Processing browning and increasing multiplication and rotting of Peach rootstock cv. Garnem by using antioxidants and plant growth regulators in vitro

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Abstract

The study aimed to find an accurate propagation program for the rootstock of peach *Prunus persica* cv. Garnem. The apical shoots and nodes of this rootstock were used as explants for the stages of initiation and multiplication, and they were planted on MS medium after adding different concentrations of Active Charcoal (AC), Polyvinyl Pyrrolidone (PVP), and Benzyl adenine (BA). The shoots were planted for the purpose of rooting on MS medium containing different concentrations of salts (1/4 MS, 1/2 MS and full MS salts) with different concentrations of IBA. In the establishment phase, the apical shoots grown on medium supplied with AC were given the highest response rate, the highest average length of plantlet, and the highest number of leaves. The best multiplication of explants in terms of the number of shoots and their length were obtained when cultivated on a medium supplemented with BA at 1 and 2 mg.L^{-1}, whereas the application of BA at 1 mg. L-1 to the cultivated nodes gave the highest number of leaves. The best rooting percent was obtained when plantlets were s treated with 2 mg. L^{-1} was applied to 1/4 MS medium. The highest number of roots was obtained from plantlets grown in 1/2 MS medium treated with IBA at 3 mg.L^{-1}, whereas the longest root was obtained from plantlets grown in 1/2 MS medium treated with 1 mg.L^{-1}IBA.

Keywords**:** Garnem, Antioxidant, Micropropagation, Cytokines, PVP

Introduction

Garnem is a hybrid of Spanish almonds Garfi *Prunus amygdalus* Batsch, syn. *P. dulcis* (Mill) DA Webb by selecting as mother and north American Nemaguard peach [*P. persica* L. Batsch] as pollen donors, these new origins are characterized by being highly active, more tolerant to biotic and abiotic stresses, calcareous soils, and are tolerant and resistant to root-knot nematodes, which is the ideal rootstock for increasing the efficiency and productivity of orchards (12 and 25). Browning is a common problematic phenomenon in plant tissue cultures, and it has been recorded in many fruit studies such as peaches (20), pears (22), and berries (34). Browning in plant tissue culture refers to a phenomenon carried out by the excised plant that releases brown matter or phenols into the medium from its tissues during the process of de-differentiation and/or differentiation (14). Added cytokinins to the nutrient media in the initiation and multiplication phases with the aim of stimulating cell division and encouraging the opening of the axillary buds to give new shoot after eliminating the phenomenon of apical dominance(18 and 25), as cytokinin is one of the most important factors determining the success of plant tissue culture, especially in the proliferation stage, as they play multiple roles in growth and development of plants, such as stimulating cell division, cell expansion, protein building, in addition to their role in the effectiveness of the activities of some enzymes(13 and 26).Auxines are plant hormones that are used in many aspects of plant growth and development, and one of the auxins used in this field is indole-3-butyric acid IBA, which is the first plant hormone to be used for enhancement of rooting cuttings, currently used commercially to root many types of plants all over the world (9 and 16). Numerous studies have been conducted in this field, including the findings of Singh and Patel (29) through cultivation of the pomegranate plant (*Punica granatum* L.) on MS medium supplemented with 100, 200, and 300 mg.L^{-1}AC and 5, 10, and 15 mg.L^{-1}PVP and observed a decrease in the appearance of phenols (browning) at 200 mg.l^{-1}AC and 15 mg.L^{-1}PVP. Noticing from his experiment (32) on the kiwi plant Dingdong cultivated stem sections of this plant containing two buds in MS medium supplemented with PVP at 0, 100, 200, and 300 mg.$L^{-1}$and AC 0, 50, 10, 0, and 200 mg.$L^{-1}$and it was found that the growth rate increased when using PVP at a concentration of 300 mg.$L^{-1}$and reached 50%, the lowest percentage of browning appeared in farms containing AC at 100 mg.$L^{-1}$was 3.33%.

It was referred, by Ak *et al*. (4) in their experiment on the peaches rootstock Garnem that using the apical shoots and nodes of this plant and growing them on MS medium to which different concentrations of BA were added (0.5, 1, and 2 mg.L^{-1}) which the best multiplication was achieved when cultivating the nodes in the media that contained 2 mg.L^{-1}, as the number of shoots reached to 4.63 shoots.explant^{-1}. As for apical shoots, the proportion of the parts suitable for taking and replanting them reached 21.30% at the same concentration. It was explained by Humdy and Obaid that a node of peach rootstock Garnem containing one axillary bud was used and cultured on MS supplemented with BA (0, 0.5, 1, 2 mg.L^{-1}) and found that BA in 2 mg.L^{-1} gave the best multiplication reached to 2 shoots.explant^{-1}, the highest length reached 2.42 cm, and the highest number of leaves reached 5.27 leaves explant^{-1}. Referred Salman (27) in their experiments on Myroblan plum and Garnem rootstock that using MS medium supplemented with 2mg.L^{-1} BA gave the

highest number of the shoot (11.34 shoots.explants^{-1}). Using 1/2 MS supplemented with IBA(1) for rooting peach rootstock Nemaguard obtained the highest rooting rate of 90% in this medium enhanced with a concentration of 3 mg.L^{-1}compared to the hormone-free half-strength media, and also obtained the highest number of roots, the longest root length was 8.5 cm at the same concentration. It was included (4) from their experiment on peach rootstock root Garnem that the highest rooting percentage was in MS medium at half strength containing a concentration of 2 mg.L^{-1}IBA reaching 42.8%, while a concentration of mg.L^{-1} for the same medium gave the highest number of roots reached 3.10 root.shoot^{-1}.Using MS medium supplemented with IBA (15) for rooting of Kiwifruit *Actinidia deliciosa* and found that concentration 1 mg.L^{-1} gave the highest number of roots 6.10 roots. Shoots-1 and the highest length of roots is 2.45 cm.

