

Factors affecting rhizogenesis *in vitro* and acclimatisation of three cherry rootstocks

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Key words: Cherry rootstocks, growth regulators, micropropagation, tissue culture.

Summary: Factors affecting rhizogenesis *in vitro* and acclimatisation of three rootstocks of cherry, i.e. Mahaleb, Maxma-14 and Weiroot-10 were investigated.

Rooting was easily achieved within 2–4 weeks on MS-based liquid or agar-gelled media containing auxins IBA at conc. 0.49 or 2.45 μM or NAA at conc. of 0.49 μM . On liquid media with 2.45 μM IBA, a maximum rooting efficiency of 95–100% was obtained. However, high concentrations of auxin delayed the time of root initiation for 3–5 days.

Rooted plantlets were transplanted into pots with a mixture of 3:1 (v/v) peat:perlite and acclimatised gradually to field conditions with efficiency of 60%.

Abbreviations: MS- Murashige & Skoog medium (1962), IBA- indole-3-butyric acid, NAA- o-naphthaleneacetic acid, IAA- indole-3-acetic acid, BA- benzyladenine, Kin.- kinetin

Introduction

Micropropagation of horticultural plants is practically accepted industry. The *in vitro* cloning is a significant procedure mainly in floriculture and nurseries of arboriculture (i.e. fruit growing) all over the world, in advanced as well as in developing countries, in the temperate climate as well as in the tropics and subtropics (Fari et al., 1996). Rooting, however, is one of the most critical steps of the propagation technique (Németh, 1981, Jones & Hoopgood, 1979, Druart, 1980). Additionally, successful rooting and vigorous growth of plantlets transplanted from *in vitro* conditions are essential for utilization of micropropagation procedures as part of an efficient production system (Karhu & Ulvinen, 1995).

Cherry is considered among the difficult-to-root species. There are limited reports on the successful application of vegetative propagation of cherry by root cuttings (Ghani & Cahalan, 1991), or stem cuttings (Al-Barazi & Schwabe, 1985). However, many authors refer that most cherry rootstocks are difficult-to-root by cuttings (Feucht & Dausend, 1976, Hartman & Kester, 1983, Ranjit & Kester 1988a,b, Ranjit et al., 1988). However, barriers to adventitious root formation in woody species can be eliminated by micropropagation (Rugini & Verma, 1982, Sriskandarajah et al., 1982).

Several investigations have been carried out to refine conditions for high rooting percentage of cherry shoots

raised *in vitro* (Ivanicka & Pretova, 1986, Branka & Jelaska, 1987, Chvojka et al. 1986, Feucht & Dausend, 1976, Paul & Feucht, 1985, Ranjit & Kester, 1988a,b).

The primary cherry rootstock widely used in cherry orchards is Mahaleb seedling rootstock (*P. mahaleb* L.). However, it has many disadvantages. Mahaleb trees do not grow satisfactorily in heavy, wet soils with high water tables due to their susceptibility to *Phytophthora* root rot. Further, trees on Mahaleb are relatively short-lived (Hartman & Kester 1983). On the other hand, the cherry rootstocks Maxma-14 and Weiroot-10 are a semi-dwarfing rootstocks and can improve the precocity of scion cultivars (Edin, et al. 1996, Hrotko, & Füzessey, 1996a,b, Hrotko et al., 1997, Kappel & Lichou, 1994, Soltész, 1998).

This paper describes *in vitro* rooting and acclimatization system of three commercially important cherry rootstocks and studies a range of factors that influence their root initiation and acclimatization of rooted plantlets.

