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EFFECT OF EXPLANT SOURCES AND DIFFERENT CONCENTRATIONS OF PLANT GROWTH REGULATORS ON *IN VITRO* SHOOT PROLIFERATION AND ROOTING OF AVOCADO (*PERSEA AMERICANA* MILL.) CV. "FUERTE"

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Abstract

In vitro shoot proliferation and rooting capacity of avocado cv. Fuerte was tested by using two different types of explant sources i.e., apical and axillary buds with varying concentrations of BAP and IBA on MS media. Explants exhibited a different response towards shoot proliferation with highest shoot number by axillary buds (2.50). By contrast, the apical buds yielded fairly positive results for shoots length (2.16 cm) than axillary buds. A good response was observed in axillary buds with 1.0 mg l^{-1} BAP (T_3) which lead to the best rate of shoot multiplication (4.80), whereas 1.5 mg l^{-1} BAP (T_4) favored the good shoot length development (4.06 cm) with apical buds. Among various BAP treatments, both T_3 (1.0 mg l^{-1}) and T_4 (1.5 mg l^{-1}) had a promotory effect on mean shoot number (3.23) and shoot length (2.91 cm). Rooting study revealed that explants also showed a variation in their rooting potential as apical buds proved to be superior with maximum 29.19% rooting, 2.15 roots per explant and 2.11 cm root length however, axillary buds did not have a significant affect on these parameters. Apical buds in interaction with 1.0 (T_3) and 1.5 mg l^{-1} IBA (T_4) gained a higher rate of 53% rooting and 3.2 roots per explant while at 0.5 mg l^{-1} IBA (T_2) they resulted in an outcome of 3.58 cm root length. Within IBA treatments; T_2 (0.5 mg l^{-1}), T_3 (1.0 mg l^{-1}) and T_4 (1.5 mg l^{-1}) gave 3.4 cm root length, 40% rooting and 2.81 mean root number, respectively.

Introduction

Avocado (*Persea americana* Mill.) is a major fruit crop of tropics and subtropics, which is highly important from economical point of view with a luxurious nutritional vlaue. Various attempts have been made to multiply avocado by tissue culture techniques (Premkumar *et al*., 2003); however *in vitro* propagation of this species has been proved quite difficult in a number of studies (Castro *et al*., 1995; Premkumar *et al*., 2003; Nhut *et al*., 2007). An understanding of the factors controlling *in vitro* developmental process is essential for the establishment of an efficient regeneration system. Shoot proliferation ability of plant tissues and the formation of adventitious roots depend upon the interaction of several endogenous and exogenous factors (Ahmed, 2002). Physiological status of an explant, concentration of plant growth regulators in culture medium and their mutual interaction are one of the major determinants among these factors (Palanisamy & Kumar, 1997; Litz *et al*., 2005). Ontogeny of explants and their position on mother plant greatly affects the *In vitro* development and according to Chern *et al*., (1993), different explant sources have different growth potential due to differences in age, endogenous metabolic status and differential genome. Shoot regeneration is considerably difficult to achieve in avocado from shoot tips and nodal explants with limited shoot proliferation, elongation of existing buds and formation of scaly leaves (Barringer *et al*., 1996).

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Problems of necrosis and vitrification have also been reported particularly with mature tissues (Ahmed *et al*., 1997) while the use of juvenile material has given the positive results (Barcelo-Munoz *et al*., 1999). Moreover, *In vitro* root formation is a major problem in avocado which has also been reported to be correlated with the position of buds on a tree and the existence of juvenility gradient in them (Palanisamy & Kumar, 1997). In addition to the explant source, plant growth regulators also have stimulatory effects on shoot regeneration and root induction capacity of plants. Therefore, in the present study an attempt was made to develop a protocol for *In vitro* propagation of avacado cv. "Fuerte" with the aim to identify the most suitable explant source (apical or axillary buds) and the best concentration of growth regulators for successful shoot proliferation and rooting.

Materials and Methods

Apical and axillary buds of Avocado cv. "Fuerte" were obtained during spring 2007 from the germplasm unit of Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi. Apical and axillary buds, about 2-3 cm in size were placed under running tap water for about half an hour to clean the surface dirt and dust (Hutchinson, 1984). Then plant material was disinfected by using Sodium hypochlorite (NaOCl) solution @ 0.5, 1.0, 1.5 & 2.0% (w/v), with continuous agitation for 10 minutes. The sterilizing solution was decanted and the explants were given 3 rinses (5 min., each) with distilled autoclaved water, using flamed aseptic forceps. The larger outer leaves and most of the brown portions of the explants were cut off without damaging the growing buds, making the final size about 1.0-1.5 cm in length (Zimmerman, 1984). Explants after surface disinfection were cultured individually in 25 ml test tubes containing 8 ml of modified MS medium (Murashige & Skoog, 1962) with macro & micro salts at 75% strength, supplemented with MS vitamins, 30 g l^{-1} sucrose and 6.5 g l^{-1} agar. The pH of the culture medium was adjusted to 5.8 before autoclaving at 121°C for 15 minutes. Observations were recorded for the percentage of necrosis, infection and survival.

