ISSN 1110-0419

Annals Of Agric. Sci., Moshtohor,
Vol. 47(2): Ho. 327-337, (2009).

MICROPROPAGATION OF FANTASY SEEDLESS GRAPEVINES

BY

Abdrabboh, G.A.* AND El-Khafafy, S.H.**

* Department of Horticulture, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

** Department of Botany, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

ABSTRACT

Fantasy seedless grapevine cultured in vitro by using MS medium supplemented with different concentrations of BA, 2, 4-D and Picloram either alone or in combinations. Shoot tips and leaf disks were evaluated as explants. We found that addition of 0.1 mg/L 2, 4-D to the culture MS medium encouraged callus production compared to other 2, 4-D treatments. Picloram (pic) also caused callus formation in both used leaf disk and shoot tip explants of Fantasy Seedless grapevine especially at 0.5 mg/L. However, addition of BA at any concentration to MS medium initiated only shoots when shoot tip explants were used while leaf disks did not respond. The combination of 0.5 mg/L Picloram (pic) plus 1.0 mg/L BA gave the best callus formation in comparison to other treatments. In most cases, leaf disk explants as well as combination of Pic. and BA were superior than shoot tip explants and combinations of 2, 4-D and BA. The best shoot number and shoot length were obtained when MS medium was supplemented with 2.0 mg/L BA. Moreover, maximum root number and root length were occurred when MS medium was supplemented with combination of both NAA and IBA at 0.1mg/L for each.

INTRODUCTION

Grape (*Vitis vinifera* L.) is a major fruit crop used as Fig. grape in Egypt. It ranks on 2nd position after citrus. Grape (*Vitis vinifera* L.) is propagating mainly through conventional method such as stem cutting, layering and grafting. Conventional method of propagation is sometimes hampered by seedling heterozygosis, space and time consuming, cutting dormancy and limited yield. Also most of serious disease such as fungi, viral and bacterial diseases is spread by infected source of propagation materials (Jaskani *et al.*, 2008). The improvement in production and quality of grapes can be achieved through incorporation of unconventional propagation method like tissue culture which is adopted an established method for the commercial propagation of grape varieties (Chee and Pool, 1985; Gray and Klein, 1987; Cholvadova 1989; Lee and Wetzstein, 1990; Lewandowski, 1991, Abdrabboh, 1995, Abdrabboh, 2002 and Jaskani *et al.*, 2008). Micropropagation of woody plant species is an increasingly applied technique and its feasibility depends on the shoot multiplication rate from subculturing shoots and percentage of roots per shoot. Rapid micropropagation of grapevine can be carried out by combining shoot proliferation and rooting of new shoots. The present study describes the in vitro propagation of grape cv. Fantasy seedless using shoot tip and leaf disks from field grown vines as initial explants.

MATERIALS and METHODS

This work was carried out in 2006 and 2007 period in tissue culture lab at Horticultural Department, Faculty of Agriculture, Al-Azhar University, to study the effect of some growth regulators on micropropagation of Fantasy seedless grapevines. Different explants from Fantasy Seedless grape were used i.e. shoot tips (0.3-0.5cm) and leaf disks ($0.5cm^2$) were dissected. The explants sterilized using 10% solution of Sodium hypochlorite (NaOC1) "Clorox" for 15 min then rinsed three times in sterile distilled water. Therefore,

the explants were cultured in Petri dishes (100 x 20 ml),filled with 40 ml of MS (Murashige and Skoog, 1962) basal medium supplemented with 30 g^{-1} sucrose, 7g^{-1} agar and adjusted to pH 5.7 before being autoclaved at 121°C for 15 minutes. The cultured petri dishes were incubated for 2 weeks under dark conditions before shifting light for 16 hrs at 27 ± 2°C. These cultures were used as a mother stock for the subsequent proliferation experiments.

The following experiments were done.

1. Initiation stage.

1.1. Effect of growth regulators on callus production and plant regeneration.

To check for the effect of growth regulators on callus induction and plant regeneration of Fantasy Seedless grapevines, MS medium supplemented with auxins such as 2, 4- Dichlorophenoxy acetic acid (2, 4-D) at 0.0, 0.1 and 1.0 mg/L or 4-amino 3,5,6- trichloropicolinic acid (picloram) at 0.0, 0.5 and 0.75 mg/L and Benzyladenin (BA) as a source of cytokinins at 0.0,0.2 and 1.0 mg/L either alone or in combinations were used in initiation stage. In this regard, five explants per plate and five replicated Petri dishes per treatment were used. Calli and shoot fresh weight (g/petri dish) were recorded as a growth parameter.

2. Multiplication stage.

2.1. Effect of BA on shoot multiplication.

This experiment was conducted to study the effect of BA on shoot multiplication of Fantasy seedless grapevines. So, shoots which were obtained from the initiation stage were transferred to test tubes containing MS medium supplemented with BA at concentrations of 0.0, 0.5,1.0 and 2.0 mg/L . Shoots number and length (cm) / explant were recorded as growth parameters. Shoot cultures were subjected to light with photoperiod of 16 h /day. One explant per tube and 9 replicated test tubes were used.

3.Rooting stage.

3.1.Effect of growth regulators on root formation.

This experiment was carried out to study the effect of adding both Naphthalene acetic acid (NAA) and Indole butyric acid (IBA) at 0.0, 0.1 and 1.0 mg/L either individually or in combinations on root number and length of Fantasy Seedless grape variety. One explant (shoot) per test tubes and 9 replicated test tubes were used. Root number and length (cm) were recorded as root growth parameters. A complete randomized block design was adopted in the experiment.

Statistical analysis.

The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1968) using Mstat program. Least significant differences (L.S.D) were used to compare between means of treatments according to Waller and Duncan (1969) at probability of 5%. Means in each row followed by the same letter(s) are not significantly different.

RESULTS AND DISCUSSION

1. Callus production.

1.1. Effect of growth regulators on callus production and plant regeneration.

1.1.a. Effect of 2,4– D.

