - | 0.5 | - | - |
| SRM35 | 4 | - | - | 0.5 | - | - |

| Medium | Medium composition (MS supplemented with) | | | | | |
|---|---|---|---|---|---|---|
| | BAP ($mgl^{-1}$) | NAA ($mgl^{-1}$) | Kinetin ($mgl^{-1}$) | IBA ($mgl^{-1}$) | IAA ($mgl^{-1}$) | 2,4-D ($mgl^{-1}$) |
| SRM36 | 4.5 | - | - | 0.5 | - | - |
| SRM37 | 5 | - | - | 0.5 | - | - |
| SRM38 | 1 | - | - | 1 | - | - |
| SRM39 | 2 | - | - | 1 | - | - |
| SRM40 | 3 | - | - | 1 | - | - |
| SRM41 | 4 | - | - | 1 | - | - |
| SRM42 | 5 | - | - | 1 | - | - |
| SRM43 | 1 | - | - | 0.2 | - | - |
| SRM44 | 2 | - | - | 0.2 | - | - |
| SRM45 | 3 | - | - | 0.2 | - | - |
| SRM46 | 4 | - | - | 0.2 | - | - |
| SRM47 | 5 | - | - | 0.2 | - | - |
| SRM48 | 1 | - | - | - | 0.1 | - |
| SRM49 | 2 | - | - | - | 0.1 | - |
| SRM50 | 3 | - | - | - | 0.1 | - |
| SRM51 | 4 | - | - | - | 0.1 | - |
| SRM52 | 5 | - | - | - | 0.1 | - |
| SRM53 | 1 | - | - | - | 0.5 | - |
| SRM54 | 2 | - | - | - | 0.5 | - |
| SRM55 | 3 | - | - | - | 0.5 | - |
| SRM56 | 4 | - | - | - | 0.5 | - |
| SRM57 | 5 | - | - | - | 0.5 | - |

| Medium | Medium composition (MS supplemented with) | | | | |
|---|---|---|---|---|---|
| | BAP ($mgl^{-1}$) | NAA ($mgl^{-1}$) | Vitamins ($mgl^{-1}$) | Casein hydrolysate (400 mg $l^{-1}$) | Activated charcoal (g $l^{-1}$) |
| SRM58 | 0.75 | 0.25 | $B_5$ Vitamins | - | - |
| SRM59 | 1 | 0.25 | $B_5$ Vitamins | - | - |
| SRM60 | 2 | 0.25 | $B_5$ Vitamins | - | - |
| SRM61 | 3 | 0.25 | $B_5$ Vitamins | - | - |
| SRM62 | 4 | 0.25 | $B_5$ Vitamins | - | - |
| SRM63 | 5 | 0.25 | $B_5$ Vitamins | - | - |
| SRM64 | 0.75 | 0.1 | $B_5$ Vitamins | - | - |
| SRM65 | 1 | 0.1 | $B_5$ Vitamins | - | - |
| SRM66 | 2 | 0.1 | $B_5$ Vitamins | - | - |
| SRM67 | 3 | 0.1 | $B_5$ Vitamins | - | - |

| Medium | Medium composition (MS supplemented with) | | | | |
|---|---|---|---|---|---|
| | BAP ($mgl^{-1}$) | NAA ($mgl^{-1}$) | Vitamins ($mgl^{-1}$) | Casein hydrolysate (400 mg $l^{-1}$) | Activated charcoal (g $l^{-1}$) |
| SRM68 | 4 | 0.1 | $B_5$ Vitamins | - | - |
| SRM69 | 5 | 0.1 | $B_5$ Vitamins | - | - |
| SRM70 | 1 | 0.5 | $B_5$ Vitamins | - | - |
| SRM71 | 2 | 0.5 | $B_5$ Vitamins | - | - |
| SRM72 | 3 | 0.5 | $B_5$ Vitamins | - | - |
| SRM73 | 4 | 0.5 | $B_5$ Vitamins | - | - |
| SRM74 | 5 | 0.5 | $B_5$ Vitamins | - | - |
| SRM75 | 0.75 | 0.25 | - | 400 | - |
| SRM76 | 1 | 0.25 | - | 400 | - |
| SRM77 | 2 | 0.25 | - | 400 | - |
| SRM78 | 3 | 0.25 | - | 400 | - |
| SRM79 | 4 | 0.25 | - | 400 | - |
| SRM80 | 5 | 0.25 | - | 400 | - |
| SRM81 | 0.75 | 0.1 | - | 400 | - |
| SRM82 | 1 | 0.1 | - | 400 | - |
| SRM83 | 3 | 0.1 | - | 400 | - |
| SRM84 | 4 | 0.1 | - | 400 | - |
| SRM85 | 5 | 0.1 | - | 400 | - |
| SRM86 | 1 | 0.5 | - | 400 | - |
| SRM87 | 2 | 0.5 | - | 400 | - |
| SRM88 | 3 | 0.5 | - | 400 | - |
| SRM89 | 4 | 0.5 | - | 400 | - |
| SRM90 | 5 | 0.5 | - | 400 | - |
| SRM91 | - | - | - | - | 1 |
| SRM92 | - | - | - | - | 2 |
| SRM93 | - | - | - | - | 3 |

