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The effect of plant growth regulators and their concentration *in vitro* on mass poropagation of Myrobalan 29C rootstock

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Myrobalan 29C is one of the most important rootstocks that is widely used for plum and apricot trees. This study was conducted to determine the most suitable media culture and regulators of plant growth for micropropagation of Myrobalan 29C in Khorasan Natural Resource and Agricultural Research Centre Mashhad, Iran. 10 explants treated were sterilized in 70% Ethanol for 1 min, Mercuric chloride (0.1%) for 1, 2 and 3 min and sodium hypochlorite (3 and 10%) for 10, 20 and 30 min. Results showed that 10% sodium hypochlorite (30 min) with 2% decay was the best treatment. In this experiment, proliferation and rooting were performed in three kinds of culture media: Murashige and Skoog (MS), McCown and Lioyd (WPM) and Driver and Kuniyuki (DKW). They were supplemented with plant growth regulators (benzyl amino purine (BAP) and thiadizuron (TDZ)) of 0, 1, 2, 3, 4 mg l^{-1} in all treatments of the proliferation; and with indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) of 0, 1, 2, 3 mg l^{-1} in the rooting step. Results showed that the highest number and length of shoot respectively were 5.58 and 2.50 cm in MS medium with 2 mg l^{-1} BAP concentrations. The DKW medium in 1 mg l^{-1} of NAA, the highest percent of rooting (100%) and root length were about 14.5 cm in MS medium with 2 mg l^{-1} of NAA respectively. The acclimatization of plantlets was successful in greenhouse conditions. The survival percent in substrateperlite (100% V) was about 80%.

Key words: Tissue culture, disinfection, micropropagation, acclimatization.

INTRODUCTION

Myrobalan 29C (*Prunus cerasifera* L.) has been widely used as a fruit tree rootstock for plum and apricot because of its rusticity and adaptability to several soil conditions (Plopa et al., 2012). This rootstock is suitable for high density plum orchard. In addition, Myrobalan has also been used as a parent in several rootstock and edible plum breeding programs (Arbeloa et al., 2006). It has also a good grafting compatibility where majority of

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the new Romanian plum varieties resulted from. When propagated by softwood cuttings, the results are very good in respect to rooting ability (75 to 95% rooted cuttings); but a lot of cuttings on the rooting beds blossom. This finally results in very weak rooted cuttings, not suitable for grafting in nursery field. That is the reason why this valuable rootstock might be micropropagated on commercial scale. The use of *in vitro* propagation technique allows for the efficiency of propagation, but for some species the propagation efficiency depends on some factors. Micropropagation is used as a useful method for propagation of clonal rootstocks (Hossini et al., 2010).

The most proliferation of Myrobalan 29C was obtained in MS medium supplemented with 1 mgl^{-1} benzyl adenine (BA) and 0.1 mgl^{-1} IBA (Ruzick, 2013; Plopa, 2012). Myrobalan 29C in MS culture media containing 0.5 mg l^{-1} benzyl adenine (BA) had the most multiplication rate (Movsiuw, 2011). Dardi et al. (2011) reported that Mahaleb rootstock in MS medium containing BAP and NAA showed the highest proliferation and rooting. Bonjak (2012) showed that BA and TDZ concentration had significantly affected the proliferation rate of Gisela5. In the micro-propagation of *Prunus avium*, the combination of 0.5 mg l^{-1} BAP and 0.05 mg l^{-1} TDZ were suitable for proliferation and a medium containing 0.3 mg l^{-1} IBA was desirable for rooting (Hossini et al., 2010; Nazeri et al., 2010). *In vitro* micropropagation of Chinese plum in 1/2 MS media showed the highest percentage rooting and acclimatization of rooted plantlets to greenhouse conditions (Ying-Ning, 2010; Mikhilov et al., 2008; Tian et al., 2007). Tatari (2013), Xiaomei (2008) and Yao et al., 2011) reported that rooted plantlet were acclimatized after 6 weeks of growth in the laboratory and 76 to 84% of rooted plantlets survived after acclimatization in the greenhouse.

Due to the importance of achieving an efficient protocol for the mass propagation of Myrobalan 29C, this study was conducted with the purpose of evaluating the most efficient and effective micropropagation protocol for Myrobalan 29C rootstock in Iran. This would be useful in future research work as a reference for the cultivation of economically important rootstock.