Data in Fig.(1) clear that both leaf disks and shoot tip explants of Fantasy seedless grape produced callus when cultured on MS medium supplemented with 2, 4-D at either 0.1 or 1.0 mg/L, while negatively responded on a medium free from 2, 4-D. In this regard, leaf disk explants showed higher callus fresh weight (g) at all given 2, 4-D concentrations than shoot tip explants. The data in Fig.(1) also showed that 2, 4-D at 0.1 mg/L gave the greatest callus fresh weight for all tested explants. Moreover, increasing concentration of 2, 4-D up to 1.0 mg/L decreased callus fresh weight. These results are in somewhat agreed with that obtained by Cheng and Reish, (1989). They cleared that grape explants produced callus when cultured on Nitsch and Nitsch (NN) medium supplemented with 0.1 or 0.2 µM 2, 4-D. Also with Abdrabboh, (2002) who confirmed these results whereas he declared that only callus was obtained when shoot tips of some grape varieties and rootstocks cultured on MS medium supplemented with 0.1 or 1.0 mg/L 2, 4-D.

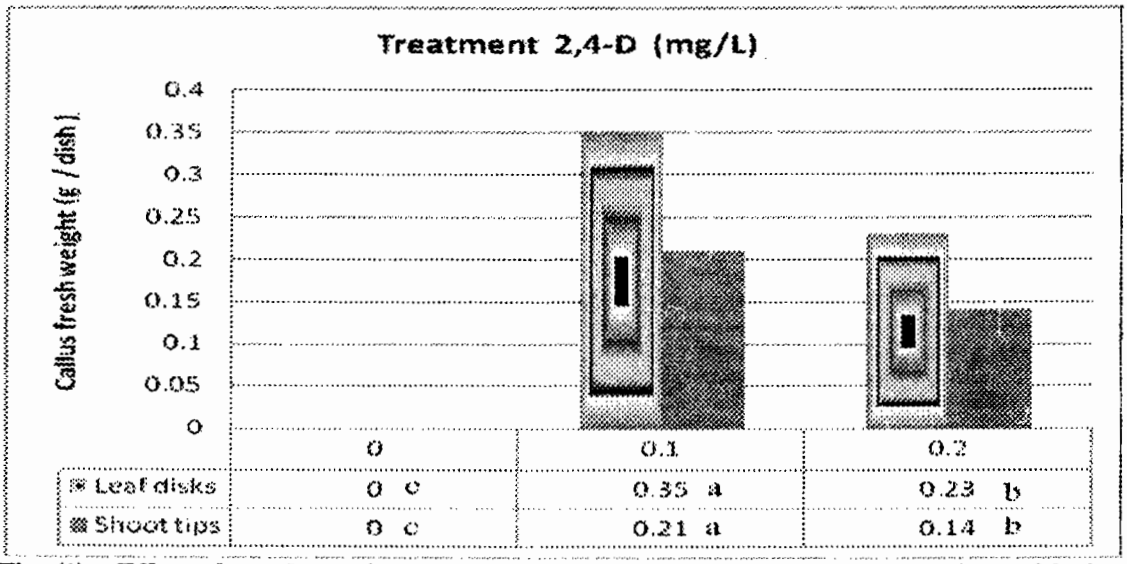

Fig. (1): Effect of supplementing the culture medium with different concentrations of 2, 4-D on callus production of Fantasy seedless grape .

1.1.b. Effect of picloram (Pic).

Data in Fig. (2) indicate that MS medium supplemented with picloram induced callus production from different tested explants at all used concentrations while the control treatment did not respond. Increasing picloram concentration led to decrease in callus fresh weight in all tested explants. These results are in agreement with the obtained by Furmanowa *et al.* (1997). They claimed that *Taxus* var. hatfidii formed callus when cultured on WR medium supplemented with 10 µM picloram. The data in Fig.(2) also cleared that leaf disk explant was superior than shoot tip explants in callus production. Abdrabboh, (2002) reported that shoot tip explants of some vine varieties and rootstocks produced callus when cultured on MS medium supplemented with picloram.

1.1.c. Effect of BA.

Data in Fig. (3) clarify that supplementing of MS medium with BA level encouraged raising shoots from shoot tip explants at all given concentrations while the leaf disk explant did not respond. Meanwhile, shoot formation expressed as (g/dish) increased progressively by increasing BA concentrations in the culture medium. These results are in harmony with the findings of Abdrabboh, (2002) who reported that shoot tip explants of some vine varieties and rootstocks gave only shoots when cultured on MS medium supplemented with BA at different concentrations.

1.1.d. Effect of combinations between 2, 4-D plus BA.

Data in Fig. (4) show that all combinations of 2, 4-D and BA concentrations enhanced callus production in all tested explants. 2, 4-D at 0.1mg/L gave callus greater than that obtained with 2, 4-D at high concentration 1.0 mg/L plus BA at 0.2mg/L or 1.0mg/L. Also, 2, 4-D at higher concentration (1.0mg/L) plus BA at different concentrations caused a reduction in callus production in all tested explants. Leaf disk explants showed an increase in callus fresh weight (g/dish) in comparison to that of shoot tip explants. Moreover, 2, 4-D at 0.1mg/L plus BA at 1.0 mg/L gave the best results regarding callus formation in both leaf dish and shoot tip explants. Leaf disk explants were superior to shoot tip explants in callus production. Control treatment did not respond for callus production. These results are in coordination with the findings of Lebrun *et al.* (1985). They claimed that anthers of *Vitis rupestris* cv. Rupestris St. George formed callus when cultured on NN medium supplemented with 6µM 2, 4-D and 1.0µM BA. The results also are in agreement with that of Salunkhe *et al.* (1997). They reported that anthers of *Vitis latifolia* L. cultured on NN medium formed callus when the medium supplemented with 20µM 2, 4-D and 9µM BA.

