DOI : 10.5958/0974-0112.2020.00003.1

Indian J. Hort. 77(1), March 2020: 48-55

Assessment of callus induction and plant regeneration potential of leaf explants derived from *in vitro* and *in vivo* shoot of strawberry

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ABSTRACT

The present investigation was carried out to study the plant regeneration using leaf explants obtained from *in vitro* and *in vivo* produced shoots of strawberry cv. Chandler. Both explants formed callus and multiple shoots. Highest callus induction (86.66%) and shoot regeneration (63.33%) was obtained with the calli of *in vitro* leaf explants on MS medium supplemented with BAP and NAA. The time required for callus induction and regeneration by the leaf explants from shoots grown under *in vitro* was less as compared to shoots produced under *in vivo* conditions. The number of shoots and shoot length obtained from *in vivo* leaf explants were also lesser as compared to *in vitro* conditions. The *in vitro* leaf derived calli was transferred to root initiation media containing different concentration of IBA and NAA, supplemented with activated charcoal after 8 weeks. Highest root initiation (91.33 per cent) was recorded on MS media supplemented with 1.5 mg/L of IBA and 200 mg/L of activated charcoal. The newly regenerated plantlets were sterilized and hardened in field conditions. The study inferred that *in vitro* explants takes less time for callus induction and regeneration, along with production of more number of shoots with increased shoot length and more number of roots and higher root induction as compared to *in vivo.*

Key words: *Fragaria* × *ananassa*, hardening, inoculation, micropropagation, proliferation.

INTRODUCTION

Plant tissue culture is based on the cell doctrine that states, the cell is totipotent and is capable of autonomy. Regeneration protocols are species specific with respect to regeneration capacity (Passey *et al*., 13) and different combinations of growth regulators have been used for the regeneration of shoots from various explants (Biswas *et al*., 1). Selection of the proper hormone combination, explants and cultivar are pre-requisites for successful regeneration and multiplication. Leaf tissue of strawberry has been studied and shown to have the greatest regeneration capacity (Passey *et al*., 13). Callus production is also more prolific from the leaf tissue. Different hormonal combinations and leaf disc explants sources influence the number of regenerated plants. A pretreatment in darkness is vital for callus induction and plantlet regeneration (Popescu *et al*., 14).

Propagation of strawberry is achieved either by runners or by *in vitro* micropropagation. Plant regeneration from strawberry callus was reported for first time by Jones *et al*. (4) and it had been observed that leaf tissue have the greatest regeneration capacity. Conventional propagation of strawberries is slow, laborious, and expensive with many limitations and may not be recommended for effective and commercial multiplication. Regeneration of strawberry is influenced by explants, hormonal combinations, light and season of the crop grown. Plant cell culture has become an excellent method for plant cell differentiation as well as a supplementary technique for plant breeding programmes through the use of new and expanded genetic variability. It has become increasingly evident in the recent past that a considerable amount of useful genetic variation can be recovered among tissue culture propagated plants through somaclonal variations.

Callus induction using leaf disc of *in vitro* grown plantlets as explants exhibited higher regeneration compared to greenhouse-grown plants (Khan and Spoor, 6). Besides somaclonal variations are more frequent among the regenerates from callus and cell suspension cultures compared to meristem and shoot apex cultures. For the plantlets regeneration, callus induction is of prime significance and thus, different tissues produce callus of different size, nature and time taken for callus induction also vary. The present study aimed at development of an efficient regenerative protocol for callus induction and regeneration from leaf discs of strawberry in the shortest possible time with the optimum concentration of α-napthaleneacetic acid (NAA) and 6-benzyl

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aminopurine (BAP) for selecting desirable plantlets for commercial cultivation.

MATERIALS AND METHODS

Leaf explants (leaf disks) for callus culture were excised from the strawberry shoots grown under *in vivo* and *in vitro* conditions (Fig. 1). The explants from the young leaves were excised from the actively proliferating *in vitro* grown shoots. Fully mature expanded leaves were excised from shoots grown under *in vivo* conditions. No sterilization was required in case of explants (leaf disks) which were taken from the *in vitro* growing shoots but the explants (leaf disks) taken from *in vivo* plantlets were washed thoroughly in tap water for 20 minutes to remove the surface contaminants. Thereafter, treated with Tween-20 (2-3 drops/100 ml of H_2O) for 4-5 minutes and washed again thoroughly with double distilled water. The washed leaf disks were then treated with 0.5 per cent bavistin for 6-7 minutes and then washed 3-4 times with distilled water. Under aseptic conditions, the sterilization procedures were carried out in a laminar-air flow cabinet model No: CAH 0600. The leaf disks were subjected to surface sterilization using 0.1 per cent mercuric chloride for 4 minutes. At the end, the leaf discs were washed with sterile distilled water repeatedly.

