Comparative Organogenic Response of Six Clonal Apple Rootstock Cultivars

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Abstract. The organogenesis potential is different among cultivars and must be optimized for individual genotype. Shoot organogenesis capacity from in vitro leaves and root organogenesis capacity of in vitro shoots in six clonal apple rootstock cultivars were compared. The shoot organogenesis capacity was highly genotype dependent. ‘GM256’ was found to be the most responsive genotype for shoot regeneration from leaf explants among the cultivars, showing high regeneration percentage on all tested media. The effects of basal medium composition and cytokinins on shoot regeneration were different depending on rootstock genotype. Optimum regeneration occurred on Murashige and Skoog (MS) basal medium for ‘71-3-150’, and optimum regeneration occurred on Quoirin and Lepoivre (QL) basal medium for ‘60-160’ and ‘PIB’. Thidiazuron (TDZ) was more effective than 6-benzylaminopurine (BA) for *Malus prunifolia* (Y), whereas TDZ and BA were not significantly different for the other cultivars. All rootstock cultivars showed high root organogenic capacity. The percentage of rooting reached more than 90% and the mean root number per plantlet ranged from three to five. The optimum rooting medium was different for different rootstock cultivars. Optimum root organogenesis occurred on half-strength QL medium for ‘GM256’ and ‘Y’, and for ‘PIB’ and ‘JM7’ on one-quarter-strength MS medium.

Fruit trees are commonly propagated by grafting scions onto rootstocks. Rootstocks have a special value for fruit production, as they can effectively influence precocity, tree size, fruit quality, yield efficiency, mineral uptake, and the ability to withstand adverse environmental conditions. Current apple resistance breeding also includes apple replant disease (Cousins, 2005; Fazio et al., 2013; Laurent et al., 2010). Genetic engineering is

a modern tool for plant breeding, especially for vegetatively propagated rootstocks. Genetic modification has been proven to be efficient for improving apple rootstocks M.26 and M.9 and pear rootstock BP10030, for which only one or a few traits need to be improved (Smolka et al., 2010; Zhu et al., 2001, 2003). In fruit crops, genetically modified rootstocks will probably be accepted more easily by the public and the industry than genetically modified scion cultivars if no translocation of the transgene from rootstock to scion occurs, because the fruits are eaten by people directly from scion cultivar and not from the rootstock. The perceived safety problem will be avoided by the combination of transgenic rootstock and nontransgenic scion cultivars. Smolka et al. (2010) investigated the effects of *rolB* transgenic apple

rootstocks M.26 and M.9 on nontransgenic scion cultivars, indicating that there was no translocation of the transgene or its messenger RNA from rootstocks to scions. An efficient regeneration system is a prerequisite for the genetic modification by transformation, and also is a basis for the use of somaclonal variation by in vitro induced mutation. Efficient in vitro rooting of shoots is a critical step in rapid micropropagation of rootstocks. Losses at this step will have vast economic consequences.

There have been several reports describing regeneration from in vitro leaf explants of apple rootstock cultivars M.9 and Ottawa 3 (Welandar and Maheswaran, 1992), M.26 (Predieri and Malavasi, 1989), M.M.106 (Ancherani et al., 1990; Modgil et al., 2005), and M.9/T337 (Hohnle and Weber, 2010). Published results indicate that the ability to regenerate is highly genotype dependent. In several studies, various rooting media were used, and rooting capacity of different apple rootstocks varied (Bahmani et al., 2012; Briand and Hicks, 1989; Sharma et al., 2007; Yassen et al., 2009).

In the present study, the shoot regeneration response and rooting capacity among six new clonal apple rootstock cultivars, GM256, 71-3-150, 60-160, PIB, JM7, and Y, were compared. The effects of various basal medium and growth regulator combinations on shoot regeneration were examined. The effect of basal medium composition on rooting capacity was also investigated. The optimal conditions of shoot regeneration and the optimal rooting medium for individual genotype were established. The regeneration protocols can be applied to the future genetic transformation and induction of somaclonal variation of these six rootstock cultivars. The establishment of a high-efficiency root organogenesis system will provide enough rootstock plants with better root systems for experimental trials and production.

