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EVALUATION OF GROWTH PARAMETERS OF BOTH DOG RIDGE, SALT CREEK ROOTSTOCKS AND SHOOT TIP MICRO-GRAFTING OF SUPEROIR AND THOMPSON SEEDLESS CULTIVARS Through *in vitro* CULTURE

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ABSTRACT

This investigation was carried out to establish a suitable growth media for two grape rootstocks namely, Salt Creek and Dog Rige and to evaluate micrografting of Thompson seedless and superior cultivars on these stocks through *in vitro* culture. Plant height, leaves and shoot number increased with increasing the concentrations of 2ip from 3 to 7μM. Different concentrations of BAP and GA_3 showed that rootstock responded higher height than concentration of BAP only, except 4 μM BAP associated with Dogridge which had significant effect than all combinations. On the other hand, Salt Creek grown in media supplemented with (0.5 μM IBA) had the lowest root length.

Micrografting of Thompson seedless and Superior cultivars were evaluated using two methods of micro-grafting (shoot-tip and cleft) under *in vitro* conditions on the studied rootstocks. Shoot-tips had higher successful (take) of graft union compared to cleft method which failed to introduce any successful plantlet. Micrografting derived from shoot-tip explants of the two grapevine cultivars showed satisfactory growth characteristics (plantlet height, leaf number and shoot number). Whereas, significant difference between the cultivars and the rootstocks regarding successful graft union and subsequent growth related traits were obtained. Salt Creek rootstock gave the higher rate of success of two scions, respectively if compared to Dog Ridge for both scions. Moreover, shoot length of Thompson seedless scion on Salt Creek rootstock was higher than Thompson seedless scion on Dog Ridge rootstock. In addition, superior cultivar on both of Salt Creek and Dog Ridge showed the same trend. In conclusion, micro-grafting proved to be an alternative suitable propagation method leading to vigorous growth potential of grafted cultivars.

INTRODUCTION

Grapevine is a major horticultural crop with great applications in food and many industries (Azami *et.al.,*2010). Total acreage of grapevine reached 163670 feddan, which produce 1,300,000 tons of fruits according to the statistics of Ministry of Agriculture of Egypt, (2010).

Majority of grapevine orchards are un-grafted on resistant rootstocks. However, these are highly sensitive to insects, fungal diseases, nematodes; especially Phylloxera, leading to losses in their fruit yield and quality. Grafting of disease-free explants is an appropriate alternative for successful propagation of grapevine cultivars (Kim *et al*., 2005). This procedure was able to overcome some of physiological and anatomical problems encountered in some species and cultivar combinations (Degratias *et al*., 1968; Navarro *et al*., 1992 and Estrada-Luna *et al*., 2002).

The producers of world's finest quality of grapes with highest yield/unit area, one such tool, which is poised to make a significant contribution and play a vital role, is mainly the vine rootstocks. The choice of rootstocks is more difficult than generally believed. This is due to interaction between the rootstock, environment and the scion. Fortunately, for the growers of the twin problems of Phylloxera and nematodes and so also the problem of viruses affecting the vine industry elsewhere in the world, does not pose any threat. This situation has helped to a large extent in narrowing down the difficult task of selection.

Among the many rootstocks introduced and tested, two rootstocks have shown their potential, Dog Ridge and Salt Creek. These rootstocks today have been used most successfully on soils of low fertility, their vigorous growth nature is found to be more useful under saline soils. Thompson Seedless grafted on these rootstocks has shown an excellent vigour, resulting in ideal canopy when they were grafted on Dog Ridge or Salt Creek. Also, these rootstocks are known to possess drought tolerance, in addition to greater nutrient uptake and also nematode resistant (Azami *et.al.,*2010).

