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EVALUATION OF SOME FACTORS AFFECTING *IN VITRO* MICROPROPAGATION OF FEW SEEDED FRUIT GUAVA TREES

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ABSTRACT: This study was carried out during the period from 2013 to 2016 in the Plant Tissue Culture Laboratory Hort. Dept., Fac. Agric., Zagazig University, Egypt to create a protocol for propagating few seeded fruit guava trees throughout the various stages of tissue culture technique. During the establishment stage, explants (nodal explants and shoot tips) were excised from 6- month-old seedlings grown in the green house. The excised explants were cultured in Murashige and Skoog (MS) medium supplemented with 1mg/l benzyl adenine (BA) combined with 100 mg/l polyvinylpyrrelidone (PVP). Results showed that nodal explants proved to be better than shoot tips concerning growth parameters. Different BA concentrations *i.e.* 0.0, 0.5, 1 or 2 mg/l proved the efficiency of 2mg/l BA for shoot proliferation from nodal explants, followed by 1mg/l BA. Inoculation nodal explants during different dates revealed that spring season (mid April) produced the best growth parameters. Obtained shoots about 1cm length from establishment stage were cultured in MS medium provided with BA or kinetin (Kin.), at different concentrations (0.0, 0.5, 1, 2, 4, or 6 mg/l). Results showed that BA recorded the highest multiplication rate compared with Kin., which produced one shoot, only, whereas 2mg/l BA gave the highest number of shoots/ explant (10.62 shoots/ explant). Addition of sucrose at 20, 30 or 40 g/l to MS medium supported with 2mg/l BA enhanced the efficiency of 40 g/l sucrose concerning number of shoots/explant (12 shoots/explant). The combination of 2mg/l BA and different auxins (NAA, IAA or IBA) at low concentrations showed that 2 mg/l BA combined with 0.5 mg/l IBA gave the highest number of shoots/ explant (11.20 shoots/ explant). During rooting stage, using each of NAA, IBA or IAA at 0, 1, 2 or 4 mg/l in MS medium, resulted in shoot rooting. Although NAA resulted in more number of roots/ shoot, IBA at 1 or 2 mg/l was adequate for producing better rooting shoots for acclimatization stage. The obtained rooted plantlets could be successfully acclimatized in peat moss medium incorporated with sand at 1:2 (*V/V*), since it gave 100% survival.

Key words: Micropropagation, *in vitro* culture, few seeded fruit guava trees, nodal explants, shoot tips, benzyl adenine (BA), kinetin (Kin.), sucrose, auxins, IBA.

INTRODUCTION

Guava, (*Psidium juagava* L.), belongs to the family Myrtaceae. The origin of guava is tropical America from Mexico to Piro. Guava is a popular fruit crop, cultivated in many countries such as USA, Brazil, Colombia, India, Egypt, South Africa, and Saudi Arabia due to its favorite taste and high fruit vitam. C content. Guava includes tree strain-types as follows:

Seeded Strains (Balady)

Fruit set of these strains occurs simultaneously through self and cross pollinations. Balady strains are containing large numbers of seeds. Vegetative and fruiting traits show clear segregation due to the heterozygous genotype. Balady strains bear, usually, heavy crops, but their main defect is the high fruit seed content. Also, the seedling trees have different responses to the environmental factors and required different horticultural practices.

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Strains with Few Seeds in Fruits

North Egypt zone regions have guava strains with fewer number of seeds and have local names like: Rashidi, Baraka,… *etc*. Flower bagging, to insure self pollination, is usually used to obtain true to type plants by seed propagation.

Seedless Guava: (Banaty)

Fruit set of seedless guava is parthenocarpic. Fruits are relatively large and lower sweeter, but the trees usually bear lower yields compared to other guava strains, particularly seeded ones.

Plant growth regulators are numerous chemical substances that profoundly influence the growth and differentiation of plant cells, tissues and organs. Auxins stimulate division and elongation of cell. On the other hand, cytokinins are able to stimulate cell division and induce shoot bud formation in tissue culture.

Mishra *et al.* (2007) mentioned that culturing nodal segments of guava cv. Pant Prabhatin in MS media containing 4.0 mg/l BA and 0.2 mg/l IBA resulted in higher shoot proliferation. Also, Usman *et al.* (2012) worked on micropropagation of Elite guava (*Psidium guajava* L.) strains of cv. Safeda, found that enhancing sucrose supplement to 45 g/1 improved plant growth and development of shoot and root length as well as leaf area compared to 30 g/l of sucrose in the media. Likewise, Rabeh *et al.* (2015) performed a study on micropropagation of guava cvs. Banaty, Sabahy and Mobaker , and mentioned that BA at 1.0 mg/l gained the best effect on shoot production per explant of the three guava cultivars.

The main goal of this study was evaluating the effect of some treatments affecting micropropagation of few seeded fruit guava trees to achieve guava plantations of the same origin.

MATERIALS AND METHODS

This study was carried out in the Plant Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt throughout the period from 2013 to 2016.

The used basal medium was Murashige and Skoog (MS) (1962). The considered medium was supplemented with the other tested additions according to the aim of each experiment. pH medium was adjusted to 5.8. Afterwards, 50 ml of the medium was poured into 370 ml jars, which were immediately plugged with polypropylene closures. The medium was then autoclaved at 121°C and 1.1 kg/cm^2 pressure for 20 minutes. All cultures of the different experiments were maintained in a growth room chamber at 25 ±2°C and exposed to 16 hr., day photoperiod and 8 hr., darkness at an intensity of 2000 Lux from cool white fluorescent lamps.