Materials and Methods

Plant materials and culture conditions

The six apple rootstock cultivars used in this study were GM256, 71-3-150, 60-160, PIB, JM7, and Y. Rootstocks ‘71-3-150’ (semidwarf), ‘60-160’, and ‘PIB’ (dwarf), which are cold hardy, were introduced to our institute from the Michurinsk State Agricultural University, Russia according to mutual agreement. ‘GM256’ is a cold-hardy and dwarfing apple rootstock, which was released by the Jilin Academy of Agricultural Science, Jilin, China (Wang et al., 1993). ‘JM7’ is a disease-resistant and dwarfing rootstock which was released by Apple Research Center, National Institute of Fruit Tree Science, Japan (Soejima et al., 2010). ‘Y’ (*Malus prunifolia* Borkh var. ringo Asami) was released by Japan, and has strong resistance to low temperatures and drought and wide adaptability (Bai and Gao, 2006). Trees of these six rootstocks were grown at the experimental orchard at Tianping Lake, Taian, Shandong, China. Young shoot tip

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explants (1–2 cm long) were excised from these plants. Shoot cultures of these six cultivars were proliferated and maintained on subculture medium (SM) of QL (Quoirin and Lepoivre, 1977) with 0.5 mg·L⁻¹ BA, 0.05 mg·L⁻¹ indole-3-butyric acid (IBA), 3% sucrose, and 0.6% agar. All cultures were kept in a growth chamber at 25 ± 2 °C, under 16-h light photoperiod of 40 μmol·m⁻²·s⁻¹ photon flux. These conditions were maintained throughout the course of the study, except when otherwise specified.

Shoot organogenesis from leaf explants of different rootstock cultivars

For shoot induction, expanding young leaves were excised from the top and mid-portions of 4- to 5-week-old in vitro cultivated shoots, wounded by several transverse cuts, and placed in glass vessels (100 mL volume) containing 50 mL of shoot induction medium (SIM). For each cultivar, three vessels were prepared for each treatment, and each vessel contained 10 leaves. Each experiment was performed twice. Leaf explants of all treatments were cultured 3 weeks in darkness before exposure to a 16-h light photoperiod. At the end of 7 weeks, the shoot regeneration frequency (number of leaf explants regenerating shoots (or buds)/number of leaf explants) was determined.

Expt. 1. In a preliminary experiment, shoot regeneration ability of six cultivars was evaluated on one SIM: MS (Murashige and Skoog, 1962) with 5 mg·L⁻¹ BA, 0.3 mg·L⁻¹ IBA, 3% sucrose, and 0.6% agar (Treatment T1).

Expt. 2. To choose an appropriate SIM for the three cultivars that exhibited poor regeneration in Expt. 1, seven SIM treatments were tested (Table 1: Treatments T2–T8). Together with the preliminary experiment, the eight SIM treatments consisted of two basal media MS, QL, two growth regulator factorial combinations: BA at 5 and 7 mg·L⁻¹ and TDZ at 1 and 2 mg·L⁻¹, each with 0.3 mg·L⁻¹ IBA, 3% sucrose, and 0.6% agar.

Adventitious shoots subculture and root organogenesis

Adventitious shoots (or buds) were transferred to SM for proliferation and elongation.

Elongated, well-developed individual shoots (1.5 cm or longer) were excised from the proliferated clumps of all apple rootstocks and were transferred to two rooting media: half-strength QL (half QL) or one-quarter-strength MS (one-quarter MS), each with 0.5 mg·L⁻¹ IBA, 3% sucrose, and 0.6% agar. The shoots were cultured in the dark for 1 week before exposure to 16-h light photoperiod according to our previous study (Sun et al., 2014). For each cultivar, three vessels were prepared for each treatment, and each vessel contained 10 shoots. Each experiment was performed twice. At the end of 3 weeks of each experiment, the rooting rate and root number was determined.

