

Effect of plant growth regulators and type of media on multiplication and rooting of peach rootstock (*Prunus persica* L.) Garnem *In vitro*

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Abstract

Experiments were conducted in one of the private laboratories of Al-Fahama / Baghdad for August 2020 to June 2022 period. This study aimed to achieve an appropriate protocol for propagation the roortstock of peach Garnem (*Prunus persica* cv. Garnem) *In vitro*. The shoot tips and nodes of this rootstock were used as explant for the stages of emergence it was sterilized with several levels of sodium hypochlorite NaOCl (0.5 and 1%) for three periods of 15, 20 and 25 minutes cultured on MS media with free hormone, after which an experiment was conducted to find out the appropriate medium for multiplication, so it was used WPM and MS media added to it the best concentration of Kin. which he reached from previous experiments by interfering with concentrations of BA(0, 1 and 2 mg l⁻¹) in order to multiply the shoots. Use concentrations of IBA for rooting shoots are 0, 0.5, 1, 2 and 3 mg l⁻¹ added to media1/2 WPM and WPM. The results of the study were analyzed statistically as factorial experiments using a completely randomized design (CRD), and the statistical program SAS (2003) was used to analyze the results, and the averages of the coefficients were compared according to Duncan's multiple test in the probability level of 0.05. The results showed efficient use NaOCl in the sterilization of peach rootstock shoot tip Garnem, the best multiplication was achieved when the shoot tips were grown on WPM media added to it 1 and 2 mg l⁻¹BA. also, the highest number and length of shoots were obtained at 1 mg l⁻¹. It gave shoots planted on the 1/2WPM to which a concentration of 2 mg l⁻¹ was added IBA the highest rooting percentage, the highest number of roots, and the highest length of roots.

Keywords: *In vitro*, Sterilization, WPM, Multiplication, Garnem.



Introduction

Garnem rootstock is a hybrid between the Spanish Garfi almond [*Prunus amygdalus* Batsch, syn.p.dulcis (Mill) DA Webb] selected as a mother and the North American Nemared peach [*P. persica* L. Batsch] as pollen donors. These new origins are characterized by their high activity, more tolerant of biotic and abiotic stresses, it tolerates calcareous soils and is resistant to root-knot nematodes and is the ideal rootstock for increasing the efficiency and productivity of orchards (16 and 24). New cultivars are being produced for rootstocks that possess diverse vegetative characteristics for stone fruit crops including peaches, through the use of public and private breeding programs(16 and 9).

Pollution constitutes a real problem that opposes the progress and development of tissue culture technology(31). The surface of plant tissues in its nature contains different types of microbes (bacteria, fungi, molds, etc.), and since the micro propagation technique requires that the explants should be free of any surface pollutants before starting cultivation or before establishing tissue cultures, because these pollutants can grow in the middle of the farm making it inefficient(14). Used(2) four types of sterilizers are sodium hypochlorite NaOCl (5, 10 and 15% for 5 and 10 minutes), hydrogen peroxide H₂O₂ (5 and 10% for 10 and 20 minutes) and fungicide Captan 50% (1, 2, 3 and 4% for 5 minutes) and boric acid 3(COH) at 1 and 2% for 5 and 10 minutes to study the percentage of contamination percentage, survival rate for shoot tips and nodes of peach (*P. persica* L. Batsch) during initiation stage, and it was found that sodium hypochlorite

had the most effective effect with a survival rate of 50% at concentration 15% for 5 minutes and 60% at concentration 10% for 10 minutes. The buds continued to grow strongly and the multiplication stage began. Explain (30) from his experiments through using four sterilization agents on peach root DO-1 *P. domestica*, namely HgCl₂, NaOCl, H₂O₂, and AgNO₃ to reduce the percentage of contamination of axillary buds, and obtained the highest survival rate of 91% when treated with hypochlorite NaOCl at a concentration of 2% for 15 minutes, while the lowest survival rate was 23.33% when treated with mercury chloride HgCl₂ at a concentration of 0.5% within ten days. Added Cytokinins to the nutrient media in stages and multiplication with the aim of stimulating cell division and encouraging the opening of axillary buds to give new branches after eliminating the phenomenon of apical dominance(23 and 15), as cytokinins are among the most important determining factors in the success of plant tissue culture, especially in the reproductive stage proliferation, as it plays multiple roles in plant growth and development, such as stimulating cell division, cell expansion, and protein building, in addition to its role in the effectiveness of the activities of some enzymes (25 and 11). The composition of the nutrient media is one of the main factors that control the success of any tissue culture, and in order to develop any plant part outside the living body after it is separated from the mother plant, the necessary nutritional needs for its growth must be provided, due to the inability of the cultivated parts or tissues to perform the process of photosynthesis in addition to the different nutritional needs according to the type of plant, so the quantities that must be available of



