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INFLUENCE OF MICROCUTTING SIZES AND IBA CONCENTRATIONS ON *IN VITRO* ROOTING OF OLIVE cv. 'DOLCE AGOGIA'

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Abstract

Different microcutting sizes and various levels of IBA @ 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75 and 2.0 mg l^{-1} were investigated for their effect on *In vitro* rooting of olive cv. 'Dolce Agogia' using modified olive medium. Among the various sizes of microcuttings, tetranodal was the most suitable with highest rooting percentage (55.85%), number of roots (3.48), root length (1.76 cm) and survival percentage (45.33). The interaction of tetranodal microcuttings was much influenced with 1.25 mg l^{-1}IBA as it resulted in a promising outcome of 95 .33% rooting, 5.61 roots per microcutting, 3.40 cm root length and survival percentage of 90.33%. IBA @ 1.25 mg l^{-1} (T_6) was superior to other treatments with fairly alluring response towards rooting. An ascending trend was observed in rooting with increasing IBA concentration up to 1.25 mg $l^{-1}$and in the same way, increase in the microcutting size was positively correlated with most favorable root development. Tetranodal microcuttings gave the best performance for *ex vitro* survival in comparison to uninodal, bi nodal and tri nodal microcuttings.

Introduction

Achievement of rooting continues to be a major constraint in vegetative propagation of many commercially important woody perennials (Durzan, 1988). In olive (*Olea europaea* L.), mist propagation of leafy stem cuttings is traditionally a prevailing technique for the production of self rooted trees. However, several problems affect its conventional propagation including seasonal constraints as the cuttings do not root properly out of a specific growing season; moreover, rooting capacity vary greatly among different cultivars (Lambardi, 1999). *In vitro* propagation techniques provide an opportunity for the rapid and large scale production of such cultivars which are difficult to produce by traditional methods together with the production of disease free stock material (Sharma *et al.,* 2003). Adventitious root formation of micropropagated shoots is an obligatory phase in plant regeneration and determines the efficacy of any *In vitro* plant production systems. The auxin treatment was one of the first factors to attract the attention of researchers for the rooting of shoots (Lambardi & Rugini, 2003) as it is known to be involved in rooting since long periods of time (Weaver, 1972) and the positive role of auxins on the induction and development of root primordia is well documented (Carboni *et al*., 1997; Frett *et al*., 2001). Among various auxins, IBA is most commonly used to stimulate rooting in microcuttings due to its weak toxicity and great stability (Weisman *et al.*, 1988; Hartmann *et al.,* 2007). Another irrefutable factor which affects the production of a well developed root system is the size of microcuttings as from the viewpoint of Eugene *et al.,* (2007), the initial size of microcutting is highly correlated with *In vitro* root development. Moreover, *In vitro* culturing requires enough monetary inputs that should be rationalized and it is essentially needed to use the explants more efficiently to achieve economic benefits. Hence, *In vitro* rooting of microcuttings is

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followed by an important step of *ex vitro* acclimatization because the benefit of micropropagation system can only be fully realized by successful acclimatization of plantlets to ambient field conditions, which is strongly affected by the quality of root system developed *In vitro*. Considerable efforts have been directed to optimize the conditions for different stages of micropropagation, but the process of acclimatization of micropropagated plants to the soil environment has not been fully studied. Consequently, the transplantation stage continues to be a major bottleneck in the micropropagation of many plants (Hazarika, 2003). Keeping in view all these factors, the present study was formulated to standardize a protocol for *In vitro* rooting and subsequent survival of olive cv. 'Dolce Agogia' under the autotrophic conditions.