Sterilized leaves (*in vivo*) were cut into strips of size 0.5 mm × 0.5 mm. The leaves (*in vitro*) of size 0.2 mm without the midrib were also taken. Growth regulators, α-napthaleneacetic acid (NAA), and 6-benzyl aminopurine (BAP) were dissolved in sodium hydroxide (NaOH) and added to semi-solid Murashige and Skoog (MS) media placed axial side down with different bio-regulators (Murashige and Skoog, 11). The leaf derived explants were excised into small pieces (3 mm^2) and cultured on MS media with 27 different combinations and concentrations of 2,4-D and BAP (Sigma) and 675 test tubes/plant for each treatment were maintained for each treatment and 12 test tubes /plant were maintained for each replication in every treatment including C_1 (MS medium + 2, 4-D 1mg/L+ BAP 0.5 mg/L); C_2 (MS medium + 2,4-D 1.5 mg/L+ BAP 0.5 mg/L); C_3 MS medium + 2,4-D 2 mg/L+ BAP 0.5 mg/L); C_4 (MS medium + 2,4-D 2.5 mg/L+ BAP 0.5 mg/L); C_5 MS medium + 2,4-D 3 mg/L+ BAP 0.5 mg/L); C_6 (MS medium + 2,4-D 1 mg/L+ BAP 1 mg/L); C_7 (MS medium + 2,4-D 1.5 mg/L+ BAP 1 mg/L); C_8 (MS medium + 2,4-D 2 mg/L+ BAP 1 mg/L); C_9 (MS medium + 2,4-D 2.5 mg/L + BAP 1 mg/L); C_{10} (MS medium + 2,4-D 3 mg/L+ BAP 1 mg/L); C_{11} (MS medium + BAP 0.5 mg/L+ NAA 0.5 mg/L); C_{12} (MS medium + BAP 0.5 mg/L+ NAA 1.0 mg/L); C_{13} (MS medium + BAP 0.5 mg/L+ NAA 1.5 mg/L); C_{14} (MS medium + BAP 1 mg/L+ NAA 0.5 mg/L); C_{15} (MS medium + BAP 1 mg/L+ NAA 1 mg/L); C_{16} (MS medium + BAP 1 mg/L+ NAA 1.5 mg/L); C_{17} (MS medium + BAP 2 mg/L+ NAA 0.5 mg/L); C_{18} (MS medium + BAP 2 mg/L+ NAA 1 mg/L); C_{19} (MS medium + BAP 2 mg/L+ NAA 1.5 mg/L); C_{20} (MS medium + BAP 3 mg/LNAA 0.5 mg/L); C_{21} (MS medium + BAP 3 mg/L+NAA 1 mg/L); C_{22} (MS medium + BAP 3 mg/L+NAA 1.5 mg/L); C_{23} (MS medium + NAA 3 mg/L+BAP 0.5 mg/L); C_{24} (MS medium + NAA 3 mg/L+BAP 1.5 mg/L); C_{25} (MS medium + NAA 3.5 mg/L+BAP 0.5 mg/L); C_{26} (MS medium + NAA 3.5 mg/L +BAP 1.5 mg/L); C_{27} (MS medium + NAA 4 mg/L+BAP 0.5 mg/L). The pH was adjusted to 5.5 by adding 0.1 M of either sodium hydroxide (NaOH) or hydrochloric acid (HCl). Finally, the media were set to pH 5.7 before addition of agar-agar and autoclaved at 15 Psi^2 at 121°C for 20 minutes. Subsequently, the actively growing callus from the initial leaf explants was sub-cultured onto fresh MS medium. Visual observations on callus growth including type and its colour were also recorded. The callus was again sub-cultured onto the medium for its further multiplication. The callus was allowed to grow for 60 days, the half of it was further sub-cultured for callus multiplication, whereas, the remaining was cultured for shoot regeneration. The sterilized media were poured into $1/3^{rd}$ of 60 mL sterile culture vials under darkness. All cultures were incubated in a culture room at 25±2ºC, followed by four weeks of 16 hr light/day photoperiod by white florescent tubes. Subcultures were performed every 21–28 days to provide new and fresh nutrients under the same conditions.

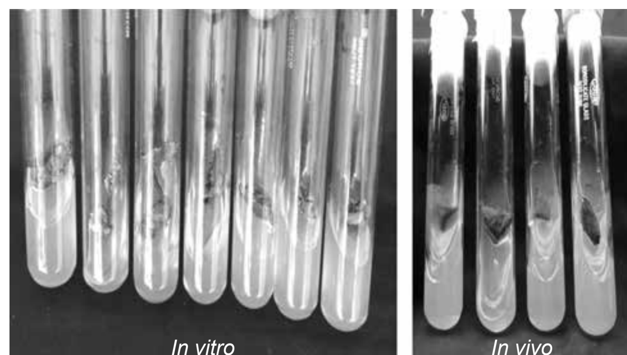

Fig. 1. Callus initiation in MS media supplemented with each of 1 mg/L BAP and NAA after 21 days of incubation

After callusing, leaf derived calli from both *in vitro* and *in vivo* grown shoots were divided into small segments of 1-1.5 cm diameter and further sub-cultured for shoot initiation in MS medium. The regenerated shoots were then multiplied on the standardized MS medium contained BAP (2 mg/L) and GA_3 (2 mg/L). Root formation was observed from

the regenerated shoot in MS medium supplemented with BAP and IBA. The multiplied shoots of size 2.5-3.0 cm were transferred singly to culture tube each containing about 15 ml of root initiation medium. Once the root system was fully developed (after 5 weeks), the plantlets were taken out of the culture tube, washed thoroughly and placed in 0.5 per cent bavistin for 15-20 minutes. The plantlets were transferred to the plastic pots containing pre-sterilized mixture of coco peat: perlite: vermiculite in the ratio of 1:1:1. The plantlets were watered and kept under high humidity conditions. After that the plantlets were transferred to the mixture of soil: farm yard manure (1:1) in the glass house conditions.