The objectives of this study were to find out the best method for decreasing the browning problem and increasing the multiplication performance of Garnem peach rootstock explants *in vitro*.

Materials and Methods

Tissue culture experiments were carried out in one of private the tissue culture laboratories in Baghdad / Al-Fahhama, as they used stem cuttings of plants cultivated in the Taj Al-Din station of El-ssaouira growing under field conditions, the cuttings with a length of 5 cm were taken from the new growths, their leaves were removed, and they were divided into two types of explants, the apical shoots with a length of 1±0.2 cm and single nodes with a length of 0.5–1±0.2 cm from the second and third nodes after the developing apex. Explants were sterilized with sodium hypochlorite solution NaOCl 6% concentration (diluted 1 ml per 9 ml sterile distilled water) for 20 minutes with continuous stirring, then washed with sterile double distilled water 4 times for 5 minutes each time to remove traces of the sterilizing substance (28). AC (Activated Charcoal) was used at a concentration of 1.5 mg.L^{-1}and PVP (Polyvinyl Pyrrolidone) at 600 mg.L^{-1}by 10 replications in each vial, culturing an apical shoot and node to see its effect on the response rate, plant length and a number of leaves. Different concentrations of BA (6-benzyl adenine) were used (0, 0.5, 1, and 2 mg.L^{-1}) with 6 repetitions. Apical shoots and nodes were planted in each bottle to see its effect on the response percentage, the length of plantlets, the number of shoots, shoot length, and the number of leaves. As for rooting, a medium was used. 1/4MS quarter, 1/2, MS and a full concentration of salts added to it IBA at different concentrations of 0, 0.5, 1, 2, and 3 mg.L^{-1}to see its effect on the response to rooting, the number and length of roots, and the percentage of callus formed, with 5 replications. Use prepared nutrient medium MS (23) in experiments, then distributed in glass bottles of 300 ml capacity, at the rate of 30 ml for each vial, and adjusted the pH number 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 degrees±1m^{o} the illumination intensity was 2000 lux for 16 hours of light and 8 hours of darkness on Day, and the results were taken after 4, 6, and 8 weeks of cultivation. Use complete random design CRD (Completely Randomized Design) in the implementation of the experiments, and the comparison between the averages was made according to Duncan's multiple range test, at the level of probability 5% (7), and the ready-made program was used for data analysis (29).

Results and Discussion

The effect of antioxidants on apical shoots and nodes response

Results presented Table1 indicate there were no significant differences between the apical shoots and nodes in terms of their response to growth, and with regard to the effect of AC and PVP, it turns out that the medium to which AC was added gave the highest response rate of 100%, outperforming the medium PVP and medium without addition (comparison), in which the response rates were 70 and 60% respectively. As for the effect of the interaction between antioxidant substances and the type of explant, it appears from the results presented below that there are clearly significant differences between the treatments. circles added to it PVP which recorded a similar rate of 100% for them, while the nodes planted on media free from any addition (comparison) gave the lowest response percentage of 40% and didn't differ significantly from the apical shoots and nodes cultivated on a medium equipped with a substance PVP.

The results presented in Table 1 show that the apical shoots were significantly superior in giving them the long length of the plantlets, amounting to 8.585 mm, outperforming the nodes that recorded the lowest length of 6.818 mm. PVP is noted in the same table that media added to AC were significantly superior as it gave the highest increase in the length of new growths amounting to 9.066 mm, while the media devoid of any addition recorded the least increase in the length of new growths amounting to 6.220 mm. Table 1 indicates that there are significant differences in the interaction between the type of explant and the antioxidants. Also, it was noticed that the highest increase in the length of new growths was when apical shoots were cultivated on media with AC and reached to10.23 mm, while the least increase in the length of the new growths occurred when the nodes were cultivated on media free of any addition (comparison) and amounted to 5.321 mm.

The results presented in Table 1 show that there are significant differences between the apical shoots and nodes in terms of the number of leaves, as it reached 7.741 leaf plantlet^{-1} for the apical shoots, while it was 6.625 leaf plantlet^{-1} for the node. Regarding the effect of adding AC and PVP for the same quality, it is noted that the superiority of these two treatments on media without addition (comparison) amounted to 7.600 and 7.771 leaf plantlet^{-1}, while it reached 6.179 leaf plantlet^{-1} in media without addition. The same table shows the interaction effect between the type of explant and the addition of AC and PVP it is noted that the highest number of leaves was when growing the apical shoot on a medium to which AC was added, and it reached 8,700 leaves, while the nodes grown on a medium devoid of additives gave the least number of leaves, amounting to 5.500 leaves.

As for the effect of the explant, it may be attributed to the difference in the degree of maturity and the differentiation of the cells that make up the tissues of the explant and the consequent difference in their hormonal and nutritional contents [12], as for the effect of AC, it may be attributed to the effectiveness of various antioxidants in controlling the browning phenomenon, which varies according to the plant and its type, and this may be attributed to the specificity of these chemicals for some plants and species. AC is characterized by being a strong adsorbent for phenol, as it absorbs toxic substances and phenols, in addition to its adsorption of growth regulators and nutrients in the medium(14 and 35). Its use in certain concentrations has a positive effect on reducing the phenomenon of browning (brown color) that the explant released into the medium, and it decreased significantly when it was included in the medium. The effectiveness in overcoming this phenomenon may be

due to the association of phenols by AC and the PVP (24 and 33). These results are in agreement with the results of both Singh and Patel (29) from their experiment on pomegranate and Sui *et al.* (31) from their experiment on the kiwi plant.

