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IN VITRO PROPAGATION OF THREE ALMOND CULTIVARS AND THE ALMOND – PEACH HYBRID ROOT-STOCK"HANSEN"

[31]

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ABSTRACT

Stem node explants of three almond (*Prunus dulcis* Mill) cultivars (Om – Elfahm, M-Dalt and Ne Plus Ultra) and the hybrid rootstock "Hansen" cv. were successfully established and proliferated on Murashige and Skoog medium (MS) supplemented with benzyl adenine (BA) at 0.0 - 2.0 mgl $^{-1}$ and indol butyric acid (IBA) at 0.0 - 0.1 mgl $^{-1}$ .The highest bud development percentage of stem node explant was obtained with medium contained BA at 2 mgl $^{-1}$ with or without IBA at 0.1 mgl $^{-1}$ for M – Dalet and Ne Plus Ultra almond cultivars compared with other treatments. The longest shoot during establishment stage was recorded by "Hansen" rootstock cultured in MS with BA at 2.0 mgl $^{-1}$ plus IBA at 0.01 mgl $^{-1}$.Using BA at 1.0 mgl $^{-1}$ plus IBA at 0.1 mgl $^{-1}$ and BA at 2.0 mgl $^{-1}$ plus 0.05 or 0.1 mgl $^{-1}$gave the highest number of proliferated shoots for Om – Elfahm, M-Dalt and Ne Puls Ultra cvs. and "Hansen", respectively in the 3rd subculture. The best average proliferated shoot length was achieved with medium supplemented with BA at 1.0 mgl $^{-1}$ plus IBA at 0.05 mgl $^{-1}$ or 0.1 mgl $^{-1}$ for M-Dalt, Ne Puls Ultra and "Hansen" respectively, whereas, using BA at 2.0 mgl $^{-1}$ plus IBA at 0.1 mgl $^{-1}$ recorded the longest average proliferated shoot for Om- Elfahm cultivar. Microshoots were rooted in modified Bourgin and Nitsch medium supplemented with IBA or NAA at 1.0 mgl $^{-1}$ only or combination (0.2 IBA mgl $^{-1}$ + NAA 0.4 mgl $^{-1}$, IBA 0.4 mgl $^{-1}$ + NAA 0.8 mgl $^{-1}$ and IBA 0.5 mgl $^{-1}$ + NAA 1.0 mgl $^{-1}$). The large callus (more than 10 mm diameter) was recorded with "Hansen" rootstock and Om – Elfahm treated by IBA at 0.4 mgl $^{-1}$ plus NAA at 0.8 mgl $^{-1}$. The greatest rooting percentage (66.67%) was obtained with medium contained IBA at 0.2 or 0.4 mgl $^{-1}$ plus NAA at 0.4 or 0.8 mgl $^{-1}$ for "Hansen" rootstock and Ne Plus Ultra shoots.

Key words: Propagation, Almond cvs., BA, IBA, Multiplication, Subculture, Rooting.

INTRODUCTION

Almond is one of the most important nut crops worldwide. It is propagated by T – budding on seedling rootstocks ,and stem cutting which has given a slight success **(Hartmann *et al* 1990).** The major rootstocks used are peach and almond-

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peach hybrids **(Channuntapipat *et al* 2003)**. Hansen was selected from a seedling population of the cross "almond B x peach selection 1-8-2".The Hansen clones immune to root-Knot nematode and phytophthora crown. Hansen is most useful in calcareous soils and under marginal or stressful conditions assuming good drainage is available **(Hansen *et al* 1981 and Kester and Asay 1986)**. The vegetative propagation of almond clones as own–rooted plants (without any rootstocks) is difficult except with specific cultivars. On the other hand micropropagation has been difficult for almond cultivars, although some success has been achieved with two almond genotypes (cvs. Supernova and rootstock Sel.M51) **(Hartman *et al* 1990 and Caboni & Damiano, 1994).**

Variations in necessity of benzyl adenine (BA) and indol butric acid (IBA) in the media used for the different almond cultivars were obvious in the different reports. For example, **Rugini and Verma (1983)** reported that high concentration of BAP promoted shoot proliferation, while low concentration encouraged shoot elongation for almond cultivars. Whereas **Gurel and Gulsen (1998)** found that during multiplication stage, BAP was essential for shoot development, but high levels (2 or 3 mgl $^{-1}$) caused vetrifications and callus formation. In the mean time, the combination of 0.1 mgl $^{-1}$ IBA and 1.0 mgl $^{-1}$ BAP was the most effective in terms of new shoot production and rate of shoot growth in two almond cultivars 'Texas" and "Nonpareil" under study. **Saeed (1998)** found that the best survival percentage was noticed on MS medium supplemented with IBA at 0.1 mgl $^{-1}$ or BA at 0.5 mgl $^{-1}$ or both and the highest proliferation rate for two almond cultivars (Ne Plus Ultra and Nonpareil) was achieved on MS medium supplemented with BAP at 2.0 mgl $^{-1}$. Also, the same results were obtained for Non Pareil 15-1 and Ne Plus Ultra using 3 – 5 Mµ BAP for shoot proliferation respectively and 1 Mµ BAP for shoot elongation. However, a low concentration of IBA at 0.049 Mµ was necessary for the almond cultivars to promote longer shoots when compared to the same medium without IBA **(Channuntapipat *et al* 2003).**

Almond is a difficult to root species. Rooting is also poor *in- vitro* , however, satisfactory rooting was obtained only in a few cultivars and peach x almond rootstock "Hansen" (**Rugini & Verma 1983 and Wanas, 1999). Caboni and Damiano (1994)**. Induced root in two almond genotypes on Bourgin and Nitisch medium , containing 50 g / liter sucrose , with IBA (10 µM) or IAA (10 µM).The dark treatment and the type of auxin used played an important role in the rooting of almond genotypes.

Accordingly, this work aimed to determine the effect of different levels of BA and IBA during the stages of micropropagation for three almond cultivars (Om-Elfahm, M–Dalet and Ne Plus Ultra) and the hybrid rootstock "Hansen 536". In addition, methods are reported for successful rooting.

MATERIAL AND METHODS

This work was carried out during two successive years 2003 and 2004 in the Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo. This study was performed for *In vitro* propagation of almond cultivars and Hansen rootstock. Three almond cultivars (Om–Elfahm, M-

Dalt, Ne Plus Ultra) and the hybrid root-stock "Hansen" were used through out this study.

