

EFFECT OF MEDIA CULTURES AND BENZYLADENINE (BA) CONCENTRATIONS IN MICROPROPAGATION OF THREE TYPES OF CHERRY ROOTSTOCK (ESTABLISHMENT AND MULTIPLICATION)

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ABSTRACT

This experiment was carried out in plant tissue culture laboratory of the Horticulture Department, Faculty of Agriculture, University of Duhok, Iraq, during 2014, using two types of explants (Shoot tips and nod segments) in the establishment stage. The highest percentage of shoot /explant (100%) was obtained from Stockton Morello rootstock on both media, while 96.88% was recorded with WPM (Woody plant medium) from nodal segment in F12/1 Mazzard and (87.5%) was observed from shoot tip in Mahaleb. While the highest shoot number with three rootstocks (24.50, 24.38 and 22.25 shoot /explant) with Stockton Morello, F12/1 Mazzard and Mahaleb respectively, were recorded from nodal segment with WPM. While the lowest number of shoots (19.63 shoots /explant) was formed when the shoot tips were cultured on MS medium. At the multiplication stage, the maximum number of shoot per explant (9.50 and 9.17 shoots /explant) in Stockton Morello rootstock was recorded with WPM and MS medium provided with 3mg l⁻¹ BA. The interaction of Stockton Morello with WPM and MS medium having 1.5 mg l⁻¹ BA produced an average of shoot length (3.58 and 3.52 cm) and the increased of shoot length was significantly compared with all treatment without the treatment in combination of Stockton Morello rootstock in WPM medium supplemented with 3 mg l⁻¹ BA. Also the highest length of shoot per explant with three rootstocks 3.58 and 2.98 cm in Stockton Morello and F12/1 Mazzard were recorded in WPM having 1.5mg l⁻¹ BA and in Mahaleb rootstock (2.92 cm) was recorded on MS medium containing 1.5mg l⁻¹ BA.

Keywords: Micropropagation, Cherries Rootstock, Culture Medium Composition and BA Concentrations.

باني وآخرون

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تأثير الوسط الغذائي وتراكيز البنزلادين (BA) في مرحلة النشوء لثلاثة أنواع من اصول الكرز في مرحلتين النشوء والتضاعف

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المستخلص

اجريت هذه الدراسة في مختبر زراعة الانسجة التابع لقسم البستنة، جامعة دهوك، اقليم كردستان العراق، خلال 2014، استعمل نوعين من الاجزاء النباتية (القلم النامية والعقد المفردة) في مرحلة النشوء. إنشاء نباتات جديدة في المختبر من اصول الناضجة لـ (Stockton Morello، F12/1Mazzard، Mahaleb)، حيث ان مصدر الجزء النباتي يلعب دورا أساسيا في كلا المرحلتين النشوء والتضاعف على دراسة النباتات في المختبر باستخدام اوساط غذائية مختلفة. تم الحصول على افضل النتائج في مرحلة النشوء وكانت أعلى نسبة من الافرع / جزء النباتي 100% من اصل Stockton Morello. بينما 96.88% تم تسجيلها من العقد المفردة لاصل F12 / 1 Mazzard المزروعة على وسط WPM و87.5% لوحظ من القلم النامية لاصل Mahaleb. المزروعة على نفس الوسط الغذائي. في حين أن أعلى عدد من الافرع لثلاثة اصول الكرز 24.50، 24.38 و 22.25 فرع / جزء نباتي Stockton Morello، F12 / 1 Mazzard و Mahaleb على التوالي والتي سجلت، من العقد المفردة المزروعة على وسط WPM. في حين شكلت أقل عدد من الافرع 19.63 فرع / جزء نباتي من القلم النامية المزروعة على وسط MS. في مرحلة التضاعف، تم تسجيل أكبر عدد ممكن من الافرع / جزء نباتي 9.50 و 9.17 فرع / جزء نباتي من اصل Stockton Morello المزروعة على وسط MS المحتوي بـ 3 ملغم/ لتر BA. التداخل بين Stockton Morello و الاوساط الغذائية WPM و MS المزود بـ 1.5 ملغم / لتر BA أنتجت معدل طول الفرع 3.58 و 3.52 سم وكانت الزيادة معنوية مقارنة بجميع المعاملات ما عدا المعاملة التي احتوت تداخل بين كل من اصل Stockton Morello المزروعة على وسط WPM المزود بـ 3ملغم/لتر BA. بلغ اعلى معدل لطول الافرع 3.58 و 2.98 سم لاصل Stockton Morello و F12 / 1 Mazzard والتي سجلت على وسط WPM المزود بـ 1.5 ملغم / لتر BA و اصل Mahaleb سجلت طول للافرع 2.92 سم على وسط MS المجهز بـ 1.5 ملغمتر .BA

الكلمات المفتاحية: الاكثار الدقيق، اصول الكرز، تركيب الوسط الغذائي و تراكيز الـ BA .