Table 1. Effect of antioxidants AC and PVP, types of explant and their interaction on the multiplication of Garnem peach explants after 4 weeks of culturing *in vitro*.

Traits / Treatments		Shoot response %	Plantlet length mm	Number of leaves/explant
Explant effect				
Apical shoot		73.333 a	8.585 a	7.741 a
Nodes		80 a	6.818 b	6.625 b
Antioxidant effect				
Without addition		60 b	6.220 c	6.179 b
AC		100 a	9.066 a	7.600 a
PVP		70 b	7.819 b	7.771 a
Antioxidant effect X Explant effect				
Without addition	Apical shoot	80 a	7.118 c	6.858 bc
	Nodes	40 b	5.321 d	5.500 d
AC	Apical shoot	100 a	10.230 a	8.700 a
	nodes	100 a	8.108 bc	6.500 cd
PVP	Apical shoot	40 b	8.615 b	7.666 ab
	nodes	80 a	7.024 c	7.875 ab

*Coefficients share the same letters for each factor or their overlaps, there are no significant differences between them according to Dunkin's multiple range test at level of probability 5%

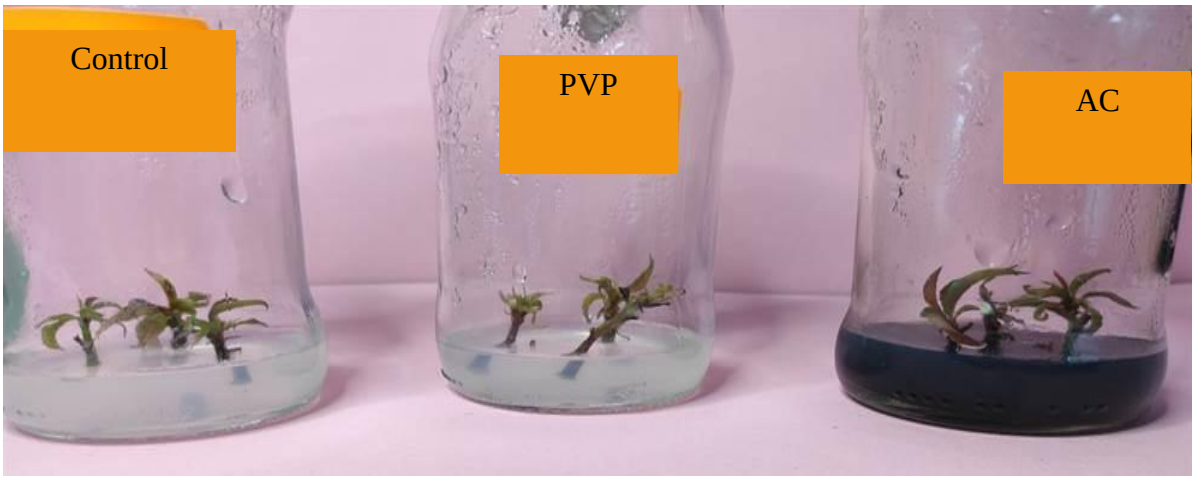

Figure 1. Effect of AC and PVP on growth of the apical shoots and nodes of the peach rootstock Garnem after 4 weeks of cultivation

Effect of BA on the apical shoots and nodes multiplication of the peach rootstock Garnem.

The results in Table (2) show that there are no significant differences between apical shoots and nodes in terms of the response rate, and with regard to the effect of BA it was noted that 1 mg.l^{-1}was significantly superior, which gave the highest response rate of 100%, and didn't differ significantly from the hormone-free medium (comparison), which gave a response rate of 90%, while the concentration of 0.5 mg.L^{-1}recorded lowest percentage response 50%. For the effect type of explant and BA where added to the media, it is noted highest response rate of 100% was when growing apical shoots on a medium added to it BA at 1 mg.L^{-1}, it didn't differ significantly from the apical shoots and nodes grown on media to which the rest of the concentrations were added, except for the apical shoots grown on medium added to it BA at 0.5 mg.L^{-1}, which recorded the lowest response rate 20%.

The results explain (Table 2) that there are significant differences between the apical shoots and nodes with regard to the characteristic of plant length (mm), it was 18.06 mm for the apical shoots while it was 12.875 mm for the nodes BA to the nutrient medium, it is noted from the results that the highest plant length was at 0.5 mg.L^{-1}and reached to 19.005 mm, which was significantly superior to the free hormone used, except for the media to which the hormone was not added (comparative), which gave the lowest length of plants amounted to 11.598 mm. The same table shows the effect of the interaction between the type of explant and plant concentrations of BA that were used and found that apical shoots planted on media added to it BA at 0.5 mg.L^{-1}gave the long length of the plantlet reaching 22.678 mm, significantly superior to the media with free hormone, while the nodules cultured on a hormone-free medium recorded the lowest length of plantlet amounted to 7.438 mm. The results presented in Table 2 indicate that there are no significant differences between apical shoots and nodes in the number of shoots.

