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A reproducible protocol for adventitious shoot regeneration from leaves of apple rootstock Merton793 and assessment of genetic stability

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ABSTRACT

The present studies were aimed to develop an efficient and reproducible method for shoot regeneration from leaf explants of apple rootstock Merton793. The influence of two cytokinins BA and TDZ with three auxins IAA, IBA and NAA in different combinations were evaluated for shoot induction using light and dark incubation. Direct and healthy shoots were obtained from leaves on MS medium with BA-NAA and BA-IAA combinations in light incubation and 100% regeneration was achieved on medium with 4 mg/l BA and 1 mg/l NAA. Both direct and indirect shoot regeneration was obtained on TDZ-NAA and TDZ-IBA. Maximum shoot regeneration was promoted on 0.8 & 0.6 mg/l TDZ with 1 mg/l NAA, however, it showed abnormal, vitrified, small and rosette type of shoots. Dark treated leaf explants showed low rates of shoot induction with TDZ combinations. Healthy adventitious shoots regenerated directly from leaves were multiplied, rooted and hardened successfully. RAPD analysis of regenerants revealed that all the banding profiles from directly originated shoots were monomorphic and similar to those of the mother plant, while shoots originated through intervening callus revealed 61.2% polymorphism. The results show that genetic fidelity of directly produced adventitious shoots was maintained, and thus, our regeneration protocol can be an useful method in transformation research in Merton793 without the induction of variation.

Keywords: *Malus* × *domestica*, plant growth regulators, cytokinins, auxins, genetic fidelity, RAPD.

INTRODUCTION

Merton 793, a cross between 'Northern Spy' × M.2 raised in 1920 by John Innes Horticulture Institute in Merton, England, has been proved to be an excellent propagating stock because it is vigorous, adaptable to different soil types, resistant to woolly apple aphid and collar rot, and tolerant to replant diseases. It was introduced in Himachal Pradesh in 2000, from New Zealand and reported to be suitable for replanting apple orchards at old sites of H.P. However, it is one of the clonal rootstocks which are difficult to root and thus, affects the survival rate. Our future aim is to transfer root inducing gene to enhance rooting in Merton793 to enable better establishment in the field. Before carrying out the genetic transformation experiments, standardization of high frequency shoot regeneration process from somatic tissues of different types of organs has been considered a prerequisite and has application of most modern genetic approaches to crop improvement.

Though, fruit tree species were recognized to be difficult to regenerate *in vitro*, yet several reports have been published on the development of regeneration systems in apple (Hohnle and Weber, 4; Wei *et al*., 17; Zhang *et al*., 20). The most important and frequently used cytokinins in apple regeneration are benzyladenine and thidiazuron but their efficiency strictly depends upon the genotype, auxin combination and concentration applied. In addition, several other factors controlling the organogenesis like size, type and position of explants, dark period and light intensity etc. have also been evaluated in various studies on apple (Jin *et al.,*6; Magyar-Tabori *et al*., 9; Mitic *et al.,*12; Zhang *et al.,*19,20) and resulted in valuable outcomes.

Regeneration of shoots via intermediate callus is more frequent adventitious propagation method as compared to the direct organogenesis from explants in woody plant species like apple. In order to avoid the somaclonal variations, it is better to look for the direct shoot regeneration with apple plants. As gross morphological variations are expected to occur at a much lower frequency than variations at DNA level, the absence of visible variation does not preclude the absence of all types of variations among the *in vitro* raised progeny. John et al. (7) considered somaclonal variation highly undesirable phenomenon because it may complicate studies on genetic transformation and transmission. Therefore, it is necessary to determine the genetic fidelity of adventitiously raised s hoots before carrying out the gene transfer experiments. There are a number of DNA markers like RAPD, SSR and RFLP available

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for screening of such type of genetic variation among regenerants, because they are more informative and not developmentally regulated. RAPD analysis has emerged to be a simple, quick to perform and powerful technique to determine sequence polymorphism (McMeans *et al.,* 11) and has successfully been utilized to detect genetic similarity or dissimilarity among adventitiously regenerated shoots/ plants in a number of plant species.

