Effect of Interaction between Different Plant Growth Regulators on *In Vitro* Shoot Proliferation of Some Citrus Rootstocks.

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ABSTRACT

The major purpose of this study was to see how growth regulators and sucrose content affected *in vitro* propagation of four citrus rootstocks: Volkameriana Lemon, Sour orange, trifoliate orang, and Cleopatra mandarin. So, the goal of this research was to see how the impacts of MS media supplemented with Sucrose at 30, 45 and 60 g /L either individually or in combination with BA and NAA at different concentrations on *in vitro* seed germination of four tested citrus rootstocks. Our data cleared that adding Sucrose at 30 g/L to MS media gained the highest seed germination of all tested citrus rootstocks compared with other concentrations of sucrose. Through initiation stage, adding1.5 mg/L BA and 0.01 mg /L NAA to MS media caused an increase shoot number and shoot fresh weight while the highest shoot length and leaves number of all tested rootstocks possessed when the explants were cultured on MS media accomplished with both BA and NAA at 0.5 mg /L and 0.01mg /L respectively. Results showed that, the highest shoots number and shoot fresh weight through multiplication stage obtained when the micro-shoots of all tested rootstocks were cultured on MS media supplemented with BA and KIN at 1.0 mg /L of all tested rootstocks. MS media complemented with1.0 mg /L of IBA and NAA caused the maximum roots number of all tested rootstocks. Volkameriana lemon and Cleopatra mandarin were superior on other tested rootstocks regarding the effect of MS complemented with BA and IBA either individually or in combination between sucrose at different concentrations on growth parameters.

Keywords: Micropropagation, Citrus, Germination, PGR, Rootstocks.

INTRODUCTION

Citrus is a popular name for a genus of flowering plants in the *Rutaceae* family. All citrus species have 2n = 18, and their karyotypic form and size are remarkably similar. Furthermore, the Rutaceae family is a huge, mostly tropical and subtropical family with 150–162 genera and 1500–2096 species (Kubitzki *et al.,* 2011; Guerra *et al.,* 1997). At any time, the citrus industry's major breeding aims include improving fruit quality and minimizing biotic and abiotic stresses. (Ruixing *et al.,* 2007). Sexual and asexual reproduction are using methods to Citrus varietals. Although most commercial types are propagated asexually through various methods, rootstocks are mainly propagated sexually by seeds. (Chaudhary, 1994). Traditional citrus plant vegetative replication takes time and is heavily reliant on the season and availability of plant material, making new cultivars difficult to accept and replace. (Rathore *et al.,* 2007) Furthermore, these outdated processes put people at risk of contracting infections. By boosting fruit quality and tolerance to infections and environmental difficulties, the in vitro micropropagation technique can aid citrus improvement and cultivation. (Gresser, 1994). Production of disease resistance planting material requires a lengthy time in the traditional process (Dagnew *et al.,* 2014). In vitro propagation is a method for resolving such issues. When compared to traditional methods, it can generate planting material on a relatively big scale (Savita *et al.,* 2010). In vitro culture prevents infections and is faster than traditional techniques of dissemination (Savita *et al.,* 2010). For a variety of citrus species and explant sources, tissue culture and micropropagation techniques have been published (Pérez-Tornero *et al*., 2010).

MATERIALS AND METHODS

Plant material and description.

The genotypes used for this study were Volkameriana lemon; Sour orange; Trifoliata orang and Cleopatra mandarin. The experiments were carried out at the laboratory of plant tissue culture, Department of Horticulture, Faculty of Agriculture Al-Azhar University during the period from 2019 to 2021. Fruit of four tested citrus rootstocks were collected from orchard in Horticulture department farm -Faculty of agriculture- Al-Azhar University – Naser City – Cairo- Egypt. The fruits of the four tested rootstocks were chosen carefully to be free of infection by any fruits born-pathogen and free of fungus. The fruits were sterilized where dipped in tap

water with supplemented soap for 30 min., to eliminate dust. Thereafter, with dipping in 99 % Ethanol for (1/4 min) then flavedo player of fruits was pruned on flame under laminar flow condition for 5 seconds and then fruits sterilized were placed on the paper sterilized then seeds were removed from bulb in order to be cultured. Sterilized seeds were cultured on different treatments. The seeds were placed on Murashige and Skoog (MS 1962) agar medium supplemented with 30,45,60 g/l of sucrose and different type of plant growth regulators such as BA at 0.0, 0.5 and 1.0 combined with NAA at 0.0, 0.01 and 0.1 mg /L for each concentration of sucrose for seed germination. Then Ph of the medium was adjusted to 5.8 by addition of 0.1 N HCl or 0.1 N NaOH. All media were sterilized autoclaving at 121°C and 1.1 kg/cm for 20 min. Cultures were incubated under 25± 2°C in the growth room under darkness conditions. The Measurements was as follows: Percentage of contamination, Percentage of germination and Percentage of dead seeds were recorded after one month. After germination stage shoots were dissected segment with 7 – 8 mm with one node. Segments were sterilized with 25% commercial hypochlorite sodium for 50 min then were rinsed in sterile distilled water 4 – 5 times. Explants were culture on MS (Murashige and Skoog 1962) media supplemented with some plant growth regulators such as BA at 0.0, 0.5, 1.0 and 2.0 mg/L either individually or in combination between with NAA at 0.0, 0.01 and 0.1 mg /L. All of media were adjusted to 5.8 pH then were autoclaved at 120ºC. Media were distributed in jar containing 25 ml containing two explants with 3 replicates. The mean number shoots, shoot length (cm), leaves number and shoot fresh weight (g) all parameters were recorded after one month. The micro-shoots of four tested citrus rootstocks were culturing on MS media supplemented with different concentrations from BA and KIN at 0.0, 0.5, 1.0 and 2.0 mg /L through multiplication stage. The mean number of shoots, shoot length (cm), leaves number and shoot fresh weight (g) all parameters were recorded after one month. The micro-shoots of all tested citrus rootstocks were re cultured on ½ MS media complemented with IBA and NAA both at 0.0, 0.5, 1.0 and 2.0 mg/L through rooting stage. The mean of root formation percentage, roots number and root length (cm) were recorded after one month later. The data of all parameters were input into Co-Stat software, then the replicated average values of all parameters data were subjected to analysis of variance (ANOVA) to determine the significance of measured parameters. LSD at 5% test was used for statistical Analysis according to Stern (1991).

