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Optimized Protocol for Micropropagation of Cadaman and Garnem Peach Rootstocks

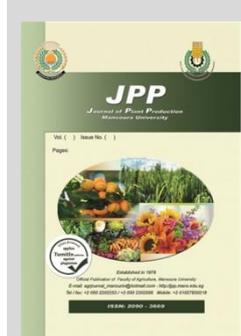
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ABSTRACT

The present study aimed to develop a micropropagation protocol for Cadaman (*P. persica* x *P. davidiana*) and Garnem (*P. persica* x *P. dulcis*) peach rootstocks. Stem nodal segments with one axillary bud were used as explants. MS medium supplemented with different concentrations of BA with or without IBA was studied to determine the best for multiplication stage. The results revealed that MS medium supplemented with 2.0 mg/l BA+0.1 mg/l IBA was optimal for shoot proliferation of Cadaman rootstock, whereas MS containing 2.0 mg/l BA+ 0.0 IBA was advisable to Garnem peach rootstock. Concerning the effect of auxin type and concentration on the rooting stage, IBA at 1.0 mg/l was more effective on rooting induction of Cadaman shoots, whereas in case of Garnem rootstock both IBA and NAA auxins had a similar effect on the rooting process at the concentration of 1.0 mg/l individually. After acclimatization, survival rate was 83% and 63.3% for Cadaman and Garnem rootstocks, respectively.

Keywords: Micropropagation; BA; IBA; Garnem; Cadaman

INTRODUCTION

Peach (*Prunus persica* L. Batsch) is one of the most important deciduous fruit crops grown in Egypt, with a harvest area of 15748 ha and total production of about 358012 tons according to FAOSTAT, 2019. There are many peach varieties that have been adapted and grown in Egypt, but still, there is a lack of peach rootstocks. Peach orchards in Egypt are declining in a short time due to many problems, the main one is the root system infection with root-knot nematodes which affecting its growing area expansion, especially in sandy soil (Abd Alhady, 2018; Eliwa *et al.*, 2018).

Rootstock is playing a major role in determining the ecological fitness of the tree, the health status of critical tree physiological stages, and tree sensitivity to pests and diseases (Holb,2002). Moreover, the rootstock affects the efficiency of pests and diseases management programs and the profitability of fruit production of the grafted cultivar (Holb *et al.*, 2003; Racsko *et al.*, 2004). Therefore, commercial peach cultivation requires new cultivars alongside with vegetative rootstocks suitable to various production conditions (Balla and Mansvelt, 2013).

Cadaman and Garnem rootstocks were introduced to Egypt in 2013 as tissue culture seedlings from Italy (Eliwa and Hagag, 2021). They are suited to many cultivated peach varieties, and highly immune to root-knot nematode (Pinchet *et al.*, 1999), most vigorous and iron chlorosis resistant rootstocks (Jimenez *et al.*, 2011). However, they are difficult to be propagated on large scale through cuttings due to poor rooting capacity (Ammer, 1999; Alsalihi *et al.*, 2004). The conventional propagation methods are quite difficult in peach (Stylianides *et al.*, 1989). Moreover, the sexual propagation is not preferred due to the occurrence of

undesired segregation (Davies and Duray, 2011). Development and application of micropropagation technique played a major role in the advancement of breeding practices in prunus (Martinez-Gomez *et al.*, 2005). Tissue culture techniques can provide new cultivars and rootstocks to meet market demands in a short period of time. Therefore, the aim of this investigation was to establish a protocol for rapid and economical micropropagation of Cadaman and Garnem peach rootstocks to limit their imports from abroad and save hard currencies to our country.

MATERIALS AND METHODS

This study was conducted at the Tissue Culture Laboratory of Horticulture Department, Faculty of Agriculture, Kafrelsheikh University, Egypt, from October 2017 to January 2019.