As far as the authors know, there is relatively little research on shoot regeneration from leaf tissue of apple rootstock Merton793. Because apple is prone to mutations, it is necessary to develop transgenics true to type except the inserted trait. Keeping in view, objective of the present study was to evaluate the effects of different PGRs and dark treatment to get high efficiency and reproducible adventitious shoot regeneration from leaf explants and to assess the genetic fidelity of the regenerants arose directly and indirectly.

MATERIALS AND METHODS

First four apical, young and unfolded leaves excised from one month old *in vitro* proliferating shoots established by Soni *et al*. (15) were used as the source material of leaf explants. Explants were prepared by cutting away the petioles and apex of leaves and then by making cuts across the veins. Prepared explants were randomly placed with adaxial side up on shoot regeneration medium contained in Erlenmeyer flasks of 100 ml capacity, which consisted of MS (Murashige and Skoog, 13) salts and vitamins supplemented with cytokinins and auxins like 1-5 mg/l 6-benzyladenine (BA) or 0.2-1mg/l thidiazuron (TDZ) in combination either with α-naphthalene acetic acid (NAA) or indole-3-acetic acid (IAA) or 3-indolebutyric acid (IBA) each with 0.5- 1 mg/l in sixty different combinations (treatments). The media was solidified with 8 g/l agar after adjusting pH (5.8) and autoclaved at 121^0C for 20 mins. For each treatment, half of the cultures were incubated in the darkness at 25 -27^0C for the initial one week and then transferred to light conditions under a 16 h photoperiod provided by cool white fluorescent lamps (32-40 µmol m^{-2} s^{-1}), while other half was incubated under continuous light. Twelve flasks each with six explants totaling 72 were used per treatment. After fifty days of incubation, observations like frequency of regeneration, number of adventitious shoots per regenerating leaf explant, length of shoots and shoot quality were recorded. All the experiments were repeated at minimum once.

Adventitious shoots developed directly from leaves without callus as well as through callus were excised, kept separate, marked and transferred to multiplication MS medium supplemented with 0.5 mg/l BA, 0.1mg/l GA_3 (gibberellic acid) and 0.05 mg/l IBA. Shoots of about 2cm long were separated from proliferating cultures and transferred to rooting medium having ½ MS medium with 2 % sucrose and 0.5 mg/l IBA. The rooted plantlets were transferred to sterilized cocopeat and soil (1:1) and maintained for hardening in glasshouse under shade.

For data analysis, regeneration percentage was calculated as the number of explants that developed shoots divided by the total number of explants. Data was transformed by the arcsine square root transformation and analysis of variance (ANOVA) was performed on the data with mean separation by Duncan's test using SPSS data program.

To test the genetic stability of regenerated shoots, young leaves were collected from mother plant of apple rootstock Merton 793 growing in the field of Department of Pomology, Dr, YSP, University of Horticulture and Forestry, Nauni, Solan (H.P.), and from randomly selected *in vitro* raised regenerants obtained through direct and indirect regeneration. Total genomic DNA from leaves mentioned above were extracted following a method described by VirscekMarn *et al*. (16) and subjected to RAPD analysis. A set of five random decamer primers i.e. OPA-11, 19, 20, and OPB-11, 12, which produced good amplifications in a number of apple genotypes, were used in the present experiment. PCR reaction mixture consisted of 1U Taq DNA polymerase, 1X Taq DNA polymerase buffer containing 1.5 mM $MgCl_2$, 10 pmol random decamer primer, 2.5 mM deoxynucleotide triphosphate (dNTPs), and 50 ng template DNA.The PCR reaction was run in a Perkin Elemer Thermal Cycler. The temperature profile used for the DNA amplification of all the samples was preliminary denaturation at 94° C for 4 min followed by 35 cycles at 94° C for 1 min, primer annealing at temperature according to primer (kept usually around $T_m \pm 2$ °C) for 30 sec, initial elongation at 72° C for 2 min and final extension at 72° C for 8 min.