Micro-grafting is a well recognized propagation procedure capable to use in most plant species leading to promising results. In this method, shoot-tips of 0.1- 0.8 mm in length are accurately grafted on cultured seed or in vitro culture derived plantlets. Micro-grafting is a commercial practice for production of virus free plant materials, especially in test tubes, since 1970s (Bitters *et a*l., 1972; Murashige *et al*.,1972 and Navarro *et al*.,1975). Micrografting as a clonal propagation method for production of virus and other disease-free plant materials hold some advantage if compared with thermotherapy, meristem culture or integrated thermo-treated meristem culture. *In vitro* culture condition has an advantage that it is not dependent upon growing season. Therefore, it is possible to perform micro-grafting at any desired time. Another principal benefit of in vitro culture condition over in vivo grown plants is attaining the high number of shoot-tip explants owing to possible frequent subcultures of individual shoots under controlled conditions (Kim *et al*., 2005). Unlike other asexual propagation methods, micro-grafting produces disease-free, especially virus-free plants, with possible benefits of scion rootstock combinations (Valat *et al.*, 2003 and Youssef *et al.,*2009). The aim of the present experiment was to establish *in vitro* protocol for both Dog Ridge and Salt Creek propagation and evaluate the success of micro-grafting response of Thompson seedless and Superior cultivar scions under *in vitro* conditions on these stocks.

MATERIALS AND METHODS

Two rootstocks, namely Dog Ridge and Salt Creek were subjected to in vitro culture to introduce the best media for proliferation and rooting as follows:

The plant materials were sterilized in 70% ethanol for 10 minute, and then transferred to 20% Cholorax solution for 15 minutes followed rinsed 3 times with sterilized double distilled water.

270 segments were used per rootstock, each treatment was replicated three times with 15 segments of each. A full strength media of WPM was supplemented with 30 g/L sucrose, 2 g/L charcoal, 2 mg/L glycine, 100 mg/L L-tyrosine, 100 mg/L myo-inositol and solidified with 0.8% agar. The pH was adjusted to 5.7.

Proliferation stage:

Surviving shoots were transferred to the indicated media (half strength of WPM) plus different growth regulators as the following treatments:

a) 6-Benzylaminopurine (BAP) at (0.5, 1, 2 and 4 μM)

b) Gibberellic acid (GA_3) at (0.5 and 1 μM)

c) Benzyladenine (BA) at (4, 8 and 10 μM)

d) Kinetin (Kin) at (8, 10 and 12 μM)

e) 2-isopentenyladenine (2ip) at (3, 5 and 7 μM).

f) Combination between each concentration of BAP and GA_3. Each treatment consisted of three replicates, 10 shoots for each replicate. After 4 and 8 weeks from culture the following parameters were recorded:

-Number of shoots/explant -Shoot length in cm/explant -Leaf number/explant.

Rooting stage:

After the prementioned period (proliferation stage) approximately 5-6 cm long shoots were harvested from the mass of shoots produced and transferred to culture tubes (150 x 15 mm with polypropylene lids) filled with 20 ml of MS (Murashige and Skoog, 1962) medium supplemented with:

a)Indole 3-butyric acid (IBA) at (0.5, 1, 2 and 4 μM)

b)α-napthaleneacetic acid (NAA) at (0.5 and 1 μM).

c)Combination between each concentration of these auxins.

Each treatment consisted of three replicates, 10 shoots for each replicate. After 4 and 8 weeks from culture time number of roots/shoot and root length in cm/shoot were recorded.

Shoot tip micro-grafting:

In order to obtain scions of Thompson seedless and Superior, shoot tips taken from the scion were sterilized as previously mentioned, then they were cultured on ½ MS (half strength of Murashige and Skoog, 1962) medium. The shoot tips were subcultured every 3-4 weeks.

Methods of grafting :

Salt Creek and Dog Ridge rootstocks which were grown *in vitro*, were decapitated just below the terminal bud. Apical meristems 0.1-0.2 mm in length of scion containing 2-3 leaf primodia were cut from the shoot tips and then placed on cambial zone on the cut surface of each rootstocks. Another method was tried. The tops were cut with a scalpel producing a small longitudinal cleft. In the cleft, a small scion with one bud from the in vitro cultivars was inserted. This was cultured again in vitro on ½ MS semi solid media + ½Mμ NAA. After 8 weeks, the following parameters were recorded: 1. No. of grafting 2- No. of successful grafts 3. Rate of success 4. Scion length /plantlet. 5. Leaf number/plantlet

Acclimatization of plantlets:

A healthy plantlets (5-7 cm in height, 2-4 roots and 4-5 leaves) from aseptic culture conditions were transferred to the growth room conditions and ultimately to final location. The plantlets must become autotrophic, thus they were transferred to pots filled with the sterilized soil mixture that was consisted of peatmoss + clay + sand (1:1:1 by volume).