MATERIALS AND METHODS

Plant materials

The explants were collected from shoots of Myrobalan 29C rootstock maintained in the experimental greenhouse of Khorasan Razavi Agricultural and Natural Resources Research Centre (Mashhad, Iran), on 25 June, 2013. The shoots were transferred to the laboratory where the tips and axillary buds were dissected with a scalpel and used as a source for explants. The explants were washed with water and dishwashing liquid to remove surface contamination and then divided into parts containing one bud. Then they were sterilized with 70% ethanol for 1 min, Mercuric chloride (0.1%) for 1, 2 and 3 min, sodium hypochlorite (3 and 10%) for 10, 20 and 30 min; and then washed 3 times with sterile distilled water and cultured in a medium. Each treatment disinfection consisted of three replications and each replicate had five samples.

Proliferation

Proliferation was performed in three kinds of culture media: MS (Murashige and Skooge, 1962), WPM (Lloyd and McCown, 1980) and DKW (Driver and Kuniyuki, 1984). They were supplemented with plant growth regulators (benzyl amino purine (BAP) and thiadiazuron) (TDZ) of 0, 1, 2, 3 and 4 mg l^{-1}. In the rooting step after three subcultures (21 days between each subculture), the numbers, length and quality of the shoots were measured. This stage was carried with four replications and each replicate had five samples.

Rooting

For rooting, *in vitro* developed shoots (2 to 3 cm long) were placed on three culture media (MS, DKW and WPM) supplemented with indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) at four levels (0, 1, 2 and 3 mgl^{-1}). The explants were transferred to the culture growth chamber at a temperature of 22°C and night temperatures of 16°C with a photoperiod of 16 h light and 8 h dark with light from 2000 to 2500 Lux. This stage was carried out in four replications and each replicate had three samples. After rooted explants were determined as the best cultured media and combination of rooting growth regulators, number and root length, leaf number and stem length and quality of explants were recorded.

Acclimatization

All plants with properly developed roots were transferred into the growth substrate coco peat and perlite (50%: 50% V) and perlite (100% V). The transferred roots were carefully washed under tap water to remove all remnants of agar. The acclimatization of the transferred plants was conducted in a mist chamber inside the greenhouse. The experiment was carried out based on completely randomized design (CRD) with four replications and each replicates had five samples. Statistical analysis of the data was carried out by using JMP software and difference among treatment means was compared by using least significance difference test (LSD).

RESULTS

Surface sterilization

The best result was achieved from the sterilization of the explants in 10% sodium hypochlorite for 30 min. The results showed that among the sterilization treatments, 3% sodium hypochlorite for 10, 20 and 30 min had the highest contamination, and among other treatments, the percentage of the contamination was 2%; sodium hypochlorite (10%) for 30 min had the least explants of active sterile (Table 1).

Proliferation

The proliferation results showed that the MS media

Table 1. The effects of surface sterilization treatments on contamination of explants Myrobalan 29C.

Treatment	Active explants (%)	Contamination (%)
Ethanol, 70%, 1 min.	8.5[c*]	80.0[ab]
Mercuric chloride, 0.1%, 1 min.	4.5[c]	5.5[c]
Mercuric chloride, 0.1%, 2 min.	5.5[c]	5.5[c]
Mercuric chloride, 0.1%, 3 min.	6.5[c]	5.5[c]
Sodium hypochlorite, 3%, 10 min.	7.5[c]	100.0[a]
Sodium hypochlorite, 3%, 20 min.	8.1[c]	100.0[a]
Sodium hypochlorite, 3%, 30 min.	1.2[c]	100.0[a]
Sodium hypochlorite, 10%, 10 min.	66.6[ab]	4.5[c]
Sodium hypochlorite, 10%, 20 min.	66.6a[b]	4.5[c]
Sodium hypochlorite, 10%, 30 min.	100.0[a]	2.0[c]

* Mean values followed by the same letters within a column are not significantly different according to least significance difference test (LSD) at 5% level.

culture supplemented with 2 mg l^{-1} BAP with mean of 5.58 numbers of shoot, 2.5 cm shoot length and 11.33 numbers of leaf is the most suitable treatment (Figures 1, 2 and 3). There was no significant difference in the quality of plantlet in all three media; however, best quality was observed in the media MS. Moreover, the considerations showed that DKW with 2 mg l^{-1} TDZ has the least numbers and length of shoot (Figures 2 and 3).

Rooting

The best root formation was observed in DKW media containing 1 mg l^{-1} NAA, which produced roots readily with 100% efficiency compared to other rooting media cultures (Table 2). The mean of numbers and length of roots in this culture media were 6.75 and 14.3 cm, respectively (Table 2). The results showed that DKW culture media without growth regulators having mean of 0.3 root numbers and the MS culture media without growth regulators having mean of 1 cm root length had the least number and length of root, respectively. However, the highest length of roots, length plant, number of leaf and plantlet quality were observed in the MS media (Table 2 and Figure 4).