Data analysis

Treatment means and descriptive statistics were computed with DPSv3.01 analysis software (Lu, 2005). Analyses of variance (ANOVA) were performed using SAS PROC MIXED in SAS® version 9.4. Residuals were generated by the OUTP option. Normality of residual distributions was tested by the Shapiro–Wilk test, distribution plot, box plot, and probability plot performed by SAS PROC UNIVARIATE. Equality of variances was determined visually by residual vs. predicted plots using SAS PROC PLOT, and the relationship of predicted means and variances was determined by Spearman's correlation coefficient using SAS PROC CORR. All data were analyzed as randomized complete block experiments with replication as a random effect. Rootstock and medium were considered fixed effects. For the percentage of regeneration, cytokinin and concentration were also analyzed as fixed effects. Concentrations for BA and TDZ were recoded as “low” or “high.” Because the purpose of the experiment was to choose the best medium and cytokinin concentration combination, these factors were combined as eight treatments (Table 1), and the data were also analyzed as a treatment by rootstock factorial design and separately by treatment.

The percentage regeneration data fit a normal distribution, as indicated by the Shapiro–Wilk test ($W = 0.995$, $Pr < W = 0.51$) and the plots. Variances appeared to be relatively

equal, with only a slight tendency to smaller variances at the extremes ($r = 0.17$, $Pr > |r| = 0.004$). Therefore, the percentage regeneration data were analyzed without transformation. Examination of plots and skewness and kurtosis values for percentage of rooting suggested a slight deviation from a normal distribution, although the Shapiro–Wilk test indicated normality ($W = 0.98$, $Pr < W = 0.26$). The variances appeared fairly equal, but the correlation of means and variances was significant ($r = 0.39$, $Pr > |r| = 0.0008$). Therefore, the data were reanalyzed after arcsin square root transformation, which reduced the correlation of means and variances ($r = 0.18$, $Pr > |r| = 0.13$). The root number data were slightly skewed, but the Shapiro–Wilk test did not indicate a significant deviation from normality ($W = 0.97$, $Pr < W = 0.13$). The plots indicated fairly equal variances, but the correlation of means and variances was significant ($r = 0.31$, $Pr > |r| = 0.01$). Square root transformation resulted in non-significance of the correlation ($r = 0.17$, $Pr > |r| = 0.16$), and therefore, the square root transformed data were subjected to ANOVA.

Results

Summary of ANOVA of shoot organogenesis.

The ANOVA revealed a complex pattern of results, characterized by significant main effects, but also many significant interactions. The main effects of rootstock, medium, cytokinin, and concentration were all significant. Most two-way interactions, with the exception of medium by cytokinin and medium by concentration were significant. Among the three-way interactions, all but medium by cytokinin by concentration were significant. The four-way interaction of rootstock by medium by cytokinin by concentration was also significant, revealing that the best choice of a medium for each rootstock requires examining all factors.

Effect of genotype on shoot organogenesis.

In the preliminary experiment (T1 treatment in Table 1), rootstock had a significant effect ($Pr > F < 0.0001$) on shoot organogenesis. Shoot regeneration rate of different cultivars was markedly different on the

Table 1. Shoot regeneration ability comparison of different rootstock cultivars on different shoot regeneration medium.