Plastic pots were placed in the growth room and irrigated with half strength of Knop media and covered with polyethylene bags (to minimize moisture loss, protect the plant from desiccation) for two weeks, about 6 pores were made on each bag, which were opened after two weeks. The survival rate was calculated on 2 months after transfer**.**

3.8.Statistical analysis:

All experiments were repeated two times, moreover, the data were statistically analyzed by Duncan multiple range test (5%) to differentiate means (Duncan, 1955).

RESULTS AND DISCUSSION

The effect of growth regulators on vegetative growth:

1-Cytokinins(BA, Kin, 2ip):

As shown in Table (1), after 4 or 8 weeks from culturing the combinations of Salt Creek or Dog Ridge with 4 μM BA or 8 μM BA presented markedly higher plant height as compared to the control or the combinations of Kin for Salt Creek or Dog Ridge which had the lowest effect.

On the other hand, after 4 weeks from culture, the significantly highest leaf number resulted from the combination of Salt creek with 4 μM BA and Dogridge with 4 μM BA or 8 μM BA, these were followed by the association of Salt creek or Dogridge with each of 10 μM BA and the all 2ip concentrations with respect to the control. Moreover, after 8 weeks from culture the combinations of Salt Creek with 4 μM BA, 8μM BA, 5 μM 2ip or Dog Ridge with each of 4 μM BA or 5 μM 2ip resulted in marked effect on the leaf number followed by the combinations of Salt creek with 10 μM BA or Dogridge with each 8 μM BA or 10 μM BA. In addition, all the combinations in concern Salt Creek or Dog Ridge with the different concentrations of Kin proved to be the lowest effect.

The media supplemented with 4 μM BA resulted in the highest values of shoot number for Salt creek or Dogridge, moreover, the differences between this combination and the other association reached the limit of significance. In view of the combinations, the best shoot number in descending order, was BA, 2ip and at last Kin. These results revealed that plant height, leaves and shoot number increased with increasing the concentrations 2ip from 3 to 7μM, while Meiners *et al*. (2007) on nodal segments of *Vaccinium corymbosum* observed that high doses of 2ip had been shown to be phytotoxic.

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2- Growth regulators (BAP, GA_3):

After 4 or 8 weeks from culture 4 μM BAP followed by (0.5 μM GA_3 + 0.5 μM BAP) and (1 μM GA_3 + 4 μM BAP) had remarkable effect than the control, thus the treatment had an increased height than the control by 146.81, 104.26, and 117.02% after 4 weeks from culture as well as by 118.57, 78.57 and 74.29% after 8 weeks from culture as shown in Table (2). Moreover, data showed that the media supplemented with different concentrations of BAP and GA_3 showed that rootstock had higher height than concentration of BAP except 4 μM BAP associated with Dog Ridge which had significant effect than all combinations. However, after 4 weeks from culture both of Salt Creek or Dog Ridge associated with 4 μM BAP, (1 μM GA_3 + 0.5 μM BAP), (1 μM GA_3 + 1 μM BAP), (1 μM GA_3 + 2 μM BAP), and Saltcreek with each of (0.5 μM GA_3 + 1 μM BAP) and (1 μM GA_3 + 4 μM BAP) had similar and significant effect than the other treatments, while the control of Saltcreek or Dogridge presented the lowest leaf number with respect to other treatments. After 8 weeks from culture 4 μM BAP, (1 μM GA_3 + 2 μM BAP), and (1 μM GA_3 + 4 μM BAP) with each of Saltcreek or Dogridge as well as Saltcreek associated with (0.5 μM GA_3 + 1 μM BAP), (1 μM GA_3 + 0.5 μM BAP) and (1 μM GA_3 + 1 μM BAP) had equivalent and marked effect than the other treatments. These results are in agreement with those found by Mon *et al*. (2008), they stated that BAP (4 μM) and GA_3 (2 μM) proved to be the best for shoot multiplication.