Materials and methods

Initiation and establishment of aseptic cultures

Shoot tip and axillary shoot cultures of cherry rootstocks Mahaleb, Maxma -14 and Weiroot-10 had been established 1 year prior to the rooting experiments and were grown and

Table 1 Rooting of Cherry rootstocks on different agar-gelled and liquid media

Code	Medium	Rootstock	Root length cm	Root No.	Rooting%
R1-A	MS+0.49µM NAA	Maxma	3.59 cd ±0.38	3.2 bc ±0.32	40
		Weiroot	3.68 de ±0.33	3.00 bc ±0.31	35
		Mahaleb	3.67 de ±0.21	3.15 bc ±1.34	40
R2-A	1/2MS+0.49µM NAA	Maxma	3.39 cd ±0.34	2.75 bc ±0.3	45
		Weiroot	3.46 de ±0.24	2.35 cf ±0.24	40
		Mahaleb	3.29 ef ±0.24	2.65 cd ±0.24	45
R2-L	1/2MS+0.49µM NAA	Maxma	3.52 cd ±0.39	3.45 b ±0.48	60
		Weiroot	3.55 de ±0.21	3.50 bc ±0.25	55
		Mahaleb	3.45 cf ±0.24	3.40 bc ±0.31	60
R3-L	1/2MS+0.49µM IBA	Maxma	4.95 b ±0.3	2.35 cd ±0.23	60
		Weiroot	4.87 bc ±0.38	2.51 fg ±0.32	60
		Mahaleb	4.97 bc ±0.30	2.30 de ±0.23	65
R4-A	1/2MS+2.45µM IBA	Maxma	5.23 ab ±0.39	3.55 b ±0.31	75
		Weiroot	5.26 ab ±0.71	3.60 b ±0.35	85
		Mahaleb	5.56 ab ±0.43	3.50 b ±0.29	90
R4-L	1/2MS+2.45µM IBA	Maxma	6.32 a ±0.68	4.85 a ±0.38	95
		Weiroot	6.21 a ±0.32	5.55 a ±0.33	95
		Mahaleb	6.44 a ±0.54	4.70 a ±0.24	95
R5-A	MS+0.49µM IBA	Maxma	3.61 cd ±0.6	3.6 b ±0.24	45
		Weiroot	3.70 de ±0.33	3.30 bc ±0.31	45
		Mahaleb	3.73 de ±0.26	3.45 b ±0.32	50
R6-A	1/2MS+0.49µM IBA	Maxma	3.53 cd ±0.31	3.0 bc ±0.5	50
		Weiroot	3.51 de ±0.33	2.70 cd ±0.31	50
		Mahaleb	3.58 de ±0.24	3.00 bc ±0.28	55
R7-L	MS+0.49µM IBA	Maxma	4.38 bc ±0.53	3.0 bc ±0.21	70
		Weiroot	4.33 bc ±0.38	2.95 bc ±0.31	80
		Mahaleb	4.48 cd ±0.34	3.10 bc ±0.27	80
R0-A	MS	Maxma	2.58 d ±0.19	3.05 bc ±0.33	20
		Weiroot	2.77 c ±0.30	2.50 d ±0.26	25
		Mahaleb	2.65 f ±0.26	3.10 bc ±0.38	30
LSD 0.05%		Maxma	1.21	0.92	
		Weiroot	1.07	0.33	
		Mahaleb	0.90	0.78	

Notes: Data are the means of 20 replicates after 4 weeks on rooting media ± SE
 In each column, means with different letters are significantly different (p= 0.05).
 Agar-gelled medium
 L- Liquid medium with perlite substrate

Table 2 Effect of auxin type on rooting criteria of cherry rootstocks studied

Code	Medium	Rootstock	Root length cm	Root No.	Rooting%
R1-A	MS+0.49µM NAA	Maxma	3.59 cd ±0.38	3.2 bc ±0.32	40
		Weiroot	3.68 de ±0.33	3.00 bc ±0.31	35
		Mahaleb	3.67 de ±0.21	3.15 bc ±1.34	40
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		Weiroot	3.46 de ±0.24	2.35 cf ±0.24	40
		Mahaleb	3.29 ef ±0.24	2.65 cd ±0.24	45
R5-A	MS+0.49µM IBA	Maxma	3.61 cd ±0.6	3.6 b ±0.24	45
		Weiroot	3.70 de ±0.33	3.30 bc ±0.31	45
		Mahaleb	3.73 de ±0.26	3.45 b ±0.32	50
R6-A	1/2MS+0.49µM IBA	Maxma	3.53 cd ±0.31	3.0 bc ±0.5	50
		Weiroot	3.51 de ±0.33	2.70 cd ±0.31	50
		Mahaleb	3.58 de ±0.24	3.00 bc ±0.28	55
R0-A	MS	Maxma	2.58 d ±0.19	3.05 bc ±0.33	20
		Weiroot	2.77 c ±0.30	2.50 d ±0.26	25
		Mahaleb	2.65 f ±0.26	3.10 bc ±0.38	30