DISCUSSION

In the present study, sampling date, genotype and different concentrations of sodium hypochlorite affected surface sterilization. Vujovic (2012) has reported sterilization of Gisela5, apricot and plum buds with 10% sodium hypochlorite for 15 min to be more effective than other treatments. Sulusoglu and Covusoglu (2013) investigated that rootstock micropropagation (*Prunus laurocerasus*) with 5% sodium hypochlorite for 14 min disinfection of treated explants was considered. Nacheva and Gercheva (2009) obtained 85 to 100% sterile explants after sterilization with calcium hypochlorite. For surface sterilization of explants, 70% ethanol, sodium hypochlorite (10 to 50%) and mercuric chloride were used (Asadi et al., 2009; Sana et al., 2006; Jang et al., 2008). Sodium hypochlorite causes the oxidation of cells, microorganisms and affects major components of cells, such as lipids, proteins and DNA. Ethanol damages cell membranes, proteins and causes more rapid disintergration of cell metabolism (George, 2008). Therefore, one can conclude that alternating the use of these two substances that deplete cells and bacterial pathogens is better; and the higher the concentration and time of treatment, the more its effectiveness improved. The use of mercuric chloride with minimum contamination, compared to sodium hypo-chlorite has 0% active buds. Perhaps due to the toxicity and lethality of mercuric chloride, it penetrates into plant tissue and destroys the buds in active meristem (Ruzic and Vujovic, 2013).

A significant interaction between media and growth regulators regarding shoot regeneration percentage was found at $p<0.05$. This study showed that number of shoot was increased as concentration of BAP increased to certain amounts. There is a positive correlation between BAP concentration (2 mgl) and number of shoots. The increasing concentrations of BAP inhibited shoot proliferation (Sulusoglu and Cavusoglu, 2013). The highest shoot multiplication was obtained in 2 mg l^{-1} BAP added to MS medium. Erbenova (2009) and Sulusoglu (2012) reported 50% increase in multiplication rate of the dwarf rootstocks of *Prunus* in MS media culture containing 1.5 mg l^{-1} BAP. George (2008) reported that cytokinins when added to media promote cell division. Nordstrom et al. (2004) suggested that auxins may also play a direct regulatory role in the balance of cytokinins levels by suppressing both the synthesis rate and pool size of cytokinins. These results can be related to the manipulation of exogenous cytokinins concentrations that may cause an increase in endogenous auxin concentrations.

Table 2. The effects different media, IBA and NAA concentrations on rooting parameters of Myrobalan 29C.

