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Micropropagation of Blackberry (*Rubus fruticosus*.) cv. Karaka Black

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THIS work aimed to establish an *in vitro* protocol for Blackberry "Karaka Black" micropropagation. Stem node explants were experimented on three types of medium salts (WPM, MS and B5) in establishment stage. Also, three types of cytokinins were tested for multiplication i.e. BA (0.0, 0.4, 0.8 and 1.2 ppm), TDZ (0.0, 0.1, 0.5 and 1.0 ppm) and Kin (0.0, 1.0, 5.0 and 10.0 ppm). Two soil mixtures were used in acclimatization stage. Finally, the effect of passage time and number of subcultures on multiplication rate were investigated. The results indicated that WPM followed by MS medium gave the highest shoot length and leaf number. BA at 0.4 ppm, Kin at 5.0 ppm and TDZ at 0.1 ppm achieved the most preferable values for multiplication stage but the kinetin one gave the healthiest plantlets. The most microshoots got well-formed roots in multiplication phase. Consequently, rooting stage was not required. The multiplication rate slightly decreased after the 3rd subculture and three months of culture date proved to be the most suitable passage time among subcultures. Peatmoss and sand mixture gave the highest plantlet quality during acclimatization.

Keywords: Blackberry, *Rubus* sp., Micropropagation, *In vitro* Propagation, Acclimatization.

Introduction

Blackberries often termed "Brambles" are a diverse group of species and hybrids in the genus *Rubus* (Family: *Rosaceae*). Blackberry is a shrub with erect, semi erect or creep grown habit, and most cultivars have thorny stems (Bobrowski et al., 1996). It is commercially grown due to berry delicious taste, pleasant flavor and nutritional profile. The shrub is believed to have its origin in *Rubus armeniacus*, and is now distributed throughout Europe, Asia, Oceania and North and South America (Hummer and Janick, 2007). Blackberries are typically purchased for consumption as fresh, individually quick frozen (IQF), or as a further processed product incorporating them into jams, syrups, wines, teas, juices, concentrates, and purees. Many potential health benefits from consuming blackberries or blackberry products are attributed to their metabolites. Metabolites also directly and indirectly influence processing regimes, shelf life, and consumer likeability (Jungmin Lee, 2017). Blackberries contain dietary fiber, vitamin C (ascorbic acid), vitamin A, vitamin E, potassium, and calcium, along with the phenolic metabolites that are a source of possible health benefits. Sensory attributes, typically used to describe the taste and flavor of blackberries, include fresh fruit, cooked fruit, cooked berry, strawberry, raspberry, vegetal, stemmy, and earthy (Du and Qian 2010). Cultivars Black Butte and Karaka Black had the heaviest fruit, above 6.0 g, in 2006. Their fruits are cylindrical, elongated and glossy with very attractive appearance. (Wójcik-Seliga and Wójcik-Gront, 2013). Tip layering and stem cuttings are the common way to propagate thornless Blackberry cultivars (Caldwell, 1984) Tip layering propagation requires a large spacing for the layering bed, few tips are available per plant, and weed control among the layers is a problem. Propagation by hardwood stem cuttings is simple but rooting is not always satisfactory. Softwood cuttings root readily but require considerably more care for successful plant production (Broome and Zimmerman, 1978). The

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blackberry can be propagated by seeds, layering, offsets, root cuttings, stem cuttings, division of shoots or grafting and it can also be propagated very effectively *in vitro* (Botez et al., 1984). The micropropagation of Blackberry was studied by several researchers (Fira et al, 2014, Kefayeti et al, 2019, Bobrowski, et al*,* 1996). Generally *in vitro* formation of axillary shoots was achieved on culture media supplemented with cytokinins and auxins, followed by the excision and *in vitro* rooting of axillary shoots on culture media in the presence or absence of auxins and then the plantlets which were rooted *in vitro* were transferred *ex vitro* and acclimatized in various soil mixtures. (Fira et al, 2014) This study aimed to propagate Blackberry "Karaka Black" plant as a new promising fruit crop in Egypt. Due to the limitation of plant material, the *in vitro* propagation will be the most suitable technique to increase the number of available plants. Moreover, Establishing a micropropagtion protocol for the studied cultivar in Egypt.

Material and Methods

These experiments were conducted in the Plant Tissue Culture Laboratory of Horticulture Department, Faculty of Agriculture, Ain Shams University during years 2020 to 2022.

The mother plants (one year old planted in plastic pots) were maintained in saran house located in the fruit trees farm nursery, Faculty of Agriculture, Ain Shams University, Shoubra El-Khiema. The mother plants were periodically fertilized using NPK fertilizer and sprayed with micro-nutrients foliar fertilizer keep them healthy.

Stem pieces 1-2 cm in length bearing one bud were prepared from 2-3 weeks old shoots collected from the mother plants. After the removal of the leaves with short part of petiole left, the explants were subjected to continuous flow of tap water with adding few drops of liquid soap for an hour. The explants were surface sterilized by shaking in 0.5% sodium hypochlorite (active ingredient) NaOCl solution (prepared by Clorox dilution) for 10 min with adding few drops of Tween 20 as a surfactant then the explants rinsed three times with sterile distilled water five minutes for each. After that the explants were planted into culture media.

The media used in the micropropagation of blackberry included **MS** (Murashige and Skoog, 1962), **B5** (Gamborg et al., 1968) and **WPM** (Lloyd and McCown 1980). Carbon and energy source was sucrose at 3% in all media and propagation stages, also, myo-inositol at 100 mgl^{-1} was added as well. The hormonal supplements were differed according to the requirements of the specified experiments as will be mentioned later.

Media salts and sucrose were dissolved in distilled water using the magnetic stirrer. The pH was adjusted to 5.70 - 5.79 using KOH and HCl, then the media were solidified with purified agar (agar-agar) at 5 gl^{-1} and phytagel at 2 gl^{-1} as solidifying agents which liquefied on 90°C using hot plate and stirrer. Media were dispensed in glass jars (400 ml) capped with polypropylene lids received 35-40 ml medium then autoclaved at 100 K.pa (15 P.S.I) and 121°C for 20 min. afterwards, the media left to cool and stored at 26± 2°C for 5 days before being used.