Notes: Data are the means of 20 replicates after 4 weeks on rooting media ± SE
 In each column, means with different letters are significantly different (p= 0.05).
 A- Agar-gelled medium
 L- Liquid medium with perlite substrate

subcultured monthly onto 30-40 ml of MS medium (Murashige & Skoog 1962) supplemented with 4.4 µM BA or Kin., 0.49 µM IBA, 87.6 mM sucrose and 7 g/l Difco Bacto Agar. Cultures were maintained under 16/8 hr. photoperiod under cool white fluorescent light at a photon flux of 54 µmol m⁻² s⁻¹ at bench height, at 24 °C (Al-Sabbagh et al., 1999).

In vitro induction of adventitious roots

For root initiation, 2 to 3-cm -long shoot tips were excised from 3-weeks-old proliferating cultures grown on media mentioned above, and grown on full or half strength of MS macroelements with 0.5 or 2.45 µM IBA or 0.5 µM NAA

gelled with 0.7% agar or on liquid media with perlite substrate to support microcuttings (Table 1). Rooting was expressed after 4 weeks of culture on rooting media as: rooting percentage, number of adventitious roots per rooted culture and length of the longest root.

The pH of all media was adjusted to 5.7–5.8 before autoclaving at 1.3 kg. cm⁻² at 121 °C for 20 min. All components of the media were autoclaved together.

Acclimatisation

Rooted cuttings were transferred after 2–4 weeks of maintaining the shoots on rooting media to a 3:1 peatmoss: perlite (v/v) mixture in 7-cm diameter plastic pots and covered with plastic bags to maintain high humidity and gradually hardened off by cutting increasingly large holes in the bags until the bags were removed completely after about 4 weeks. During this period, they were kept in a controlled growth chamber at 20 °C with 80% RH and 16 hr. photoperiod under cool white fluorescent light (45 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux). They were fertilized weekly with 1/10 MS inorganic salts. Then, they were kept in a green house for the first year, then transplanted in a nursery.

The following factors were examined in relation to their effects on acclimatisation of plantlets raised in vitro: 1) time of transferring rooted plantlets from rooting media to plastic pots, 2) growth regulators in the multiplication medium (BA, Kin.) immediately before transferring shoots to rooting media and 3) agar or liquid media.

Culture conditions Proliferating cultures were incubated in a culture room at 23–24 °C with a 16/8 hr. photoperiod under cool white fluorescent light at a photon flux of 54 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at bench height. For rooting, shoots were incubated at 25 °C in complete darkness for the first 7 days, and then to the normal conditions in the culture room under 16/8 hr. light regime for further 3 weeks after which the roots were scored.

Statistical design and analysis of data:

Rooting data for all experiments were taken after 4 weeks of transfer of cuttings to rooting media. Frequency of rooting was summarized as percentage. Data for number and length of primary roots were assessed by analysis of variance using the statistical evaluation program MSTAT. Twenty replications were used per treatment. All experiments were repeated at least twice on the same rooting media.

Results

Rooting

Effect of auxin type and concentration on rooting

Root initiation started within 2 weeks of culturing shoots on rooting media on all media tested, with no further root