inorganic salts depend mainly on the type of tissue or plant part grown and its developmental stage (27 and 18). Used (26) BA with the aim of stimulating the multiplication of peach shoots Garnem and Myroplan Plum and it obtained the best multiplication at 2 mg l^{-1} , as the number of shoots reached to $11.34 \text{ shoot.explant}^{-1}$. Conducted (19) from their study on the pear plant, Othoman variety, by cultivating the shoot tips on two types of nutrient media: MS and WPM by adding different concentrations of BA (0, 1, 2, 3 and 4 mg l^{-1}) and Kin. At 0, 2, 4, 6 and 8 mg l^{-1} , it was clear from this study that WPM media was significantly superior to MS medium in the traits of the number of shoots which achieved to $5.60 \text{ shootsexplant}^{-1}$, and the number of leaves ($37.01 \text{ leaves explant}^{-1}$) after 8 weeks from cultivation, as for adding the Kin. It was clear from the results that there were no significant differences between the two mediums except for the lengths of shoots, in which the MS medium recorded the highest length of the shoots was 2.60 cm. Recorded(6) through his experiment on the peach rootstock GF677 by cultivating the nodes of this rootstock on three types of nutritional media: MS, WPM, and DKW, to which different concentrations of BA (0, 0.5, 1, and 2 mg l^{-1}) were added, the best multiplication it reached 100% when growing the shoots on MS medium supplied with 2 mg l^{-1} , and did not differ significantly with the concentration 2 mg l^{-1} that was added to WPM medium, and the percentage reached 87.5%. shoots length (2.4 cm). Complete the formation of roots on the shoots resulting from the multiplication stage by stimulating the initiation of adventitious roots from the base of the shoots (13), as the resulting vegetative growths can be invested in new

propagation stages after cutting them and planting them on auxin-containing media, and thus the numbers of these plants can be increased according to a numerical sequence, and the selection of a suitable growth regulator for rooting is taken into account, often IBA(4). Indicated(1) in his experiment on pear plant (Osmani variety), that $1/2 \text{ WPM}$ medium was superior to the other media that had been used in the percentage of rooting, the number of roots and their length, which reached to 40%, $2.81 \text{ root shoot}^{-1}$ and 1.72 cm, respectively. Demonstrated (7) from their study which used three types of nutrient media (MS, $1/2 \text{ MS}$ and WPM) plus different concentrations of IBA (0, 0.5, 1, 2 and 3 mg l^{-1}) to find out their effect on rooting of peach rootstock Garnem and it was found that the highest number of roots were obtained in $1/2 \text{ MS}$ supplemented with 2 mg l^{-1} IBA, as the number of roots reached $6.2 \text{ root shoot}^{-1}$, and the highest average length of roots was in the same medium except that it contained 1 mg l^{-1} IBA. Reported(12) from their experiment on kiwi using concentrations of IBA the best rooting was at 1 mg l^{-1} , it gave the highest number of roots reaching to $6.10 \text{ root shoot}^{-1}$ and the highest length of roots was 2.45 cm.

The aims of this study is to find a suitable protocol for peach rootstock propagation Garnem through using of appropriate combinations of nutrient media and hormones to increase multiplication and rooting In vitro.

Materials and Methods

This experiment was conducted in one of the private laboratories in Baghdad / Al-Fahhama, as they used cuttings of juicy stems of plants cultivated in the Taj Al-Din station of El-ssaouira and



growing under field conditions for the period from August 2020 to June 2022, Control program was used for mother plants using 1 ml l⁻¹ of pesticides Beltanol at a rate of one watering every 10 days. Pans with a length of 5 cm were taken from the new growths, their leaves were removed and divided into two types of explants, shoot tips with a length of 1 ± 0.2 cm and nodes with a length of 0.5 - 1 ± 0.2 cm from the second and third nodes after developing summit. The explants were washed with water and liquid soap to ensure the removal of dirt, dust and suspended materials, then left to wash with running water for half an hour and then transferred to the laminar air flow table to start the surface sterilization process, The explants were sterilized with sodium hypochlorite solution NaOCl concentration at 0.5% (dilute 0.5ml each 9.5ml sterile distilled water) and 1% (at a dilution of 1 ml per 9 ml distilled water) for three periods 15, 20 and 25 minutes with continuous stirring of the explants and then washed with sterile double distilled water for 4 times to 5 minutes each time to remove traces of the sterile substance(27). The shoots were obtained from previous experiments were taken and cultured on two types of nutrient MS media and WPM added to them the best concentration of Kin. which was reached from previous experiments overlapping with several concentrations of BA 0, 1 and 2 mg l⁻¹ to determine the best medium and the best combination in multiplying the cultivated explants, represented by calculating the number of shoots for each cultivated explants, the length of the shoots (mm), the number of total leaves, the percentage of multiplication, the number of shoots and their length, and the number of leaves after 4 weeks of cultivation. Then, the shoots resulting from the