BA results showed in the same Table that the highest number of shoots recorded when BA was added at 1 and 2 mg l^{-1}, it reached 2,800 and 3,000 shoots. explant^{-1}, and they didn't differ significantly between them, but they differed significantly on the comparison treatment, which didn't give any shoots, either with regard to the interaction between type of explant and BA where added, it gave the nodes planted on media added to it BA at 1 and 2 mg.L^{-1} the highest number of shoots reached to 3,600 and 4,000 shoots. Explant-outperforming the rest of the concentrations, while the hormone-free media didn't give any shoots.

Table 2 shows that there are significant differences between apical shoots and the nodes in relation to the characteristic of the length of shots (mm), as it was 6.749 mm for the apical shoots, while the nodes had 5.264 mm. And the effect of concentrations BA the superior concentration of 1 and 2 mg.L^{-1}was noticed by giving them the longest shoot length 8.383 and 8.793 mm compared to media without addition which didn't give any shoots. Regarding the effect of type of explant and concentrations of BA, it is noted that apical shoots cultivated on media equipped with 2 mg.L^{-1}were superior, as the length of shoots reached to 10.858 mm, while the explant didn't give

any branches when cultivated on media free of the hormone. The results presented in Table 2 indicate that there are significant differences between apical shoots and the nodes in terms of the total number of leaves, as it amounted to 23,764 leaves for the nodes, while it was less for apical shoots and amounted to 19,300 leaves. Explant-1

Table 2. Impact of benzyl adenine (BA) concentration, type of explant and their interaction on the multiplication of Garnem peach explants after 8 weeks of culturing *in vitro*.

Traits / Treatments		response %	Plantlet length Mm	Leaves number/ explant	Shoots number/ explant	Shoots length Mm
explant type						
Apical shoot		75 a	18.062 a	19.300 b	1.300 b	6.749 a
Nodes		85 a	12.875 b	23.764 a	2.275 b	5.264 b
BA mg.L^{-1}						
0		90 a	11.598 c	9.750 b	0.000 c	0.000 c
0.5		50 b	19.005 a	25.730 a	1.350 b	6.848 b
1		100 a	15.881 b	25.350 a	2.800 a	8.383 a
2		80 ab	15.388 b	25.297 a	3.000 a	8.793 a
BA X Explant type						
0	**Apical shoot**	100 a	15.758 c	8.000 f	0.000 c	0.000 e
	nodes	80 a	7.438 d	11.500 f	0.000 c	0.000 e
0.5	**Apical shoot**	20 b	22.678 a	23.600 cd	1.200 b	7.798 bc
	nodes	80 a	15.332 c	27.860 b	1.500 b	5.898 d
1	**Apical shoot**	100 a	13.103 c	18.100 e	2.000 b	8.338 bc
	nodes	100 a	18.660 b	32.600 a	3.600 a	8.428 b
2	**Apical shoot**	80 a	20.708 ab	27.500 bc	2.000 b	10.858 a
	nodes	80 a	10.068 d	23.094 d	4.000 a	6.729 cd

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

Concerning the effect of concentrations BA added to the nutrition media, the results showed in the same table that the concentrations of BA the used one gave the highest number of leaves at the concentration of 0.5 mg.L^{-1}and reached 25,730 leaves.explant^{-1}, and didn't differ significantly between them, except that it differed over the comparison treatment, which recorded the lowest number of

leaves amounting to 9,750 leaves.explant^{-1}. Regarding for the effect of interaction between the type of explant and BA hormones, the used nodes when planted on media added to it BA at 1 mg.L^{-1}, recorded the highest number of leaves reached to 32,600 leaves.explant^{-1}, while the number was less when growing apical shoots on a hormone-free medium, and reached to 8,000 leaves.explant^{-1}.

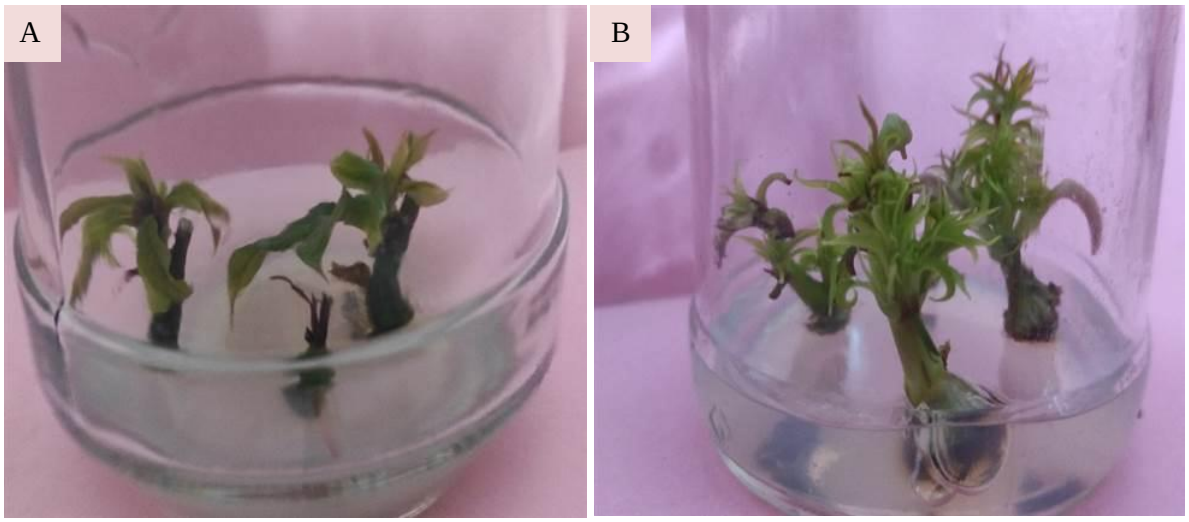

Figure 2. a- BA at 0.5 mg l^{-1} b- BA at 2 mg l^{-1} after 8 weeks of cultivation