Cultures of all experiments were incubated at 242°±C under 16 hr light using LED lamps (2 lamps per shelf) alternated with 8 hr dark photoperiod of 2000 - 2500 lux light intensity at cultures level.

Establishment stage

Effect of medium type on the criteria of establishment.

This experiment aimed to find out the best basal medium for establishing the cultures of blackberry cv. Karaka black. Stem node explants were prepared and inoculated on three well known types of free hormone media at full strength. These media were MS, B5 and WPM. Average number of proliferated shoots per explants (all the proliferated shoots were counted regardless of its length), average proliferated shoot length (cm) and leaf number per proliferated shoot were recorded three months after culture date. This experiment contained 3 treatments (one cultivar × 3 medium types) in 5 replicates each one consisted of 2 culture jars contained 2 explants for each.

Multiplication stage

Effect of cytokinin type on some proliferation and rooting characteristics during the first subculture.

These experiments were carried out to discover the proper cytokinin type for multiplying the cultures of blackberry cv. Karaka black. Well established cultures were subcultured by dividing

the proliferated shoots into stem nodes and the adventitious shoots after being them defoliated) on full strength WPM medium supplemented with BA (6-Benzylaminopurine or benzyl adenine) or Kin (6-furfurylaminopurine) or TDZ (Thiadizeron) at different concentrations each individually in four treatments. Average number and length (cm) of proliferated shoots per explants and average leaf number per proliferated shoot were recorded eight weeks after subculture date. Each experiment contained 4 treatments (one cultivar × 4 cytokinin concentrations) in 5 replicates each one consisted of 2 culture jars contained 2 explants for each.

Effect of BA concentrations on some proliferation characteristics during the first subculture.

This experiment was conducted to determine the best concentration of BA for the multiplication of the blackberry cultures. Well established cultures were subcultured on full strength WPM medium supplemented with BA at 0, 0.2, 0.8, 1.2 mgl^{-1} in four treatments. Contamination, %, chlorosis %, number and length (cm) of proliferated shoots per explants, average leaf number per proliferated shoot and rooting % were recorded eight weeks after subculture date. This experiment contained 4 treatments (one cultivar × 4 Kin concentrations) in 5 replicates each one consisted of two culture jars contained 2 explants.

Effect of TDZ concentrations on some proliferation characteristics during the first subculture.

This experiment was conducted to determine the best concentration of TDZ for the multiplication of the blackberry cultures. Well established cultures of them were subcultured on full strength WPM medium supplemented with TDZ at 0, 0.1, 0.5, 1.0 mgl^{-1}in four treatments. Average number and length (cm) of proliferated shoots per explants and average leaf number per proliferated shoot were recorded eight weeks after subculture date. This experiment contained 4 treatments (one cultivar × 4 TDZ concentrations) in 5 replicates each one consisted of two culture jars contained 2 explants.

Effect of Kin concentrations on some proliferation characteristics during the first subculture.

This experiment was conducted to determine the best concentration of Kin for the multiplication of the blackberry cultures. Well established cultures of them were subcultured on full strength WPM medium supplemented with Kin at 0, 1, 5, 10 mgl^{-1}in three treatments. Average number and length (cm) of proliferated shoots per explants and average leaf number per proliferated shoot were recorded eight weeks after subculture date. This experiment contained 4 treatments (one cultivars × 4 Kin concentrations) in 5 replicates each one consisted of two culture jars contained 2 explants.

Determination of the proper passage time under the multiplication conditions.

This experiment aimed to detect the best passage time which accomplish the most possible biomass of cultures with no negative effects on the further propagation stages. Blackberry explants were subcultured on WPM medium supplemented with 5 mgl^{-1} kinetin and 126 mgl^{-1} Phloroglucinol. Average number and length (cm) of proliferated shoots per explants and average leaf number per proliferated shoot were recorded after one, three and six months. This experiment contained 3 treatments (one subculture × 3 passage time) in 5 replicates each one consisted of two jars contained 2 explants.

Influence of subculture number on the multiplication characteristics.

This experiment was made for chasing the effect of subculture number on the multiplication rate of the cultures of blackberry cv. Karaka black. Full strength WPM medium supplemented with 5 mgl^{-1} kinetin and 126 mgl^{-1} Phloroglucinol was the medium which used during five successive subcultures, three months as passage time. Average number and length (cm) of proliferated shoots per explants and average leaf number per proliferated shoot were recorded three months after every subculture. This experiment contained 5 treatments (one cultivar × 5 subcultures) in 5 replicates each one consisted of two jars contained 2 explants.

Acclimatization of blackberry plantlets

The *ex-vitro* conditions have substantially lower relative humidity, higher light level and septic environment that are stressful to micropropagated plants compared to *in vitro* conditions. The plantlets produced *in vitro* require an acclimatization process in order to ensure that sufficient number of plants survive and grow well when transfer to soil.

The plantlets were washed gently under continuous flow of water directly after pulling them from the culture jars in order to remove the media residues and then the plantlets were

dipped in solution of fungicides mixture for 30 min before sticking in the pots. Acclimatization was carried out on 3 months old blackberry plantlets. Soil mixture of peatmoss + perlite 1:1 (v/v) and peatmoss + sand 1:1 (v/v) mixtures were autoclaved under 15 P.S.I and 121°C for 30 min. Plastic pots were washed using water and liquid soap then soaked in chlorox solution for 2 hours then rinsed using tap water and dried. Afterwards, these pots were filled with sterile soil mixture and the plantlets were planted in the pots which kept inside glass champer in the incubation room for a month before being transferred to the plastic house. Survival percentage, shoot length, leaf dimension, leaf area and SPAD values were measured 5 weeks later. Leaf area was measured according to the equation:

LA = a + b LW constant (a)=0.90, Fitted coefficient (b)=0.70, length (L) and width (W) (Fallovo et al. 2008)

Data analysis

In all the experiments, the obtained data were statistically analyzed in a completely randomized design through variance analysis. Mean comparisons were carried out by Tukey (1977) multiple range test at $p \leq 0.05$. Data were statistically analyzed using the analysis of variance adopting a SAS package version 9.1.3 Service Pack 4.