Materials and Methods

Microcuttings of Olive (*Olea europea* L.) cv. 'Dolce Agogia' taken from stock cultures were proliferated on modified olive medium (Micheli *et al*., 2007). Different microcuttings sizes *viz.,* uninodal, binodal, trinodal and tetranodal; prepared from first four apical nodes of proliferated shoots were used as experimental material. To evaluate the rooting potential of these microcuttings, modified olive medium (half macro & micro elements) was supplemented with 100 mg l^{-1} brilliant black dye and with IBA @ 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75 and 2.0 mg l^{-1} to determine the comparative influence of these IBA levels on rooting aptitude of microcuttings. pH of the rooting medium was adjusted to 5.8 before autoclaving. It was a bifactorial experiment (growth regulator x microcutting sizes) laid out in completely randomized design (CRD) with three replications per treatment and 15 shoots per replication. Cultures were incubated at 25 ± 1^{o}C under 16-h photoperiod (2,000 lux) with white fluorescent tubes (Philips TL 40W / 54). Data pertaining to rooting percentage (%), number of roots per microcutting and root length (cm) was recorded after four weeks of culturing. *Ex vitro* acclimatization of rooted microcuttings was achieved by transfer to the pots containing mixture of soil: sand (1:1), placed in glass house having light intensity of 4000-10,000 lux. During acclimatization period maximum temperature recorded was 24.8 to 32.7^{o}C while minimum temperature was in the range of 15.2 to 22^{o}C with relative humidity of 90-95%. Statistical analysis of the data was done 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 Discussion

Rooting percentage: Significant differences were observed among various sizes of microcuttings at $p<0.05$ in terms of rooting rate (Table 1). Maximum rooting percentage (55.85%) was recorded by the tetranodal microcuttings followed by trinodal and binodal microcuttings, which produced 51.22 and 50.18% rooting respectively. While, minimum rooting percentage (46.77%) was recorded by uninodal microcuttings. The most probable reason for good rooting response by the tetranodal microcuttings might be due to more carbohydrate reserves as compared to uninodal, binodal and trinodal microcuttings. The formation of adventitious roots is a highly energy requiring process, which involves cell division, in which predetermined cells switch from their morphogenetic path to act as mother cells for the root primordia; hence, need more carbohydrates for root initiation (Aeschabacher *et al.,* 1994). Moreover, Haissig (1982) also reported that carbohydrate reserves in the microcuttings are the principle source of energy that helps root primordia initiation.

Tetranodal microcuttings showed best interaction with 1.25 mg l^{-1} IBA (T_6) and recorded 95.33% rooting and remained significantly superior to uninodal, binodal and trinodal microcuttings. While tri nodal microcuttings showed good interaction with 1.50 mg l^{-1} IBA (T_7) resulting in 85.66% rooting, followed by binodal and uninodal microcuttings, which showed good interaction at concentration of 1.75 mg l^{-1} IBA (T_8) and produced 76 and 66.66% rooting respectively. Tetranodal and trinodal microcuttings produced good results and may be preferable for rooting. This might be due to the synthesis of endogenous rooting co-factors in good amount such as morphogen (Wilson, 1994) and rhizocaline (Bouillenne & Bouillenne, 1955) that may assume to stimulate the formation of root initials at the optimum concentration of IBA. Maximum rooting by tetranodal microcuttings at 1.25 mg l^{-1} showed that, this optimum concentration of IBA along with endogenous root inducing co-factors might become a threshold level at which it starts to be metabolized to signal the process of root initiation (Nanda & Kochnar, 1987). Results also showed that uninodal, binodal and trinodal microcuttings produced maximum rooting percentage at greater concentration of IBA (>1.25 mg l^{-1}) as compared to tetranodal microcutting, which showed that level of endogenous root inducing factors, might be low in uninodal, binodal and trinodal microcuttings, therefore, they required more concentration of IBA for root primordia initiation.

IBA treatments were also significantly different for rooting percentage as exhibited by Table 1. Maximum rooting (66.83%) was obtained with 1.25 mg l^{-1} IBA (T_6), followed by 1.50 mg l^{-1} IBA (T_7) and 1.75 mg l^{-1} IBA (T_8) both of which produced 61.83 and 60.08% rooting respectively. Maximum rooting percentage with 1.25 mg l^{-1} is probably due to the reason that optimum concentration of IBA might be responsible to increase the cambial growth at the base of microcuttings that result in differentiation of root primordia. It is clear from the results that 1.50 and 1.75 mg l^{-1} IBA also proved good for rooting to the same extent because these two concentrations showed slightly better interaction with uninodal, bi nodal and tri nodal microcuttings. Achievement of minimum rooting (37.00 %) in control treatment (T_1) rather than complete inhibition of rooting indicates that endogenous auxin along with some root inducing factors might occur naturally within the microcuttings that may help for root primordia initiation. It is reported by Nordstrom & Eliasson (1991) that auxin is produced in the apex and moves basipetally to trigger the rhizogenesis. Furthermore, root inducing factors are believed to be essential for rooting, which combine with auxin to form a complex that directs RNA to activate enzymes that cause root initiation (Hartmann *et al.,* 2007).

