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Propagation of Aronia (*Aronia melanocarpa*) with Tissue Culture

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ABSRACT

Aronia (*Aronia melanocarpa L.*), known as both small fruit and ornamentally valuable plant, could be propagated by seeds although this method is not recommended. The most easy method to propagate it is using *in vitro* techniques by following micro propagation technology. In this study semi-hardwood buds from aronia plant the explant length 1cm and, each of explant contains one lateral bud figure 2 (explant –a). Based in this study to reproduce aronia in *in vitro* by tissue culture technology and by using micro propagation was obtained on suitable culture medium, where the highest shoot length (14.60 mm) achieved at MS basal medium which combination of different concentrations of growth regulators (1.0 mg l^{-1} BA +0.02 mg l^{-1} IAA 0.1 mg l^{-1} GA_3) at treatment 8. The highest shoot number (64 unit) were obtained at MS basal medium containing a combination of growth regulators at different concentrations (2.0 mg l^{-1} BA +0.01 mg l^{-1} IAA+0.1 mg l^{-1} GA_3) at treatment 6. The highest callus rate achieved at treatments (3, 4, 10) respectively, all the procedures in *in vitro* was obtained within 4 weeks, and so this include rooting process where the highest root number (9.5 unit) at MS control at 0.0 mg l^{-1} concentration of IBA. The most suitable MS basal medium for root length containing 1.0 mg l^{-1} concentration of IBA in length (18 mm) and the highest plant length (33 mm) at 2.0 mg l^{-1} concentration of IBA. Concerninig results of infection, two subcultures considered best subculture compared to other subcultures from where number of death plants and infected plants particularly free of bacterial infections, where the percentage of healthy plants (99%).

1. Introduction

Among the rest of small fruits, aronia is one of the most important small fruits in terms of containing the highest percentage of antioxidants. *Aronia melanocarpa* is a Rosacea family species and has two types commonly of native North American shrubs: *Aronia arbutifolia* (L.) Pers. (red chokeberry) and *Aronia melanocarpa* (Michx.) Ell. (black chokeberry) (Kokotkiewicz ve ark., 2010). There are another species of aronia called *Aronia prunifolia* (Purple chokeberry) contained the highest amount of total anthocyanins, phenolics, and proanthocyanidins (Bräunlich, 2014). It is original home North America. *Aronia melanocarpa* classified as ornamental's shrub as well as decorative autumn coloration that making her very popular (Hirvi and Honkanen, 1985). The use of tissue culture for production of commercial plants especially for plants that has economically importance is due to modern and sophisticated developments of this field in biotechnology. Consider new methods to produce uniform planting material of high quality and diseases–free plants. Biotechnology tools such as micropropagation and *in vitro* culture provide a valuable alternative in the fight against viruses and management of genetic resources. Aronia berry classified between most fruit in terms of antioxidants, where the antioxidants percentage in aronia is higher than that is in apple, banana, elderberries and others. The high levels of flavonoids and anthocyanin in aronia is higher than those found in cranberries and five times more. As well as the chemicals content in aronia it has been alleged that it reduce some disease such as the potential cancer and heart disease. Commercially, aronia is fundamentally used for juice. Food coloring, tea, syrup and fruit spread coloring all these uses includes other uses for aronia. In Russia apple and aronia juices are combined and fermented to producing or giving red wine. Either in Europe the juice often blended with apple juice to give juice a blush (Smith and Ringenberg, 2003). For aronia

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propagation methods if there are one or more type from aronia plants, can propagate those plants by vegetative methods. The most common species that reproduce by vegetative reproduction are Viking and Nero. This includes propagation by micropropagation, and semi-hardwood and hardwood stem cuttings, layering. As long as aronia produce a high percent of apomictic seed, its cultivars can also be grown from seed, they will come true from seed. Most cultivars are also nearly seedless, so it is difficult to get many seed (Anonymous, 2018a). For aronia benefits prevent urinary tract infection and weight control, there are others benefits relate in aronia fruit such as treat inflammations, hypertension as well as can be very beneficial in cases of arthritis, cardiovascular conditions and other diseases. Aronia also contributes to strengthening immunity, blood vessels, lower blood pressure levels, and aronia also delays the natural aging process (Anonymous, 2018b). Therapeutically they show positive effect in the anti-inflammatory and anti-oxidative activity and, also in the treatment several of neoplasms (Kowalczyk et al., 2003).

The purpose of the research was to obtain homogeneous plants from aronia as well as high rate of shoot production and obtain on plant free of fungal and bacterial infections. The search aim is to find an efficient way to multiply the aronia in large scale numbers for expanding the areas cultivation in order to meet our local needs. In addition, the search also aims to determine the best media culture for large scale numbers at low cost.