For shoot proliferation, both apical and axillary buds about 1-1.5 cm in length, from established cultures were individually implanted in culture jars containing MS medium (75% macro and micro salts), supplemented with MS vitamins, 40 mg l^{-1} arginine and glutamine each, 30 g l^{-1} sucrose and 6.5 g l^{-1} agar. BAP was added to the media @ 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg l^{-1} to compare the effect of BAP levels on both apical and axillary buds regarding the number of shoots per explant and shoot length (cm). The pH of the culture medium was adjusted to 5.8 before autoclaving at 121°C for 15 minutes. Experiment was a two factor design (explant x BAP concentration) laid out in Completely Randomized Design (CRD) with three replications per treatment and five shoots per replication.

To achieve rooting, uniform sized shoots (1.5 cm) bearing two leaves from both apical and axillary buds were excised from proliferated cultures. Individual shoots were transferred to culture jars having MS medium (75% macro and micro salts), supplemented with MS vitamins, 30 g l^{-1} sucrose, 6.5 g l^{-1} agar and with IBA @ 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg l^{-1} to study the comparative role of IBA doses in rooting of apical and axillary buds. The pH of the rooting medium was adjusted to 5.8 before autoclaving. Data were recorded for the rooting percentage, number of roots per explant and root length (cm) after four weeks of culturing. The rooting experiment was also bifactorial (explant x IBA concentration) randomized in CRD with three replications per

treatment and 5 shoots per replication. All cultures were incubated at 25±ºC under 16/8 hr photoperiod (2,000 lux) with white fluorescent tubes (Philips TL 40W/54). Statistical analysis of the data was carried out by using analysis of variance (ANOVA) technique and differences among treatment means were compared by using Least Significance Difference (LSD) test at 5% probability level (Steel *et al*., 1997).

Results and Discussions

Effect of different NaOCl concentrations on establishment and disinfection of explants: Prior to *in vitro* culture establishment, it is necessary to remove foreign contaminants including bacteria and fungi; however, it is very difficult to obtain sterile plant material completely free from contamination. It becomes more problematic while dealing with woody plant material (Niedz & Bausher, 2002). In fact, woody plants are grown in soil for many years under ambient conditions and hence are routinely infected heavily with microorganisms both endogenously and exogenously, which are often difficult to control *in vitro* (Ahmad *et al.*, 2003). For *in vitro* culture establishment, apical and axillary explants of avocado were disinfected with various concentrations of NaOCl. It is evident from the data (Table 1) that 1% NaOCl significantly increased the survival percentage of apical and axillary buds i.e, 56% and 48% respectively. It was observed that contamination rate was highest in apical (66%) and axillary buds (72%) at 0.5% NaOCl. As its concentration increased up to 1.5%, contamination rate drastically fell to 19% in apical and 24% in axillary buds and survival percentage was 26% and 16% in apical and axillary buds respectively. Whereas at 2.0% NaOCl contamination rate became negligible but due to necrosis, 100% mortality was recorded. Results depicted that NaOCl effectively control the rate of contamination; however, its higher concentration badly damages the explants. Similar results were reported by Moutia & Doukin (1999) that high concentration of NaOCl proved to be toxic resulting in 100% necrosis and death of explants. For *in vitro* culture establishment, it is of prime importance to find safe sterilization agent that can remove the fungus and bacteria from the explant tissue (Ahmad *et al*., 2003). Thus it can be concluded from the results that 1% NaOCl found to be more effective for control of contamination in both explants, with minimum detrimental effects.

In vitro shoot proliferation

Number of shoots per proliferated explant: Analysis of the data revealed that axillary buds proved to be better than apical ones for the mean number of shoots per proliferated explants (Table 2). Maximum number of shoots (2.50) was observed in axillary buds which was significantly higher than that produced by apical buds (1.58) at $p<0.05$. Different responses shown by different explants may be due to the activation of shoot multiplication signal (SMS) in different sites. SMS was identified as a branching factor in highly branched mutants of petunia (Snowden *et al.*, 2005). Conversely the presence of higher levels of auxins inhibits the action of SMS and act as a shoot branching inhibitor (Foo *et al.*, 2005). Hence, it may be possible that in apical buds, SMS act as a shoot branching inhibitor due to the presence of high endogenous levels of auxins. On the other hand, SMS associated with lower concentration of auxin in axillary buds may act as a shoot branching factor.

Table 1. Effect of different levels of NaOCl on percentage of necrosis, infection and survival of cultures established from apical and axillary buds of avocado cv. Fuerte.

Explant source	NaOCl solution (% w/v)	Necrosis (%)	Infection (%)			Survival (%)
			Bacterial	Fungal	Total	
Apical buds	0.5	6	45	20	66	28
	1.0	10	22	12	34	56
	1.5	55	12	7	19	26
	2.0	98	3	2	5	0
Axillary buds	0.5	8	47	25	72	20
	1.0	12	25	15	40	48
	1.5	60	15	9	24	16
	2.0	100	5	4	9	0

Table 2. Effect of different explant sources and BAP concentration on number of shoots per explants and shoot length (cm) of avocado cv. Fuerte.