All the experiments were repeated thrice and 25 replicates were used. The effect of different treatments was quantified as standard error of mean at 5% level of significance (Panse and Sukhatme, 12).

RESULTS AND DISCUSSION

The data presented on callus induction in Table 1 reveals that the time taken for callus induction (swelling of leaves) was more in explants taken from shoots grown under *in vivo* conditions (45 days) as compared to *in vitro* conditions (28 days). The shoots grown under *in vitro* conditions registered maximum callus induction (52.38%), whereas, it was minimum (43.61 %) in leaf explants taken from shoots grown under *in vivo* conditions. These findings are in close conformity with Munir *et al.* (10) who recorded the callogenic responses in strawberry. The high quality of callusing of strawberry cultivar 'Chandler' was obtained *in vitro* conditions compared to *in vivo* conditions. Among different treatments, maximum callus induction (81.1%) was obtained on MS medium supplemented with1 mg/L of BAP used along with 1 mg/L of NAA which was at par with MS medium containing BAP (1mg/L) along with NAA (1.5mg/L), whereas, it was minimum (19.99%) on MS medium enriched with 2, 4-D (2mg/L) and BAP (0.5mg/L). Earlier studies also recorded the highest callus formation when MS medium was supplemented with BAP (2mg/L) and 0.5 mg/L of NAA (Mahmoud and Kosar, 2013). Most effective auxin for callus induction from leaf explant was NAA followed by BAP (Biswas *et al.,* 2). Kaushal *et al.* (5) also observed that conjoint use of BAP and NAA was best for the induction and multiplication of callus in strawberry cv. Chandler and Fern. Our results are in conformity with some of the earliest studies on strawberry, which showed good callus induction responses in MS media with BAP. The interaction effects were also significant and the maximum callus induction (86.66%) was observed in leaf explant from shoots grown under *in vitro* conditions on MS medium containing BAP (1mg/L)

Table 1. Leaf derived callus induction from *in vivo* and *in vitro* Chandler strawberry.

Culture media (C)	Callus induction (%)		Mean
	in vivo	*in vitro*	
C_1	0.00 (0.005)*	0.00 (0.005)	0.00 (0.005)
C_2	0.00 (0.005)	0.00 (0.005)	0.00 (0.005)
C_3	0.00 (0.005)	39.99 (39.17)	19.99 (19.58)
C_4	26.66 (30.95)	33.32 (35.17)	29.49 (32.75)
C_5	42.21(40.46)	55.55 (48.22)	48.88 (44.34)
C_6	33.33 (35.17)	39.99 (39.02)	36.66 (37.10)
C_7	39.99 (39.17)	44.44 (41.73)	42.21 (39.06)
C_8	51.10 (45.61)	53.33 (46.90)	52.21 (46.42)
C_9	53.33 (46.90)	59.99 (50.78)	56.66 (48.84)
C_{10}	60.88 (50.78)	64.44 (53.49)	62.66 (52.38)
C_{11}	51.10 (45.66)	62.21(52.22)	56.66 (48.94)
C_{12}	59.99 (50.78)	66.66 (54.78)	63.33 (52.78)
C_{13}	59.99 (50.78)	68.88 (56.28)	64.44 (53.53)
C_{14}	66.66 (54.78)	73.33 (59.00)	69.99 (56.89)
C_{15}	75.55 (60.39)	86.66 (68.98)	81.10 (64.69)
C_{16}	71.10 (57.61)	79.99 (63.61)	75.55 (60.61)
C_{17}	46.66 (43.05)	53.33 (46.90)	49.99 (44.98)
C_{18}	51.11 (45.61)	55.55 (48.22)	53.33 (46.92)
C_{19}	39.99 (39.17)	44.44 (41.78)	42.21 (40.48)
C_{20}	45.55 (42.42)	51.10 (45.61)	48.33 (44.02)
C_{21}	15.53 (22.90)	33.33 (35.17)	24.43 (29.04)
C_{22}	39.99 (39.17)	42.20 (40.50)	41.10 (39.83)
C_{23}	41.11 (39.18)	48.88 (44.33)	44.99 (42.07)
C_{24}	46.66 (43.05)	53.33 (46.90)	49.99 (44.98)
C_{25}	54.44 (47.54)	64.44 (53.46)	59.44 (50.56)
C_{26}	55.55 (48.17)	65.55 (54.32)	60.55 (51.28)
C_{27}	66.66 (54.78)	73.33 (59.39)	69.99 (57.08)
Mean	43.61(39.70)	52.38 (45.40)	