2. Materials and Methods

Plant material

Aronia cuttings obtained from Yalova Atatürk Central Horticultural Research Institute. There is one cultivar of aronia plants that is 'Viking' have been used during this study. Providing certain additional information here about donor plants (growth stage, age, fruting, etc), sampling procedure (part of explant source, one year old branch etc), sample transporting (in cold box, etc) and explant preparation would let readers informed easily.

Tissue culture conditions and media

Surface Sterilization of Explants and transfer to culture media

Aronia nodal explants were dipped in 1.25% sodium hypochlorite solution for 30 min and rinsed with sterile distilled water. The nodal explants were washed in ethanol (70%) for 5 min, rinsed with sterile distilled water, and sterilized for 20 min in a solution of 15% sodium hypochlorite containing 2 drops of Tween 20. After rinsing with sterile distilled water three times, the nodal explants were transferred on MS medium as in table 1 with 1.0 mg l^{-1} GA_3, 3% sucrose, MS vitamins and 8 g l^{-1} Agar culture tubes containing 10 ml medium. Cultures were incubated in a growth room at 25±1 °C, with 75 µmol m-2 s-1 cool white fluorescent light.

After 21 days, new aronia shoots will be transferred media containing different BA (0.0, 1.0, 2.0 and 3.0 mg l^{-1}) and IAA (0.0, 0.01 and 0.02 mg l-1) combination. All BA and IAA combination will contain GA_3 (100mg l^{-1}).

Culture media of proliferation

1. Control
2. 1.0 mg l^{-1} BA + 0,1 mg l^{-1} GA_3
3. 2.0 mg l^{-1} BA + 0,1mg l^{-1} GA_3
4. 3.0 mg l^{-1} BA + 0,1 mg l^{-1} GA_3
5. 1.0 mg l^{-1} BA +0.01 mg l^{-1} IAA + 0,1 mg l^{-1} GA_3
6. 2.0 mg l^{-1} BA +0.01 mg l^{-1} IAA + 0,1 mg l^{-1} GA_3
7. 3.0 mg l^{-1} BA +0.01 mg l^{-1} IAA + 0,1 mg l^{-1} GA_3
8. 1.0 mg l^{-1} BA +0.02 mg l^{-1} IAA + 0,1 mg l^{-1} GA_3
9. 2.0 mg l^{-1} BA +0.02 mg l^{-1} IAA + 0,1 mg l^{-1} GA_3
10. 3.0 mg l^{-1} BA +0.02 mg l^{-1} IAA + 0,1 mg l^{-1} GA_3

After three sub-cultures of proliferation, plantlet without roots will be transferred to rooting MS media containing IBA (0.0, 1.0, 2.0, 3.0 mg L^{-1}). In order to rooting, plantlets will be in MS rooting media for 30 days.

In all proliferation stages, callus rates, number of sister plants per plant, total proliferation rates, number of death plant and number of infected plants will be calculated and measured. In rooting stages, root number, root length will be measured and calculated after 30 days.

Table 1
Culture medium (Murashige ve Skoog, 1962)

Micro Elements		
$CoCl_2.6H_2O$	0.025 mg/l	0.11 µm
$CuSO_4.5H_2O$	0.025	0.10 µm
FeNaEDTA	36.70	0.10 mM
H_3BO_3	6.20	0.10 mM
KI	0.83	5.00 µm
$MnSO_4.H_2O$	16.90	0.10 mM
$Na_2MoO_4.2H_2O$	0.25	1.03 µm
$ZnSO_4.7H_2O$	8.60	29.91µm
Macro Elements		
$CaCl_2$	332.02 mg/l	2.99 mM
KH_2PO_4	170.00	1.25 mM
KNO_3	1900.00	18.79 mM
$MgSO_4$	180.54	1.50 mM
NH_4NO_3	1650.00	20.61mM
Vitamins		
Glycine	2.0 mg/l	26.64 µm
myo-Inositol	100.00	0.56 µm
Nicotinic acid	0.50	4.06 µm
Pyridoxine HCl	0.50	2.43 µm
Thiamine HCl	0.10	0.30 µm

***Statistical analysis*:** The experiment was repeated three times at 10 replicates per propagation treatment, and the data were subjected to ANOVA1 analysis. The results were analyzed using the statistical programs jump and SPSS. The averages were compared with the least significant differenceThe LSD has a confidence level of 0.05.