Treatment BAP (mg l^{-1})	Number of shoot per explant		Mean	Shoot length (cm)		Mean
	Apical buds	Axillary buds		Apical buds	Axillary buds	
T_1 0.0	1.0 j	1.0 j	1.00 E	1.06 d	1.00 d	1.03 C
T_2 0.5	1.20 i	1.43 h	1.31 D	1.70 cd	1.23 d	1.46 C
T_3 1.0	1.66 g	4.80 a	3.23 A	2.90 b	2.43 bc	2.66 AB
T_4 1.5	2.50 d	3.96 b	3.23 A	4.06 a	1.76 cd	2.91 A
T_5 2.0	1.83 f	2.73 c	2.28 B	2.46 bc	1.63 d	2.04 B
T_6 2.5	1.63 g	2.30 e	1.96 C	1.56 d	1.50 d	1.53 C
T_7 3.0	1.26 i	1.26 i	1.26 D	1.36 d	1.36 d	1.36 C
Mean	**1.58 B**	**2.50 A**		**2.16 A**	**1.56 B**	
$LSD_{5\%}$	Explant	Interaction	Treatments	Explant	Interaction	Treatments
	0.04	0.11	0.09	0.29	0.078	0.55

Means followed by the same letter are not statistically different.

Statistical analysis showed that the interaction between the explant sources (apical and axillary buds) and BAP was significant at $p<0.05$ for number of shoots per proliferated explants (Table 2). In axillary buds maximum number of shoots per proliferated explant (4.8) was recorded at 1.0 mg l^{-1} BAP (T_3); while in apical bud maximum number of shoots (2.50) per proliferated explant was recorded at 1.5 mg l^{-1} BAP (T_4) as shown in Fig. 1a & b. Poor results were obtained at BAP free media (T_1) with no adventitious shoot in the apical and axillary buds. This shows that BAP is necessary for shoot development and multiplication. The possible reason of early response of axillary buds toward BAP might be that the axillary buds are rich in endogenous BAP so they show better response at relatively lower concentration of BAP (1.0 mg l^{-1}). Cytokinin is being synthesized by roots and travels acropetally; therefore, axillary buds are rich in cytokinins due to presence at relatively lower position on the mother plant in contrast to apical buds.

Treatments differed significantly with regards to their effects on shoot number. Maximum number of shoots per proliferated explant (3.23) was obtained at 1.0 and 1.5 mg l^{-1} BAP (T_3 & T_4 respectively). At very low concentration of BAP (0.5 mg l^{-1}) or at very high concentration of BAP (3.0 mg l^{-1}) the shoot number was less. The shoots exhibited slightly different morphology at the high concentration of BAP (3.0 mg l^{-1}) as both apical and axillary explants gave dense clumps of new shoots with a lot of axillary buds but showed no shoot elongation (Fig. 2a, b). Identical symptoms were recorded in *Gerenia jasmonoides* by Chuenboonngarm *et al*., (2001). Hu & Wang (1983) provided evidence in this concern and stated that higher concentration of cytokinin reduced the number of shoots in micropropagation. Results show that by increasing BAP concentration shoot number of both explants (apical and axillary buds) increased up to T_4

(1.5 mg l^{-1} BAP), showing a positive relation between BAP and shoot number after which it starts declining with further increase in BAP concentration. Therefore, selection of proper concentration of plant growth regulator is critical to shoot regeneration. An optimal concentrartion of cytokinin results in marked increase not only in RNA but also in DNA and protein synthesis leading to initiation of shoot primodia (Mok & Mok, 2001).

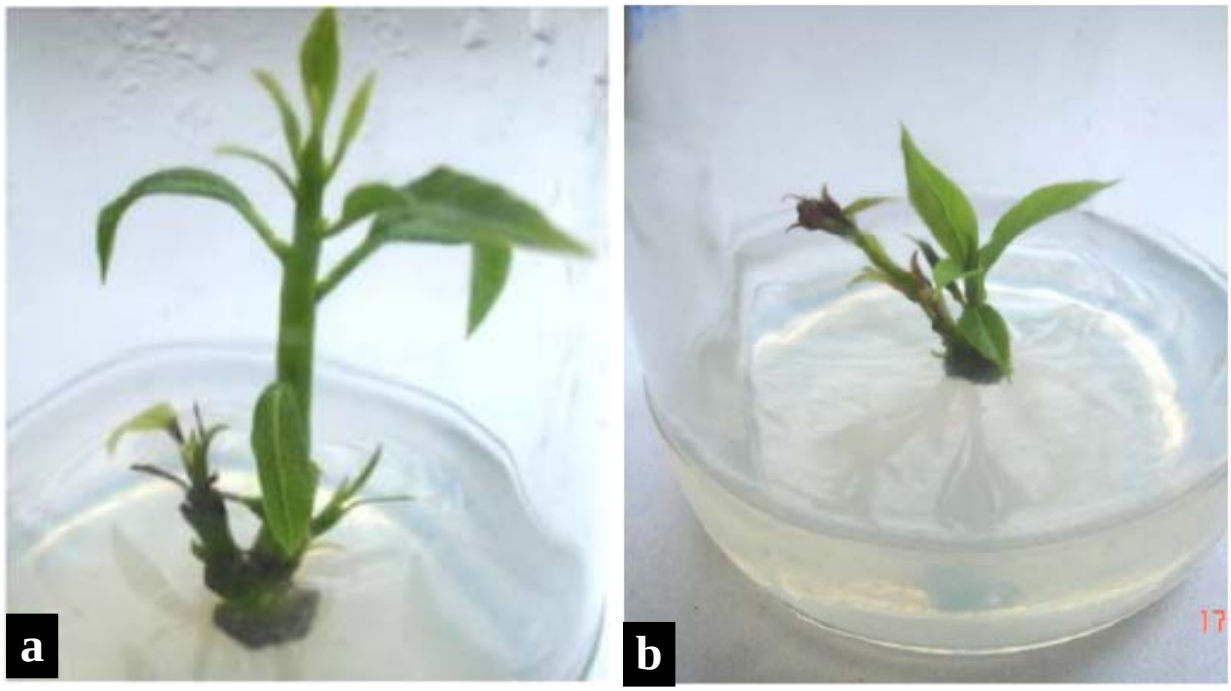

Fig. 1. (a): Highest number of shoots per proliferated explant produced by axillary buds at T_3 (1.0 mg l^{-1} BAP) (b): Maximum number of shoots per proliferated explant given by apical buds at T_4 (1.5 mg l^{-1} BAP) which is comparatively less than axillary buds.