Results and Discussion

Data in Table 1show that stem node explants of Blackberry planted on B5 medium recorded the lowest contamination percentage significantly followed by those of WPM and MS media respectively. Concerning the survival percentage, all explants planted on the three experimented media had been survived without significance among them. Similarly, there was no significant difference among the three experimented media in the number of shoots per explant. While the highest average shoot length was in favor of the explants on MS medium significantly followed by those of WPM then B5 medium with significant difference among them. Whereas, the stem nodes planted on WPM medium surpassed those of MS and B5 media regarding the average leaf

TABLE 1. Effect of medium type on the criteria of establishment of Blackberry cv. Karaka Black stem node cultures.

Medium Type	Contamination ٪	survival %	Average number of Shoots per explant	Average Shoot length (cm)	Average Leaf number	Notes
MS	30.77A	100.00A	1.00A	0.90A	2.67AB	Leaf edge chlorosis
B5	2.56C	100.00A	1.00A	0.17C	1.67B	Leaf general chlorosis
WPM	10.25B	100.00A	1.00A	0.50B	3.33A	Green

Means of each column having the same letter (s) are not significantly different at 5% level.

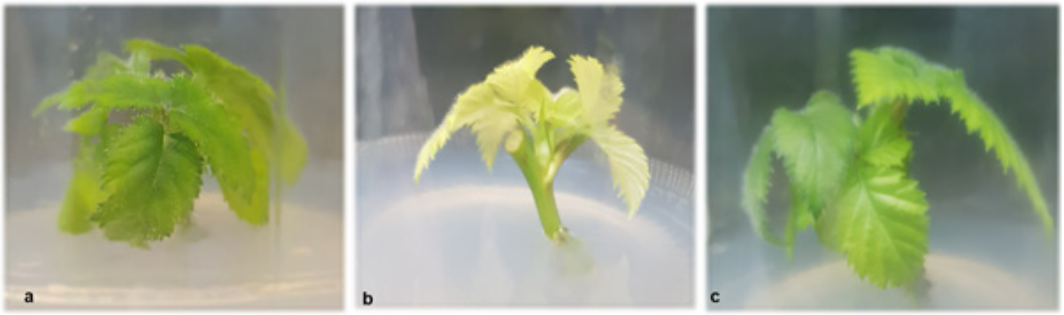

Fig. 1. Effect of medium salts on the response of Blackberry explants cv. Karaka black (a) MS medium (b) B5 medium (c) WPM medium.

number per explant with significant difference in comparison with those of B5 medium.

One can conclude that WPM proved to be the most suitable medium for establishment of Blackberry explants upon the obtained results and personal observations regarding the quality of growth and the health of shoots grown on it. (Fig. 1)

In this concern, Borodulina et al., 2019 advocated that medium composition had a clear effect on the behavior of Blackberry explants as MS medium was better than QL medium as the first one gave shoots longer two times than the latter one. Moreover, the adding of $Ca(NO_3)_2$ instead of $CaCl_2$ in the medium led to significant increase in the average length of microprobe, that may interpret the superiority of WPM medium which included $Ca(NO_3)_2$ in its composition while MS and B5 media had no such component. Also, the absence of glycine in B5 medium may play a role in shoot discoloration and weakness compared to MS and WPM media, as reported by Asad et al., 2009 glycine was very effective to stimulate somatic embryogenesis and maximum shoot regeneration in Sugarcane cultures.

Data in Table 2 declare that chlorosis symptoms were detected in some shoots of survived ones in the four experimented concentrations of BA, the lowest number of them was significantly recorded in control and 0.4 ppm treatments in comparison with those of 0.8 and 1.2 ppm treatments. Concerning shoot number, the medium supplemented with 0.4 ppm BA encouraged the proliferation of the highest number of shoots significantly compared to the other concentrations.

On the same trend, the shoots proliferated on the medium supplemented with 0.4 and 1.2 ppm BA scored the highest significant shoot length. On the contrary, the medium supplemented with 1.2 ppm BA scored the highest leaf number per shoot significantly followed by 0.8 ppm then 0.4 ppm and 0.0 ppm, respectively. Although the 0.4 ppm treatment scored low leaf number in the comparison, but the formed leaves were full expanded, green and healthy compared to those of the other treatments. On the other hand, well-formed adventitious roots were detected on some shoots, 0.4 ppm treatment significantly got the most, compared to the other three treatments. (Fig. 2)

Our findings went in parallel with those of Fira et al., 2014 who reported that the optimal concentration of BA for Blackberry multiplication was 0.5 mg/l. This concentration achieved high multiplication rate and vigorous plants. Also,

TABLE 2. Effect of Benzyladenine (BA) concentration on the multiplication of Blackberry cv. Karaka Black.

BA concentration (ppm)	Chlorosis %	Shoot number per explant	Shoot length (cm)	Leaves number per shoot	Rooting%
0.0	5.67B C	1.00 B	0.90 B	3.33 C	0.00 C
0.4	4.00 C	5.33 A	2.73 A	6.62 B	22.22 A
0.8	8.33 A	3.00 B	1.37 B	7.33 B	11.11 B
1.2	6.67 AB	2.00 B	2.17 A	11.00 A	11.11 B

Means of each column having the same letter (s) are not significantly different at 5% level.