Apical parts of shoots (10–15cm) long were taken from adult trees during the growing season .The trees were about 8 years old planted in the orchard of Faculty of Agricultural, Ain Shams University , Shoubra El-Kiema , Cairo .

2. 1. Culture procedure

After removing leaves the shoots were put under running tap water for about one hour then sterilized under laminar flow hood condition .Sodium hypochlorite solution was prepared using commercial bleach "Clorox" (5.25 % available chlorine) at 10 % concentration. The shoots were dipped for 8 minutes in such solution before rinsing three times in sterile distilled water 10 minutes for each rinsing. After that stem nodes were prepared and cultured in establishment culture media. The pH of different media was adjusted at 5.7 before autoclaving at 100 K. pa (15 P.S.I) and 121^{o}C for 20 minutes, then left to cool and harden for 24 hours before being used of the.

The cultures of the different experiments were incubated at temperatures almost maintained between 24±2°C and photoperiods of 16 hour day and 8 hour night supplied by fluorescent lamps (two lamps per shelf to provide light intensity of 2200 – 2400 lux at explants level 30 cm from light).

2.1.1. Establishment experiment

Each stem node explant was separately cultured in test tubes (150 x15 mm) filled with 10 ml of **Murashige and Skoog (1962)** (MS) full strength medium plus 30 g sucrose, 7gl^{-1} agar and supplemented with benzyl adenine (BA) at 0.0, 0.5, 1.0 and 2.0 mgl^{-1} either alone or in combination with indole butyric acid (IBA) at 0.0, 0.01, 0.05 and 0.1 mgl^{-1}.

Bud development percentages, and shoot length (cm) were determined after four weeks .The experiment was repeated three times at least.

The experiment was arranged in a completely randomized design with 16 treatments for each cultivar (4 BA treatments x 4 IBA treatments) in a factorial experiment with five replicates (three explants for each replicate).

2 .1.2. Multiplication experiment

Approximately uniform growing shoots (about 1.5 cm in length) were aseptically transferred after four weeks to proliferation medium which was consisted of MS salts and vitamins plus one of the following BA and IBA levels :

1. BA 0.0+ IBA 0.0 mgl^{-1} (control)
2. BA 1.0+ IBA 0.05 mgl^{-1}
3. BA 1.0+ IBA 0.1 mgl^{-1}
4. BA 2.0+ IBA 0.05 mgl^{-1}
5. BA 2.0+ IBA 0.1 mgl^{-1}

The shoots were cultured into glass jars (350 mm) filled with 30 ml of medium. The proliferated shoots were subcultured into fresh medium after four weeks and another two subcultures on the same media were performed every four weeks. Average number and length of shoots (new proliferated shoots) were recorded each subculture during the multiplication stage.

The experiment was arranged in a completely randomized design with 15 treatments for each cultivar [5 treatments of (BA+IBA) x 3 subcultures] in a factorial experiment with five replicates (three explants for each replicate).

2.1.3. Rooting experiment

Uniform proliferated shoots (about 1-2 cm in length) were transferred to test tubes 150 x15 mm filled with 10ml of rooting medium, which consisted of modified full strength **Bourgin and Nitsch (1967)** medium supplemented with 30g sucrose, 7 gl^{-1} agar and IBA or NAA at 1.0 mgl^{-1} only or combination (0.2 IBA mgl^{-1} + NAA 0.4 mgl^{-1}, IBA 0.4 mgl^{-1} + NAA 0.8 mgl^{-1} and IBA 0.5 mgl^{-1} + NAA 1.0 mgl^{-1}). During rooting experiment the shoots were incubated in constant dark for one week then transferred to normal light regime for three weeks. The following measurements were recorded after 4 weeks on rooting medium, callus formation scale and rooting percentage.

Rooting percentage =

$$\frac{\textit{Number of rooted shoots}}{\textit{Total number of shoots}} \times 100$$

The experiment was arranged as a factorial experiment in a completely randomized design (5 treatments x 4 cultivars x 3 replicates) with three shoots for each replicate. Duncan's multiple range test at 5% level was used in the three studied experiments. to verify the differences between means of the treatments **(Sendecor and Cochran, 1982).**

RESULTS AND DISCUSSION

3.1. Effect of growth regulators (IBA and BA) during establishment stage

3.1.1. Effect on bud development percentages and shoot length of Om–Elfahm almond cv.

Data in Table (1) illustrated the effect of IBA and BA levels on bud development percentages and shoot length of Om–Elfahm almond cv. during establishment stage.

Table 1. Effect of BA and IBA levels on bud development percentages and shoot length of Om–Elfahm almond cv. during establishment stage.

BA mgl^{-1} / IBA mgl^{-1}	Bud development%				Mean
	0.0	0.5	1.0	2.0	
0.0	20.10 d	60.00 ab	46.67 bc	60.00 ab	46.60 A
0.01	46.67 bc	73.34 a	53.34 bc	66.67 ab	60.00 A
0.05	53.34 bc	33.34 cd	53.34 bc	46.67 bc	46.67 A
0.1	33.34 cd	46.67 bc	60.00 ab	33.34 cd	43.34 A
Mean	38.35 A`	53.34 A`	35.34 A`	51.67 A`	
BA mgl^{-1} / IBA mgl^{-1}	**Shoot length (cm)**				**Mean**
	0.0	0.5	1.0	2.0	
0.0	1.28 g	1.50 c-e	1.58 b-d	1.71 b	1.52 A
0.01	1.23 g	1.63 bc	2.05 a	1.57 b-d	1.62 A
0.05	1.38 e-g	1.45 d-f	2.13 a	1.61 b-d	1.64 A
0.1	1.30 fg	1.68 b	1.73 b	1.48 c-e	1.55 A
Mean	1.30 C`	1.57B`	1.87 A`	1.59 B`	

* Means followed by the same letter (s) are not significantly different from each other at 5 % level .

Data revealed that the highest mean bud development was noticed with IBA at 0.01 mgl^{-1}. Insignificant differences were noticed among all the used IBA concentrations on bud development percentage.