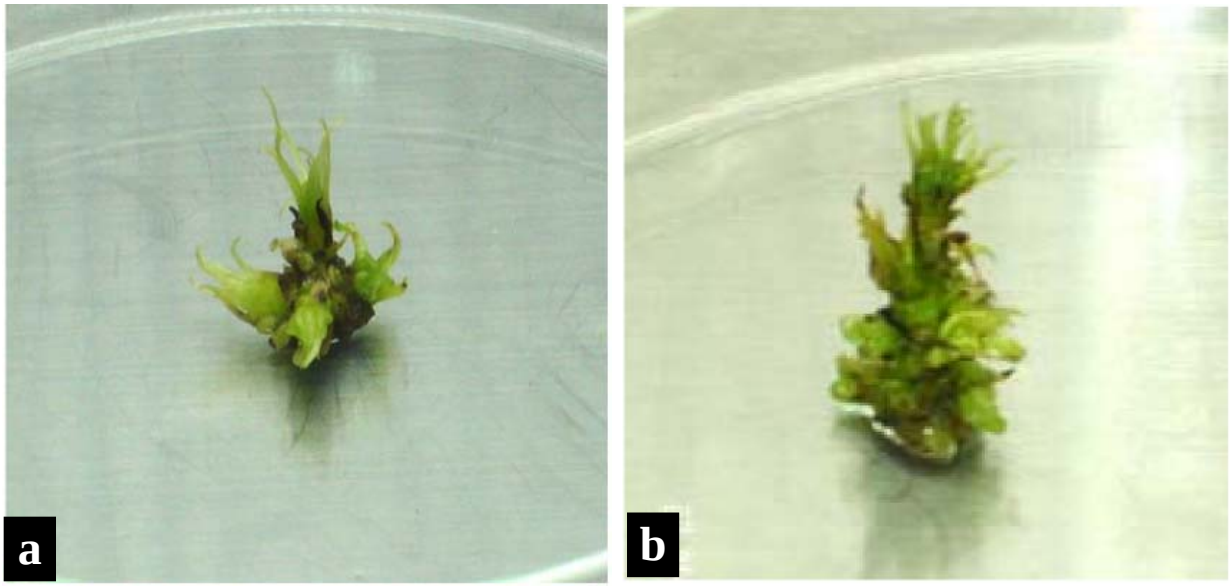

Fig. 2. Change in shoot morphology at 3.0 mg l^{-1} BAP (T_7). (a): Axillary bud showing swollen shoot with lateral buds having no shoot elongation. (b). Apical buds with dense clumps of axillary buds with no shoot elongation.

Shoot length (cm): Statistical analysis showed significant differences between the responses of apical and axillary buds for the mean shoot length at $p<0.05$ (Table 2). Apical buds showed significantly better response (2.16 cm) with respect to mean shoot length as compared to axillary buds (1.56 cm). The differential response of apical and axillary buds may be due to the difference in morphogenetic potential and their inherent capabilities. Likewise, Palanisamy & Kumar (1997) and Barcelo-Munoz *et al*., (1999) reported that the choice of an appropriate explant is critical for success in morphogenesis and the ability of various types of tissues to respond for regeneration depends on their inherit capabilities. Moreover, Han *et al*., (1997) compared the *in vitro* reactivity of explants of black locust from crown branches with basal sprouts of the same mature tree. They concluded that shoots derived from the top branches showed more rapid growth than the shoots derived from epicormic branches. Differential meristematic activity within different plant parts with respect to position, may also suggest the different regeneration potential of the buds. It is probable that the better response of apical buds may be due to higher meristematic activity as compared to axillary buds. In this respect, Piccioni & Standardi (1995) stated that meristematic activity in meristem of apical buds is greater than axillary buds. Further, it may be hypothesized that accumulation of different inhibitors may occur in axillary buds leading to reduced length. Suzuki (1990) observed that buds situated lower on mulberry shoots are more inhibited as compared to the buds present on the top. He detected abscisic acid and phenolic compounds accumulation in the axillary buds and attributed the growth inhibition to these inhibitors; which generally induce a shortening of the internode of higher plants *In vivo* (Hazarika, 2003). Furthermore, Monaco *et al*., (1995) reported that phenolic compounds frequently develop browning and are well known to be inhibitory to plant's cellular growth.