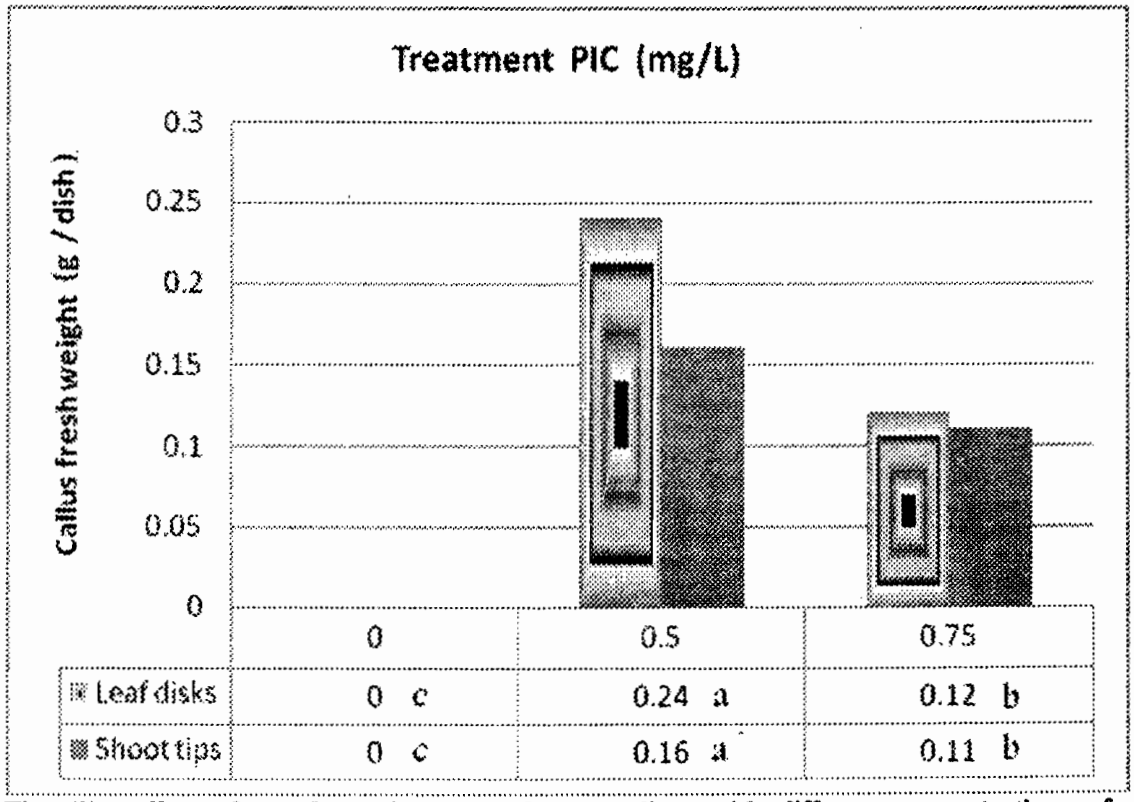

Fig. (2): Effect of supplementing the culture medium with different concentrations of picloram on callus production of Fantasy seedless grape.

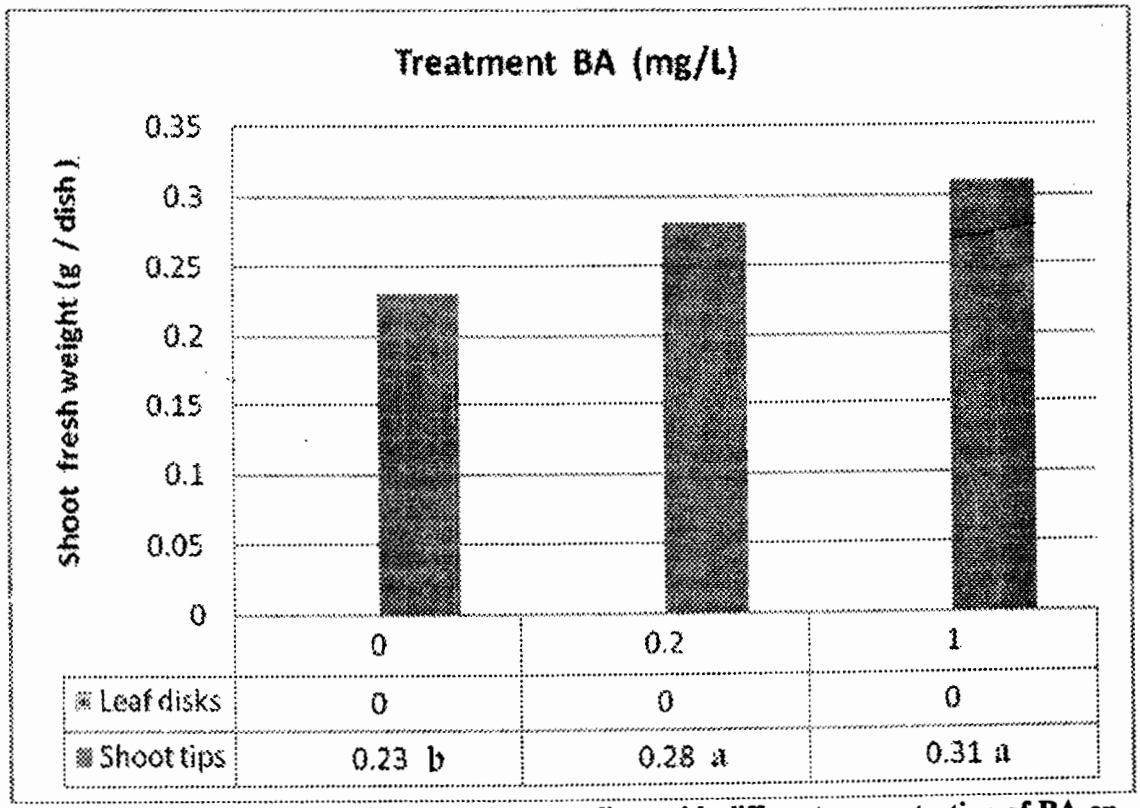

Fig. (3): Effect of supplementing the culture medium with different concentration of BA on shoot formation of Fantasy seedless grape.