RESULTS AND DISCUSSION

Seeds germination:

Data in Figs 1 to 3 and Fig.7 showed the effect adding sucrose from 30 up to 60 g /L to MS media combined with either BA at 0.0,05 and 1.0 mg /L or NAA at 0.0,0.01 and 0.1 mg /L on germination parameters of four tested citrus rootstocks. It was clear that the germination percentage, shoots number, shoot length (cm), leaves number, death percentages and survival percentage as parameters indicating the success of seed germination were enhanced by culturing seeds on MS media. MS media, free of plant growth regulators and amino acids, significantly increased seed germination percentage and gained the highest survival percentage while the least death percentage of all tested rootstocks were gained when cultivated on MS media accomplished with 0.5 mg / L of BA either individually or in combination with NAA at 0.01 compared with those of other treatments, while when seeds of all investigated rootstocks were cultivated on MS media complemented with high levels of BA and NAA, either individually or in combination, the lowest seed germination % was obtained when seeds of all examined rootstocks were cultivated on MS media with simply a high amount of BA, the maximum number of shoots were produced when compared to control and other treatments in comparison to control and other treatments, adding BA at 0.5 mg/L to the growth MS media resulted in the maximum shoot length and leaf number of all rootstocks examined. All treatments have significant effect on germination parameters of all tested rootstocks where Trifoliata orange was superior on residual teste rootstocks. Adding different concentrations of sucrose 30,45 and 60 g /L either individually or in combination between BA and NAA to MS media significantly affected germinations parameters in all tested rootstocks where MS media containing all different concentrations of sucrose that individually gained the highest germination percentage in seeds of all tested rootstocks. When immature seeds were cultured on MS media complemented with 30g/L sucrose followed descending order by 45 g/L and 60 g/L of sucrose, the maximum germination percentage occurred, compared to MS media

supplemented with BA and NAA individually or in combination with all concentrations of sucrose.. The results are in agreement with Hassanine and Azoz (2003) found that The maximum seed germination % was found in MS media augmented with 0.5 mg/L of BA and sucrose at 30g/L of *Citrus reticulata* Blanco compared with other treatments. Hansuek *et al.,* (2018) discovered that When seeds of neck orange (*Citrus reticulate* Blanco) were cultivated on MS free media (without plant growth regulators), they gained the maximum seed germination percentage that was observed when compared to other treatments**.** At the same time, when neck orange seeds (*Citrus reticulate* Blanco) were cultivated on MS media accomplished with 0.5 mg/L of BA, the maximum shoot length was attained when compared to control and other treatments. In comparison to control, adding 2.0 mg/L BA to MS media resulted the most shoots number of neck orange (*Citrus reticulate* Blanco).

Initiation stage:

Data in Figures 4 and 7 showed the effect of MS media accomplished with BA from 0.5 up to 1.5 mg /L either separately or in combination between NAA at 0.0,0.01 and 0.1 mg /L on proliferations of some citrus rootstocks. It was clear that adding BA and NAA either individually or in combination between all of them significantly enhanced shoot growth of all tested citrus rootstocks compared with control. The maximum shoots number and shoot fresh weight achieved when the micro-shoots of all tested citrus rootstocks were cultivated on MS media complemented with both BA and NAA 1.5 and 0.01 mg /L respectively followed by 1.0 and 0.5 BA combined with NAA at 0.01 mg /l respectively in comparison with those of control and other treatments. In comparison to other treatments and without any plant growth regulators, adding BA and NAA at 0.5 and 0.01 mg/L to the growth MS media resulted in an increase in shoot length and leaf number of all evaluated citrus rootstocks.. BA was superior on NAA in regarding to above shoot growth of all tested rootstocks. Both Cleopatra mandarin and Volkameriana lemon were superior than other tested citrus rootstocks in regarding to all previous vegetative parameters that were affected by explant of all tested rootstocks that were cultured on MS media provisioned with BA either individually or in combination with NAA. These results are agreement with some researches such as Haripyaree *et al.* (2011) who reported that the best proliferation percentage obtained the explants of *Citrus megaloxycarpa* were cultured on MS media augmented with BA at 0.25 mg /L either individually or in combinations between with 0.05 mg /L of NAA compared to those of the control and other treatments. Also, Goswami et al. (2013) discovered that adding 0.1 mg/L BA or 0.5 mg/L KIN to MS medium increased lemon (*Citrus limon* L. cv. Kaghzi Kalan) shoot growth as compared to control and other treatments. In terms of the number of days it took for a lemon blossom to break, BA outperformed kinetin (*Citrus limon* L. cv. Kaghzi Kalan). The physiological purpose of BAP is now the most extensively used cytokinin in the micropropagation business due to its effectiveness and affordability. (Bairu *et al.,* 2007).

Multiplication stage:

The effect of MS media supplemented with BA either individually or in combination with both KIN from 0.0 up to 2.0 mg /L on multiplication phase of some tested citrus rootstocks showed in Fig. 5 and Fig.7. It was obvious that supplementing MS media with either BA or KIN at varied concentrations, either singly or in combination, increased shoot growth of all citrus rootstocks examined. When the micro-shoots of all tested citrus rootstocks were cultivated on MS media complemented with BA and Kin 1.0 mg /L for them, followed by either 1.0 or 2.0 mg /L of BA interacted with KIN at 0.5 mg /L respectively, the maximum shoots number and shoot fresh weight were obtained, as compared to the control and other treatments.. MS media achievement with both BA and KIN at 0.5 and 1.0 mg /L caused an increase in shoot length and leaves number of all tested citrus rootstocks in comparison with control and those of other treatments. BA gave the highest above shoot parameters rather than KIN of all tested rootstocks. Volkameriana lemon and Sour orange possessed the best value in previous vegetative growth rather than the remaining of tasted citrus rootstocks in regarding to the effect of MS media complemented with different concentrations from 0.0 up to 2.0 mg /L either or individually or in combination between both BA and KIN in all tested citrus rootstocks. These results are in agreement with some researches In comparison to the findings of the control and other treatments, Chamandoosti. F (2017) discovered that explants of *Citrus latifolia* Tan. (persian lime) cultivated on MS medium supplemented with 4.44 M BA + 0.053 M NAA and 4.44 M BA + 0.049 M IBA obtained the highest number of shoots per explant. Also,

MS media complemented with BA at 4.44 µM and IBA 0.049 µM possessed the highest shoot length in comparison with those of control and other treatments. Increasing BA concentrations from 8.8 µM to 26.6 µM alone caused positive effect on shoot multiplication in *Chlorophytum borivilianum* (Mehdi Farshad *et al.,* 2014) Also, Kour and Singh (2012) illustrated that MS media accomplished with 1.5 mg /L BA led to an increase in shoots number and shoot length of rough lemon (Citrus jambhiri Lush.) compared with control and other treatments. In addition, Menamo *et al.,* (2016) revealed that the highest shoots number achieved when the micro-shoots of Lemon (*C. limon*) and Macrophylla (*c macrophylla*) were cultivated on MS media complemented with 2.5 mg /L of BA compared with control and other treatments. Similarly, Goswami *et al.,* (2013) discovered that MS media with 1.0 mg/L BA and 0.5 mg/L KIN produced the most lemon (*Citrus limon* L. cv. Kaghzi Kalan) shoots when compared to other treatments.