Establishment Stage

Effect of explant type on micropropagation during establishment stage of few seeded fruit guava trees

Shoot tips (with two lateral buds) and nodal explants of about 1cm length and 2-3 mm thickness were obtained from sprouted shoots of 6 months old seedlings grown in the green house. The explants were soaked in a soap solution for 5 min., then washed under running water for 1 hr. The explants were then soaked in rhizolex fungicide solution (2 g/l) for 30 min., followed by washing with distilled water three times then transferred to aseptic conditions inside the culture cabinet (laminar air flow cabinet), and then sterilized with the mercuric chloride at 0.1% for 3 min., followed by rinsing explants three times with sterilized distilled water, then soaked in Clorox solution (NaOCl, 5.25% free chlorine) at 5% for 5 min., followed by rinsing explants three times with sterilized distilled water under the same conditions. The sterilized explants were cultured in glass jars (100 ml) containing MS medium amended with 1mg/l benzyl adenine (BA). In establishment stage experiments, the medium was amended with 100 mg/l polyvinylpyrrelidone (PVP) to prevent browning.

Effect of some benzyl adenine (BA) concentrations during establishment stage on nodal explants of few seeded fruit guava trees

The sterilized nodal explants were cultured on MS medium supplemented with different concentrations of BA (0.0, 0.5, 1, or 2 mg/l).

Effect of nodal explant inoculation date during establishment stage of few seeded fruit guava trees

Nodal explants were inoculated during spring (mid-April), summer (mid-July), autumn (mid-October), and the winter seasons (mid-January) then sterilized as previously mentioned and cultured on MS medium supplemented with 1 mg/l BA.

The data recorded for all experiments of establishment stage after six weeks of culture period were: 1. Survival percentage; 2. Number of shoots/explant; 3. Shoot length; 4. Number of leaves/shoot and 5. Other observations.

Multiplication stage

In this stage 1 cm length growing shoots, previously obtained from nodal explants cultured on MS medium with 1mg/l BA, were used in the following experiments of multiplication stage :

Effect of benzyl adenine (BA) concentration during multiplication stage of few seeded fruit guava trees

1cm length growing shoots previously obtained from nodal explants, were cultured on MS medium supplemented with different concentrations of BA (0.0, 0.5, 1, 2, 4, or 6 mg/l).

Effect of kinetin (Kin.) concentration during multiplication stage of few seeded fruit guava trees

1 cm length growing shoots previously obtained from nodal explants, were cultured on MS medium provided with different concentrations of Kin. (0.0, 0.5, 1, 2, 4, or 6 mg/l).

Effect of sucrose concentration in MS medium during multiplication stage of few seeded fruit guava trees

1 cm length growing shoots previously obtained from nodal explants, were cultured on MS medium amended with 2mg/l BA and different concentrations of sucrose (20, 30, or 40 g/l).

Effect of BA and auxins during multiplication stage of few seeded fruit guava trees

1 cm length growing shoots previously obtained from nodal explants, were cultured on MS medium supplemented with 2mg/l BA alone or combined with 0.5 or 1 mg/l of α- naphthalene acetic acid (NAA), indol-3- acetic acid (IAA), or indole -3- butyric acid (IBA).

Number of shoots/explant, average shoot length (cm), average number of leaves/shoot and other observations were determined six weeks later of culture period.

Rooting Stage

Effect of different auxins on rooting stage of few seeded fruit guava trees

1.5 cm length growing shoots which were previously obtained from multiplication stage, were cultured on MS medium supplemented with 0.0, 0.5, 1, 2 or 4 mg/l of α- naphthalene acetic acid (NAA), indol-3- acetic acid (IAA), or indole -3- butyric acid (IBA) beside control (without growth regulators).

Rooting percentage, number of roots/shoot, total length (cm) of roots/shoot, shoot length (cm), number of leaves/shoot and other observations were determined six weeks later of culture period.

Acclimatization stage

The objective of this stage was to adapt the plantlets, obtained *in vitro* before transferring to the open field. The produced plantlets were washed with tap water, and then disinfected by immersing the roots in rhizolex solution (1.0 g/l) for 10 minutes. The plantlets were then transferred to plastic pots (9 × 7 cm) containing: sand + peat moss at 1:1, 2:1 and 3:1(*V/V*), or Peat moss alone. The cultured pots were covered with polyethylene bags to keep high relative humidity around the plantlets during incubation at 25± 2°C and 2000 Lux photoperiod in a growth room. Survival percentage; plantlet length (cm); and number of leaves / shoot were determined six weeks later of acclimatization stage.

Statistical Analysis

The statistical layout of all the above mentioned experiments during the whole work was the complete randomized design. The obtained data were statistically analyzed according to Snedecor and Cochran (1980). The means were compared using the Duncan's (1955) multiple range test at 0.05.

RESULTS AND DISCUSSION

Establishment Stage

Effect of explant type on some micropropagation parameters during establishment stage of few seeded fruit guava trees

Results in Table 1 and Photo 1 show that survival percentage was 100% in shoot tips and nodal explants. The highest number of shoots/ explant (6.17 shoots/explant), the longest shoot (2.30 cm), and the highest number of leaves/ shoot (10.09 leaves/shoot) were produced by nodal explants with significant differences against values of shoot tips explant (2.67, 1.16 and 7.36, respectively).

Effect of benzyl adenine (BA) concentration on some growth parameters of nodal explants during establishment stage of few seeded fruit guava trees

Results in Table 2 and Photo 2 show that BA at 2 mg/l produced the highest number of shoots/ nodal explant (7.83 shoots/explant) accompanied with less significant shoot length (2.06 cm) and higher number of leaves/ shoot (11.87 leaves/shoot) comparing to lower BA concentrations (0.5 and 1 mg/l). On the other side, control treatment recorded the lowest number of shoots/nodal explant and number leaves/shoot (1.20 and 6.20, respectively) and the highest shoot length (3.45 cm).

Effect of nodal explant inoculation date on some growth parameters during establishment stage of few seeded fruit guava trees

Results in Table 3 and Photo 3 demonstrate that there was no contamination during spring (mid- April) and summer (mid- July) inoculations, where it reached 52.9% and 60% during autumn (mid- October) and winter (mid-January) inoculations, respectively. Number of shoots/ nodal explant was the highest in spring inoculation (6.18 shoots/nodal explant), followed by summer inoculation (4.33 shoots/ nodal explant), where it was the least in each of autumn and winter inoculation (2.00 shoots/ nodal explant). Average shoot length followed similar trend, while the number of leaves/shoot did not show significant differences in its response to nodal explant inoculation date.

