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Micropropagation of Peach, *Prunus persica* (L.) BATSCH. cv. Garnem

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The present study aimed to develop micropropagation procedure for *Prunus persica* cv. Garnem. Shoot induction and multiplication was found from nodal explants cultured on MS medium fortified with different concentrations of two cytokinins (6-benzyladeninepourine and Kinetin) alone and in combination with 0.5 mg/l gibberellic acid and 0.01 mg/l indole-3-butyric acid. Microplantlets were cultured to root on ½ strength MS medium supplemented with different concentrations of indole-3-butyric acid and α-naphthalene-acetic acid alone. 6-Benzyladeninepourine (0.5 mg/l) with indole-3-butyric acid (0.01 mg/l) and gibberellic acid (0.5 mg/l) was found to be the optimum combination treatment for shoot initiation (100%). 6-Benzyladeninepourine (2.0 mg/l) with indole-3-butyric acid (0.01 mg/l) and gibberellic acid (0.5 mg/l) was found to be optimum for maximum shoot number per explant (7.67). The maximum rooting (42.86%), maximum root number/shoot (6.33) and longest rooting (7.17 cm) were found at 1.5 mg/l indole-3-butyric acid. α-naphthalene-acetic acid was found to be not effective. The plantlets were acclimatized in the glasshouse and survival percentage was 73.3% at potting mixture of autoclaved river sand, forest soil and manure in a 2:1:1 (v/v/v) ratio.

Key words: Acclimatization, auxin, cytokinin, micropropagation, *Prunus persica*.

INTRODUCTION

Peach (*Prunus persica* L. Batsch) belongs to the *Prunoideae,* a subfamily of *Rosaceae,* with 8 basic and 16 somatic chromosome numbers (2n = 16) (Hesse, 1975). *Prunus* includes several approximately 400 species adapted primarily to the temperate regions of the northern hemisphere (Krussmann, 1986). China is the native home for peach, which was domesticated there 4000-5000 years ago (Aranzana et al., 2010).

The introduction of temperate fruits especially of peach to Ethiopia and North Africa took place during the era of exploration and colonization in sixteenth and seventeenth century by Europeans (Scorza and Sherman, 1996). Because of its early introduction, peach is relatively well established in many highland areas and is introduced

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much earlier than apple and plum. From recently (2011) introduced, Garnem is one of the peach rootstock cultivars introduced from Spain to the country. The growth, productivity, and longevity of a peach tree are greatly influenced by the selection of an appropriate rootstock. The cultivar Garnem (*Prunus persica* cv. garnum) is highly immune to root-knot nematode *Meloidogyne javanica* (Treub.) Cjotwood (Pinochet et al., 1999), the most vigorous, and iron chlorosis resistant rootstock (Jimenez et al., 2011). It is a Spanish rootstock and is ranked third following GF677, and Seedling (Reighard, 2011). Jimenez et al. (2011), noted that Garnem significantly affects tree size, as measured by Trunk Cross Sectional Area (TCSA). In 2009 the 'Calrico' scion when grafted on Garnem and PADAC 9970-23 rootstocks exhibited higher yield per tree bigger fruit size and showed the higher TCSA values (239.6 and 239.0 cm^2, respectively). Garnem is the most invigorating rootstock, in agreement with genotype description (Felipe, 2009) and field evaluation (Zarrouk et al., 2005). However, it is difficult to be multiplied on mass scale through cuttings because of very low rooting percentages (Ammer, 1999). The use of conventional propagation methods in peach is quite difficult because it results low multiplication rate and also quite difficult in peach (Stylianides et al., 1989).

Recently, breeding practices in *Prunus* have been advanced by the development and application of micropropagation (Martinez-Gomez et al., 2005). There has been no report on micropropagation of *P. persica* cv. garnum in Ethiopia. The purpose of this study was therefore to find out the following objectives: to assess the effects of different types and concentrations of cytokinin on shoot induction and multiplication; to assess the effects of different types and concentrations of auxin on root induction; and to acclimatize *in vitro* seedlings.

MATERIALS AND METHODS

The study was conducted at the Plant Tissue Culture Laboratory, National Agricultural Biotechnology Laboratory, Holeta Agricultural Research Center from October 2013 to January 2014.