Rooting stage:

Data in Fig. 6 and Fig.7 showed the effect of MS media augmented with both IBA and NAA at 0.0, 0.5, 1.0 and 2.0 mg /L individually or in combination between them on rooting production of some citrus rootstocks. It was clear that adding all concentrations of IBA and NAA either or individually or in combination significant enhanced root growth of all tested citrus rootstocks compared with control. When the micro-roots of all tested citrus rootstocks were cultured on MS media supplemented with IBA and NAA at 1.0 and 1.0 mg/L, respectively, followed by 2.0 and 0.5 IBA combined with NAA at 0.5 and 10 mg/l, respectively, the maximum root number achieved was compared to those of control and other treatments. In comparison to control and other treatments, the maximum root length attained when all evaluated rootstocks were micro-cultured on MS media complemented with 2.0 mg/L NAA and 1.0 mg/L IBA. IBA possessed the highest roots number rather than NAA while the root length enhanced when added NAA to MS media rather than IBA in all tested rootstocks. Volkameriana lemon was superior than other tested citrus rootstocks in regarding to all previous vegetative parameters were affected by explant of all tested rootstocks were cultured on MS media supplemented with IBA either individually or in combination with NAA. These findings are in line with those of other studies. According to Menamo *et al.,* (2016), MS media accomplished with 1.0 mg/L NAA resulted in the highest rooting percentage and quantity of roots for Lemon (*C. limon*) and Macrophylla (c.macrophylla) when compared to control and other treatments**.** In addition, Haripyaree *et al.,* (2011) found that MS media supplemented with 2.0 mg/L IBA resulted in the highest root production percentage of *Citrus megaloxycarpa* when compared to control and other treatments. The physiological role of auxins may be responsible for the rise in root development. Many plant species have shown that IBA has a favourable influence on roots. It's possible that the lack of rooting morphogenesis is due to a lack of cell sensitivity to morphogenesis (Amri *et al.,* 2010).

CONCLUSION:

The best germination percentage achieved when seeds of all tested rootstocks were cultivated on MS media containing sucrose at 30g /L. The best shoot proliferation of four tested rootstocks obtained when the explants were cultured on MS media supplemented with BA at 1.0 or 1.5 mg /L combined with 0.01 mg /L of NAA. Adding, 1.0 mg /L of both BA and KIN led to the maximum shoots number through multiplication stage. In rooting stage, the best root percentage obtained when the micro-shoots were cultivated on MS accomplished with IBA and NAA at 1.0 mg /L. Volkameriana Limone and Cleopatra mandarin were superior on other tested rootstocks.

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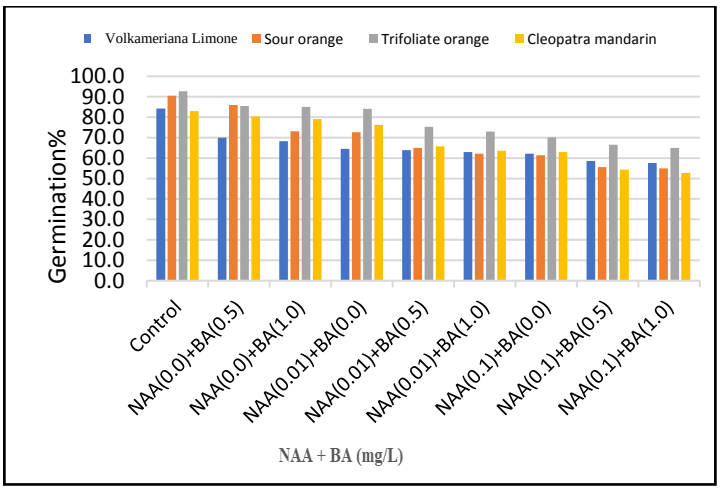
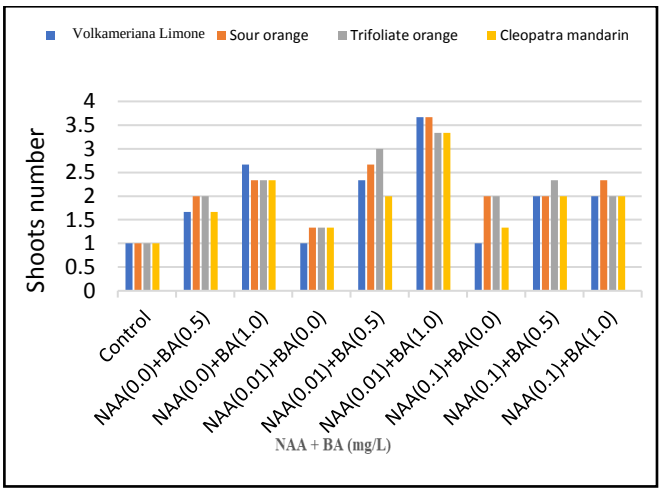


L.S.D at 5% = R =3.17, T= 4.76 and RxT = 9.52



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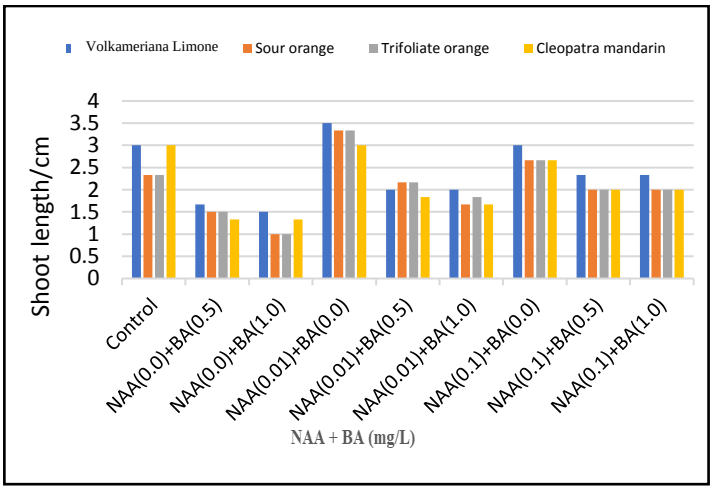