Regarding BA specific effect, BA at 0.5 mgl^{-1} exhibited the highest bud development also in significant differences among all tested BA levels on bud development were existed. Interactions between the two studied variables, cleared that the highest significant bud development percentage was achieved with BA at 0.5 mgl^{-1} plus IBA at 0.01 mgl^{-1}, followed by BA at 2.0 mgl^{-1} plus IBA at 0.01 mgl^{-1} without significant differences between them. Whereas, control treatment (medium without BA or IBA) showed the least significant bud development (20.0%)

Concerning shoot length, there were no significant differences among all IBA treatments on shoot length. Whereas, BA at1.0 mgl^{-1} showed the highest value (1.87 cm) of shoot length, followed by BA levels at 2.0 and 0.5 mgl^{-1} without significant differences among them. On the contrary, the lowest value (1.3 cm) was noticed with control medium (BA at 0.0 mgl^{-1}). The interactions between IBA and BA levels, showed the highest significant shoot length (2.13 and 2.05 cm) when BA was 1.0 mgl^{-1} plus IBA at 0.01 mgl^{-1}or 0.05 mgl^{-1}, respectively without significant differences between them. The lowest significant shoot length (1.23 cm) was recorded with media without BA and supplemented with IBA at 0.01 mgl^{-1}, respectively.

3.1.2. Effect on bud development percentages and shoot length of M - Dalet almond cv.

Data presented in Table (2) showed the effect of IBA and BA levels on bud development percentages and shoot length of M-Dalet almond cv. during establishment stage.

Table 2. Effect of BA and IBA levels on bud development percentages and shoot length of M –Dalet almond cv .during establishment stage.

BA mgl^{-1} / IBA mgl^{-1}	Bud development%				Mean
	0.0	0.5	1.0	2.0	
0.0	46.67 c	53.30bc	53.33bc	53.33bc	51.65 A
0.01	53.34 bc	60.00a-c	66.67a-c	60.00a-c	60.00 A
0.05	60.00a-c	66.67a-c	73.33ab	53.33bc	63.33 A
0.1	53.34bc	66.67a-c	53.33bc	80.00 a	63.33 A
Mean	53.34 A`	61.66 A`	61.66A`	61.66A`	
BA mgl^{-1} / IBA mgl^{-1}	Shoot length (cm)				Mean
	0.0	0.5	1.0	2.0	
0.0	1.00 g	2.15 c	2.41 ab	1.85 de	1.85 A
0.01	1.15 fg	2.19 c	2.42 a	1.67 e	1.86 A
0.05	1.25 f	2.23 a-c	2.16 c	1.94 d	1.90 A
0.1	1.32 f	2.21 bc	2.29 a-c	1.78 de	1.90 A
Mean	1.18 C`	2.19 A`	2.32 A`	1.81 B`	

* Means followed by the same letter(s) are not significantly different from each other at 5 % level .

Specific effect of IBA levels cleared that IBA at either 0.05 or 0.1 mgl^{-1} showed the highest values with no significant differences detected among all the used IBA concentrations on bud development percentage. Also, means of BA levels, showed insignificant differences among all tested BA levels which gave higher values for bud development compared to control. Regarding interaction between the two studied factors, data showed that bud development percentage exhibited significant differences in the most cases, and the highest value of bud development percentage was noticed with BA at 2.0 mgl^{-1} plus IBA at 0.1 mgl^{-1}, whereas, the least significant value was noticed with the untreated shoots.

Regarding effect of IBA levels on shoot length, insignificant differences were noticed among all used IBA concentrations. As for the effect of BA levels on shoot length, data showed that the highest significant shoot length (2.32 and 2.19 cm) were obtained by BA at (1.0 mgl^{-1} and 0.5 mgl^{-1}, respectively without significant differences between them. On the other hand, the least significant shoot length was noticed with control shoots. Concerning the interaction between the two studied variables, the highest mean of shoot length was obtained with (BA at 1.0 mgl^{-1} either alone or plus IBA at 0.01 mgl^{-1}. Whereas, the lowest value was recorded by media without BA and IBA.

3.1.3. Effect on bud development percentages and shoot length of Ne Plus Ultra almond cv

Data in Table (3) showed that effect of IBA and BA levels on bud development percentages and shoot length of Ne Plus Ultra almond cv. during establishment stage IBA at 0.0 or 0.05 mgl^{-1} showed the highest bud development %.

Table 3. Effect of BA and IBA levels on bud development percentages and shoot length of Ne Plus Ultra almond cv. during establishment stage.

BA mgl^{-1} / IBA mgl^{-1}	Bud development %				
	0.0	0.5	1.0	2.0	Mean
0.0	53.34 bc	66.67 ab	53.34 bc	80.00 a	63.34A
0.01	53.34 bc	60.00 bc	66.67 ab	60.00 bc	60.00 A
0.05	60.00 bc	66.67 ab	73.34 ab	53.34 bc	63.34 A
0.1	40.00 c	53.34 bc	66.67 ab	53.34 bc	53.34 A
Mean	51.67A`	61.67A`	65.00A`	61.67A`	
BA mgl^{-1} / IBA mgl^{-1}	**Shoot length (cm)**				
	0.0	0.5	1.0	2.0	Mean
0.0	1.20 fg	1.36 f	1.89 bc	1.62 de	1.51 B
0.01	1.00 h	1.58 de	2.30 a	1.89 bc	1.70 A
0.05	1.12 gh	1.63 de	1.97 b	1.75 cd	1.62 AB
0.1	1.15 gh	1.88 bc	1.56 e	1.53 e	1.53 B
Mean	1.61 B`	1.92 A`	1.70 B`	1.70 B`	

* Means followed by the same letter (s) are not significantly different from each other at 5 % level .

No significant differences were noticed among all used IBA concentrations and control treatment in mean bud development percentages. Data, also showed that BA at 1.0 mgl^{-1} exhibited the highest bud development percentage without significant differences among all the tested BA levels and control one .The interactions between the IBA and BA levels, showed that the highest significant bud development percentage was noticed with BA at 2.0 mgl^{-1} without IBA.

Meanwhile, the least value was recorded with IBA at 0.1 mgl^{-1} without BA.

The specific effect of IBA levels on shoot length cleared that the greatest significant length (1.70 cm) was obtained with IBA at 0.01 mgl^{-1}, followed by IBA 0.05 mgl^{-1}, without significant differences between them. The least shoots length (1.51 and 1.53 cm) were noticed with IBA at 0.0 and 0.1 mgl^{-1} without significant differences between them.

Regarding the effect of BA levels, data showed that the highest significant shoot length (1.92 cm) was recorded with BA at 0.5 mgl^{-1}. On the other hand, control treatment gave the lowest shoot length (1.61 cm). Data exhibited a significant interaction between the two studied factors, where the greatest shoot length was recorded with medium supplemented with BA at 1.0 mgl^{-1} plus IBA at 0.01 mgl^{-1} and the least value was noticed by medium supplemented with IBA at 0.01 mgl^{-1} without B.