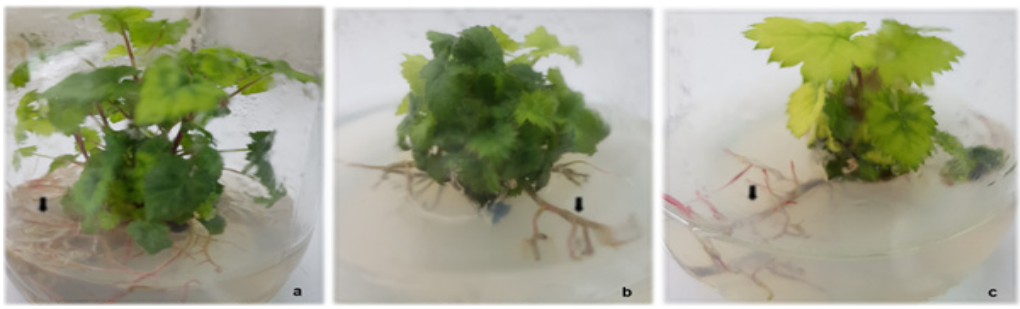

Fig. 2. Effect of BA concentration in multiplication medium of Blackberry cv. Karaka black (a) 0.4ppm (b) 0.8ppm (c) 1.2ppm (Arrows refer to the adventitious roots in all tested concentrations)

Kereša et al., 2019 noticed that the highest number of shoots/explant, but the shortest shoots at the same time, were produced on medium containing 1 mg L^{-1} BAP. While, maximum shoots length was obtained on medium containing 0.3 mg L^{-1} BAP. Also, Schuchovski and Biasi 2017 advocated that BAP was the best among four types of cytokinin tested on the multiplication of 'Brazos' blackberry *in vitro*.

Data in Table 3 demonstrate that the medium supplemented with 0.1 ppm TDZ surpassed significantly the other media supplemented with TDZ at the concentration of 0.5, 1.0 and 0.0 ppm regarding the average shoot number per explant, average shoot length and total leaf number per culture. Whereas, the proliferated shoots on 0.5 ppm TDZ medium recorded the highest average leaf number per shoot followed by those on 0.1 ppm TDZ medium with no significant difference between them while those on 1.0 ppm TDZ medium were the lowest. No adventitious roots were detected but basal callus was observed in all microshoots of all test concentrations of TDZ except 0.0 ppm treatment but, the rate of callus formation was gradually increasing and the growth of shoots decreasing in parallel with the level of TDZ in medium (Fig. 3). Similar results were obtained by Schuchovski and Biasi 2017 on 'Brazos' blackberry *in vitro* multiplication as Thidiazuron was not preferable because of shoot malformation and massive callus formation.

Data in Table 4 prove that the explants planted on medium supplemented with 1.0 and 5.0 ppm Kin showed the highest shoot number, shoot length and leaf number insignificantly when compared to those planted on 0.0 and 10.0 ppm Kin medium. All Kin treatments had higher significant values compared with control. (Fig. 4)

Regarding the rooting percentage, one can say that medium supplemented with Kin at 1.0 or 5.0 or 10.0 ppm motivated the subcultured shoots to form adventitious roots in all experimented concentrations. Consequently, one can conclude that using kinetin in the multiplication media may be enough for both shoot proliferation and rooting so no need to do rooting as a separate stage (Fig. 5). In the

TABLE 3. Effect of Thidiazuron (TDZ) concentration on the multiplication of Blackberry cv. Karaka Black.

TDZ concentration (ppm)	Average shoot number per explant	Average shoot length (cm)	Total Leaves number per culture	Average leaf number per shoot	Rooting%
0.0	1.00 C	0.90 BC	3.33 C	3.33 AB	0.00 A
0.1	5.33 A	4.33 A	31.67 A	5.93 A	0.00 A
0.5	2.67 B	1.67 B	13.67 B	6.33 A	0.00 A
1.0	1.67 BC	0.27 C	4.00 C	2.50 B	0.00 A

Means of each column having the same letter (s) are not significantly different at 5% level.

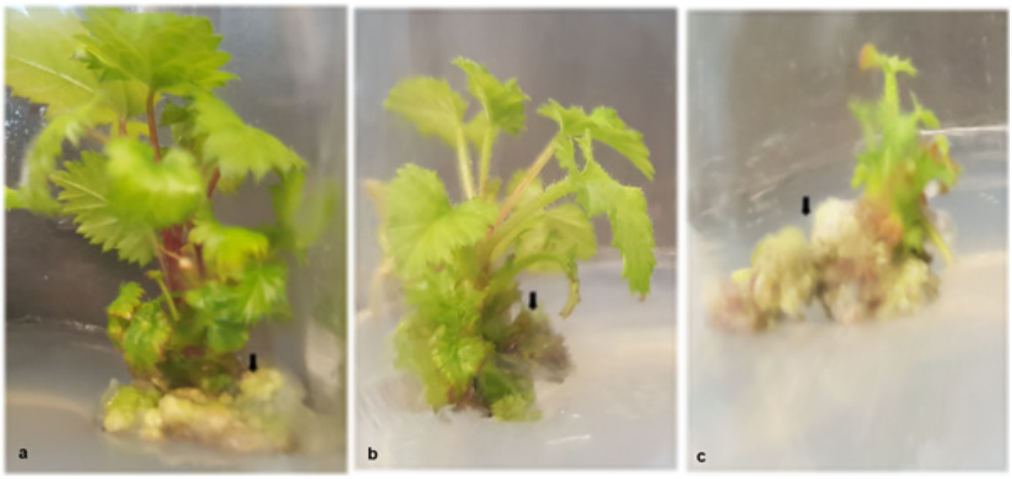

Fig. 3. Effect of TDZ concentration in multiplication medium of Blackberry cv. Karaka black (a) 0.1ppm (b) 0.5ppm (c) 1.0ppm (Arrows refer to the basal callus which increased in parallel with TDZ concentration increase)

TABLE 4. Effect of Kinetin (Kin) concentration on the multiplication of Blackberry cv. Karaka Black.

Kin concentration (ppm)	Average shoot number per explant	Average shoot length (cm)	Average leaf number per shoot	Rooting%
0.0	1.00B	0.90C	3.33B	0.0 B
1.0	5.00A	6.33A	7.18A	100A
5.0	4.33A	5.33A	6.92A	100A
10.0	2.33B	2.67B	6.28A	100A

Means of each column having the same letter (s) are not significantly different at 5% level.