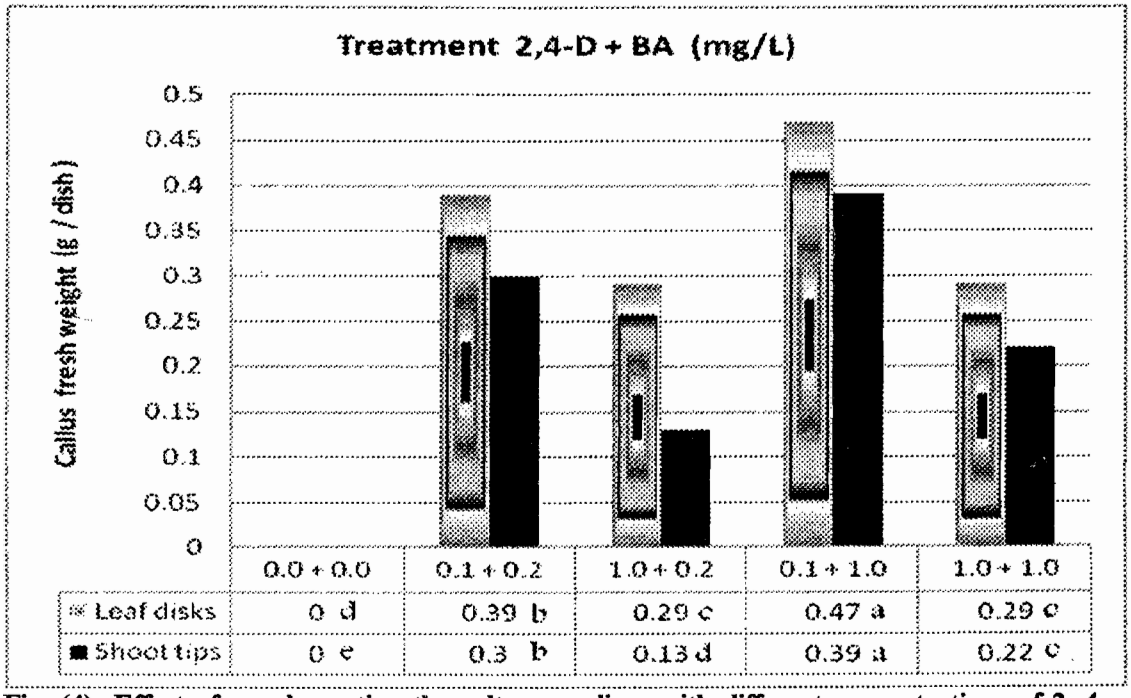

Fig. (4): Effect of supplementing the culture medium with different concentrations of 2, 4-D and BA on callus production (g/dish) of Fantasy seedless grape.

1.1.e. Effect of combination between picloram plus BA.

Data in Figs.(5 and 10) cleared that MS medium supplemented with picloram plus BA was effective in increasing callus production in all tested explants. In this regard, the best callus formation was obtained when MS medium was supplemented with 0.5 mg/L picloram and 1.0 mg/L of BA (Fig. 10 A). Meanwhile, explants of leaf disks reflected higher callus formation than that of shoot tip explants. Control treatment did not produce any callus. The results also cleared that increasing the concentration of picloram from 0.5 mg/L to 0.75 mg/L plus BA at any given concentration decreased the callus fresh weight in all tested explants. These results are in harmony with that of Reisch, (1986) who claimed that picloram at 0.1 or 0.25 µM plus 1.0 µM BA led to an increase in callus production at the base of explants which cultured on MS medium. The results are also agreed with that obtained by Abdrabboh, (2002) who cleared that best callus formation of some vine varieties and rootstocks was obtained when MS medium was supplemented with 0.5 mg/L plus BA at 1.0 mg/L.

2. Proliferation of shoots

2.1. Effect of BA

Data in Figs.(6 and 10) indicate that shoot number / explants increased progressively by increasing BA concentration up to 2.0 mg /L BA where it attired a maximum number then decreased by 1.0,0.5 and 0.0 (control) treatments. These results are coordinated with the findings of Rieach, (1986) who reported that MS medium supplemented with BA at 0.0 1.0 2.5 5.0 7.5 or 10.0 µM were stimulate shoot proliferation from shoot tip explants of four grape cultivars. Data in Fig.(6) also verify that shoot length was progressively increased by increasing concentration of BA in the medium. In this regard, the highest value of shoot length (cm) was obtained when MS medium supplemented with 2.0 mg/L BA compared with the other treatments including control. These results are in agreement with those obtained by Baydar,(2000) who reported that the highest shoot formation of grape - explants was obtained when MS medium was supplemented with 2.0 mg/L BA. Also with Abdrabboh (1995 and 2002) who confirmed these results as he stated that shoot length of the tested vine varieties and rootstocks was increased by increasing BA in MS medium whereas it attained maximum value at 2.0 mg/L BA.