3.1.4. Effect on bud development percentages and shoot length of Hansen rootstock

Data in Table (4) declared the effect of IBA and BA levels on bud development percentages and shoot length of Hansen rootstock (almond x peach) during establishment stage.

Concerning the effect of IBA levels, data showed that IBA at 0.01 mgl^{-1} gave the highest percentage of bud development without significant differences among all IBA levels and (the control). As for BA levels it is obvious that increasing BA concentration resulted on insignificant increase in bud development percentages where the highest value was achieved by BA at 2.0 mgl^{-1}. The interactions between the two studied factors showed significant high bud development percentage with BA at 2.0 mgl^{-1} plus IBA at 0.01 mgl^{-1}. Whereas, control (medium without BA and IBA) showed the least significant value.

Regarding the specific effect of IBA levels on shoot length, the highest percentage of shoot length was obtained by IBA at 0.01 mgl^{-1}, followed by IBA at 0.0 and 0.05 mgl^{-1} without significant differences among them. Concerning the effect of BA levels, BA at 1.0 and 2.0 mgl^{-1} exhibited the highest significant shoot length. Meanwhile ,the least shoot length was found in control explants. The interactions between IBA and BA levels showed a significant effect, where (BA at 1.0 mgl^{-1} plus IBA at 0.01 mgl^{-1}) produced the highest shoot length in Hansen rootstock .On the contrary, BA at 0.0 mgl^{-1} under all IBA levels produced the shortest shoot length without significant differences.

The results during establishment stage showed that the highest bud development percentages were obtained with medium contained BA at 0.5 mgl^{-1} plus IBA at 0.01 mgl^{-1} for Om- Elfham, BA at 2.0 mgl^{-1} plus IBA at 0.1 mgl^{-1} for M–Dalet and Hansen, while Ne Plus Ultra recorded the highest bud development with BA at

Table 4. Effect of BA and IBA levels on bud development percentages and shoot length of Hansen rootstock (almond x peach) during establishment stage.

BA mgl^{-1} / IBA mgl^{-1}	Bud development %				
	0.0	0.5	1.0	2.0	Mean
0.0	33.43 c	53.34 a-c	66.67 ab	66.67 ab	55.00 A
0.01	66.67 ab	60.00 ab	60.00 ab	73.34 a	65.00 A
0.05	46.67 bc	66.67 ab	60.00 ab	53.34a-c	56.67 A
0.1	66.76 ab	46.67 bc	66.67 ab	66.67 ab	61.67 A
Mean	53.34A`	56.67 A`	63.34 A`	65.00 A`	
BA mgl^{-1} / IBA mgl^{-1}	Shoot length (cm)				
	0.0	0.5	1.0	2.0	Mean
0.0	1.15 g	1.87 ef	2.38 ab	2.17 cd	1.90 AB
0.01	1.09 g	1.87 ef	2.51 a	2.31 bc	1.94 A
0.05	1.25 g	2.20 b-d	1.77 f	2.23 bc	1.86 AB
0.1	1.25 g	1.90 ef	2.03 de	1.93 ef	1.78 B
Mean	1.19 C`	1.96 B`	2.17 A`	2.16 A`	

* Means followed by the same letter(s) are not significantly different from each other at 5 % level

2.0 mgl^{-1} without IBA. The longest shoot was recorded by BA at 1.0 mgl^{-1} plus IBA at 0.01 mgl^{-1} for all the considered cultivars.

As far as the previous work in such subject concern, just one report from those dealing with almond micropropagation has mentioned the importance of the addition of 0.5 mgl^{-1} BA plus 0.1mgl^{-1} IBA on the survival percentages and growth parameters of almond. **(Saeed 1998)** In addition **Eldeen *et al* 1998** found that MS medium supplemented by IBA 0.1 mgl^{-1} plus BAP at 0.5 mgl^{-1} gave the best survival percentages and growth parameters for two almond cultivars and two rootstocks of peach.

3.2. Effect of growth regulators (IBA and BA) and number of subcultures on multiplication rate

3.2.1. Effect on average number and length of proliferated shoots / explant for Om – Elfahm almond cv.

Data illustrated in Tables (5 and 6) showed the effect of IBA, BA levels and number of subcultures on average number and length of proliferated shoots / explant for Om – Elfahm almond cv. during multiplication stage.

Concerning the effect of BA and IBA levels, data cleared that the highest

Table 5. Effect of BA, IBA levels and number of subcultures on average number of proliferated shoots/ explant of almond cv Om- Elfahm during multiplication stage

Subcultures / Treatments	1st sub-culture	2nd sub-culture	3rd sub-culture	Mean
BA 0.0 +IBA 0.0 (control)	1.00 f	1.05 f	1.20 f	1.08 C
BA 1.0+ IBA 0.05 mgl^{-1}	2.93 e	6.10 c	7.32 ab	5.45 A
BA 1.0+ IBA 0.1 mgl^{-1}	3.40 e	6.10 c	7.51 a	5.67 A
BA 2.0+ IBA 0.05 mgl^{-1}	2.86 e	5.48 d	7.10 ab	5.15 B
BA 2.0+ IBA 0.1 mgl^{-1}	3.06 e	5.48 d	6.80 b	5.09 B
Mean	2.65 C`	4.83 B`	5.98 A`	

* Means followed by the same letter(s) are not significantly different from each other at 5 % level

Table 6. Effect of BA, IBA levels and number of subcultures on average length of proliferated shoots (cm) / explant of almond cv Om- Elfahm. during multiplication stage

Subcultures / Treatments	1st sub-culture	2nd sub-culture	3rd sub-culture	Mean
BA 0.0 +IBA 0.0 (control)	1.77 f	2.54 e	3.07 d	2.46 B
BA 1.0+ IBA 0.05 mgl^{-1}	2.59 e	4.84 c	5.51 ab	4.31 A
BA 1.0+ IBA 0.1 mgl^{-1}	2.66 de	4.71 c	5.63 ab	4.33 A
BA 2.0+ IBA 0.05 mgl^{-1}	2.32 e	4.76 c	5.32 b	4.133 A
BA 2.0+ IBA 0.1 mgl^{-1}	2.16 ef	4.57 c	5.58 a	4.20 A
Mean	2.30 C`	4.29 B`	5.08 A`	

Means followed by the same letter(s) are not significantly different from each other at 5 % level .

significant mean number of shoots / explant (5.67and 5.45) were achieved with BA at 1.0 mgl^{-1} plus IBA at 0.1 or 0.05 mgl^{-1} without significant differences between them, followed by BA at 2.0 mgl^{-1} plus IBA at 0.1 or 0.05 mgl^{-1} also, without significant differences between them. The least value of average number of shoots / explant was recorded with medium without BA or IBA.