Plant material

Healthy and vigorous shoots about 10 cm long containing 4-8 axillary buds were excised from three years old Cadaman [*P. persica* x *P. davidiana* (Carr.) Franch] and Garnem [*P. dulcis* (Mill.) 109 D.A. Webb x *P. persica*] peach rootstocks trees growing in a greenhouse at the Educational Nursery of Agriculture Faculty, Damietta university and defoliated with sterile scissors and used as explant material.

Stem nodal segments of about 1.5-2 cm, having one axillary bud, were prepared from the shoots and washed in running tap water for 15 min then with liquid soap to remove the dust particles and fungal and bacterial spores. Then were rinsed for 10 min with distilled water. Explants were surface sterilized under aseptic conditions inside the laminar airflow cabinet by immersing in 70% ethanol for 30 sec followed by treatment for 20 min in 50% commercial bleach (5.25%

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sodium hypochlorite NaOCl) and rinsed three times in sterile distilled water, 5 min for each. The explants placed immediately in multiplication media.

Multiplication stage:

Sterilized explants were cultured in glass jars (350ml) containing 50 ml MS media (Murashige and Skoog, 1962) supplemented with different concentrations of Benzyl adenine (BA) alone or in combination with various concentrations of Indole-3-butyric acid (IBA) as shown in Table (1), 30 g/L sucrose and 7g/L agar.

Table 1. Combinations of BA and IBA (mg/l) in multiplication media treatments (T)

IBA	BA			
	0.0	0.5	1.0	2.0
0.0	T1 (control)	T2	T3	T4
0.1	-	T5	T6	T7
0.5	-	T8	T9	T10

MS medium without plant growth regulators (PGRs) (T1) was used as a control. All shoots were cultured on multiplication media for 8 weeks with two subcultures, then number of shoots per explant, shoot length (cm)/explant and number of leaves/shoot were recorded. Each treatment consisted of 10 replicates with 20 jars/replicate and 3 explants/jar.

Rooting stage:

For rooting individual shoots 2-3 cm in length were excised from the multiplication stage and cultured on MS basal medium at full strength supplemented with 30 g/L sucrose, 7g/L agar, and different combinations of IBA and NAA as shown in Table (2) to determine the best rooting performance for each rootstock. The MS medium without PGRs (T1) was used as a control.

Table 2. Combinations of IBA and NAA (mg/l) in rooting media treatments (Tr)

Treatments	IBA	NAA
Tr1 (control)	0.0	0.0
Tr2	1.0	0.0
Tr3	0.0	1.0
Tr4	0.5	0.5

Each treatment consisted of 50 test tubes in five replicates, 10 tubes/replicate with one plantlet/tube. After 4 weeks, rooting %, number of roots/shoot, and average root length (cm) were recorded.

The pH of the media was adjusted to 5.8 before autoclaving at 121° C at 1.2 kg/cm² steam pressure for 20 min.

Acclimatization:

Plantlets with well-developed shoots and roots were picked up from rooting media then washed in sterile water and transplanted on plastic pots filled with a sterilized soil mixture of peat moss and vermiculite (1:1) and kept in growth chamber room (22±1° C, 70 % RH).The potted plants were irrigated and covered with polyethylene bags (to reduce light intensity, minimize moisture loss, protect plantlets from desiccation and provide a gradual conditioning to greenhouse environment) for four weeks then bags were gradually removed and plantlets were maintained under greenhouse conditions. The successfully acclimatized plantlets were counted and survival percentage was calculated.

Statistical analysis:

Experiments were repeated thrice to confirm the results and subjected to a completely randomized design. Results were statistically analyzed using CoStat Computer Software (version 6.311). Differences between means were evaluated using the Least Significant Difference (LSD) test at p≤0.05. according to Steel et al., (1997)

RESULTS AND DISCUSSION

Multiplication stage:

Effect of various combinations of BA with or without IBA on shoots multiplication of Cadaman peach rootstock is presented in Table 3. Results show that, the highest number of shoots/explant (3.25) and length of shoot (3.13cm) with the highest number of leaves/shoot (10.93) were obtained on the media supplemented with 2.0 mg/l BA + 0.1 mg/l IBA (T7), whereas insignificant differences were found in shoots number and average shoot length/explant among the other treatments. Leaves number/shoot was the highest with T7 followed by T4 and T6 (10.93, 10.67 and 10.33, respectively) with insignificant differences, while T5 recorded the lowest value (1.35 leaves/shoot). The other treatments had intermediate values with insignificant differences.