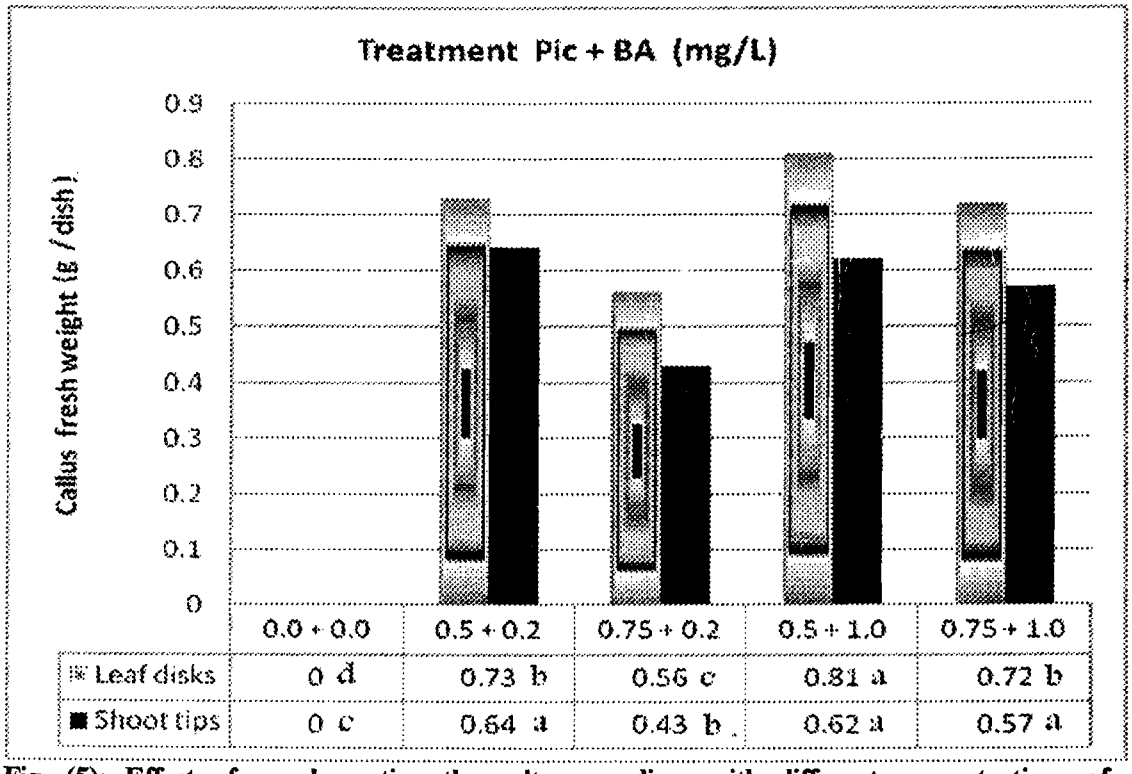

Fig. (5): Effect of supplementing the culture medium with different concentrations of Picloram and BA on callus production (g/dish) of Fantasy Seedless grape.

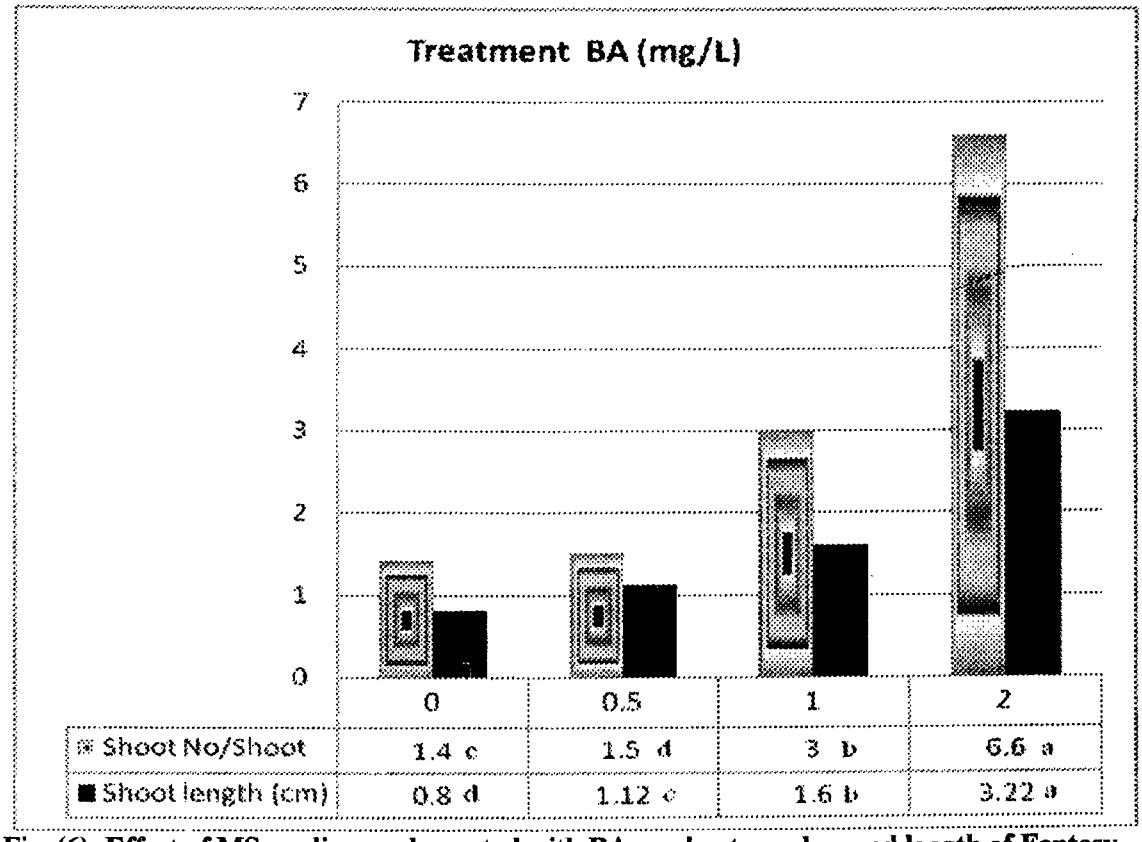

Fig. (6): Effect of MS media supplemented with BA on shoot number and length of Fantasy seedless grape variety.

3. Rooting of shoots.

3.1. Effect of growth regulator on root formation.

3.1.a. Effect of NAA.

Data presented in Fig. (7) show that the greatest number of roots per shoot was obtained when MS was supplemented with 0.1 mg/L NAA in comparison with other treatments including control. The roots number decreased as NAA concentration increased in MS medium from 0.1 to 1.0 mg/L. However, control treatment did not respond whereas no roots were obtained on MS medium without NAA. Moreover, root length of shoots of Fantasy seedless grape variety increased when MS medium supplemented with NAA at 0.1 or 1.0 mg/L. Maximum root length was occurred when MS medium was supplemented with NAA at 0.1 mg/L compared with other treatments including control. The results also cleared that root length of shoots decreased when NAA concentration in the growth medium increased from 0.1 mg/L to 1.0 mg/L. These results are in agreement with that obtained by Abdrabboh,(2002) who claimed that shoots of some vine varieties and rootstocks possessed the greatest root length when cultured on MS medium supplemented with 0.1 mg/L NAA.

3.1.b. Effect of IBA.

Data presented in Fig. (8) indicate that IBA had the same trend of NAA where the highest average root number / shoot was obtained when MS medium was supplemented with 0.1 mg/L IBA. However, the root number / shoot was decreased by increasing IBA concentration in the medium. On the other hand, control treatment did not give any response. These results are in agreement with that of Abdrabboh, (2002) who reported that shoots of some vine varieties and rootstocks had the best root formation expressed as root number / shoot cultured on MS medium supplemented with IBA at 0.1 mg/L. He also claimed that root number/shoot was decreased by increasing IBA concentration in the medium, while no roots were obtained when shoots were cultured on MS medium free from IBA. Jaskani *et al.* (2008) supported these results as they claimed that maximum rooting percentage (80%) occurred when MS medium was supplemented with 10 um IBA.