Statistical analysis revealed that the interaction between the explant source (apical and axillary buds) and BAP concentrations was significant at $p<0.05$ for shoot length (Table 2). The maximum shoot length i.e., 4.06 cm was achieved from apical buds (Fig. 3a) at 1.5 mg l^{-1} BAP (T_4). However, in axillary buds maximum shoot length of 2.43 cm (Fig. 3b) was recorded when the concentration of BAP was used at 1.0 mg l^{-1} (T_3). Poor response was given by apical and axillary buds both at lower concentration of 0.5 mg l^{-1} BAP (T_2) as well as at the higher concentration of 3.0 mg l^{-1} BAP i.e., T_7 (Figs. 4a, b & 5a, b). Results directed towards a conclusion that both the explants show better response towards BAP with maximum attainment of shoot length but after reaching an optimum concentration, shoot length of both explants declined. It may be possible that cells with in different explants are at different stages of differentiation. Cheristianson & Warnick, (1988) reported that cells in different differential stages mediated differently by plant growth regulators. Moreover, Rinne *et al*., (1994) declared that level of plant hormones varied from basal to apical portions. Apical buds have comparatively higher endogenous auxins as compared to axillary buds (Palanisamy & Kumar, 1997). Similarly, Taiz & Zeiger (2002) stated that shoot apex serves as a primary source of auxins and play a role in shoot elongation. Another possible cause for better response of apical buds may be due to the reason that endogenous auxin synergistically interact with the exogenously applied BAP at 1.5 mg l^{-1}(T_4), which implies that at this concentration cytokinin : auxin ratio is most appropriate to fulfill the requirements of shoot elongation. In contrast with axillary buds, maximum shoot length (2.43 cm) was achieved at low concentration of BAP (1.0 mg l^{-1}), because of the reason that axillary buds have comparatively higher endogenous cytokinins and lower auxins (Taiz & Zieger, 2002). The possible reason of deprived shoots in axillary buds may be due to the deficiency of auxins which in turn is responsible for cell enlargement and consequent shoot elongation. Furthermore, Gomez & Segura (1994) stated that apical explants are more responsive to cytokinin than the axillary explants.

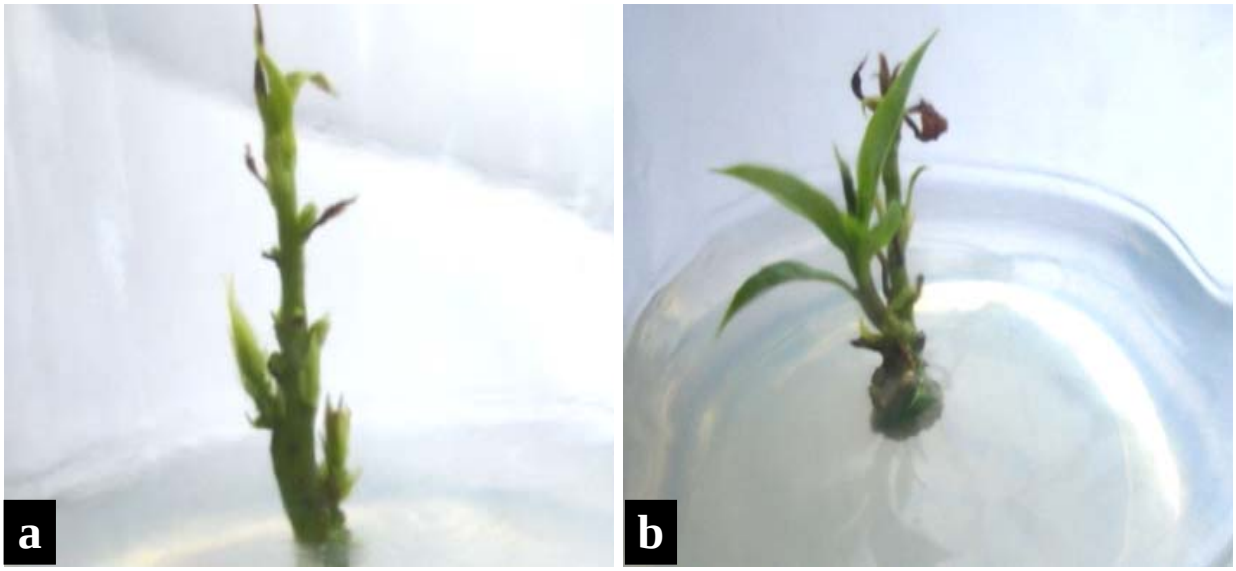

Fig. 3. (a). Showing better shoot length achieved by apical buds at T_4 (1.5 mg l^{-1} BAP). (b): Gain of diminutive shoot length by axillary buds in comparison to apical buds at T_3 (1.0 mg l^{-1} BAP).

