Turkish Journal of Agricultural and Natural Sciences
Special Issue: 1, 2014

TÜRK
TARIM ve DOĞA
BİLİMLERİ DERGİSİ

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.turkjans.com

Effects of agar types on rooting performance in tissue culture: Sample of Quince A rootstock cultures

[a,b]Melekber SULUSOGLU[*]

[a]Kocaeli University, Arslanbey Agricultural Vocational School, TR-41285, Kocaeli/Turkey.

[b]Kocaeli University, Graduate School of Natural and Applied Sciences, Department of Horticulture, TR-41380, Kocaeli/Turkey

[*]Corresponding author: meleksl@kocaeli.edu.tr

Abstracts

This study investigated the effects of the four commercial gelling agents from Merck, Bacto, Oxoid, and Gelrite on *in vitro* rooting of, one of the popular pear rootstocks, Quince A. IBA or NAA, at 0.5 gL^{-1} concentration, was added in the rooting media. Different rooting rates were observed according to used agar and auxin types. The highest rooting rate was recorded in the medium containing 9 gL^{-1} Oxoid agar. Root number and length were best in Bacto agar, especially at its 7 gL^{-1} concentration. Gelrite was unsuitable for rooting of Quince A. Optimum concentrations for Merck (7 gL^{-1}), Bacto (7 gL^{-1}), and Oxoid (7 & 9 gL^{-1}) agar were selected in the first experiment, then they were supplemented with 0.5 gL^{-1} IBA or 0.5 gL^{-1} NAA in the second experiment. IBA was more effective on root number and root length than NAA. Merck agar (7 gL^{-1}) was the most economical, Bacto agar (7 gL^{-1}) was the most effective on root quality of Quince A.

Keywords: Agar types, Quince A, *in vitro* rooting, NAA, IBA, cost analysis

Agar tiplerinin doku kültüründe köklenme başarısına etkileri: Quince A anacı köklenme örneği

Özet

Çalışmada 0.5 gL^{-1} IBA veya NAA eklenen dört farklı ticari agar tipinin, Merck, Bacto, Oxoid, ve Gelrite, bilinen bir armut anacı olan Quince A'nın köklenmesi üzerine incelenmiştir. Çalışmada kullanılan agar tipine ve konsantrasyonuna bağlı olarak farklı köklenme oranları oluşmuştur. En yüksek köklenme oranı 9 gL^{-1} Oxoid agar içeren ortamda kaydedilmiştir. Kök sayısı ve uzunluğu Bacto agar eklenen ortamlarda en iyi olarak belirlenmiş, özellikle 7 gL^{-1} konsantrasyonu öne çıkmıştır. Gelrite Quince A'nın köklenmesi için uygun bulunmamıştır. İlk çalışmada, Merck (7 gL^{-1}), Bacto (7 gL^{-1}), ve Oxoid (7 & 9 gL^{-1}) agar konsantrasyonları en iyi olarak belirlenmiş, çalışmanın ikinci kısmında bu agar konsantrasyonlarına 0.5 gL^{-1} IBA veya 0.5 gL^{-1} NAA ilave edilmiştir. IBA kök sayısı ve kök uzunluğu üzerinde daha etkili bulunmuştur. Ekonomiklik açısından değerlendirildiğinde Merck agar (7 gL^{-1}) öne çıkarken, Bacto agar (7 gL^{-1}) Quince A'nın kök kalitesi bakımından en iyi sonuçları vermiştir.