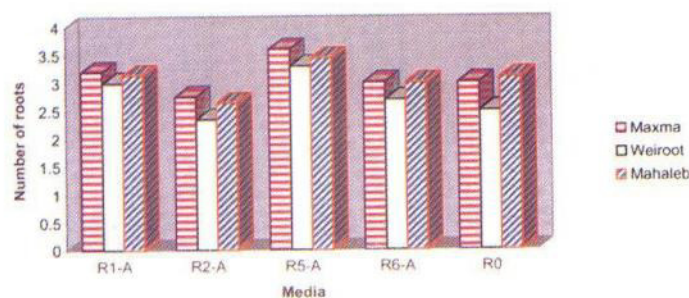


Figure 1 Effect of auxin type on root number of cherry rootstocks

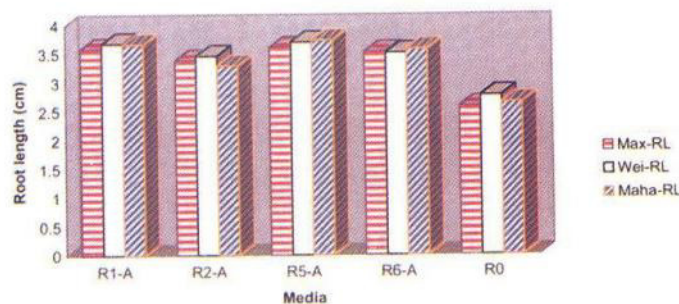


Figure 2 Effect of auxin type on root length of cherry rootstocks

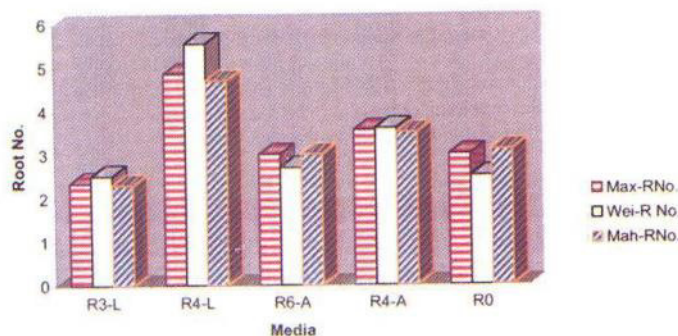


Figure 3 Effect of auxin conc. on number of roots formed in vitro/cutting in cherry rootstocks

differentiation occurred after that. However, emerged roots continued their development.

Rooting potential of the studied rootstocks was high (20–30%) even without IBA treatment, but IBA or NAA was essential for good rooting.

Auxin type and conc. affected the rooting percentages and the number of roots as well as root length in the rooted shoots of all cherry rootstocks studied with IBA being slightly superior to NAA. However, the differences of the effect of auxin type (IBA, NAA) on number of roots formed and their length were not statistically significant (Table 1, Figs. 1, 2). On the other hand, the effects of auxin concentrations on root number and length were significantly different (Table 1, Figs. 3, 4). However, it could be observed that higher IBA concentrations delayed the time of root initiation for 3–5 days.

Effect of agar-gelled versus liquid media

The best root formation was observed on 1/2 MS-based liquid media with 2.45 μM IBA which produced roots readily with 95% efficiency. Liquid media with perlite substrate gave higher rooting percentage (95%) than that of agar-gelled media (75%) (Table 1). Liquid media significantly increased the number and length of primary roots formed per microcutting (Figs. 5, 6). The microcuttings also produced more lateral roots and root hairs in liquid media than on agar media.

Effect of salt concentration on rooting

Decreasing MS macro salt concentrations by half resulted in slight increase in rooting% (5%) irrespective of auxin used (Table 1). Similar effects also were observed in terms of root number and length. However, these differences were not statistically significant (Table 1, Figs. 7, 8).

Acclimatisation

Acclimatisation was affected directly by rooting conditions. Survival was best when plantlets were transferred to pots when roots reached 1–2 cm in length on rooting media. Further maintenance on rooting media gave rise to longer roots but resulted in poor survival. Multiplication medium was also important for successful acclimatisation, since shoots transferred to rooting media from that with Kinetin where the shoots had better leaf expansion and better quality in general, resulted in better acclimatisation and survival than that derived from media with benzyladenine. Furthermore, plantlets rooted on liquid media had better survival than that rooted on agar-gelled media.

Acclimatisation of *in vitro* propagated cherry rootstocks proved to be difficult at times with losses occasionally amounting to more than 80% of the plantlets (in the preliminary experiments). However, acclimatisation rate could be improved considerably up to 60% survival, so that these acclimatised plantlets could be transplanted to a nursery or field. These plantlets were healthy, vigorous and free of any morphological abnormalities and are planted in the field under natural conditions.