The nutrient media used was Murashige and Skoog (1962). To prepare a ready use MS media, the MS stock solutions were mixed along with plant growth regulators (PGR) and sucrose (3%). Magnetic stirrer was used to mix the solution well. The pH of the medium was adjusted at 5.75 using 0.1N NaOH or 0.1N HCl before adding 0.4% agar (Agar-Agar, Type 1), and heated till the agar melts properly. About 10 and 50 ml of the medium was dispensed in each culture tube (150 mm long and 25 mm diameter) and jar (250 ml). The culture tubes and jars containing the medium were plugged tightly with non-absorbent cotton wool plugs and autoclavable lids prior to autoclaving at 121°C with 0.15 KPa pressure for 20 min. Since, Gibberellins are rapidly degraded by elevated temperatures, and the biological activity of a freshly prepared solution of GA_3 was reduced by more than 90% as a result of autoclaving (Van Bragt and Pierik, 1971), it was sterilized by filtration through a sieve or a filtration assembly using filter membranes of 0.22 to 0.45 µm size. The filter sterilized GA_3 was then added into the autoclaved MS media before solidified.

Young and healthy shoots (4-6 cm long), containing axillary buds (third, fourth and fifth nodes; from shoot apex), were excised and collected from three years old Garnem fruit crop by cutting with sterile scissor and used as explant. Actively growing shoots (juvenile plants) were taken as they are more responsive to shoot regeneration and proliferation than shoot explants from adult forms as reported by Naghmouchi et al. (2008). The explants were washed with tap water 3-5 times followed by liquid soap for 30 min with agitation to physically remove most microorganisms, and treated in 70% ethanol for 30 s and then in NaOCl (0.25% w/v) containing one drop of 'Tween 20' per 50 ml solution for 15 min.

Shoot initiation

Sterilized explants' were cultured in test tubes containing MS (Murashige and Skoog, 1962) medium fortified with 3% sucrose, 0.4% agar (Agar-Agar, Type 1) and varying level of BAP (SIGMA) (0.5, 1.0, 2 and 4 mg/l) alone and in combination with 0.01 mg/l IBA (HIMEDIA) and 0.5 mg/l GA_3. In addition, Kn (UNI-CHEM) (0.5, 1.0, 2 and 4 mg/l) alone and in combination with 0.01 mg/l IBA and 0.5 mg/l GA_3 (Table 1) was the other treatment combination for the initiation experiment. MS medium without Plant Growth Regulators (PGRs) was used as control. For each initiation treatment seven test tubes were lined up randomly in Completely Randomized Design (CRD) with three replications. All test tubes with cultured explants were properly sealed with cotton and parafilm and maintained in the growth room at standard conditions (22 ± 1°C and 16/8 h light/dark using cool-white fluorescent lamps (photon flux density, 40 µmol m^{-1} s^{-1} irradiance) and relative humidity (RH) of 70-80%. Number of days to shoot initiation and number of explants initiated were recorded after four weeks of culturing, and shoot initiation percentage was computed.

Shoot multiplication

To avoid the carry over effect of shoot initiation media on shoot multiplication, initiated shoots were maintained on PGRs free MS medium with 1 g/l activated charcoal for two weeks. Aseptically initiated 1.0-1.5 cm long shoots with 7- 10 nodes were trimmed at both sides and placed horizontally and lightly pressed into the medium in 200 ml jars each with 50 ml MS medium supplemented with 3% sucrose, 0.4% agar and varying levels of BAP (0.5, 1.0, 2 and 4 mg/l) alone and in combination with 0.01 mg/l IBA, 0.5 mg/l GA_3. The other treatment combination was Kn (0.5, 1.0, 2 and 4 mg/l) alone and in combination with 0.01 mg/l IBA, 0.5 mg/l GA_3 (Table 1). MS medium without PGRs was used as control. For each treatment four jars (each with 5 shoots) were lined up randomly in CRD with three replications. All shoots were incubated on multiplication medium for 6 weeks then data on number of shoot per explant, number of leaves per shoot, and shoot length (cm) were recorded.