Anahtar kelimeler: Agar tipleri, Quince A, *in vitro* köklenme, NAA, IBA, maliyet analizi

Introduction

Micropropagation technology has been widely applied for the production of a large number of economically important plants including trees, horticultural crops, and medicinal plants. Use of true type gelling agent is very important for the success of tissue cultures and it also affects the cost. Agar is the most frequently used solidifier in plant tissue culture media (Afrasiab and Jafar, 2011; Lima et al., 2012) owing to its stability, high clarity, and non-toxic nature (Henderson and Kinnersly, 1988). Agar types and different concentrations affect growth and development of *in vitro* cultures (Stoltz, 1971). Agar-products from different manufacturers are different in terms of contained impurities, level of solidification and composition. In a culture medium, the gelling agent is the costliest ingredient compared to others (Ezekiel, 2010; Mengesha et al., 2012). The study on the selection of agar sources is important, as it might considerably affect the plant cultures. There are some studies that determined the best agar brands (Scholten and Pierik, 1998). Researchers have been trying to prepare the media containing agar and other gelling agents at

different concentrations to reduce the cost (Zimmerman et al., 1995; Mohamed et al., 2009).
Reducing the total cost of *in vitro* multiplication depends on the optimization of suitable type and concentration of the gelling agents. In the present study, we have investigated the effects of agar types and concentrations on *in vitro* rooting of Quince A rootstock.

Material and Methods

In this study, microshoots of Quince A rootstock were used as the plant material. Quince A is one of the semi-dwarf rootstocks of pear that can attain 3-4 m height in productive stage (Campbell, 2003; Kumar-Cauhan et al., 2012; Anonymous, 2014a). This rootstock is compatible or semi-compatible with most of the pear cultivars (Davarynejad et al., 2008). Quince A microshoots were produced *in vitro* as described earlier (Gülşen et al., 1997). About 1 cm long shoots were removed from the proliferation medium and planted in the rooting medium that included MS basal medium at full strength. Following concentrations, 5, 7, and 9 gL^{-1}, of agar from Merck (agar-agar ultrapure-granulated), Bacto (Difco-Bacto, agar granulated), and Oxoid (agar no.: Technical 3); and 2.5, 3.5, and 4.5 gL^{-1} from Gelrite (Gellan Gum) were added into the culture media for solidification. Gelrite provides the same apparent gel firmness at half concentrations then other agar types. As observed earlier, 0.5 gL^{-1} IBA was added into culture media to maximize root formation of Quince A (Dumanoğlu and Gülşen, 1994). Subsequently, four agar concentrations (7 gL^{-1} Merck, 7 gL^{-1} Bacto, 7 gL^{-1} Oxoid and 9 gL^{-1} Oxoid agar) were selected to use and were supplemented with 0.5 gL^{-1} IBA or NAA. Hormone free medium was found to be unsuitable, therefore, was not considered in the study. Sucrose (30 gL^{-1}) was used in all experiments and pH of the media was set to ~5.5 before autoclaving at 121 ºC under 1.2 kg/cm^2 pressure for 20 min. Tubes of 10 mm diameter, containing 10 ml media, were used to plant one microshoot per tube. The cultures were grown for four weeks at 25 ±1 ºC with 16 hours photoperiods provided by warm white fluorescent lights.

Study was conducted as a completely randomized plot design with three replicates, where each replicate contained 20 micro plants. Rooting rate was recorded by counting the roots with a length of ≥0.5 cm. The length of the microshoot was recorded at the end of the rooting period since it was important for the acclimatization stage. The rate of callus formation and its size was also observed. Statistical analysis was performed using Minitab 17 Statistical program. One-way ANOVA was used for the analysis and mean values were compared by employing Duncan's multiple range test at $p<0.05$ probability level. The percent values were transformed to arcsine square root values before the statistical analysis. A cost benefit analysis for the four gelling agents used for Quince A *in vitro* rooting was made. The cost of gelling agent required to prepare one litter culture medium was calculated. Then, agar types were ranked as per economical point of view and effects on rooting quality.

Results

Rooting rate and quality in Quince A changed according to agar type and concentrations. Agar type vs. concentration interactions were statistically significant for all the examined criteria. The highest rooting rate (43.33%) occurred in Oxoid agar at 9 gL^{-1}, and then in Bacto agar at 7 gL^{-1} (Table 1). Root formation was not observed in 2.5 gL^{-1} Gelrite. Rooting differences among the agar types are given in Figure 1.