INTRODUCTION

There are many rootstocks used for cherry production Yousif,(33). However, it has many disadvantages. Rootstocks trees (Stockton Morello.F12/1 Mazzard. Mahaleb), do not grow satisfactorily in heavy, wet soils, weak compatibility between rootstocks and cultivars, sensitive to nematodes and difficult propagation by traditional methods Hammatt and Grant (12).The rootstocks Stockton Morello, F12/1 mazzard and Mahaleb are most commonly used for sweet and sour cherries production and are effective in size-controlling; growing satisfactorily in heavy and wet soils, early and good production. Cherry rootstocks are traditionally propagated either by relatively slow and labor-intensive vegetative methods (cuttings and suckers), or from seeds which need long time for stratifications and often results in non-uniform material. The application of plant tissue culture for vegetative propagation of temperate fruit rootstocks started in the mid-70s, and a considerable number of improved protocols were developed ever since. Generally, the goals of micro-propagation are obtaining rapid, large-scale and low-cost production of genetically identical, physiologically uniform and pathogen-free plant Rathore *et al.*,(24).Successful *in vitro* clonal propagation methods were reported in many rootstocks, including cherry (29, 26, 31). The micropropagation is used as a useful method for propagation of vegetative rootstock, (3). There is no universal medium for *in vitro* culture, since plant species and cultivars are genetically specific with regard to different components of medium, which include not organic substances, but also contained mineral elements (10). Erbenova *et al.*, (9),reported a 50% increase of multiplication rate on the dwarf rootstocks of sweet cherries cultured on MS medium containing 1.5 mg l^{-1} BAP. Ruzic *et al.*, (27) reported that the MS and MS (double macro-salts) media culture containing $4.4 \mu\text{M}$ BA, $0.5 \mu\text{M}$ NAA and $0.3 \text{ m}\mu$ GA3 are

a suitable culture media for propagation of cherry. In a study on micropropagation of *Prunusavium*, the combination of 0.5 mg l^{-1} BAP and 0.05 mg l^{-1} TDZ was suitable for multiplication and a culture media having 0.3 mg l^{-1} IBA is desirable for the rooting (7). Carolina *et al.*,(4), cultured nodal segments of *Prunusserotina* in MS medium culture supplemented with $4.4 \mu\text{M}$ BA, $0.49 \mu\text{M}$ IBA and $0.29 \text{ m}\mu$ GA3. The aim of this study is to investigate the effects of different culture media and plant growth regulator on micropropagation of three types of cherry rootstocks in depending on their role to size growth controlling of cherry trees and difficulty propagation by common methods.

MATERIALS AND METHODS

Three contemporary cherry rootstocks, including 1-Stockton Morello. 2-F12/1 Mazzard. 3-Mahaleb, were collected from the Agriculture college field. The present work was carried out in plant tissue culture laboratory of the Horticulture Department, Faculty of Agriculture, University of Duhok, Iraq, during the period from March to September 2014. Initial cultures of all rootstocks were established using actively growing shoot from collage field. Shoot were packed in plastic and were transferred to the laboratory. First of all, the explants were washed by tap water and dishwashing liquid to remove surface contamination. Then they were divided into some parts containing more than one node and were pre-sterilized by immersion in 70% ethanol for 30 sec. Followed by a rinse in sterile distilled water, and 5 min soaking in 0.1% (W/V) of HgCl_2 mercuric chloride, followed by triple rinsing with sterile distilled water (1-1.5cm) were isolated and onto WPM (16) and MS medium Murashige and Skoog (20) containing 2 mg l^{-1} BA for 4 weeks. After all, they were transferred to the different culture media for multiplications stage. Upon establishing of aseptic culture, uniform single shoots were multiplied on MS and WPM media of constant hormonal composition. Shoot multiplication of cherry rootstock was monitored on the medium supplemented with different concentrations of BA (1, 1.5 and 3 mg l^{-1}) + WPM and MS medium. All

multiplication media contained 30g l⁻¹ sucrose and 8g l⁻¹ agar. The pH value was adjusted to 5.7± 0.1 before autoclaving at 121 °C, 150 KPa for 20 min. Establishment and multiplication parameters i.e. Shoot percentage, Number of shoots, Number of leaves and Shoot length (cm). Shoot cultures were grown in 250 ml culture vessels containing 25ml of establishment and multiplication media at 23 ±1 °C and 16 hour photoperiods (Light intensity, 1000 Lux.). On culture surface provided with cool white fluorescent tubes 40W, 6, 500 °K and temperature was 23±1°C. The experiments were set up in a completely randomized design (CRD) three times. Each treatment included 8 replicates (with 4 explants in each 250cm³ jar). The data were analyzed with computer using SAS program SAS (28). The differences between various treatment means were tested with Duncan Multiple Range test at 5% level Duncan, (8).

RESULTS AND DISCUSSION:

Establishment stage parameters:

Effect of various culture media on three types of cherry rootstock (1-Stockton Morello. 2-F12/1 Mazzard. 3-Mahaleb) from two types of explant

1.Percentage of Shoot / explant:

The establishment of aseptic culture is the first critical step in *in vitro* propagation process. There are many reports on successful application of mercuric chloride at 0.1% at 5 min for surface sterilized of initial explants Mansseri-Lamrioui *et al.*, (18) and Hosseini *et al.*, (13). It is notable on Table 1 Fig 1 that the rootstocks were significantly various in their response on the percentages of shoot development and the highest percentage of shoot was from the Stockton Morello 100% as compared with F12/1 Mazzard and Mahaleb rootstock. However the mean value of different explants recorded and the highest percentage of shoots 93.23 % from nodal segments compared with shoot tip 92.19% but the increases were not significant. While the mean value of different media gave the highest percentage 93.75% which observed with WPM as compared with MS medium which gives 91.67%. Concerning the combination between rootstocks and media, the highest percentage