Generally, for the establishment stage, nodal explants are preferred than shoot tips where it gave the highest number of shoots/explant, shoot length, and the highest number of leaves/ shoot. In this regard Amin and Jaiswal (1988) found that culturing nodal explants of guava (*Psidium guajava* L.) cv. Chittidar on Murashige and Skoog (MS) medium containing 1 mg/l benzyl adenine, axillary buds grew out within 3–4 weeks. On transfer to fresh medium of the same composition, these shoots attained 3–5 cm in length and had 4–6 nodes after 4 weeks following culture. Likewise, Mishra *et al.* (2007) mentioned that culturing nodal segments of guava cv. Pant Prabhatin in MS media containing 4.0 mg/l BA and 0.2 mg/l IBA resulted in higher shoot proliferation.

Concerning effect of BA treatment, 2mg/l BA treatment followed by 1mg/l was found to be the best concentrations for shoot induction compared with the other tested BA concentrations (0.0 and 0.5 mg/l). Likewise, Usman *et al.* (2012) cleared that shoot induction of guava cv. Safeda was enhanced up to 83% with 3.5 to 4.25 shoots per single node cutting and shoot tip explants, respectively using higher levels of BAP (1.5 and 2 mg/l) in MS medium. Among inoculation dates, the spring (mid-April) was the best time for taking explants, which resulted in the highest parameters followed by the summer season (mid- July). The obtained results are in harmony with Meghwal *et al.* (2010) worked on micropropagation of guava cv. Allahabad Safeda and found that the nodal explants excised during April-June were better for culture initiation.On the other side, Mohsin *et al.* (2008) found that when explants of Bari guava were cultured on February, growth parameters such as survival percentage, number of shoots per explant, shoot length, and number of leaves/shoot were higher, followed by March and April. This might be attributed to the varietal differences and different temperatures.

Table 1. Effect of explant type on some micropropagation parameters during establishment stage of few seeded fruit guava trees

Explant type	Survival percentage	Number of shoots / explant	Shoot length (cm)	Number of leaves/shoot
Shoot tips	100	2.67 b	1.16 b	7.36 b
Nodal explants	100	6.17 a	2.30 a	10.09 a

Means followed by the same letter (s) within each column are not significantly different according to Duncan's multiple range test (P= 0 .05).

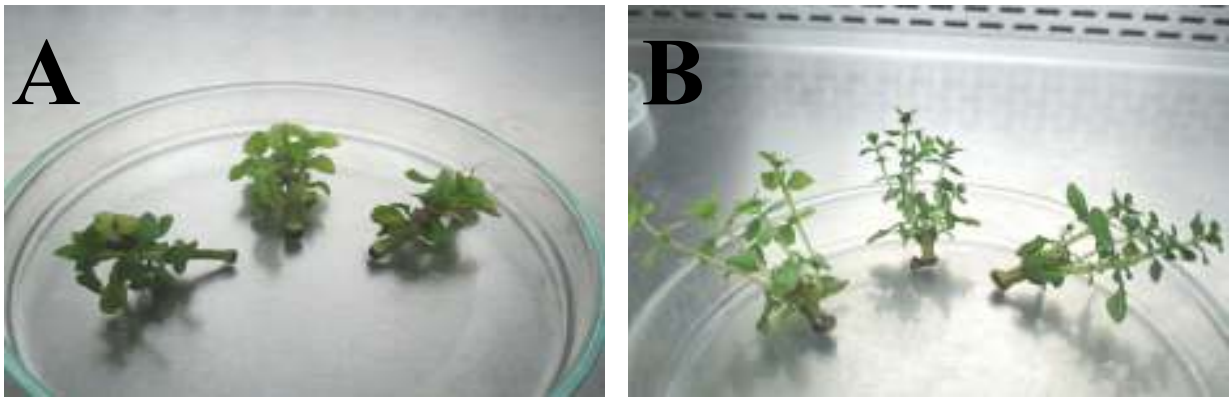

Photo 1. Effect of explant type on some micropropagation parameters during establishment stage of few seeded fruit guava trees

A. Shoot tips B. Nodal explants.

Table 2. Effect of benzyl adenine (BA) concentration on some growth parameters of nodal explants during establishment stage of few seeded fruit guava trees

Treatment	Number of shoots/nodal explant	Shoot length (cm)	Number of leaves/shoot
Control	1.20 d	3.45 a	6.50 b
0.5 mg/l BA	4.00 c	3.00 ab	12.15 a
1.0 mg/l BA	6.17 b	2.30 ab	10.08 a
2.0 mg/l BA	7.83 a	2.06 b	11.87 a

Means followed by the same letter (s) within each column are not significantly different according to Duncan's multiple range test (P= 0 .05).

Photo 2. Effect of benzyl adenine (BA) concentration on some growth parameters of nodal explants during establishment stage of few seeded fruit guava trees

A. Control. B. 1 mg/l BA. C. 2 mg/l BA.

Table 3. Effect of nodal explant inoculation date on some growth parameters during establishment stage of few seeded fruit guava trees

Explant inoculation date	Contamination percentage	Number of shoots/explant	Shoot length (cm)	Number of leaves/ shoot
Spring (mid –April)	0.00	6.18 a	2.30 ab	10.09 a
Summer (mid- July)	0.00	4.33 b	2.48 a	9.18 a
Autumn (mid- October)	52.90	2.00 c	1.00 c	8.40 a
Winter (mid- January)	60.00	2.00 c	1.80 b	9.00 a

Means followed by the same letter (s) within each column are not significantly different according to Duncan's multiple range test (P= 0 .05).