$LSD_{0.05}$ Explant-1.47 Medium-5.41 Interaction-7.66

Figures in parentheses are arc sine transformed values; C_1, MS medium + 2, 4-D 1mg/L+ BAP 0.5 mg/L; C_2, MS medium + 2,4-D 1.5 mg/L+ BAP 0.5 mg/L; C_3, MS medium + 2,4-D 2 mg/L+ BAP 0.5 mg/L; C_4, MS medium + 2,4-D 2.5 mg/L+ BAP 0.5 mg/L; C_5, MS medium + 2,4-D 3 mg/L+ BAP 0.5 mg/L; C_6, MS medium + 2,4-D 1 mg/L+ BAP 1 mg/L; C_7, MS medium + 2,4-D 1.5 mg/L+ BAP 1 mg/L; C_8, MS medium + 2,4-D 2 mg/L+ BAP 1 mg/L; C_9, MS medium + 2,4-D 2.5 mg/L + BAP 1 mg/L); C_{10}, MS medium + 2,4-D 3 mg/L+ BAP 1 mg/L; C_{11}, MS medium + BAP 0.5 mg/L+ NAA 0.5 mg/L; C_{12}, MS medium + BAP 0.5 mg/L+ NAA 1.0 mg/L; C_{13}, MS medium + BAP 0.5 mg/L+ NAA 1.5 mg/L; C_{14}, MS medium + BAP 1 mg/L+ NAA 0.5 mg/L; C_{15}, MS medium + BAP 1 mg/L+ NAA 1 mg/L); C_{16}, MS medium + BAP 1 mg/L+ NAA 1.5 mg/L; C_{17}, MS medium + BAP 2 mg/L+ NAA 0.5 mg/L; C_{18}, MS medium + BAP 2 mg/L+ NAA 1 mg/L; C_{19}, MS medium + BAP 2 mg/L+ NAA 1.5 mg/L); C_{20} (MS medium + BAP 3 mg/LNAA 0.5 mg/L; C_{21}, MS medium + BAP 3 mg/L+NAA 1 mg/L; C_{22} MS medium + BAP 3 mg/L+NAA 1.5 mg/L; C_{23}, MS medium + NAA 3 mg/L+BAP 0.5 mg/L; C_{24}, MS medium + NAA 3 mg/L+BAP 1.5 mg/L; C_{25}, MS medium + NAA 3.5 mg/L+BAP 0.5 mg/L; C_{26} MS medium + NAA 3.5 mg/L +BAP 1.5 mg/L; C_{27}, MS medium + NAA 4 mg/L+BAP 0.5 mg/L

in combination with NAA (1mg/L) which was at par with leaf explants from shoots grown *in vitro* on MS medium contained BAP (1mg/L) in combination with 1.5 mg/L of NAA (Fig. 2).

The calli obtained with BAP and NAA were cut to segments of size of 1.0-1.5 cm and were subsequently maintained on the same medium sub-culturing after every 4 weeks for callus regeneration (Table 2). Sixteen different combinations of growth regulators were obtained for callus regeneration. Among different growing conditions of leaf explants, the leaf derived calli from shoots under *in vitro* registered maximum callus regeneration (22.08%) compared to *in vivo* (14.79%). In the present study, the regeneration was more in leaf-derived calli from shoots grown under *in vitro* than from shoots grown under *in vivo* conditions. Mohamed *et al.* (9) also reported the direct shoot regeneration from leaf discs to be higher in cultures initiated *in vitro* than greenhouse derived leaf discs. Jones *et al.* (4) also reported more callus regeneration under *in vitro* conditions as compared to greenhouse leaves in strawberry. Calluses induced from leaf disc explants of *in vitro* grown plants exhibited higher regeneration potential compared to those induced from greenhouse-grown plants (Khan and Spoor, 6). Among different treatment combinations, maximum callus regeneration (59.99%) was obtained on MS medium supplemented with BAP (2 mg/L) in combination with NAA (0.5 mg/L) and kinetin (Kn, 0.5mg/L). Minimum callus regeneration of 3.33 per cent was registered on MS medium supplemented with BAP (2 mg/L) used in combination with IBA (0.5 mg/L). These results are in conformity with Biswas *et al.* (2). Sakila *et al.* (16) also showed the best medium for regeneration of strawberry was MS medium containing BAP (1.5 mg/L) and Kn (0.5 mg/L). The callus differentiation in *Saussurea obvallata* was obtained on BA and NAA (Dhar and Joshi, 2005).

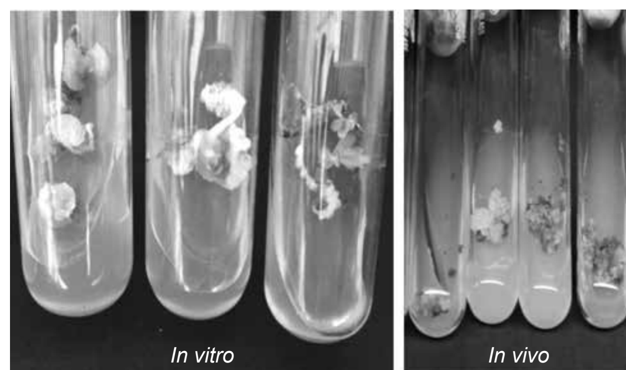

Fig. 2. Callus induction in MS media supplemented with each of 1 mg/L BAP and NAA after45 days of incubation

Table 2. Shoot regeneration from *in vivo* and *in vitro* leaf derived calli of Chandler strawberry.