Number of roots: Various microcutting sizes were statistically different from each other (p<0.05) regarding the number of roots per microcutting (Table 2). Tetranodal microcuttings produced maximum (3.48) number of roots followed by trinodal and binodal microcuttings, which developed 3.27 and 3.21 roots per microcutting respectively. Contrarily, uninodal microcuttings produced minimum number of roots i.e., 2.74. Tetranodal microcuttings produced more roots as compared to uninodal, binodal and trinodal microcuttings probably due to the possession of more number of nodes and axillary buds present on nodes. Breen & Muraoka (1974) have also reported that auxin responsible for root growth and development is one of the rooting cofactors produced in the nodes. It is further reported by Vander & Lek (1925) that hormone like substances are formed in the nodes and transported through phloem to the base of microcuttings, where they stimulate the root formation and development.

Table 1. Effect of microcutting size and different concentrations of IBA on rooting percentage of Olive cv. Dolce Agogia.

Treatment IBA (mg l^{-1})		Microcutting size				Mean
		Uninodal	Binodal	Trinodal	Tetranodal	
T_1	0.00	30.66 m	35.00 klm	39.33 jklm	45.00 ghij	37.00 F
T_2	0.25	34.00 lm	39.00 jklm	44.33 hijk	47.33 efghij	41.16 EF
T_3	0.50	39.33 jklm	43.66 hijk	49.00 defghi	51.33 defghi	45.83 DE
T_4	0.75	43.33 ijkl	47.00 fghij	52.33 defghi	53.00 defgh	48.91 CD
T_5	1.00	48.00 defghij	52.66 defghi	54.00 defg	56.00 def	52.66 C
T_6	1.25	57.33 cd	57.33 cd	57.33 cd	95.33 a	66.83 A
T_7	1.50	56.00 def	56.00 def	85.66 b	56.66 de	61.83 B
T_8	1.75	66.66 c	76.00 b	48.00 defghij	49.66 defghi	60.08 B
T_9	2.00	45.66 ghij	45.00 ghij	48.00 defghij	48.33 defghij	46.75 D
Mean		**46.77 C**	**50.18 B**	**51.22 B**	**55.85 A**	

$LSD_{5\%}$, Microcutting sizes = 3.19, Interaction (T x S) = 9.57, Treatment = 4.78
Means followed by the same letter are not significantly different at $p<0.05$

Table 2. Effect of microcutting size and different concentrations of IBA on number of roots of Olive cv. Dolce Agogia.

Treatment IBA (mg l^{-1})		Microcutting size				Mean
		Uninodal	Binodal	Trinodal	Tetranodal	
T_1	0.00	1.15 s	1.25 s	0.74 t	0.78 t	0.98 H
T_2	0.25	2.19 pq	2.28 p	2.06 q	1.76 r	2.07 G
T_3	0.50	2.76 o	2.83 no	2.86 no	3.75 hij	3.05 F
T_4	0.75	3.20 lm	2.84 no	2.99 mn	3.83 hij	3.21 E
T_5	1.00	3.20 lm	3.86 ghij	3.17 lm	4.06 c	3.71 D
T_6	1.25	3.70 hij	3.89 ghi	4.13 def	5.61 a	4.33 A
T_7	1.50	3.75 l	3.93 fgh	5.00 b	4.16 de	4.20 B
T_8	1.75	4.29 d	4.56 c	4.00 efg	3.83 hij	4.17 B
T_9	2.00	3.65 ijk	3.80 hij	3.89 ghi	3.51 k	3.71 C
Mean		2.74 C	3.21 B	3.27 B	3.48 A	

$LSD_{5\%}$, Microcutting sizes = 0.07, Interaction (T x S) = 0.21, Treatment = 0.10
Means followed by the same letter are not significantly different at $p<0.05$

Table 3. Effect of microcutting size and different concentrations of IBA on root length (cm) of Olive cv. Dolce Agogia.