(>/= 5.0 mg/l) whole of the callus turned brown and finally died. When the MS basal medium containing different combinations of BAP + NAA was modified by adding $B_5$ vitamins instead of the vitamins of MS medium the results were similar to those obtained on using vitamins of MS basal and there was no regeneration. An addition of casein hydrolysate at the rate of 400 mg/l could not induce regeneration and most of the calli turned brown. When IBA and IAA were used as auxins instead of NAA, after 4-5 days of inoculation all the calli turned brown and finally died. The combination of BAP + kin and BAP + 2,4-D also failed to induce regeneration. It was observed that only the combination of BAP 2.0 mg/l + NAA 0.25 mg/l + kin 0.5 mg/l was able to induce

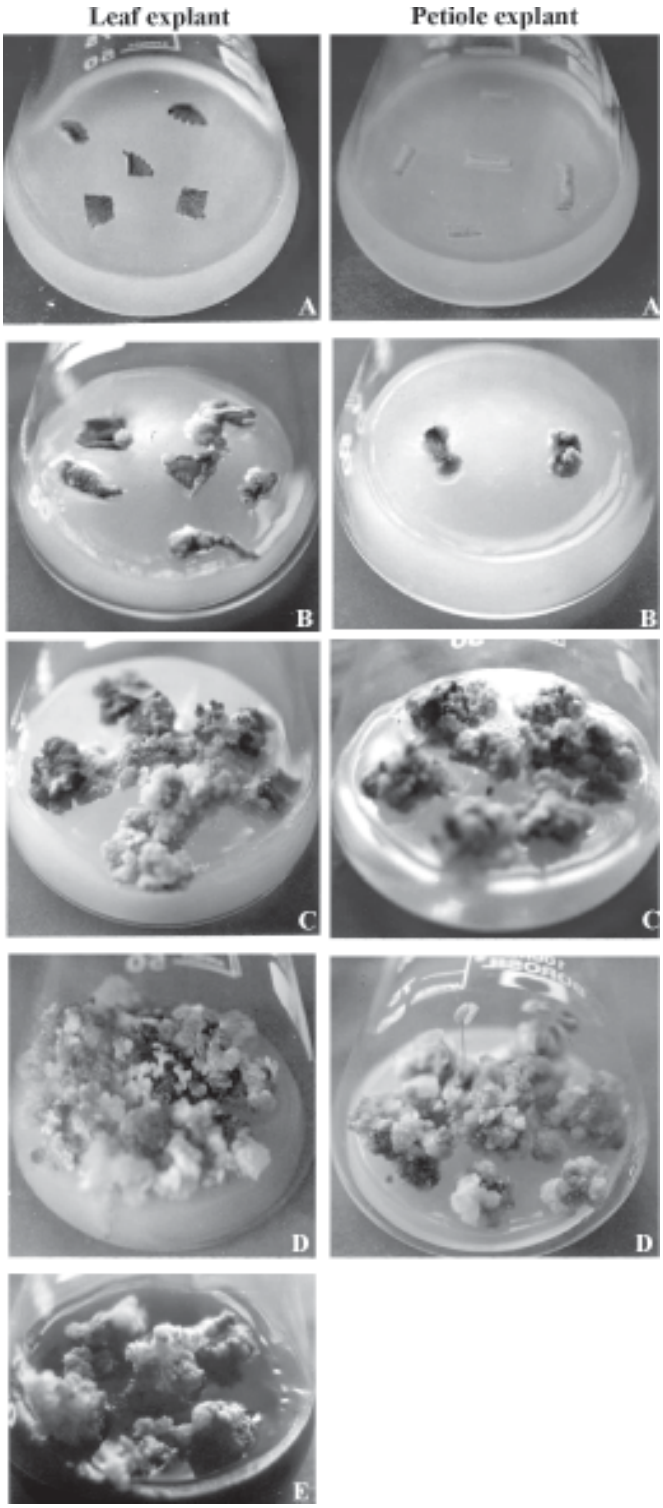


**Fig. 1 Callus initiation, multiplication and shoot regeneration in strawberry cv. Chandler. (A) Explant, (B) Callus initiation, (C) Callus multiplication, (D) Shoot regeneration, (E) Effect of activated charcoal on shoot regeneration**