mentioned multiplication experiments, 1-2 cm long, were taken and cultivated on WPM media with different concentrations of salts (1/2 WPM and WPM) plus different concentrations of IBA 0, 0.5, 1, 2 and 3 mg l⁻¹ with 5 replications for each treatment and one shoot for each replicate to show their effect on the number of days required for rooting, the rooting percentage, the number of roots, the length of roots (mm), the rate of callus formation and the size of callus after 6 weeks of cultivation. Use prepared nutrient medium MS (20) and WPM in experiments, then it was distributed in glass bottles of 300 ml capacity, at the rate of 30 ml for each bottle, and the pH number was adjusted to 5.7 – 5.8, then it was sterilized at a temperature of 121 C and a pressure of 1.04 kg cm⁻², using an autoclave for 15 minutes. All experiments were incubated in the growth chamber at a temperature of 25 ± 1 °C and a light intensity of 2000 lux for 16 hours of light and 8 hours of darkness on Day⁻¹, and the results were taken after 4, 6 and 8 weeks of cultivation. The CRD (Completely Randomized Design) was used in the implementation of the experiments, and the averages were compared according to Duncan's multiple limit test at the probability level of 0.05(3), and the ready-made program SAS (28) was used to analyze the data.

Results and Discussion

Effect of surface sterilization and its duration on the percentage of contamination and the survival buds of explants, The results in Table 1 indicate the efficiency of the sterilization process when using different concentrations of commercial bleach, as it is noted that there are no significant differences between the



concentrations of bleach used and periods in the percentage of contamination,. Table 1 also shows the effect of the concentration of the minor on the duration of soaking, and it is noted that there are significant differences between the treatments in in the same table in survival buds, as

the concentration of 5% with a soak for 20 minutes recorded the highest percentage of survival buds 100%, while the lowest percentage of survival buds was obtained when the treatment with 10% for a period of 25 minutes of soaking reached to 55%,

Table 1. Effect of different concentrations of NaOCl In the percentage of contaminated and the survival buds (%) for the shoot tips and nods of the peach rootstock Garnem after 4 weeks have passed from cultivation on MS media hormone free

Treatments & exposure times (minutes)	Contaminated buds %	Survival buds %
0.5% NaOCl for 15 minutes	5.000 a	95.00 ab
0.5% NaOCl for 20 minutes	0.000 a	100.00 a
0.5% NaOCl for 25 minutes	0.000 a	70.00 bcd
1% NaOCl for 15 minutes	0.000 a	65.00 cd
1% NaOCl for 20 min	0.000 a	85.00 abc
1% NaOCl for 25 min	5.000 a	55.00 d

* Coefficients that share the same letters for each factor or their overlaps, there are no significant differences between them according to Dunkin's multinomial test at the probability level of 0.05

The reason for the varying response of the plant parts may be due to the fact that increasing the concentration of the active substance and increasing the duration of exposure has a harmful effect on the plant

parts, and this harmful effect of sodium hypochlorite is due to acid Hypochlorous (HOCl) which is a strong oxidizing substance, as this acid is formed as a result of the solubility of chlorine with water(22)

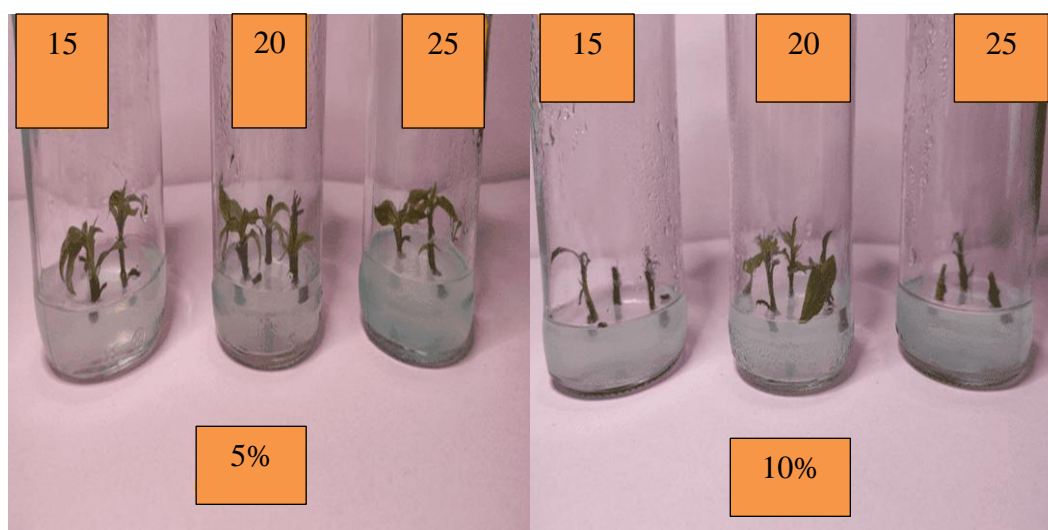
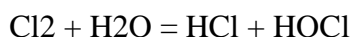