Discussion

The right choice of the auxin type and concentration for rooting are very important in influencing rooting ability of microcuttings *in vitro*. In our studies, the rootstocks tested differed in their auxins response on rooting *in vitro*. Rooting potential was relatively high (20–30%) even without auxins. This might be ascribed to the role of subculturing on rejuvenation. Similar observations have been reported in apple (Sriskandarajah & Mullins, 1981, Sriskandarajah et al., 1982).

Recently, Jám bor-Benczúr et al., 1999, found out the best period and medium for *in vitro* rooting of *Prunus* x

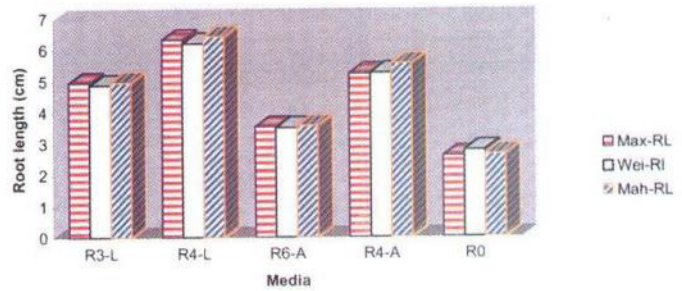


Figure 4 Effect of auxin conc. on root length of cherry rootstocks

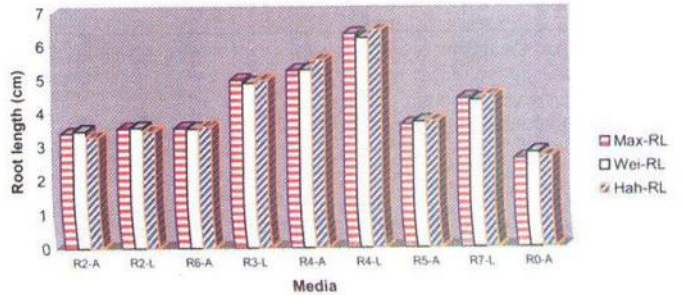


Figure 5 Effect of agar-gelled vs. liquid media on number of roots/microcutting of cherry rootstocks

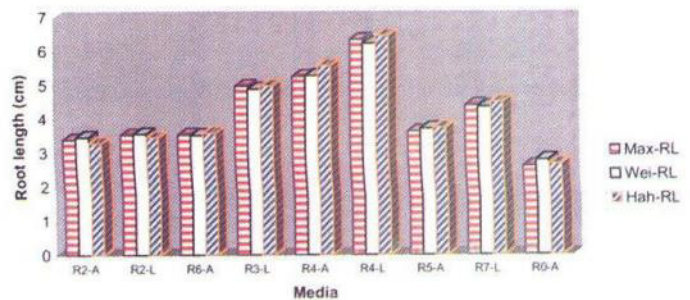


Figure 6 Effect of agar-gelled vs. liquid media on roots length of cherry rootstocks

Davidopersica 'Piroska', -the difficult-to-root *ex vitro* cultivar. They achieved best results in autumn on media with 0.2 mg/l IBA and 30 g/l glucose. They concluded that the best period for rooting is spring with IBA or NAA in low 0.1 or 0.2 concentration. They further described the anatomical changes of the leaves and roots during the *in vitro* and *ex vitro* phase (Jám bor-Benczúr et al., 1999). However, in the present study, roots were induced even on medium free of any auxins. However, auxin addition was necessary to raise rooting percentage and the quality of roots and rooted shoots too. Using 0.49 μM IBA which is equal to 0.1 mg/l used by Jám bor-Benczúr et al., a rooting frequency of 60–65% could be obtained. It could also be observed that the physical properties of the substrate used during *in vitro* rhizogenesis can affect the initiation, growth and number of roots of the plantlets. Liquid media caused significantly increase of the num-