Amplified DNA was electrophoresed using DNA gel electrophoresis system (GeNei) in 1.2% agarose gel and run at 80V. 3 kb DNA ladder (Sigma) was used as size marker. Images of the amplified DNA were taken in Alpha- imager gel documentation system. All the treatments were tested twice. Well resolved and consistently reproducible bands were scored as present. The primers producing the scorable bands with all the regenerants and mother plant were used to score similarities/ dissimilarities.

RESULTS AND DISCUSSION

In the present study, the effect of BA with different auxins were evaluated on shoot regeneration capacity of Merton 793. It has been seen that leaf

explants cultured on medium with nine combinations of BA and NAA developed healthy shoots directly from the petiolar base and tip of leaves (Figs.1, 2). Adventitious shoot regeneration frequency achieved on media containing 1 mg/l NAA in combination with 3, 4 and 5 mg/l BA were 80.33, 100 and 50% respectively. 4 mg/l BA and 1 mg/l NAA resulting in 100 percent shoot induction was found optimal with 4.5 average number of shoots per regenerating explant (Fig.3, Table 1). On the other hand, BA in combination with 0.5 mg/l NAA resulted in low regeneration rates (5.8-35%) and 2-3 shoots per explant. It is clear that shoot regeneration frequency increased with increase in BA level upto 3 mg/l BA, then showed a decrease.

It has further been observed that medium with BA and IBA combinations did not produce any adventitious shoots. When BA was added with IAA in ten combinations, adventitious buds were seen in 2nd wk in eight combinations from the basal and tip portions of the leaf explants without intervening callus, which elongated into shoots during 4th wk. Highest shoot regeneration (62.5%) was observed on medium containing 3 mg/l BA and 0.5 mg/l IAA followed by 41.93% on 3 mg/l BA and 1 mg/l IAA (Table 1). Average number of shoots formed were 4.8 and 4 respectively. However, highest level of BA (5 mg/l) with 0.5 mg/l IAA resulted in longer and enhanced number of shoots to 8 but showed only 33.10% regeneration (Fig. 4). Here, 0.5 mg/l was found better concentration of IAA as compared to 1 mg/l whereas1 mg/l NAA was found more effective than 0.5 mg/l. Between two auxins, NAA proved better than IAA because it promoted highest induction of healthy regenerated shoots in Merton793. Very few studies have reported the use of IAA with BA.

It appears from the present results that shoot regeneration ability was strongly influenced by BA concentration and its combination with auxins, and a high concentration of BA was capable to promote better shoot regeneration. The results are consistent with the findings obtained with other apple rootstocks and cultivars that a high cytokinin-low auxin ratio was required to induce regeneration in leaf explants (Gamage *et al*.,3; Wei *et al.,*17; Zhang *et al*.,19). It has been found that high BA upto 2 mg/l and low IBA combination yielded good number of shoots in apple rootstock (Zhang *et al.,*20). Jamil and Khan (5) also achieved similar results in apple cultivars by culturing internodes and leaves with low levels of NAA (0.2&0.5 mg/l) and BA (0.5-2.0 mg/l). From all these studies, it is suggested that the optimal concentration of BA and NAA depends upon the genotype.

In this investigation, the effects of TDZ were compared with BA on the organogenetic potential of leaves. Significant differences were found in shoot regeneration frequencies and number of shoots per explant in different combinations of TDZ with IBA or NAA. It was observed that white, nodular callus developed in 2ndwk and then adventitious buds appeared in 4thwk from which shoots were regenerated on seven combinations of TDZ and IBA out of ten. Best regeneration rates of 85.83-88.17% were obtained on media containing 0.6 and 0.8 mg/l

Table 1. Regeneration of adventitious shoots from leaves in Merton 793 on MS medium with BA and IAA or NAA, incubated in light.