Treatment IBA (mg l^{-1})		Microcutting size				Mean
		Uninodal	Binodal	Trinodal	Tetranodal	
T_1	0.00	0.70 t	0.93 st	1.12 qrs	1.00 rst	0.93 G
T_2	0.25	1.27 mno	1.44 kl	1.15 qrs	1.65 ghij	1.37 EF
T_3	0.50	1.31 mno	1.49 jk	1.25 nop	1.70 fgh	1.43 E
T_4	0.75	1.36 lmn	1.60 ghij	1.35 mno	1.85 de	1.54 D
T_5	1.00	1.37 lm	1.64 fghi	1.59 jk	1.86 de	1.61 D
T_6	1.25	1.82 def	1.65 fgh	1.90 de	3.40 a	2.19 A
T_7	1.50	1.85 de	1.67 efg	3.05 b	1.81 def	2.03 B
T_8	1.75	1.93 d	2.12 c	1.27 nop	1.55 ijk	1.72 C
T_9	2.00	1.47 kl	1.49 hijk	1.22 opq	1.08 pqr	1.31 F
Mean		1.43 C	1.53 B	1.54 B	1.76 A	

$LSD_{5\%}$, Microcutting sizes = 0.04, Interaction (T x S) = 0.14, Treatment = 0.07
Means followed by the same letter are not significantly different at $p<0.05$

Tetranodal microcuttings had the best interaction with 1.25 mg l^{-1} IBA (T_6) and produced 5.61 numbers of roots followed by trinodal microcuttings which showed good interaction with 1.50 mg l^{-1} IBA (T_7) and developed 5.00 roots per shoots. While uninodal and binodal microcuttings showed relatively good interaction with 1.75 mg l^{-1} IBA (T_8) and recorded 4.29 and 4.56 roots respectively. Best interaction by tetranodal microcuttings with 1.25 mg l^{-1} IBA suggested that, at this optimum concentration cells of the root apical meristem may continue to divide and differentiate into specialized competent cells that lead to the development and elongation of root apical meristem. Furthermore, optimum concentration of IBA may enhance the rooting *via* increased internal level of free IBA that modify the action of endogenous IAA by increasing its ability to promote cell division and elongation and leads to enhance the tissue sensitivity towards rooting (Vander *et al.*, 1993). It is also clear from the results that uninodal, binodal and trinodal microcuttings showed positive interaction at slightly higher concentration of IBA as compared to tetranodal microcuttings. This might be due to presence of fewer nodes on them, which may have insufficient endogenous auxin for the root formation and development. Therefore, need more concentration of IBA for cell division and differentiation of root apical meristem.

Among different treatments of IBA, maximum number of roots (4.33) was obtained with 1.25 mg l^{-1} IBA (T_6) followed by 1.50 mg l^{-1} IBA (T_7) and 1.75 mg l^{-1} IBA (T_8), which produced 4.20 and 4.17 roots respectively. The results suggested that 1.25 mg l^{-1} IBA (T_6) is most favorable for stimulation of rhizogenesis. It is reported by Bellamine *et al.*, (1998) that IBA has its primary role in the root formation due to successive and interdependent phases. Troncose *et al.*, (1972) found that optimum concentration of IBA enhance the cell wall extensibility with the release of H^+ that lowers the pH to accelerate the cell division and differentiation for the growth and development of root primordia. Further, Haissig (1982) found that the optimal concentration of IBA have an indirect influence by enhancing the speed of translocation and movement of sugar to the base of microcuttings and subsequently enhance the rooting process. The results also indicated that there is an increasing trend of rooting with increase IBA concentration up to a certain limit (1.25 mg l^{-1}), then showed a decline in root number beyond the optimum concentration. It is further reported by Baker & Wetzstein (2004) that higher concentration of IBA induces higher level of degradative metabolites in tissues which might lead to the blockage of root formation process.