The medium containing 7 gL^{-1} Bacto agar resulted in dense growth of fine lateral roots, where average number of roots was 5.57 per microshoot, it was followed by Merck agar at 7 gL^{-1} concentration (Table 2). Root elongation increased at higher concentrations of Merck agar while decreased at higher concentrations of Bacto agar (Table 3).

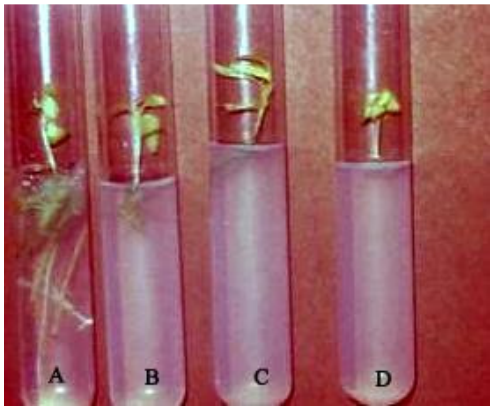

Figure 1. Rooting of Quince A microshoots in A: 9 gL^{-1} Oxoid; B: 7 gL^{-1} Bacto; C: 7 gL^{-1} Merck; and D: 4.5 gL^{-1} Gelrite.

Table 1. Effects of agar types and concentrations on rooting of Quince A (%)

Agar concentration (gL^{-1})	Agar type				
	Merck	Bacto	Oxoid	Gelrite	Average
5 (2.5)	11.67 aBC	33.33 aA	21.67 abAB	0.00 bC	
7 (3.5)	16.67 aAB	35.00 aA	16.67 bAB	6.67 abB	17.64
9 (4.5)	10.00 aB	3.33 bB	43.33 aA	13.33 aB	
Average	12.68	23.88	27.22	6.67	

*Values within the same column (rooting rate differences among agar concentrations on the same agar type) with different lower-case letters are significantly different; values in the same row (rooting rate differences among agar types at the same concentration) with different upper-case letters are significantly different ($p<05$).

Table 2. Effects of agar types and concentrations on root number of Quince A (%)

Agar concentration (gL^{-1})	Agar type				
	Merck	Bacto	Oxoid	Gelrite	Average
5 (2.5)	0.67 cA	1.50 bA	2.83 aA	0.00 aA	
7 (3.5)	3.50 aAB	5.57 aA	1.67 aBC	0.33 aC	1.92
9 (4.5)	2.67 bAB	0.33 bB	3.26 aA	0.67 aAB	
Average	2.28	2.47	2.59	0.33	

Values within the same column (rooting number differences among agar concentrations on the same agar type) with different lower-case letters are significantly different; values in the same row (rooting number differences among agar types at the same concentration) with different upper-case letters are significantly different ($p<05$).

Table 3. Effects of agar types and concentrations on root length of Quince A (%)

Agar concentration (gL^{-1})	Agar type				
	Merck	Bacto	Oxoid	Gelrite	Average
5 (2.5)	0.33 bB	3.40 aA	1.72 aAB	0.00 aB	
7 (3.5)	1.95 abAB	3.21 aA	1.39 aAB	0.43 aB	1.59
9 (4.5)	2.63 aA	0.27 bB	3.04 aA	0.73 aB	
Average	1.64	2.29	2.05	0.39	

Values within the same column (root length differences among agar concentrations on the same agar type) with different lower-case letters are significantly different; values in the same row (root length differences among agar types at the same concentration) with different upper-case letters are significantly different ($p<05$).