Table 3. Effect of BA and IBA on shoots number, shoot length/explant(cm) and leaves number/shoot of Cadaman rootstock.

Treatment(mg/l)	Shoots No./explant	Shoot length /explant(cm)	Leaves No./shoot
T1 (0.0 BA+0.0 IBA) control	2.00b	2.33 ab	4.53 ab
T2 (0.5 BA+ 0.0 IBA)	2.33 b	2.46 ab	4.97 ab
T3 (1.0 BA+ 0.0 IBA)	2.00b	2.57 ab	5.50 ab
T4 (2.0 BA+ 0.0 IBA)	1.83 b	2.64 ab	10.67 a
T5 (0.5 BA+0.1 IBA)	1.67 b	1.46 ab	2.53 b
T6 (1.0 BA+0.1 IBA)	2.17 b	2.69 ab	10.33 a
T7 (2.0 BA+0.1 IBA)	3.25 a	3.13 a	10.93 a
T8 (0.5 BA+0.5 IBA)	1.25 b	2.01 ab	4.67 ab
T9 (1.0 BA+0.5 IBA)	1.75 b	2.11 ab	6.17 ab
T10 (2.0 BA+0.5 IBA)	1.83 b	2.16 ab	6.72 ab

Means within each column followed by the same letter are not significantly different at P≤ 0.05 according to LSD test.

Concerning the effect of BA and IBA combinations on Garnem explants during multiplication stage, data presented in Table 4 show that T4 (2.0 mg/l BA+ 0.0 mg/l IBA) medium gave the highest shoot no./explant (2.00) compared to the control treatment which gave the lowest number of shoots (0.67), the average shoot length recorded the best value (2.45cm) with the combination of 1.0 mg/l BA+0.1mg/l IBA (T6 treatment) followed by T4 and T7 (2.24 cm for each) with insignificant differences, the shortest shoot length (1.72 cm) was obtained by the control and T2 treatments. It's clear from the obtained values that, T7 recorded the highest number of leaves/shoot (5.63) followed by T4 (5.27), whereas the control (T1) and T5 treatments gave the lowest number of leaves/shoot (2.22 and 2.30, respectively).

Table 4. Effect of BA and IBA on shoots number, shoot length/explant(cm) and leaves number/shoot of Garnem rootstock.

Treatments (mg/l)	Shoots No./explant	Shoot length /explant(cm)	Leaves No./shoot
T1 (0.0 BA+0.0 IBA) control	0.67 d	1.72b	2.22 c
T2 (0.5 BA+ 0.0 IBA)	1.22 bc	1.72b	4.77 abc
T3 (1.0 BA+ 0.0 IBA)	1.32 bc	1.99 ab	4.73 abc
T4 (2.0 BA+ 0.0 IBA)	2.00 a	2.42 a	5.27 ab
T5 (0.5 BA+0.1 IBA)	0.92 cd	1.69 b	2.30 c
T6 (1.0 BA+0.1 IBA)	1.00 bcd	2.45 a	2.43 bc
T7 (2.0 BA+0.1 IBA)	1.25 bc	2.42 a	5.63 a
T8 (0.5 BA+0.5 IBA)	0.89 cd	1.63 b	3.5 abc
T9 (1.0 BA+0.5 IBA)	1.17 bcd	1.94 ab	4.57 abc
T10 (2.0 BA+0.5 IBA)	1.5 ab	1.92 ab	3.00 abc

Means within each column followed by the same letter are not significantly different at $P \leq 0.05$ according to LSD test.

Briefly, the current study revealed that MS medium supplemented with 2.0 mg/l BA+0.1mg/l IBA (T7) was optimal for Cadaman peach rootstock multiplication. Whereas, T4 treatment (2.0 mg/l BA+ 0.0 IBA) was advisable to Garnem peach rootstock (Fig. 1).