of shoots 100% was obtained from Stockton Morello rootstock in WPM and MS media. While the lowest percentage was formed in Mahaleb rootstock 85.94% in WPM and MS media. However, regarding the interaction between rootstock and explants, the nodal segments and shoot tip from Stockton Morello rootstock gave the highest shoot percent 100% compared with other two rootstocks and the lowest shoot percentage 84.38% was produced from nodal segments of Mahaleb rootstock. Interaction between media and explants showed that the explants in the WPM medium produced high percentage of 93.75% from nodal segments and shoot tip. While the lowest percentage of shoots 90.63% was produced from nodal segments when cultured on MS medium. The results of the three combinations (rootstocks, culture media and explants) show that the combination of nodal explants and shoot tips from Stockton Morello rootstock with all media WPM and MS medium produced 100% as compared with all treatments but the shoot percentage was not increased significantly. The highest percentage of shoot /explant 100% was obtained from Stockton Morello rootstock on both media. While 96.88% was recorded with WPM from nodal segment in F12/1 Mazzard and 87.5% was observed from shoot tip in Mahaleb. While the lowest shoot percentage 84.38% was obtained from nodal segment in Mahaleb rootstock. The findings of the present study are in conformity with those many others where BA performed better than Kinetin, BA has the advantage over other Cytokinin in inducing *in vitro* shoot production in woody plant like Jak fruit (*Artocarpus heterophyllus*), Amin and Jaiswal (1). Erbenova *et al.*, (9), in their study on the dwarf cherry rootstock found out that the concentration of 1.5 mg l⁻¹ will have very effective results. Moreover, Roozban *et al.*, (25) in their study on 9 kinds of pear observed that the concentration 2mg l⁻¹ BAP was the best treatment.

Table 1. Effect of different types of explants culture on different type of media on shoot percentage of three cherry rootstocks after 4 weeks.

| Type of rootstocks | Type of media | Type of Explant | | Rootstock X Media | Mean of Rootstock |
|---------------------|---------------|-----------------|-----------|-------------------|-------------------|
| | | Node segment | Shoot tip | | |
| Stockton Morello | WPM | 100 a | 100 a | 100 a | 100 a |
| | MS | 100 a | 100 a | 100 a | |
| F12/1 Mazzard | WPM | 96.88 a | 93.75 a | 95.31 b | 92.19 b |
| | MS | 87.5 a | 90.63 a | 89.06 b | |
| Mahaleb | WPM | 84.38 a | 87.5 a | 85.94 b | 85.94 b |
| | MS | 84.38 a | 87.5 a | 85.94 b | |
| Rootstock X Explant | Stockton M. | 100 a | 100 a | Mean of Media | |
| | F12/1Mazzard | 92.19 ab | 92.19 ab | | |
| | Mahaleb | 84.38 b | 87.5 b | | |
| Media X Explant | WPM | 93.75 a | 93.75 a | 93.75 a | |
| | MS | 90.63 a | 92.71 a | 91.67 a | |
| Mean of explants | | 93.23 a | 92.19 a | | |

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test Duncan (8)

2. Number of Shoot /explant:

Table (2) Fig (1) showed that in all rootstocks, different media and different explants an increase in the shoot number/explant was found. The mean value of different rootstocks was not significantly affected. The highest number of shoot/explant 23.63 was obtained from Stockton Morello rootstock as compared with both other rootstocks 22.69 and 20.88 number of shoot/explant from F12/1 mazzard and Mahaleb, respectively. On the other hand, the mean value of explants show that the highest number of shoot/explant formed from nodal segment 23.23 compared with shoot tip which given 21.56 shoot/explant. However the mean value of media recorded the highest number of shoot on WPM compared with MS medium 22.85 and 21.94 shoot/explant respectively, but the increases of shoot was not significant. The results of the interaction between rootstocks and media was not significant, were the highest number of shoots in Stockton Morello and F12/1 Mazzard 23.88 and 23.50 shoots /explant were obtained on WPM respectively. While the lowest number of shoots was formed in Mahaleb when the explants were cultured the explants on MS medium. Regarding the interaction between rootstocks and explants, the nodal explant from Stockton Morello and F12/1 Mazzard rootstock produced the highest number of shoot 24.19 and 23.63 shoots/explant. While the lowest shoots number 19.88 shoot/explant

was obtained from Mahaleb's shoot tip. In case of the interaction between different media and different explants, the nodal segments culture on WPM produced the highest shoot number 23.71 shoots/explant compared with all treatments but the increase was non-significant. Concerning the combination between three factors, the results revealed that the shoots could be observed with used WPM containing fixed concentration of BA 2 mg l^{-1} . The highest shoot number with three rootstocks 24.50, 24.38 and 22.25 shoot/explant with Stockton Morello, F12/1 Mazzard and Mahaleb respectively, were recorded from nodal segment with WPM. While the lowest number of shoots 19.63 shoots/explant was formed when the shoot tips were cultured on MS medium. On the basis of the obtained results it may be concluded that there are differences in uptake of cytokinins, recognition by the cell, or mechanisms of action of the cytokinin compound. However, cytokinins in our experiment can be divided into two groups: very active group only BA, which was more effective, i.e. more shoots of cherry cv. Lapins formed, whereas TDZ, 2ip and kin exhibited rather weak effects on multiplication. These results agree with results obtained by Kadota and Niimi, (15) with pear (*Pyrus pyrifolia*), they suggest that BAP displayed more noticeable effect than TDZ and kintin, i.e. BA is more suitable for shoot multiplication of pear. It is well known that high concentration of cytokine of adenine type is often necessary for growth and differentiation of tissue culture.