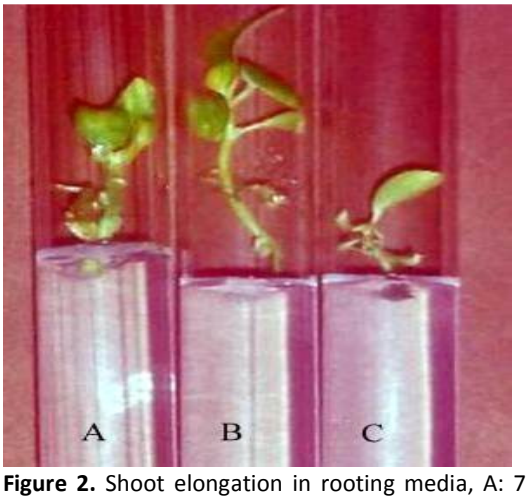

Figure 2. Shoot elongation in rooting media, A: 7 gL^{-1} Bacto; B: 9 gL^{-1} Oxoid; C: 7 gL^{-1} Merck

The analysis of variance showed that solidifying agents and concentration interactions were statistically significant. The shoot length was maximum in the media containing 5 gL^{-1} Bacto agar and 2.5 gL^{-1} Gelrite (2.89 cm and 2.79 cm, respectively). Gelrite was the worst effective agar for rooting; though, shoot elongation occurred. Shoot length was 2.53 cm in 9 gL^{-1} Oxoid agar (Table 4 and Figure 2).

Our findings showed that the microshoots placed in the medium congaing 9 gL^{-1} Oxoid agar exhibited the greatest development with a morphologically superior root and shoot system. Shoot elongation was limited in Merck agar in 5 and 7 gL^{-1} concentrations.

Callus formation was observed in unrooted microshoots mostly on basal sides, with size change from 0.5 to 0.65 cm (data not shown). Bacto agar was the most effective gelling agent on callus formation as per the average values (Table 5). Gelrite at concentration 4.5 gL^{-1} resulted in a very low rate of rooting and the roots were very fragile with callus formation.

Table 4. Effects of agar types and concentrations on shoot length of Quince A (%)

Agar concentration (gL^{-1})	Agar type				
	Merck	Bacto	Oxoid	Gelrite	Average
5 (2.5)	1.2 aB	2.89 aA	1.60 bB	2.79 aA	
7 (3.5)	2.06 aA	1.84 bA	1.82 abA	2.02 aA	2.02
9 (4.5)	1.20 aB	1.81 bAB	2.53 aA	2.51 aA	
Average	1.49	2.18	1.98	2.44	

Values within the same column (shoot length differences among agar concentrations on the same agar type) with different lower-case letters are significantly different; values in the same row (shoot length differences among agar types at the same concentration) with different upper-case letters are significantly different ($p<05$).

Table 5. Effects of agar types and concentrations on callus formation of Quince A microshoots (%)

Agar concentration (gL^{-1})	Agar type				
	Merck	Bacto	Oxoid	Gelrite	Average
5 (2.5)	10.55 bB	60.00 aA	55.00 aA	60.00 abA	
7 (3.5)	58.33 aAB	76.67 aA	41.67 aB	46.67 bB	51.71
9 (4.5)	51.67 aBC	58.33 aAB	28.33 aC	73.33 aA	
Average	40.18	65.00	41.67	60.00	

Values within the same column (callus rate differences among agar concentrations on the same agar type) with different lower-case letters are significantly different; values in the same row (callus rate differences among agar types at the same concentration) with different upper-case letters are significantly different ($p<05$).

Among all, only four agar concentrations (7 gL^{-1} Merck, 7 gL^{-1} Bacto, 7 gL^{-1} Oxoid, 9 gL^{-1} Oxoid agar) showed the highest performance and were used in the next study. 0.5 gL^{-1} IBA or NAA was added in the culture media. Rooting rate (49.17%) was maximum in the media containing 9.0 gL^{-1} Oxoid agar compare to 7.0 gL^{-1} Oxoid agar (17.50%). Rooting rate differences among selected agar concentrations were statistically significant. NAA (0.5 gL^{-1}) increased the rooting rate in 7 gL^{-1} Merck agar and 9 gL^{-1} Oxoid agar, but the effects with IBA and NAA hormones were not statistically significant (Figure 3).