The difference in the ideal concentration in the multiplication of apical shoots and nodes may be attributed to the difference in the degree of maturity and differentiation of the cells that make up the tissues of the explant such as the terminal and lateral buds, and the consequent difference in their hormonal and nutritional contents (11 and 6). As for the effect of BA, the superiority of some circles equipped with it may be attributed to its role in increasing the building of RNA and proteins, and enzymes inside the cell, which encourages the division of plant cells in addition to reducing the phenomenon of apical dominance and stimulating the growth of lateral shoots and increasing the vascular differentiation of the shoots, which facilitates their growth and branching(8 and 18) and is also attributed to its role in attracting and collecting metabolites at the site of lateral buds and stimulate the transfer of nutrients and other growth materials to start the growth of buds and the growth of the vegetative system (10) and (31). These results are in agreement with Ak *et al*. (4), Abo Elyazid *et al*. (2), and Salman (26).

The effect of salts strength and auxins in rooting the shoots by *in vitro*

The results presented in Table 3 refer to the characteristic of the number of days for rooting that there are significant differences between the salts of the nutrient medium, as it gave a quarter and a half of the strength of the medium salts MS rooting during 10.58 and 11.93 days of cultivation, while no roots were observed when cultured on media with full concentration of salts. The same table also shows the effect of using concentrations of IBA in the number of days for rooting, it was found that the concentration of 2 mg.L^{-1}gave rooting after 22.41 days of cultivation, and the concentration of 1 mg.L^{-1}gave rooting within 3,000 days of planting. Regarded the effect of interaction between IBA with the salts of the nutrient medium, the results showed that the concentration of 2 mg.L^{-1}added to media with a quarter of the salts concentration gave rooting within 40.25 days of cultivation, while the shoots planted on media with half the concentration of the salts added to it gave rooting. IBA at a concentration of 1 and 3

mg.L^{-1}rooted during 9,000 days of cultivation, while the media with full concentration of salts didn't give any rooting with or without the presence of all the used concentrations of the hormone.

Results in Table 3 indicate that there are no significant differences with regard to the rooting percentage trait between the two mediums MS a quarter and a half of the strength of the salts, as the planted shoots recorded a rooting rate of 20 and 24%, outperforming the average MS at a full concentration of salts, in which no roots were observed. As for the effect of concentrations IBA, the same table shows the concentration 2 mg.L^{-1}was superior by giving it the highest rooting percentage of 33.33%, and it didn't differ significantly from the concentrations 0.5 and 3 mg.L^{-1}, outperforming the comparison treatment that didn't give any percentage of rooting. It is noted in the same table the effect of overlapping IBA with the salts of the nutrient medium, it was found that the concentration of 2 mg.L^{-1}added to the media MS with a quarter of the concentration of salts, the highest rooting rate was recorded at 80%, and it didn't differ significantly from concentration 0.5 mg.L^{-1}added to the media. MS with half the concentration of salts, in which the rooting rate was 60%, while the hormone-free nutrient medium salts didn't give any percentage of rooting, nor did it differ significantly with the media. MS at full concentration of added salts IBA all of which didn't record any percentage of rooting when planting.

Results in Table3 show significant differences between the salts of the nutrient medium used in relation to the number of roots, as the medium was superior MS with half the strength of the salts by giving it the highest number of roots formed, it reached 3,800 roots shoot^{-1}, while it is noted that no roots are formed when the shoots are planted in a media MS in the full strength of its salts. As for the effect of concentrations IBA results show that there were significant differences, 3 mg.L^{-1}gave the highest number of roots, reaching 5,000 roots. Shoot-1 outperformed the rest of the concentrations while the comparison treatment didn't give any roots. As for the effect of medium salts with IBA, the results show that the concentration of 3 mg.L^{-1} added to the medium was superior to MS significantly half the strength of its salts, as the number of roots in it reached 10,000 roots.shoot^{-1}, while the salts of the medium with its different levels didn't give any roots with or without the presence of the hormone.

Results presented in Table 3 show the superiority of MS with half the concentration of salts in the root length characteristic was significant and amounted to 38.17 mm, while no was given MS medium in a full concentration of salts any roots, as for the effect of concentrations IBA the results show superiority 2 mg.L^{-1}concentration by giving it highest average root length of 29.04 mm. The same table also shows the effect of overlap between concentrations of IBA and the salts of nutrient medium, and it turned out that the concentration of 1 mg.L^{-1}added to media with half the concentration of salts gave the highest average root length of 68.81 mm, outperforming the rest of the treatments, while no was given MS media with different concentrations of its salts, to which the hormone has not added any roots of the cultivated branches, as it didn't differ from the shoots grown on media MS with the full concentration of salts added to it IBA in various concentrations.

Notes from results presented in Table 3 superiority of the MS with half strength of its salts significantly by giving it the highest percentage of secondary root formation which amounted to 16.00% compared to the medium MS with the full concentration of salts, which didn't record any percentage of root formation. As for the effect of concentrations of IBA, the

results showed that there were no significant differences between the treatments with regard to the percentage of formation of secondary roots. The same table also shows the effect of IBA and salts of the medium, and it was found there were no significant differences between the treatments.