Rooting of shoots

Microshoots 1.5 cm long were cultured on half MS medium supplemented with 3% sucrose, 0.4% agar, 1 mg/l activated charcoal and different concentrations of IBA (0.75, 1.5, 3.0 and 6.0 mg/l) and NAA (0.75, 1.5, 3.0 and 6.0 mg/l) (Table 2). The MS medium without PGRs was used as control. For each treatment seven test tubes, each with one plantlet, were lined up randomly in CRD with three replications. After the shoots were incubated on the rooting medium for 4 weeks, data on number of rooted microshoots, number of roots per microshoot, and average root length (cm) were recorded.

Table 1. Treatment combination for initiation and shoot multiplication experiments.

Cytokinin	IBA	GA_3	Treatment code	
			Initiation	Multiplication
BAP				
0	0	0	IT01	MT01
0.5	0	0	IT02	MT02
1	0	0	IT03	MT03
2	0	0	IT04	MT04
4	0	0	IT05	MT05
0.5	0.01	0.5	IT06	MT06
1	0.01	0.5	IT07	MT07
2	0.01	0.5	IT08	MT08
4	0.01	0.5	IT09	MT09
Kn				
0.5	0	0	IT10	MT10
1	0	0	IT11	MT11
2	0	0	IT12	MT12
4	0	0	IT13	MT13
0.5	0.01	0.5	IT14	MT14
1	0.01	0.5	IT15	MT15
2	0.01	0.5	IT16	MT16
4	0.01	0.5	IT17	MT17

Table 2. Treatment combination for the rooting experiment.

Treatment code	IBA
RT1	½ MS + 0
RT2	½ MS + 0.75
RT3	½ MS + 1.5
RT4	½ MS + 3.0
RT5	½ MS + 6.0
	NAA
RT6	½ MS + 0.75
RT7	½ MS + 1.5
RT8	½ MS + 3.0
RT9	½ MS + 6.0

Acclimatization

Plantlets with well-developed shoots and roots were transplanted on a tray containing a mixture of autoclaved river sand, forest soil and manure in a 2:1:1 (v/v/v) ratio and transferred to greenhouse for hardening. The transplanted plantlets were kept in greenhouse under shade of polyethylene sheets and red cheese cloth, to reduce light intensity and maintain moisture, for one week and were sprayed with water two to three times every day. After 15 days, percentage of plantlets that were successfully acclimatized was recorded.

Statistical analysis

Shoot length, root number and root length data were transferred into square root value for statistical analysis. Rooting percentage data was also transferred into arc sine value for statistical analysis. Significance of the treatment effects was determined by analysis of variance SAS computer software (version 9.1), employing a completely randomized design. Variations among treatment means were assessed by Duncan's Multiple Range Test (DMRT) ($P = 0.05$) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Shoot Initiation

Analysis of variance revealed that concentration of BAP and Kn alone and in combination with IBA and GA_3 had highly significant effect ($P \leq 0.0001$) on days for shoot initiation, shoot initiation percentage and percent of usable shoots (vigor) (Table 3).

The nodal explants started to initiate after a week of culturing on most of the media. Shoot initiation was observed in all treatments including the control, hormone free MS medium (81% initiation) (Table 4), indicating that the Garmem cultivar has enough endogenous cytokinin and auxin combination for initiation. However, the length of initiated shoots and usable initiated shoots, differ in different treatments (Figure 1). Maximum shoot initiation

Table 3. ANOVA for treatment effects on days for initiation, percent shoot initiation, % usable shoots, shoot number per explant, shoot length and leaf number per shoot.

Source of variation	DF	Mean square of					
		Days for initiation	% shoot initiation	% usable shoot	Shoot no/ explant	shoot length	leaf no/ shoot
Treatments	16	6.09***	389.11***	1949.02***	19.79***	0.05***	15.83***
error	34	0.19	48	27.94	0.23	0.001	0.25
R^2		0.94	0.79	0.97	0.97	0.96	0.97
CV (%)		3.51	8.71	20.3	15.08	2.70	7.21

***, highly significant (P≤ 0.0001) at α=0.05 significant level; R^2 = coefficient of determination; CV = coefficient of variation; DF = Degree of freedom

Table 4. The effect of BAP and Kn alone and in combination with GA_3 and IBA on shoot initiation percentage, percent of usable shoot initiation, mean number of days for shoot initiation, shoot number per explant, shoot length and leaf number per shootlet.