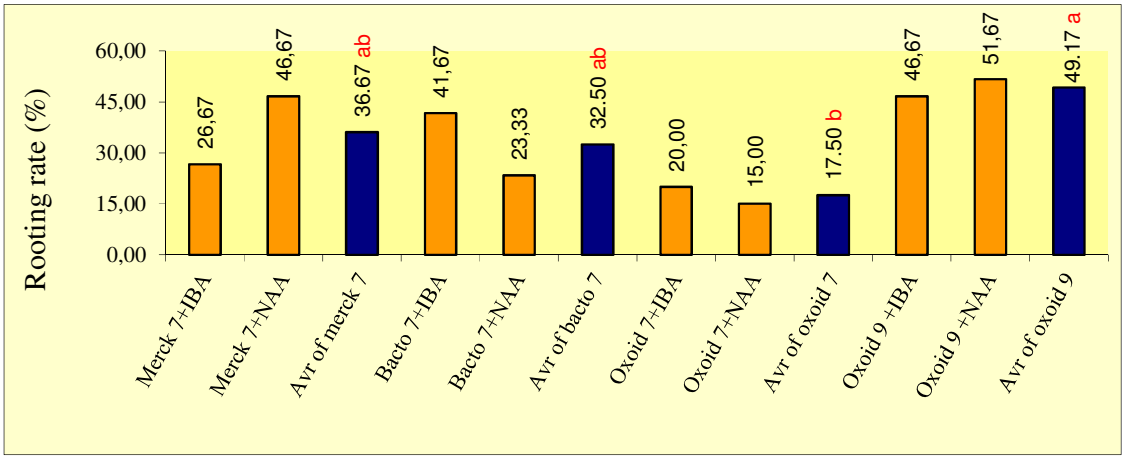

Figure 3. Rooting rate of Quince A microshoots in the selected agar concentrations supplemented with 0.5 gL^{-1}IBA or 0.5 gL^{-1}NAA.

IBA at a concentration of 0.5 gL^{-1} resulted in maximum statistically significant root numbers in comparison to NAA at 0.5 gL^{-1} concentration. Root number was highest in the media solidified with Bacto agar. Root number was lowest in media containing Oxoid agar. The differences in root length between the selected agar concentrations were statistically significant. The media solidified with 7 gL^{-1} Bacto agar and supplemented with 0.5 gL^{-1} IBA provided maximum root number (5.70

roots per explant) (Figure 4). Generally root elongation was higher with 0.5 gL^{-1} IBA than with 0.5 gL^{-1} NAA in the used culture media. The maximum root length (3.7 cm) was observed in 7 gL^{-1} Oxoid agar supplemented with 0.5 gL^{-1} IBA (Figure 5). Bacto agar at 7 gL^{-1} concentration supplemented with 0.5 gL^{-1} NAA and Oxoid agar at 9 gL^{-1}concentration supplemented with 0.5 gL^{-1} IBA showed similar effects (Figure 5). Elongation of microshoots was highest for the explants in the medium solidified with 9.0 gL^{-1} Oxoid agar supplemented with 0.5 gL^{-1} IBA or NAA (2.58 and 3.51 cm, respectively).