Table 3 shows that there are significant differences between the salts of the nutrient medium, as it was given a half MS the highest average number of secondary roots was 2 secondary roots shoot^{-1}, while no secondary roots were observed in the rest of the media. And the effect of concentrations IBA results show there were significant differences between the concentrations used, as the concentration of 0.5 mg. L^{-1} gave the highest average number of secondary roots reaching 1.33 secondary roots.shoot^{-1}, while the hormone-free media didn't give any secondary roots. The same table also shows the effect of interaction between concentrations of IBA and medium salts. The table shows that the highest average number of roots was when the shoots were grown on half MS the concentration of salts and added to it IBA at a concentration of 0.5 mg.L^{-1}, in which the number of secondary roots was 4 secondary roots.shoot^{-1}, while the media with different strengths of salts didn't give any secondary roots, and it didn't differ from the MS medium with the full concentration of salts added to it IBA in various concentrations.

The results in Table 3 refer to the effect of medium salts on the average length of secondary roots, MS By half strength gave the highest average length of secondary roots, which reached 3.614mm, compared to MS with full strength which didn't record any formation of secondary roots. As for the effect of concentrations of IBA the concentration of 0.5 mg. L-1 was superior by giving it the highest average length of secondary roots it reached 2.063 mm compared to hormone-free media (comparison), in which secondary roots were not observed.

The same table also shows the effect of overlap between IBA concentrations and the salts of medium and it turned out that the concentration 0.5 mg.L^{-1}added to the 1/2MS of the salts, the highest average length of secondary roots was given, which reached 6.190 mm, while the formation of secondary roots was not observed in all medium salts free of the hormone.

The explanation for the positive effect of half-strength medium salts on rooting may be due to the fact that reducing the ratio of nutrient medium salts to half caused a decrease in the carbohydrate-to-nitrogen ratio to the extent that the best conditions for rooting were provided compared to the rest of the medium salts (5 and 14), and it may also be due to the fact that keeping the crops in a dark atmosphere for a short period before transferring to light conditions can enhance the ability of the shoots to root by *In vitro* because the Photoreceptor active in the dark, which is one of the factors affecting the growth process(3 and 17). As for the effect of IBA in improving the rooting process to its role in increasing levels of IBA inside the plants or modification through the build process, IAA is synergistically internal (18).

In addition to the division of rootstock cells Root initial cells Depends on the concentration of auxin, whether internal or added to the nutrient medium, and the increased number and length of roots may be due to the suitability of this concentration for greater stimulation of the division of cambium cells and their increase in elongation (21 and 22). These results are in agreement with Ak *et al*.(4), but they didn't agree with the findings of Abd Alhady(1) and Humdy and Obaid (15) in their experiments on peaches Nemaguard which concentration of 3 mg.L^{-1} was the best in rooting and Kiwifruits that

concentration of 1mg.L^{-1} is the best for routings.

Table 3. Effect of salt strength, IBA concentration and their interaction on rooting of micro shoots of Garnem peach rootstock explants after 6 weeks of culturing *in vitro*.

Traits / Treatments		Days to root	Root percentage	Number of roots/ explant	Roots length mm	Secondary rooting percentage	Secondary rooting number	secondary root length mm
Effect of explant type								
1/4 MS		10.58 A	20.00 A	1.500 b	5.958 b	0.000 B	0.000 b	0.000 B
1/2 MS		11.93 A	24.00 A	3.800 a	38.17 a	16.00 A	2.000 a	3.614 A
MS		0.000 B	0.000 B	0.000 c	0.000 c	0.000 B	0.000 b	0.000 B
Effect of IBA mg.L^{-1}								
0		0.000 C	0.000 B	0.000 d	0.000 e	0.000 A	0.000 e	0.000 E
0.5		4.889 Bc	20.00 Ab	1.000 c	13.05 c	6.667 A	1.333 a	2.063 A
1		3.000 Bc	6.670 B	1.667 b	22.93 b	6.667 A	0.333 d	1.013 D
2		22.41 A	33.33 A	1.1667 c	29.04 a	6.667 A	1.000 b	1.493 B
3		7.667 B	13.33 Ab	5.000 a	8.52 d	6.667 A	0.666 c	1.453 C
IBA X Explant type								
0	1/4 MS	0.000 d	0.00 c	0.000 e	0.000 e	0.000 A	0.000 e	0.000 e
	1/2 MS	0.000 d	0.00 c	0.000 e	0.000 e	0.000 A	0.000 e	0.000 e
	MS	0.000 d	0.00 c	0.000 e	0.000 e	0.000 A	0.000 e	0.000 e
0.5	1/4 MS	0.000 d	0.00 c	0.000 e	0.000 e	0.000 A	0.000 e	0.000 e
	1/2 MS	14.66 c	60.00 ab	3.000 c	39.16 b	20.00 A	4.000 a	6.190 a
	MS	0.000 d	0.00 c	0.000 e	0.000 e	0.000 A	0.000 e	0.000 e
1	1/4 MS	0.000 d	0.00 c	0.000 e	0.000 e	0.000 A	0.000 e	0.000 e
	1/2 MS	9.000 cd	20.00 bc	5.000 b	68.81 a	20.00 A	1.000 d	3.040 d

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

	MS	0.000 d	0.00 c	0.000 e	0.000 e	0.000 A	0.000 e	0.000 e
2	1/4 MS	40.25 a	80.00 a	2.500 c	17.81 c	0.000 A	0.000 e	0.000 e
	1/2 MS	27.00 b	20.00 bc	1.000 d	69.33 a	20.00 A	3.000 b	4.480 b
	MS	0.000 d	0.00 c	0.000 e	0.000 e	0.000 A	0.000 e	0.000 e
3	1/4 MS	14.00 c	20.00 bc	5.000 b	11.98 d	0.000 A	0.000 e	0.000 e
	1/2 MS	9.000 cd	20.00 bc	10.00 a	13.58 d	20.00 A	2.000 c	4.360 c
	MS	0.000 d	0.00 c	0.000 e	0.000 e	0.000 A	0.000 e	0.000 e

Effect of salts strength and auxins on callus formation of Garnem shoots *In vitro*

Table 4 shows the percentage of callus formation for the tissue branches, it was noted that the concentration of 2 and 3 $mg.l^{-1}$added to an MS medium with a quarter and a half of the strength gave the highest percentage of callus formation, which reached 60%, but it didn't record the appearance of callus among MS at a full concentration of salts and at all

Table 4. Interaction effect of salts strength and IBA concentration on callus formation (%) of Garnem peach explants after 6 weeks of culturing on MS medium.