Treatments			Shoot initiation (%)	Usable shoot initiation (%)	days for initiation	Shoot no/ explant	Shoot length	Leaf no/ shoot
BAP	**GA_3**	**IBA**						
0	0	0	81±8.24^{cde}	0 ± 0^{e}	11.3±0.6^{e}	1.3±0.6^{e}	0.50 ± 0^{i}	4.7±0.6^{f}
0.5	0	0	81±8.24^{cde}	14.26±0^{d}	12.3±0.6^{d}	4.3±0.6^{c}	0.73±0.06^{fg}	7.7±0.58^{d}
1	0	0	85.7± 0^{bcd}	28.6 ± 0^{c}	10.3±0.6^{f}	6.0 ± 0^{b}	0.87±0.06^{cde}	8.0 ± 0^{cd}
2	0	0	85.7± 0^{bcd}	52.4±8.2^{b}	11.0±0^{ef}	7.3±0.6^{a}	0.67 ±0.06^{gh}	8.0 ± 0^{cd}
4	0	0	76.2±8.2^{def}	0 ± 0^{e}	9.0 ± 0^{g}	2.7±0.6^{d}	0.53± 0.06^{i}	5.3±0.58^{f}
0.5	0.5	0.01	95.2±8.3^{ab}	76.2±8.2^{a}	13.3±0.6^{bc}	4.7±0.6^{c}	1.0 ± 0^{ab}	9.3±0.6^{ab}
1	0.5	0.01	100± 0^{a}	81±8.24^{a}	13.3±0.6^{bc}	5.7±0.6^{b}	0.93±0.12^{bc}	8.7±0.6^{bc}
2	0.5	0.01	85.7 ± 0^{bcd}	57.14±0^{b}	14.3±0.6^{a}	7.7±0.6^{a}	0.80 ± 0^{def}	8.3±0.6^{cd}
4	0.5	0.01	71.43± 0^{efg}	28.57±0^{c}	13.3±0.6^{bc}	6.3±0.6^{b}	0.83±0.06^{cdef}	8.0 ± 0^{cd}
Kn	**GA_3**	**IBA**						
0.5	0	0	66.7±8.3^{fg}	9.5±8.2^{de}	13.3±0.6^{bc}	1.7±0.6^{e}	0.77±0.06^{efg}	8.7±0.6^{bc}
1	0	0	81±8.24^{cde}	14.26±0^{d}	13.00±0^{cd}	1.3±0.6^{e}	0.90±0.1^{bcd}	7.7±0.6^{d}
2	0	0	90.5±8.3^{abc}	28.6 ± 0^{c}	13.00 ±0^{cd}	1.0 ± 0^{e}	1.10 ± 0.2^{a}	9.7±0.6^{a}
4	0	0	81±8.2^{cde}	14.26±0^{d}	13.3±0.6^{bc}	1.0 ± 0^{e}	0.57±0.06^{ih}	6.7±0.6^{e}
0.5	0.5	0.01	52.4± 8.2^{h}	14.26±0^{d}	12.3±0.6^{d}	1.0 ± 0^{e}	0.50 ± 0.06^{i}	5.3±0.6^{f}
1	0.5	0.01	76.2±8.2^{def}	9.5±8.2^{de}	13.00±0^{cd}	1.3±0.6^{e}	0.73±0.06^{fg}	6.7±0.6^{e}
2	0.5	0.01	76.2±8.2^{def}	9.5±8.2^{de}	14.0± 0^{ab}	1.3±0.6^{e}	0.73±0.06^{fg}	6.3±0.6^{e}
4	0.5	0.01	66.7±8.3^{gh}	4.8±8.2^{de}	14.0± 0^{ab}	0 ± 0^{f}	0 ± 0^{j}	0 ± 0^{g}
CV (%)			8.74	20.3	3.51	6.82	2.64	3.55

For each parameter, values followed by different letter are significantly different according to Duncan Multiple Range Test at α=0.05 significant level; CV = coefficient of variation.

on a culture medium augmented with BAP alone was 85.7% and its maximum usable shoot was 52.4%.