Culture media	Growth regulators (mg l^{-1})	Rooting (%)	Root length (cm)	Number of root	Plantlet length (cm)	Number leaf	Plantlet quality
	IBA(0)	3.5^{c}	0.26^{f}	0.4^{de}	3.00^{defghi}	10.00^{abc}	1.66^{ab}
	IBA(1)	50.00^{ab}	11^{ab}	2.25^{abcde}	5.5^{bcdefg}	13.00^{a}	1.25^{ab}
	IBA(2)	72.08^{ab}	10.50^{abc}	3.25 ^{ab}	3.87^{cdefghi}	9.25^{abc}	2.00^{a}
	IBA(3)	68.75^{ab}	13.75^{a}	2.50^{abcde}	6.75^{abc}	13.25^{a}	2.00^{a}
MS	NAA(0)	50.00^{ab}	8.50^{bcd}	1.75^{abcde}	2.75^{fghi}	10.00^{abc}	2.00^{a}
	NAA(1)	91.66^{a}	10.50^{abc}	3.25^{ab}	9.75^{a}	12.75^{a}	2.00^{a}
	NAA(2)	75.00^{ab}	14.50^{a}	3.25^{ab}	9.75^{a}	12.5^{ab}	2.00^{a}
	NAA(3)	87.50^{a}	12^{ab}	3.75^{a}	8.75^{ab}	12.75^{a}	2.00^{a}
	IBA(0)	14^{c}	0.37^{f}	0.3^{e}	2.5^{fghi}	10.00^{abc}	1.00^{b}
	IBA(1)	66.25^{ab}	2.87^{ef}	3.00^{abc}	4.75^{cdefghi}	10.25^{abc}	1.75^{ab}
	IBA(2)	77.08^{ab}	4.50^{def}	2.25^{abcde}	6.25^{bcde}	9.75^{abc}	1.00^{b}
	IBA(3)	52.94^{ab}	3.75^{ef}	1.75^{abcde}	4.5^{cdefghi}	10.5^{abc}	1.00^{b}
DKW	NAA(0)	66.50^{ab}	2.12^{ef}	1.50^{abcde}	2.37^{fghi}	9.50^{abc}	1.00^{b}
	NAA(1)	100^{a}	6.00^{cde}	4.00^{a}	6.25^{abcd}	12.75^{a}	1.00^{b}
	NAA(2)	68.75^{ab}	2.25 ^{ef}	2.25^{abcde}	3.87^{cdefghi}	9.50^{abc}	1.00^{b}
	NAA(3)	81.25^{ab}	3.62 ^{ef}	2.75^{abcd}	5.75^{bcdef}	10.50^{abc}	1.00^{b}
	IBA(0)	62.5	1.00 ^{f}	0.75^{bcde}	1.87^{hi}	6.00^{cd}	2.00^{a}
	IBA(1)	25.00^{ab}	1.12^{f}	1.00^{bcde}	1.75^{hi}	6.00^{cd}	2.00^{a}
	IBA(2)	50.00^{ab}	2.00^{ef}	1.75^{abcde}	2.37^{fghi}	6.00^{cd}	2.00^{a}
	IBA(3)	43.75^{ab}	2.12^{ef}	1.75^{abcde}	2.12^{ghi}	7.25^{bcd}	1.50^{ab}
WPM	NAA(0)	25.00^{ab}	1.5^{ef}	0.50^{cde}	1.87^{hi}	5.25^{cd}	1.50^{ab}
	NAA(1)	93.75^{a}	3.20^{ef}	3.75^{a}	3.00^{efghi}	6.75^{cd}	2.00^{a}
	NAA(2)	68.75^{ab}	2.62^{ef}	2.75^{abcd}	2.5^{fghi}	6.00^{cd}	2.00^{a}
	NAA(3)	54.16^{ab}	1.25 ^{f}	2.00^{abcde}	1.25^{i}	3.50^{d}	1.25^{ab}

[a]– explants strong growth, with no signs of verification, necrosis of leaf are yellowing terminal meristem, [b]– less than15% have the symptoms of verification, necrosis of leaf are yellowing terminal meristem, and [c]– explant weak, 15-30% have the symptoms of verification, necrosis of leaf are yellowing terminal meristem.

This is probably induced by an inhibition of free IAA conjugates due to the presence of exogenous cytokinins (Jaramillo et al., 2008; George (2008).

It is observed that the auxin to cytokinins ratio represents an important signal in the formation of cell phenotype and also in the onset and maintenance of the process of cell division. Since auxins are capable of imitating cell division involved in the formation of meristems, giving rise to either unorganized tissue or defined organs.

Tatari and Mosavi (2013) reported that low shoot growth with necrotic leaf could be due to the effects of high concentrations of BAP and hormonal imbalances in the explants. Considering the MS medium contrast, WPM and DKW media have no Cl^- in the culture media while chlorotic plants increased stability. The medium increases the amount of Cl^- as plants balance is disturbed at different nutrient absorption. The MS media culture contains more calcium than the two