Treatment	Media (mg·L ⁻¹) ^a	Mean regeneration % ± SE ^b							Means ± SE
		‘GM256’	‘71-3-150’	‘60-160’	‘IIB’	‘JM7’	‘Y’		
T1	MS BA 5	97.8 ± 2.2 aA	79.8 ± 2.9 abB	10.5 ± 2.8 bD	6.4 ± 3.6 bcD	84.2 ± 0.8 abAB	30.6 ± 3.9 cC	51.5 ± 13.9 d	
T2	MS BA 7	96.7 ± 3.3 aA	55.3 ± 0.8 cdC	6.8 ± 0.7 dE	0.0 ± 0.0 cC	75.8 ± 0.8 cB	20.8 ± 3.6 cD	42.6 ± 13.6 e	
T3	MS TDZ 1	95.0 ± 1.7 aA	72.5 ± 1.9 abB	5.8 ± 1.7 dC	0.0 ± 0.0 cC	94.5 ± 1.1 abA	81.1 ± 4.5 aB	58.1 ± 15.1 bc	
T4	MS TDZ 2	95.0 ± 1.7 aA	84.1 ± 6.3 aA	4.7 ± 2.0 dC	0.0 ± 0.0 cC	95.0 ± 1.7 aA	55.8 ± 4.2 bB	55.8 ± 15.1 bcd	
T5	QL BA 5	91.7 ± 5.0 aA	54.1 ± 2.1 cdB	30.8 ± 0.8 bC	26.4 ± 3.6 aC	95.2 ± 1.8 aA	30.6 ± 2.8 cB	54.8 ± 10.9 cd	
T6	QL BA 7	93.3 ± 6.7 aA	33.9 ± 2.8 eB	25.0 ± 1.7 bcBC	12.8 ± 3.9 bC	87.7 ± 0.7 abA	18.6 ± 3.1 cC	45.2 ± 12.4 e	
T7	QL TDZ 1	95.0 ± 1.7 aA	65.8 ± 2.4 bcBC	50.8 ± 0.9 aC	16.1 ± 5.6 abD	92.2 ± 4.5 abA	71.9 ± 0.3 aBC	65.3 ± 10.1 a	
T8	QL TDZ 2	91.7 ± 5.0 aA	49.3 ± 0.4 dBC	46.7 ± 0.0 aC	12.5 ± 6.4 bD	96.7 ± 0.0 aA	67.8 ± 2.8 abB	60.8 ± 10.9 ab	
Rootstock means ± SE		94.5 ± 0.7 A	61.8 ± 5.3 B	22.6 ± 5.8 D	9.3 ± 3.0 E	90.1 ± 2.2 A	47.2 ± 7.8 C		

^aBasal medium, cytokinin, and concentration. All media with 0.3 mg·L⁻¹ indole-3-butyric acid and 3% sucrose.

^bMeans followed by different lower case letters within the same column were significantly different at $P < 0.05$ by least significance difference (LSD) test with Tukey's multiple comparison adjustment. Means followed by different upper case letters within a row were significantly different at $P < 0.05$ by LSD test with Tukey's multiple comparison adjustment.

MS = Murashige and Skoog; BA = 6-benzylaminopurine; TDZ = thidiazuron; QL = Quoirin and Lepoivre.

same SIM (T1). 'GM256' had the highest shoot organogenesis capacity (98%), followed by 'JM7' (84.2%), '71-3-150' (79.8%), and 'Y' (30.6%), whereas '60-160' (10.5%) and 'ΠB' (6.4%) were very low. The shoot numbers per leaf explant of 'GM256', '71-3-150', 'JM7', and 'Y' (Fig. 1A–C and F) were higher than that of '60-160' and 'ΠB' (Fig. 1D and E). When all treatments (medium–cytokinin–concentration combinations) were analyzed, the ranking of rootstock was the same.

Effects of the kinds and concentrations of cytokinin on shoot organogenesis. Because of the previous poor regeneration rates of cultivars 60–160, ΠB, and Y (T1 treatment in Table 1), the effects of different cytokinins and concentrations on shoot regeneration were examined. The effects of both cytokinin and concentration overall were significant ($P > F < 0.0001$), and the interaction of cytokinin and concentration was also significant ($P > F = 0.0006$). Generally, when the concentration of BA was increased from 5 mg·L⁻¹ to 7 mg·L⁻¹, the regeneration frequency decreased ($P > F < 0.0001$), and within each basal medium, exclusive of 'GM256' on QL. Within rootstocks, the decrease was significant for '71-3-150' on both MS and QL basal media. As the concentration of TDZ increased from 1 mg·L⁻¹ to 2 mg·L⁻¹, regeneration frequency was averagely decreased (61.7% vs. 58.3%). Within each basal medium, the difference was not significant. Within rootstocks, the decrease was significant only for '71-3-150' when QL was the basal medium and for 'Y' when MS was the basal medium. Therefore the concentration of BA or TDZ had no significant effect on shoot regeneration for the other four rootstock cultivars.