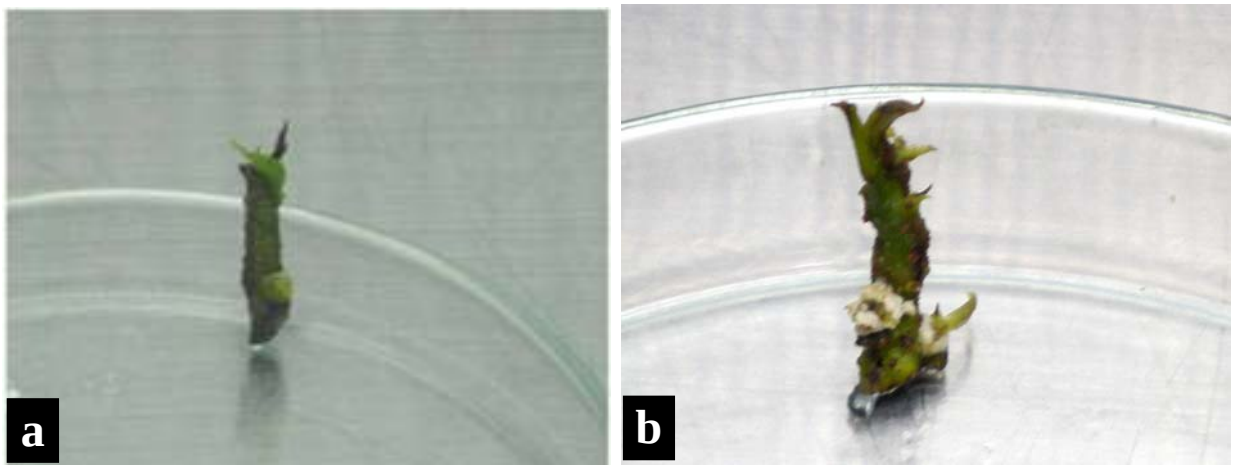

Fig. 4. (a): Poor results regarding shoot development in apical buds at low concentration of 0.5 mg l^{-1} BAP (T_2) (b): stunted growth of apical buds at high concentration of 3.0 mg l^{-1} BAP (T_7).

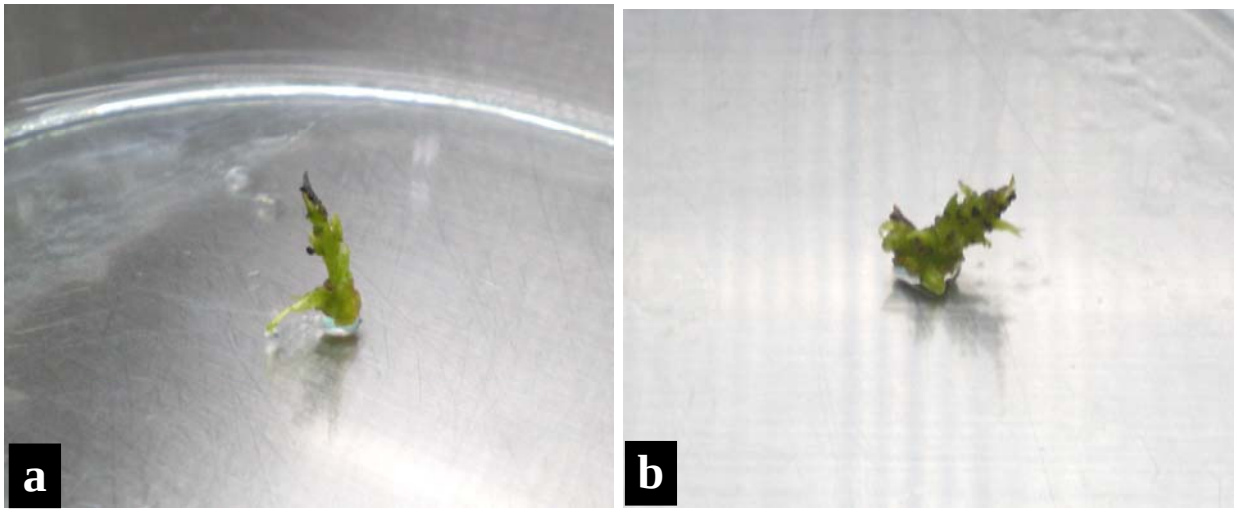

Fig. 5. Abnormal shoot development by axillary buds at (a): 0.5 mg l^{-1} BAP (T_2) and (b): 3.0 mg l^{-1} BAP (T_7).

Among the different treatments, 1.5 mg l^{-1} BAP (T_4) showed the best results (2.91 cm) than other treatments. There was an increasing trend in the shoot length up to 1.5 mg l^{-1} BAP (T_4) however, further increase in the BAP concentration showed negative effect on shoot elongation. It may be inferred from the above results that cytokinins play a critical role in the regeneration process; however, its either low or higher than optimal concentrations show the poor interaction with both explants (apical and axillary buds). Different concentrations of cytokinin significantly influence the shoot elongation by their effect on cell division and cell expansion. Reduction in shoot length by high concentration of BAP might be due to the toxic effects of ethylene, produced at high cytokinin concentration. These results are in line with those of Thomas *et al*., (2004) who reported that the production of ethylene by the excessive cytokinins caused the inhibition of internode elongation and number of regeneration of tobacco disc and suggested this as the example of the interdependence of hormonal regulatory pathways.

In vitro rooting

Rooting percentage: Statistical analysis revealed that apical buds proved to be more responsive toward rooting treatments than axillary buds (Table 3). Apical buds gave 29.19% rooting while axillary buds exhibited comparatively low rooting percentage (19%). Similarly Cheng *et al*., (1992) also compared the activity of apical and axillary buds for rooting capacity and found higher rooting percentage in apical buds as compared to axillary ones. Variation in the response of apical and axillary buds may be due to the slight change in the endogenous phytogradients between the explants. It may be possible that apical buds are rich in phytogradients that enhance rooting percentage. Gomez *et al*., (1995) found peroxidase activity in apical buds of avocado and its involvement in the regulation of auxin during rooting process was highlighted by Berthon *et al*., (1989). They also found that peroxidase activity is associated with the cambial cell division and differentiation which are primary events in the process of root formation.