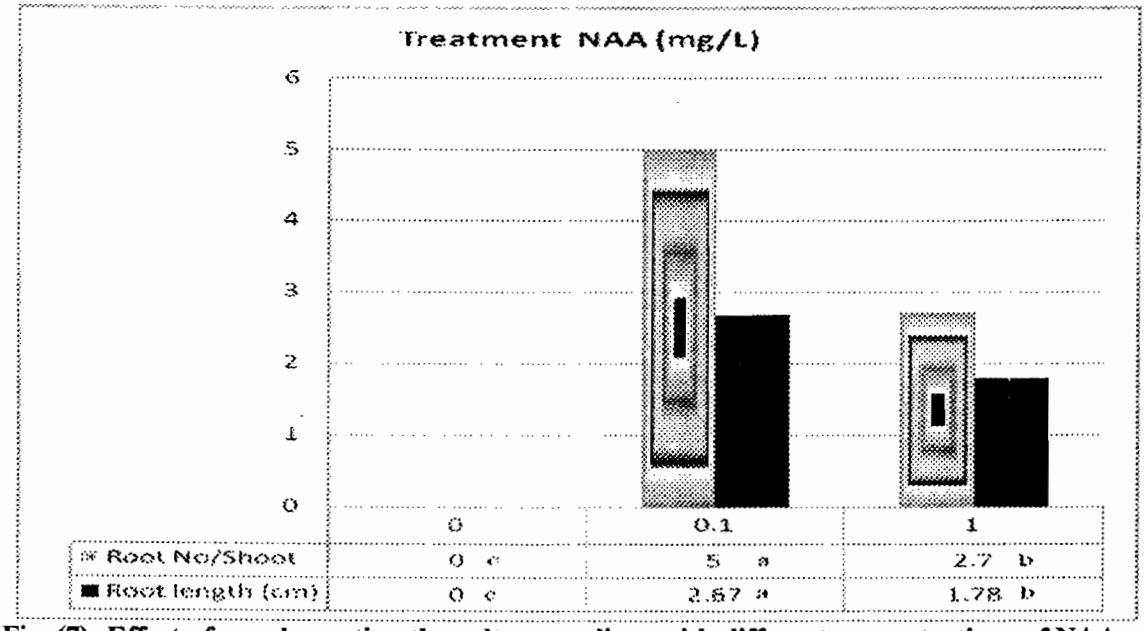

Fig. (7): Effect of supplementing the culture medium with different concentrations of NAA on root number of Fantasy seedless grape.

Results in Fig.(8) also cleared that shoots of Fantasy seedless formed roots when cultured on MS medium supplement with IBA at different concentration. Root formation expressed as root length (cm) was occurred on MS medium supplemented with IBA at 0.1 or

1.0 mg/L in comparison to control treatment which did not respond. The results also cleared that increasing IBA concentration in the medium from 0.1to 1.0 mg/L led to a decrease in shoot length.

3.1.c. Effect of combination between NAA and IBA

Results in Figs. (9 and 10) showed that root number /shoot of Fantasy seedless grape variety increased when the MS medium supplemented with combination of NAA and IBA at different concentrations in comparison to control. Root number / shoot attained the maximum value when MS medium supplemented with combination of 0.1 mg/L NAA and 1.0 mg/L IBA (Fig 9and Fig. 10 C). However, MS medium free of auxins did not respond. These results are go in line with that obtained by Lewandowski (1991) who claimed that shoots of *Vitis labrusca L* . cv. Delawere had the best root formation expressed as roots number /shoot when cultured on half strength MS medium supplemented with 0.001 mg/L NAA and 0.005 mg/L IBA.

Also, Abdrabboh, (2002) reported that the best root formation expressed as root number/shoot of some vine varieties and rootstocks was occurred when MS medium supplemented with combination of 0.1 mg/L NAA and 1.0 mg/L IBA. Also it is cleared that MS medium supplemented with combination of NAA and IBA increased root length (cm) in comparison to control treatment. The best results regarding root lengths were obtained when MS medium was supplemented with combination of NAA and IBA at 0.1 mg/L for each. The data also showed that increasing the concentration of NAA from 0.1mg/L to 1.0mg/ l combined with 0.1 or 1.0 mg/ l IBA led to a reduction in root lengths as compared with that obtained at 1.0mg/L NAA in the combination. Shoots cultured on MS medium free from auxins failed to form roots. These results can be confirmed by the findings of Lewandowski (1991) who cleared that shoots of *Vitis labrusca* cv Delaware were formed roots when cultured on half strength MS medium supplemented with 0.001mg/L NAA plus 0.005mg/L IBA. Also, Abdrabboh, (2002) reported that shoots of some vine varieties and rootstocks possessed the greatest root length (cm) when cultured on MS medium supplemented with 0.1 mg/L NAA and 0.1mg/L of IBA. He also added that increasing the combination from 0.1 of each auxin to1.0 mg/L led to a reduction in root length.