Effect of auxin (IBA, NAA) on rooting:

Data in Table (3) revealed that, after four weeks from culture, the rooting media supplemented with (0.5 μM IBA + 0.5 μM NAA) presented the highest root length of Salt creek, moreover, Dogridge treated with (0.5 μM IBA + 0.5 μM NAA) or (1 μM IBA + 1 μM NAA) as well as (0.5 μM IBA + 1 μM NAA) combined with each of Salt creek and Dogridge had equivalent effect. The previous treatments had marked effect than the other treatments, hence after 8 weeks from culture Salt creek associated with (0.5 μM IBA + 0.5 μM NAA) had marked effect than Dogridge associated with the same doses have presented the significant highest root length than the other treatments. Generally, Salt creek grown in media supplemented with (0.5 μM IBA) had the lowest root length.

The combining 0.5 μM IBA + 0.5 μM NAA with each of Salt Creek or Dog Ridge resulted in significant highest root number with respect to the other treatments, while after 8 weeks from culture, Salt Creek grown in media supplied with (0.5 μM IBA + 0.5 μM NAA) had marked effect than Salt creek associated with (0.5 μM IBA + 1 μM NAA) or Dogridge combined with (0.5 μM IBA + 0.5 μM NAA), both of them had marked effect than the other treatments. On the other hand, the two concentrations of NAA presented the lowest root number. Mon *et al*., (2008) stated that, 3 μM NAA presented the highest number of roots after two weeks while at 1μM gave the highest number of roots after 3 weeks. Meanwhile, Skiada *et al*. (2010) found that 0.5 μM IBA improved root formation and resulted in a higher rooting percentage

(>95%) and proved to be more beneficial for the overall morphological appearance of the plantlet of 'Malagouzia'.

Micrografting:

Two methods have been used in micrografting, and only shoot tip method resulted in attaining plantlet. Meanwhile, the cleft method failed to raise any plantlet. The rate of success depends upon the stock and the cultivar. Regarding the effect of Salt creek and Dogridge rootstocks on Thompson seedless and superior scion varieties, the data presented in Fig. (1) and illustrated in photos (1) revealed that Saltcreek rootstock gave higher rate of success (70 and 63%) of two scions Thompson seedless and superior, respectively. In the meantime Dog Ridge gave 40 and 57% for both scions, respectively. Moreover, the data presented in Fig.(2) showed that shoot length of Thompson seedless scion on Salt Creek rootstock was higher than Thompson seedless scion on Dog Ridge rootstock by 48%. In addition, superior cultivar on both of Salt Creek and Dog Ridge showed the same trend. The data also showed that leaf number of Thompson seedless scion on Salt Creek rootstock was higher than Thompson seedless scion on Dog Ridge rootstock by 154.5%, moreover, superior cultivar on both of Salt Creek and Dog Ridge showed the same trend. In this respect, Azami *et al*.(2010) stated that grafted assemblies had significant differences concerning shoot length, leaf number and shoot fresh and dry weight. Moreover, *In vitro* culture derived shoot-tips had better response compared to in vivo derived counterparts. These findings are consistent with the results in apple (Deogratias *et al.*, 1991) peach, plum and apricot (Edriss *et al*.,1984 and Estrada-Luna *et al*.,2002).

It's clear that, Salt creek as rootstock is considered more successful for Thompson seedless and superior scion varieties than Dogridge one, as it gave a high rate of successful of grafting, shoot number and leaf number of scions. In conclusion, in grapevine, micro-grafting is a high-tech method for production of disease- free material for cultivation as well as in breeding programs for detection of virus infections. This method of propagation yields a homogenous clonal disease-free population of plants, capable of high establishment and performance potential under field conditions.

Fig.(1): Effect of rootstocks and scion cultivars on the success of *in vitro* micro-grafting.

Fig.(2): Effect of rootstocks and scion cultivars on shoot characters

Dogridge (Grafting)

Photo (1): Effect of rootstocks on shoot characters of shoot tip micro-grafting.

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تقييم طبيعة النمو لاصلى السولت كريك والدوجردج والتطعيم الدقيق لهما بطعمى التومسون سيدلس والسوبريور من خلال زراعة الانسجة

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اجرى هذا البحث بغرض تحديد افضل بيئة لنمو كل من اصلى عنب الدوجردج والسولت كريك حيث انهم من الاصول الصعبة التجذير بالاضافة الى تقييم التطعيم الدقيق لهم مع كل من صنفى التومسون سيدلس والسوبريور باستخدام تكنيك زراعة الانسجة.

