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PLANT REGENERATION OF *ZIZIPHUS SPINA - CHRISTI* BY *IN VITRO* METHODS

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ABSTRACT

These procedures were conducted for the micro propagation of *Ziziphus spina - christi* by using shoot tips. Explants were obtained from young plants grown in the greenhouse which were initiated from seed germination and were cultured on MS medium at different salt strength, (full strength ,3/4 ,1/2 ,1/4,1/8,1/10, 1/20 and 1/40) free from growth regulators Murashige and Skoog,(1962).Effect of BA, ZT, 2ip, Kin and IBA, were investigated at various concentrations (0.0 ,0. 5 ,1.0 ,1.5 ,2.0 , and 2.5 mgl^{-1}) for each to study their effects on growth and development of *Ziziphus spina-christi* shoots. In the rooting stage the effect of IBA at concentrations of 4.9 and 9.9 uM of IBA was investigated to detect its effects on rooting of shoots. Results showed that shoot growth at the establishment stage required MS control (growth regulators free),and shoot multiplication required MS medium supplemented with either 1.0 mgl^{-1} BA, 2.0 mgl^{-1} ZT, 2.5 mgl^{-1}2ip, 1.5 mgl^{-1} Kin or 2.0 mgl^{-1} IBA. Shoot length was significantly affected by the presence of 2.0 mgl^{-1}IBA or MS free medium. The rooting of shoots required MS control (growth regulators free).Rooted shoots were acclimatized and successfully transferred to soil (peat: sand 1:1 v/v) with 68.75 % the surviving of plants.

Key Words: *Ziziphus spina - christi.* Establishment, Multiplication, Rooting and Acclimatization stage.

INTRODUCTION

Nabk tree, *Ziziphus spina – christi* is a multipurpose tree species belonging to Family Rhamnaceae. Its fruit is highly nutritious; it is consumed fresh, dried and canded rich in vitamin.

The leaves contain essential ingredients and used as a crude drug for various cures Yagi *et al*, (1978).the presence of the anti-tumour oestrogen beta- stitosterol Perdue and Hartwell, (1976), the alkaloids amphibian A.E. F. mauritine A, and C and four saponin glycosides in the plant have been reported Mahran *et al,* (1993). The antinociceptive activity of aqueous leaf extracts of this species has been studied Epfraim *et al.* (1998). Nabk is said to be analgesic, antipyretic, antitumor, astringent, collyrium, demulcent, depurative, emolient, laxative, pectoral, stomachic, tonic., abscess, arthrosis, bronchosis, cancer, cold, constipation, cough, dermatosis, fever, furuncle, hepatosis, high blood pressure, measles, ophthalmia, pain, rheumatism, snakebite, sore, toothache, tuberculosis and tumor, James, (2002). It is also, used for toothaches and tumors. The powdered seeds mixed with lemon juice are administered for liver complaints, the flower infusion is used as an eye wash and febrifuge, the boiled bark is used to treat viral diseases, the cathartic raw root juice is used for arthritis and rheumatism, and the fruits are used for bronchitis, coughs and tuberculosis Hutchens, (1973).The leaves can be used as an excellent leaf fodder and the bark can be used as a source of tannin, and hard heavy wood. Nabk trees are considered one of the most drought- resistant fruit crop adapted to the arid and semi arid regions. Since it is a cross pollinated tree, a wide range of genetic variability exists in nature, vegetative propagation by grafting is used with a limited success Kim *et al*, (2006).*In vitro* propagation of elite clones assumes important for mass and clonal propagation. Micropropagation via axillary shoot proliferation has been applied to commercial propagation of fruit tree, since the beginning of 1980 Zimmerman, (1991).

MATERIALS AND METHODS

This work was carried out in Applied Research Center of Medicinal Plants and Natural Products in Tissue Culture Laboratory. National Organization for Drug Control and Research (NODCAR), during the period from 2006-2007.

Plant material and explants source

Shoots about 2.5- 3.0 cm in length. Shoot tips (1-1.5cm in length) were used as a source of explants. The explants were surface sterilized by quick dipping in 70 %ethanol containing Tween 20, then sterilized by using 0.1% $MgCl_2$ for 5.0 min after that rinsed for 3 times for 1.0 min in sterilized distilled water for washing prior to transfer to culture medium Kumar *et al,* (2003).