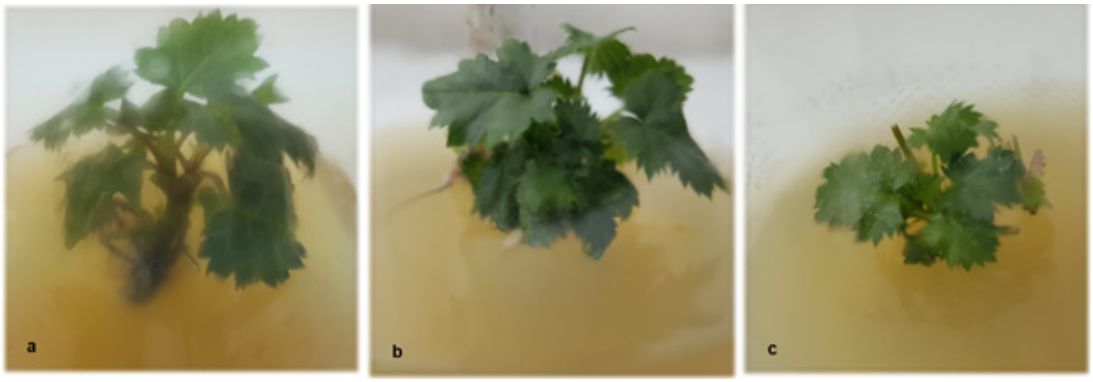

Fig. 4. Effect of Kin concentration in multiplication medium of Blackberry cv. Karaka black after one month of culture date (a) 1.0ppm (b) 5.0ppm (c) 10.0ppm.

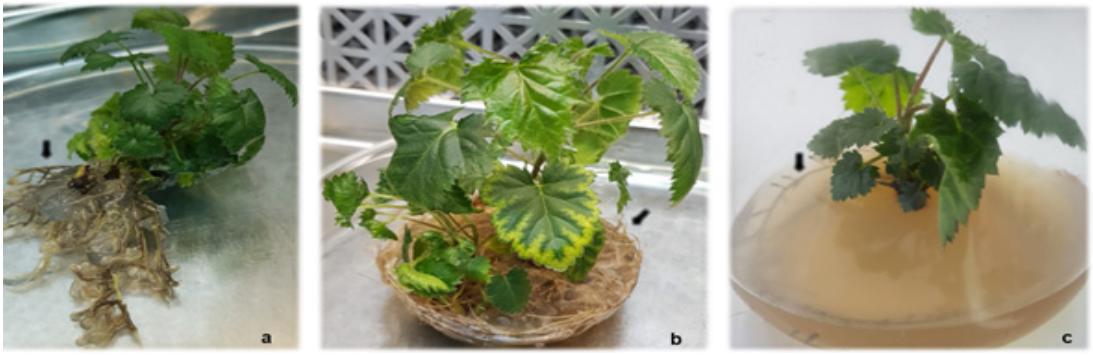

Fig. 5. Effect of Kin concentration in multiplication medium of Blackberry cv. Karaka black after three month of culture date (a) 1.0ppm (b) 5.0ppm (c) 10.0ppm (Arrows refer to the adventitious roots)

same trend, Borodulina et al., 2019 revealed that in the control hormone-free variant, after 14 days of culture, up to 85% of Blackberry microshoots took root, 3-4 roots were formed on each one. Also, Da Silva and Biasi 2022 advocated that all the Ébano Blackberry explants in the multiplication experiment had roots. Also, Kereša et al., 2019 stated that the rooting percentage was higher (61%) on hormone free rooting medium.

Data in Fig. 6 show the effect of passage time on the multiplication criteria of Blackberry cultures. Regarding the contamination percentage, the Blackberry cultures significantly showed no contamination after the second and third passage times while those after the first passage time showed low contamination percentage. It was obvious that Blackberry cultures after one month of planting date demonstrated the lowest number of shoots per explant, shoot length, leaf number and no rooting significantly. On the other hand, the same cultures after three months of planting date proved a significant improvement in all mentioned parameters. Whereas, the same Blackberry cultures after three months of planting date showed a significant improvement in favor of shoot length and leaf number while the shoot

number per explant recorded few increases and the rooting percentage was the same. It's worthy to mention that the general status of cultures after six months was not enough satisfied because of chlorosis and weakness of the plantlets. One can recommend that passage time of three months was the most suitable regarding the measured parameters and healthiness of cultures. Most researchers who worked on the micropropagation of Blackberry stated that period of 4 or 6 weeks as a passage time among subcultures (Fira et al, 2014, Gomes et al., 2017 and Aly et al., 2022). On the other hand, Gomes et al., 2017 reported that Blackberry cultures could survive for 15 months under the conditions of minimal growth conservation then regenerated successfully.