The use of TDZ resulted in significantly more regeneration than BA (60% vs. 52%, respectively; $P > F < 0.0001$). The effect of cytokinins on shoot regeneration was different depending on rootstock cultivars (cytokinin by rootstock interaction: $P > F < 0.0001$). There were also significant three-way interactions of cytokinin by rootstock by medium ($P > F < 0.0001$) and cytokinin by rootstock by concentration ($P > F = 0.0005$). For 'Y', TDZ significantly improved shoot regeneration percentage over BA (69.2% vs. 25.1%, respectively), independent of basal medium composition. For '60-160', TDZ significantly improved shoot regeneration percentage over BA overall (27% vs. 18.3%), because of the large increase on QL basal medium. For '71-3-150', TDZ also significantly increased regeneration (67.9% vs. 55.8%). For 'JM7', TDZ significantly improved regeneration at the highest concentration on MS basal medium, but not on QL basal medium. For the other three cultivars, TDZ and BA did not show significant differences. Therefore, the effectiveness of TDZ was influenced by the genotype and basal medium composition.

However, TDZ influenced adventitious shoot formation (adventitious bud elongation), and shoot number per leaf, with the exception of 'ΠB'. In general, TDZ-induced

Fig. 1. Shoot regeneration behavior of six rootstock cultivars on different shoot induction medium. (A–F) MS + 5 mg·L⁻¹ BA + 0.3 mg·L⁻¹ IBA; (G–J) MS + 1 mg·L⁻¹ TDZ + 0.3 mg·L⁻¹ IBA; (K, L) QL + 0.5 mg·L⁻¹ BA + 0.05 mg·L⁻¹ IBA; (M–R) QL + 5 mg·L⁻¹ BA + 0.3 mg·L⁻¹ IBA; (A, G, O) rootstock 'GM256'; (B, H, P) rootstock '71-3-150'; (C, J, N) rootstock 'Y'; (D, M) rootstock '60-160'; (E, Q) rootstock 'ΠB'; (F, I, R) rootstock 'JM7'. MS = Murashige and Skoog; BA = 6-benzylaminopurine; IBA = indole-3-butyric acid; TDZ = thidiazuron; QL = Quoirin and Lepoivre.

more adventitious buds per explant than BA for most cultivars, but TDZ hindered the conversion of buds into shoots (Fig. 1G–J).

The conversion of adventitious buds into shoots was improved when TDZ was removed in a subsequent stage of culture (Fig. 1L).

Effect of basal medium composition on shoot regeneration of different cultivars. Regeneration on QL medium resulted in a small, but significant, increase in regeneration over MS (56.5% vs. 52.0%, respectively; $P > F = 0.008$), when averaged overall rootstocks. The effects of the composition of basal medium on shoot regeneration were different depending on rootstock cultivar (Table 1; $P > F = 0.0002$). Compared with MS, QL significantly improved shoot regeneration percentage for '60-160' (exclusive of BA at 5 mg·L⁻¹) and 'IIB' in all treatments, whereas QL showed a decrease of shoot regeneration percentage for '71-3-150'. There was no significant difference between QL and MS for 'GM256', 'Y', and 'JM7' overall.

These results showed that 'GM256' was an easy-to-regenerate cultivar, which did not exhibit stringent culture requirements, displaying high regeneration ability on a wide range of medium compositions. 'IIB' was a difficult-to-regenerate cultivar, which exhibited stringent culture requirements, displaying regeneration ability only on a few medium compositions with low regeneration percentages. These results showed that successful shoot regeneration on leaf explants mainly depended on the rootstock cultivar, and that the interaction between rootstock cultivar and SIM also was very important. In the present study, 'GM256' reached a high percentage of shoot regeneration on all tested media. 'JM7' also obtained a high percentage of shoot regeneration on seven SIM treatments, except treatment T2. The highest percentage was obtained on MS with 2 mg·L⁻¹ TDZ for '71-3-150', on QL with 1 mg·L⁻¹ TDZ for '60-160', and on MS with 1 mg·L⁻¹ TDZ for 'Y' and on QL with 5 mg·L⁻¹ BA for 'IIB'.

Effects of media treatments on regenerated shoot growth. Growth behavior of adventitious buds produced from TDZ and BA was different. BA-induced adventitious buds were able to elongate and develop into shoots directly on SIM (Fig. 1A–C and F), exclusive of '60-160' and 'IIB' on MS (Fig. 1D and E). TDZ-induced adventitious buds were unable to elongate to form shoots on SIM (Fig. 1G–J), it was necessary to transfer the buds to SM containing BA to develop into shoots (data not shown). These results revealed that adventitious buds produced by BA were easy to elongate on the

same SIM, whereas adventitious buds produced by TDZ were difficult to elongate on the same SIM. The problem of shoot elongation was overcome by transferring bud cultures to a new medium lacking TDZ but containing BA (data not shown).