Figure 1. Concentrations NaOCl and the duration of exposure to the shoot tips and nodes of the peach rootstock Garnem4 weeks after cultivation

Media type influence and BA in multiplication of shoot tips and nodes of peach rootstock Garnem Multiplication ratio (%): The results are shown in the Table2 superiority WPM media recorded the highest multiplication ratio reached to 83.33% compared to 50% for MS media, and about impact the explant the shoot tips

recorded the highest multiplication ratio reached to 100%, compared to 33.33% for the contract, and with regard to the effect of BA the concentrations 1 and 2 mg l⁻¹ were successful in obtaining multiplication ratio, which amounted to 75% for each of them, while the media free from it failed to multiply.

Table 2. Effect of explant and type of nutrient media provided with the best concentration of Kin. in addition to different concentrations of BA in multiplication ratio for explants of peach rootstock Garnem4 weeks after cultivation

Type of media	Type of explant	BA concentration mg l ⁻¹			Type of media * Type of explant
		0	1	2	
WPM	Shoot tip	100 a	100 a	100 a	100 a
	Nod	0.000 b	100 a	100 a	66.67 b
MS	Shoot tip	100 a	100 a	100 a	100 a
	Nod	0.000 b	0.000 b	0.000 b	0.000 c
					Effect of media
Type of media * BA mg l ⁻¹	WPM	50 b	100 a	100 a	83.33 a
	MS	50 b	50 b	50 b	50.00 b
					Effect of explant
Type of explant * BA mg l ⁻¹	Shoot tip	100 a	100 a	100 a	100 a
	Nod	0.000 c	50 b	50 b	33.33 b
Effect of BA mg l ⁻¹		50 b	75 a	75 a	

* Coefficients that share the same letters for each factor or their overlaps, there are no significant differences between them according to Dunkin's multinomial test at the probability level of 0.05

It may be attributed to the superiority of the shoot tips planted on the 1/2WPM media over those planted on MS media for all concentrations BA to nutrient and hormonal balance of the shoot tips grown in 1/2WPM, it may also be due to the different types of salts and their concentrations between the two mediums, as indicated (17) the importance of nutrient salts and plant

hormones as they are considered among the determinants of plant growth and development, and that the mutual influence between them plays a major role in guiding growth and development. These results are in line with what he found (19) from their experiment on pear plant, Osomani variety, which showed moderate superiority WPM on MS media in



multiplication and(6) from his experiment on the origin of peaches GF677 which indicated that the best multiplication was in WPM to which a concentration of 2 mg l⁻¹ was added BA.

Number of shoots: Table 3 explains that a significant increase was obtained when using WPM media in compare with MS media that gave the highest number of shoots, which reached 3.097 shoot explant⁻¹ WPM media compared to 1.833 shoots explant⁻¹ for MS

media, as for the effect of the type of explant, the shoot tips gave the highest number of shoots, amounting to 3.430 shoot explant⁻¹ compared to 1.500 shoot explant⁻¹ for the node, and with regard to the effect of concentrations of BA the concentration 1 mg l⁻¹ recorded the highest number of shoots, reaching 3.125 shoot explant⁻¹, outperforming the treatment of medium devoid of BA and the medium supplemented with a concentration 2 mg l⁻¹, which gave the least number of shoots reached 2.125 shoot explant⁻¹.