Root length (cm): Tetranodal microcuttings produced longest roots (1.76 cm) as compared to uninodal (1.43 cm), binodal (1.53 cm) and trinodal (1.54 cm) microcuttings. Maximum root length in tetranodal microcuttings might be due to the ability of their cells to undergo more division and differentiation as compared to uninodal, binodal and trinodal microcuttings, most probably due to the development of more number of competent cells. It is reported by Marta *et al.*, (1995) that a mass of activated cells is required for root apical meristem division and elongation. This cell division and elongation is responsible for new tissues in the root elongation zone that leads to the regeneration of root cap around the periphery of quiescent zone resulting in overall increase in root length (Hopkins, 1995). Further, it is reported by Weier *et al.*, (1982) that in microcuttings with more number of nodes, the competent cells of cambium region divide actively and continuously that ultimately leads to the root development and elongation. The tetranodal microcuttings possessed the more number of leaves as compared to other microcuttings, which may also be responsible for root elongation and growth (Fig. 1). Hartmann *et al.*, (2007) documents that, leaves manufacture some root promoting substances which move downward to the base of stem, where they promote

root elongation. The longest root length by tetranodal microcuttings may also be due to more number of roots produced by these microcuttings, which absorbs more nutrients and ultimately produce more growth as compared to uninodal, binodal and trinodal microcuttings, which produced less number of roots, as root initiation and elongation both are energy requiring process that could occur only in the presence of carbohydrate reserves (Haissig, 1982; Thrope, 1982). The energy reserves in uninodal, binodal and trinodal microcuttings may be utilized only for root initiation and remain insufficient for elongation. On the other hand, tetranodal microcuttings due to their large size may have sufficient carbohydrate reserves required for both root initiation and elongation hence produced longer roots.

Statistically, a significant interaction was observed between microcutting sizes and different concentrations of IBA with regards to root length. Tetranodal microcuttings showed best interaction with 1.25 mg l^{-1} IBA (T_6) and formed the longest roots of 3.40 cm (Fig. 2), followed by trinodal microcuttings which produced 3.05 cm long roots at relatively higher concentration of 1.50 mg l^{-1} IBA (T_8) than tetranodal microcuttings. While uninodal and binodal microcuttings interact with 1.75 mg l^{-1} IBA (T_8) and produced 1.93 cm and 2.12 cm long roots respectively. Results clarify that uninodal and bi nodal microcuttings showed their positive interaction at comparatively higher concentration of IBA as compared to tetranodal microcuttings. It is confirmed by Blazich (1988) that redistribution of nutrients in the microcuttings during rooting was accelerated by optimum concentration of IBA. Moreover, Blazich (1988) state that mobilization and redistribution of nutrients leads to the synthesis of nucleic acid and proteins, which are necessary for root growth and development.

IBA treatments indicate significant differences regarding the length of roots. 1.25 mg l^{-1} IBA (T_6) produced the longest root (2.19 cm) followed by 1.50 mg l^{-1} IBA (T_7) and 1.75 mg l^{-1} IBA (T_8), which formed 2.03 cm and 1.72 cm long roots respectively. It is evident from the data that 1.25 mg l^{-1} IBA proved to be optimum concentration for producing longer roots. This might be due to the reason that tetranodal microcuttings gave maximum output at this concentration. The most favorable concentration of IBA induces cell enlargement by extruding protons actively into the cell wall region and resulting in a decrease "pH activating wall loosening enzyme" that promotes the breakage of key cell wall bonds and increase cell wall extensibility; hence, causing an increase in cell size and elongation (Taiz & Zeiger; 2006: Cleland, 1995). It is noticed that effect of IBA on root length is promotory up to 1.25 mg l^{-1} IBA and after that it shows a declining effect. This inhibition of elongation is currently associated with higher concentration of IBA (Mudy & Haworth, 1994) with the formation of lacunae and aerenchyma, which are often ascribed to increase ethylene biosynthesis in response to supraoptimal auxin concentration (Armstrong & Jackson, 1994). Further, Hopkins (1995) found that low levels of auxin are required for root elongation whereas at higher concentration it acts as a root growth inhibitor.