Table 2. Effect of different types of explants cultured on different types of media on shoot number of three cherry rootstocks after 4 weeks.

| Type of rootstocks | Type of media | Type of Explant | | Rootstock X Media | Mean of Rootstock |
|---------------------|------------------|-----------------|-----------|-------------------|-------------------|
| | | Node seg | Shoot tip | | |
| Stockton Morello | WPM | 24.50 a | 23.25 a | 23.88 a | 23.63 a |
| | MS | 23.88 a | 22.88 a | 23.38 a | |
| F12/1 Mazzard | WPM | 24.38 a | 22.63 a | 23.50 a | 22.69 a |
| | MS | 22.88 a | 20.88 a | 21.88 a | |
| Mahaleb | WPM | 22.25 a | 20.13 a | 21.19 a | 20.88 a |
| | MS | 21.50 a | 19.63 a | 20.56 a | |
| Rootstock X Explant | Stockton Morello | 24.19 a | 23.06 a | Mean of Media | |
| | F12/1 Mazzard | 23.63 a | 21.75 a | | |
| | Mahaleb | 21.88 a | 19.88 a | | |
| Media X Explant | WPM | 23.71 a | 22.00 a | 22.85 a | |
| | MS | 22.75 a | 21.13 a | 21.94 a | |
| Mean of explant | | 23.23 a | 21.56 a | | |

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test Duncan (8)

3. Number of leaves / explant:

Table 3 Fig 1 showed the effect of different media on different explants of three types of rootstocks of cherry and this table showed that in all rootstocks, different culture media and different type of explants (shoot tip and node segment) resulted in an increases in the number of leaves per explant. The mean value of rootstocks show that the highest number of leaves 28.34 was observed from Stockton Morello rootstock and 26.94 was found from F12/1 Mazzard rootstock and it was significantly increases as compared with the Mahaleb rootstock which gave 24.75 leaves/explant. On the other hand, the mean value of the explant, the highest leaves number 27.08 leaves /explant was obtained from nodal segment compared with shoot tip 26.27 leaves /explant, but the increased was not significantly. However, the mean value of media show the large number of leaves 27.31 leaves /explant was observed on WPM compared with MS medium. Concerning the interaction between rootstocks and media, the highest leaves number per explant 28.88 from Stockton Morello rootstock when the explant cultured on WPM and the increased was significantly as compared with Mahaleb rootstock which gave 26.06 and 24.44 leaves /explant when explants were cultured on WPM and MS medium respectively, but non significantly with F12/1 Mazzard when cultured on WPM and MS media 28.00 and 25.88 leaves /explant respectively. While the combination between rootstocks and explant,

the highest number of leaves per explant 28.81 was found on nodal segments of Stockton Morello and it was significantly increased with some treatments. While the lowest leaves 24.50 was observed from nodal segments of Mahaleb. In case of interaction between media and explant, the nodal segment cultured on WPM medium produced an average of 28.21 leaves /explant and shoot tip on WPM medium produced 26.42 leaves /explant. While the lowest leaves number 25.96 leaves /explant was obtained from nodal segments when cultured on MS medium.

The interaction between three factors revealed that the more leaves could be obtained with WPM and MS media supplemented with BA at 2 mg l⁻¹. The highest number of leaves per explant with three rootstocks 29.88, 29.50 and 25.25 leaves /explant was obtained from nodal segment of Stockton Morello, F12/1 Mazzard and Mahaleb respectively when the nodal explant cultured on WPM. On the other hand, the lowest number of leaves was formed on Mahaleb rootstock 23.75 leaves /explant when shoot tip cultured on MS medium. While the highest number of leaves of Stockton Morello and F12/1 Mazzard 27.88 and 26.38 leaves /explant respectively were recorded in MS medium when shoot tip and nodal segment were cultured on MS medium respectively. Reducing salt concentration in MS medium resulted in poor performance with regard to percent establishment, shoots number, leaves number and shoots length as compared with WPM. Some plant gave similar response with all media, while other show preference for specific media for explants McCown and Sellmer (19).

Table 3. Effect of different type of explant cultured on different type of media on leaves number of three cherry rootstocks after 4 weeks.

| Type of rootstocks | Type of media | Type of Explant | | Rootstock X Media | Mean of Rootstock |
|---------------------|------------------|-----------------|-----------|-------------------|-------------------|
| | | Node seg | Shoot tip | | |
| Stockton Morello | WPM | 29.88 a | 27.88 abc | 28.88 a | 28.34 a |
| | MS | 27.75 abc | 27.88 abc | 27.81 ab | |
| F12/1 Mazzard | WPM | 29.50 ab | 26.50 abc | 28.00 ab | 26.94 a |
| | MS | 26.38 abc | 25.38 abc | 25.88 abc | |
| Mahaleb | WPM | 25.25 abc | 24.88 bc | 25.06 bc | 24.75 b |
| | MS | 23.75 c | 25.13 abc | 24.44 c | |
| Rootstock X Explant | Stockton Morello | 28.81 a | 27.88 ab | Mean of Media | |
| | F12/1 Mazzard | 27.94 ab | 25.94 abc | | |
| | Mahaleb | 24.50 c | 25.00 bc | | |
| Media X Explant | WPM | 28.21 a | 26.42 a | 27.31 a | |
| | MS | 25.96 a | 26.13 a | 26.04 a | |
| Mean of explant | | 27.08 a | 26.27 a | | |

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test Duncan (8).