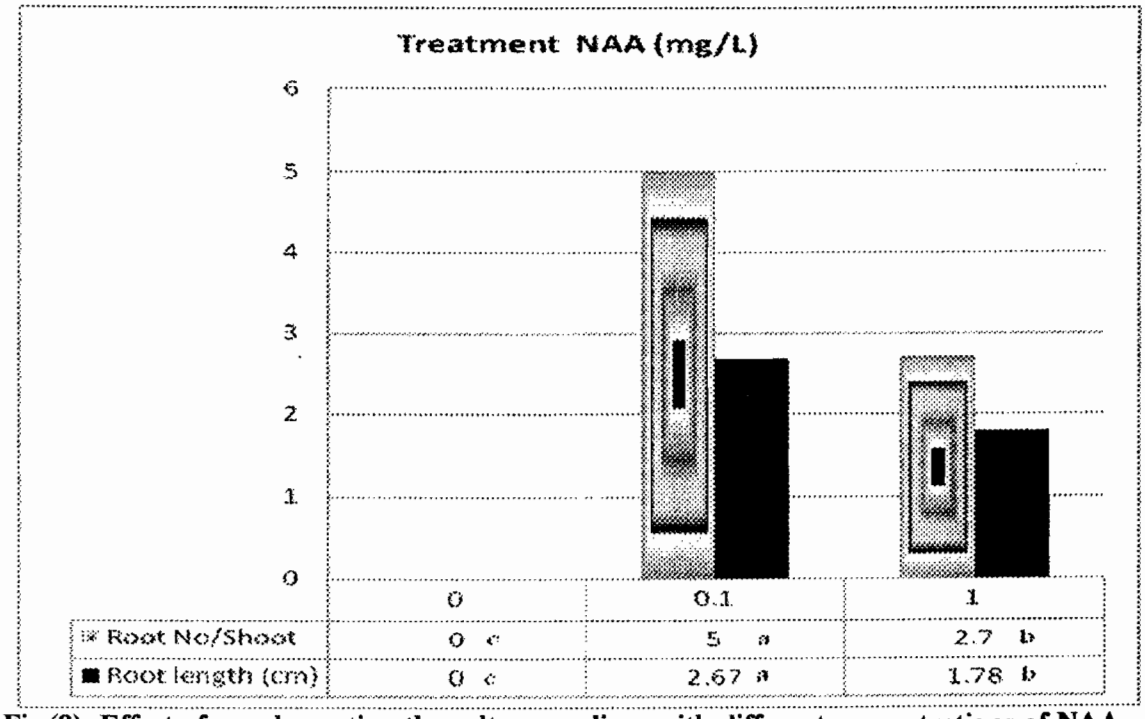

Fig.(8): Effect of supplementing the culture medium with different concentrations of NAA on root number of Fantasy seedless grape variety.

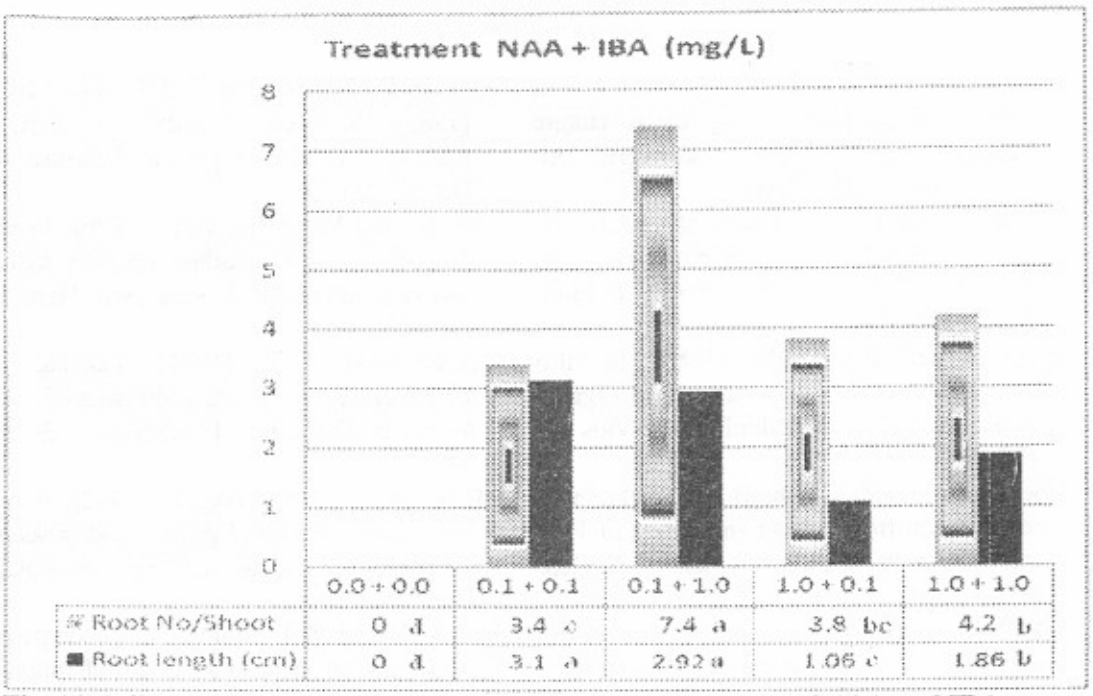

Fig. (9): Effect of supplementing MS medium with combinations of NAA and IBA on root number of Fantasy seedless grape.

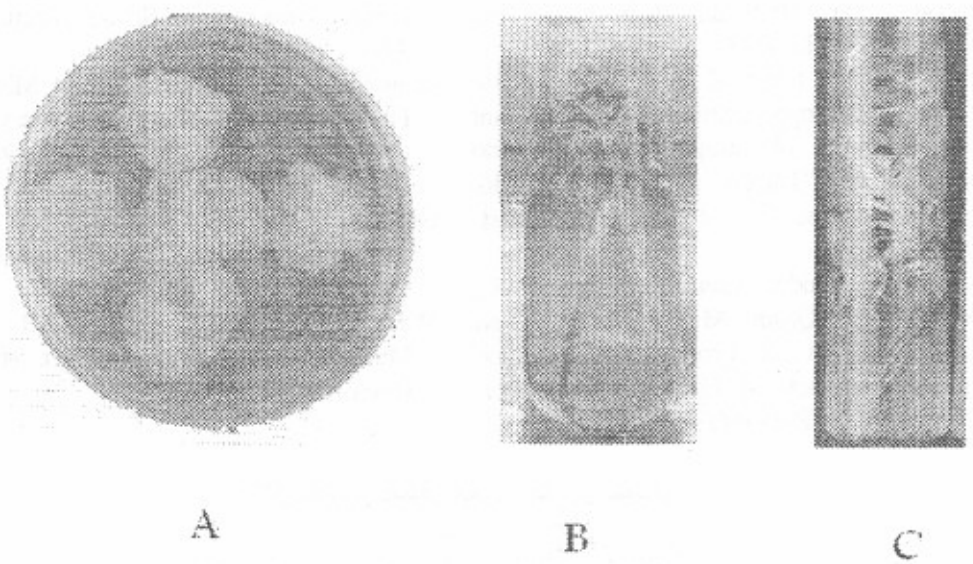

Fig. (10): Callus production on 0.5 mg/ l picloram and 1.0 mg/L BA; B. Shoot multiplication on 2.0 mg/ l BA; C. Root development on 0.1 NAA and 1.0 mg/L IBA.