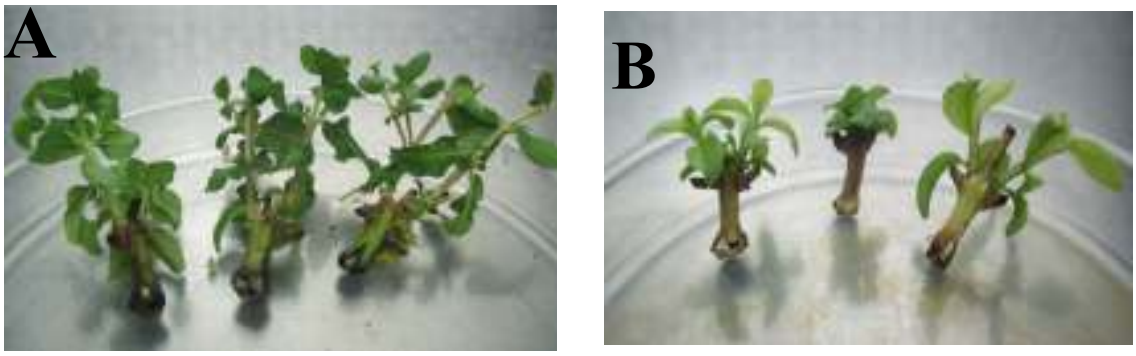

Photo 3. Effect of nodal explant inoculation date on some growth parameters during establishment stage of few seeded fruit guava trees

A. Nodal explants inoculated during summer season. B. Nodal explants inoculated during autumn season.

Multiplication Stage

Effect of benzyl adenine (BA) concentration on some growth parameters during multiplication stage of few seeded fruit guava trees

Results in Table 4 and Photo 4 illustrate that BA at 2 mg/l gained the highest number of shoots/ explant (10.62 shoots/explant) accompanied with less significant shoot length (1.54 cm) and number of leaves/shoot (10.07 leaves/ shoot). The results cleared also that control explants produced 90% rooting with considerable root length 4.39 cm, that was not obtained for BA concentrations since it depressed rooting process.

Effect of kinetin (Kin.) concentration on some growth parameters during multiplication stage of few seeded fruit guava trees

Table 5 reveal that kinetin was not promising in enhancing number of shoots / explant and significantly depressed shoot length without any significant effect on number of leaves/shoot. On the other side, rooting percentage was decreased as kinetin concentration increased, while number of roots and root length were decreased with all kinetin concentrations comparing to control. The highest values for those parameters were belong to control.

Effect of sucrose concentration in MS medium supplemented with 2 mg/l BA on multiplication stage of few seeded fruit guava trees

Results in Table 6 and Photo 5 show that increasing sucrose concentration up to 40 mg/l resulted in more significant shoots number/ explant than 20 or 30 mg/l sucrose. However, both shoot length and number of leaves/shoot were not significantly affected with sucrose concentration.

Table 4. Effect of benzyl adenine (BA) concentration on some growth parameters during multiplication stage of few seeded fruit guava trees

Treatment	Number of shoots/explant	Shoot length (cm)	Number of leaves/shoot	Rooting percentage	Number of roots/shoot	Root length (cm)
Control	1.80 d	3.10 a	12.07 a	90.00	2.80 a	4.39 a
0.5 mg/l BA	3.22 c	2.18 b	9.99 b	10.00	0.30 b	0.10 b
1.0 mg/l BA	5.88 bc	1.84 bc	9.96 b	0.00	0.00 b	0.00 b
2.0 mg/l BA	10.62 a	1.54 c	10.07 b	0.00	0.00 b	0.00 b
4.0 mg/l BA	8.66 ab	1.88 bc	10.33 b	0.00	0.00 b	0.00 b
6.0 mg/l BA	6.90 b	1.94 bc	11.25 ab	0.00	0.00 b	0.00 b

Means followed by the same letter (s) within each column are not significantly different according to Duncan's multiple range test (P= 0 .05).