Basal medium composition also influenced the growth of shoot buds when containing the cytokinin BA. Adventitious shoots produced on QL showed better elongation than on MS for '60-160' and 'Y' (Fig. 1M and N). Adventitious shoot growth did not show differences between MS and QL for the other four cultivars (Fig. 1O–R).

In conclusion, basal medium composition and the type of cytokinin both influenced shoot organogenesis from leaf explants. The optimal basal medium and cytokinin for shoot organogenesis was different depending on rootstock cultivar. When TDZ and BA did not show significant differences in regeneration percentage, BA was recommended because BA could induce adventitious buds to form shoots directly on SIM.

Root organogenesis of in vitro shoots. The regenerated shoots or buds (from TDZ) both grew very well (data not shown) on SM (Fig. 1K and L). In vitro shoots of all apple rootstock cultivars showed high root organogenesis capacity. The rooting frequency of the six apple rootstock cultivars all reached more than 90% on at least one basal medium, and the mean root number per plantlet ranged from 2.4 to 4.9 (Table 2). There were significant differences between two media for mean rooting percentage ($P > F = 0.01$), but there were no significant differences in mean number of roots. However, the medium for obtaining the highest rooting frequency was different depending on rootstock cultivar ($P > F = 0.01$). For 'GM256' and 'Y', half QL was significantly higher than one-quarter MS; for 'IIB' and 'JM7', one-quarter MS was significantly higher than half QL; and for '71-3-150' and '60-160', one-quarter MS and half QL were not significantly different. There were no significant differences in root number per plantlet due to rootstock or medium, and no significant interaction between rootstock and medium (Table 2). A difference of root growth on half QL or one-quarter MS was not found (Fig. 2A–L) in all tested cultivars. These results revealed that all rootstock cultivars tested in this paper

showed high root organogenesis capacity when on the proper medium for each individual rootstock cultivar, especially for 71-3-150 and '60-160'. Both were easy-to-root cultivars which did not exhibit stringent culture requirements and showed high root organogenesis capacity on either half QL or one-quarter MS.

Discussion

Regeneration efficiency of apple rootstock is dependent on cultivar, basal medium, and hormonal combinations, so the components of the regeneration medium should be optimized for each cultivar. In this study, shoot regeneration frequency among six apple rootstock cultivars was significantly different. This result is in conformity with the previous report that on the same induction medium. Shoot regeneration capacity was significantly different among seven genotypes in *Melia* (Vila et al., 2004), among twenty-four genotypes in *Pyrus* sp. (Bell et al., 2012), among six genotypes in *Lycopersicon esculentum* Mill (Gubis et al., 2003), and among eleven genotypes in *Chrysanthemum* (Lim et al., 2012). The difference in shoot regeneration capacity of the different rootstock cultivars confirmed that genotype remained a key determinant for shoot organogenesis in plants. In this paper, rootstock cultivar 'GM256' showed the highest regeneration capacity, and 'IIB' showed the lowest regeneration capacity. Similar results have been reported in other apple rootstocks, for example, M.9 showed a higher regenerative capacity than Ottawa 3 (Welandar and Maheswaran, 1992).

The types of basal medium significantly affected shoot regeneration efficiency. The optimal basal medium for shoot regeneration was in some cases different depending on rootstock cultivar. In this study, MS exhibited the highest shoot regeneration efficiency for '71-3-150', and QL showed the highest shoot regeneration percentage for '60-160' and 'IIB'. The responses of the other rootstocks were not significant. The responses from different media for the same rootstock cultivar probably are a result of the nutritional differences in media. The most prominent difference between MS and QL is the difference of ratio of $\text{NH}_4^+/\text{NO}_3^-$ (Abu-Qaoud et al., 1991;

Table 2. Root organogenesis capacity comparison of different rootstock cultivars on different rooting medium.