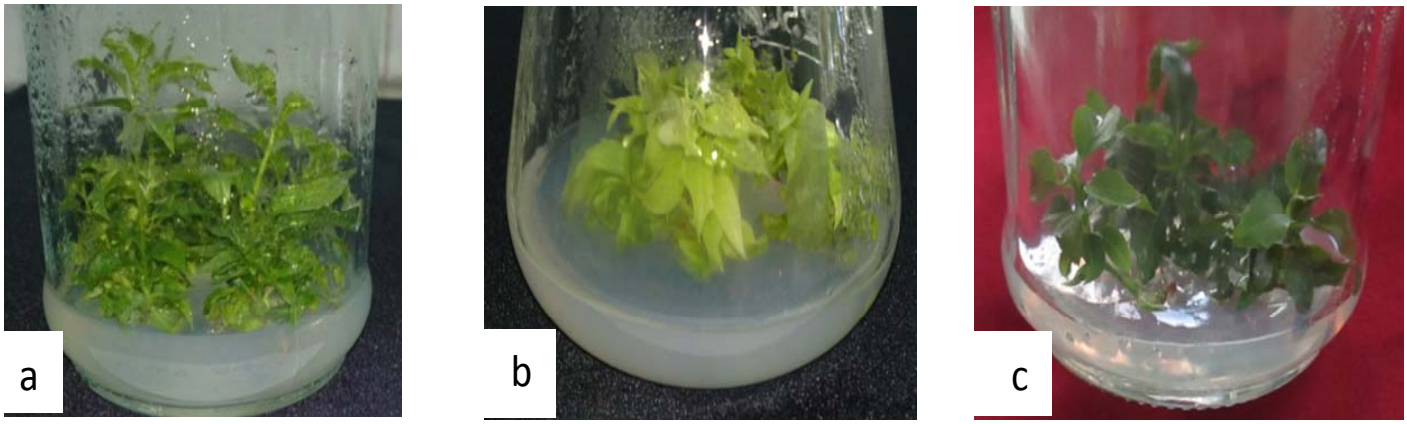

Figure 1. Effects of media and plant growth regulator on proliferation of Myrobalan29C (a) (MS), (b) (DKW) and (c) (WPM).

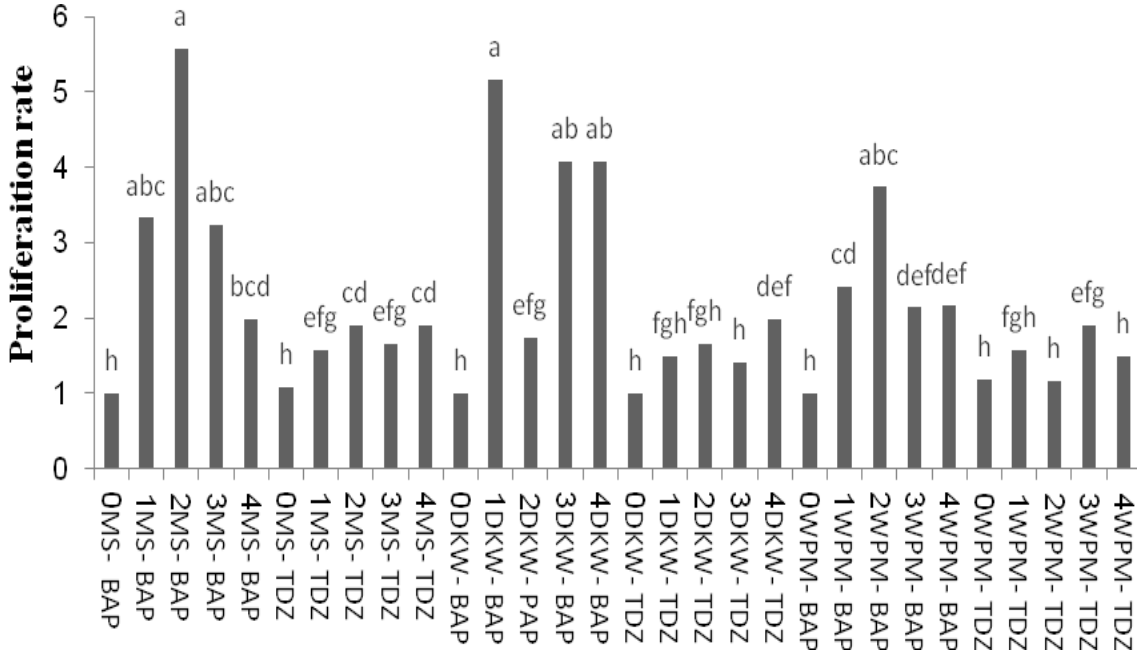

Figure 2. The effects of different media, BAP and TDZ concentrations on propagation coefficient of Myrobalan 29C.

other media which play an important role in the stability and strength of the plantlet. Nutrient concentration of N, K, Mg and macro nutrients is lower in WPM and DKW media than in MS media which is effective in reducing the proliferation
of the media culture.

The best root formation was observed in DKW media containing 1 mg l^{-1} NAA. Roots formation in tissue culture can be induced by exogenous auxins such as IBA, NAA and IAA and their interaction with endogenous auxins (Thorpe et al., 2008). Among the three auxins, NAA was found superior to IAA and IBA, though thick and callused rooted were obtained in one- step procedure. Low levels of NAA resulted in the highest rooting, whereas high concentration gave rise to more root initials, which eventually developed into callus rather than rootlets. They are only required at an early stage to emerge new formed roots. The MS culture media with reduced concentration of macro and microelements by ½ gave a maximum rooting percentage of 100% (Plopa et al., 2012). Vujovic et al. (2012) reported that the rooting rates in media containing 1 mg/L IBA or NAA (65 and 70%,