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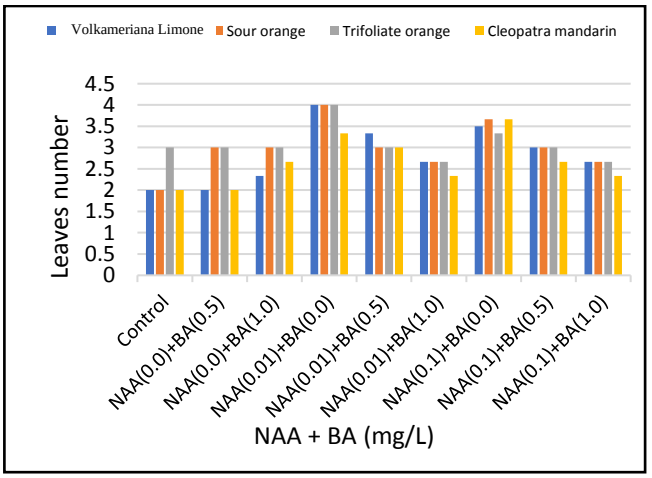

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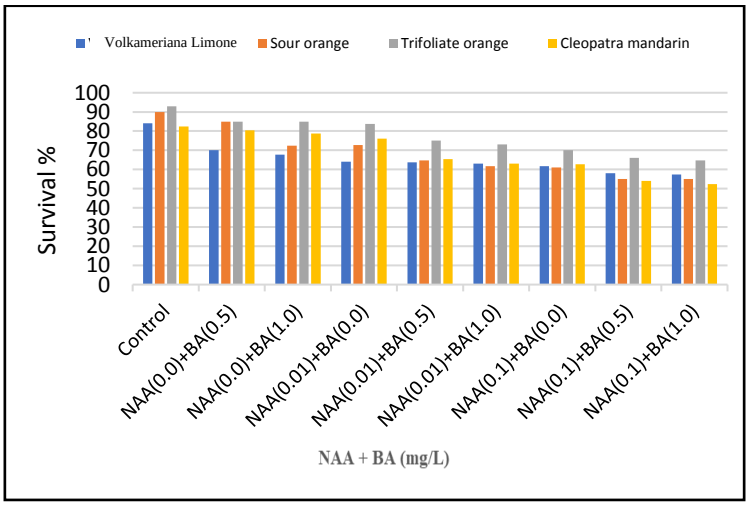
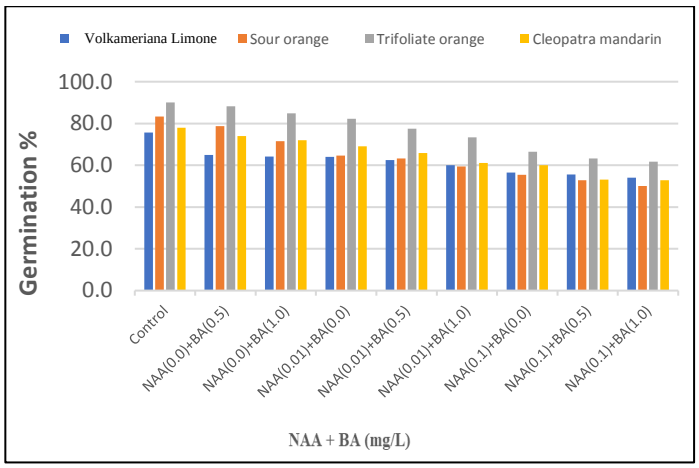
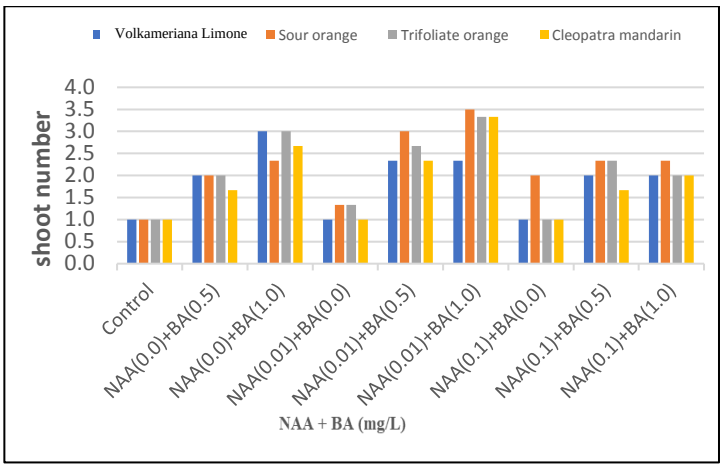


L.S.D at 5% = R2.06, T=3.10 and RxT= 6.20

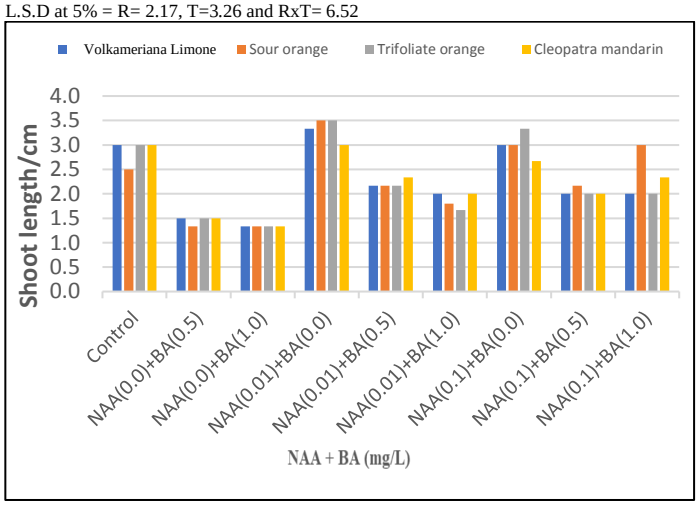
Figure 1: Effect of MS media complemented with Sucrose at 30 g/L either individually or in combination with BA and NAA at different concentrations on germination parameters of some citrus rootstocks.