REFERENCES

Abdrabboh, G.A.E., (1995): Tissue Culture as a Potential Tool in Obtaining Salt Tolerant Plants From Grapes"*Vitis vinifera*. M.Sc. Thesis, Fac. Agric., Al-Azhar Univ., Cairo, Egypt. PP.1-30.

Abdrabboh, G.A.E., (2002): Selection of salt tolerant grape plants using tissue culture techniques. Ph.D. Thesis, Fac. Agric., Al-Azhar Univ., Cairo, Egypt.

Baydar, N.G. (2000): Study of adventitous shoot formation in leaves of grape *Vitis spp.* Turkish J. Biol. 24(3). 645-656. C. F. Hort. Abst. 70 (11)1226.

Chee, R. and Pool, R.M. (1985): In vitro propagation of Vitis. the effects of organic substances on shoot multiplication. Vitis 24. 106-118.

Cheng, Z.M. and Reisch, B.I. (1989): Shoot regeneration from petiols and leaves of *Vitis labruscana L* 'Catawba' Plant Cell Rep, 8(7). 403-406.

Cholvadova, B. (1989): Cultivating of meristem cultures of the grape-vine (*Vitis vinifera* L.), Physiologia Plantarum, 24. 31-44).

Furmanowa, M.K. Glowniak.; Sylkowska – Baranek, K. (1997): Effect of picloram and methyl jasmonate on growth and taxane accumulation in callus cultures of taxus x medium var. Hatfieldii. Plant Cell, Tiss. Org. Cult, 49(1). 75-79.

Gray, D.J. and Klein, C.M (1987): In vitro shoot micropropagation and plant establishment of grape cvs. "Orlando seedless" and "Tampa" rootstock. Proc. Flo. State Hort. Soc. 100. 308-309 (Hort Abst. 67. 3741).

Jaskani, M.; Haider Abbas, J; Sultana, R.; Khan, M.M.; Qasim, M.and Iqrar, A. **Khan** (2008): Effect of growth hormones on micropropagation of *Vitis vinifera* L. cv. Perlett. Pak. J. Bot.40 (1) 105- 109.

Lebrun, L.; Rajasekaran K. MC. and Mullins (1985): Selection in vitro for NaCl – tolerance in *Vitis rupestris* Scheele, Ann Bot, 56.733-739.

Lee, N. and Wetzstein, H.Y. (1990): In vitro propagation of Muscadine grape by axiliary shoots proliferation. J. Amr. Sco. Hort, 115 (2). 324-329.

Lewandowski, V.T. (1991): Rooting and acclimatization of micropropagated *Vitis labrusca* Delaware. HortScience, 26.586-589.

Murashige, T. and Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures physiology. Plant. 15. 478-479.

Reisch, B.I. (1986): Influence of genotype and cytokinins on in vitro shoot proliferation of grapes. J. Amer. Soc. Hort. Sci., 111(1). 138-141.

Rout, G.R.; Samantaray, S. and Das, P. (1999): Root induction in microshoots of *Simarouba glauca* L. in vitro. Peroxidase as a marker for rooting. Silvae Genetica 48(1). 14-17.

Salunkhe, C.K.; Rao, P.S. and Mhatre, M. (1997): Induction of somatic embryogenesis and plantlets in tendrils of *Vitis vinifera* L. Plant Cell Rep., 17.65-67.

Snedecor, G.W. and Chochran, W.G. (1968): Statistical Methods. 6th Ed., The Iowa State Univ. Press, Iowa, U.S.A.

Waller, H.R. and Duncan, D.B. (1969): Multiple range and multiple F- test. Biometrics,11. 1-42.

الإكثار الدقيق لصنف العنب فانتازى سيدلس

جمال عبدربه السيدعبدربه*، السيد حسن الكفافى**.
* قسم البساتين و** قسم النبات، كلية الزراعة جامعة الازهر – القاهرة

أجرى هذا البحث فى الفتره من عامى ٢٠٠٦ و ٢٠٠٧ لدراسة تأثير بعض منظمات النمو على الإكثارالدقيق صنف العنب فانتازى سيدلس وفى هذا الصدد فانه قد تم اكثار هذا الصنف باستخدام بيئة موراشيجى و سكوج الاساسية مع تزويدها بالاوكسينات مثل ٢و٤ داى كلورد فينوكس استيك اسيد (2, 4-D) و البيكلورم وكذلك البنزايل أدنين بصورة فردية أو فى صورة مخاليط بينهما.

- أدى استخدام ال(2, 4-D) الى تكوين كلس فقط فى كل الاجزاء المستخدمة سواء كانت قمم نامية أو اجزاء من الورقة وقد كان أفضل تركيز فى هذا الاتجاه هو ٠,١ مليجرام/ لتر.
- ادى تزويد بيئة موراشيجى وسكوج بالبيكلورم بتركيزات مختلفة الى الحصول على نسيج الكلس فقط فى كل الاجزاء النباتية المنزرعة وقد كان التركيز ٠,٥ مليجرام هو الافضل فى هذا الاتجاه .

- أدى استخدام البنزايل أدنين فى بيئة الزراعة وعند كل التركيزات المستخدمة الى تكوين نموات خضرية فقط.
- ساعداستخدام خليط من البكلورم بتركيز ٠,٥ مليجرام/ لتر و ٠,١ مليجرام/ لتر من البنزايل أدنين ادى الى الحصول على أكبر كمية من نسيج الكلس بالمقارنة ببقية المعاملات.
- تفوق خليط البكلورم بتركيز ٠,٥ مليجرام/ لتر+ ١,٠ مليجرام/ لتر من البنزايل ادينين على خليط ال 2, 4-D + البنزايل أدنين.
- شجع اضافة البنزيل أدنين على زيادة عدد النموات المتحصل عليها وكذالك طول النموات وتوصلت الى افضل النتائج عند استخدام البنزايل أدنين بتركيز ٢ مليجرام/ لتر.
- تم الحصول على أكبر عدد من الجذور وكذلك أ طولها عند اضافة خليط من النفتالين أستيك أسـيد و الاندول بيوترك أسيد الىالبيئة بتركيز ٠,١ مليجرام لكل منها.