MS salts / IBA $mg.L^{-1}$	1/4 MS	1/2 MS	MS	Effect of IBA
0	0.00 b	0.00 b	0.00 b	0.00 b
0.5	0.00 b	20.00 ab	0.00 b	6.67 b
1	0.00 b	0. 00 b	0.00 b	0.00 b
2	60.00 a	42.00 ab	0. 00 b	33.33 a
3	60.00 a	60.00 a	0. 00 b	40.00 a
MS salts	24.00 a	24.00 a	0.00 b	

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

Concentrations of IBA were added. As for the average effect IBA it appears from the table that the concentrations of 2 and 3 $mg.L^{-1}$were significantly superior, giving the highest percentage of callus formation, which amounted to 33.33 and 40% respectively, while no callus was recorded in hormone-free media (comparison). The same table also shows the average effect of medium salts and notes the superiority of MS medium with a quarter and a half of strength by giving them the highest percentage of callus formation, which amounted to 24% for both of them, while a medium with full concentration of salts didn't give any callus formation.

Conclusion

From the results, we conclude that the activated charcoal was useful in giving good vegetative growth and a good response, and for using BA we found that the concentration 1 and 2 $mg.L^{-1}$ gave the best multiplication for explants, and the

best rooting was obtained when the shoots were grown on 1/4 MS medium supplied with a concentration 2 mg.L^{-1} and given 1/2 MS medium supplemented with a concentration of 3 mg.L^{-1} the largest number of roots.

Figure 3. a- Concentration of 1 mg L^{-1} IBA in the 1/2 MS b- Concentration of 0.5 mg l^{-1} IBA in the 1/2 MS c- Concentrate 3 mg l^{-1} in 1/2 MS

Figure 4. Rooting and Callus formation after 6 weeks of cultivation on 1/2 MS medium

Conflict of Interest

The authors have no conflict of interest.

References

1- **Abd Alhady, M. R. A.2018.** *In vitro* propagation for peach rootstock (Nemaguard). Egyptian Journal of Desert Research, 68(1):1-13. DOI: 10.21608/ejdr.2018.39085.

2- **Abou Elyazid, D. M; M. S. Gawish and Eliwa, G. I. 2021.**Optimized Protocol for Micropropagation of Cadaman and Garnem Peach Rootstocks. Journal of Plant Production, 12(7):731-735. DOI: 10.21608/JPP.2021.83432.1038

3- **Aghaye, R. N. M. and A. Yadollahi. 2012.** Micropropagation of GF 677 rootstock. Journal of Agricultural Science, 4(5):131.138. DOI: 10.5539/jas.v4n5p131.

4- **Ak, B. E.; P. K. Kiyar; I. H. Hatipoglu and Dikmetaş, B.2021.** Effects of different BA and IBA concentrations on proliferation and rooting of 'GARNEM' rootstock *in vitro* propagation. International Journal of Agriculture Environment and Food Sciences, 5(4):470-476. https://doi.org/10.31015/jaefs.2021.4.6

5- **Al-Dabbagh, F. M. and A. S. Muhammad.2000.**The vegetative propagation of loam *Eriobotrya*

japonica Lindle Using the technique of plant tissue culture, vegetative propagation, rooting. Iraqi Journal of Agricultural Sciences (special *issue)*, 5 (2), 152-163.

6- **Al-Jawary, M. A. K.2005.**The emergence and multiplication of Sidr branches *Zizyphus spinachristi* willing Classify Malasi and Pampawi in vivo using branch tips. M.Sc. thesis. College of Agriculture, University of Baghdad. Republic of Iraq.

7- **Al-Rawi, K. and A. M. Khalaf.1980.** Design and Analysis of Agricultural Experiments. Dar Al-Kutub Foundation for Printing and Publishing, University of Mosul. Ministry of Higher Education and Scientific Research. Iraq

8- **Al-Rifai, A. T. and A. A. Samir.2002.** 21st Century Techniques for Plant Improvement Using Tissue Culture, Dar Al-Fikr Al-Arabi. Cairo. Egypt.

9- **Davies, P. J. (Ed).1995.**Plant Hormones. Physiology, Biochemistry and Molecular Biology. Kluwer Academic Publishers. Dordrecht. Netherland. ISBN 0-7923-2985-6.DOI: http://dx.doi.org/10.1006%2Fanbo.1996.0152

10- **Devlin, R. M. and F. H. Witham F. H.1983.** Plant Physiology. 4th. Wadsorth Publishing Company, Belmont. California, U S A.

11- **El-Gendy, S. A.; M. E. Hashim; A. M. Hosni and Lasheen, F. F. 2001.** In vitro shoot proliferation of Gladiolus from either terminal and Lateral buds or segments of corms. Arab Universities Journal of Agricultural Sciences (Egypt).