Maximum rooting percentage (53) was recorded in apical buds when low concentration of 1.0 mg l^{-1} IBA (T_3) was used; however, higher rooting rate of 41% was observed in axillary buds at high concentration of IBA i.e., 1.5 mg l^{-1} (T_4). Apical buds were perceived as early responsive towards IBA. The possible reason behind the different response of explants may be due the fact that different explants have different potential to respond for growth hormones. Kulkarni *et al*., (2000) stated that the threshold concentrations of growth regulators required for organogenetic induction and optimal response differed for different explant types.

Among the different treatments, IBA at the concentration of 1.0 mg l^{-1} (T_3) and 2.0 mg l^{-1} (T_4) proved better for rooting percentage. Lowest rooting rate was recorded at 3.0 mg l^{-1} (T_7) which gave only 13% rooting; however, control condition (T_1) resulted in all the cuttings to be rootless. The results revealed that auxins play a major stimulatory role in induction of roots. The optimum concentration of auxins are known be to involved in cell enlargement, thought to be the controlling factor in rooting process (Tsipouridis *et al*., 2006); however, either its sub optimal or supra optimal concentration may badly effect the rooting percentage. According to Hartmann *et al*., (1997), sub optimal concentration of IBA results in an inhibition of free endogenous IAA activity by IAA oxidase with a subsequent decrease in out growth of root meristemoides. In the same way high concentration (3.0 mg l^{-1}IBA) also leads to inhibition of root primodium organization.

Table 3. Effect of different concentrations of IBA on rooting percentage (%) of apical and axillary buds of avocado cv. Fuerte.

Treatments IBA (mg l^{-1})	Rooting percentage (%)		Mean
	Apical buds	Axillary buds	
T_1 (0.0)	0.0 l	0.0 l	0.0 E
T_2 (0.5)	25 f	19 h	22 C
T_3 (1.0)	53 a	28 e	40 A
T_4 (1.5)	40 b	41 b	40 A
T_5 (2.0)	38 c	22 g	30 B
T_6 (2.5)	31 d	14 j	22 C
T_7 (3.0)	17 j	9 k	13 D
Mean	**29.19 A**	**19.05 B**	
$LSD_{5\%}$	Explant	Interaction (E×T)	Treatments
	0.48	1.27	0.90

Means followed by the same letter are not significantly different at $p<0.05$

Table 4. Effect of different explant sources (apical and axillary buds) and IBA concentrations on number of roots per rooted explant of avocado cv. Fuerte.

Treatments IBA (mg l^{-1})	Number of roots per explants		Mean
	Apical buds	Axillary buds	
T_1 (0.0)	0.0 i	0.0 i	0.0 E
T_2 (0.5)	1.8 g	1.4 h	1.66 D
T_3 (1.0)	3.2 a	2.2 ef	2.71 A
T_4 (1.5)	2.83 b	2.8 bc	2.81 A
T_5 (2.0)	2.6 c	2.4 d	2.5 B
T_6 (2.5)	2.3 de	1.8 g	2.1 C
T_7 (3.0)	2.16 f	1.33 h	1.75 D
Mean	2.15 A	1.72 B	
$LSD_{0.05}$	Explant	Interaction (E×T)	Treatments
	0.06	0.18	0.12

Any two means not sharing a letter differ significant at $p<0.05$

Number of roots per explant: Apical buds showed significantly ($p<0.05$) better root development, manifesting superiority over axillary buds with respect to root number (Table 4). The average root number per rooted explant obtained in apical bud was 2.15, while axillary bud gave only 1.72 roots per explant. Different response of apical and axillary buds may be linked to some physiological differences between them; moreover, variations in the biochemical status of explants may also influence their morphogenetic potential (Han *et al*., 1997). Palanisamy & Kumar (1997) compared the apical and axillary bud performance for root formation and achieved higher root number in apical buds compared to axillary ones. They attributed this better response of root formation in apical buds to higher concentration of endogenous auxins.

Observations regarding the interaction between various IBA concentrations and explant types revealed that apical buds gave maximum number of roots per rooted explant (3.2) when IBA was used at concentration of 1.0 mg l^{-1} i.e., T_3 (Fig. 6a). While in axillary buds maximum root number per rooted explant (2.8) was attained at relatively higher concentration (1.5 mg l^{-1}; T_4) of IBA (Fig. 6b). However, rooting was not observed in the absence of IBA application, either in apical or axillary buds. It is inferred from the results that in both explants (apical and axillary buds), root number per rooted explant showed the tendency to increase with increasing concentration of IBA up to 1.5

mg l^{-1} (T_4) and 1.0 mg l^{-1} in axillary and apical buds respectively. However, the higher concentration (>1.0 mg l^{-1} in apical and >1.5 mg l^{-1} in axillary bud) than the optimal exerted the inhibitory effect on the number of roots produced per rooted explant. The possible reason of this better response of apical buds at relatively low concentration of IBA (1.0 mg l^{-1}) may be due to the fact that apical buds are already rich in endogenous auxins as compared to axillary buds.