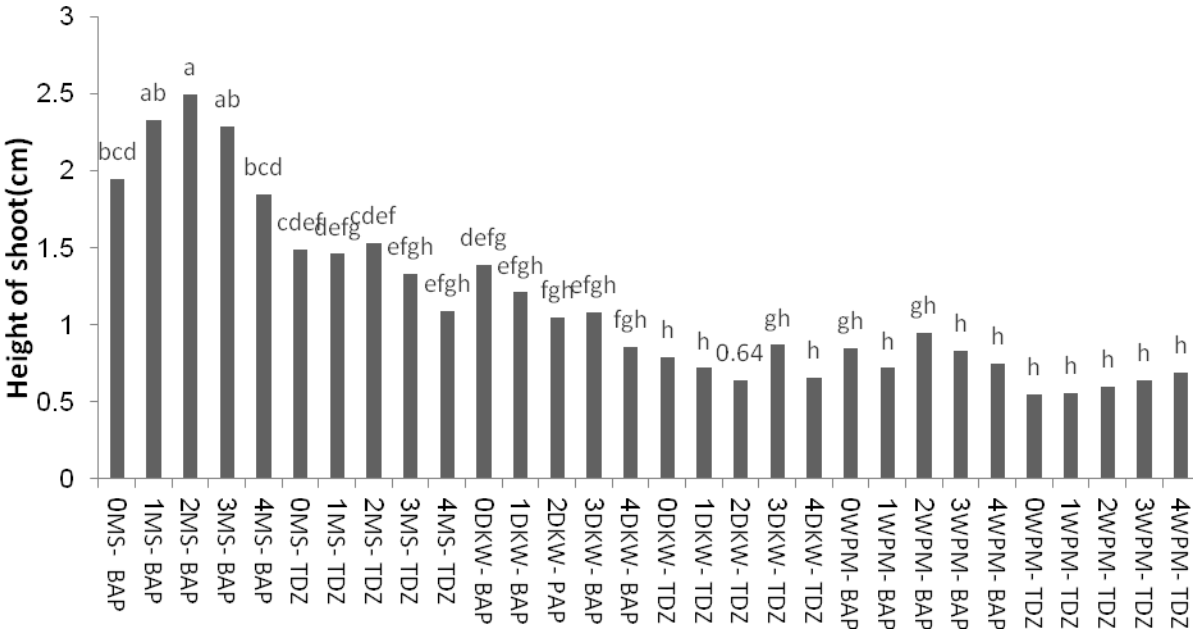

Figure 3. Effects BAP and TDZ concentrations on shoot proliferation and elongation of Myrobalan 29C.

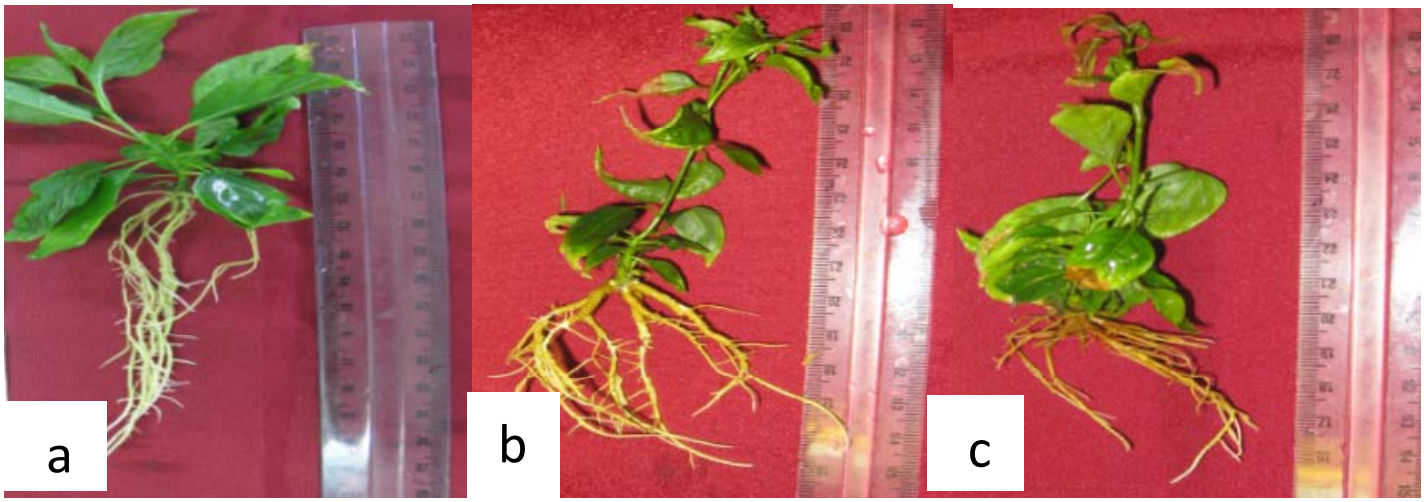

Figure 4. Root formation of Myrobalan29c. (a) (MS), (b) (DKW) and (c) (WPM).