The explants of the two rootstocks responded positively to the addition of BA to the MS medium in the multiplication stage. All studied parameters increased by increasing BA concentration in the media from 0.5 to 2.0 mg/l without the addition of IBA (T2, T3 and T4) comparing with the control treatment (free of growth regulators). Adding IBA in combination with BA enhanced the responses of explants to multiplication media especially with 0.1 mg/l concentration. However, further increment of IBA concentration in the media than 0.1 mg/l had insignificant effect on the explant multiplication parameters in both studied rootstocks.

Generally, these results are in line with previously revealed by Kalinina and Brown (2007); Mansseri-Lamrioui et al., (2011), they obtained multiple shoots formation in several ornamental *Prunus* species using 1.0 mg/l BA among the other studied cytokinins. Balla and Mansvelt (2013), reported that adding 0.05 mg/l auxin (like NAA) for the multiplication of Cadaman rootstock is required, while BA and adenine-sulfate concentration should be reduced to 0.3mg/l, they also found that peach cultivars propagated in their laboratory (e.g. Babygold 6, Biscoe, Creshaven, Fantasia, Frederica, Redhaven and Suncrest) require a BA concentration of 1mg/l and IBA at 0.05 mg/l. Felek et al., (2017), found that BA (2.0 mg/l) + IBA (0.01 mg/l) and GA3 (0.5mg/l) was optimum for maximum shoot number/explant for Garnem rootstock. The effectiveness of BA in initiations, proliferation and elongation of shoots maybe due to its ability to be metabolized in plant tissues or inducing other natural hormones (Zaerr and Mapes, 1982). However, high concentrations of BA at multiplication stage proved to have inhibiting effect on shoot multiplication and growth in peach (Felek et al., 2017) and other plants (Ramage and Williams, 2004; Tiwari et al., 2002).

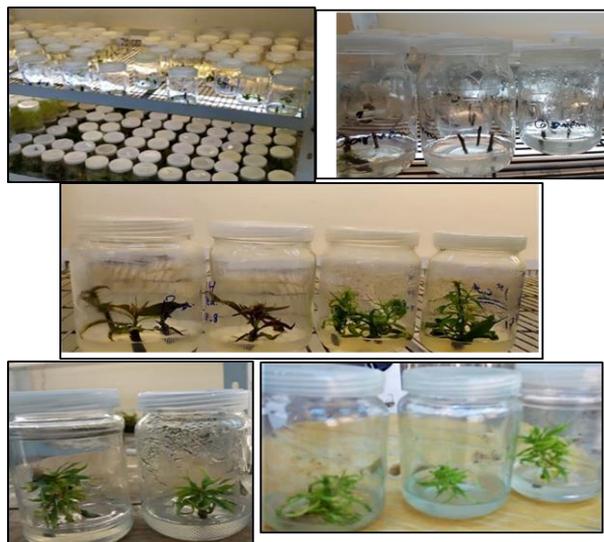


Fig. 1. Multiplication stage of Cadaman and Garnem peach rootstocks

Rooting stage:

Data presented in figures 2 and 3 show the effect of IBA and NAA auxins on the rooting of Cadaman shoots. It is clear that the highest rooting percentage (91%) with largest number of roots/shoot (5.3) and longest root length (5.5cm) were obtained with Tr2 (1.0 mg/l IBA), followed by Tr4 rooting medium (0.5mg/IBA + 0.5mg/l NAA), which achieved 68% rooting percentage with average of 5.3 roots/shoot and 4 cm for root length. Meanwhile, MS medium without any auxin Tr1(control) showed the lowest values of the studied parameters.