Plant growth regulators (mg/l)		Explants regenerating (%)	Average number of shoots per explant	Plant growth regulators (mg/l))		Explants regenerating (%)	Average number of shoots per explant
BA	IAA			BA	NAA		
1	1	0.00^{g}	0.00	1	1	17.20^{i}	3.00
2	1	26.33^{d}	3.63	2	1	41.53^{d}	1.40
3	1	41.93^{b}	4.00	3	1	80.33^{b}	4.00
4	1	10.33^{f}	2.33	4	1	100.30^{a}	4.50
5	1	0.00^{g}	0.00	5	1	50.00^{c}	3.00
1	0.5	10.00^{f}	2.33	1	0.5	5.80^{j}	2.00
2	0.5	34.50^{c}	1.25	2	0.5	30.00^{g}	2.00
3	0.5	62.50^{a}	4.80	3	0.5	35.23^{f}	2.80
4	0.5	22.40^{e}	1.77	4	0.5	20.00^{h}	2.00
5	0.5	33.10^{c}	8.00	50.5		0.00^{k}	0.00
SE		0.51	0.52			1.28	0.57
$CD_{0.05}$		1.52	1.52			2.68	1.67

Different letters in the same column (superscript) denote significant differences at P< 0.05 by Duncan's test (n=30)

TDZ each with 1 mg/l IBA and resulted in 5.4 and 7.47 average number of smaller shoots developed through callus (Fig.5, Table 2), whereas highest number of shoots (9.33) was obtained directly on 0.4 mg/l TDZ (Fig.6). Though higher TDZ resulted in the highest shoot regeneration rate but produced vitrified, rosette type and abnormal shoots (Figs.5, 6). Treatments containing TDZ and 0.5 mg/l IBA resulted in granular calli from which a few vitrified shoots (36-42.6%) were observed to originate.

It has further been observed from TDZ and NAA combinations that 75% of the leaf explants were regenerated through callus with 15.27 average number of shoots per explants on MS medium containing 0.4 mg/l TDZ with 1 mg/l NAA (Fig.7) and 66% with 8.53-9.6 shoots on 0.2 and 0.4 mg/l TDZ each with 0.5 mg/l NAA (Table 2). Higher number of shoots (13.6) were obtained when 0.2 mg/l TDZ was combined with 1 mg/l NAA resulting in 62.17% regeneration. It was noticed that on TDZ and NAA combinations, shoots were of rosette type and hyperhydric though only a few looked normal. In some treatments, bunches of small rosette type shoots with deeply dissected and translucent leaves originated (Fig.7) which were not able to differentiate into nodes and internodes, though their number increased to approx18. Inspite of the good regeneration rates and number of shoots in Merton793,TDZ inhibited shoot elongation and led to fasciated and vitrified shoots which were not desirable.