The shoot initiation percentage was increased when BAP was combined with 0.5 mg/l GA_3 and 0.01 mg/l IBA. The maximum shoot initiation percentage was 100%, with 81% usable shoot (Figure 2, IT07) on MS medium containing 1.0 mg/l BAP + 0.5 mg/l GA_3 and 0.01 mg/l IBA. However, this treatment was at par with 0.5 mg/l BAP + 0.5 mg/l GA_3 and 0.01 mg/l IBA (Table 4). The shoot initiation percentage was declined when BAP concentrations were decreased and increased in concentration lower and higher than 1 mg/l. The addition of GA_3 and IBA on BAP fortified MS medium increased shoot initiation percentage than BAP alone supplemented MS medium. The combinations of BAP, GA_3 and IBA at definite proportions were very critical and found to be essential in *P. persica* for shoot initiation from nodal explants.

The maximum shoot initiation percentage was recorded on the culture medium containing Kn alone, and it was 90.5% with 28.6% usable shoots. Shoot initiation percentage was declined when Kn concentrations were lower and higher than 2 mg/l. The addition of 0.5 mg/l GA_3 and 0.01 mg/l IBA on MS medium containing Kn did

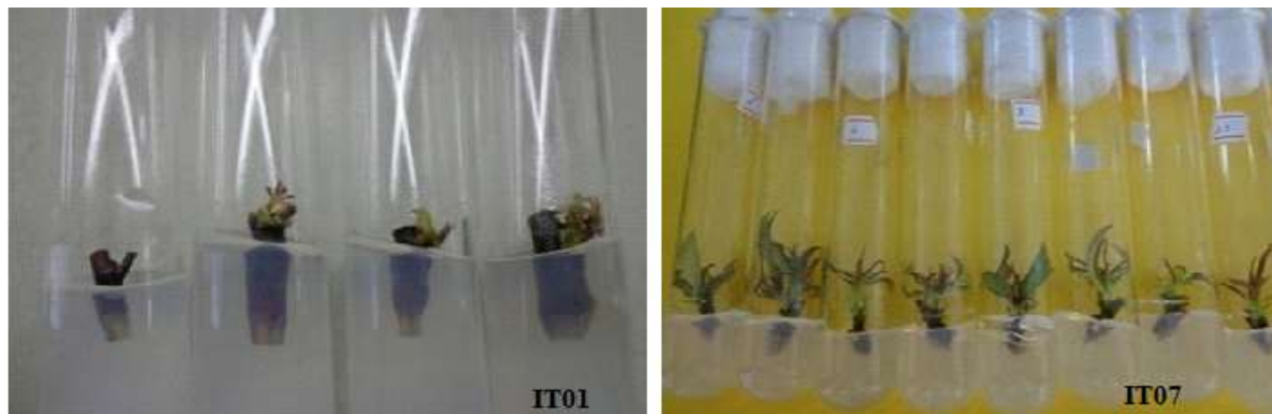

Figure 1. The effect of BAP in combination with GA_3 and IBA on shoot initiation of nodal explants. Note: IT01 = hormone free (control); and IT07 = 1.0 + 0.5 + 0.01 mg/l BAP + GA_3 + IBA.