3. Results

Table 2

Result of effect 10 different concentrations of plant growth regulators on length and number of shoots as well as percentage both of infection and callus on the Viking cultivar of aronia.

Medium´s symbol	Shoot length (mm)	Shoot number (mm)	İnfection rates (%)	Callus rates (%)
MScontrol	1.03c	0.33c	3.33	0.00 c
MS2	13.00ab	52.80ab	0.00	90.00 ab
MS3	10.70ab	52.27ab	0.00	100.00 a
MS4	9.76b	54.10ab	0.00	100.00 a
MS5	12.02ab	48.07 ab	0.00	93.33 ab
MS6	11.54ab	64.00a	0.00	83.33 b
MS7	12.03ab	48.43ab	0.00	96.67 ab
MS8	14.60a	44.13b	10.00	93.33 ab
MS9	10.33ab	44.73b	3.33	93.33 ab
MS10	10.75ab	40.37 b	6.67	100.00 a
LSD	4.81	17.83	insignificant	16.34

3.1. Shoot proliferation

3.1.1. Shoot length

The effect of proliferation application are giving according to that different concentrations each of BA, IAA and GA_3 for the shoots length and number as well as infection percentages result founded statistically significant, whereas the percentage of infections statistically insignificant (Table 2). The highest shoot length achieved at MS8, which containing 1.0 mg L-1 BA +0.02 mg L-1 IAA +0.1 mg L-1 GA_3 in rate (14.60 mm). The culture medium at 1.0 mg L-1 BA had significant effect on shoot length. The lowest shoot length was observed at $MS_{control}$ in rate (1.03 mm), following that MS4 in rate (9.76 mm). We conclude from our study that the better BA concentration was 1.0 mgL-1 where consider the most suitable concentration for obtain highest shoot length.

3.1.2. Shoot number (per-explant)

Table 2 shows the superiority of MS6 medium for shoot number significantly, which containing 2.0 mg l-1 BA supplemented with 0.01 mg l-1 IAA+0.1 mg l-1 GA3 in rate (64.00 units) on others mediums(Figure 1). The lowest shoot number was obtained at $MS_{control}$ in 0.0 concentrations of plant growth regulators where rate growths were (0.33 units) (Figure 1). There is a gradual decrease in the shoot number during the experiments from the first stage to the final stage after three months of culture. In multiplication stage concluded the most suitable culture that containing 2.0 mg L-1 concentration of BA plus 0.01 mg L-1 concentration IAA as well as 0.1 mg L-1 GA_3. In the result that culture suitable to use this BA level to micro propagation tissue culture technology in in vitro on WAP such as aronia plant.

3.1.3. Infection Rates

According to the statistical analysis percentage of infection plants had insignificant as shows in (Table 2). Fungal infections were very limit and little compared to bacterial infections.

3.1.4. Callus Rates

The highest callus percentage achieved at MS3, MS4, and MS10 mediums respectively callus percentage 100% each one of them that means in all stage high concentration of the cytokinin and auxins had effect on callus formation. The lowest callus percentage was observed at MScontrol, following MScontrol the second lower medium at MS6 as shows in the (Table 2).