Table 3. Effect of explant and type of nutrient media provided with the best concentration of Kin. in addition to different concentrations of BA in average number of shoots peach rootstock Garnem4 weeks after cultivation

Type of media	Type of explant	BA concentration mg l ⁻¹			Type of media * Type of explant
		0	1	2	
WPM	Shoot tip	4.250 b	5.000 a	3.331 cd	4.194 a
	Nod	1.000 f	3.000 d	2.000 e	2.000 c
MS	Shoot tip	2.331 e	3.500 c	2.168 e	2.666 b
	Nod	1.000 f	1.000 f	1.000 f	1.000 d
					Effect of media
Type of media * BA mg l ⁻¹	WPM	2.625 b	4.000 a	2.666 b	3.097 a
	MS	1.666 c	2.250 bc	1.584 c	1.833 b
					Effect of explant
Type of explant * BA mg L ⁻¹	Shoot tip	3.291 b	4.250 a	2.749 b	3.430 a
	Nod	1.000 d	2.000 c	1.500 cd	1.500 b
Effect of BA mg l ⁻¹		2.145 b	3.125 a	2.125 b	

* Coefficients that share the same letters for each factor or their overlaps, there are no significant differences between them according to Dunkin's multinomial test at the probability level of 0.05

Branches Length (mm): Table 4 shows the effect medium type used and notes the superiority WPM media which giving it the longest length for the shoots, it reached 9.266 mm, compared with 7.465 mm for MS media, as for

the effect of the explant, the nodes excelled by giving it the highest length of the shoots, which reached 8.837 mm, compared to 7.895 mm for the shoot tips.as for the effect of BA it is noted that the concentration 2 mg l⁻¹



was superior in recording the highest length of the shoots, which reached 9.333 mm, while the media with free hormone gave the lowest length of shoots reached 7.497 mm. Number of leaves: Table (5) shows impact the type of media in terms of the number of leaves, and it is noted that there are no significant differences between MS media and WPM media, as for the impact the vegetative part has outgrown the tips of the shoots, the contract recorded the highest number of leaves, amounting to 23.70 leaves, explant⁻¹ for 14.42 sheets to contract, concerning impact BA concentration was higher than 2 mg l⁻¹ by recording the highest number of leaves, it reached 21.64 leaf, while the lowest number was in media free from BA reached 6.73 leaves explant⁻¹. The difference in the response of explant may be due to multiplication when cultivated on MS and WPM media to lower nitrogen concentration in the WPM media which is approximately a quarter of its concentration in the MS media which made it more efficient to multiply shoot tips(5), as well as the high concentrations of BA in the

multiplication stage, it has an inhibitory effect on the multiplication and growth of peach shoots^[8], as the positive effect of multiply is the result of an interaction between the salts of the WPM media and BA at 1 mg l⁻¹, it may be due to the explant gaining the optimal physiological state of activity from this overlap if taking into account the different quality and composition of the salts of the media and their different concentrations, such as the formula of nitrogen and calcium constituting a WPM media represented by a substance Ca(NO₃) which are more ready for the plant compared to NH₄NO₃ and CaCl₂ located in the MS media(29), Or it may be due to the positive effect of the presence of cytokinins in the medium, which led to increased branching through its role in reducing apical dominance and thus encouraging buds to grow through its main and important contribution to the production of nucleic acids and amino acids because it increases the production of transporter DNA (rRNA) it also reduces acid hydrolysis by decreasing enzyme activity RNase (15 and 21) .

Table 4. Effect of plant part and type of nutrient media provided with the best concentration of Kin. in addition to different concentrations of BA to average length of shoots (mm) peach rootstock Garnem 4 weeks after cultivation

Type of media	Type of explant	BA concentration mg l ⁻¹			Type of media * Type of explant
		0	1	2	
WPM	Shoot tip	9.709 bc	9.119 c	7.310 d	8.713 ab
	Nod	5.260 f	10.46 b	13.74 a	9.820 a
MS	Shoot tip	7.810 d	7.120 de	6.301 e	7.077 c
	Nod	7.210 de	6.370 e	9.980 bc	7.853 bc
					Effect of media
Type of media * BA mg l ⁻¹	WPM	7.484b	9.789 a.d	10.525 a.d	9.266 a
	MS	7.510 b.d	6.745b	8.141b	7.465 b
					Effect of explant
Type of explant * BA mg l ⁻¹	Shoot tip	8.759 b	8.119 b	6.806 c	7.895 b
	Nod	6.235 c	8.415 b	11.86 a	8.837 a
Effect of BA mg l ⁻¹		7.497 c	8.267 b	9.333 a	

* Coefficients that share the same letters for each factor or their overlaps, there are no significant differences between them according to Dunkin's multinomial test at the probability level of 0.05

These results are consistent with the findings of [19] from their experiment on pear plant, Osmani variety, which

showed moderate superiority WPM on medium MS in multiplication and (6) from his experiment on the peaches

Table 5. Effect of explant and type of nutrient medium provided with the best concentration of Kin. in addition to different concentrations of BA average number of leaves for peach root cultivar Garnem4 weeks after cultivation