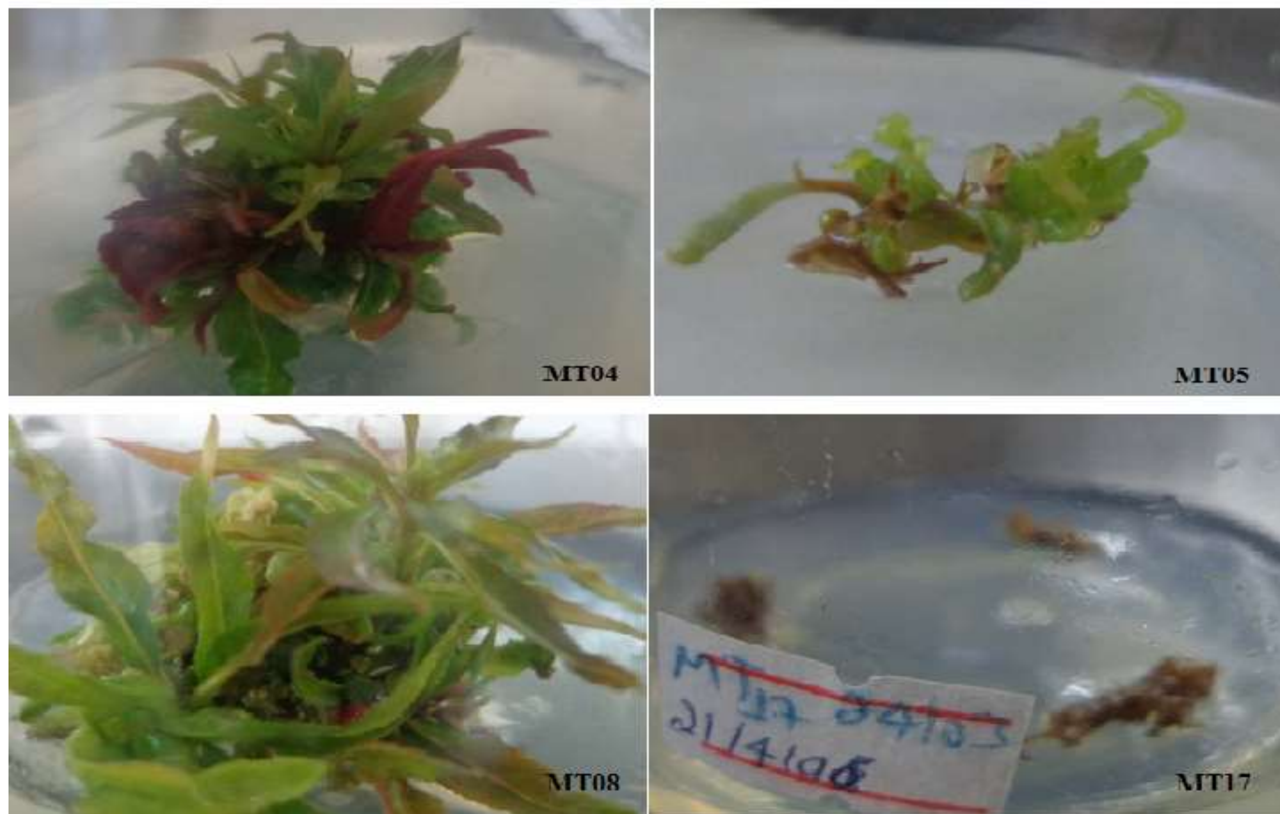

Figure 2. The effect of BAP and Kn alone and in combination with GA_3 and IBA on shoot number per explant, shoot length and leaf number per shoot. Note: MT04 = 2.0 mg/l BAP; MT05 = 4.0 mg/l BAP; MT08 = 2.0 + 0.5 + 0.01 mg/l BAP + GA_3 + IBA; and MT17 = 4.0 + 0.5 + 0.01 mg/l Kn + GA_3 + IBA.

not produce better shoot initiation than Kn alone. The best shoot initiation on culture medium fortified with Kn in combination with GA_3 and IBA was 76.2% with 14.3% usable shoots initiation. Shoot initiation of 81% was recorded, on hormone free culture medium although their length was too short (<0.5 cm) (Figure 2, IT01), and these shoots were dried when sub-cultured. Most of the initiated shoots from the culture medium fortified with BAP in combination with GA_3 and IBA were longer than those shoots regenerated on the culture medium fortified

with BAP and Kn alone and Kn in combination with GA_3 and IBA (Table 4). Kinetin showed poor response to shoot regeneration as compared to BAP. Subbu et al. (2008) reported that BAP (82%) was more effective than Kn (16%) on shoot regeneration of *Saraca asoca*. Mansseri-Lamrioui et al. (2011) also described that 2ip and Kn had lower results in comparison with BAP on *Prunus avium*.

The synergistic effect of BAP, gibberellic acid and auxin has been demonstrated in many plants including *Prunus persica x P. amygdalus* (Fotopoulos and Sotiropoulos, 2005) who noted that BAP and GA_3 in combination produced shoots possessing better macroscopic appearance. Vaghari-Azar et al. (2012) also reported that the addition of GA_3 to the culture medium showed a positive influence on shoot length, confirming the results reported by Dejampour et al. (2007) on peach x almond and apricot x plum inter-specific hybrids. Also, Tsipouridis and Thomidis (2003) reported that exogenous application of GA_3 cause a significant increase in sprouting of resetting plantlets of GF677 (peach x almond hybrid). The hormonal balance: BAP (2 mg/l), IBA 0.1 mg/l and GA_3 0.1 mg/l with 1 mM phloroglucinol showed its positive influence in *P. avium* (Hammatt and Grant, 1997). On the other hand, studies by Nagaty (2012) reported a different result where media containing 3.6 µM TDZ with 2.5 µM IBA was more effective than other TDZ levels in inducing shoot initiation in some other *Prunus* spp. This difference might be due to the genotype effect as reported by various authors (Hammatt and Grant, 1998; Ainsley et al., 2000; Gentile et al., 2002; Perez-Tornero et al., 2000).