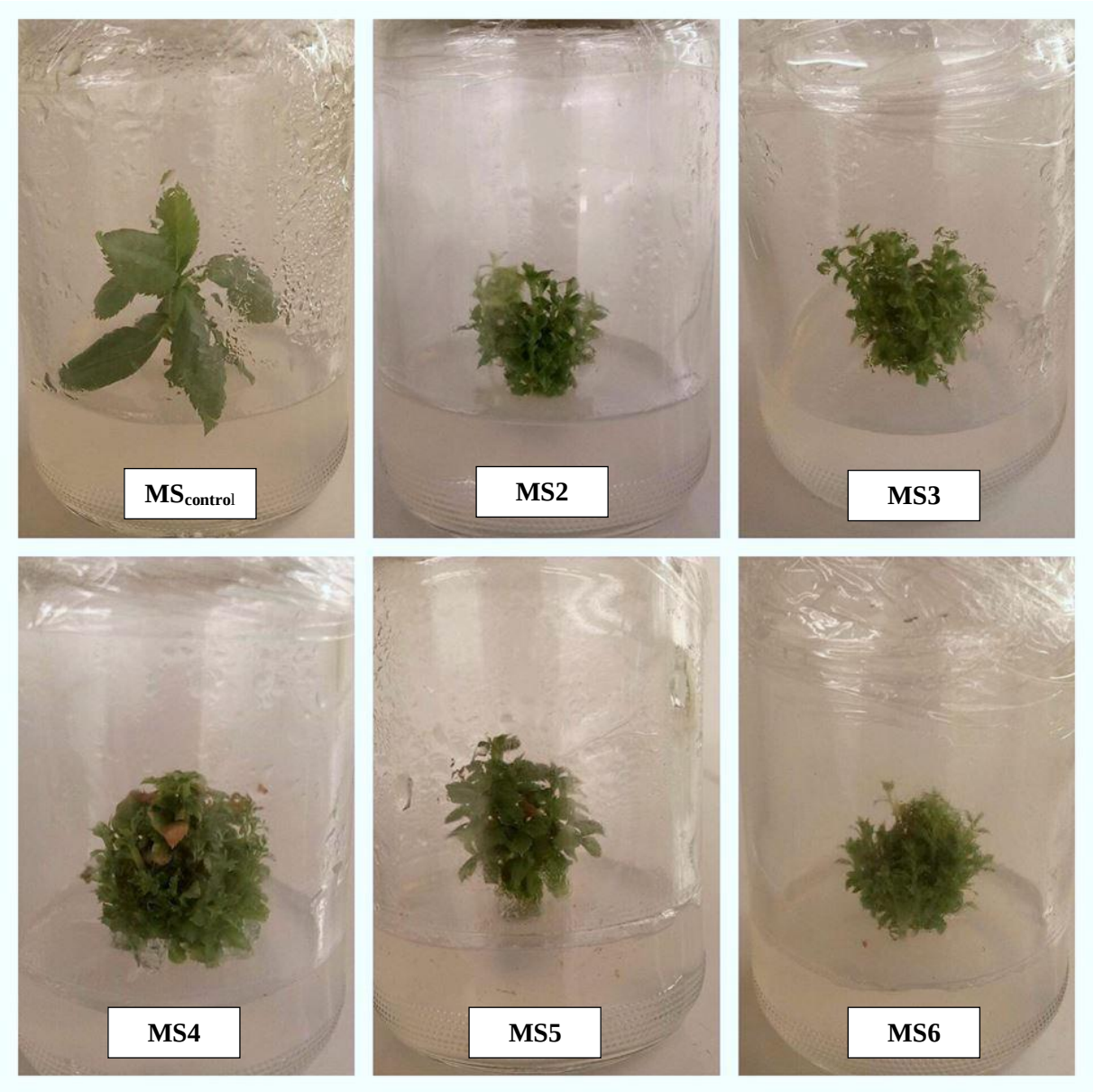

Figure 1

The Effect of Growth Regulators on the micro Propagation of Aronia melanocarpa L.

As shown from (Figure 1) MS6 and MS8 both of them superiority on other mediums on the one hand shoots number and shoot length respectively