Regarding the effect of the number of subcultures, data in Tables (5, 6, up to 12) showed that the highest significant average number and length of shoots / explant were noticed during 3rd subculture. With advanced number of subcultures average number and length of shoots / explant increased . On the other hand, the first subculture exhibited the least significant number and length of shoots.

The interactions between the two studied factors, showed a significant differences in most cases, the highest number of shoots / explant (7.51) was obtained with BA at 1.0 mgl $^{-1}$ plus IBA at 0.1 mgl $^{-1}$ during the 3rd subculture with slight difference between this treatment and (BA at 1.0 mgl^{-1} plus IBA at 0.05 mgl^{-1} and BA at 2.0 mgl^{-1} plus IBA at 0.1 mgl^{-1}). The lowest values of average number of shoots / explant (1.0, 1.05 and 1.20) were achieved with control treatment without significant differences in the first, second and third subcultures respectively .As for the effect of BA and IBA levels on average means shoot length there was no significant differences among all the used BA and IBA levels on the average length of proliferated shoot. However, control treatment recorded the least average proliferated shoot length.

The interactions between the two studied variables, showed that the highest significant value of the average proliferated shoot length (5.58cm) was noticed with shoot treated by BA at 2.0 mgl^{-1} plus IBA at 0.1 mgl^{-1} during the 3rd subculture .On the other hand, the lowest values were recorded with all used treatments (BA and IBA levels)during the 1st subculture.

3.2.2. Effect on average number and length of proliferated shoots / explant for M-Dalet almond cv.

Data in Tables (7 and 8) showed the effect of IBA, BA levels and number of subcultures on average number and length of proliferated shoots / explant for M-Dalet almond cv. during multiplication stage.

Table 7. Effect of BA, IBA levels and number of subcultures on average number of proliferated shoots/ explant of almond cv M- Dalet during multiplication stage

Treatments \ Subcultures	1st sub-Culture	2nd sub-culture	3rd sub-culture	Mean
BA 0.0 +IBA 0.0 (control)	1.00 g	1.07 g	1.05 g	1.04 E
BA 1.0+ IBA 0.05 mgl^{-1}	2.58 e	3.65 d	4.31 bc	3.51 C
BA 1.0+ IBA 0.1 mgl^{-1}	2.00 f	2.99 e	3.94 cd	2.98 D
BA 2.0+ IBA 0.05 mgl^{-1}	3.00 e	4.20 bc	5.15 a	4.12 B
BA 2.0+ IBA 0.1 mgl^{-1}	3.58 d	4.59 b	5.52 a	4.56 A
Mean	2.43 C`	3.30 B`	3.99 A`	

Means followed by the same letter(s) are not significantly different from each other at 5 % level .

Table 8. Effect of BA, IBA levels and number of subcultures on average length of proliferated shoots (cm) / explant of almond cv of M- Dalet. during multiplication stage

Treatments \ Subcultures	1st sub-culture	2nd sub-culture	3rd sub-culture	Mean
BA 0.0 +IBA 0.0 (control)	1.80 d	1.92 d	2.18 d	1.97 C
BA 1.0+ IBA 0.05 mgl^{-1}	3.06 c	4.17 b	4.70 ab	3.98 A
BA 1.0+ IBA 0.1 mgl^{-1}	3.35 c	4.15 b	4.81 a	4.10 A
BA 2.0+ IBA 0.05 mgl^{-1}	3.38 c	4.74 ab	4.75 ab	4.29 A
BA 2.0+ IBA 0.1 mgl^{-1}	3.00 c	3.46 c	4.44 ab	3.63 B
Mean	2.91 C`	3.69 B`	4.17 A`	

Means followed by the same letter(s) are not significantly different from each other at 5 % level .

The specific effect of BA and IBA levels showed the highest mean number of shoot / explant (4.56) with BA at 2.0 mgl^{-1} plus IBA at 0.1 mgl^{-1} followed by BA at 2.0 mgl^{-1} plus IBA at 0.05 mgl^{-1} with significant differences between them. On the contrary, the least mean number of shoots were noticed with control treatment. An evident increase in number of shoots / explant took place with increasing IBA concentration when used with 2 mgl^{-1} BA .

The interactions revealed that medium supplemented with BA at 2.0 mgl^{-1} plus IBA at 0.05 or 0.1 mgl^{-1}during the 3rd subculture exhibited the highest average number of shoot with insignificant differences between them. Lower values were recorded using control treatment during the 1st, 2nd and 3 rd subcultures.

As for the effect of BA and IBA levels on the average length of M- Dalet cv., data showed that no significant differences between the treatments which contained BA at 1.0 mgl^{-1} plus IBA at 0.05 or 0.1 mgl^{-1} and that one containing BA at 2.0 mgl^{-1} plus IBA at 0.05 mgl^{-1} which exhibited the longest average shoot length, whereas control treatment recorded the lowest shoot length.

The interactions between the two studied factors showed the highest significant value of shoot length (4.81cm) with medium contained BA at 1.0 mgl^{-1} plus IBA at 0.1 mgl^{-1} during the 3rd subculture, followed by (4.75 and 4.74 cm) with BA at 2.0 mgl^{-1} plus IBA at 0.05 mgl^{-1} during the 3rd and 2nd subcultures. The least values (1.80,1.92 and 2.18 cm) were noticed with control treatment during 1st, 2nd and 3rd subcultures, respectively, it means that the effect of subculture was not significant unless balanced levels of BA and IBA are existe.

3.2.3. Effect on average number and length of proliferated shoots / explant for Ne Plus Ultra almond cv.

Data in Tables (9 and 10) showed the effect of IBA, BA levels and number of subcultures on average number and length of proliferated / explant for Ne Plus Ultra almond cv. during multiplication stage.

From the data on the effect of BA and IBA levels, it seems that the highest average number of shoots/ explant (4.31) was obtained when treating shoot by BA at 1.0 mgl^{-1} plus IBA at 0.05 mgl^{-1} .Data ,also, showed that the least mean number of shoots was noticed with control (zero BA and IBA)(1.02).