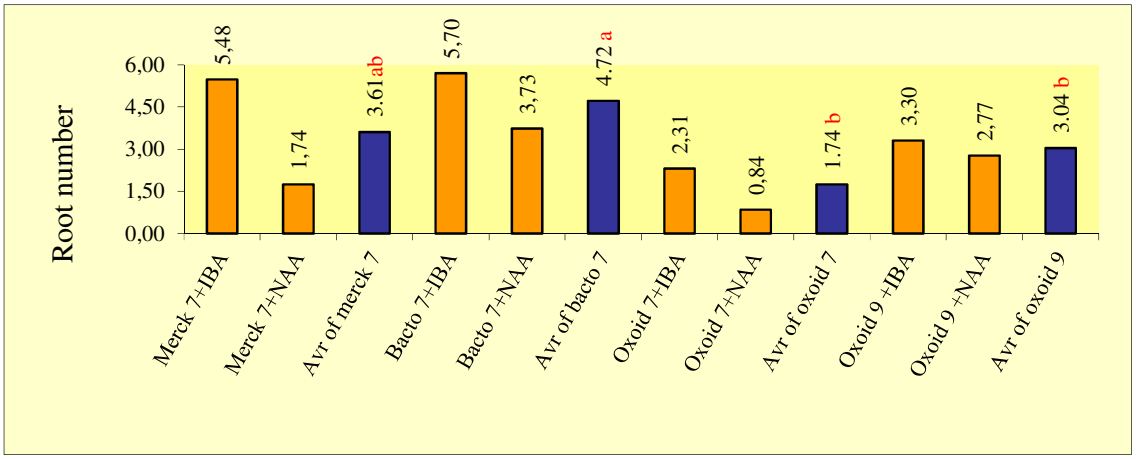

Figure 4. Root number of Quince A microshoots at the selected agar concentrations supplemented with 0.5 gL^{-1}IBA or 0.5 gL^{-1}NAA.

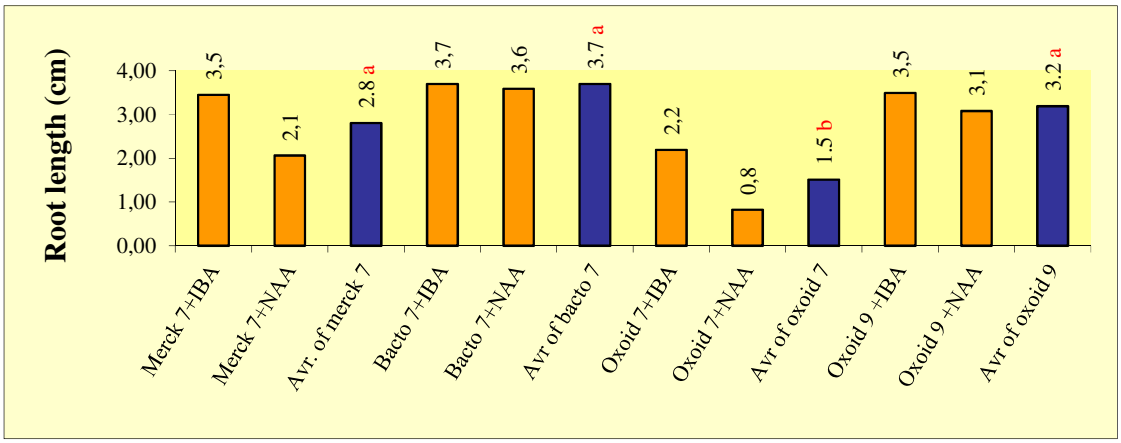

Figure 5. Root length of Quince A microshoots in the selected agar concentrations supplemented with 0.5 gL^{-1} IBA or 0.5 gL^{-1} NAA.