حيث أدت زيادة تركيز الـ 2- ايزوبنتيل ادينين من 3الى7 ميكرومول الى زيادة ارتفاع النبات وعدد الاوراق الناتجة وكذلك عدد الافرع.

استخدام تباينات مختلفة من تركيزات البنزيل امينوبيورين مع الجبرلين ادت الى زيادة فى ارتفاع النبات مقارنة باستخدام البنزيل امينوبيورين منفرداً باستثناء أصل الدوج ردج الذى ارتبط زيادة ارتفاع النبات فيه بتركيز 4-ميكرومول من البنزيل امينوبيورين مقارنة بباقى التباينات الاخرى المستخدمة بالدراسة. من ناحية أخرى وجد ان تركيز 0.5 ميكرومول من الاندول بيوتريك اسيد فى بيئة زراعة السولت كريك انتجت اقل طول لنمو الجذور.

من ناحية اخرى تم تقييم نجاح التطعيم الدقيق لكل من صنفى التومسون سيدلس والسوبريور على كل من اصلى الدوج ردج والسولت كريك الناتجة من زراعة الانسجة من خلال استخدم طريقتين للتطعيم (التطعيم القمى والتطعيم بالشق). لوحظ عدم نجاح طريقة التطعيم بالشق حيث لم يتم الحصول على اى نباتات من هذة الطريقة. كانت صفات النمو للاصناف المدروسة جيدة (ارتفاع النبات وعدد الاوراق وعدد الافرع) عند استخدام التطعيم القمى. كما كان هناك اختلاف معنوى بين الاصناف والاصول فى التاثير على مدى نجاح التحام الاصل بالطعم وانعكس ذلك على صفات النمو. حيث وضح أن نسبة نجاح التومسون سيدلس والسوبريور كانت عالية فى حالة السولت كريك عنها على الدوج ردج .

كما ان ارتفاع النبات التومسون سيدلس على السولت كريك اعلى منه فى حالة التطعيم القمى الدقيق على الدوج ردج. فى حين ان صنف السوبريور لم يعطى اى فروق معنوية نتيجة اختلاف الاصول وأخذت النتائج نفس الاتجاه مع كل من الاصلين.

قام بتحكيم البحث

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Table (1):Effect of different cytokinins form on vegetative growth of Salt Creek and Dog Ridge explants after four and eight weeks of culturing.

Characters / Treatments	After four weeks						After eight weeks					
	Plant height (cm)		Leaf number		Shoot number		Plant height (cm)		Leaf number		Shoot number	
	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge
Control	2.6 ef	2.8 de	2.00 c	2.10 c	2.00 d	2.20 cd	3.0 ef	3.5 e	3.60 c	3.40 c	3.10 cd	3.00 d
4 μM BA	3.4 abc	3.8 a	4.10 a	3.80 a	3.50 a	3.20 a	5.7 a	6.2 a	5.00 a	5.00 a	4.70 a	4.50 a
8 μM BA	3.5 abc	3.7 ab	3.00 b	3.00 b	2.40 c	2.50 bc	4.7 b	5.1 b	5.00 a	4.50 b	3.50 c	4.00 b
10μM BA	3.1 cd	3.1 cd	2.70 b	3.80 a	2.40 c	2.00 d	4.0 cd	4.0 cd	4.10 b	4.30 b	3.20 cd	3.50 c
8 μM Kin	2.1 f	2.3 ef	2.10 c	2.00 c	2.40 c	2.20 cd	2.6 f	2.7 f	2.70 e	2.90 d	4.10 b	3.10 cd
10 μM Kin	2.0 f	2.4 ef	2.00 c	2.10 c	2.10 d	2.20 cd	3.0 ef	3.5 e	3.00 cd	3.00 cd	3.20 cd	3.10 cd
12 μM Kin	1.9 f	2.1 f	2.00 c	2.00 c	2.20 cd	2.20 cd	2.6 f	2.8 f	2.90 d	2.70 e	3.20 cd	3.00 d
3 μM 2ip	3.0 cd	3.2 bcd	2.30 c	2.20 c	2.30 cd	2.40 c	3.8 cd	3.7 de	3.40 c	3.80 c	3.10 cd	3.10 cd
5 μM 2ip	3.1 cd	3.4 abc	3.10 b	3.00 b	2.50 bc	2.10 d	3.9 cd	4.2 c	5.30 a	5.40 a	3.20 cd	3.30 cd
7 μM 2ip	3.2 bcd	3.3 abc	3.00 b	3.10 b	2.80 b	2.10 d	3.9 cd	3.7 de	3.60 c	3.40 c	3.40 c	3.30 cd
Mean-R	2.79A	3.01A	2.63A	2.71A	2.46A	2.31A	3.72A	3.94A	3.86A	3.84A	3.47A	3.39A