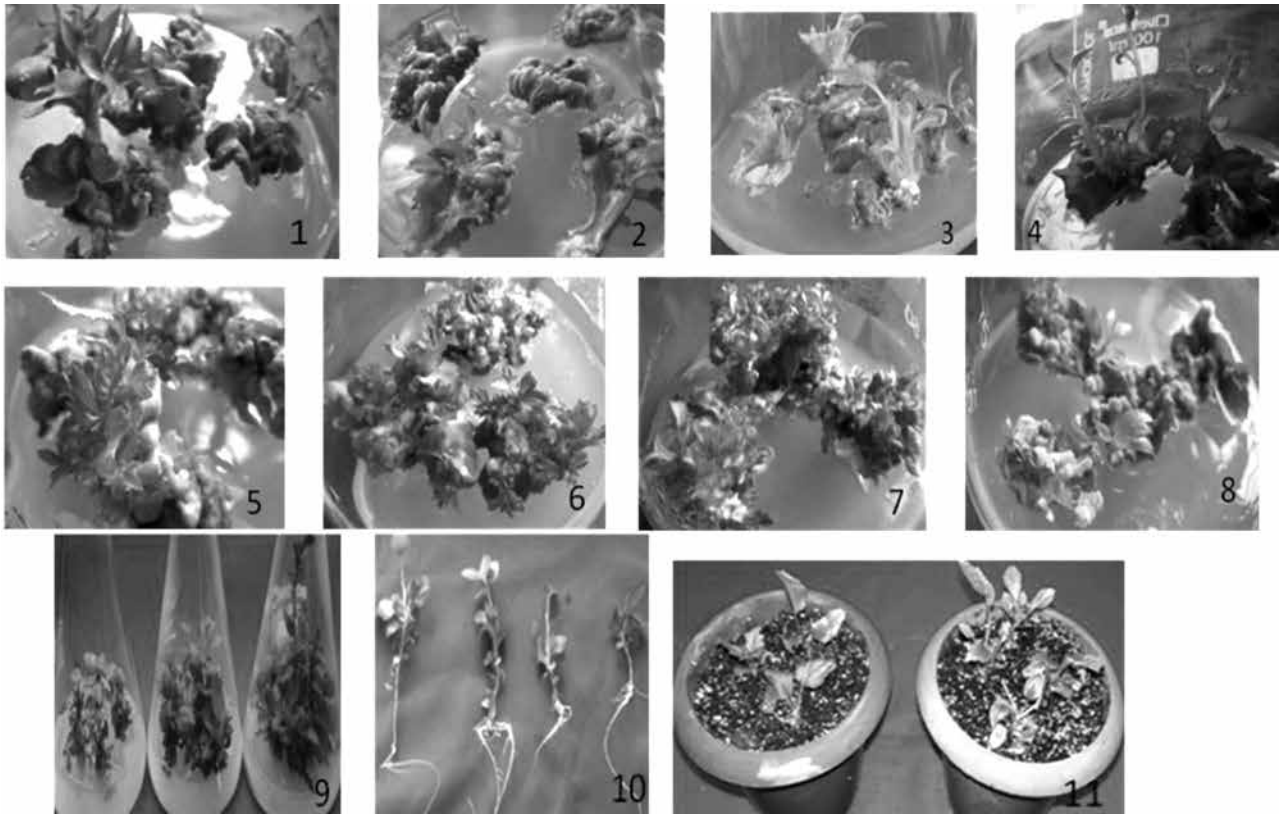
The present studies agree with Magyar-Tabori *et al.* (9) that both regeneration pathways can be detected in apple regeneration systems. Direct shoot regeneration was reported by Pawlicki and Walender (14) in apple rootstocks 'Jork 9, indirect in cultivar 'Queen Cox'(Wilson and James,18) while both types of regeneration were reported in leaf and stem explants of wild apple *Malus seiversii* where shoots were originated through callus phase within 60 days (Zhang *et al.,*19). Whereas, it took four weeks to induce shoots from callus in the present investigation. Our results are somewhat similar to UK apple cultivar 'Queen Cox' where TDZ levels above 1 mg resulted in abnormal and stunted shoots that did not develop further (Wilson and James, 18). But such type of observations of hyperhydricity were recorded at lower concentrations of TDZ in presently studied M793. It is clear that the optimal TDZ level largely depended on genotype and auxin type and its concentration. On the contrary, there are studies which showed that very low levels of TDZ (0.44µM) promoted regeneration for 'Alkmene', 'Greensleeves', 'Idared' and 'M9' (Magyar-Tabori *et al*., 9) and a wide range of TDZ concentrations (5-20 µM) resulted in 100% regeneration rate in 'Royal Gala'.

In our studies, we have also determined the influence of dark incubation on shoot formation and found that Merton793 leaves could not induce shoots in any of the BA combinations. It is interesting to note that leaves responded to TDZ the same way for regeneration as in light, but with reduced frequencies of shoot formation. It has been observed that seven combinations of TDZ and IBA induced shoots through intermediate callus after 4wks. Highest regeneration

Table 2. Effect of TDZ and IBA on adventitious shoot development from leaves of the rootstock M 793, incubated in light and initial darkness.