Establishment stage

The explants were placed on MS medium, Murashige and Skoog, (1962) at different salt strengths free from growth regulators, (full strength, 3/4, 1/2, 1/4, 1/8, 1/10, 1/20 and1/40).The pH was adjusted to 5.7 using 0.1N NaOh or 0.1N HCl before autoclaving (121C°for 20 min) 50 ml of medium were dispensed into culture jars.

Multiplication stage

For multiplication induction, excised micro shoots (2.0cm in length) were transferred to MS medium supplemented with different concentrations of cytokinin, benzyladenine (BA), zeatin (ZT), 2-isopentenyl aminopurine (2-ip), kinetin (Kin) and indole butyric acid (IBA), were investigated at various concentrations (0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 mgl^{-1}) for each to study there effects on the growth and development of plants. Two excised shoots were placed in a jar containing 50 ml of culture medium.

Rooting stage

For root induction, excised micro shoots (4.0 cm length) were transferred to solidified MS medium supplemented with IBA at concentrations of 4.9 and 9.9 uM of IBA. Two excised shoots were placed in a jar containing 50 ml of culture medium.

The incubation conditions

All the cultures were incubated at 25±2C° under 16/8h day photoperiod light condition and darkness respectively from white fluorescent lamps .The shoots were maintained for 4 weeks in the culture medium.

Acclimatization stage

Rooted shoots were transplanted in polyethylene pots (12 cm diameter) filled with a mixture of peat moss and sand (1:1 v/v) as 16 explants were used per treatment and transferred to greenhouse. The

data were pertaining to mean percentage of cultures regeneration. The experiment was implemented in a randomized complete design with four replicates for each treatment. The mean percentage of starting, multiplication and rooting stages were statistically analyzed by Duncan multiple range test, (1955).

RESULTS AND DISCUSSION

Establishment stage

The explants of *Ziziphus spina-christi* were cultured on MS medium free from growth regulators and at different salt concentrations, the initiated shoots were healthy and grew vigorously on MS full salt strength medium. Shoot tips explants possessed the highest significant mean of shoot number (1.43 shoot /explant), also the shoot length was significantly affected by MS. The highest mean of shoot length (10.67 cm) and the highest average of leaf number (10.73 leaf /explant) were obtained on MS medium, as shown in (Table,1).This result was in agreement with Wondvifraw and Wannakrairoj (2004) who stated that axillary bud explants of *Aframomum corrorima* Braun Jansen, (an important culinary and medicinal plant species native to Ethiopia), was developed from rhizome cultured on MS medium without any growth regulators and MS medium proved to be the best for the establishment stage.

Table (1): Effect of MS medium salt strength on the growth and development of shoot tips of *Ziziphus spina-christi*.

Treatments	Shoot number (explant)	Shoot length (cm)	Leaf number (explant)	G.V
MS	1. 43 A	10. 67A	10. 73 A	5. 00 A
¾ MS	1. 00A	6. 78 B	8. 65 B	3. 40 B
½ MS	0. 00 B	0. 00 C	0. 00 C	2. 00 C
¼ MS	0. 00 B	0. 00 C	0. 00 C	2. 00 C
⅛ MS	0. 00 B	0. 00 C	0. 00 C	2. 00 C
1/10 MS	0. 00 B	0. 00 C	0. 00 C	2. 00 C
1/20 MS	0. 00 B	0. 00 C	0. 00 C	2. 00 C
1/40 MS	0. 00 B	0. 00 C	0. 00 C	2. 00 C

Means within a column followed by the same letter are not significantly different($p = 0.05$) according to Duncan's multiple range test.
Growth vigor (G.V): 2=weak, 3=good, 4=very good and 5= excellent.

Multiplication stage

The explants of *Ziziphus spina- christi* were cultured on MS medium growth regulators free as a control treatment and at different concentrations of BA, ZT, 2ip, Kin and IBA. The recorded data were as follows.

1- Effect Benzyladenine of (BA)

Data as shown in Table (2) showed the effect of different concentrations of BA on average shoots number per shoot, shoot length (cm) and leaf number per shoot. Addition of BA (0.5 -2.5 mgl^{-1}) increased the number of shoots per shoot, where MS medium supplemented with 1.0 mgl^{-1} BA induced the highest mean of shoot number (2.85) per shoot. The highest shoot length was obtained on MS control treatment (10.87cm). MS control treatment induced the highest average of leaf number per shoot, (12 .08). However increasing BA concentrations above to 1.5 -2.5 mgl^{-1} did not showed any response, where BA at 2.5mgl^{-1} gave the lowest value. This result was in agreement with Hussain (2003) who found that shoot tip and stem node explants of *Zizyphus spina-christi* native tree species were established on MS basal medium salts at full strength containing BA at 0.01-1.0 $mgl^{-1}$and Whipkey (1992), reported that shoot proliferation of *Artemisia annua* L. was achieved in modified Murashige and Skoog medium supplemented with 1.0 mgl^{-1} 6-benzylamino purine (BA).