4. Shoot length:

Data in Table 4 revealed that the length of shoot formed on three rootstocks of cherry. The mean value of the rootstocks show that the longest shoot 2.61 cm was recorded on Stockton Morello rootstock and gave significantly longer shoot than F12/1 Mazzard and Mahaleb rootstocks 1.97 and 1.66 cm respectively. While the mean value of explants, the longest of shoot formed from shoot tips 2.21 cm compared with the nodal segments which gave 1.95 cm. However the mean value of the different media, the high length of shoot 2.10 cm was observed on WPM than MS medium which gave 2.06 cm. Regarding the combination between rootstocks and media, the explant in the combination of WPM and MS medium produced the high length of 2.59 and 2.63 cm respectively, from the Stockton Morello rootstocks, and which gave the significantly longer shoot than F12/1 Mazzard and Mahaleb 2.00 and 1.78 cm on

MS medium and WPM respectively. However, the interaction between rootstock and explant, the longest shoot/explant 2.74 and 2.47 cm were obtained from Stockton Morello rootstock when shoot tip and nodal segment cultured on WPM medium. While the lowest length of shoot was found with Mahaleb rootstock from nodal segments. Concerning the combination between the rootstocks and explants, the nodal segment and shoot tip from Stockton Morello rootstock gave significant longer shootlet than the F12/1 Mazzard and Mahaleb rootstock on both two explants. In the case of interaction between media and explants, revealed that the length of shootlets on the media WPM and MS medium, shoot tip was inversely related to culture media at fixed concentration of BA 2 mg l^{-1} . The longest shoot 2.30 and 2.12 cm was recorded with WPM and MS medium from shoot tips respectively. While the lowest length of shootlet was formed 1.90 cm from nodal segments with WPM medium. The results of three combination (different type of rootstocks, different media and different explants), revealed that the more shoot length could be found with WPM and MS medium enriched with fixed concentration of BA at 2 mg l^{-1} . The combination of Stockton Morello and F12/1 Mazzard with WPM medium produced an average of 2.81 and 2.24 cm was obtained from shoot tips compared with 2.68 and 2.08 cm was found from shoot tips in MS medium respectively. While the high length of shoot in three rootstocks 2.68, 2.08 and 1.85 cm in Stockton Morello, F12/1 Mazzard and Mahaleb respectively, were recorded with WPM medium when used shoot tip compared with nodal segment on WPM and MS medium.

While the lowest length of shoot or shortest of shoot were found on Mahaleb nodal explant 1.49 cm in MS medium containing fixed concentration of BA 2 mg l⁻¹ and the shortest of shoot were observed on Stockton Morello and F12/1 Mazzard 2.58 and 1.65 cm in MS and WPM medium respectively, when used nodal explant for culture in the different

media. In general decrease in shoot length in all rootstocks of cherries with different media containing fixed concentration of AB 2mg l⁻¹ is in agreement with the finding of Hamad and Taha (11) also reported that the subculture improved shoot elongation at short-lasting incubation 30 or 45 day.

Table 4. Effect of different type of explants cultured on different types of media on shoot length of three cherry rootstocks rootstocks after 4 weeks.

| Type of rootstocks | Type of media | Type of Explant | | Rootstock X Media | Mean of Rootstock |
|---------------------|------------------|-----------------|-----------|-------------------|-------------------|
| | | Node seg | Shoot tip | | |
| Stockton Morello | WPM | 2.36 abc | 2.81 a | 2.59 a | 2.61 a |
| | MS | 2.58 ab | 2.68 a | 2.63 a | |
| F12/1 Mazzard | WPM | 1.65 cd | 2.24 a-d | 1.94 b | 1.97 b |
| | MS | 1.93 bcd | 2.08 a-d | 2.00 b | |
| Mahaleb | WPM | 1.70 cd | 1.85 bcd | 1.78 b | 1.66 b |
| | MS | 1.49 d | 1.61 cd | 1.55 b | |
| Rootstock X Explant | Stockton Morello | 2.47 ab | 2.74 a | Mean of Media | |
| | F12/1 Mazzard | 1.79 cd | 2.16 bc | | |
| | Mahaleb | 1.59 d | 1.73 cd | | |
| Media X Explant | WPM | 1.90 a | 2.30 a | 2.10 a | |
| | MS | 2.00 a | 2.12 a | 2.06 a | |
| Mean of explant | | 1.95 a | 2.21 a | | |

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test Duncan (8).

In contrast, Norton and Norton (22) recorded a decrease in shoot length and leaf size after several subcultures, which indicate that besides being affected by external factors, growth is highly influenced by genotype. Also the result obtain with *L. Corniculatus* shoots seedling showed that the even in the lowest concentration 0.08 and 0.22 μ M cytokinin retarded elongation (Nikolić *et al.*, (21). While cytokinin Kin mainly influenced shoot growth of cherry cv. Lapins, whereas it made little impact on multiplication. Some species, such as *Tabernaemontana fuschsia efolia* L. (Apocynaceae), exhibited higher multiplication rate on media with Kin that BAP De Oliveira *et al.*, (6).