respectively); although number of roots and plant height was significantly higher when IBA was supplemented. Hossini et al. (2010) reported that IBA concentration increase caused shortening of the root; 80% rooting and 2.9 cm root length were obtained in culture medium with the addition of 0.5 mg l^{-1} IBA and greater percentage of rooting (88.6%) was attained when rooting medium was supplemented with 1.0 mg l^{-1} NAA. Root percentage of the regenerated shoots was 38.2% by addition of 10.74 µM NAA in the medium (Demiral and Ulger, 2008; Mohamed, 2012).

Balla and Kirilla (2006) reported that following a nice growth during the multiplication and elongation phases, difficulties arose during the rooting phase and large differences were found in the nutrient demand of the rooting phase. In spite of the high percentage of rooting, widespread shoot tip necrosis was detected and optimal levels of different macro elements, iron and sugar had to be determined in a series of experiments for the clonal rootstock cultivars.

Figure 5. Acclimatization of Myrobalan29c rootstock on *in vivo* condition.

Acclimatization was affected directly by rooting conditions. Survival was best when plantlets were transferred to pots after a short period of root emergence on rooting media. The plants with older roots and brown colour were better adapted than new plantlets with white roots. The results showed that after 2 months of acclimatization, the best substrates, perlite (100%V) was about 80% (Figure 5). Therefore, acclimatization directly affected rooting plants that have high quality; best rooting had rate induction (Mahdaviyan et al., 2010). Rooted plantlets survived Gisela 6 after acclimatization with the greenhouse was successful (80 and 79.2%) (Hossini et al., 2010; Sulusoglu and Cavusoglu, 2013).

Conclusion

The results of this research showed that Myrobalan 29C rootstock can be reproduced *in vitro*. According to this research, MS and DKW media including BAP with 2 mg l^{-1} and NAA with 1 mg l^{-1} growth regulators are most suitable for micro propagation.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Arbeloa A, GarcíaE, Andreu P, Marín J.A (2006). Rootstock Breeding Programme for Apricot through Interspecific Crosses of Myrobalan x Apricot: Significant Effect of Accidental Pollinations. Acta Hortic. (717): 133-136.

Asadi AA, Vedadi C, Rahimi M, Naserian B (2009). Effect of plant growth hormones on root and shoot regeneration in rose (*Morrasia*) under *in-vitro* conditions. Biosci. Res. 6:40-45.

Balla I, Kirilla Z (2006). Micropropagation of peach rootstocks and cultivars. Acta Hortic. 725:511-516.

Bonjak A, Keresa S, JercicI, Baric M (2012). The effect of cytokine type and explant orientation on axillary shoot proliferation and in vitro rooting of Gisela 5 cherry rootstock. J. Food Agric. Environ. 10:616-620.

Dardi G, Vito G, Standardi A (2011). Invitromass poropagation of eleven *Prunus mahaleb* ecotypes. Acta Hortic. 410:98-100.

Demiral S, Ulger SA (2008). Research on Propagation of Gisela 5 Cherry Rootstock by Tissue Culture. Akdeniz Univ. Ziraat Fak. 21:117–121.

Driver JA, Kunyoxi H (1984).In Vitro propagation of paradox walnut rootstocks. Hort. Science.(19): 57-509.

Erbenova M, Paprstein F, Sedlak J (2009). *In vitro* propagation of dwarfed rootstocks for sweet cherry. Acta Horticulturae.(560): 477-480.

George EF, Hall MA, De Klerk GJ (2008). Plant propagation by tissue culture. Plant Cell. Reprod. 186-113.

Hossini AD, Moghadam EG, Anahid S (2010). Effects of media Cultures and Plant Growth Regulators in Micropropagation of Gisela6 Rootstock. Ann. Biol. Res. (1):135-141.

Jang HH, Ann SH, Kim MD, Kim CW (2008). Use of hydrogen peroxide as an effective disinfectant to Actinobacillus ureae. Process Biochem. 43:225-228.

Jaramillo EH, De Forero A, Cancino G, Moreno AM, Monsalve LE, Acero W (2008). *In vitro* regeneration of three chrysanthemum (*Dendrathema grandiflora*) varieties “via” organogenesis and somatic embryogenesis. Univ. Sci. (13): 118-127.