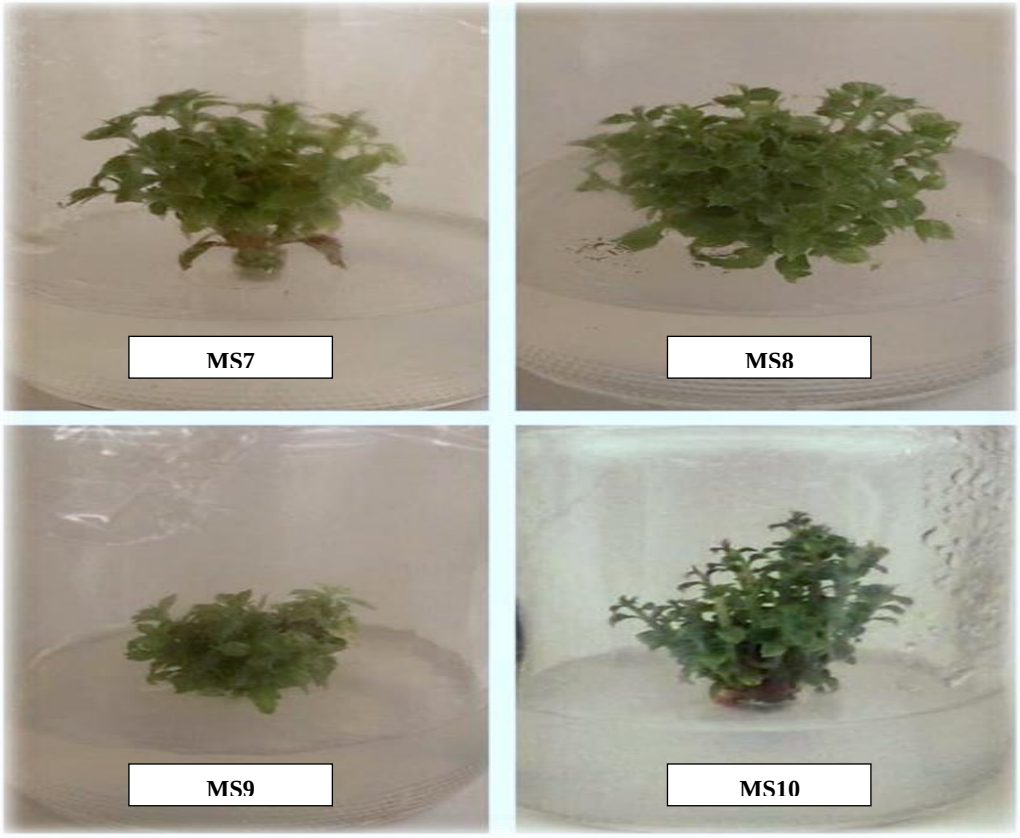

Figure (A) first stage of culture explant

Figure (B) second stage after one month of culture explant

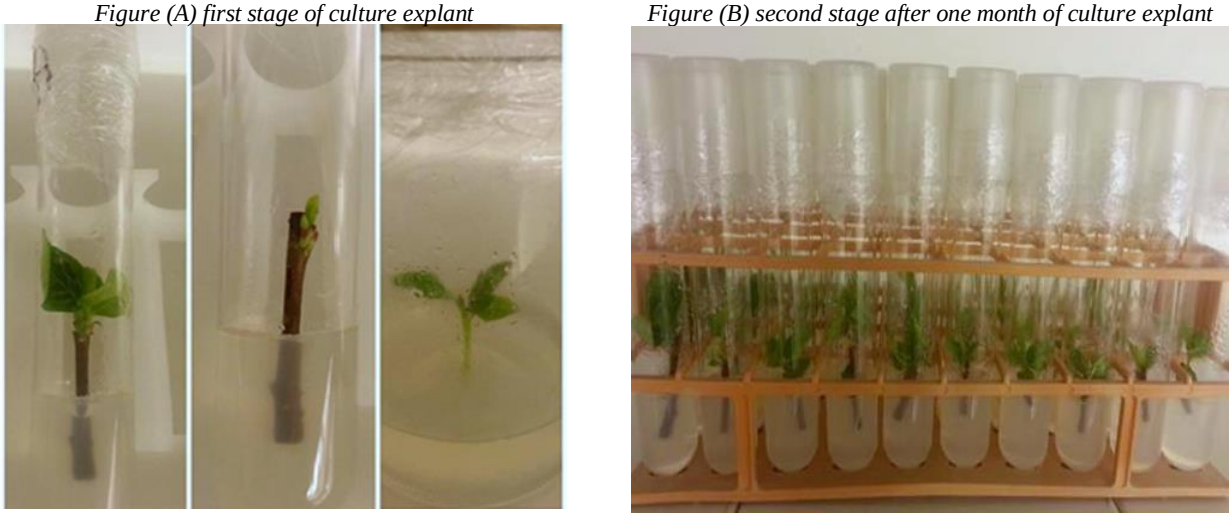

Figure 2
(A, B, C, and D) the effect plant growth regulators of *Aronia melanocarpa L.* growth stages from explant stage to per-explant formation.

3.2. Rooting

Table 3
Result of effect different concentrations of plant growth regulators on number and length roots as well as plant length.

Treatments	Medium's symbol	Root number	Root length	Plant length
1- 0.0 mg l^{-1} IBA	MScontrol	9.56 a	22.61 a	28.98 ab
2- 1.0 mg l^{-1} IBA	MS2	8.00 ab	18.03 a	24.01 bc
3- 2.0 mg l^{-1} IBA	MS3	8.44 ab	8.30 b	33.44 a
4- 3.0 mg l^{-1} IBA	MS4	6.89 b	3.39 b	21.49 b
	LSD	2.84	7.77	6.91

3.2.1. Root number

The effect of rooting application is giving according to that concentration of indol butiric acid (IBA) for the number of rooting statistically significant (Table 3). The highest root number's rate (9.56 units) founded at $MS_{control}$.The lowest root number's rate (6.89 units) at MS4 in 3.0 mg L-1 concentration of IBA. We conclude from this study the suitable two IBA concentrations are founded each of MS3 and MS2 at 2.0 mg L-1, 1.0 mg L-1 concentration of IBA, respectively. The lowest root number (6.89 mm) was obtained 3.0 mg L-1 IBA application whereas this concentration considered the highest level of concentration among other levels. However $MS_{control,}$ MS3, and MS2 respectively have highest averages of root number among other mediums (Figure 6).