Multiplication parameters:

Effect of media and BA on cherry rootstock

1.Number of Shoot/ explant:

Table 5 showed that WPM was superior on increased the number of leaves and shoots length. Data in Table 5 showed that in all rootstocks, different culture media and different concentration of BA resulted in an increased the shoots number but it non-significantly only in some treatments The

mean value of differ rootstocks, the highest shoot number 7.17 shoots/explant was found from Stockton Morello compared with F12/1 Mazzard and Mahaleb which gave 6.47 and 6.17 shoots/explant respectively. While the mean value of concentrations show on the same table that the 3mg l⁻¹ BA added to the medium gave (8.58 shoots/explant) and it was significantly compared with treatment when used 1mg l⁻¹ BA which gave 3.61 shoots/explant. However, the mean value of different culture media show the highest number of shoots per explant 6.80 shoots/explant was found on WPM. This might be due to the fact that the requisite concentration of BA differs greatly according to the culture features of plant George (10). On the other hand, the combination between rootstocks and media, the maximum number of shoots 7.28 shoots /explants from Stockton Morello rootstock when culture on WPM medium but the increase was not significantly with all treatments. While the lowest number of shoot appear from Mahaleb rootstock 5.83 shoots/explant when used MS medium. Whereas, the combination between rootstocks and different concentrations of BA, the larger shoots number was recorded from Stockton Morello rootstock 9.33 shoots/explant 3mg l⁻¹ of BA. While the minimum shoot number 3.42

shoots/ explant was observed from Mahaleb rootstock when the explant cultured on medium containing 1mg l^{-1} BA. On the other hand, the combination between different culture media and concentrations of BA, the maximum shoots number 9.00 shoots/ explant was found on WPM medium having 3mg l^{-1} BA and the increased was significantly than the treatments used WPM and MS medium supplemented 1mg l^{-1} BA. While the minimum shoots number 3.50 shoots/ explant was obtained on MS medium containing 1mg l^{-1} BA. The consequence of the three combination (different type of rootstocks, different type of culture media and different concentrations of BA), revealed that the more shoots could be observed with the used of WPM and MS medium supplemented with a higher BA concentration. The maximum number of shoot per explant 9.50 and 9.17 shoots/explant in Stockton Morello rootstock was recorded with WPM and MS medium provided with 3mg l^{-1} BA. However, the minimum number of shoots formed from Stockton Morrello 3.83 shoots/explant, when the explants cultured on MS medium supplemented with 1mg l^{-1} BA. Whereas, the higher number of shoots from F12/1 Mazzard and Mahaleb 8.67 and 8.83

shoots/ explant were observed on WPM containing 3mg l^{-1} BA respectively. But the lowest number of shoots 3.17 shoots/ explant was formed from F12/1 Mazzard when the explant cultured on MS medium having 1mg l^{-1} BA. From the published reported on various systems, it is clear that the cytokinin is essential for multiple shoots induction from explant. BA-induced multiple shoot induction has been reported in cherry rootstock (PHL-A) (17). These results also showed that the medium with of cytokinin like BAP in the proliferation stage is necessary. Arab *et al.*, (2) reported *in vitro* multiplication of G × N15 (hybrid of almond × peach) vegetative rootstock, showed (7.37 ± 0.35) 1mg l^{-1} BAP is the most effective treatment. Besides, Sulusoglu and Cavusoglu, (30) stated that the effective BAP concentration for Mahaleb rootstocks (K-KKI and S-AB), F12/1 Mazzard and SL-64 are 1mg l^{-1} . Increase of BA concentration in medium MS + 0.5mg l^{-1} BA compared with MS + 1mg l^{-1} BA resulted in increase of the shoot number and decrease of the shoot length on cherry rootstock Pruski *et al.*, (23). These findings are confirmed our results.

Table 5. Effect of different type of rootstocks, different type of media and different concentrations of BA on number of shoot of cherry rootstock after 8 weeks.

| Type of rootstocks | Type of media | BA concentrations | | | Rootstock X Media | Mean of Rootstock |
|--------------------|------------------|-------------------|---------|---------|-------------------|-------------------|
| | | 0 | 1.5 | 3 | | |
| Stockton Morello | WPM | 4.17 b | 8.17 a | 9.50 a | 7.28 a | 7.17 a |
| | MS | 3.83 b | 8.17 a | 9.17 a | 7.06 a | |
| F12/1 Mazzard | WPM | 3.67 b | 7.50 a | 8.67 a | 6.61 a | 6.47 a |
| | MS | 3.17 b | 7.33 a | 8.50 a | 6.33 a | |
| Mahaleb | WPM | 3.33 b | 7.33 a | 8.83 a | 6.50 a | 6.17 a |
| | MS | 3.50 b | 7.17 a | 6.83 a | 5.83 a | |
| Rootstock X Conce. | Stockton Morello | 4.00 c | 8.17 ab | 9.33 a | Mean of Media | |
| | F12/1 Mazzard | 3.42 c | 7.42 ab | 8.58 ab | | |
| | Mahaleb | 3.42 c | 7.25 b | 7.83 ab | | |
| Media X Conce. | WPM | 3.72 b | 7.67 a | 9.00 a | 6.80 a | |
| | MS | 3.50 b | 7.56 a | 8.17 a | 6.41 a | |
| Mean of Conce. | | 3.61 b | 7.61 a | 8.58 a | | |

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test Duncan (8).