Mahdaviyan M, Bozari N, Abdolahi H (2010). Effect media culture and plant growth regulators on Proliferation and rooting of vegetative St.loci64 rootstock. Seed Plant J. (26):15-26.

Lloyd GB, McCrown BH (1980). Commerically feasible micropropagation

of mountain by use of shoot tip culture. Proc. Int. Plant Propagators Soc. 30:421-437.
Mikhilov RV, Shulga OA, Dolgov SV (2008). Genetic transformation of somatic tissues and molecular biology approach for improving resistance to sharka disease of plum (*Prunus domestica* L.) varieties. Biotechnology 944:44.
Mohamed A (2012). Establishment of Regeneration system for Taif peach (*Prunus persica* L. Batsch) cultivar (Balady cultivar) in Taif, KSA. J. Am. Sci. 8:232-239.
Movsiuw A (2011). *In vitro* propagation of virus-free Myrobalan 29C rootstock. GRI (73):101-102.
Murashige T, Skooge F (1962). A revised medium for rapid growth and bio-assays with tobacco tissue culture. Physiol. Plant 15:473-497.
Nacheva L, Gercheva P (2009). Micropropagation of Gisela 5 (cherry dwarf rootstock): The effect of the type and the concentration of the carbohydrates in the nutrient medium. ISHS Acta Hortic. 825:261–281.
Nazeri MA, Yadolahi A, Moeeni A (2010). Investigate on the effect TDZ and BA on Proliferation Gisela rootstock. Special Twelfth Iranian Genet. Congr. 5:1-5.
Nordstrom AP, Tarkowski D, Tarkowska R, Norbaek C, Astot K, Dolzel G. Sandberg (2004). Auxin Regulation of cytokinins biosynthesis in the *Arabidopsis thaliana*: A factor of potential importance for auxin cytokinins regulated development. Proc. Natl. Acad. Sci. USA 101(21):8039-8044.
Plopa C, Dutu I, Isac V, Mazilu C, Ancu S (2012). Factors influencing *in vitro* Propagation of Myrobolan Dwarf Plum Rootstock II EUFRIN Plum and Prune Working Group Meeting on Present Constraints of Plum Growing in Europe. ISHS Acta Hortic. 968.
Ruzic DV, Vajovic T (2013).The effect of cytoknins types and their concentration on *in vitro* multiplication of sweet cherry. Hortic. Sci. 35:12-21.
Sana B, Ghosh D, Saha M, Mukherjee J (2006). Purification and characterization of a salt, solvent, detergent and bleach tolerant protease from a new gamma- Proteobacterium isolated from the marine environment of the *Sundarbans*. Process Biochem. 41:208-215.
Shekafandeh A, Qasemi M (2008). Effects of BA and TDZ on bud growth and somatic embryogenesis of immature almond (*Prunus dulcis* L.) varieties blooming late 7 Shahrody. J. Hortic. Sci. 2:148-152.
Sulusoglu M (2012). Development of embryo culture protocol for cherry laurel (*Prunus laurocerasus* L.). J. Food Agric. Environ. 10:47-352.
Sulusoglu M, Cavusoglu A (2013). Micropropagation of cherry laurel *Prunus laurocerasus* L. J. Food Agric. Environ. 11:576-579.
Tatari M, Mosavi A (2013). Evaluation *in vitro* culture of vegetative Tetra, Nemagard and GF677 rootstock. Byagricultural 3:103-115.
Tian L, Wen Y, Jayasankar S, Sibbald S (2007). Regeneration of *Prunus salicina* Lindl (Japanese plum) from hypocotyls of mature seeds. *In vitro* Cell. Dev. Biol. Plant 43:343-347.
Thorpe T, Stasolla C, Yeung EC, de Klerk (2008). Plant Growth Regulators. Third Ed, Vol 1., Springer. pp. 115-173.
Vujovic T, Ruzic, D, Cerovic, R (2012). *In vitro* shoot multiplication as influenced by repeated subculturing of shoots of contemporary fruit rootstocks. Hortic. Sci. 39(3):101- 107.
Xiaomei L, Paula M (2008). Plant regeneration from in vitro leaves of mature black cherry (*Prunus serotina*). Plant Cell Tiss. Organ Cult. 94:113–123.
Yao YX, Sun YW, Li GG, Li GH (2011). Regeneration of plant from *in vitro* culture of petioles in *Prunus domestica* Lindl (European plum). Biotechnology 10:5504.
Ying-Ning Z (2010). Micropropagation of Chinese plum (*Prunus salicina* Lindl.) Using Mature Stem Segments. Agrobotany (38):214-218.

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