Table 3 Effect of auxin conc. on rooting criteria of cherry rootstocks studied

Code	Medium	Rootstock	Root length cm	Root No.	Rooting%
R3-L	1/2MS+0.49 μ M IBA	Maxma	4.95 b \pm 0.3	2.35 cd \pm 0.23	60
		Weiroot	4.87 bc \pm 0.38	2.51 fg \pm 0.32	60
		Mahaleb	4.97 bc \pm 0.30	2.30 dc \pm 0.23	65
R4-A	1/2MS+2.45 μ M IBA	Maxma	5.23 ab \pm 0.39	3.55 b \pm 0.31	75
		Weiroot	5.26 ab \pm 0.71	3.60 b \pm 0.35	85
		Mahaleb	5.56 ab \pm 0.43	3.50 b \pm 0.29	90
R4-L	1/2MS+2.45 μ M IBA	Maxma	6.32 a \pm 0.68	4.85 a \pm 0.38	95
		Weiroot	6.21 a \pm 0.32	5.55 a \pm 0.33	95
		Mahaleb	6.44 a \pm 0.54	4.70 b \pm 0.24	95
R6-A	1/2MS+0.49 μ M IBA	Maxma	3.53 cd \pm 0.31	3.0 bc \pm 0.5	50
		Weiroot	3.51 de \pm 0.33	2.70 cd \pm 0.31	50
		Mahaleb	3.58 de \pm 0.24	3.00 bc \pm 0.28	55
R0-A	MS	Maxma	2.58 d \pm 0.19	3.05 bc \pm 0.33	20
		Weiroot	2.77 e \pm 0.30	2.50 de \pm 0.26	25
		Mahaleb	2.65 f \pm 0.26	3.10 bc \pm 0.38	30

Notes: Data are the means of 20 replicates after 4 weeks on rooting media \pm SE
 In each column, means with different letters are significantly different ($p=0.05$).
 A- Agar-gelled medium
 L- Liquid medium with perlite substrate

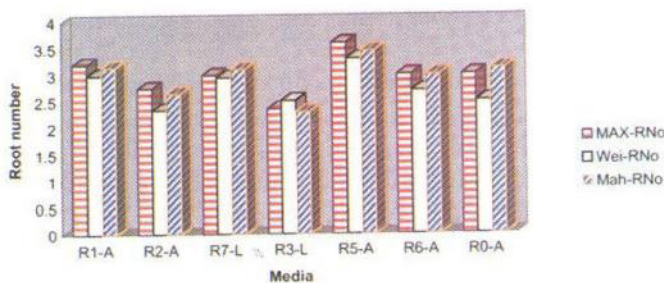


Figure 7 Effect of salt conc. on number of roots formed/microcutting of cherry rootstocks

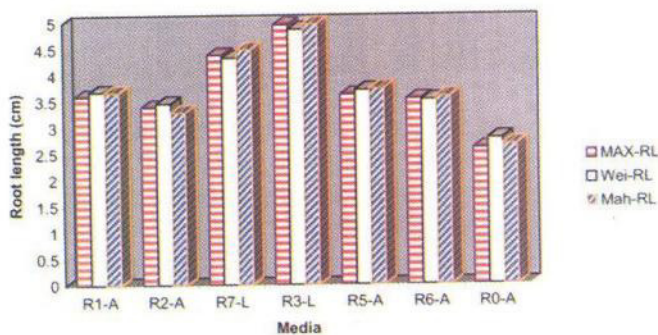


Figure 8 Effect of salt conc. on root length of cherry rootstocks

ber and length of primary roots formed per microcutting. This might be due to the better access to the nutrients and auxins and better aeration in the liquid media compared to agar-gelled media.

No data are available in the literature concerning these factors that affect successful acclimatisation of cherry rootstocks. In the present study, however, it was shown that acclimatisation was directly influenced by rooting conditions. Microcuttings from liquid media produced more lateral roots and root hairs than on agar-gelled media. Also, roots from

liquid media are not injured during transplantation, while those from agar media are injured while washing away the agar debris. Survival was also better when plantlets of shorter roots (1–2 cm) were transplanted than that of longer roots. This might be ascribed to the fact that longer roots were injured during transplantation into pots, while the shorter ones were easily handled, not injured and continued their development in the pot mixtures. This results agrees with the findings by Jámbor-Benczúr that the shorter roots are better from the point of view of acclimatisation (Jámbor-Benczúr et al. 1999).