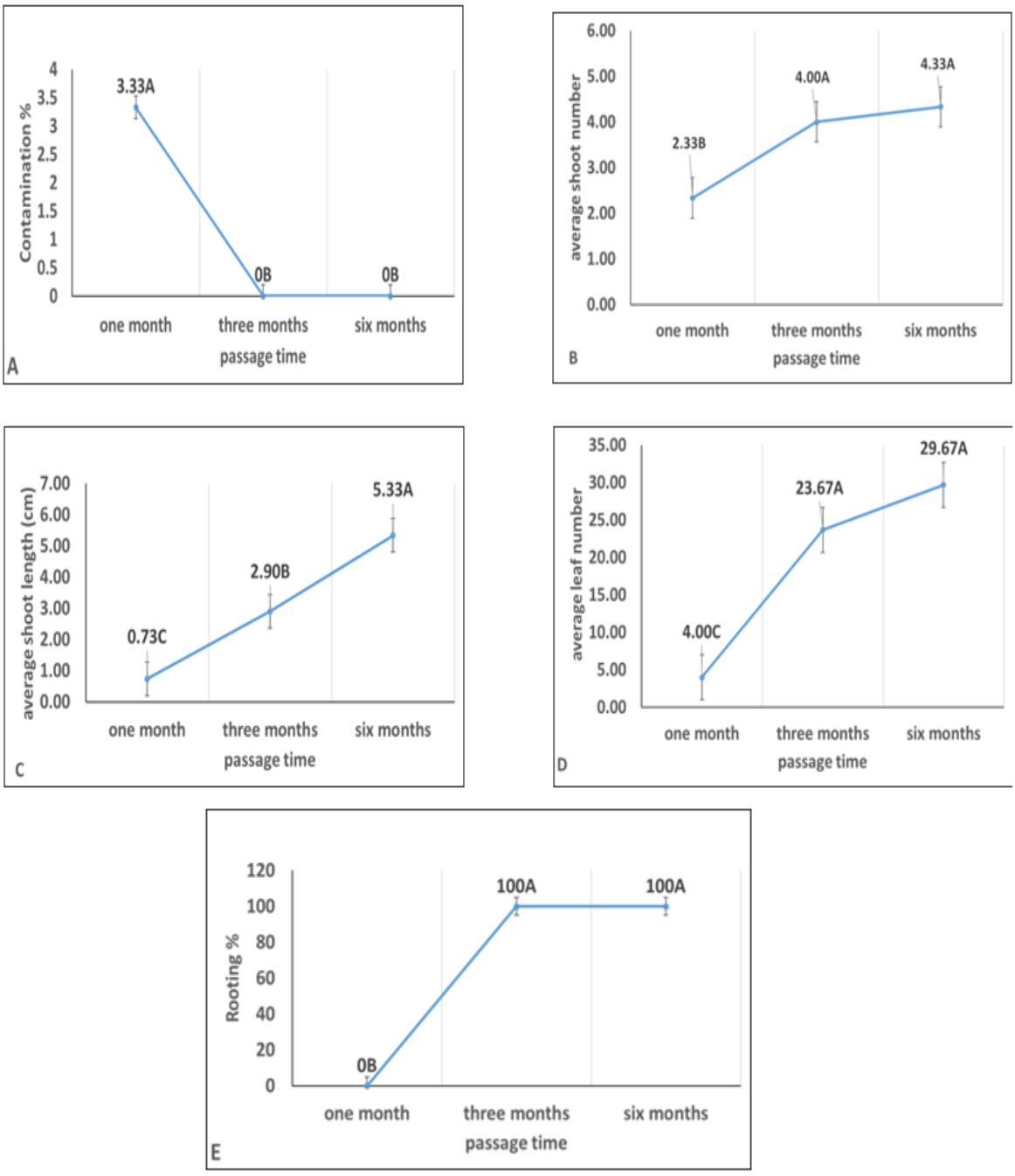

Fig. 6. Effect of passage time on A) contamination percentage B) average shoot number C) average shoot length (cm) D) average leaf number E) rooting percentage of Blackberry cultures.

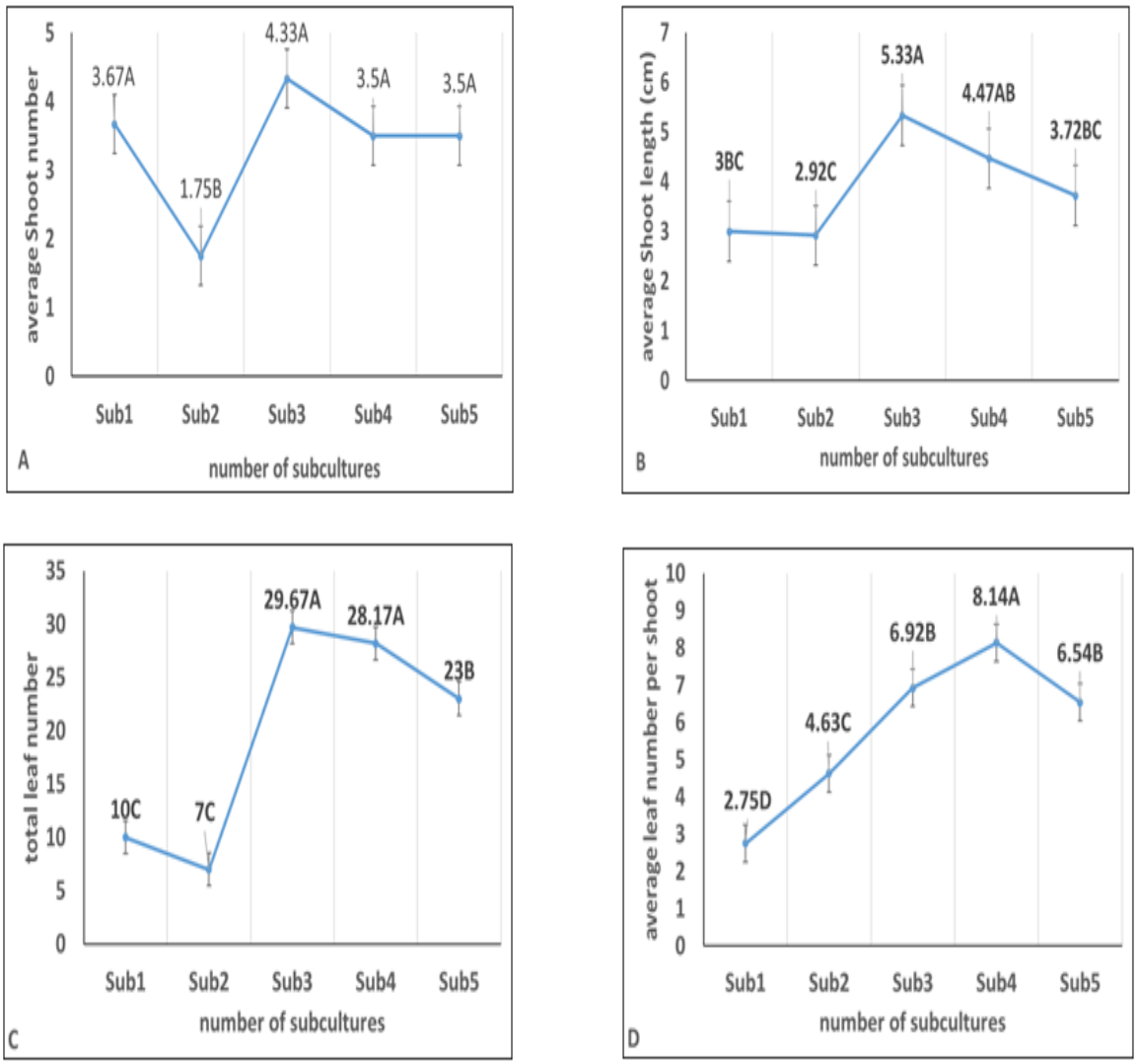

Fig. 7. Effect of subculture number on A) average shoot number B) average shoot length (cm) C) total leaf number D) average leaf number per shoot of Blackberry cultures.