Rootstock	Mean rooting % $\pm$ SE ^z		Rootstock means	Mean root no.		Rootstock means
	One-quarter MS	Half QL		On-quarter MS	Half QL	
'GM256'	69.5 $\pm$ 6.8 b ^y	90.8 $\pm$ 8.2 a	80.2 $\pm$ 3.3 B ^y	3.2 $\pm$ 0.2 a	4.2 $\pm$ 0.3 a	3.7 $\pm$ 0.2 A
'71-3-150'	94.2 $\pm$ 6.8 a	96.7 $\pm$ 5.2 a	95.6 $\pm$ 1.1 A	3.4 $\pm$ 1.2 a	3.3 $\pm$ 0.6 a	3.3 $\pm$ 0.1 A
'60-160'	92.0 $\pm$ 7.8 a	96.0 $\pm$ 4.7 a	94.0 $\pm$ 1.4 A	4.9 $\pm$ 1.8 a	4.2 $\pm$ 1.4 a	4.6 $\pm$ 0.3 A
'IIB'	98.3 $\pm$ 2.7 a	82.3 $\pm$ 4.8 b	90.3 $\pm$ 2.4 AB	4.8 $\pm$ 0.9 a	2.4 $\pm$ 0.4 a	3.6 $\pm$ 0.4 A
'JM7'	95.6 $\pm$ 5.2 a	85.4 $\pm$ 8.9 b	90.5 $\pm$ 1.5 AB	4.8 $\pm$ 0.7 a	2.9 $\pm$ 0.5 a	3.8 $\pm$ 0.3 A
'Y'	70.4 $\pm$ 6.0 b	98.6 $\pm$ 3.4 a	84.5 $\pm$ 4.1 AB	4.5 $\pm$ 0.9 a	4.6 $\pm$ 0.7 a	4.5 $\pm$ 0.1 A
Medium means $\pm$ SE	86.7 $\pm$ 2.6 b	91.6 $\pm$ 1.3 a		4.3 $\pm$ 0.2 a	3.6 $\pm$ 0.2 a	

^zAll rooting media with 0.5 mg·L⁻¹ indole-3-butyric acid and 3% sucrose.

^yFor each trait, means followed by different letters within the same row were significantly different at $P < 0.05$ by least significant difference (LSD) with Tukey's multiple comparison adjustment. Rootstock means within a column followed by different bold letters were significantly different at $P < 0.05$ by LSD with Tukey's multiple comparison adjustment.

MS = Murashige and Skoog; QL = Quoirin and Lepoivre.

Fig. 2. Rooting behavior of six rootstock cultivars on two rooting media. (A, C, E, G, I, K) Rooting medium was one-quarter MS with 0.5 mg·L⁻¹ IBA and 3% sucrose; (B, D, F, H, J, L) Rooting medium was half QL with 0.5 mg·L⁻¹ IBA and 3% sucrose. (A, B) rootstock 'GM256'; (C, D) rootstock '71-3-150'; (E, F) rootstock '60-160'; (G, H) rootstock 'TIE'; (I, J) rootstock 'JM7'; (K, L) rootstock 'Y'. MS = Murashige and Skoog; IBA = indole-3-butyric acid; QL = Quoirin and Lepoivre.

Bell and Reed, 2002), although other differences in the media cannot be excluded as contributing to the response. The composition of MS is 20.6 mM NH₄⁺ and 39.4 mM NO₃⁻,

whereas that of QL is 5 mM NH₄⁺ and 33 mM NO₃⁻. In addition, the total molarity of QL is lower than MS, 68.5 vs. 94 mM, respectively. These results indicated that selection of the

composition of basal medium is critical, as previously reported. Woody plant medium (WPM) was more effective than MS in *Buddleia globosa* (Aboshama, 2011), MS was more effective than WPM and B5 in *Populus alba* × *Populus berolinensis* (Wang et al., 2008), MS was better than N6 in Iranian native dwarf rootstock of apple (*Malus domestica* Borkh cv. Gami Almasi) (Rustae et al., 2007), half MS was better than MS in *Pyrus communis* cultivars Louise Bonne and Seckel, and MS was better than half MS in *Pyrus ×bretschneideri* cultivar Crystal Pear (Chevreau et al., 1989).