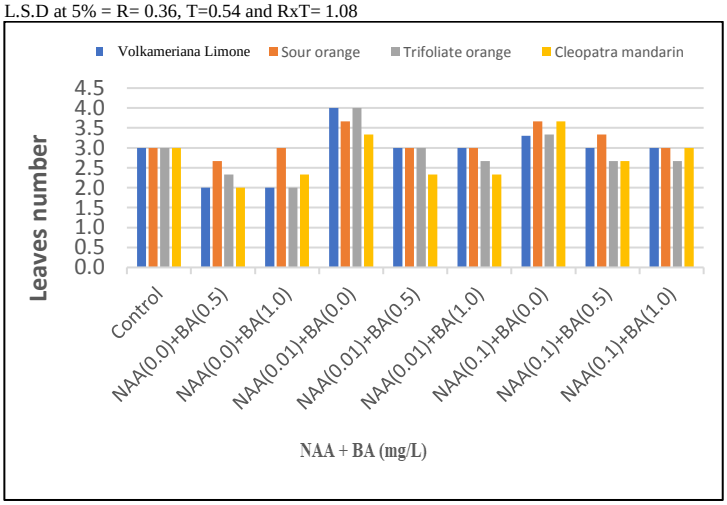
L.S.D at 5% = R= 2.17, T=3.26 and RxT= 6.52



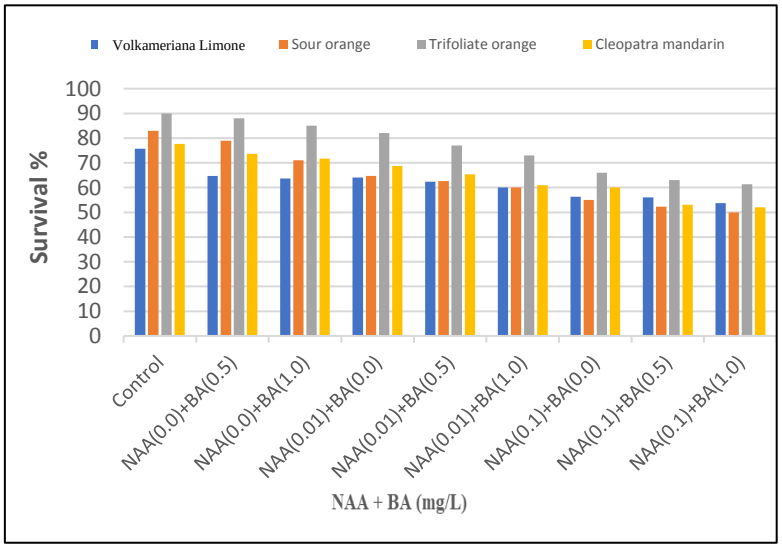
L.S.D at 5% = R= 0.36, T=0.54 and RxT= 1.08



L.S.D at 5% = R= 0.29, T=0.47 and RxT= 1.48

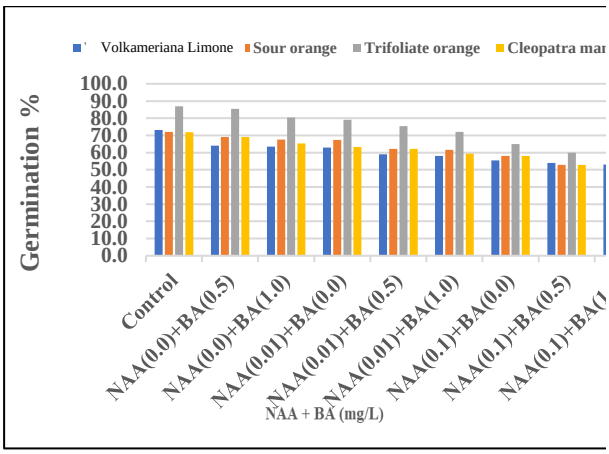


L.S.D at 5% = R= 0.57, T=0.86 and RxT= 1.72

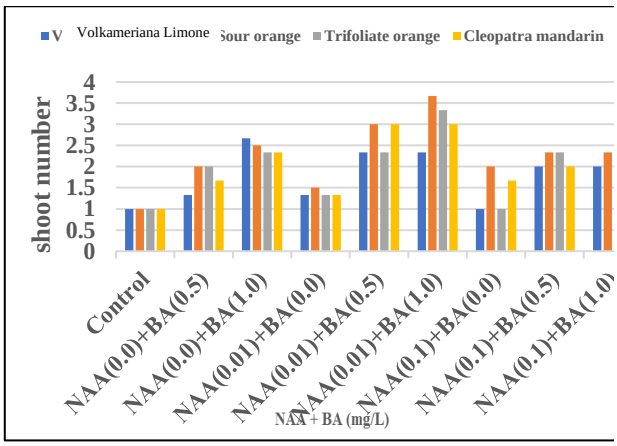


L.S.D at 5% = R= 1.69, T=2.54 and RxT= 5.08

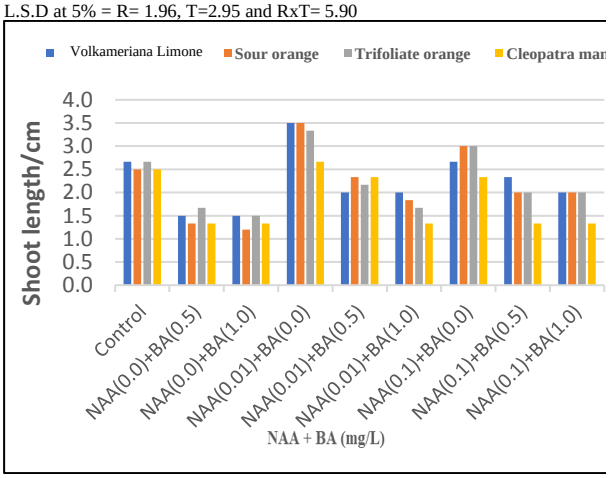
Figure 2: Effect of MS media supplemented with Sucrose at 45 g/L either individually or in combination with BA and NAA at different concentrations on germination parameters of some citrus rootstocks.