Culture media (C)	Frequency of callus regeneration (%)		Mean
	in vivo	*in vitro*	
C_1	0.00 (0.005)	0.00 (0.005)	0.00 (0.005)
C_2	10.00 (14.99)	13.33 (21.13)	11.66 (18.06)
C_3	13.33 (21.13)	23.33 (28.76)	18.33 (24.95)
C_4	16.66 (23.35)	26.33 (30.77)	21.66 (27.06)
C_5	20.00 (26.06)	30.00 (32.98)	25.00 (29.52)
C_6	0.00 (0.005)	0.00 (0.005)	0.00 (0.005)
C_7	23.33 (28.76)	33.33 (35.20)	28.33 (31.59)
C_8	20.00 (26.00)	36.33 (37.12)	28.16 (31.59)
C_9	10.00 (14.99)	16.66 (23.35)	13.00 (19.17)
C_{10}	0.00 (0.005)	0.00 (0.005)	0.00 (0.005)
C_{11}	0.00 (0.005)	0.00 (0.005)	0.00 (0.005)
C_{12}	0.00 (0.005)	20.00 (26.06)	10.00 (13.03)
C_{13}	0.00 (0.005)	6.66 (12.28)	3.33 (6.14)
C_{14}	30.00 (32.69)	40.00 (39.13)	35.00 (35.91)
C_{15}	36.66 (37.12)	43.33 (41.05)	39.99 (39.09)
C_{16}	56.66 (48.91)	63.33 (53.04)	59.99 (50.97)
Mean	14.79 (17.13)	22.08 (23.80)	

$LSD_{0.05}$ Explant-2.76 Medium-7.81 Interaction-NS

Figures in parentheses are arc sine transformed values; NS, non-significant; C_1, MS medium + BAP 1 mg/L; C_2, MS medium + BAP 2 mg/L; C_3, MS medium + BAP 3 mg/L; C_4, MS medium + BAP 1 mg/L+ NAA 0.25 mg/L; C_5, MS medium + BAP 1 mg/L+ NAA 0.5 mg/L; C_6, MS medium + BAP 1 mg/L+ NAA 1.0 mg/L; C_7, MS medium + BAP 2 mg/L+ NAA 0.25 mg/L; C_8 (MS medium + BAP 2 mg/L+ NAA 0.5 mg/L; C_9, MS medium + BAP 2 mg/L+ NAA 1 mg/L; C_{10}, MS medium + BAP 1 mg/L+IBA 0.5 mg/L; C_{11}, MS medium + BAP 1.0 mg/L+IBA 1.0 mg/L); C_{12} (MS medium + BAP 2 mg/L+IBA 0.5 mg/L); C_{13} (MS medium + BAP 2.0 mg/L+IBA 1 mg/L; C_{14}, MS medium + BAP 1 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L; C_{15}, MS medium + BAP 1.5 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L; C_{16}, MS medium + BAP 2.0 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L

The interaction between explants and treatments were non-significant. Maximum callus regeneration (63.3%) was observed in leaf derived calli from shoots under *in vitro* on MS medium supplemented with BAP (2 mg/L) used in combination with NAA (0.5 mg/L) and Kn (0.5mg/L) followed by leaf derived calli from shoots under *in vivo* on MS medium supplemented with BAP (2 mg/L) used along with NAA (0.5 mg/L) and 0.5mg/L of Kn (Fig. 3). The regeneration started when some of the calli started turning brown in case of leaf derived calli from shoots grown under *in vivo* condition while leaf derived calli from shoots grown under *in vitro* condition turned cream brown to light