3.2.2. Root length

The effect on root length of the application according to statistically analysis is significant. Where the high rate for root length was founded at control group (22.61 mm). This rate considered highest rate among others when the concentration level of IBA was 0.0 mgl-1 at $MS_{control}$, but root thickness was very thin in the $MS_{control}$ compared to other mediums (Figure 4). Hovewer $MS_{control}$ and MS2 have highest averages of root length among other mediums. The lowest root length (3.39 mm) was obtained 3.0 mg L-1 IBA application whereas this concentration considered the highest level of concentration among other levels.

3.2.3. Plant length

The effect on plant length of the application according to statistically analysis is significant. The highest plant length rate (33mm) was obtained at MS3 when IBA concentration were 2.0 mg L-1 this result considered optimum among $MS_{control}$, MS2, and MS4 results. As well as the lowest plant length (21.48mm) were founded at 3.0 mg L-1 of IBA concentration.

Table 4
The nember of infected, health, and death plants during rooting stage.

Meduims	Infected plants	Health plants	Death plants
$MS_{control}$	(1) becterail infection	9	0
MS2	0	10	0
MS3	(1) fungel infection	9	0
MS4	(1)becterail infection + (3) fungel infection	6	0

3.2.4. Rooting result after one month

After one month of culture per-explant for the purpose rooting the nember of infected, health, and death plants was achieved, where the number of infected plants was (1 unit) becterail infection at $MS_{control}$, following $MS_{control}$

The second medium at MS2 was free of infections. Fungal infections were noted each of MS3 and MS4 at (1+3) respectivelly, whereas in the last medium MS4 the number of becterail infection was (1).The number of health plants in all mediums $MS_{control}$, MS2, MS3, and MS4 was (9, 10, 9, and 6) respectivelly.The number of death plants in all mediums was (0 unit). However MS2 was free of becterail and fungal infections that indicate all plants was health where its number was (10 units) as well as death plant was (0 unit) as shown in (Table 4).

Figure 3
Effect (IBA) on the initial rooting stage of Aronia melanocarpa L.

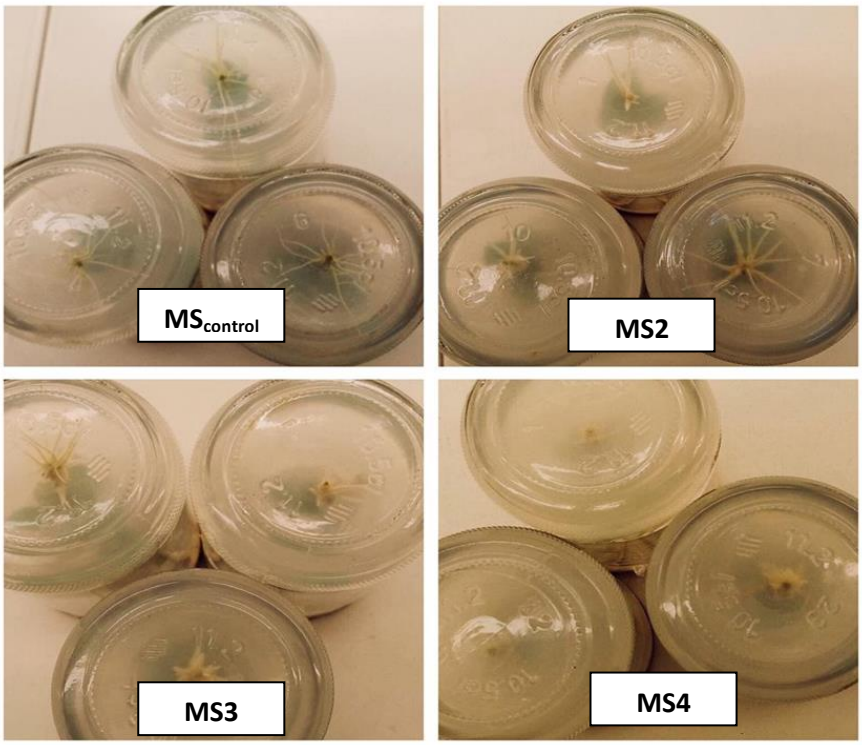

Figure 4

Effect (IBA) on the rooting of Aronia melanocarpa L.

3.3. Sterilization

That is the most important step and fundamental process in tissue culture by culture plant in in vitro where surface sterilization apply such as first step on the plant portion used.