12- **Felipe, A. J.; J. Gomez-Aparisi and Socias, R. 1997.** Breeding almond x peach hybrid rootstocks at Zaragoza (Spain). In II International Symposium on Pistachios and Almonds 470 (pp. 195-199). DOI: 10.17660/ActaHortic.1998.470.26

13- **Fernando, S. C.; E. S. Santha and Hewarathna, D. J. A. 2010.** Activated coconut shell charcoal as a component of tissue culture media of Cocos nucifera L. Journal of the National Science Foundation of Sri Lanka, 38(3).:181-183.

14- **George, E. F.; M. A. Hall and De Klerk, G. J. 2008.** Plant Propagation by Tissue Culture. 3rd Edition. The Back Ground Springer. Netherland.

15- **Hamdiy, M. A. and A. A. Obaid 2018.**Effect of benzyladenine and brassinolid and indol butyric acid on micropropagation of Kiwifruit *Atinidia deliciosa.* Diyala Agricultural Sciences Journal, 10(2): 87-99.

16- **Hartmann, H. T.; D. E. Kester and Davies, F. T.1990.** Plant Propagation Principles and Practices.Englewood Cliffs. N. J. Prentice-Hall. USA. pp. 246-247.

17- **Housman J.2003.**Changes in peroxidase activity, auxin level and ethylene production during root formation by poplar shoots raised in vitro. Plant Growth Regulators, 13(3): 263-268. DOI: https://doi.org/10.1007/BF00024847

18- **Jundia, H.2003.**Physiology of Fruit Trees (the Latest Methods in Treating the Problems of Cultivation, Breeding and Production of Fruit Trees in different Lands). Arab House for Publishing and Distribution. Cairo. Egypt.

19- **Lee, C. I., and W. P. Hackett.1976.** Root Regeneration of Transplanted *Pistacia chinensis* Bunge Seedlings at Different Growth Stages1. Journal of the American Society for Horticultural Science, 101(3):236-240. DOI: https://doi.org/10.21273/JASHS.101.3.236.

20- **Li G. R.; L. Sun and Sun J. F. 2005.** Study on Prevention of Browning in Tissue Culture of Nectarine. Anhui Agricultural Sciences, 33(5): 827-828.

21- **Liu J, Zhang X, Kumar P B, et al. 2008.** Influencing factors and preventive measures of browning of pear explants. Journal of Fruit Science, 25(5): 727-731.

22- **Muhammad, A. K.1985.**Plant Physiology. The Second Part, Directorate of Dar Al-Kutub for Printing and Publishing. University of Mosul. Iraq.

23- **Nguyen, T. V.; Thu. T. Thanh; M. Claeys and Angenon, G.2007.** Agrobacterium-mediated transformation of sorghum (Sorghum bicolor (L.) Moench) using an improved in vitro regeneration system. Plant Cell, Tissue and Organ Culture, 91(2): 155-164. DOI: https://doi.org/10.1007/s11240-007-9228-1

24- **Reighard, G. L. and F. Loreti.2008.** Rootstock development. The peach: botany, production and uses (In Layne DR, Bassi D (eds.). pp, 193-220).

25- **Ružić, D. V. and T. I. Vujović.2008.** The effects of cytokinin types and their concentration on in vitro multiplication of sweet cherry cv. Lapins (*Prunus avium* L.). Horticultural Science, 35(1), 12-21.

26- **Salman, M. A. 1988.** Fundamentals of Cultivation of Plant Cells and Tissue, Dar Al-Kutub for Printing and Publishing. University of Mosul. Iraq.

27- **Salman, M. A. and Salih, M. I. 2016.** Effect of Brassinolide, Benzyl adenine and Auxines on *In vitro* propagation of Myroblan plum and Garnem rootstock. Diyala Agricultural Sciences Journal. Vol.8 (2):142-150.

28- **SAS. Copyright (2002-2003).** Institute Inc. Cary, NC. 27513, USA.

29- **Singh, P. and R. M. Patel.2016.** Factors affecting in vitro degree of browning and culture establishment of pomegranate. Afr J Plant Sci 10: 43–49.DOI:https://doi.org/10.5897/AJPS2013.1119

30- **Sivanesan, I.; M. Y. Lim and Jeong, B. R. 2012.** Micropropagation and greenhouse cultivation of Scrophularia takesimensis Nakai, a rare endemic medicinal plant. Pakistan Journal of Botany (Pakistan)., 44(5):1657-1662.

31- **Sui, L.; L. Kong; X. Liu and Zhang, Y. 2020.** Anti-browning in Tissue Culture of 'Donghong'Kiwifruit. In IOP Conference Series: Materials Science and Engineering (Vol. 740, No. 1, p. 012195). IOP Publishing. DOI:10.1088/1757-899X/740/1/012195

32- **Wang, Q.; H. Tang; Y. Quan and Tang, Y. Q. 1994.** Phenol induced browning and establishment of shoot-tip expiants of'Fuji'apple and'Jinhua'pear cultured in vitro. Journal of Horticultural Science, 69(5), 833-839.

33- **Yu, Y. M.; B. Wang; H. Y. Yu *et al*. 2018.** Preventive measures for vitrification browning vitrification in blueberry tissue culture. *Modern* Agricultural Science and Technology, (10): 105-106.

34- **Zhou, B.; X. Wei; R. Wang and Jia, J.2010.** Quantification of the enzymatic browning and secondary metabolites in the callus culture system of *Nigella glandulifera*. Freynt Sint. Asian J. tradit. Med., *5*(3):109-116.