Type of media	Type of explant	BA concentration mg l ⁻¹			Type of media * Type of explant
		0	1	2	
WPM	Shoot tip	18.92 d	22.50 c	22.00 c	21.14 b
	Nod	7.000 g	17.30 d	27.75 b	17.35 c
MS	Shoot tip	31.50 a	23.50 c	23.83 c	26.28 a
	Nod	9.500 fg	12.00 ef	13.00 e	11.50 d
Effect of media					
Type of media * BA mg l ⁻¹	WPM	12.96 c	19.90 ab	24.88 a	19.25 a
	MS	20.50 ab	17.75 bc	18.42 b	18.89 a
Effect of explant					
Type of explant * BA mg l ⁻¹	Shoot tip	25.21 a	23.00 ab	22.92 ab	23.71 a
	Nod	8.250 d	14.65 c	20.38 b	14.43 b
Effect of BA mg l ⁻¹		16.73 c	18.83 b	21.65 a	

* Coefficients that share the same letters for each factor or their overlaps, there are no significant differences between them according to Dunkin's multinomial test at the probability level of 0.05

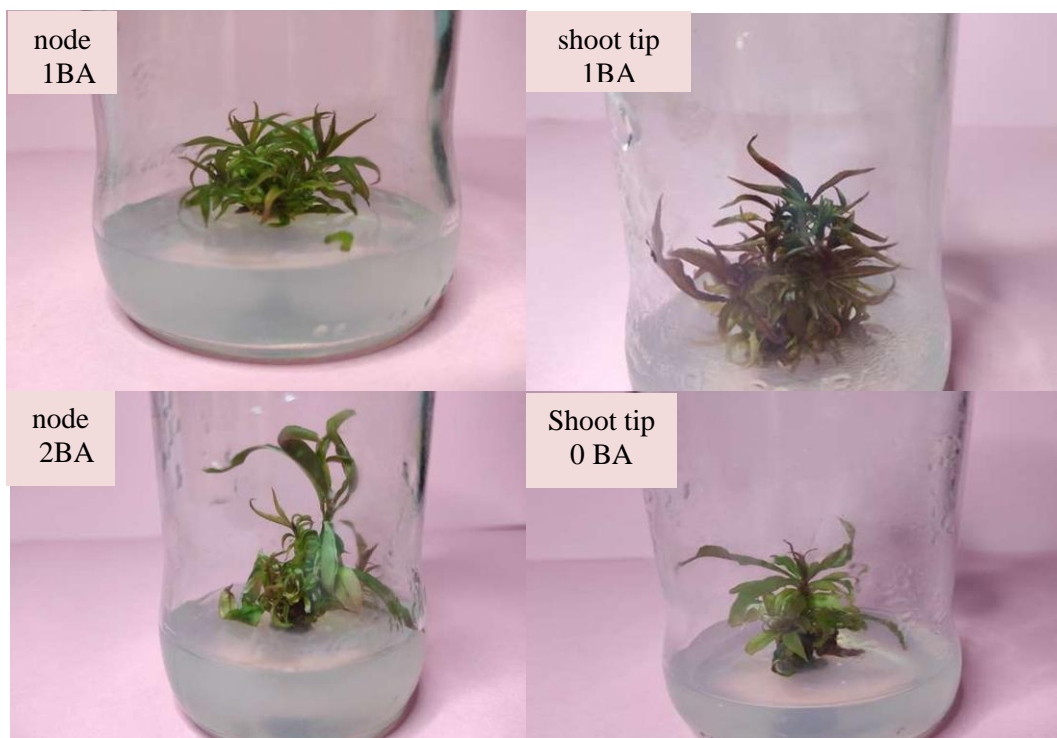


Figure 2. the shoot tips and the nodes of the peach rootstock Garnem planted in WPM media adding Kin. at 6 mg l⁻¹ with BA in 1 and 2 mg l⁻¹ after 4 weeks of cultivation

rootstock GF677 which indicated that the best multiplication was in the WPM media to which a concentration of 2 mg l⁻¹ was added BA. It is also consistent with his findings (26) in their experiment on the peaches rootstock Garnem and Myroblan plum to be better multiply was when using BA at 2 mg l⁻¹.