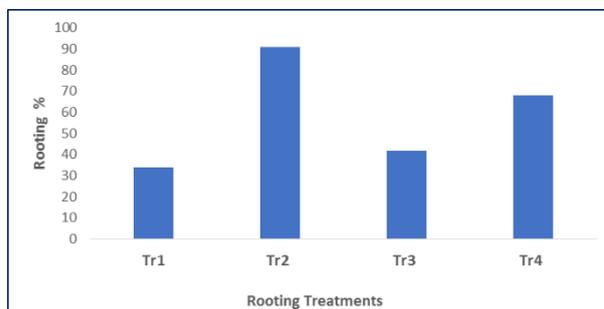


Fig. 2. Effect of IBA and NAA on Rooting percentage of Cadaman peach rootstock.

Rooting treatments (mg/l): Tr1(0.0 IBA+0.0 NAA), Tr2(1.0 IBA+0.0 NAA), Tr3 (0.0 IBA+1.0 NAA), Tr4 (0.5 IBA+0.5 NAA)

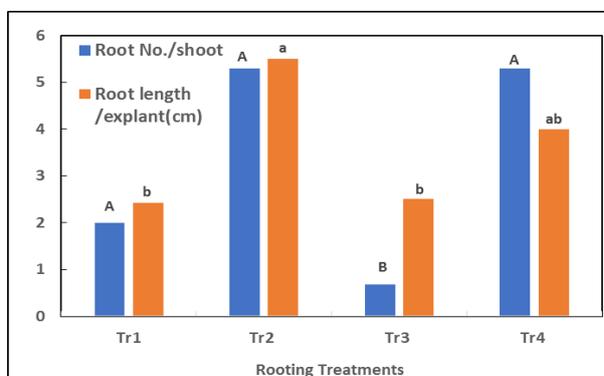


Fig. 3. Effect of IBA and NAA on roots number/shoot and root length/explant(cm) of Cadaman rootstock.

Rooting treatments (mg/l): Tr1(0.0 IBA+0.0 NAA), Tr2(1.0 IBA+0.0 NAA), Tr3 (0.0 IBA+1.0 NAA), Tr4 (0.5 IBA+0.5 NAA)

Data in Figures 4 and 5 demonstrate that Garnem shoots showed different responses to rooting treatments. The MS medium supplemented with 1.0 mg/l from NAA or IBA (Tr3 and Tr2 rooting treatments) recorded 50% rooting, with insignificant differences in root No./shoot and root length. The free auxins rooting treatment (T1) gave the lowest rooting percentage (16%). Moreover, Tr4 treatment (0.5 IBA+0.5 NAA) have the lowest root no./shoot (0.33). While, the differences in shoot length among the treatments were not statistically significant.

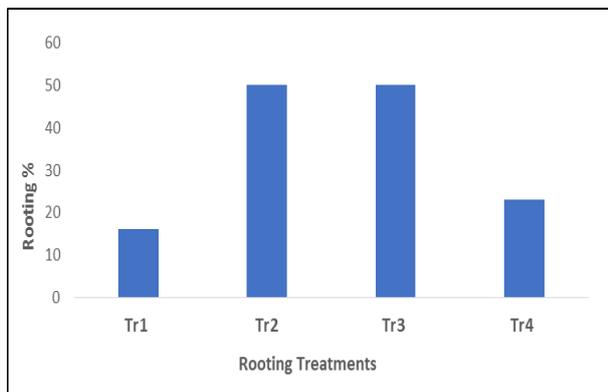


Fig. 4. Effect of IBA and NAA on rooting percentage of Garnem peach rootstock.

Rooting treatments (mg/l): Tr1(0.0 IBA+0.0 NAA), Tr2(1.0 IBA+0.0 NAA), Tr3 (0.0 IBA+1.0 NAA), Tr4 (0.5 IBA+0.5 NAA)

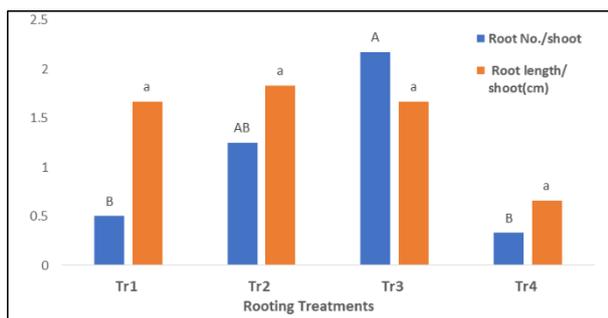


Fig .5. Effect of IBA and NAA on root number/shoot, and root length/explant of Garnem rootstock