regeneration. An average of 4.66 shoots per callus piece in petiolar callus (Fig. 1.2 d) and about and 4.07 shoots per callus piece in leaf derived callus (Fig. 1.1d) were obtained. Regeneration started when some of the calli started turning brown. It took 30-35 days for initiation of shoot regeneration in petiolar callus and 35-40 days in case of leaf-derived calli. The regeneration percentage was higher in petiolar callus (83.33%) than leaf derived callus (81.67%). The well-developed shoots in both leaf and petiolar calli were obtained after 50-55 days. The shoots were transferred to standardized multiplication medium (MS basal + BAP 0.5 mg/l + kin 0.5 mg/l + $GA_3$ 1.0 mg/l). In the rest of the concentrations of BAP + NAA + kin, the calli turned green but failed to regenerate even after 60-70 days.

Activated charcoal was added to the basal medium at the rate of 1.0 – 3.0 g/l to leach out the excess of hormones and to induce shoot regeneration. The callus turned green (Fig. 1.1 e) but regeneration was not there and when the calli were kept for more than 7 days on this medium they started turning brown and eventually died. Even after 7 days of transferring these calli to the medium containing various combinations of BAP + NAA + kin, they failed to regenerate and turned brown. After a desirable rate of multiplication was achieved, the multiplied shoots of size 2.5 – 3.0 cm were excised and transferred to half strength MS basal + IBA 1.0 mg/l + activated charcoal 0.2 g/l. Root initiation started after about 7 days of transfer in rooting media and well-developed root system was obtained in 4-5 weeks (Fig. 2.1a). Maximum number of roots i.e. 5.33/ shoot and root length 3.44 cm were obtained after 5 weeks on the rooting medium and when the roots were well developed the plantlets were taken out of the culture tubes and hardened (Fig. 2.1b, 2.1c, 2.1d). The per cent survival obtained after 5 weeks of transplanting was 92%. The hardened plants were then transferred to the field (Fig. 2.1 e, 2.1 f).

## DISCUSSION

So far, in strawberry various explants like leaf blades (Nehra *et al*. 1990), petioles (Jones *et al*. 1988), anthers (Rosati *et al*. 1975), stipules (Rugini and Orlando 1992) have been used for the establishment of callus cultures. Similar browning of leaf explants was reported in cv.

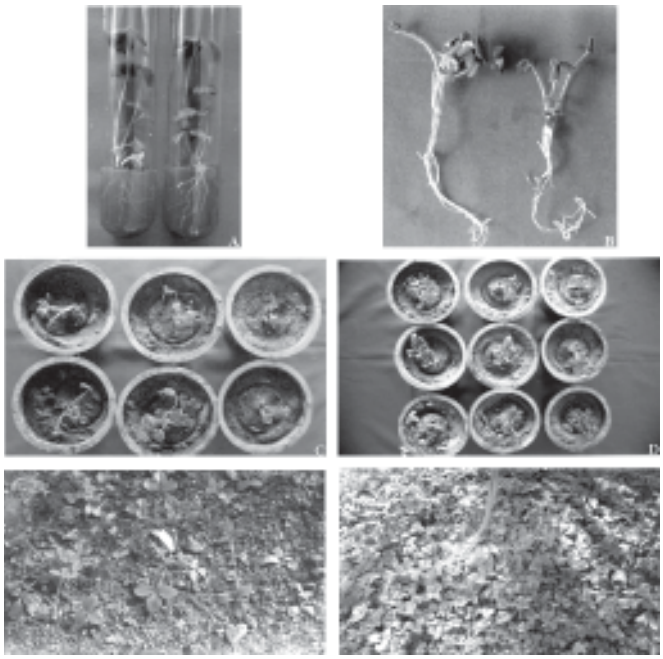

**Fig. 2 Hardening and transplanting of *in vitro* raised plants of strawberry cv. Chandler. (A) *In vitro* rooting, (B) Complete plantlet, (C) One week after hardening, (D) One month after hardening, (E) One month of field transplanting, (F) Four months of field transplanting**

Redcoat when cultured on the combination of BA and NAA (Nehra *et al*. 1990). The use of MS medium was found to be quite satisfactory for the induction and multiplication of calli. In past also many workers (Propesco *et al*. 1997, Nehra *et al*. 1990, Jones *et al*. 1988) used MS medium for the induction of callus cultures in strawberry. Green compact calli in leaf explants were obtained from *in vitro* and green house grown plants of cv. Redcoat on MS medium supplemented with 5 µM each of BA and 2,4-D (Nehra *et al*. 1990). The difference in the growth response from earlier findings may be attributed to the varietal difference in strawberry or to their growth conditions. Stipules along with a portion of petiole was found to be highly competent for organogenesis (Rugini and Orlando 1992), but in contrast stipules devoid of the petiolar portion failed to produce callus in the present studies. Darkness was found to be essential for the initiation of callus (Rugini and Orlando 1992, Blando *et al*. 1993).