The effect of the strength of the salts of the nutrient media WPM and levels IBA in the rooting of micro shoots

Number of days for rooting (day): It is noted from the results in Table 6 that the 1/2 WPM it gave rooting within 9,300 days of cultivation while in WPM media gave rooting within 14.92 days of cultivation, and with regard to the effect of concentrations of IBA a concentration of 0.5 mg l⁻¹ recorded rooting within 11.25 days of cultivation, and a concentration of 3 mg l⁻¹ gave rooting within 21.50 days of cultivation, while the shoots grown on hormone-free media failed to stimulate rooting. As for the effect of the interaction between concentrations of IBA and salts of the nutrient media WPM it formed shoots cultured on WPM in addition to the hormone, at 3 mg l⁻¹, roots were formed within 19 days of cultivation, and the shoots grown on WPM the hormone added to it at a concentration of 1 mg l⁻¹ took root within 31 days of cultivation, while the shoots grown on hormone-free media were not recorded in stimulating rooting. The same applies to the shoots grown on WPM media added IBA At a concentration of 0.5 mg l⁻¹, as well as the shoots grown on 1/2 WPM media to

which the concentration of 1 and 2 mg l⁻¹ was added IBA.

Rooting ratio: Data in Table 6 indicate that the shoots grown on WPM media at full strength, its salts gave the highest rooting rate 18%, and it did not differ significantly from 1/2 WPM media in which the rate of rooting reached 12%, as for the effect of concentrations IBA the concentration of 2 mg l⁻¹ recorded the highest rooting rate 35%, outperforming the treatment of 0.5 mg l⁻¹ and the comparison treatment, in which no root emergence rate was recorded, and the effect of the interaction between the concentrations of IBA and salts of the nutrient medium WPM the highest rooting percentage reached 50% for the shoots planted on WPM media with the addition of the hormone at a concentration of 2 mg l⁻¹, it was significantly superior to the control treatment that did not record any root formation.

number of roots: The results are shown in Table 6 of the mean superiority WPM media significantly, by giving it the highest number of roots, it reached 2.466 root shoot⁻¹, compared to 1.560 roots shoots⁻¹ for 1/2 WPM media, with regard to the effect of concentrations IBA, the concentration of 2 mg l⁻¹ recorded the highest number of roots, reaching 3,550 root shoots⁻¹, outperforming all treatments, while the hormone-free media failed to give roots to the shoots planted in it. As for the effect of the interaction between the concentrations of IBA and WPM media



it gave shoots planted on the WPM to which the hormone was added at 2 mg l⁻¹ the highest number of roots reached 7.100 roots shoot⁻¹, which was significantly superior to the rest of the treatments, while the hormone-free media failed to form roots WPM to which a concentration of 0.5 mg l⁻¹ was added IBA So is the 1/2 WPM to which the concentration of 1 and 2 mg l⁻¹ was added. Roots length (mm): Table 6 shows that the WPM media gave long root length was 4.794 mm, significantly

superior to 1/2 WPM media in which the root length reached 1.598 mm, and the effect of concentrations IBA it is noted that its addition had a significant effect on increasing the length of the roots, reaching a maximum of 5.245 mm at 2 mg l⁻¹, outperforming the control treatment, while the shoots grown on hormone-free media failed to form roots in addition to the shoots grown on WPM media added IBA at 0.5 mg l⁻¹

Table 6. Effect of the strength of the medium salts WPM and IBA levels in rooting tissue of Garnem peach rootstock after 6 weeks of cultivation

Traits Treatments	Days to roots	Root %	Number of roots	root length mm	Callus training %	
Effect of explant type						
WPM	14.92 A	12 A	2.460 A	4.794 A	20 A	
1/2 WPM	9.30 A	6 B	1.560 B	1.598 B	6 B	
Effect of IBA mg l⁻¹						
0	0.00 D	0.000 B	0.000 E	0.000 E	0.00 C	
0.5	11.25 C	10 AB	2.000 C	2.730 D	5 BC	
1	15.50 B	5 AB	1.500 D	4.475 B	5 BC	
2	12.30 C	20 A	3.550 A	5.245 A	25 AB	
3	21.50 A	10 AB	3.000 B	3.530 C	30 A	
IBA * Explant type						
0	WPM	0.00 d	0.00 b	0.000 e	0.000 f	0.00 c
	1/2 WPM	0.00 d	0.00 b	0.000 e	0.000 f	0.00 c
0.5	WPM	0.00 d	0.00 b	0.000 e	0.000 f	0.00 c
	1/2 WPM	22.50 b	20 ab	4.000 b.c	5.459 c	10 c
1	WPM	31.00 a	10 b	3.000 c	8.950 b.d	10 c
	1/2 WPM	0.00 d	0.00 b	0.000 e	0.000 f	0.00 c
2	WPM	24.60 b	40 a	7.100 a	10.490 a	50 a
	1/2 WPM	0.00 d	0.00 b	0.000 e	0.000 f	0.00 c
3	WPM	19.00 c	10 b	2.200 d	4.530 d	40 ab
	1/2 WPM	24.00 b	10 b	3.800 b	2.530 e	20 bc

*Coefficients that share the same letters for each factor or their overlaps, there are no significant differences between them according to Dunckin's multiple range test at the level of probability 0.05

as well as the shoots grown on 1/2 WPM media and the hormone added to it at 1 and 2 mg l⁻¹.