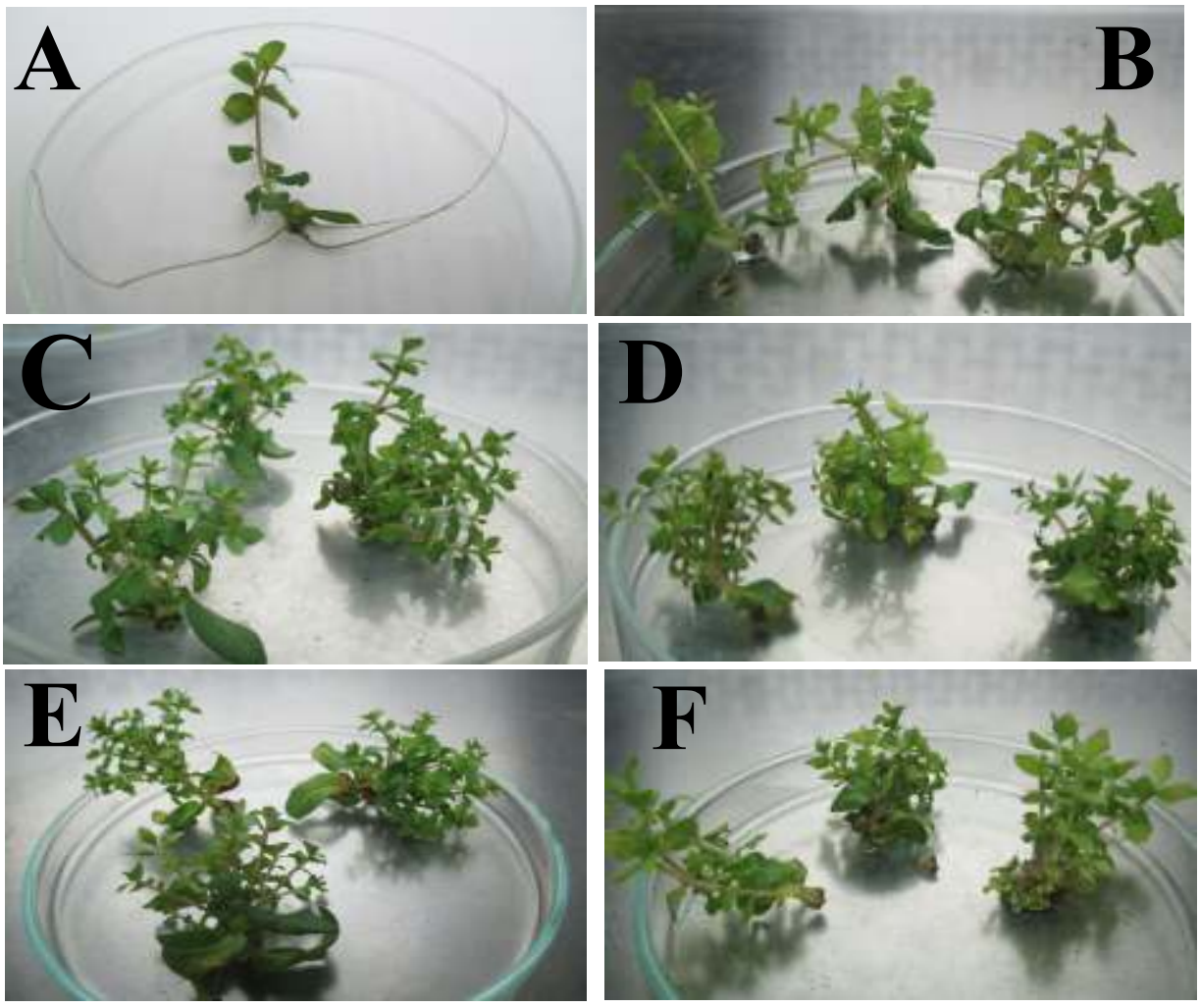

Photo 4. Effect of benzyl adenine (BA) concentration on some growth parameters during multiplication stage of few seeded fruit guava trees

A. Control. B. 0.5 mg/l BA. C. 1 mg/l BA.
D. 2 mg/l BA. E. 4 mg/l BA. F. 6 mg/l BA.

Table 5. Effect of kinetin (Kin.) concentration on some growth parameters during multiplication stage of few seeded fruit guava trees

Treatment	Number of shoots/explant	Shoot length (cm)	Number of leaves/shoot	Rooting percentage	Number of roots/shoot (cm)	Root length (cm)
Control	1.20 ab	3.58 a	13.20 a	90.00	1.60 a	4.72 a
0.5 mg/l Kin.	1.40 a	2.01 b	11.50 a	40.00	1.00 b	0.40 b
1.0 mg/l Kin.	1.10 ab	2.50 b	11.30 a	30.00	1.02 b	0.90 b
2.0 mg/l Kin.	1.00 b	2.25 b	11.50 a	20.00	0.60 b	0 .13 b
4.0 mg/l Kin.	1.00 b	2.27 b	11.20 a	10.00	0.20 b	0 .10 b
6.0 mg/l Kin.	1.00 b	2.01 b	11.10 a	10.00	0.30 b	0.13 b

Means followed by the same letter (s) within each column are not significantly different according to Duncan's multiple range test (P= 0 .05).

Table 6. Effect of sucrose concentration in MS medium supplemented with 2 mg/l BA on multiplication stage of few seeded fruit guava trees

Sucrose concentration (g/l)	Average number of shoots/ explant	Average shoot length(cm)	Average number of leaves/shoot
20	6.00 b	0.90 a	9.89 a
30 (control)	7.27 b	0.90 a	9.65 a
40	12.00 a	0.85 a	8.40 a

Means followed by the same letter (s) within each column are not significantly different according to Duncan's multiple range test (P= 0 .05).

(6.00 shoots/explant) **(7.27 shoots/explant)** **(12.00 shoots/explant)**

Photo 5. Effect of sucrose concentration in MS medium supplemented with 2 mg/l BA on multiplication stage of few seeded fruit guava trees

A. 20 g/l sucrose. B. 30 g/l sucrose. C. 40 g/l sucrose.

Effect of 2mg/l BA combined with different auxins concentrations on multiplication stage of few seeded fruit guava trees

Results in Table 7 and Photo 6 demonestrat that addition of 0.5 or 1 mg/l NAA or IAA and 1 mg/l IBA suppressed the enhancing effect of 2mg/l BA on number of shoots/explant. On the contrary, when 0.5 mg/l IBA was combined with 2 mg/l BA resulted in significant increase in number of shoots/explant than using 2mg/l BA alone. However, application of IAA or IBA combined with 2mg/l BA significantly enhanced shoot length, while the maximum number of leaves/ shoot was obtained when 2 mg/l BA combined with 1 mg/l IAA or NAA without significant differences between them.

Generally, the highest multiplication rate (number of shoots/explant) was obtained by 2 mg/l BA. Increasing BA concentration up to 6 mg/l decreased number of shoots /explant. This indicate that BA had an adverse effect when used at a higher than moderate concentration. On the other hand, using Kin. alone at 0.0, 0.5,1, 2, 4 or 6 mg/l was not effective in shoot multiplication compared with BA, and did not differ significantly than control treatment (produced approximately only one shoot/ explant). The aforementioned findings are in harmony with Rai *et al.* (2009) worked on micropropagation of guava and mentioned that BAP was more effective than Kin in inducing shoot proliferation. On medium containing Kin; only one shoot was developed per an explant or there was no shoot proliferation at all. Likewise, Strosse *et al.* (2008) found that the cytokinins thidiazuron and benzyl aminopurine stimulated multiplication of banana to a larger extent compared to zeatin, kinetin and isopentenyl adenine.

Also, shoot proliferation of lemon decreased with increasing concentration of BA alone, (Goswami *et al.*, 2013). Superiority of BA for shoot induction may be attributed to the ability of plant tissues to metabolize BA more readily than other synthetic growth regulators (Malik *et al*., 2005).