Table(2):Effect of different concentrations of benzyladenine (BA) on the growth and development of *Ziziphus spina - christi* shoots cultured on MS medium in the multiplication stage.

Treatments	Shoot number (shoot)	Shoot length (cm)	Leaf number (shoot)	G.V
MS cont	1. 46 B	10. 87 A	12. 08 A	5. 00A
0. 5 BA mgl^{-1}	1. 53 B	8. 98 B	10. 00 B	5. 00 A
1. 0 BA mgl^{-1}	2. 85 A	8. 45 B	10. 00 B	5. 00 A
1. 5 BA mgl^{-1}	1. 00 B	6. 87 C	8. 00 C	5. 00A
2. 0 BA mgl^{-1}	1. 00 B	6. 54 C	8. 00 C	4. 58 B
2. 5 BA mgl^{-1}	1. 00 B	6. 46 C	8. 00 C	4. 49B

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

Growth vigor (G.V): 2=weak, 3=good, 4=very good and 5= excellent.

2- Effect of zeatin (ZT)

Data as shown in (Table, 3) illustrated the effect of (ZT) on the average of shoots number and shoot length of proliferated shoot /shoot Addition of ZT (0.5 -2.5 mgl^{-1}) increased the number of shoots per shoot. Medium supplemented with 2.0 mgl^{-1} ZT induced the highest mean of shoot number (2.26) per shoot .The highest shoot length was obtained on MS control (10.87cm) .The treatment MS control induced the highest average of leaf number (12.08). Increasing ZT concentrations from 0.5 to 2.5 mgl^{-1} decreased the average shoot length and number of leaf per shoot compared with control treatment.

Table (3): Effect of different concentrations of zeatin (ZT) on the growth and development of *Ziziphus spina - christi* shoots cultured on MS medium in the multiplication stage.

Treatments	Shoot number (shoot)	Shoot length (cm)	Leaf number (shoot)	G.V
MS cont	1. 46 B	10. 87 A	12. 08 A	5. 00A
0. 5 ZT mgl^{-1}	1. 40 B	9. 94 B	10. 00 B	5. 00 A
1. 0 ZT mgl^{-1}	1. 60 B	9. 87 B	10. 00 B	5. 00 A
1. 5 ZT mgl^{-1}	1. 69 B	9. 62 B	10. 00 B	5. 00A
2. 0 ZT mgl^{-1}	2. 26 A	9. 54 B	10. 00 B	5. 00 A
2. 5 ZT mgl^{-1}	1. 19 B	8. 46 B	8. 00 C	4. 39B

Means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan,s multiple range test.
Growth vigor (G.V): 2=weak, 3=good, 4=very good and 5= excellent.

3- Effect of 2-isopentenyl aminopurine (2-ip):

Data as shown in (Table, 4) showed the effect of different concentrations of 2-ip on the average of shoots number and shoot length of proliferated shoots per shoot. Addition of 2-ip (0.5 -2.5 mgl^{-1}) increased the number of shoots per shoot .Medium supplemented with 2.5 mgl^{-1} 2-ip induced the highest mean of shoots number (2.63) per shoot .The highest shoot length was obtained on MS control (10.87cm) .The treatment of MS control induced the highest average of leaf number (12 .08).

Increasing 2-ip concentrations from 0.5 to2.5 mgl^{-1} decreased the average shoot length and number of leaf per shoot compared with control treatment.

Table (4): Effect of different concentrations of 2-isopentenyl aminopurine (2-ip) on the growth and development of *Ziziphus spina - christi* shoots cultured on MS medium in the multiplication stage.

Treatments	Shoot number (shoot)	Shoot length (cm)	Leaf number (shoot)	G.V
MS cont	1. 46 B	10. 87 A	12. 08 A	5. 00A
0. 5 2ip mgl-^{1}	1. 00 B	6. 28 C	8. 00 C	4. 30 B
1. 0 2ip mgl-^{1}	1. 00 B	6. 45 C	8. 09 C	4. 37 B
1. 5 2ip mgl-^{1}	1. 00 B	6. 87 C	8. 13 C	4. 49B
2. 0 2ip mgl-^{1}	1. 00 B	6. 94 C	8. 34 C	4. 58 B
2. 5 2ip mgl-^{1}	2. 63 A	7. 56 B	10. 00 B	5. 00 A

Means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan,s multiple range test.
Growth vigor (G.V): 2=weak, 3=good, 4=very good and 5= excellent.