In each column, the means followed by the same letter are not significantly different at the 5% level according to DMRT.

(1)* **After four weeks from culture**

(2)** **After eight weeks from culture**

Table (2): Effect of different growth regulators on vegetative growth of Salt Creek and Dog Ridge explants after four and eight weeks of culturing.

Characters	After four weeks						After eight weeks					
	Plant height (cm)		Leaf number		Shoot number		Plant height (cm)		Leaf number		Shoot number	
Treatments	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge
Cont.	2.3 h	2.4 h	1.30 i	1.60 i	2.10 ef	2.30 e	3.50 hi	3.5 hi	2.00 j	2.10 j	2.60 k	2.80 k
0.5 μM BAP	2.8 ef	2.8 ef	3.00 ef	3.20 cde	2.00 ef	1.80 f	3.90 h	4.8 ef	3.40 gh	4.50 cde	2.40 k	2.10 k
1 μM BAP	2.9 ef	2.9 ef	3.10 de	3.70 bc	2.20 ef	2.40 e	3.50 hi	4.0 gh	3.50 gh	4.40 de	2.50 k	3.80 i
2 μM BAP	2.7 f	2.9 ef	3.30 bcde	3.70 bc	3.67 c	2.90 d	3.40 i	3.9 h	4.30 de	4.70 bcd	4.70 jh	4.90 fgh
4 μM BAP	5.5 b	6.1 a	4.60 a	4.40 a	4.20 a	4.50 a	6.70 b	8.6 a	5.50 a	5.60 a	7.40 a	8.00 a
0.5 μM GA_3	2.7 f	2.8 ef	2.40 g	2.50 fgh	2.00 ef	2.20 ef	4.00 fg	4.5 f	2.70 i	2.80 i	3.70 i	3.90 i
1 μM GA_3	2.2 h	2.4 h	2.30 h	2.40 gh	2.20 ef	2.20 ef	3.70 hi	3.8 h	2.70 i	3.60 gh	4.50 hi	4.80 fgh
0.5 μM GA_3 + 0.5 μM BAP	4.3 cd	5.3 b	2.50 fgh	2.40 g	2.20 ef	3.10 cd	5.70 cd	6.8 b	3.00 hi	4.1 ef	4.40 hi	5.20 fg
0.5 μM GA_3 + 1 μM BAP	3.1 ef	3.2 e	4.60 a	3.70 bc	2.50 e	3.20 cd	4.00 fg	4.5 f	5.00 abc	4.5 cde	3.67 i	5.00 fg
0.5 μM GA_3 + 2 μM BAP	5.1 b	5.1 b	3.50 bcde	3.50 bcde	3.20 cd	3.30 cd	3.20 g	3.7 hg	3.70 fgh	4.0 def	4.50 hi	4.80 fgh
0.5 μM GA_3 + 4 μM BAP	4.2 cd	4.0 d	3.50 bcde	3.60 bc	3.10 cd	3.40 cd	4.60 f	4.7 f	3.50 gh	3.00 hi	5.10 fg	4.80 fgh
1 μM GA_3 + 0.5 μM BAP	4.0 d	4.5 c	4.20 a	4.30 a	3.40 cd	3.50 c	5.30 de	5.8 c	5.10 abc	4.50 cde	5.30 fg	5.70 ef
1 μM GA_3 + 1 μM BAP	4.0 d	4.3 cd	4.30 a	4.50 a	3.00 cd	3.30 cd	5.50 cde	6.2 b	5.20 ab	4.70 bcd	6.00 def	5.30 fg
1 μM GA_3 + 2 μM BAP	4.1 cd	5.2 b	4.10 a	4.20 a	3.50 c	3.50 c	5.50 cde	6.4 b	5.10 abc	5.00 abc	3.80 i	6.20 cde
1 μM GA_3 + 4 μM BAP	5.1 b	5.1 b	4.50 a	3.90 b	4.00 b	4.20 b	6.00 b	6.2 b	5.10 abc	5.00 abc	6.30 bcd	7.10 b
Mean-R	3.67 B	3.93 A	3.41 A	3.44 A	2.88 A	3.05 A	4.57 B	5.16 A	3.99 A	4.17 A	4.46 B	4.96 A