The interactions between the two studied factors, cleared that the highest significant average number of shoots (5.35, 5.16 and 5.29) were noticed with medium containing BA at 1.0 mgl^{-1} plus IBA at 0.05 mgl^{-1} and BA at 2.0 mgl^{-1} plus IBA at 0.1or 0.05 mgl^{-1}) during 3rd subculture, respectively with no significant differences among them. On the contrary, the least significant values were noticed with control treatment (BA 0.0 + IBA 0.0) during the 1st, 2nd and 3rd subcultures.

Concerning the effect of BA and IBA levels on shoot length, (Table 10) data showed a significant differences in average proliferated shoot length among all the used treatments .The highest value of average shoot length (3.63 cm) was recorded with shoots treated by BA at 1.0 mgl^{-1} and IBA at 0.05 mgl^{-1}. Increasing BA and IBA concentrations resulted in a significant decrease in average shoot length.

The effect of interactions between BA, IBA levels and number of subcultures, data showed that the greatest shoot length was cleared in medium supplemented with BA at 1.0 mgl^{-1} plus IBA at 0.05 mgl^{-1} during 3rd subculture. On the contrary, the shortest shoot was noticed with control treatment during 1st subculture.

Table 9. Effect of BA, IBA levels and number of subcultures on average number of proliferated shoots/ explant of almond cv. Ne Plus Ultra during multiplication stage

Treatments \ Subcultures	1st subculture	2nd subculture	3rd subculture	Mean
BA 0.0 +IBA 0.0 (control)	1.00 g	1.05 g	1.00 g	1.02 C
BA 1.0+ IBA 0.05 mgl^{-1}	2.93 d	4.65 b	5.35 a	4.31 A
BA 1.0+ IBA 0.1 mgl^{-1}	1.61 f	4.16 c	4.49 bc	3.42 B
BA 2.0+ IBA 0.05 mgl^{-1}	2.47 e	3.00 d	5.16 a	3.54 B
BA 2.0+ IBA 0.1 mgl^{-1}	2.27 e	3.15 d	5.29 a	3.57 B
Mean	2.05 C`	3.20 B`	4.26 A`	

Means followed by the same letter(s) are not significantly different from each other at 5 % level .

Table 10. Effect of BA, IBA levels and number of subcultures on average length of proliferated shoots (cm) / explant of almond cv. Ne Plus Ultra during multiplication stage

Treatments \ Subcultures	1st sub-culture	2nd sub-culture	3rd sub-culture	Mean
BA 0.0 +IBA 0.0 (control)	1.59 e	1.95 de	2.17 d	1.90 D
BA 1.0+ IBA 0.05 mgl^{-1}	2.38 d	3.65 c	4.85 a	3.63 A
BA 1.0+ IBA 0.1 mgl^{-1}	2.15 d	3.20 c	4.43 ab	3.26 B
BA 2.0+ IBA 0.05 mgl^{-1}	2.05 de	3.20 c	4.14 b	3.13 BC
BA 2.0+ IBA 0.1 mgl^{-1}	1.91 de	3.15 c	3.65 c	2.90 C
Mean	2.01 C`	3.03 B`	3.85 A`	

Means followed by the same letter(s) are not significantly different from each other at 5 % level .

3.2.4. Effect on average number and length of proliferated shoots / explant for "Hansen" rootstock

Data in Tables (11 and 12) indicated the effect of IBA, BA levels and number of subcultures on average number and length of shoots / explant of "Hansen" rootstock during multiplication stage.

Regarding the effect of BA and IBA levels on average number of shoots/ explant, it led to an evident increase in the average shoot number with increasing BA and IBA levels where the highest significant mean was obtained by medium contained BA at 2.0 mgl^{-1} plus IBA at 0.1 mgl^{-1}. Insignificant differences were clear between shoots treated by BA1.0 mgl^{-1} plus IBA at 0.1 mgl^{-1} and BA 2.0 mgl^{-1} plus IBA at 0.05 mgl^{-1} in the average number of shoots. Meanwhile, the control treatment exhibited the least value (1.03).

The interactions between the two studied variables revealed that medium containing BA 2.0 mgl^{-1} plus IBA at 0.1 mgl^{-1} through 3rd subculture exhibited the highest value of average number of shoots / explant (6.11) .On the contrary, shoots cultured in free hormones medium recorded the least number of shoots during the three subcultures.

As for the effect of BA and IBA levels on the average shoot length of Hansen rootstock, the longest shoot (3.57cm) was obtained with medium supplemented with BA at 1.0 mgl^{-1} plus IBA at 0.1 mgl^{-1}.No significant differences between shoots cultured in medium containing BA at 2.0 mgl^{-1} plus IBA at 0.05 or 0.1 mgl^{-1} and BA at 1.0 mgl^{-1} plus IBA at 0.05 mgl^{-1} on the average shoot length. Whereas, the shortest shoots (1.3 cm) was noticed with control treatment (zero BA and IBA).

The interactions between the two studied factors, cleared that the highest values of average shoot length (4.19, 3.95 and 3.79cm) were noticed with media supplemented with BA at1.0 mgl^{-1} plus IBA at 0.1 mgl^{-1} and BA at 2.0 or 1.0

Table 11. Effect of BA, IBA levels and number of subcultures on average number of proliferated shoots/ explant of “Hansen” rootstock during multiplication stage.

Subcultures / Treatments	1st sub-culture	2nd sub-culture	3rd sub-culture	Mean
BA 0.0 +IBA 0.0 (control)	1.00 i	1.03 i	1.05 i	1.03 D
BA 1.0+ IBA 0.05 mgl^{-1}	2.80 h	3.35 g	4.75 de	3.63 C
BA 1.0+ IBA 0.1 mgl^{-1}	4.32 ef	4.57 de	5.38 b	4.75 B
BA 2.0+ IBA 0.05 mgl^{-1}	4.00 f	4.91 b-d	5.39 b	4.76B
BA 2.0+ IBA 0.1 mgl^{-1}	4.87 cd	5.25 bc	6.11 a	5.14 A
Mean	3.40 C`	3.82 B`	4.53 A`	

Means followed by the same letter(s) are not significantly different from each other at 5 % level .

Table 12. Effect of BA, IBA levels and number of subcultures on average length of proliferated shoots (cm) / explant of “Hansen” rootstock during multiplication stage.