Application of sucrose at 20, 30 or 40 g/l to MS medium provided with 2mg/l BA declared that 40 g/l sucrose gave the highest number of shoots/explant, but produced the shortest shoots and the least number of leaves/ shoot compared with 20 or 30 g/l sucrose. In this regard, Usman *et al.* (2012) worked on micropropagation of Elite guava (*Psidium guajava* L.) strains of cv. Safeda, found that enhancing sucrose supplement to 45 g/1 improved plant growth and development of shoot and root length as well as leaf area compared to 30 g/l of sucrose in the media.

Using 2mg/l BA alone or combined with low concentrations (0.50 or 1 mg/l) of different auxins (NAA, IAA, or IBA) cleared that 2mg/l BA combined with 0.5 mg/l IBA gave the highest multiplication rate and the longest shoots compared with 2mg/l BA alone without significant difference between them in number of leaves /shoot. The obtained result was in harmony with Mishra *et al.* (2007) who revealed that higher shoot growth of guava cv. Pant Prabhat was recorded with 3.0 mg/l BA and 0.2 mg/l IBA. Likewise, Rabeh *et al.* (2015) performed a study on micropropagation of guava cvs. Banaty, Sabahy and Mobaker , and mentioned that BA at 1.0 mg/l gained the best effect on shoot production per explant of the three guava cultivars, but the better effects on shoot production were obtained when IBA at 0.02 to 2.0 mg/l combined with BA at 1-2 mg/l were used.

Rooting Stage

Effect of different auxins on some growth parameters during rooting stage of few seeded fruit guava trees.

Table 8 and Photo 7 indicate that with control treatment (without auxin addition) rooting percentage was 80%, while it increased to be 100% with all auxins concentrations. NAA concentrations significantly depressed root length while IBA concentrations (2and4mg/l) and 0.5 mg/l IAA significantly enhanced it and came in the maximum length (4.50 , 5.30 and

Table 7. Effect of 2mg/l BA combined with different auxins concentrations on some growth parameters during multiplication stage of few seeded fruit guava trees

Auxin concentration (mg/l)	Number of shoots/explant	Shoot length (cm)	Number of leaves/shoot
2BA alone	7.26 b	1.17 c	10.07 bc
2BA+ 0.5 NAA	2.56 c	0.94 d	9.89 bc
2BA+ 1 NAA	1.33 c	1.19 bc	11.26 ab
2BA+ 0.5 IAA	8.00 b	1.14 c	9.14 c
2BA+ 1 IAA	7.66 b	1.38 ab	12.54 a
2BA+ 0.5 IBA	11.22 a	1.51 a	10.87 b
2BA+ 1 IBA	7.83 b	1.49 a	10.57 bc

Means followed by the same letter(s) within each column are not significantly different according to Duncan's multiple range test (P= 0.05).

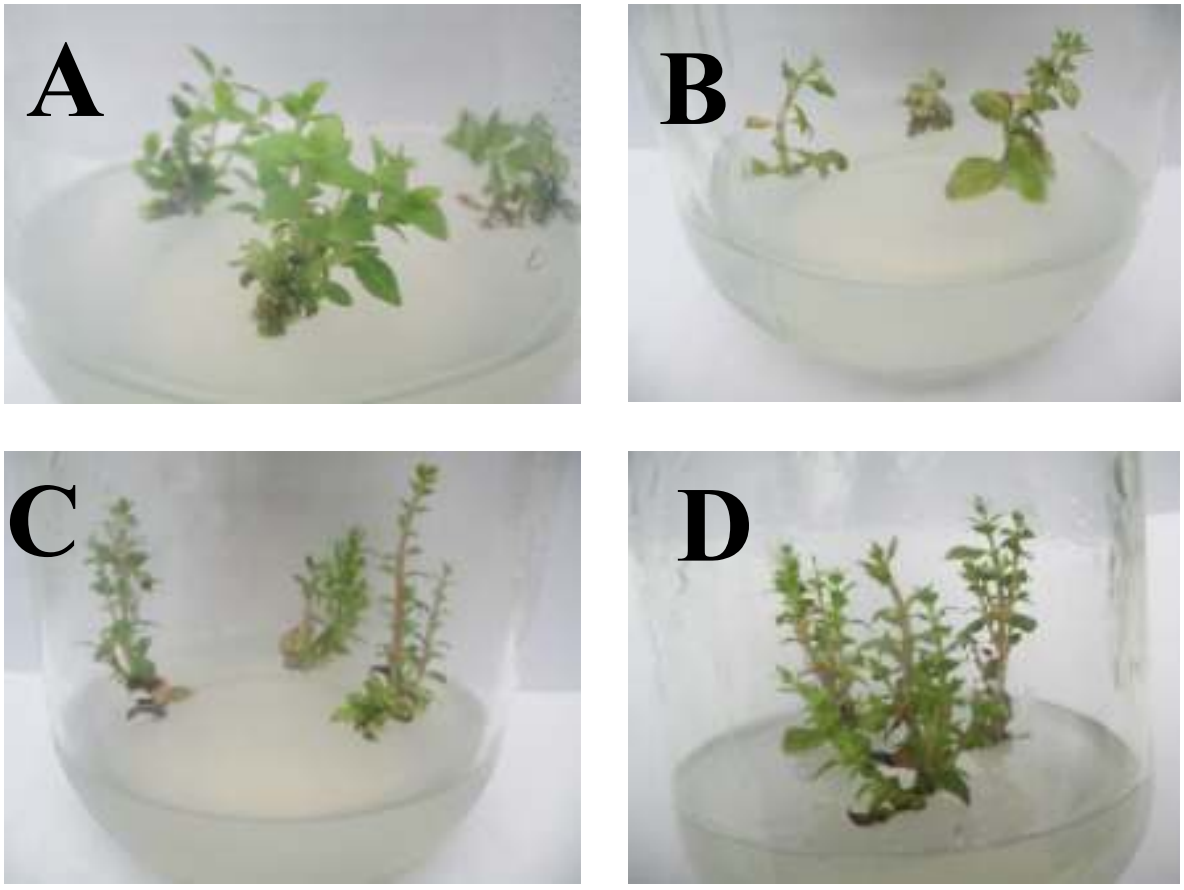

Photo 6. Effect of 2mg/l BA combined with different auxins concentrations on some growth parameters during multiplication stage of few seeded fruit guava trees