Survival percentage during acclimatization: Observations taken during the acclimatization stage revealed that there was a positive correlation between rooting percentage, number of roots, root length and survival percentage during the hardening phase. As tetranodal microcuttings remained dominant for all these parameters in comparison to uninodal, binodal and trinodal microcuttings (Table 4), consequently, they showed the highest survival percentage of 45.33% than those of uninodal (36.99%), binodal (40.18%) and trinodal (41.07%) microcuttings. Highest survival percentage in tetranodal microcuttings is certainly due to the development of more number of roots under *In vitro* conditions, which are necessary for *In vivo*

acclimatization. It is reported by Salisburry & Ross (1978) that roots help in absorption and movement of nutrients through the transportation stream with the help of water and maintain shoot-root balance, which is the most important factor for plant development *ex vitro*. The survived tetranodal microcuttings grew more quickly with lush green leaves and healthy stems up to a height of almost one feet (30 cm) within 6 months in glass house (Fig. 3). While uninodal, binodal and tri nodal microcuttings grew slowly and merely reached the stem length of 12 cm, 15 cm and 20 cm correspondingly in the same time interval (Fig. 4-a, b and c).

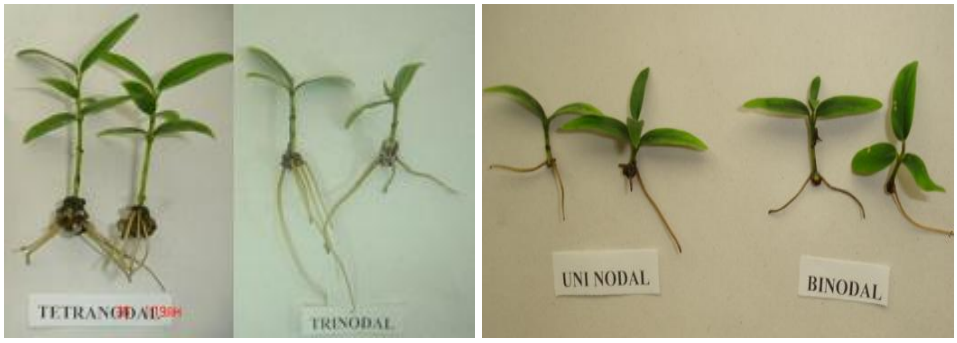

Fig. 1. Tetranodal microcuttings exhibiting more number of leaves as compared to tri nodal, bi nodal and uninodal microcuttings.

Fig. 2. Development of longest roots in tetranodal microcuttings at 1.25 mg l^{-1} IBA (T_6).

Fig. 3. Tetranodal micro cutting having 30 cm long stem with lush green leaves after six months in glass house.

There was a significant interaction between microcutting sizes and different concentration of IBA for survival percentage under the glass house conditions (Table 4). Tetranodal microcuttings showed the best interaction with 1.25 mg l^{-1} IBA (T_6) at which they had a maximum survival rate of 90.33% and proved significantly superior to all other microcuttings while trinodal microcuttings positively interacted with 1.50 mg l^{-1} IBA (T_7) to give 80.00% survived plants. Both uninodal and binodal microcuttings had

Table 4. Effect of microcutting size and different concentrations of IBA on survival percentage during acclimatization of Olive cv. Dolce Agogia.