2. Number of leaves

Table 6 shows that the WPM is better than MS medium, The highest number of leaves 15.28 leaves/ explant was found on Stockton Morello rootstock, which was not significantly than the F12/1 Mazzard and Mahaleb rootstocks 14.36

and 13.89 leaves/ explant respectively. While the mean value of different concentrations of BA, the maximum leaves number per explant 15.94 leaves/ explant in 3mg l^{-1} BA and it was significantly increased than the 1 and 1.5mg l^{-1} BA. However, the mean value of different culture media, the highest number of leaves 14.57 leaves/explant was observed on WPM compared with MS medium which gave 14.44 leaves/ explant, but the increase was non-

significant. In the case of the interaction between rootstocks and media, the explants from Stockton Morello rootstock cultured on MS medium produced the highest number of leaves 15.44 leaves/ explant. In addition, the interaction displayed that the greatest number of leaves on WPM medium and F12/1 Mazzard which gave 14.72 leaves/ explant. While the lowest number of leaves per explant 13.89 leaves/ explant was obtained from Mahaleb rootstock when cultured on WPM medium. Nevertheless, the interaction between rootstocks and BA, show that the highest number of leaves/explant 17.58 and 16.08

leaves/ explant were found on Stockton Morello and F12/1 Mazzard rootstocks when the explant cultured on medium having 3mg l^{-1} BA and it was significantly increases compared with all treatments excepted the treatment containing 1.5mg l^{-1} when Stockton Morello rootstock on culture medium. While the lowest number of leaves per explant 13.50 leaves/ explant were observed from three rootstocks (Stockton Morello, F12/1 Mazzard and Mahaleb) when cultured on medium containing 1mg l^{-1} BA and F12/1 Mazzard when cultured on medium having 1.5mg l^{-1} BA.

Table 6. Effect of different type of rootstocks, different type of media and different concentrations of BA on number of leaves of cherry rootstock after 8 weeks.

| Type of rootstocks | Type of media | BA concentrations | | | Rootstock X Media | Mean of Rootstock |
|--------------------|------------------|-------------------|----------|----------|-------------------|-------------------|
| | | 0 | 1.5 | 3 | | |
| Stockton Morello | WPM | 13.83 a | 13.67 a | 17.83 a | 15.11 a | 15.28 a |
| | MS | 13.17 a | 15.83 a | 17.33 a | 15.44 a | |
| F12/1 Mazzard | WPM | 13.33 a | 13.33 a | 17.50 a | 14.72 a | 14.36 a |
| | MS | 13.67 a | 13.67 a | 14.67 a | 14.00 a | |
| Mahaleb | WPM | 13.17 a | 13.33 a | 15.17 a | 13.89 a | 13.89 a |
| | MS | 13.83 a | 14.67 a | 13.17 a | 13.89 a | |
| Rootstock X Conce. | Stockton Morello | 13.50 b | 14.75 ab | 17.58 a | Mean of Media | |
| | F12/1 Mazzard | 13.50 b | 13.50 b | 16.08 ab | | |
| | Mahaleb | 13.50 b | 14.00 b | 14.17 b | | |
| Media X Conce. | WPM | 13.44 b | 13.44 b | 16.83 a | 14.57 a | |
| | MS | 13.56 b | 14.72 ab | 15.06 ab | 14.44 a | |
| Mean of Conce. | | 13.50 b | 14.08 b | 15.94 a | | |

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test Duncan, (8).

Whereas, the interaction between the culture media and different concentration of BA, the highest number of leaves per explants in both media 16.83 and 15.06 leaves/ explant were observed on WPM and MS medium having 3mg l^{-1} BA respectively. While the lowest number of leaves shows on WPM containing 1 and 1.5mg l^{-1} BA 13.44 leaves/explant. The interaction three factors revealed that the major leaves could be observed on WPM than MS medium supplemented with high concentration of BA (Table 6 and Figure 2, 3). The greatest number of leaves in three rootstocks 17.83, 17.50 and 15.17 leaves/explant in the Stockton Morello, F12/1 Mazzard and Mahaleb rootstocks respectively, were recorded with WPM medium having 3mg l^{-1} BA. As well as, the lowest number of leaves per explant were formed on Stockton morello explants 13.17 leaves/explant in MS medium

having 1mg l^{-1} BA and formed on Mahaleb explants 13.17 leaves/ explant, was obtained on WPM and MS medium containing 1 and 3mg l^{-1} BA. While the highest number of leaves per explant formed on Stockton Morello rootstock 17.33 leaves/explant in MS medium having 3mg l^{-1} BA and highest leaves number of leaves per explant formed on F12/1 Mazzard 14.67 leaves/explant in MS medium supplemented with 3mg l^{-1} BA. Whereas, a promotive effect at low BAP concentration has also been described in the literal are for explant of different citrus genotype Costaet al., (5). Similar results were reported by Mahdavian *et al.*, (17) who obtained the highest leaves number per explant by culturing the explants of cherry rootstocks on MS and DKW media which containing 0.5 and 1mg l^{-1} BA.

3.Shoot length :Data in table (7) revealed that the longest of shootlet formed on three rootstocks explants was inversely related to BA concentrations and different culture media. The mean value of different type of rootstocks,

the lowest of shootlet 2.94 cm was recorded on Stockton Morello rootstock and it was increased significant than the two other rootstocks 2.58 and 2.31 cm were recorded on F12/1 Mazzard and Mahaleb rootstocks respectively, and the F12/1 Mazzard increased significantly than the Mahaleb rootstock. As well as, the lowest shoot length 2.31 cm was recorded on Mahaleb rootstock. Whereas, the mean value of different concentration of BA, show in the same table, the longest of shootlet 3.04 cm was found on medium having 1.5mg l⁻¹ BA compared with the treatment contained (1 and 3 mg l⁻¹ BA) were recorded lowest shoot length 2.20 and 2.60 cm respectively and the increase was significant between treatments. Also the treatment which contained 3mg l⁻¹ BA shows significantly increases on the shoot length than the treatment having 1mg l⁻¹BA. While the mean value of different type of culture media, the maximum shoot length 2.62 cm was recorded on MS medium that the WPM medium which gave 2.60 cm, but the increased was not significant. Concerning the interaction between the different type of rootstocks and different culture media, the longest shoot 3.02 and 2.87 cm was found from the Stockton Morrelo rootstock when the explants cultured on WPM and MS medium respectively. Whereas, the lowest shoot length was formed in Mahaleb rootstock 2.22 cm on WPM and the increased of the shoot length was significant between three rootstocks, where in the length of Stockton Morello