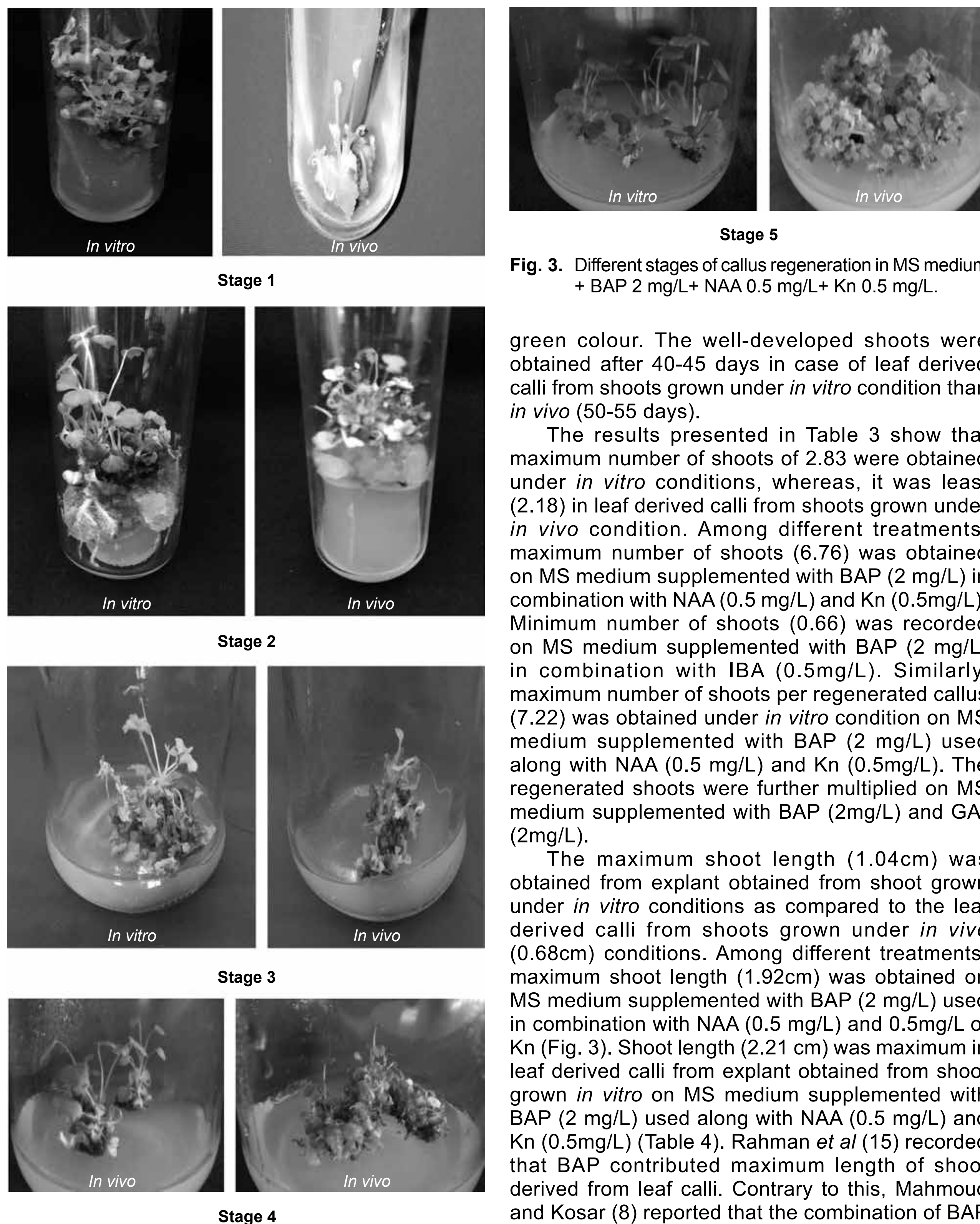

Fig. 3. Different stages of callus regeneration in MS medium + BAP 2 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L.

green colour. The well-developed shoots were obtained after 40-45 days in case of leaf derived calli from shoots grown under *in vitro* condition than *in vivo* (50-55 days).

The results presented in Table 3 show that maximum number of shoots of 2.83 were obtained under *in vitro* conditions, whereas, it was least (2.18) in leaf derived calli from shoots grown under *in vivo* condition. Among different treatments, maximum number of shoots (6.76) was obtained on MS medium supplemented with BAP (2 mg/L) in combination with NAA (0.5 mg/L) and Kn (0.5mg/L). Minimum number of shoots (0.66) was recorded on MS medium supplemented with BAP (2 mg/L) in combination with IBA (0.5mg/L). Similarly, maximum number of shoots per regenerated callus (7.22) was obtained under *in vitro* condition on MS medium supplemented with BAP (2 mg/L) used along with NAA (0.5 mg/L) and Kn (0.5mg/L). The regenerated shoots were further multiplied on MS medium supplemented with BAP (2mg/L) and GA_3 (2mg/L).

The maximum shoot length (1.04cm) was obtained from explant obtained from shoot grown under *in vitro* conditions as compared to the leaf derived calli from shoots grown under *in vivo* (0.68cm) conditions. Among different treatments, maximum shoot length (1.92cm) was obtained on MS medium supplemented with BAP (2 mg/L) used in combination with NAA (0.5 mg/L) and 0.5mg/L of Kn (Fig. 3). Shoot length (2.21 cm) was maximum in leaf derived calli from explant obtained from shoot grown *in vitro* on MS medium supplemented with BAP (2 mg/L) used along with NAA (0.5 mg/L) and Kn (0.5mg/L) (Table 4). Rahman *et al* (15) recorded that BAP contributed maximum length of shoot derived from leaf calli. Contrary to this, Mahmoud and Kosar (8) reported that the combination of BAP and NAA resulted in highest number of shoot and shoot length in strawberry. The present investigation extends confirmation where a combination of

Table 3. Shoot emergence from *in vivo* and *in vitro* leaf derived calli of Chandler strawberry.