The current study revealed that 0.5 and 1.0 mg/l BAP in combination with 0.5 mg/l GA_3 and 0.01 mg/l IBA gave maximum shoot initiation percentage (95.2-100%, respectively), and maximum percentage of usable shoots (76.2- 81%, respectively) which are at par. Therefore, 0.5 mg/l BAP in combination with 0.5 mg/l GA_3 and 0.01 mg/l IBA is recommended for maximum percentage of shoot initiation and usable shoot.

Shoot multiplication

Analysis of variance (Table 3) revealed that the treatment had highly significant effect on mean number of shoots, length of shoot, and mean number of leaves. The combination of IBA, GA_3 and BAP was more effective on shoot multiplication compared to BAP alone. Data indicated that the maximum mean number of shoots (7.3 - 7.7) per explant was found on MS medium containing 2 mg/l BAP alone, and in the combination of 2 mg/l BAP, 0.5 mg/l GA_3 and 0.01 mg/l IBA (Table 4). There was no significance difference regarding shoot number between these two treatments. However, 2 mg/l BAP, 0.5 mg/l GA_3 and 0.01 mg/l IBA showed better number of shoots (7.7) and shoot length (8.0 cm) as compared to 2 mg/l BAP. This is consistent with the findings of Demsachew (2011) on *Malus domestica* cvs. MM106 and Anna. Hammatt and Grant (1997) also reported that the hormonal balance: BAP (2 mg/l), IBA 0.1 mg/l and GA_3 0.1 mg/l with 1 mM phloroglucinol showed positive influence in *P. avium* multiplication. There was no sign of shoot multiplication on medium supplemented with 4.0 mg/l Kn, 0.5 mg/l GA_3 and 0.01 mg/l IBA. All samples cultured in this medium were dried (Figure 2, MT17). The addition of IBA and GA_3 to MS medium fortified with Kn did not produce better shoot number per explant as compared to those explants cultured on MS medium fortified with Kn alone. Shoot multiplication on Kn supplemented MS medium did not differ from shoot multiplication on control MS medium. This indicates that the use of Kn for *P. persica* cv. Garnem micropropagation is not advisable.

Further increase in the concentration of BAP beyond the optimal level reduced the number of shoots indicating an upper limit in concentration. When the concentration of BAP was increased to 2 - 4 mg/l in multiplication medium, the shoots were turned very dwarf and bushy. They also, callused and became to red color (Figure 2, MT04). Generally, higher concentration of BAP had suppressive effect on morphogenesis of *P. persica* (Figure 2, MT05). This is in harmony with the findings of Tiwari et al. (2002). Who noticed that high BAP concentration had an inhibiting effect on further shoot multiplication and growth. Ramage and Williams (2004) also reported that stunted plants were associated with increased exogenous BAP concentration.

The type and concentration of cytokinin influenced shoot multiplication. Among the two cytokinins (BAP/Kn) in combination with GA_3 and IBA, BAP was better and more effective than Kn, for shoot multiplication. This result is in agreement with previous reports of Kalinina and Brown (2007) and Mansseri-Lamrioui et al. (2011) who found multiple shoot formation in nine ornamental *Prunus* species using 1 mg/l BAP, among the studied cytokinins suck as 2ip, Kn and BAP. Muna et al. (1999) and Pruski et al. (2000) also reported that BAP could be used successfully to induce shoot multiplication in *Prunus* spp. The reason for the effectiveness of the BAP may lie in its ability to get metabolized in plant tissues or its ability to induce other natural endogenous hormones for initiation, proliferation and elongation of shoots (Zaerr and Mapes, 1982).