A. 2mg/l BA alone.

B. 2mg/l BA+0.5 mg/l NAA.

C. 2mg/l BA+1 mg/l IAA.

D. 2mg/l BA+0.5 mg/l IBA.

Table 8. Effect of different auxins on some growth parameters during rooting stage of few seeded fruit guava trees

Auxins concentrations (mg/l)	Rooting (%)	Number of roots/shoot	Root length (cm)	Shoot length (cm)	Number of leaves/shoot
Control	80.00 b	1.40 d	2.66 c	2.99 c	14.63 b
0.5 NAA	100.00 a	6.68 c	1.19 d	1.85 cd	11.78 cd
1 NAA	100.00 a	9.29 b	0.97 d	2.33 c	13.11 c
2 NAA	100.00 a	14.50 a	0.78 d	1.30 d	9.60 de
4 NAA	100.00 a	13.18 ab	0.45 d	1.35 d	9.00 de
0.5 IAA	100.00 a	1.90 d	4.79 ab	4.11 ab	11.50 cd
1 IAA	100.00 a	2.30 d	3.37 bc	4.45 ab	17.20 a
2 IAA	100.00 a	2.36 d	3.42 bc	4.02 ab	15.50 ab
4 IAA	100.00 a	2.44 d	1.17 d	4.58 a	16.29 a
0.5 IBA	100.00 a	1.60 d	1.43 d	2.03 cd	12.83 c
1 IBA	100.00 a	2.60 d	4.17 b	3.79 b	12.26 c
2 IBA	100.00 a	2.14 d	4.50 ab	2.29 c	10.58 d
4 IBA	100.00 a	2.00 d	5.30 a	1.56 cd	8.40 e

Means followed by the same letter(s) within each column are not significantly different according to Duncan's multiple range test (P= 0.05).

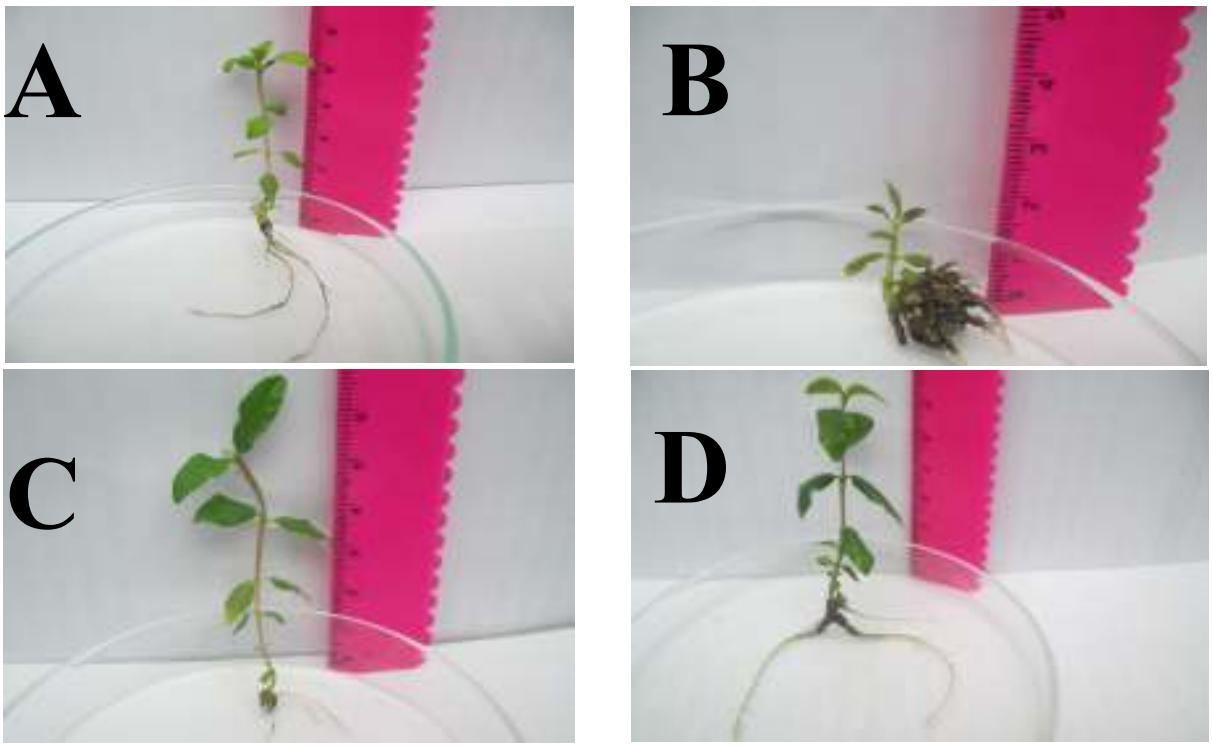

Photo 7. Effect of different auxins on some growth parameters during rooting stage of few seeded fruit guava trees

A. The control. B. 2 mg/l NAA. C. 4 mg/l IAA. D. 2 mg/l IBA.

4.79 cm, respectively). Shoot length recorded the highest values with 0.5, 1, 2, or 4 mg/l IAA, while it recorded the least significant values with 2 or 4 mg/l NAA. Also, number of leaves/ shoot followed a similar trend to that of shoot length parameters.