Culture media (C)	No. of shoot per regenerated callus		Mean
	in vivo	*in vitro*	
C_1	0.00	0.00	0.00
C_2	1.33	2.22	1.77
C_3	1.66	2.66	2.16
C_4	2.33	3.00	3.00
C_5	2.67	3.33	3.33
C_6	0.00	0.00	0.00
C_7	2.33	3.22	2.77
C_8	3.00	4.00	3.83
C_9	3.33	4.33	3.83
C_{10}	0.00	0.00	0.00
C_{11}	0.00	0.00	0.00
C_{12}	0.00	1.33	0.66
C_{13}	1.22	2.33	1.77
C_{14}	4.33	5.00	4.66
C_{15}	5.66	6.66	6.16
C_{16}	6.33	7.22	6.76
Mean	2.18	2.83	
$LSD_{0.05}$ Explant-0.21 Medium-0.58 Interaction-NS			

Figures in parentheses are arc sine transformed values; NS, non-significant; C_1, MS medium + BAP 1 mg/L; C_2, MS medium + BAP 2 mg/L; C_3, MS medium + BAP 3 mg/L; C_4, MS medium + BAP 1 mg/L+ NAA 0.25 mg/L; C_5, MS medium + BAP 1 mg/L+ NAA 0.5 mg/L; C_6, MS medium + BAP 1 mg/L+ NAA 1.0 mg/L; C_7, MS medium + BAP 2 mg/L+ NAA 0.25 mg/L; C_8 (MS medium + BAP 2 mg/L+ NAA 0.5 mg/L; C_9, MS medium + BAP 2 mg/L+ NAA 1 mg/L; C_{10}, MS medium + BAP 1 mg/L+IBA 0.5 mg/L; C_{11}, MS medium + BAP 1 mg/L+IBA 1.0 mg/L); C_{12} (MS medium + BAP 2.0 mg/L+IBA 0.5 mg/L); C_{13} (MS medium + BAP 2.0 mg/ L+IBA 1 mg/L; C_{14}, MS medium + BAP 1 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L; C_{15}, MS medium + BAP 1.5 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L; C_{16}, MS medium + BAP 2.0 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L

Table 4. Shoot length from *in vivo* and *in vitro* leaf derived calli of Chandler strawberry.

Culture media (C)	Length of shoot (cm)		Mean
	in vivo	*in vitro*	
C_1	0.00	0.00	0.00
C_2	0.84	1.11	0.97
C_3	0.94	1.20	1.07
C_4	0.96	1.21	1.08
C_5	1.07	1.26	1.16
C_6	0.00	0.00	0.00
C_7	1.10	1.38	1.24
C_8	1.17	1.44	1.31
C_9	1.00	1.12	1.06
C_{10}	0.00	0.00	0.00
C_{11}	0.00	0.00	0.00
C_{12}	0.00	0.96	0.48
C_{13}	0.80	1.10	0.95
C_{14}	1.31	1.70	1.50
C_{15}	1.54	1.96	1.75
C_{16}	1.62	2.21	1.92
Mean	0.68	1.04	
$LSD_{0.05}$ Explant-0.04 Medium-0.11 Interaction-0.16			

C_1, MS medium + BAP 1 mg/L; C_2, MS medium + BAP 2 mg/L; C_3, MS medium + BAP 3 mg/L; C_4, MS medium + BAP 1 mg/ L+ NAA 0.25 mg/L; C_5, MS medium + BAP 1 mg/L+ NAA 0.5 mg/L; C_6, MS medium + BAP 1 mg/L+ NAA 1.0 mg/L; C_7, MS medium + BAP 2 mg/L+ NAA 0.25 mg/L; C_8 (MS medium + BAP 2 mg/L+ NAA 0.5 mg/L; C_9, MS medium + BAP 2 mg/L+ NAA 1 mg/L; C_{10}, MS medium + BAP 1 mg/L+IBA 0.5 mg/L; C_{11}, MS medium + BAP 1.0 mg/L+IBA 1.0 mg/L); C_{12} (MS medium + BAP 2 mg/L+IBA 0.5 mg/L); C_{13} (MS medium + BAP 2.0 mg/L+IBA 1 mg/L; C_{14}, MS medium + BAP 1 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L; C_{15}, MS medium + BAP 1.5 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L; C_{16}, MS medium + BAP 2 mg/L+ NAA 0.5 mg/L+ Kn 0.5 mg/L

both BAP and kinetin contributed towards shoot regeneration.

It was observed that the regenerated shoots of leaf derived calli from shoots grown under *in vivo* condition grown in clusters with very less shoot length, therefore the research efforts were further continued towards shoots obtained from *in vitro* conditions only and were then transferred to the rooting media. The data pertaining to the influence of IBA and or NAA) on *in vitro* root formation are presented in Table 5. Minimum days (27.67) for root initiation were recorded on MS (half strength) medium with IBA of 1.5 mg/L and activated charcoal 200 mg/L. The frequency of root formation was highest (91.33%) on MS (half strength) medium incorporated with IBA of 1.5 mg/L and activated charcoal of 200 mg/L, which was at par with the MS (half strength) medium in combination with IBA of 1 mg/L and activated charcoal of 200 mg/L (Fig. 4-5). The results obtained in the present study are in agreement with the results of Mahajan *et al.* (7) who reported that the initiation of root was obtained on half strength MS media supplemented with IBA of 1 mg/L and activated charcoal of 200 mg/L. In the present study, the addition of activated charcoal contributed towards the promotion of the elongation of roots.