The maximum shoot length (1.1±0.2 cm) was found at 2.0 mg/l Kn and the minimum (0.5 cm) at the control treatment (Table 4). The maximum and minimum number of leaves (9.7±0.6, 4.7±0.6) per shoot was recorded at 2.0 mg/l Kn and control treatment respectively (Table 4). The number of leaves per shoot was increased with the increase in shoot length. The shoot length was higher in the Kn supplemented MS medium than BAP supplemented MS medium. This is consistent with the findings of Ndoye et al. (2003) who stated that Kn promoted shoot elongation in *Balanite aegyptiaca* but led

Table 5. ANOVA for the effect of IBA and NAA on rooting %, root number per shoot and root length.

Source of variation	DF	Mean square of		
		Rooting %	Root number per shoot	Root length
Treatments	8	528.199***	1.2***	1.37***
error	18	1.376	0.036	0.06
R^2		1.0	0.99	0.99
CV (%)		2.5	3.96	6.54

***, highly significant (P≤ 0.0001) at α=0.05 significant level; R^2 = coefficient of determination; CV = coefficient of variation; DF = Degree of freedom.

Table 6. The effect of IBA and NAA on rooting %, root number per shoot and root length.

IBA	NAA	Rooting (%)	No of roots/shoot	Root length (cm)
0	0	0^b	-	-
0.75	0	0^b	-	-
1.5	0	42.86^a	6.3	7.2
3	0	0^b	-	-
6	0	0^b	-	-
0	0.75	0^b	-	-
0	1.5	0^b	-	-
0	3	0^b	-	-
0	6	0^b	-	-
CV (%)		2.5	3.96	6.54

to a decline in shoot multiplication.

Hormone free MS medium produced less number of shoots per explant indicating that hormones are needed to power shoot multiplication. Hence, 2.0 mg/l BAP in combination with 0.5 mg/l GA_3 and 0.01 mg/l IBA is recommended for shoot multiplication of *P. persica* cv. Garnum, since this combination treatment produces the higher number of shoot per explant as well as comparable elongated shoots than the other treatments (Figure 2, MT08).

Root induction

Analysis of variance revealed that concentration of IBA and NAA had highly significant effect (P≤0.0001) on rooting %, root number per shoot and root length (Table 5). The results presented in Table 6 showed that the addition of 1.5 mg/l IBA with 1 g/l AC was the only treatment that initiated roots. The percentage of rooting (42.86%), number of roots per shoot (6.3) and root length (7.2 cm) were recorded (Table 6 and Figure 3A). Earlier studies on peach by Alanagh et al. (2010), reported that the highest rate of rooting (up to 40%) and the maximum number of roots per shootlet (2.62±0.56) were obtained in the induction medium supplemented with 2 mg/l IBA. The influence of IBA on root induction has been reported in many plants and proved effective as compared to NAA (Benelli et al., 2001; Tanimoto, 2005; Ansar et al., 2009).

The application of NAA resulted in no rooting of Garnem shoots. This might be explained due to the NAA resistance to degradation by the auxin-oxidase enzyme (Smulders et al., 1990). Nissen and Sutter (1990) have shown that, in tissue culture, media IAA is rapidly photo-oxidized (50% in 24 h), while IBA is oxidized slowly (10%) and NAA is very stable. Slow movement and delayed degradation of IBA may be the reason for the better performance as compared to IAA and NAA. IBA may also enhance rooting via increased internal free IBA or may synergistically modify the action of endogenous synthesis of IAA (Krieken et al., 1993).

Acclimatization

Well-developed rooted plantlets were gently removed from the culture tubes, washed to remove adhered agar and traces of the medium to avoid contamination. Then, they were transferred to plastic planting tray containing a mixture of autoclaved river sand, forest soil and manure in a 2:1:1 (v/v/v) ratio and transferred to greenhouse for hardening. After 15 days the survival rate was 73.3% (Figure 3 D). This result agrees with the findings of Demsachew (2011), who reported 65.7% acclimatization

Figure 3. Root formation after 30 days from cultivation on rooting medium containing 1.5 mg/l IBA (A), Polythelene sheet and cheese close covered shoots (B), shoots at the time of transplantation (C), and shoots after 15 days (D).

in apple cv. Anna. Deepa et al. (2011) also reported that the well rooted plantlets transferred to pots containing a mixture of sterilized sand, soil and vermiculate in a 2:1:1 (v/v/v) ratio. After 2 weeks, the acclimatized plantlets were looked healthy with vigorous growth and 70% survival rate was recorded.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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