3.3.1. Subculture One

Table 5

Total proliferation rates, number of death plants and number of infected plants measure of subculture one

	1-Subculture	Total proliferation rates (%)	Number of death plants	Number of infected plants	
	Number of explant	Health plants		Fungal infections	Bacterial infections
0	15	100	0	0	0
2	15	100	0	0	0
3	15	100	0	0	0
4	15	100	0	0	0
5	15	100	0	0	0
6	15	100	0	0	0
7	15	100	0	0	0
8	15	86.66	0	2	0
9	15	93.33	0	0	1
10	15	100	0	0	0
	150	97.99	0	2	1

3.3.2. Subculture Two

Table 6

Total proliferation rates, number of death plants and number of infected plants measure of subculture two

	2-Subculture	Total proliferation rates (%)	Number of death plants	Number of infected plants	
	Number of explant	Health plants		Fungal infections	Bacterial infections
0	13	92.3	1	1	0
2	13	100	0	0	0
3	13	100	0	0	0
4	13	100	0	0	0
5	13	100	0	0	0
6	13	100	0	0	0
7	13	100	0	0	0
8	13	100	0	0	0
9	13	100	0	0	0
10	13	100	0	0	0
	130	99.23	1	1	0

3.3.3. Subculture Three

Table 7

Total proliferation rates, number of death plants and number of infected plants measure of subculture three

	3-Subculture	Total proliferation rates (%)	Number of death plants	Number of infected plants	
	Number of explant	Health plants		Fungal infections	Bacterial infections
0	12	100	0	0	0
2	12	100	0	0	0
3	12	100	0	0	0
4	12	100	0	0	0
5	12	100	0	0	0
6	12	100	0	0	0
7	12	100	0	0	0
8	12	83.33	0	2	0
9	12	100	0	0	0
10	11	90.9	0	1	1
	119	97,42	0	1	1

In this study, the result of using sodium hypochlorite 15% for 20 min led to an increase in proportion of healthy samples. In subculture one, the healthy plants percentage were 98% as in table 5, where in subculture two, the percentage were 99 % as in (Table 6). While in subculture three, the percentage was 97% as in table 7. However subculture two was the best culture among others in terms of healthy plants were 99% and number of infected plants for fungal infection was 1 unit and bacterial infection was 0 units as in (Table 6).

4. Discussion

4.1. Sterilization

The most important point in tissue culture technology is sterilization process (Sökmen and Gürel, 2001). Sterilization of plant surface considered important step which the success or failure of tissue culture depends on, according to several factors that are; the time of sterilization, the type of material used in sterilization process and its concentration and, part of the plant to be sterilized (Zale et al., 2004). During surface sterilization sodium hypochlorite had used as an effective substance in the process of surface sterilization of plant material (Jones et al., 1979; Pevalek-Kozlina and Jelaska, 1985). In this study the result of sterilization were nonsignificant, where when increasing concentration of sodium hypochlorite to 15% for 20 min, led to an increased in the proportion of healthy samples. This is consistent with study carried out by (Zale et al., 2004). Callus induction and plant regeneration from mature embryo in grain sorghum (Sorghum bicolor L. Monech) where reported that the high concentration of

Sodium hypochlorite solution of 5% for 20 min, led to increase of healthy samples percentage.