It can be concluded that explants obtained from *in vitro* shoots have the capacity of maximum callus induction, regeneration with minimum time for callus

Table 5. Effect of auxins and activated charcoal on *in vitro* rooting of calli derived from leaf *in vitro* of strawberry cv. Chandler

Culture media (C)	Number of days taken for root initiation	Frequency of root formation (%)
C_1	30.66	51.66
C_2	30.33	62.33
C_3	30.00	65.66
C_4	29.33	70.00
C_5	28.66	80.33
C_6	27.67	91.33
C_7	32.33	76.00
$LSD_{0.05}$	0.94	11.42

C_1, ½ MS medium + NAA 0.5 mg/L+ activated charcoal 200 mg/L; C_2, ½ MS medium + NAA 1.0 mg/L+ activated charcoal 200 mg/L; C_3, ½ MS medium + NAA 1.5 mg/L+ activated charcoal 200 mg/L; C_4, ½ MS medium + IBA 0.5 mg/L+ activated charcoal 200 mg/L; C_5, ½ MS medium + IBA 1 mg/L+ activated charcoal 200 mg/L; C_6, ½ MS medium + IBA 1.5 mg/L+ activated charcoal 200 mg/L; C_7, ½ MS medium

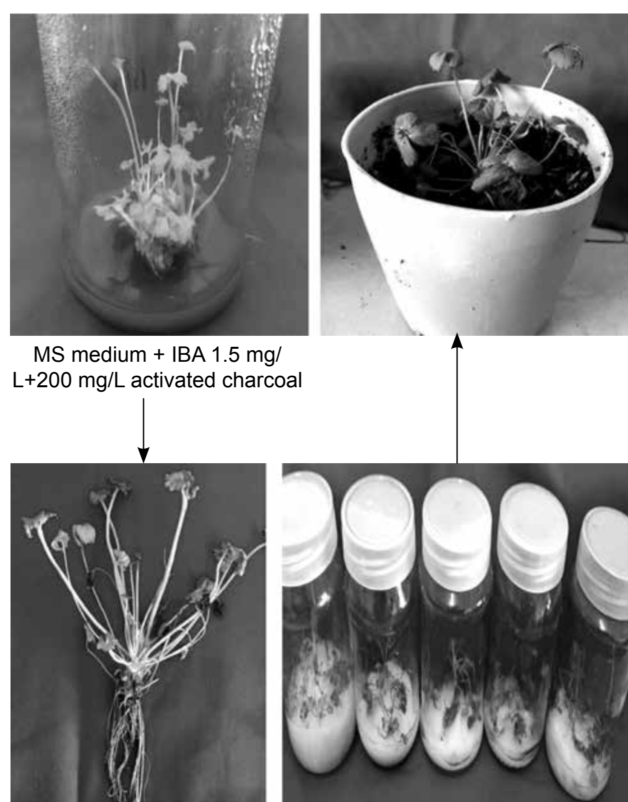

Fig. 4. Stages of root initiation and hardening.

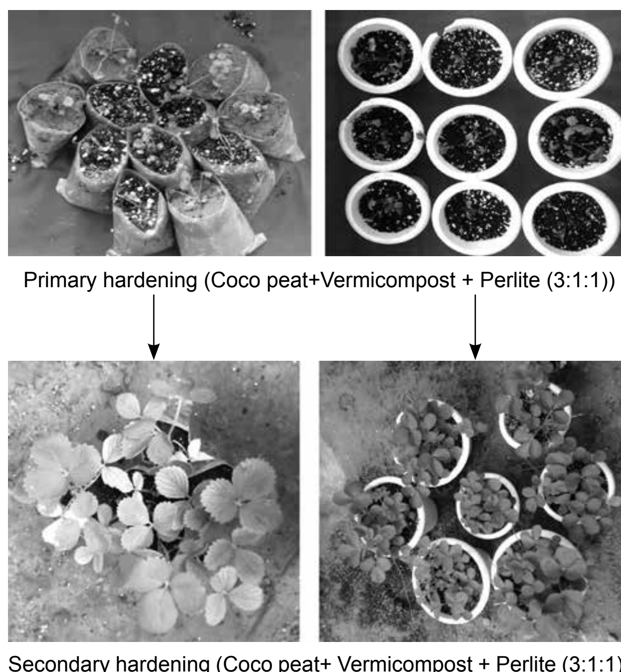

Fig. 5. Stages of hardening of callus derived plants of strawberry.

induction to produce plantlets with maximum number of shoots and shoot length. Besides, *in vitro* plantlets showed high root regeneration and root formation which is important for hardening and successful growth of plantlets under field conditions.

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Received : November, 2018; Revised : December, 2019; Accepted : February, 2020