4- Effect of kinetin (Kin):

Data as shown in (Table, 5) illustrated the effect of (Kin) on the average of shoots number and shoot length of proliferated shoots per shoot. Addition of Kin (0.5 -2.5 mgl^{-1}) increased the number of shoots per shoot. Medium supplemented with 1.5 mgl^{-1} Kin induced the highest mean of shoots number (2.00) per shoot .The highest shoot length was obtained on MS control (10.87cm) .The treatment MS control induced the highest average of leaf number (12 .08).

Table:(5) Effect of different concentrations of kinetin (Kin) on the growth and development of *Ziziphus spina-christi* shoots cultured on MS medium in the multiplication stage.

Treatments	Shoot number (shoot)	Shoot length (cm)	Leaf number (shoot)	G.V
MS cont	1. 46 B	10. 87 A	12. 08 A	5. 00A
0. 5 Kin mgl-^{1}	1. 00 B	6. 38 C	8. 00 B	4. 30 B
1. 0 Kin mgl-^{1}	1. 00 B	6. 47 C	8. 09 B	4. 37 B
1. 5 Kin mgl-^{1}	2. 00 A	8. 87 B	8. 73 B	5. 00 A
2. 0 Kin mgl-^{1}	1. 00 B	6. 94 C	8. 34 B	5. 00 A
2. 5 Kin mgl-^{1}	1. 00 B	6. 46 C	8. 00 B	5. 00 A

Means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan,s multiple range test.
Growth vigor (G.V): 2=weak, 3=good, 4=very good and 5= excellent.

5- Effect of indole -3-butyric acid (IBA):

Data as shown in (Table, 6) illustrated the effect of (IBA) on the average of shoots number and shoot length of proliferated shoots per shoot. Addition of IBA (0. 5 -2.5 mgl^{-1}) increased the number of shoots, there was no significant effect between the concentrations of IBA at 1.5, 2.0 and 2.5 mgl-1 on the average of shoots number per shoot 2.0 mgl^{1}IBA produced the highest mean of shoot length (12.54 cm). No significant effect was observed on the average of leaf number between all concentrations of IBA and MS control.

Table(6): Effect of different concentrations of indole -3-butyric acid (IBA) on the growth and development of *Ziziphus spina - christi* shoots cultured on MS medium in the multiplication stage.

Treatments	Shoot number (shoot)	Shoot length (cm)	Leaf number (shoot)	Callus	G.V
MS cont	1. 46 C	10. 87 C	12. 08 A	0. 00 D	5. 00A
0. 5 IBA mgl^{-1}	1. 53 C	8. 98 D	11. 97 A	1. 00	5. 00 A
1. 0 IBA mgl^{-1}	2. 85 B	8. 45 D	12. 00 A	2. 00 B	5. 00 A
1. 5 IBA mgl^{-1}	4. 38. A	10. 32 B	12. 13 A	2. 00 B	5. 00A
2. 0 IBA mgl^{-1}	3. 89 A	12. 54 A	12. 00 A	3. 00 A	5. 00 A
2. 5 IBA mgl^{-1}	3. 46 A	10. 46 B	12. 00 A	3. 00A	5. 00 A

Means within a column followed by the same letter are not significantly different (p=0.05)according to Duncan,s multiple range test.
*Callus size: 1=very small, 2=small, 3=medium, 4=big, 5= very big.
**Growth vigor (G.V): 2=weak, 3=good, 4=very good and 5= excellent.

This synergistic effect of cytokinin and auxin has been demonstrated in many plants including *Santolina canescens* Casado *et al*, (2002), *Bupleurum fruticosum* Fraternal *et al*, (2002)

Rooting stage

Individual shoots were excised from 4 weeks old cultures of multiplication medium and were placed on the rooting MS medium supplemented with 4.9 and 9.9 uM IBA developed roots and the following data were recorded. Results indicated that MS growth regulator free medium exhibited the highest rooting %, roots number and roots length and no callus formations was observed on such medium. MS control gave highest rooting % (62.5%), the highest average of shoots number (1.96 shoots /shoot), highest average of shoot length (12.92 cm) and highest average of leaf number (12.00).