Plant Growth regulators (mg/l)		Explants regenerating (%)		Average number of shoots per explants		Plant Growth regulators (mg/l)		Explants regenerating (%)		Average number of shoots per explants	
TDZ	IBA	Light	Dark	Light	Dark	TDZ	NAA	Light	Dark	Light	dark
0.2	1	41.33^{f}	37.17^{d}	6.00	1.73	0.2	1	62.17^{d}	37.83^{c}	13.60	3.27
0.4	1	64.17^{c}	58.03^{a}	9.33	3.83	0.4	1	75.00^{a}	50.33^{a}	15.27	3.73
0.6	1	85.83^{b}	51.00^{b}	5.40	3.47	0.6	1	50.00^{f}	0.00^{g}	12.73	0.00
0.8	1	88.17^{a}	0.00^{f}	7.47	0.00	0.8	1	0.00^{h}	0.00^{g}	0.00	0.00
1.0	1	37.50^{g}	14.07^{e}	3.53	2.00	1.0	1	50.00^{f}	0.00^{g}	12.67	0.00
0.2	0.5	0.00^{h}	14.40^{e}	0.00	1.33	0.2	0.5	66.00^{b}	28.33^{e}	9.60	2.67
0.4	0.5	0.00^{h}	0.00^{f}	0.00	0.00	0.4	0.5	66.00^{b}	25.33^{f}	8.53	3.00
0.6	0.5	36.67^{g}	44.27^{c}	3.13	2.00	0.6	0.5	50.33^{f}	25.00^{f}	9.37	3.00
0.8	0.5	0.00^{h}	0.00^{f}	0.00	0.00	0.8	0.5	52.33^{e}	41.67^{b}	11.80	4.33
1.0	0.5	42.60^{d}	15.60^{e}	3.00	3.00	1.0	0.5	43.00^{g}	33.43^{d}	12.03	3.00
SE		1.62	0.79	0.54	0.60			1.15	0.50	0.64	0.57
$CD_{0.005}$		4.78	2.34	1.61	1.77			3.40	1.48	1.88	1.69

Different letters in the same column (superscript) denote significant differences at P< 0.05 by Duncan's test (n=30)



Figs. 1-11. Adventitious shoot regeneration from in vitro leaves of apple rootstock Merton 793. 1,2. Direct shoot induction from basal and tip portions of leaves on 1&3 mg/l BA each with 1 mg/l NAA, 3. Origin of direct and healthy shoots on 4 mg/l BA and 1 mg/l NAA,4.Highest number of shoots on 5 mg/l BA and 0.5 mg/l IAA, 5. Vitrified and rosette type short shootson 0.8 mg/l TDZ and 1 mg/l IBA,6. Large number of direct, short shoots with abnormal leaves on 0.4 mg/l TDZ and 1 mg/l IBA, 7. Group of abnormal shoots arosethrough callus on 0.4 mg/l TDZand 1 mg/l NAA,8. Indirect and abnormal shoot regeneration on 0.8 mg/lTDZ with NAA incubated indark, 9. Multiplication of regenerants originated directly, 10-11. Rooted shoots and hardened plants.

of 58% was obtained on 0.4 mg/l TDZ with 1 mg/l IBA followed by 51% on 0.6 mg/l TDZ and 1mg/l IBA. Average number of shoots per explant formed were 3.83 and 3.47 respectively. TDZ with low concentrations of IBA (0.5mg/l) resulted upto 44% shoot induction (Table 2). TDZ and NAA combinations induced direct and indirect adventitious shoots, which were very short, vitrified with abnormal leaves (Fig.8). The shoot regeneration frequencies ranged from 41.67% to 50.33% on medium containing 0.8 and 0.4 mg/l TDZ with 0.5 and 1mg/l NAA respectively and the average number of shoots per explant ranged from 3.73 and 4.33.

A number of workers have used initial dark treatment for different periods to induce adventitious shoots and the evidence with respect to the effect of darkness on shoot organogenesis seems to be contradictory. Darkness increases endogenous level of auxin in tissues and on the contrary light is known to decrease free endogenous IAA. It may be possible that continuous presence of auxin in dark incubated cultures decreased the regeneration frequency in presently studied apple rootstock M793. Mitic *et al* (12) reported the increased regeneration frequency, and direct organogenesis, when dark pre-treatment was given to the explants 'Melrose' and 'Golden Delicious'. Similarly, Jin *et al*. (6) found that prior incubation of 2 weeks in dark induced the highest shoot regeneration percentage and number of shoots per explants from leaves of 'Pingyitincha' apple rootstock. However, the functional mechanism of dark incubation in promoting shoot regeneration is unclear.