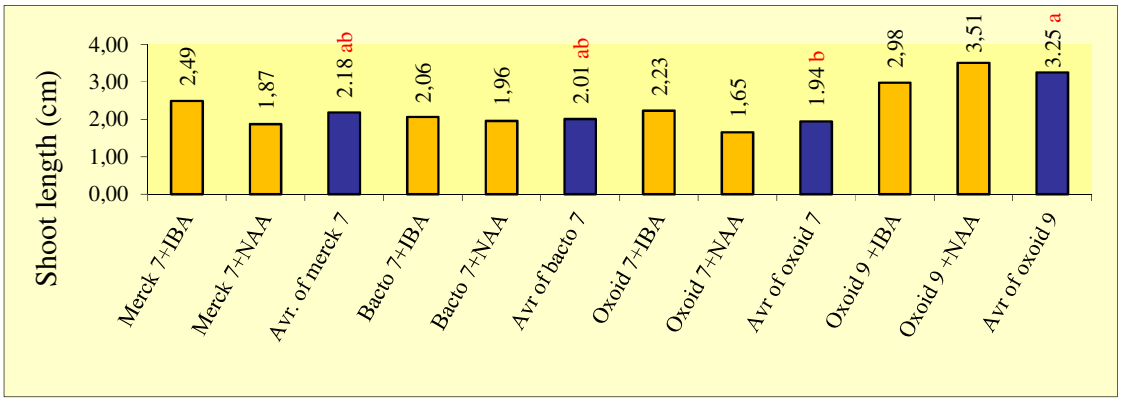

Figure 6. Shoot length of Quince A microshoots in the selected agar concentrations supplemented with 0.5 gL^{-1} IBA or 0.5 gL^{-1} NAA.

Table 5. Cost analysis of agar types

Gelling agents	Cost/kg[1] (€)	Amounts in medium[2] (gL^{-1})	Cost/liter medium[3] (€)	Ranking for economical point	Ranking for rooting criteria
Merck	209	7	1.46	1	3
Bacto	296	7	2.07	2	1
Oxoid	306	9	2.75	4	2
Gelrite	503	4.5	2.26	3	4

[1]Price of agars in August 2014.
[2]Recommended concentrations according to the study results.
[3]Calculated from the agar amount needed to prepare one liter culture medium.

According to the results, 7 gL^{-1} Merck, 7 gL^{-1} Bacto, 9 gL^{-1} Oxoid, and 4.5 gL^{-1} Gelrite agar resulted in better root or shoot quality in the culture conditions. We calculated the cost of one liter medium for each agar type according to the optimum required amount. Merck agar was determined as the most economical gelling agent, while Bacto agar was the second one. Oxoid agar was the most expensive because of need to add a higher concentration in the culture media when compared with others. Rooting rate was better in Oxoid agar but root quality (both of root number and root length) was better in the Bacto agar containing media. It can be affirmed that Gelrite is not suitable for Quince A rooting. This study clearly lays down the differences among the agar types, and establishes that choosing a suitable agar type and its concentration will affect the success of a microshoot propagation.

Discussion

This study showed effect of gelling agents on the rooting rate and the root quality. The results are in close agreement with the findings of Özel et al. (2008), who found differences in root number and root elongation when used Sigma agar, Gelrite or Phytagel alone or blend with isubgol; and cost of the culture medium varied with agar types used. On the other hand, effect of different gelling agents was investigated on *in vitro* rooting of *Vanilla planifolia*, but no significant difference was found (Mangesha et al., 2012). In our study, increasing concentrations of Bacto agar blocked the shoot elongation; similar results were obtained with same agar brand at the shoot multiplication stage in cherry rootstock 'Gisela 5' by Ruzic and Cerovic (1998). Gelrite has been reported to yield better results than agar for regeneration and shoot formation (Henderson, 1987; Welander and Maheswaran, 1992; Sharma et al., 2011), though, in our study, it decreased the rooting rate, root number, and root length.

Conclusion

Root development has a positive effect on the nutrient absorption capacity that results in better growth of microplants in the later developmental stages. This study underlines the importance of the different gelling agents in the rooting stage in plant tissue cultures, using Quince A as an experimental model. These results indicate that the type and concentration of agar has a definite effect upon the rooting and growth of the shoots. The results can be improved by investigating further the changes in root anatomy in different agar types and also the extent at which these physiological and anatomical changes influence the growth and survival *ex vitro*.

References

Afrasiab, H., Jafar, R., 2011. Effect of different media and solidifying agent on callogenesis and plant regeneration from different explants of rice (*Oryza sativa* L) varieties super basmati and IRRI-6. *Pak J Biol Sci* 43(1): 487-501.

Anonymous, 2014a. Quince A. http://www.thompson-morgan.com/fruit-rootstock-guide

Campbell, J., 2003. *Agfact H4.1.15*, first edition, p:12.

Chauhan, J.K., Sharma, U., Sharma, S., 2012. Effect of rootstock and interstock on plant vigour of pear 2(1): *Int J of Farm Sci* 2(1): 29-35.