Roots number was significantly affected by the presence of MS control without any growth regulator, highest root number (2.38 root/shoot), root length was significantly affected by the presence of MS control which gave the highest mean of root length (10.56 cm), meanwhile, IBA at 4.9 or 9.9 uM significantly decreased the rooting %, average root number and length also, enhanced callus formation at the basal end of shoots, as shown in (Table, 7).

This result in agreement with Nin *et al*, (1994), who stated that the formed shoots of *Artenisia absinthium* L. rooted easily when placed in growth regulator-free MS basal medium.

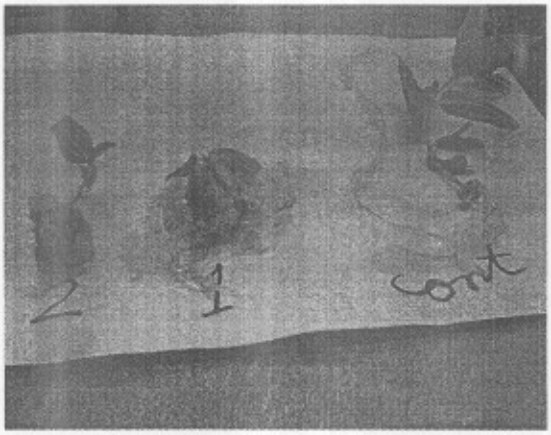
Table (7): Effect of indole butyric acid concentrations on the root formation of *Ziziphus spina -christi* shoots on MS medium during rooting stage.

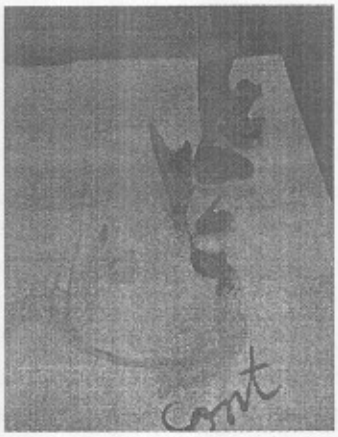
Treatments	Shoot number (shoot)	Shoot Length (cm)	Leaf number (shoot)	Root number (shoot)	Root length (cm)	Rooting %	Callus	G.V
MS cont	1.96 A	12.92 A	12.00 A	2.38 A	15.56 A	62.5%	0.00 B	5.00A
MS+4.9 uM	1.00 B	3.59 B	4.75 B	1.75 B	2.88 B	18.75 %	4.00 A	5.00A
MS+9.9 uM	1.00 B	2.06 C	4.13 B	0.85 C	0.43 C	6.25 %	5.00 A	5.00A

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

*Callus size: 1=very small, 2=small, 3=medium, 4=big, 5= very big.

**Growth vigor (G.V): 2=weak, 3=good, 4=very good, 5= excellent.





Acclimatization stage:

After 4 weeks rooted shoots of (12 cm in long) were transplanted in polyethylen pots (12 cm diameters) filled with a mixture of peat moss and sand (1:1 v/v) as 16 explants were used per treatment and transferred to greenhouse and left under mist system for 8 weeks. Result showed that Nabk plantlets were successfully acclimatized as (68.75 % survival) with peat moss and sand (1:1 v/v) under mist condition and plantlet were cultured in soil, as shown in (Table, 8).

This result was in agreement with Prasad *et al,*(2004), the *in vitro* raised plantlets of *Cryptolepis buchanani* was acclimatized successfully to pots containing a mixture of autoclaved peat moss and compost 1:1 ratio.

Table (8): Effect of transplanting media (substrates) on growth and development of *Ziziphus spina - christi* plantlets grown under mist in a controlled greenhouse.

Treatment	Survival %	Shoot number (plant)	Shoot length/plant (cm)	Leaf number (plant)
P :S (1:1 v/v)	68.75	1.00	18.57	36.00

Recommendation.

The Recommendation of this study revealed that shoot tips explant of *Ziziphus spina - christi* (Nabk tree) was successfully established on MS growth regulators free medium in the starting stage, and shoot multiplication required MS control or MS medium supplemented with either 1.0 mgl^{-1} BA, 2.0 mgl^{-1}ZT, 2.5 mgl^{-1}2ip, 1.5 mgl^{-1} Kin or 2.0 mgl^{-1} IBA. Shoot length was significantly affected by the presence of 2.0 mgl^{-1}IBA or MS free medium. Rooting was induced on MS growth regulators free medium. Rooted shoots were acclimatized and successfully transferred to soil (peat: sand 1:1 v/v) with 68.75 % of the surviving plants.