Our experience on apple tissue culture showed that the induction of shoots is influenced by the concentration and type of cytokinin applied, because their uptake, interaction with endogenous cytokinins of explant and metabolism differ between apple genotypes. Both BA and TDZ are the most commonly used cytokinins in apple regeneration systems and

their comparison was done in several studies. For example, apple rootstock G.41, 'Golden Delicious' and 'Pingyitincha'regenerated better on media with TDZ (Jin *et al.,* 6; Mittic *et al.,*12; Zhang *et al*., 20) while BA was favorable for 'Bramley' (McAdam *et al.,*10). In another study, Dobranszki *et al.* (2) reported that the number of regenerated shoots per leaf explant was the highest with 0.5 mg/l TDZ among nine cytokinins, but very high vitrification was detected which agrees with our results. Although, there are reports that increased BA concentration also promoted vitrification, but the results presented here clearly show that BA at higher levels did not develop vitrification and was found superior to TDZ which are in conformity with the previous studies on apple rootstocks M9/T337 and M26 (Hohnle and Weber, 4). It was seen that NAA responded with both cytokinins while IAA worked well with BA, and IBA with TDZ. Therefore, it is suggested that type of cytokinin, its combination with type of auxin and their concentrations determine the adventitious shoot induction in Merton 793 rootstock.

Single healthy regenerated shoots exhibited 4-7fold multiplication (Fig.9) and 70 -75% rooting of shoots (Fig.10). Around 70% plantlets survived after transplanting in pots (Fig.11). Vitrified shoots were not found suitable for multiplication and rooting.

During the assessment of genetic fidelity studies of regenerants, it was observed that five primers generated a total of 18 distinct bands and 227 amplified products ranging in size between 100-1183 bp from 14 regenerated shoots and mother plant of apple rootstock M793 (Table 3). The number of bands for each primer varied from 1-5 with an average of 3.6. It has been observed that four primers (OPA-11,19,20, OPB-12) except OPB-11 produced monomorphic banding profiles from directly originated shoots (R1-R8) which were also identical to that of the profiles of mother plant DNA (Fig.12a-c). Whereas all the primers except OPA-19 showed polymorphic bands in regenerants (R9-R14) raised through callus (Fig.12a-c) resulting in 62% polymorphism (Table 3). Among R9-R14, it was found that two regenerants R12 and R13 revealed greater polymorphism as compared to others. It is indicated here that RAPD profiles from directly regenerated shoots under assessment generated very little variation among them and their mother plant DNA, thus, ensure the genetic stability, while profiles from callus-mediated shoots showed visible variant banding patterns.

Similarly, no visible variations in RAPD patterns among regenerants of 'Gala' and 'Royal Gala' apple were found (McMeans *et al.,* 11). Jin et al. (6) also reported adventitious shoots of apple rootstock