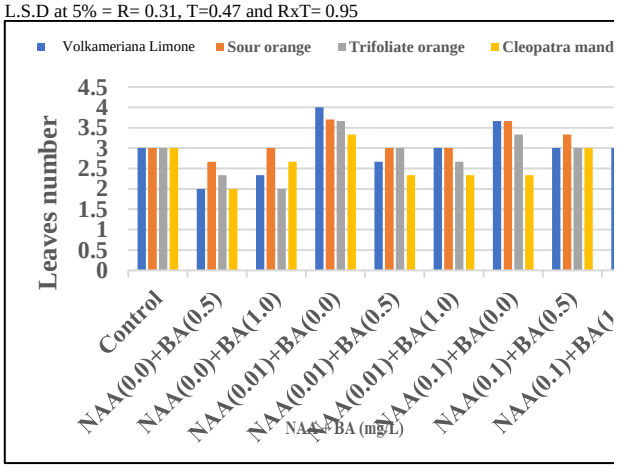
L.S.D at 5% = R= 1.96, T=2.95 and RxT= 5.90



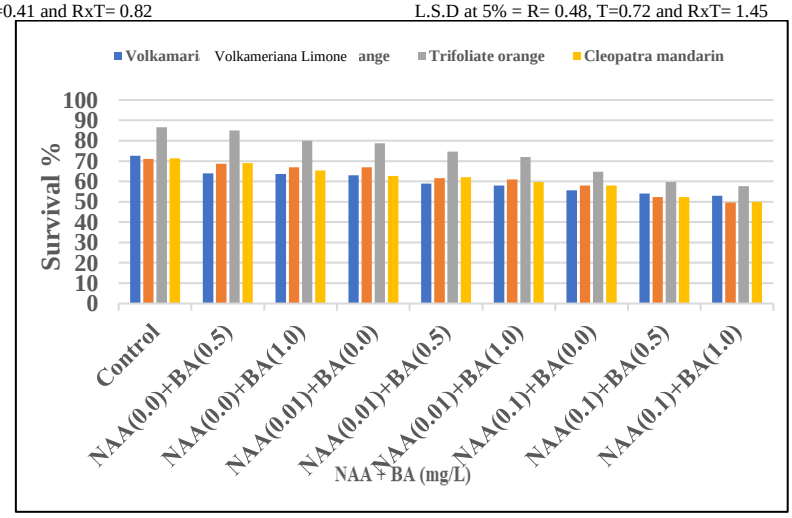
L.S.D at 5% = R= 0.31, T=0.47 and RxT= 0.95



L.S.D at 5% = R= 0.23, T=0.41 and RxT= 0.82

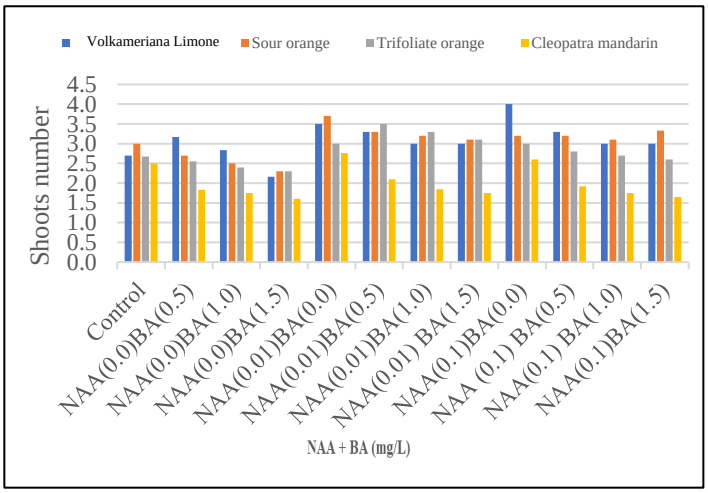


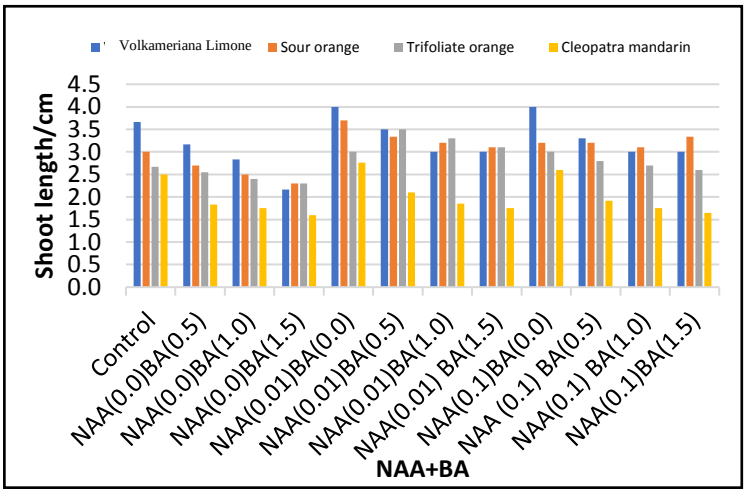
L.S.D at 5% = R= 0.48, T=0.72 and RxT= 1.45



L.S.D at 5% = R= 1.78, T=2.68 and RxT= 5.36

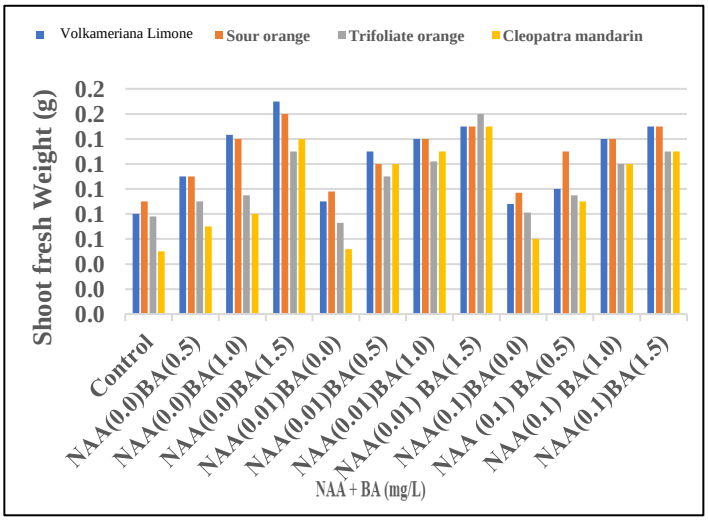
Figure 3: Effect of MS media supplemented with Sucrose at 60 g/L either individually or in combination with BA and NAA at different concentrations on germination parameters of some citrus rootstocks.