Treatment IBA (mg l^{-1})		Microcutting size				Mean
		Uninodal	Binodal	Trinodal	Tetranodal	
T_1	0.00	15.66 n	16.00 mn	18.00 lmn	25.33 klm	18.75 G
T_2	0.25	24.00 klm	26.33 kl	28.00 jk	35.33 ghij	28.41 EF
T_3	0.50	28.33 jk	29.33 ijk	32.66 hijk	40.00 fgh	32.58 E
T_4	0.75	33.33 jk	35.00 ghij	38.00 fghi	45.33 ef	37.91 D
T_5	1.00	40.00 fgh	43.00 efg	48.66 e	49.00 e	45.16 C
T_6	1.25	45.33 ef	47.33 ef	50.33 e	90.33 a	58.33 A
T_7	1.50	46.00 ef	48.66 ef	80.00 b	40.66 fgh	53.83 B
T_8	1.75	60.66 d	70.66 c	40.00 fgh	38.00 fgh	52.33 B
T_9	2.00	39.66 fgh	45.33 ef	34.00 gh	35.00 ghij	38.49 D
Mean		36.99 C	40.18 B	41.07 B	45.33 A	

$LSD_{5\%}$, Microcutting sizes = 3.19, Interaction (T x S) = 9.57, Treatment = 4.78
Means followed by the same letter are not significantly different at $p<0.05$

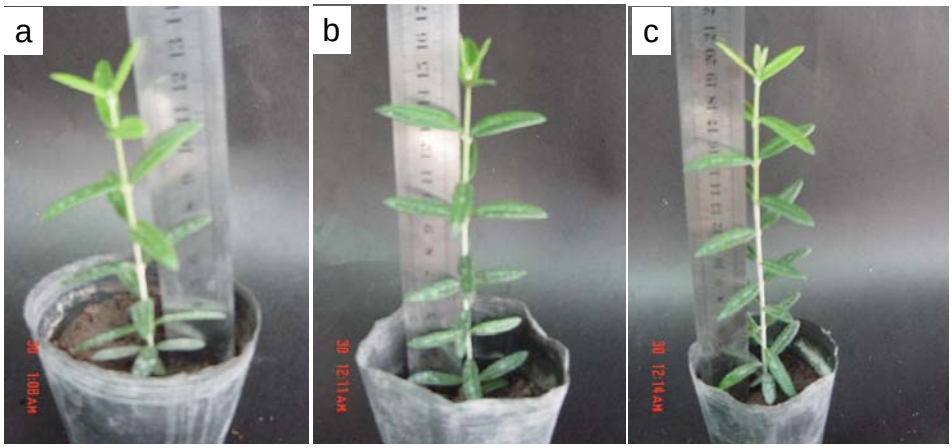

Fig. 4. Uninodal (a), binodal (b) and trinodal (c) microcuttings having 12 cm, 15 cm and 20 cm stem height after six months in glass house.

Fig. 5. Vigorous long roots produced at 1.25 mg l^{-1} IBA (T_6) consequently increasing the survival of microcuttings during acclimatization.

a quite fair interaction with 1.75 mg l^{-1} IBA (T_8) and produced 60.66 and 70.66% survived plants during the acclimatization phase. Hence, the results depict that tetranodal microcuttings rooted with 1.25 mg l^{-1} IBA proved to be more successful during acclimatization. This might be due to the reason that tetranodal microcuttings at this concentration produced highest rooting percentage (95.33%), more number of roots (5.61) and longest root (3.40 cm) under *In vitro* conditions than uninodal, binodal and tri nodal microcuttings. It is in accordance with Wiseman & Lavee (1994), who reported that survival of microcuttings during acclimatization is directly proportional to the number of roots formed *In vitro*. Moreover, the highest shoot length gained by tetranodal microcuttings during acclimatization may also be due to the possession of large number of roots in these cuttings (Table 2) as according to Hartmann *et al.*, (2007), roots are the site for synthesis of gibberellic acid (GA) which boosts up the elongation of shoots.

Different IBA treatments yielded significant results pertaining to the survival percentage of microcuttings. 1.25 mg l^{-1} IBA (T_6) gave the maximum survival percentage (58.33%), while 1.50 mg l^{-1} (T_7) and 1.75 mg l^{-1} IBA (T_8) yielded a survival rate of 53.83 and 52.33% respectively. It is clear from the results that 1.25 mg l^{-1} IBA is the most suitable concentration to achieve the highest survival percentage of the microcuttings. This might be due to the reason that IBA at this concentration produced the long vigorous roots with good quality (Fig. 5), which are important for successful growth of microcuttings *ex vitro*.

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