In the herein work, NAA was more effective in enhancing number of roots / shoot , especially at 2 and 4 mg/l, while IBA at 1, 2, or 4 mg/l was more effective in enhancing root length during rooting stage . Likewise, IAA at 0.5, 1, or 2 mg/l was the most effective in enhancing shoot length and number of leaves/shoot during rooting stage. However, it was clear that IBA at 1 or 2 mg/l was adequate during rooting stage to produce good rooting shoots for the following acclimatization stage. In this regard, Pierik (1987) pointed out that the most efficient auxins were definitely IBA and NAA. Also, Gokhale and Bansal (2009) mentioned that although all the 3 auxins (NAA, IAA, and IBA) induced rooting *in vitro* propagation of *Orxylum indicum* (L.) Vent, yet IBA responced better for all parameters of rooting. IBA is a potential auxin that induces rooting *in vitro* regenerated shoots (Iriondo *et al.,* 1995 ; Rajore and Batra, 2005). Similarly, Baskaran and Jayabalan (2005) on *Eclipta alba* mentioned that full strength MS medium fortified with 9.8 µM IBA showed better root formation compared to half strength MS medium with 9.8 µM IBA. Moreover, it promoted lengthy roots and strengthened root induction within twenty days of culture. This was true for Full MS strength for date palm explants (Badawy *et al.,* 2005).

Acclimatization Stage

Effect of media on plantlets growth after 6 weeks later of acclimatization stage

Results in Table 9 show that the best adapting medium was 2 sand: 1 peat moss (*V/V*) which gave 100% survival percentage, long plantlets (6.08 cm), and high number of leaves/ plantlet (13. 39 leaves/ plantlet), descendungly followed by 1 sand : 1 peat moss (*V/V*) with values (96.88%, 6.39 and 14.14, respectively). Peat moss alone gained similar survival percentage to that of 1sand: 1 Peat moss medium (96.88%), but it recorded the least plantlet length and number of leaves/ plantlet.

Table 9. Effect of acclimatization media on plantlets growth parameters after 6 weeks later of acclimatization stage

Acclimatization media (*V/V*)	Survival percentage	Plantlet length (cm)	Number of leaves/ plantlet
1 sand : 1 peat moss	96.88	6.39 a	14.14 a
2 sand : 1 peat moss	100.00	6.08 a	13.39 ab
3 sand : 1 peat moss	90.91	5.65 ab	12.02 b
Peat moss alone	96.88	4.99 b	12.18 b

Means followed by the same letter(s) within each column are not significantly differ according to Duncan's multiple range test (P= 0.05).

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تقييم بعض العوامل المؤثرة على الإكثار الدقيق بزراعة الأنسجة لأشجار الجوافة ذات الثمار قليلة البذور

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أجريت هذه الدراسة خلال الفترة من ٢٠١٣ إلى ٢٠١٦ في معمل زراعة الأنسجة النباتية بقسم البساتين كلية الزراعة، جامعة الزقازيق، مصر لوضع بروتوكول لإكثار أشجار الجوافة ذات الثمار قليلة البذور باستخدام تقنية زراعة الأنسجة، خلال مرحلة الإنشاء تم أخذ المنفصل النباتى (المنفصل النباتى ذات العقدة أو قمم الأفرخ) بطول ١سم من شتلات عمرها ٦ شهور نامية فى الصوبة، وتم زراعتها على بيئة موراشيج وسكوج المدعمة ب ١ مجم/لتر بنزيل أدنين متداخل مع ١٠٠ مجم/لتر بولى فينيل بيريليدون، وأظهرت النتائج كفاءة المنفصل النباتى ذات العقدة مقارنة بقمم الأفرخ بالنسبة لقياسات النمو المختلفة، أوضح استخدام تركيزات مختلفة من البنزيل أدنين (صفر، ٠,٥ ، ١ أو ٢ مجم/ لتر) أن المعاملة بتركيز ٢ مجم/لتر أعطت أفضل نمو للأفرخ يليها المعاملة بتركيز ١ مجم/ لتر، عند زراعة المنفصل النباتى خلال فصول السنة المختلفة، وجد أن المنفصلات النباتية المأخوذة فى فصل الربيع (منتصف أبريل) هى الأفضل فيما يخص قياسات النمو المختلفة، أُجريت مرحلة التضاعف للأفرخ الناتجة من مرحلة الإنشاء بطول ١سم باستخدام تركيزات مختلفة من البنزيل أدنين أوالكينتين (صفر، ٠,٥ ، ١، ٢، ٤، أو ٦ مجم/لتر)، وأوضحت النتائج تفوق البنزيل أدنين فى إعطاء أعلى معدل تضاعف للأفرخ مقارنة باستخدام الكينتين الذى أعطى فرخ واحد فقط، وكان أفضل تركيز من البنزيل أدنين هو ٢ مجم /لتر حيث أعطى أكبر عدد من الأفرخ (١٠,٦٢ فرخ/منفصل نباتى)، كما أدى إضافة السكروز بتركيز٤٠ مجم/ لتر من ضمن الإضافات المختبرة للسكروز (٢٠، ٣٠ ، أو ٤٠ مجم/لتر) لبيئة موراشيج وسكوج المحتوية على ٢ مجم/ لتر بنزيل أدنين إلى إعطاء أكبر عدد من الأفرخ (١٢ فرخ/منفصل نباتى)، أظهر التداخل بين ٢ مجم/لتر بنزيل أدنين مع تركيزات منخفضة (٠,٥ أو ١ مجم/لتر) من الأوكسينات المختلفة (نفثالين حمض الخليك، أندول حمض الخليك، أو أندول حمض البيوتريك) أن المعاملة بتركيز٢ مجم/لتر بنزيل أدنين مدعومة بتركيز ٠,٥ مجم/لتر إندول حمض البيوتريك حققت أكبر عدد من الأفرخ (١١,٢٠ فرخ/منفصل نباتى)، كما وجد أن استخدام الأوكسينات المختلفة بتركيزات صفر، ١، ٢، أو ٤ مجم/لتر فى بيئة موراشيج وسكوج أثناء مرحلة التجذير أدى إلى تجذير الأفرخ ، وبالرغم أن نفثالين حمض الخليك أعطى عدد كبير من الجذور/الفرخ، إلا أن إندول حمض البيوتريك بتركيز ١ أو ٢ مجم/لتر أعطى أفرخ مجذرة مناسبة لمرحلة الأقلمة، وقد نجحت أقلمة النبيتات الناتجة فى بيئة الرمل: البيت موس بنسبة ٢: ١ (حجمية/حجمية) بنسبة بقاء ١٠٠%.

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