Data in Fig. 7 showed the effect of subculture number on the multiplication rate of Blackberry cultures when considered three months as passage time. It's obvious that the number of proliferated shoots per explant, shoot length and total leaves number were in favor of the 3rd subculture. While the proliferated shoots of the 4th subculture surpassed the other ones in leaf number per shoot. Finally, all obtained micro-shoots of all subcultures were got adventitious roots.

Upon the obtained results, the 3rd subculture scored the highest multiplication rate according to the total number of leaves (29.67) which indicates the total number of axillary buds for the next subculture. In this concern, Gomes et al., 2017, carried out five successive subcultures on four genotypes of Blackberry, the multiplication rate of each genotype showed a fluctuation among the different subcultures. Consequently, 'karaka Black' may have its own behavior. Moreover, the decrease in multiplication rate may occur in the last subcultures due to repeated cut and carryover effect of cytokinins which may cause injury to the tissues. (Vujović et al. 2012)

Data in Table 5 indicate that using the first soil mixture (Peat moss: sand) 1:1 (v: v) or the second soil mixture (Peat moss: perlite) 1: 1 (v: v) in the acclimatization of Blackberry plantlets were recorded 100% survival percentage for both of

TABLE 5. Effect of soil mixture components on the acclimatization of Blackberry plantlets.

Soil mixture	Survival %	Plantlet length (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Leaf Area (cm^2)	SPAD reading
Peat moss + sand	100.00A	1.45A	8.25A	1.38A	1.08A	1.95A	55.25A
Peat moss + perlite	100.00A	0.78B	3.50B	0.75B	0.63B	1.22B	47.70B

Means of each column having the same letter (s) are not significantly different at 5% level.

them. Whereas, the first soil mixture surpassed its counterpart significantly regards plantlet length, leaf number, leaf length, leaf width, leaf area and chlorophyll SPAD reading. Similar results obtained by, Fira et al., 2009 experimented perlite only or in mixture with peatmoss, on the other side, Borodulina et al., 2019 tested sand only or in mixture with vermiculate, in both cases, the mixture contains the water holder (peatmoss or vermiculate) gave better survival results than individual medium (sand or perlite) only.

Conclusion and Recommendation

Regarding the aforementioned results, one can conclude that Blackberry cv. Karaka Black showed promising response to *in vitro* culture, as stem node explants achieved the highest shoot length and leaf number on WPM followed by MS medium. On the other hand, Cytokinins were good stimulators for Blackberry multiplication like BA at 0.4 ppm, Kin at 5.0 ppm and TDZ at 0.1 ppm but the kinetin gave the best plantlets. It's worthy to mention that, Blackberry micro-shoots have ability to form adventitious roots easily without special motivation as the most micro-shoots got well-formed roots in multiplication phase. Consequently, there was no need to make rooting stage as a separate experiment. The multiplication rate slightly decreased after the 3rd subculture, but the cultures appeared in good Fig. as they able to multiplicate for many further subcultures. Also, there was an important point to discuss, the duration time among subcultures, three months of culture date proved to be the most suitable passage time in terms of the number of produced nodes for the next subculture (multiplication rate) with no diminution in micro-shoots health. Finally, the existence of sand in the acclimatization mixture had a good impact compared to perlite as a ventilation factor as Peatmoss + sand 1:1 (v:v) gave the highest plantlet quality during acclimatization. Further studies are required to investigate the performance of Blackberry acclimatized plantlets *in vivo* under Egypt climate and establishing *in vitro* protocols for some other cultivars to enrich the diversity of traits.

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Conflicts of interest

There were no conflicts of interest during this work

References

Aly, A.A., El-Desouky, W. and El-Leel, O. F. A. (2022) Micropropagation, phytochemical content and antioxidant activity of gamma-irradiated blackberry (Rubus fruticosus L.) plantlets. *In Vitro Cellular & Developmental Biology-Plant*, 1-13. https://doi.org/10.1007/s11627-021-10244-7

Asad, S., Arshad, M., Mansoor, S. and Zafar, Y. (2009) Effect of various amino acids on shoot regeneration of sugarcane (Sacchrum officinarum L.). *African Journal of Biotechnology*, **8**(7),1214-1218. Google Scholar

Bobrowski, V.L., Mello-Farias, P. and Petters, J. (1996) Micropropagation of blackberries *(Rubus sp.)* cultivars. *Current Agricultural Science and Technology*, **2**(1),17-20. Google Scholar

Borodulina, I.D., Plaksina, T.V., Panasenko, V.N. and Sokolova, G.G. (2019) Optimization of blackberry clonal micropropagation. *Ukrainian Journal of Ecology*, **9**(3),339-345. Google Scholar

Botez, M., Badescu, Ghe. and Botar, A. (1984) *Cultura Arbustilor Fructiferi,* Ed. Ceres. Bucuresti, pp.180-194.

Broowe, O.C. and Zimmerman, R.H. (1978) *In vitro* propagation of blackberry. *HortScience*, **13**(2),151-153.

Caldwell, JD (1984) Blackberry propagation. *HortScience,* **2**,193-195.