Subcultures / Treatments	1st sub-culture	2nd sub-culture	3rd sub-culture	Mean
BA 0.0 +IBA 0.0 (control)	0.83 f	1.38 e	1.74 e	1.31 C
BA 1.0+ IBA 0.05 mgl^{-1}	2.22 d	3.11 c	3.79 ab	3.04 B
BA 1.0+ IBA 0.1 mgl^{-1}	2.85 c	3.68 b	4.19 a	3.57 A
BA 2.0+ IBA 0.05 mgl^{-1}	2.22 d	2.90 c	3.95 ab	3.02 B
BA 2.0+ IBA 0.1 mgl^{-1}	2.27 d	3.00 c	3.75 b	3.00 B
Mean	2.07 C`	2.81 B`	3.48 A`	

Means followed by the same letter(s) are not significantly different from each other at 5 % level .

mgl^{-1} plus IBA at 0.05 mgl^{-1} during the 3rd subculture, respectively. On the other hand, the least value of shoot length (0.83cm) was recorded by control treatment during the 1st subculture.

Results in this section indicated that increasing BA concentration up to 2.0 mgl^{-1} resulted in a significant increase in average of shoot number, whereas shoot length was decreased with BA at 2 mgl^{-1}. These results agreed with the findings of **Tabachnik & Kester (1977) and Rugini & Verma (1983)** who reported that high concentration of BAP promoted shoot proliferation, whereas low concentration encouraged shoot elongation for almond cultivars. Generally, in the present study, the results showed that the combination of IBA at 0.05 - 0.1 mgl^{-1} and BA at 1–2 mgl^{-1} gave the best shoot proliferation and shoot length with the three almond cvs. and Hansen rootstock under study .Similarly **Gurel and Gulsen (1998**) found that the combination of 0.1 mgl^{-1} IBA and 1 mgl^{-1} BAP was the most effective in terms of new shoot production and rate of shoot growth of almond . However, BAP was essential for shoot proliferation. The same results were obtained for Nonpareil 15-1 cv. using 3.0 µM BAP for shoot proliferation and 1.0 µM BAP for shoot elongation, and for Ne Plus Ultra cv. using 5.0 µM BAP for shoot proliferation and 1.0 µM BAP for shoot elongation. Low concentration of IBA at 0.049 was necessary for almond cultivars to promote longer shoots when compared to the same medium without IBA (**Channuntapipat *et al* 2003).**

Results in the current study showed an evident increase in shoot number by increasing number of subcultures till the3rd one, and these results are in harmony with **Wanas (1999**) who found that the increasing in number and length of proliferated shoots of Hansen rootstock by the 3rd and 4th subcultures was achieved in MS medium plus 0.4 mgl^{-1} IBA, 1.5 mgl^{-1} BA .However, it is worthy to perform further experiment in order to determine after how many numbers of subculture the cultures should be renewed to avoid any decrease in proliferation rate.

3.3. Effect of auxins type and concentrations on callus formation and rooting of some almond cvs and Hansen rootstock.

3.3.1. Effect on callus formation

Data in Table (13) showed the effect of IBA and NAA levels on callus formation of three almond cultivars and “Hansen” rootstock .

Regarding almond cultivars and “Hansen” rootstock effect, it is clear that Om- Elfahm cv. and Hansen rootstock exhibited the largest callus (3.22 and 3.16) (more than 10 mm diameter) without significant differences between them, followed by Ne Plus Ultra cv. which recorded 1.87 scale (5 -10 mm diameter). On the other hand, the smallest size of callus (less than 5mm diameter) 1.44 was noticed with M- Dalet cultivar.

It is clear that callus formation greatly increased with increased concentration of IBA.

The interactions between the two studied factors (cultivars and treatments) were significant in most cases. The high callus formation scale (3.89) was noticed with Om- Elfahm cv. cultured in Bourgin and Nitcsh medium supplemented by IBA at 0.4 mgl^{-1} plus NAA at 0.8 mgl^{-1}. On the contrary, medium contained NAA at 1mgl^{-1} with M -Dalet and Ne Plus Ultra

Table 13. Effect of IBA and NAA levels on callus formation of almond cvs. (Om – Elfahm, M-Dalet and Ne Plus Ultra) and Hansen rootstock during rooting stage.

Cvs. / Treatments	Om – Elfahm	M-Dalet	Ne Plus Ultra	Hansen	Mean
IBA 1.0 mgl^{-1}	3.67 ab	1.56 de	2.22 c	3.67 ab	2.78 A
NAA 1.0 mgl^{-1}	1.56 de	1.00 e	1.00 e	1.33de	1.22 C
0.2 mgl^{-1}IBA +0.4 mgl^{-1} NAA	3.44 ab	1.33 de	1.56 de	3.33 b	2.42 B
0.4 mgl^{-1}IBA +0.8 mgl^{-1} NAA	3.89 a	1.56 de	2.33 c	3.78 ab	2.84 A
0.5 mgl^{-1}IBA +1.0 mgl^{-1} NAA	3.56 ab	1.76 cd	2.22 c	3.67 ab	2.83 A
Mean	3.22 A`	1.44 C`	1.87 B`	3.16 A`	

Callus index:
0.0 - 1: no or very small callus 1.1- 2 : small callus (less than 5 mm diameter)
2.1 - 3: moderate callus (less than 5-10 mm diameter) 3.1 - 4 : large callus (more than 10 mm diameter)
Means followed by the same letter(s) are not significantly different from each other at 5 % level .

cvs. showed the least values (1.00). Generally, IBA only or IBA combined with NAA resulted in increasing callus size for Om- Elfahm cv. and “Hansen” rootstock. Meanwhile , it was ineffective for Ne Plus Ultra and M- Dalet almond cultivars.

3.3.2. Effect on rooting percentages

Data in Table (14) showed the effect of IBA and NAA levels on rooting percentages of three almond cultivars and “Hansen” rootstock.

As for auxin treatments it is clear that all the used treatments resulted in different means of the rooting except with NAA at 1.0 mgl^{-1} only. Shoots treated by IBA combined with NAA significantly increased rooting percentages .The highest rooting percentages (38.89 and 33.34 %) were obtained with Bourgin and Nitsch medium supplemented with IBA at 0.4 mgl^{-1} plus NAA at 0.8 mgl^{-1} and IBA at 0.5 mgl^{-1} plus NAA at 1.0 mgl^{-1} respectively, without significant differences between them ,followed by IBA at 0.2 mgl^{-1} plus NAA at 0.4 mgl^{-1} . On the other hand, NAA or IBA only at 1.0 mgl^{-1} exhibited the least values (0.0 and 2.78%) of rooting percentages, respectively .