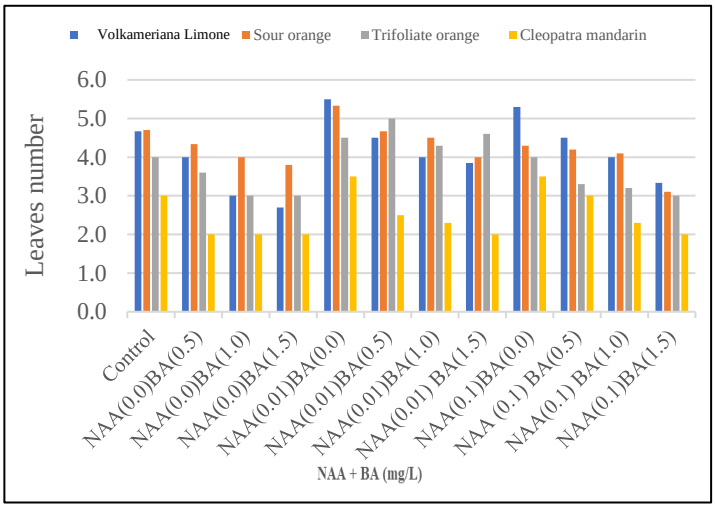




L.S.D at 5% = R= 0.34, T=0.59 and RxT= 1.18

L.S.D at 5% = R= 0.23, T=0.40 and RxT= 0.80

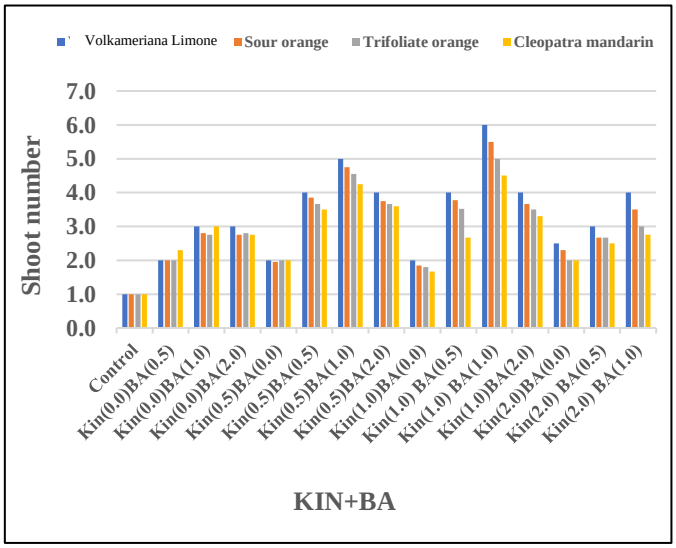


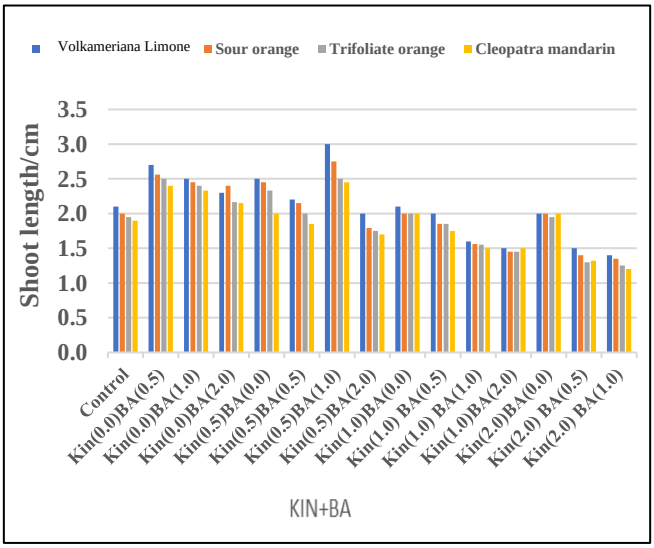


L.S.D at 5% = R= 0.009, T=0.015 and RxT= 0.030

L.S.D at 5% = R= 0.36, T=0.62 and RxT= 1.25

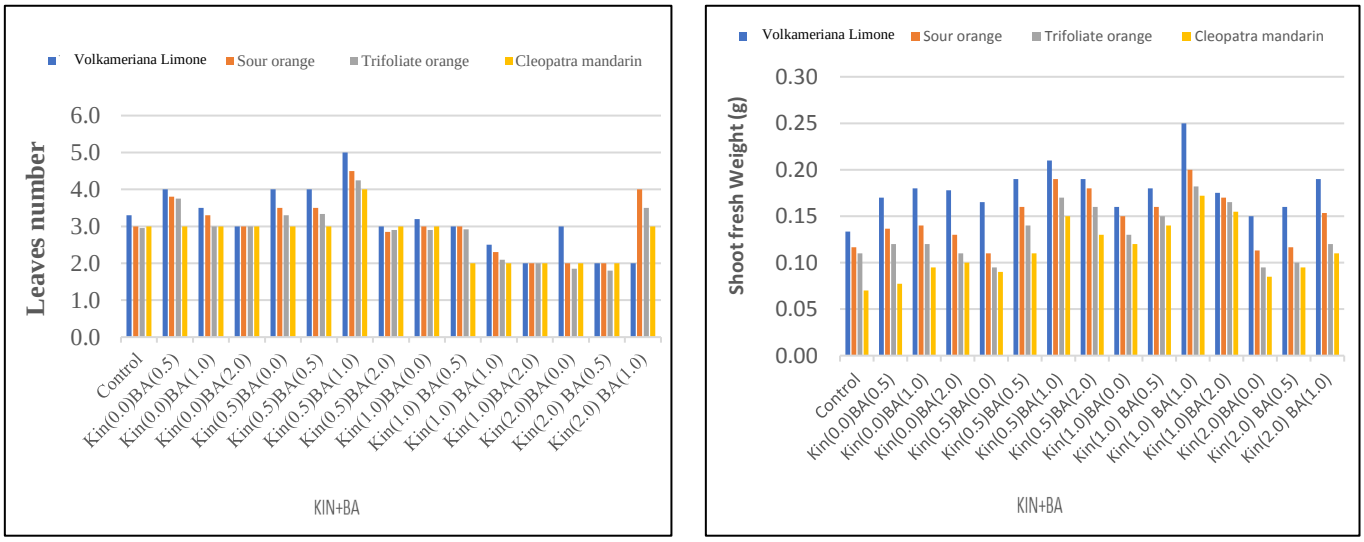
Figure 4: Effect of MS media supplemented with BA and NAA either individually or in combination between of them on morphological characteristic of some citrus rootstocks through initiation stage.