Davarynejad, G., Shahriari, F., Hamid, H., 2008. Identification of graft incompatibility of pear cultivars on quince rootstock by using isozymes banding pattern and starch. *Asian J of Plant Sci*. 7(1):109-112.

Dumanoglu, H., Gulsen, Y., 1994. *In vitro* rooting of quince A (*Cydonia oblonga* Mill.). *Acta Hort* 367:360-363.

Ezekiel, A., 2010. Viable options and factors in consideration for low cost vegetative propagation of tropical trees. *Int J Bot* 6:187-193.

Gülşen, Y., Sülüşoğlu, M., Eroğlu, B., 1999. Quince A ayva anacının mikro üretiminde bazı agar tiplerinin sürgün proliferasyonu ve kalitesine etkileri. III. Yumuşak Çekirdekli Meyveler Sempozyumu. Türkiye III. Ulusal Bahçe Bitkileri Kongresi Bildiri Kitabı, 17-21 Eylül, Ankara/Türkiye, 218-222. (abst. in English).

Henderson, J.M., 1987. The use of Gelrite as a substitute for agar in medium for plant tissue culture. *Ala. Agric* 2:5-6.

Henderson, W.E., Kinnersley, A.M., 1988. Corn starch as an alternative gelling agent for plant tissue culture. *Plant Cell Tissue Organ Cult* 15:17-22.

Kumar-Chauhan, J., Sharma, U., Sharma, S0,. 2012. Effect of rootstock and interstock on plant vigour of pear. *Int J of Farm Sci* 2(1):29-35.

Lima, G.P.P., da Silva Campos, R.A., Willadino, L.G., Camara, T.J.R., Vianello, F., 2012. Polyamines, gelling agents in tissue culture, micropropagation of medicinal Plants and Bioreactors. *Recent Adv in Plant in vitro Culture* 9:165-182 (ed: Annarita Leva and Laura M. R. Rinaldi, ISBN 978-953-51-0787-3).

Mengesha, A., Ayenew, B., Gebremariam, E., Tadesse, T., 2012. Micro-propagation of *Vanilla planifolia* using enset (*Ensete ventricosum* (Welw, cheesman)) starch as a gelling agent. *Current Research J of Bio Sci* 4(4):519-525.

Mohamed, M.A.H., Alsadon, A.A., Al-Mohaidib, M.S., 2009. Corn and potato stach as an alternative for *Solanum tuberosum* micropropagation. *Afr. J.Biotec* 8:9199-9203.

Özel, Ç.A., Khawar, K.M., Arslan, O., 2008. A comparison of the gelling of isubgol, agar and Gelrite on *in vitro* shoot regeneration and rooting of variety Samsun of tobacco (*Nicotiana tabacum* L.). *Scientia Hort* 117:174-181.

Ruzic, Dj., Cerovic, R., 1998. Influence of agar brands and concentration on *in vitro* shoot multiplication of the cherry rootstock Gisela 5. Proc. Third. Int. Cherry Sym. Ed: Jonas Ystaas. *Acta Hort* 468:209-216.

Scholten, H.J., Pierik, R.L.M., 1998. Agar as a gelling agent:differential biological effects *in vitro*. *Scientia Hort* 77:109-116.

Sharma, S., Venkatasalam, E.P., Patial, R., Latawa, J., Singh, S., 2011. Influence of gelling agents and nodes on the growth of potato microplant. *Potato J* 38(1):41-46.

Stoltz, L.P., 1971. Agar restriction of the growth of excised mature Iris embryos. *J Am Soc Hort Sci* 96(5):618-684.

Welander, M., Maheswaran, G., 1992. Shoot regeneration from leaf explants of dwarfing apple rootstocks. *J of Plant Physiol* 140: 223-228.

Zimmerman, R.H., Bhardwaj, S.V., Fordham, I.M., 1995. Use of starch-gelled medium for tissue of some fruit crops. *Plant Cell Tiss Org Cult*, 43: 207–213.