Rooting treatments (mg/l): Tr1(0.0 IBA+0.0 NAA), Tr2(1.0 IBA+0.0 NAA), Tr3 (0.0 IBA+1.0 NAA), Tr4 (0.5 IBA+0.5 NAA)

From the above-mentioned results concerning the effect of auxin type and concentration on Cadaman and Garnem rootstocks, it is clear that IBA was more effective on root induction of Cadaman shoots compared to NAA, whereas both affected equally at 1.0 mg/l concentration individually on Garnem shoots rooting. These results are in harmony with others obtained by many investigators, which confirmed that IBA was more effective on root induction compared to NAA (Komalavalli and Rao, 2000; Benelli et al., 2001; Tanimoto, 2005; Durkovic, 2008; Ansar et al., 2009 and Felek et al., 2017).

The results cleared that using NAA in combination with IBA was not effective in root initiation for both studied rootstocks. That is might be explained as Smulders et al., (1990), suggested on the resistance of NAA to degradation by the auxin-oxidase enzyme. Nissen and Sutter, (1990) also reported that IAA was rapidly photo-oxidized in tissue culture media (50%/24h), while IBA was slowly oxidized (10%/24h) and NAA was very stable, that is explaining the better performance of IBA compared to NAA and IAA is

due to its slow movement and delayed degradation, or to IBA effect on increasing internal free IBA or modify the action of endogenous synthesis of IAA, synergistically (Krieken et al., 1993).

Acclimatization

Well-developed rooted plantlets of both studied rootstocks with at least 2.5 - 3 cm root length, were gently removed from the rooting media, washed carefully and transferred to plastic pots filled with sterilized soil and covered with transparent plastic bags. Four weeks later the plastic covers were gradually removed and the plantlets were transferred to the greenhouse for hardening. After 15 days survival rate was 83% and 63.3% for Cadaman and Garnem rootstocks, respectively.

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إستحداث بروتوكول للإكثار الدقيق لأصلي الخوخ الكادامان و الجارنم

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أجري هذا البحث بهدف تطوير بروتوكول للإكثار الدقيق لأصلي الخوخ الكادامان و الجارنم بإستخدام الأجزاء الساقية البرعمية كمنفصلات نباتية ، تم إستخدام بيئة موراشيخ وسكوج (MS) في مرحلة التضاعف مضاف اليها بنزويل أدنين (BA) بتركيزات (0.0 , 0.5 , 1.0 , 2.0 مجم/لتر) مع إندول بيوتريك أسيد IBA بتركيزات (0.0 , 0.1 , 0.5 مجم/لتر) . أظهرت النتائج أن بيئة MS مضاف اليها 2مجم/لتر IBA + 0.1 مجم/لتر أعطت أفضل نتائج للتضاعف لأصل الكادامان من حيث عدد الأفرع و متوسط أطوالها و عدد الأوراق/ فرع , في حين كانت بيئة (MS) مضاف اليها 2مجم/لتر BA سجلت أفضل نتائج التضاعف لأصل الجارنم . فيما يخص مرحلة التجذير تم دراسة تأثير إضافة IBA و NAA بتركيزات مختلفة الي بيئة موراشيخ وسكوج بكامل قوتها و أظهرت النتائج أن IBA بتركيز 1.0 مجم/لتر أعطي أعلى نسبة تجذير (91%) و أعلى عدد للجذور/فرع (5.3) و بمتوسط طول (5.5سم) في الأصل الكادامان . في حين حققت بيئة MS مضاف اليها 1.0 مجم/لتر من أي من IBA أو NAA أعلى نسبة تجذير (50%) مقارنة بالمعاملات الأخرى في أصل الجارنم. سجلت النباتات عند أقلمتها معدل بقاء 83% في الأصل الكادامان و 63.3% للأصل الجارنم.