Shoot organogenesis can be induced by culturing an actively growing part (explant) of the plant on the medium supplemented with specific cytokinins and auxins. Cytokinin plays a major role in inducing shoot organogenesis in plants (Cheng et al., 2010; Gordon et al., 2007; Su et al., 2011). The synthetic cytokinin BA is used routinely in tissue culture and BA was successfully used to induce shoot organogenesis in a number of plant species (Caboni et al., 1999; Petri and Scorza, 2010; Preethi et al., 2011), including apple scion and apple rootstock cultivars (Modgil et al., 2005; Welander and Maheswaran, 1992). However, BA was not effective or was less effective for some species. TDZ, a substituted phenylurea, has an immense cytokinin potential in inducing shoot organogenesis in a number of plant species (Parveen and Shahzad, 2010; Sarwar and Skirvin, 1997; Sharma et al., 2012; Tomsone et al., 2004; Virscek-Marn et al., 1999), and TDZ was used to induce shoot organogenesis and was shown to be more effective than BA in many species, including apple cultivars (Montecelli et al., 2000; Mitic et al., 2012; Yorgancilar and Erisen, 2011). In this study, TDZ was shown to be more effective than BA for '71-3-150', '60-160', and 'Y', whereas differences for the other rootstocks were not significant. These results indicated that the effectiveness of BA or TDZ depended on the rootstock cultivar, similar to previous reports that the best regeneration was obtained in 'Gami Almasi' by using BA (Rustae et al., 2007), and in 'Royal Gala' by using TDZ (Dobranski et al., 2006). However, for some genotypes, BA and TDZ had similar effects on shoot regeneration (Yorgancilar and Erisen, 2011).

Generally, TDZ-supplemented medium resulted in larger adventitious shoots per explant and inhibited shoot elongation (Fig. 1G–J), but adventitious shoots could be induced to elongate by transferring to proliferation medium containing BA but lacking TDZ. It has been previously reported that inhibition of shoot elongation was overcome by transferring to hormone-free medium or one lacking TDZ medium (Kim et al., 1997; Peddaboina et al., 2006; Wang et al., 2007). BA-supplemented medium can induce shoot regeneration together with elongation on the same medium. As previously reported, BA was used successfully to elongate the induced shoot buds in apple (Predieri and Malavasi, 1989; Yepes and Aldwinckle, 1994). Inhibition of shoot elongation by TDZ

may be consistent with its high cytokinin activity (Huetteman and Preece, 1993).

In conclusion, shoot regeneration capacity was markedly different among the six apple rootstock cultivars. 'GM256' was easy to regenerate, and it showed high regeneration frequency on a wide range of media: the highest regeneration frequency was 97.8% on the optimal medium (MS BA 5). 'ΠΒ' was difficult to regenerate and the highest regeneration frequency was only 26.4%. Further research should be conducted to improve the regeneration frequency of 'ΠΒ'.

Adventitious root formation is a key step in micropropagation. An efficient rooting treatment should yield a high percentage of rooted shoots and a high root number, which is necessary for acclimatization. In this paper, high rooting percentages and high root numbers were obtained for all six cultivars. The medium for obtaining the highest rooting percentage varied among cultivars, as has been previously reported (Mert and Soyulu, 2010). Basal medium composition had an effect on rooting. Reduction of the mineral concentration of MS medium to half the normal value increased the rooting percentage of PR 204/84 rootstock (Fotopoulos and Sotiropoulos, 2005), and WPM was most suitable for rooting of apple rootstock P60 and P2 compared with half WPM, MS and half MS (Orlikowska, 1992). In this paper, half-strength QL was slightly, but significantly, better than half-strength MS when averaged overall six rootstocks. However, one-quarter-strength MS was better than half-strength QL for root organogenesis of rootstocks 'ΠΒ' and 'JM7', and half-strength QL was better than one-quarter-strength MS for root organogenesis of rootstocks 'GM256' and 'Y', whereas one-quarter-strength MS and half-strength QL had a similar effect on root organogenesis of rootstocks '71-3-150' and '60-160'. No significant differences were found in the mean number of roots. Further observation of plantlet acclimation and survival may be useful in studying the effect of rooting medium.

In conclusion, all the six cultivars assayed study had higher organogenesis (including root and shoot organogenesis) capacity when they were cultivated on their compatible medium respectively.

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