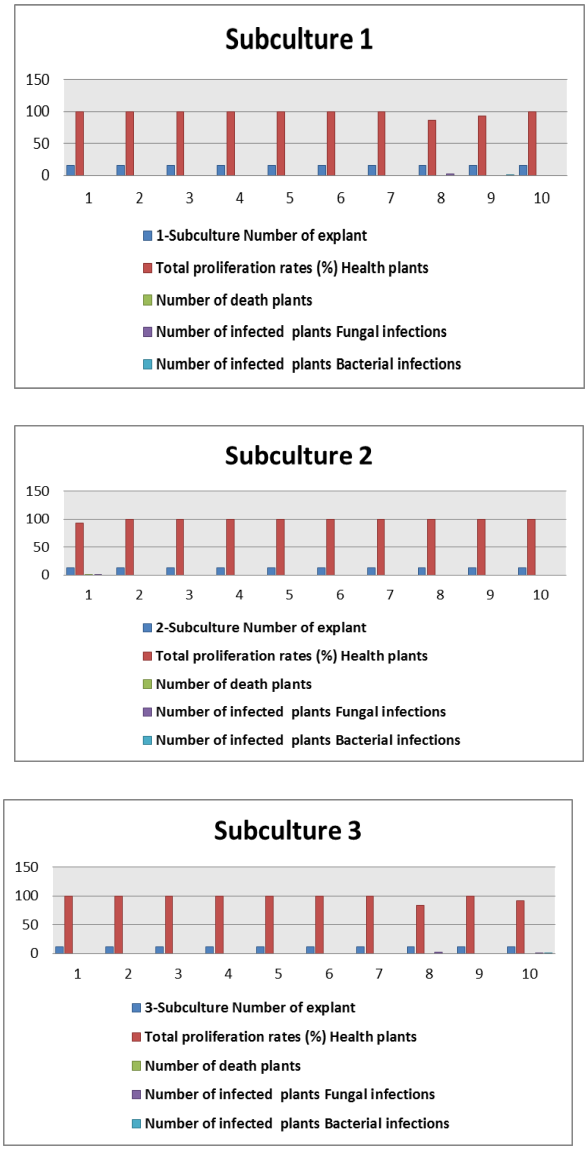

Figure 5
(sub-culture 1, 2, 3)

4.2. Proliferation

4.2.1. Shoot length

The highest shoot length (14.6 mm) was obtained at MS8 using 1.0 mg L^{-1} concentration of BA Table 2 and Figure 1. That matches with study on in vitro regeneration from callus of Rosmarinus officinalis by (Tawfik and Read, 1990). When reported that the great shoot length produce at 1.0 mg/l concentration of BA. Publically the lowest shoots number was obtained at $MS_{control}$ of all experiments where noted large leaves and poor shoot proliferation with elongation of plant, particulary when the culture medium was 0.0 mg L^{-1} concentration of plant growth regulators Table 2 and Figure 1. The same result were obtained by (Brand and Lineberger, 1986). Who reported when added 0.0 mg L^{-1} of plant hormones to the culture medium the result was large leaves and poor proliferation, using Halesia carolina L.

4.2.2. Shoot number

MS6 superior on other treatments producing shoot number (64 units) at 2.0 mg L^{-1} concentration of BA Table 2 and Figure 1 .Similar result were achieved by (Şuţan et al., 2017). Who explained that the higher average of shoot multiplication giving when increasing concentration of cytokinin by 1.50 mg×dm-3 BA in two cultivars of Aronia melanocarpa (Melrom and Niro) at treatment (4) Whereas (52 unit) of shoot number was obtained at MS2 by 1.0 mg L^{-1} of BA. However 2.0 mg L^{-1} concentration of BA considered better level to produce number of shoots in in vitro. This result achieved by (Tawfik et al., 1990). On clonal propagation of Liatris pycnostachya by in vitro culture of axillary buds when reported the shoot number was increased using 1.0 or 2.0 mg L^{-1} plus 0.5 AG3 applyed that on sweet cherry fruit.

5. Acknowledgements

Thank you to the Yalova Atatürk Central Horticultural Research Institute for the supply of plant materials.

6. Rooting

The most important point in our study that in every stage of culture medium $MS_{control}$ the root was observed each of proliferation and rooting stage, where rooting was observed very cleary on $MS_{control}$ medium without supplementation of plant growth regulators, but root thickness was very thin compared to other mediums as well as the increase of IBA concentration in the MS led to reduce number and length of root and also plant length that achieve with (Tawfik and Read, 1990). on in vitro propagation of Halesia carolina L. and the influence of explantation timing on initial shoot proliferation study when reported after root initiation the poor root growth was obtained and probably the result of high auxin concentrations (Agarwal and Chandra,1989) around the roots. At (MS2) 1.0 mgL^{-1} concentration of IBA the most suitable concentration for root length that were (18.03 mm) and, that consistent with (Sakila et al., 2007). study on micropropagation of strawberry when reported 1.0 mg mg L^{-1} considered the most suitable IBA concentration to induction root per explant and root length average that being 3.68 cm.

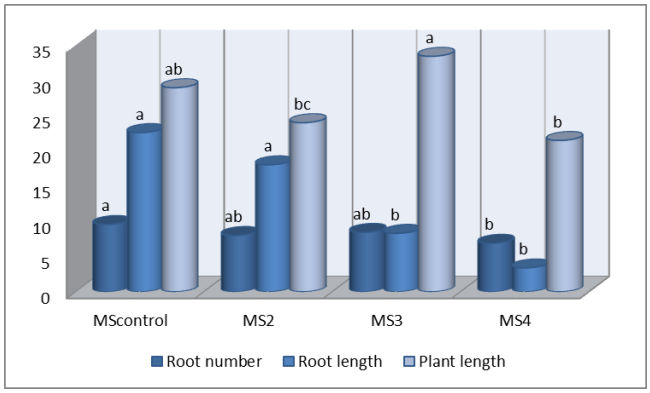

Figure 6
Root number, length, andplant leng

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