In each column, the means followed by the same letter are not significantly different at the 5% level according to DMRT.

(1)* After four weeks from culture
(2) After eight weeks from culture**

Table (3): Effect of different auxins on root length and root number of Salt Creek and Dog Ridge explants after four and eight weeks of culturing.

Characters / Treatments	After 4 weeks				After 8 weeks			
	Root length (cm)		Root number		Root length (cm)		Root number	
	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge	Salt Creek	Dog Ridge
CONT.	7.00 c	6.50 cde	3.70 def	2.80 h	9.50 de	8.80 def	4.10 e	4.70 e
0.5 μM IBA	2.70 h	3.00 k	3.50 fh	3.10 gh	4.10 L	5.30 kl	5.20 d	5.30 d
1 μM IBA	6.20 c	6.40 c-f	4.50 b	4.30 bc	8.10 e-h	7.00 hjk	6.40 c	5.90 d
2 μM IBA	6.00 ef	7.00 cd	4.00 bcde	3.90 bcde	8.1 e-h	9.00 def	6.20 c	4.10 e
4 μM IBA	6.20 c-f	5.50 f	3.80 cdef	3.80 bcde	8.2 e-h	8.80 def	4.20 e	4.90 e
0.5 μM NAA	6.00 ef	6.50 cde	3.10 fg	2.70 h	8.5 efg	8.60 def	3.70 f	3.50 f
1 μM NAA	3.00 k	4.50 fgh	2.10 jk	2.40 h	5.00 kl	6.10 k	3.30 f	3.10 f
0.5 μM IBA + 0.5 μM NAA	9.00 a	8.00 b	5.00 a	5.20 a	13.3 a	11.70 b	8.30 a	7.50 b
0.5 μM IBA + 1 μM NAA	8.00 b	8.00 b	4.20 bcd	4.30 bc	9.00 def	10.00 cde	7.40 b	6.70 d
1 μM IBA + 0.5 μM NAA	6.20 c-f	6.50 cde	4.10 bcde	4.10 bcde	7.8 f-j	8.40 e-h	5.60 d	5.80 c
1 μM IBA + 1 μM NAA	7.1 bc	8.40 b	4.10 bcde	4.10 bcde	10.00 cde	10.20 c	5.60 d	5.70 c
2 μM IBA + 0.5 μM NAA	5.30 fg	5.60 ef	3.90 cde	4.10 bcde	6.80 jk	7.90 f-j	4.50 e	5.00 d
2 μM IBA + 1 μM NAA	6.20 c-f	6.10 ef	3.80 cde	4.00 bcde	8.30 e-h	8.40 e-h	5.00 d	5.60 d
4 μM IBA + 0.5 μM NAA	4.10 gh	3.20 jk	3.60 efg	3.30 efg	5.90 k	5.70 kl	4.30 e	4.40 e
4 μM IBA + 1 μM NAA	4.40 gh	4.50 fgh	3.20 efg	3.20 efg	7.1 g-k	7.30 g-k	4.20 e	4.30 e
Mean-R	5.83 A	5.98 A	3.80 A	3.70 A	7.98 A	8.21 A	5.20 A	5.10 A

In each column, the means followed by the same letter are not significantly different at the 5% level according to DMRT.

(1)* After four weeks from culture

(2) After eight weeks from culture**