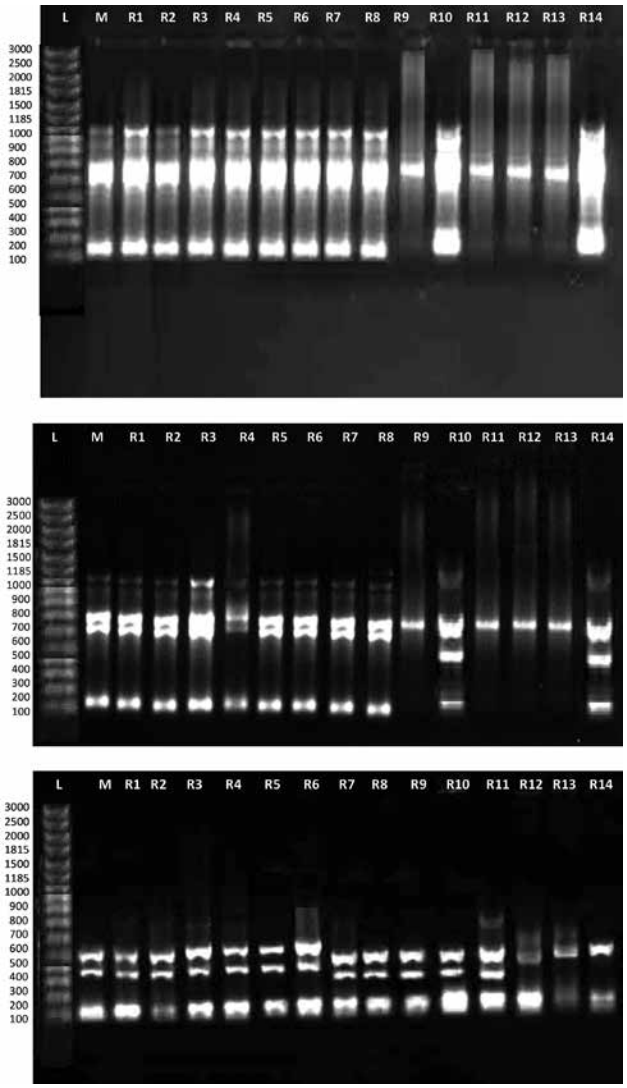


Fig.12 (a-c). RAPD profiles of regenerants of apple rootstock Merton 793 with primers OPA-11 (a), OPA-20 (b) and OPB-12 (c). L – 3kb ladder, M- mother plant, R1-R8 directly originated and R9-R14 indirectly originated regenerants.

genetically stable with SSR markers. In another study on MM111 (Dantas *et al.,*1), regenerants raised from internodes as well as leaf discs resulted in 5-20 somaclones. In the present study, variation in the RAPD patterns of some of the regenerants may be due to their origin from intermediate callus. VirscekMarn *et al*. (16) indicated that a high degree of variation in apple cultivars was connected with adventitious shoot formation from leaves. Karp (8) reported three types of changes as source of somaclonal variation, which are heritable changes, resulting from chromosome and gene mutations

Table 3. Total number and size range of amplified bands generated in directly and indirectly originated regenerants.

Sr. no.	Primer	Sequence 5'-3'	Scorable bands	Monomorphic bands in regenerants		Polymorphic bands in regenerants		Size range (bp)
				Directly originated	Indirectly riginated	Directly originated	Indirectly originated	
1	OPA-11	5'-CAATCGCCGT-3'	5	5	1	0	4	100-1000
2	OPA-19	5'-CAAACGTCGG-3'	2	2	2	0	0	300-700
3	OPA-20	5'-GTTGCGATCC-3'	3	3	2	0	1	100-600
4	OPB-11	5'-GTAGACCCGT-3'	3	2	1	1	2	300-1183
5	OPB-12	5'-CCTTGACGCA-3'	5	5	1	0	4	100-1000
		Total bands	18	17	7	1	11	
		Percentage of polymorphism				5.56%	61.2%	

and reversible changes that result from altered gene expression and nonheritable epigenetic changes, while nucleotide changes in priming sites, insertions and deletions are considered as source of RAPD polymorphism. Moreover, any changes including methylation, transposition and gene amplification, can also be the source of polymorphism in RAPD patterns of callus-mediated regenerants. Thus, callus-derived shoots may be avoided during transformation studies in apple, only direct regenerants may be considered as putative transformants.

In conclusion, we report here an efficient adventitious shoot regeneration for the first time in apple rootstock Merton793 where leaf explants demonstrated the direct, reproducible and high organogenetic potential with best shoot quality on 3-4 mg/l BA and 1 mg/l NAA supplemented medium, without dark incubation. Evaluation of genetic fidelity through RAPDanalysis indicates that direct adventitious shoot regeneration did not induce somaclonal variation thus a safe method which also recognizes the assessment of genetic uniformity an important pre- requisite in shoot regeneration before carrying out the genetic transformation experiments.

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