Among different treatments, T_3 (1.0 mg l^{-1} IBA) and T_4 (1.5 mg l^{-1} IBA) gave improved results by giving 2.7 and 2.8 number of roots per rooted explant respectively. Whereas, T_2 (0.5 mg l^{-1} IBA) and T_7 (3.0 mg l^{-1} IBA) gave poor results. Explants cultured in the medium without IBA (T_1) did not show any response to root development. Above results provide an evidence that optimum concentration of auxins is very critical for better rooting response. Likewise Preece & Read (2005) reported that optimum concentration of IBA plays an efficacious role in early dedifferentiation of xylem with subsequent development of root initials. Moreover, it stimulates the individual quiescent cells in the pericycle to differentiate and proliferate to form roots primodium. An inhibitory effect of auxins was also observed when explants were exposed to a too high concentration of IBA. Baker & Wetzstein (1994) reported that higher concentration of auxin induces the higher level of degradative metabolites in tissue thus blocking the regeneration process.

Root length: Data regarding root length of avocado is given in Table 5 which shows significant difference (p<0.05) between explant sources. Increased root length (2.11 cm) was noticed in apical buds; however, shorter root length was apparent in the axillary buds (1.88 cm). Results agree with those found in *Azadirachta indica* by Palanisamy & Kumar (1997) who revealed a positive response of apical buds towards root length as compared to the axillary buds; however, results conflict with observations made by Volkaert *et al*., (1989) who stated that rooting ability is not influenced by explant source. It is probable that varying biochemical status or physiological state may lead to the variation between apical and axillary buds in terms of root primodia elongation. Cheng *et al*., (1992) stated that root formation is influenced by the physiological and biochemical status of individual plants. Furthermore, Han *et al*., (1997) stated that morphogenetic potential is dependant on biochemical status of source explant. Higher levels of polyamines in apical buds compared to axillary ones during rhizogenesis were detected by Rey *et al*., (1994). Moreover, Burtin *et al*., (1990) reported that root formation involves intensive mitotic activity and metabolic changes accompanied by changes in polyamine level. Polyamines are involved in a wide range of important processes including cell division, protein synthesis and DNA replication and play important role in various morphogenic responses (Bais & Ravishankar, 2002). Furthermore, Friedman *et al*., (1985) found that polyamines in combination with auxins, have a regulatory role in root development in mung bean cuttings.

The trend observed in the previous parameters of rooting percentage and root number was maintained here for the interaction between explants (apical and axillary buds) and IBA concentrations (Table 5). Apical buds produced the longest roots (3.58 cm) on the medium supplemented with 0.5 mg l^{-1} IBA (T_2), followed by axillary buds (3.3cm) at the same concentration of IBA. However, IBA free medium (T_1) failed to support rhizogenesis in both the explants. Results lead us to a conclusion that apical buds were more efficient in rooting response as compared to axillary buds, with respect to root length. Differential response of apical and axillary bud may be due to the change in differential uptake of IBA. Sujatha & Reddy (1998) also demonstrated the same reason that different response of growth regulators in apical and axillary buds is due to difference in uptake, recognition by the cells, or in the mechanism of action of IBA.

Table 5. Effect of different explant sources (apical and axillary buds) and IBA concentrations on root length of avocado cv. Fuerte.

Treatments IBA (mg l^{-1})	Root length (cm)		Mean
	Apical buds	Axillary buds	
T_1 0.0	0.0 j	0.0 j	0.0 G
T_2 0.5	3.58 a	3.3 b	3.4 A
T_3 1.0	3.10 c	2.71 d	2.91 B
T_4 1.5	2.76 d	2.41 e	2.59 C
T_5 2.0	2.3 e	2.07 f	2.20 D
T_6 2.5	1.73 g	1.49 h	1.61 E
T_7 3.0	1.3 i	1.18 i	1.24 F
Mean	2.11 A	1.88B	
$LSD_{0.05}$	Explant	Interaction (E×T)	Treatments
	0.52	0.139	0.098

Any two means not sharing a letter differ significant at $p<0.05$

Fig. 6. (a): Better root development with respect to root number in apical buds at 1.0 mg l^{-1} BAP (T_3) (b): Showing maximum root number in axillary buds at 1.5 mg l^{-1} BAP (T_4).

Similar to the previous parameter, treatments differed significantly with regards to their effects on root length. It is clear from the results (Table 5) that 0.5 mg l^{-1} IBA was most effective concentration for elongation of root primodium in both explants (apical and axillary buds) by giving 3.4 cm long roots. The root length progressively decreased on further increasing the IBA concentration from the optimal concentration (0.5 mg l^{-1}). Present results highlighted the crucial role of IBA in optimum concentration in root elongation. Optimum concentration of IBA is imperative for activation of expansion enzymes, for the explant cell wall loosening and extensibility leading to increase in root length (Hasnat *et al*., 2007). Furthermore, IBA is involved in the regulation of many aspects of cambial development and its presence is essential for procambial initiation, cambial cell division and primary cell wall expansion (Taiz & Zieger, 2002). A little

variation in response of explants at low and high concentrations of IBA revealed that growth and elongation of roots is extremely sensitive to the auxin concentration and root length tend to reduce with higher concentration than optimum level (0.5 mg l^{-1}). Inhibition of root elongation at supra optimal concentration may be linked with enhancement of ethylene biosynthesis. Hopkin (1995) stated that root length is particularly sensitive to excessive auxins because it inhibits root length due to ethylene production, a root growth inhibitor. Results corroborate with that of Ahmad *et al*., (2003) who indicated that root elongation phase was very sensitive to auxin concentrations and was inhibited by higher concentration.

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