This paper describes a detailed system for the root initiation, acclimatisation and establishment of *in vitro* rooted three commercially important cherry rootstocks, i.e. Maxma-14, Weiroot-10 and Mahaleb. The described method has potential to produce thousands of healthy and uniform true-to-type plants of the cherry rootstocks in a short time.

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Table 4 Effect of agar-gelled and liquid media on rooting criteria of cherry rootstocks studied

Code	Medium	Rootstock	Root length cm	Root No.	Rooting%
R2-A	1/2MS+0.49µM NAA	Maxma	3.39 cd±0.34	2.75 bed±0.3	45
		Weiroot	3.46 de±0.24	235 ef ±0.24	40
		Mahaleb	3.29 ef ±0.24	2.65 cd±0.24	45
R2-L	1/2MS+0.49µM NAA	Maxma	3.52 cd±0.39	3.45 b ±0.48	60
		Weiroot	3.55 de±0.21	3.50 bc±0.25	55
		Mahaleb	3.45 ef ±0.24	3.40 bc±0.31	60
R3-L	1/2MS+0.49µM IBA	Maxma	4.95 b ±0.3	2.35 cd±0.23	60
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		Mahaleb	6.44 a ±0.54	4.70 a ±0.24	95
R5-A	MS+0.49µM IBA	Maxma	3.61 cd±0.6	3.6 b ±0.24	
		Weiroot	3.70 de±0.33	3.30 bc±0.31	45
		Mahaleb	3.73 de±0.26	3.45 b ±0.32	50
R6-A	1/2MS+0.49µM IBA	Maxma	3.53 cd±0.31	3.0 bc ±0.5	50
		Weiroot	3.51 de±0.33	2.70 cd±0.31	50
		Mahaleb	3.58 de±0.24	3.00 bc±0.28	55
R7-L	MS+0.49µM IBA	Maxma	4.38 bc±0.53	3.0 bc±0.21	70
		Weiroot	4.33 bc±0.38	2.95 bc±0.31	80
		Mahaleb	4.48 cd±0.34	3.10 bc±0.27	80
R0-A	MS	Maxma	2.58 d ±0.19	3.05 bc±0.33	20
		Weiroot	2.77 e ±0.30	2.50 de±0.26	25
		Mahaleb	2.65 f ±0.26	3.10 bc±0.38	30
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		Mahaleb	3.29 ef ±0.24	2.65cd ±0.24	45
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		Weiroot	4.87 bc±0.38	2.51 fg±0.32	60
		Mahaleb	4.97 bc±0.30	2.30 de±0.23	65
R5-A	MS+0.49µM IBA	Maxma	3.61 cd±0.6	3.6 b ±0.24	45
		Weiroot	3.70 de±0.33	3.30 bc±0.31	45
		Mahaleb	3.73 de±0.26	3.45 b ±0.32	50
R6-A	1/2MS+0.49µM IBA	Maxma	3.53 cd±0.31	3.0 bc ±0.5	50
		Weiroot	3.51 de±0.33	2.70 cd±0.31	50
		Mahaleb	3.58 de±0.24	3.00 bc±0.28	55
R7-L	MS+0.49µM IBA	Maxma	4.38 bc±0.53	3.0 bc±0.21	70
		Weiroot	4.33bc ±0.38	2.95 bc±0.31	80
		Mahaleb	4.48 cd±0.34	3.10 bc±0.27	80
R0-A	MS	Maxma	2.58 d ±0.19	3.05 bc±0.33	20
		Weiroot	2.77 e ±0.30	2.50 de±0.26	25
		Mahaleb	2.65 f ±0.26	3.10 bc±0.38	30

Notes: Data are the means of 20 replicates after 4 weeks on rooting media ± SE

In each column, means with different letters are significantly different (p= 0.05).

A- Agar-gelled medium

L- Liquid medium with perlite substrate

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