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تجديد نمو نبات النبق بطريقة زراعة الأنسجة

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مركز الدراسات التطبيقية لبحوث النباتات الطبية التابع للهيئة القومية للرقابة والبحوث الدوائية

أجرى هذا البحث في خلال الفترة من سنة 2006 إلى سنة 2007 في معمل زراعة الأنسجة بمركز الدراسات التطبيقية لبحوث النباتات الطبية التابع للهيئة القومية للرقابة والبحوث الدوائية. كان الهدف هو إكثار نبات النبق بطريقة زراعة الأنسجة وتم استخدام القمة النامية كمنفصل نباتي في الزراعة و أخذ ت القمم النامية من نباتات حديثة النمو ناشئة من إنبات بذور النبق الصيني داخل الصوبة الزجاجية وتم تعقيم المنفصل النباتي بطريقة الغمس السريع في الكحول 70% لمدة عدة ثواني ثم التعقيم بالغمس في محلول كلوريد الزئبقيق السليمانى بتركيز 1, مليجرام/ لتر لمدة 5 دقيقة ثم الشطف ثلاث مرات بماء مقطر معقم . وتمت زراعة المنفصل النباتي في وضع رأسي داخل البرطمانات المعقمة المحتوية على تركيزا ت مختلفة من قوة أملاح بيئة موراشيجى وسكوج الخالية من أي منظمات نمو (قوه كاملة ،4/3 ،2/1 ،4/1 ،8/1 ،10/1 ، 20/1 و 1/ 40 من قوة تركيز الأملاح) وتم تحضين هذه الأجزاء داخل غرف التحضين تحت 25 درجه مئوية لمدة 4 أسابيع وباستخدام لمبات فلوريسنت بيضاء ذات شدة إضاءة 2000 لكس و لفترات إضاءة 16 ساعة يوميا وفترات إظلام 8 ساعات على التوالي. و في مرحلة التضاعف تم أخذ النموات الخضرية الناتجة من مرحلة البداية و زراعتها مرة أخرى على أفضل تركيز من الأملاح ثبت نجاحه من مرحلة البداية وهو تركيزا لقوة الكاملة من أملاح بيئة موراشيجى و سكوج ولقد تم التحضين تحت نفس الظروف السابق ذكرها في مرحلة البداية . ولقد تمت دراسة تأثير كل من منظمات النمو البنزايل أدينين ، الزياتين ، 2- بنزبل امينو بيورين، كينيتين و اندول حمض البيوتريك ، بالتركيزا ت التالية 0.0 ، 0.5 ، 1.0 ، 1.5، 2.0 ، 2.5 مليجرام في اللتر عند إضافتها إلى بيئة موراشيجى و سكوج بقوة أملاح كاملة على نمو و تطور النبتات الناتجة من مرحلة البداية . وفى مرحلة التجذير تمت دراسة تأثير ا ندول حمض البيوتريك بتركيز 4.9 ، 9.9 ميكرو مول على تكوين الجذور على نبتات النبق. أوضحت النتائج أن القمة النامية المستخدمة كمنفصل نباتي للزراعة في مرحلة الإنشاء والبداية تحتاج إلى أملاح بيئة موراشيجى وسكوج بقوة كاملة و الخالية من أي منظمات نمو وفى مرحلة التضاعف فان النبتات الصغيرة النامية تحتاج إلى تركيز 1.0 مليجرام في اللتر من البنزايل أدينين ، 2.0 مليجرام في اللتر من الزياتين ، 2.5 مليجرام في اللتر 2- بنزبل امينو بيورين، 1.5مليجرام في اللتر كينيتين أ و2.0 مليجرام في اللتر من إندول حمض البيوتريك . استطالة البادرات تحتاج إلى أملاح بيئة موراشيجى وسكوج الخالية من أي منظمات نمو بقوه كاملة أو 2.0 مليجرام في اللتر من ا ندول حمض البيوتريك. أما تجذير البادرات يتطلب املاح بيئة موراشيجى وسكوج بقوه كاملة و الخالية من أي منظمات نمو. تمت أقلمة النبتات الصغيرة بسبة نجاح 68.75 % من النبتات الحية عند زراعتها في أصص مقاس 12 سم محتوية على خليط من الرمل والبيت بنسبة 1: 1.