rootstock increased significantly when cultured on both media than the F12/1 Mazzard and Mahaleb rootstock. While the longest of shoot on F12/1 Mazzard and Mahaleb 2.59 and 2.40 cm was formed from shoot on MS medium respectively, but the increased of shootlet was not significantly. However, the combination between rootstocks and different concentrations of BA, show that the longest of shootlets produced in Stockton Morello 3.55 cm when the explants were cultured on medium having 1.5mg l⁻¹ BA and the increased was significantly than all treatments, while the lowest shoot length were produced from Mahaleb and F12/1 Mazzard 2.16, 2.18 and 2.19 cm when the explants cultured on media contained 1 and 3 mg l⁻¹ BA respectively. Where the combination of BA show that the explants in combination of MS medium with 1.5mg l⁻¹ BA produced on an average length of 3.13 cm, and explants in WPM medium having 1.5 mg l⁻¹ BA produced an average length of 2.94 cm and the shortest 2.18 cm were produced when the explants culture on MS medium contained 1 mg l⁻¹ BA. In case of the combination of three factors (different type of rootstocks, different type of media and different concentrations of BA), revealed that more shoot length could be found with the use of WPM and MS medium enriched with BA concentration (Figures 2, 3) (Table 7).

Table 7. Effect of different type of rootstocks, different type of media and different concentrations of BA on shoots length of cherry root stock after 8 weeks.

| Type of rootstocks | Type of media | BA concentrations | | | Rootstock X Media | Mean of Rootstock |
|--------------------|------------------|-------------------|----------|----------|-------------------|-------------------|
| | | 0 | 1.5 | 3 | | |
| Stockton Morello | WPM | 2.38 b-f | 3.58 a | 3.08 ab | 3.02 a | 2.944 a |
| | MS | 2.17 f | 3.52 a | 2.98 abc | 2.87 ab | |
| F12/1 Mazzard | WPM | 2.10 f | 2.98 abc | 2.63 b-f | 2.57 bc | 2.58 b |
| | MS | 2.28 c-f | 2.95 a-d | 2.53 b-f | 2.59 bc | |
| Mahaleb | WPM | 2.17 f | 2.27 def | 2.23 ef | 2.22 c | 2.311 c |
| | MS | 2.15 f | 2.92 a-e | 2.13 f | 2.40 c | |
| Rootstock X Conce. | Stockton Morello | 2.25 c | 3.55 a | 3.03 b | Mean of Media | |
| | F12/1 Mazzard | 2.19 c | 2.97 b | 2.58 bc | | |
| | Mahaleb | 2.16 c | 2.59 bc | 2.18 c | | |
| Media X Conce. | WPM | 2.22 d | 2.94 ab | 2.65 bc | 2.60 a | |
| | MS | 2.18 d | 3.13 a | 2.55 cd | 2.62 a | |
| Mean of Conce. | | 2.20 c | 3.04 a | 2.60 b | | |

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test Duncan, (8).

The interaction of Stockton Morello with WPM and MS medium having 1.5 mg l⁻¹ BA

produced an average of shoot length 3.58 and 3.52 cm and the increased of shoot length was significantly compared with all treatment without the treatment in combination of Stockton morello rootstock in WPM medium supplemented with 3mg l⁻¹ BA. The highest

length of shoot per explant with three rootstocks 3.58 and 2.98 cm in Stockton Morello and F12/1 Mazzard were recorded in WPM having 1.5mg l^{-1} BA and in Mahaleb rootstock 2.92 cm was recorded on MS medium containing 1.5mg l^{-1} BA. On the other hand, the shortest shoots were formed on Mahaleb rootstock 2.13 and 2.15 cm in MS medium supplemented with 1 and 3mg l^{-1} BA, also the shortest of shoots on F12/1 Mazzard rootstock 2.17 cm was found on MS medium contained 1mg l^{-1} BA. The general decreased in shootlets length in all rootstocks with increasing BA concentration in the culture medium is in agreement with the finding of Mansseri-Lamrioui *et al.*, (18) on wild cherry (*Prunusaium* L.) and (Vujović *et la.*, (32) on fruit rootstocks. Hossini *et al.*, (14) also reported that the different concentration of BA improved shoot elongation at 1mg l^{-1} BAP when added to the MS medium and 1mg l^{-1} BAP when added to the LS medium. In contrast, (Hosseini *et al.*, (14) recorded a decrease in shoot length when added a higher concentration of BA 2mg l^{-1} but the longest of shoots finding in this report when added 0.2mg l^{-1} BA to the MS medium.



Figure 2. Mass production of Cherry Rootstock (1-Stockton Morello. 2- F12/1 Mazzard. 3- Mahaleb) on Proliferation media containing the best conce. Of BA + MS after 8 weeks



Figure 3. Mass production of Cherry Rootstock (1-Stockton Morello. 2- F12/1 Mazzard. 3- Mahaleb) on Proliferation media containing the best conce. Of BA + WPM after 8 weeks

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