Concerning the varietal differences between almond cultivars and Hansen rootstock, the greatest rooting percentage (31.12%) was cleared with Hansen hybrid rootstock followed by (20.0%) for Ne Plus Ultra cultivar with insignificant difference between them. Whereas, the lowest value of rooting percentage (6.67%) was recorded for Om –Elfahm cultivar.

The interactions between treatments and cultivars , showed the highest value of rooting percentage (66.67%) with

Table 14. Effect of IBA and NAA levels on rooting percentages of almond cvs. (Om–Elfahm, M-Dalet and Ne Plus Ultra) and Hansen hybrid rootstock .

Cvs. / Treatments	Om–Elfahm	M-Dalet	Ne Plus Ultra	Hansen	Mean
IBA 1.0 mgl^{-1}	0.0 d	0.0 d	0.0 d	11.11 cd	2.78 C
NAA 1.0 mgl^{-1}	0.0 d	0.0 d	0.0d	0.0 d	0.0 C
0.2 mgl^{-1}IBA+0.4 mgl^{-1} NAA	0.0d	0.0 d	11.11 cd	66.67 a	19.45 B
0.4 mgl^{-1}IBA+0.8 mgl^{-1} NAA	33.34 bc	55.56 ab	33.33 bc	33.33 bc	38.89 A
0.5 mgl^{-1}IBA+1.0 mgl^{-1} NAA	0.0 d	33.34 bc	55.56 ab	44.45 ab	33.34 A
Mean	6.67 C`	17.78BC`	20.0 AB`	31.12 A`	

Means followed by the same letter(s) are not significantly different from each other at 5 % level.

Hansen shoots treated by IBA at 0.2 mgl^{-1} plus NAA at 0.4 mgl^{-1}. No significant differences between M-Dalet shoot treated by IBA at 0.4 mgl^{-1} plus NAA at 0.8 mgl^{-1} and Ne Plus Ultra or Hansen shoots treated by IBA at 0.5 mgl^{-1} plus NAA at 1.0 mgl^{-1}. However, no rooting was noticed with IBA at 1.0 mgl^{-1} for Om–Elfahm, M-Dalet and Ne Plus Ultra or by NAA at 1.0 mgl^{-1} for all used cultivars or shoot treated by IBA at 0.2 mgl^{-1} + NAA 0.4 mgl^{-1} for Om–Elfahm and M-Dalet cultivars and 0.5 mgl^{-1} IBA+1.0 mgl^{-1} NAA for Om –Elfahm .

Results indicated that the best rooting percentages were obtained with medium contained IBA at 0.2 mgl^{-1} plus NAA at 0.4 mgl^{-1} for "Hansen" rootstock and IBA at 0.5 mgl^{-1} plus NAA at 1.0 mgl^{-1} for Ne Puls Ultra cv. and IBA at 0.4 mgl^{-1} plus NAA at 0.8 mgl^{-1} for M-Dalet cv.

As far as the previous work in such concern, Bourgen and Nitsch medium is mostly used. This was found with **Caboni and Damiano (1994)** who used Bourgin and Nitsch medium supplemented by 10µM IBA or 10µM IAA for rooting shoots of two almond genotypes. Also, **Saeed (1998)** obtained highest rooting percentage for two almond cvs. (Ne Plus Ultra and Nonpareil) by using Bourgin and Nitsch medium supplemented by IBA+ NAA .On the contrary, **Wanas (1999)** found that using ½ MS medium plus 0.2 mgl^{-1} IBA + 0.4 mgl^{-1} NAA gave 75% rooting in Hansen rootstock.

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الإكثار المعملي لثلاثة من أصناف اللوز والأصل الجذري "هانسن"

[31]

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1– **قسم البساتين – كلية الزراعة – جامعة عبن شمس– شبرا الخيمة – القاهرة – مصر**

زرعت العقد الساقية لثلاث من أصناف اللوز (أم الفحم، إم – دالت ،نابلس الترا) والأصل الجذري الهجين" هانسن" على بيئة موراشيج وسكوج المضاف إليها تركيزات بنزيل أدنين (صفر-2) ملليجم / لتر وأندول حمض البيوتريك بتركيزات (صفر– 0.1) ملليجم/لتر.

وقد أشارت النتائج أن أعلى نسبة لتطور البراعم كانت مع الصنفين إم–دالت ونابلس الترا مع المعاملة المحتوية على 2ملليجم/ لتر فقط أو مضاف إليها 0.1 ملليجم /لتر من أندول حمض البيوتريك، وقد أعطى الأصل الج`ري هانسن أفضل طول للفرع عند إضافة 2 ملليجم/لتر بنزيل أدنين مع 0.01 ملليجم

/لتر أندول حمض البيوتريك وذلك خلال مرحلة التأسيس.

استخدام بنزيل أدنين بمعدل 1 ملليجم مع أندول حمض البيوتريك بمعدل 0.1ملليجم أعطى أعلى نسبة تفريع للصنف أم الفحم في حين أن استخدام 2 ملليجم بنزيل أدنين مع 0.1، 0.05 ملليجم أندول حمض البيوتريك أعطى أعلى نسبة تفريع للأصناف اللوز إم دالت – نابلس الترا والهجين الجذري هانسن خلال عملية إعادة الزراعة الثالثة.

أعلى متوسط لطول الفرع الناتج تم الحصول عليه عند استخدام بنزيل أدنين بمعدل 1 ملليجم / لتر مع أندول حمض البيوتريك بمعدل 0.1 ملليجم /لتر مع أصناف أم – دالت – نابلس ألترا – الهانسن بينما الصنف أم الفحم سجل أعلى طول للفرع الناتج عند استخدام 2 ملليجم /لتر بنزيل أدنين مع 0.1 ملليجم/لتر أندول حمض البيوتريك.

سجل الأصل الجدري هانسن والصنف أم الفحم أكبر حجم للكالس (>10مم قطر) عند معاملة الأفرع 0.4 ملليجم/ لتر أندول حمض البيوتريك+ 0.8 ملليجم / لتر نفتالين حمض الخليك.

أفضل نسبة تجذ ير تحققت مع الأصل الهانسن المعامل 0.2 ملليجم / لتر أندول حمض البيوتريك+ 0.4 ملليجم / لتر نفتالين حمض الخليك والصنف نابلس الترا المعامل 0.4 ملليجم / لتر أندول حمض البيوتريك 0.8 ملليجم/ لتر نفتالين حمض الخليك.

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