Da Silva, I.A.O. and Biasi, L.A. (2022) Double-phase culture medium and plant growth regulators in the micropropagation of blackberries. *Comunicata Scientiae*, **13**,1-7.https://doi.org/10.14295/cs.v13.3613

Du, X. and Qian, M. (2010) Fractionation and Identification of Aroma-Active Constituents in Thornless Trailing 'Black Diamond'Blackberry. In *Flavor and Health Benefits of Small Fruits*. *American Chemical Society*. **1035**,45-61. http://dx.doi.org/10.1021/bk-2010-1035.ch004

Fallovo C., Cristofori, V., de-Gyves, E.M., Rivera, C.M., Rea, R., Fanasca, S., Bignami, C., Sassine, Y. and Rouphael, Y. (2008) Leaf area estimation model for small fruits from linear measurements. *HortScience*, Dec 1,**43**(7), 2263–2267. https://doi.org/10.21273/HORTSCI.43.7.2263

Fira, A., Clapa, D. and Simu, M. (2014) Studies regarding the micropropagation of some blackberry cultivars. *Bulletin UASVM Horticulture*, **71**(1), 22-37. Google Scholar

Gamborg, O.L., Miller, R.A. and Ojima, K. (1968) Plant cell cultures. I. Nutrient repuirements of suspension cultures of soybean root cells*, Exp. Cell Res*., **50**,150-158. https://doi.org/10.1016/0014-4827(68)90403-5

Gomes,H.T.,Bartos,P.M.C.,Andrade,M.T.D.,Almeida, R.F., Lacerda, L.F.D. and Scherwinski-Pereira, J.E. (2017) In vitro conservation of blackberry genotypes under minimal growth conditions and subsequent large-scale micropropagation. *Pesquisa Agropecuária Brasileira*, **52**, 1286-1290. http://dx.doi.org/10.1590/s0100-204x2017001200018

Hummer, K.E. and Janick, J. (2007) Rubus iconography: Antiquity to the renaissance. Acta *Horticulturae,* **759**,89-106. http://dx.doi.org/10.17660/ActaHortic.2007.759.6

Jungmin, L. (2017) Chapter 4. Blackberry fruit quality components, composition, and potential health benefits. In: Hall H.K., Funt R.C. (Ed.) Blackberries and Their Hybrids. pp.49-62. http://dx.doi.org/10.1079/9781780646688.0049

Kefayeti, S., Kafkas, E. and Ercisli, S. (2019) Micropropagation of'Chester thornless' Blackberry Cultivar using Axillary Bud Explants. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **47**(1), 162-168. https://doi.org/10.15835/nbha47111280

Kereša, S., Habuš Jerčić, I., Batelja, Lodeta, K., Barić, M., Pecina, M., Bošnjak Mihovilović, A., and Zec, M. (2019) Influences of different types of sugar and cytokinin on micropropagation of blackberry cultivar 'Reuben'. *Glasnik Zaštite Bilja*, **42**(3),14-21. https://doi.org/10.31727/gzb.42.3.3

Lloyd, G. and McCown, B. (1980) Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia* by use of shoot tip culture. *Combined Proceeding of the International Plant Propagators' Society*, **30**, 421-427. http://www.scienceopen.com/document?vid=ccd74c91-e730-4965-86f5-e823ebb0e7a8

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, **15**,473- 497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Schuchovski, C.S. and Biasi, L.A. (2017) Development of an efficient protocol for 'Brazos' blackberry in vitro multiplication. *Acta horticulturae*, **1224**, 157-164.https://doi.org/10.17660/ActaHortic.2018.1224.21

Tukey JW (1977) *Exploratory Data Analysis*. Addison-Wesley, chapter 1. Exploratory data analysis, pp. 5- 23. Google Scholar

Vujović, T., Ružić, D.J., and Cerović, R. (2012) In *vitro* shoot multiplication as influenced by repeated subculturing of shoots of contemporary fruit rootstocks. *Horticultural Science*, **39**(3), pp.101-107. http://dx.doi.org/10.17221/208/2011-HORTSCI

Wójcik-Seliga, J. and Wójcik-Gront, E. (2013) Evaluation of blackberry and hybrid berry cultivars new to Polish climate – Short communication. *Hort. Sci. (Prague),* **40**(2), 88–91. https://doi.org/10.17221/1/2012-HORTSCI

الاكثار الدقيق للبلاك بيري *Rubus fruticosus* صنف Karaka Black

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يهدف هذا العمل إلى وضع بروتوكول معملي للإكثار الدقيق للبلاك بيري صنف Karaka Black. تم اختبار منفصلات العقد الساقية على ثلاثة انواع من املاح البيئة (بيئة النباتات الخشبية وموراشيجي وسكوج وبيئة جامبورج) في مرحلة التأسيس. ايضا ثلاث انواع من السيتوكينينات تم اختبارها للتضاعف هي البنزيل ادنين BA (٠,٠، ٠,٤، ٠,٨، ١,٢ جزء في المليون)، الكينيتين Kin (٠,٠، ٠,٠، ١,٠، ٥,٠، ١٠,٠ جزء في المليون) وثيديآزورون TDZ (٠,٠، ٠,١، ٠,٥، ١,٠ جزء في المليون). كما تم دراسة تأثير موعد النقل وعدد النقلات على معدل التضاعف. أجريت مرحلة التأقلم في مخلوط بيتموس ورمل ومخلوط بيتموس وبيرليت (١/١ حجم). أشارت النتائج إلى أن بيئة WPM يليها مباشرة بيئة MS أعطت أعلى طول للأفرع وعدد الأوراق. حققت معاملة البنزيل ادنين عند تركيز ٠,٤ جزء في المليون، الكينيتين عند ٥,٠ جزء في المليون والثيديآزورون عند ٠,١ جزء في المليون أفضل القيم لمرحلة التضاعف، ولكن إضافة الكينتين قد أعطت نبتات أكثر صحة. انتجت معظم الأفرع الناتجة أثناء مرحلة التضاعف جذور جيدة التكوين وبالتالي لم يكن من الضروري إجراء مرحلة التجذير. انخفض معدل التضاعف بشكل طفيف بعد النقلة الثالثة وكان أنسب وقت بين النقلات هو ثلاثة أشهر من تاريخ النقل. وقد أعطى خليط البيتموس والرمل أعلى جودة للنباتات أثناء مرحلة التأقلم.

الكلمات الدالة: بلاك بيري، زراعة الانسجة، الزراعة المعملية، التضاعف، التأقلم.