L.S.D at 5% = R= 0.27, T=0.55 and RxT= 1.10

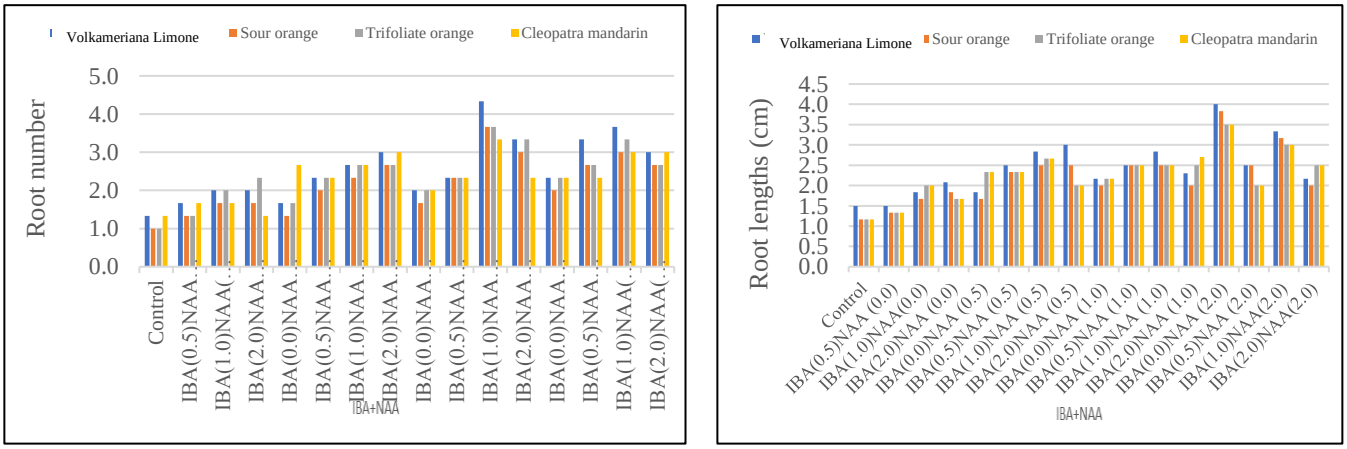
L.S.D at 5% = R= 0.17, T=0.35 and RxT= 0.71



L.S.D at 5% = R= 0.27, T=0.54 and RxT= 0.711

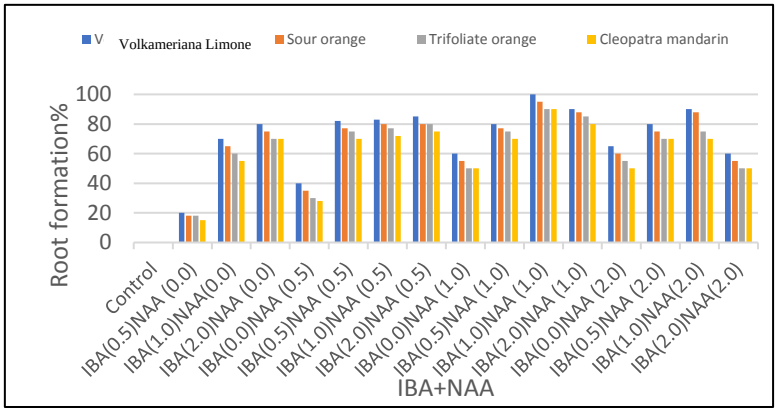
L.S.D at 5% = R= 0.008, T=0.017 and RxT= 0.035

Figure 5: Effect of MS media supplemented with BA and KIN either individually or in combination with of them on morphological characteristic of some citrus rootstocks through multiplication stage.



L.S.D at 5% = R= 0.31, T=0.42 and RxT= 0.82

L.S.D at 5% = R= 0.26, T=0.52 and RxT= 0.76



L.S.D at 5% = R= 2.58, T=3.68 and RxT= 5.97

Figure 6: Effect of MS media supplemented with IBA and NAA either individually or in combination with of them on morphological characteristic of some citrus rootstocks through rooting stage.

Figure 7: *In vitro* propagation of some citrus rootstocks.

تأثير التفاعل بين منظمات النمو المختلفة على الإكثار الدقيق لبعض أصول الموالح

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الملخص العربي :

الهدف الأساسي من هذه الدراسة دراسة مدى تأثير بيئة موراشيجي وسكوج المضاف اليه تركيزات مختلفة من السكروز وبعض منظمات النمو المختلفة على الإكثار الدقيق لبعض أصول الموالح. أفضل نسبة إنبات لأصول الدراسة ظهرت عند زراعة البذور على بيئة موراشيجي وسكوج مضاف إليها السكروز ب تركيز ٣٠ جرام / لتر مقارنة بتركيزات السكروز المختلفة والمعاملات الأخرى. أفضل نمو لأصول الموالح محل الدراسة ظهرت عند زراعة المنفصلات النباتية على بيئة موراشيجي وسكوج المعدلة بإضافة البنزيل ادنين بتركيز من ١,٠ الى ١,٥ مليجرام / لتر مجتمعا مع نفثالين حمض الخليك بتركيز ٠,٠١ مليجرام / لتر مقارنة بالكنترول والمعاملات الأخرى. أفضل معدل تضاعف لنمو أصول الدراسة عند زراعة النبتات الصغيرة على بيسئة موراشيجي وسكوج المضاف إليها تركيز ١,٠ مليجرام / لتر لكل من البنزيل ادنين والكينتين مقارنة بالكنترول والمعاملات الأخرى. أوضحت النتائج أن أفضل تجذير لأصول الدراسة عند زراعة النبتات الصغيرة على بيئة موراشيجي وسكوج مضافاً إليها كل من نفثالين حمض الخليك مع اندول حمض الخليك بتركيز ١,٠ مليجرام / لتر. سجل أصلي الفولكا واليوسفي كليوباترا أفضل النتائج مقارنة بالأصول الأخرى.

الكلمات الاسترشادية: الإكثار الدقيق، الموالح، الإنبات، منظمات النمو النباتية، الأصول.