It was reported that the first signs of regeneration were production of anthocyanins and necrosis in some sectors of calli (Damiano *et al*. 1995). The ability of the callus to regenerate was strongly influenced by genotype and growth conditions. It was reported by other workers that the percentage of regenerating calli from the leaf laminae, petiole and root explants was low and cultivar dependent (Rugini and Orlando, 1992). Cultivar Gea showed the highest regeneration capacity and regenerated 32% from leaf laminae, 16% from petiole and root cultures, followed by cv. Addie with 12 per cent from leaf lamina only, while others failed to regenerate from callus derived from these tissues. Other workers however, reported that shoot regeneration was more from leaf lamina than petiole (Jones *et al*. 1988, Propescu *et al*. 1997). The *in vitro* shoots in the present studies were maintained on cytokinin rich medium prior to explant preparation. Thus, the poor shoot regeneration in leaf explants taken from *in vitro* shoots may possibly be due to hormonal imbalance caused by accumulation of cytokinins in the leaf tissue (Nehra *et al*. 1990). Addition of casein hydrolysate at the rate of 400- 600 mg/l stimulated shoot production (Liu and Sanford 1988), whereas in our research the use of casein hydrolysate could not induce regeneration. It was also reported that addition of activated charcoal to maintenance media before regeneration enhanced the plant regeneration capability in long-term embryogenic callus cultures of red *Fescue* (*Festuca rubra* L. ) (Zaghmout Ousama *et al*. 1988). Pre-treatment with activated charcoal increased the level of precocious germination during culture and significantly increased shoot and root formation after transfer to regeneration media. Other workers also reported that the addition of activated charcoal promotes the elongation of both shoots and roots (Damiano *et al*. 1995).

## REFERENCES

Blando, F., Niglio, A. and Frattarelli, A. (1993). Cell suspension cultures in strawberry: growth characteristics and variability. *Acta Hort.* **336:** 257-262.

Dale, A. and Sjulin, T.M (1990). Few cytoplasms contribute to north American strawberry cultivars. *Horti Sci,* **25:** 327-329.

Damiano, C., Ascarelli, A., Frattareli, A., Lauri, P., Nishio, T. and Dore, C.L. (1995). Adventitious regeneration and genetic variability in strawberry. *Acta Hort.* **392:** 107-114.

Gould, A.R. (1986). Factors controlling generation of variability *in vitro*. In: K.I. Vasil (ed.), Cell Culture and Somatic Cell Genetic of Plants, pp. 549. Academic Press, New York.

Jones, O.P, Walter, B.J. and Beech, M.G. (1988). The production of strawberry plants from callus cultures. *Plant Cell Tissue Organ Cult.* **12:** 235-241.

Kaushal, K. (2002). Studies on somaclonal variations in strawberry (*Fragaria* x *ananassa* Duch). Ph.D Thesis, Dr Y.S.Parmar University of Horticulture and Forestry, Nauni, Solan.

Liu, Z.R. and Sanford, J.C. (1988). Plant regeneration by organogenesis from strawberry leaf and runner tissue. *Hort. Sci.* **23:** 1057-1059.

Luby, J.J., Hancock, J.F. and Comerson, J.S. (1991). Expansion of the strawberry germplasm in North America. In: A. Dale and J.J. Luby (eds). The Strawberry into the 21st Century, pp. 65. Timber Press, Portland Oregon.

Luby, J.J. and Stahler, M.M. (1993). Collection and evaluation of *Fragaria virginiana* in North America. *Acta Hort.* **345:** 49-53.

Nehra, N.S., Stushnoff, C and Kartha, K.K. (1990). Regeneration of plants from immature leaf derived callus of strawberry (*Fragaria* x *ananassa*), *Plant Sci. Limerick* **66:** 119-126.

Propescu, A.N., Isac,V.S., Coman, M.S. and Radulescu, M.S (1997). Somaclonal variation in plants regenerated by organogenesis from callus culture of strawberry (*Fragaria* x *ananassa*). *Acta Hort.* **439:** 89-96.

Rosati, P., Devreux, M., and Laneri, U. (1975). Anther culture of strawberry (*Fragaria* x *ananassa* Duch). *Hort. Sci.* **10:** 119-120.

Rugini, E and Orlando, R. (1992). High efficiency shoot regeneration from calluses of strawberry (*Fragaria* x *ananassa* Duch) stipules of *in vitro* shoot cultivars. *J. Hort. Sci.* **67:** 577-582.

Zaghmout Ousama, M.F. and Torello William, A. (1988). Enhanced regeneration in long term callus culture of red *Fescue* by pre-treatment with activated charcoal. *Hort. Sci.* **23:** 615-616.