Callus ratio: The results are shown in Table 6 to mean that WPM The highest rate of callus formation was recorded at 20%, surpassing that of medium 1/2 WPM in which the percentage of callus formation was 6%, as for the effect of concentrations IBA the concentration of 3 mg l⁻¹ gave the highest rate of callus formation amounting to 30%, while no callus formation was observed in the shoots grown on a hormone-free medium. As for the effect of the interaction between the concentrations of IBA and WPM it is noted that the concentration of 3 mg l⁻¹ added to the WPM it gave the highest percentage of callus formation reaching 40%, while callus formation was not observed in the hormone-free media as well as WPM full strength of its salts with the hormone added at a concentration of 0.5 mg l⁻¹ in addition to 1/2 WPM media to which the concentration of 1 and 2 mg l⁻¹ was added IBA. The positive effect of rooting may be attributed to reducing the nitrogen

concentration. On the other hand, the total nitrogen content in the nutrient medium affects the phenotypic composition WPM few when compared to its concentration in the MS media it is noted that the dilution of the media has made the nitrogen concentration less than the normal value for it and thus led to a decrease in nitrogen to less than the appropriate concentration (10 and 11), as well as the presence of auxin in the nutrient medium, which may make the interaction of factors combined lead to an increase in the rate of the number of roots formed in the medium, or to the harmony of the added concentrations of IBA with the existing hormones in the shoots, which contributed to the formation of the highest number of roots for each shoots (1). These results are in line with what have reached (7) in its experiment on the origin of peach Garnem and (1) from his experiment on pear plant, Osomani variety, who concluded that the best rooting percentage was the result of using a WPM media it is also in line with the findings of (12) when using IBA at a concentration of 1 mg l⁻¹ in rooting kiwi shoots.

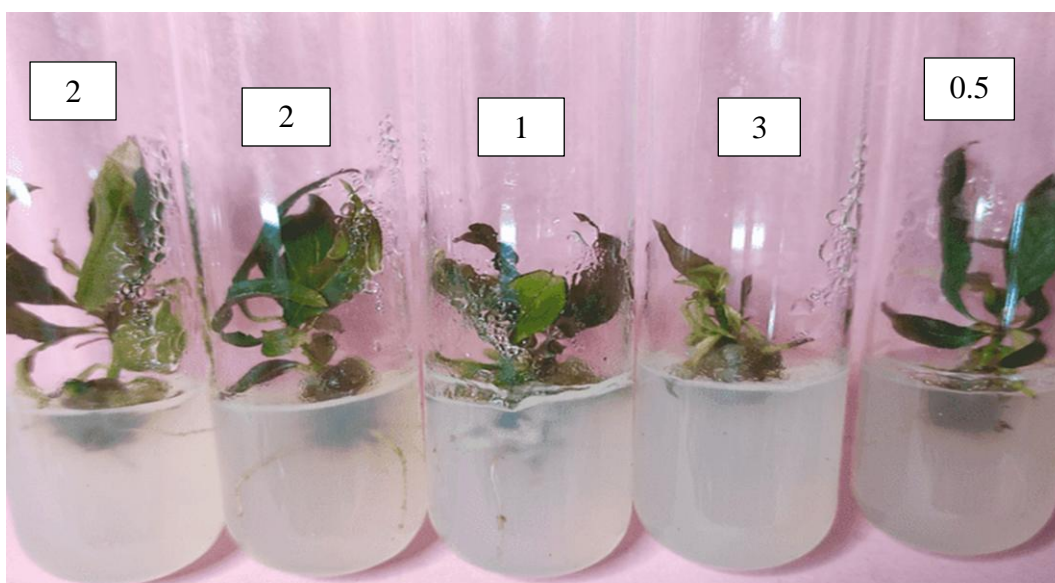


Figure 3. Micro shoots cultured on WPM media added concentrations of IBA 6 weeks after cultivation

Conclusion

There are avariable effects among BA concentration on shoot multiplication, so we recommended using BA at 1 mg l⁻¹ interfering with Kin. at 6 mg l⁻¹ for multiplication using WPM media. For rooting stage we recommend using IBA at 2 mg l⁻¹ addition to WPM media.

Conflict of interest

The authors declare no conflict of interest.

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