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ASSESSMENT OF APPLE ROOTSTOCKS M 9 AND M 26 FOR *IN VITRO* ROOTING POTENTIAL USING DIFFERENT CARBON SOURCES

MEHWISH YASEEN, TOUQEER AHMAD, NADEEM AKHTAR ABBASI[*]
AND ISHFAQ AHMED HAFIZ

Department of Horticulture,
Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.

Abstract

A study was accomplished to evaluate the effect of different carbon sources for the *In vitro* rooting of apple rootstocks M 9 and M 26. Significant differences were exhibited by carbon sources, apple rootstocks as well as by the interaction of these two factors. Among the various carbon sources tested, the best rooting response was obtained with 35 g l^{-1} sorbitol (T_9) both in terms of mean root number (5.0) and root length (3.84) while 45 g l^{-1} sorbitol (T_{10}) was the optimum concentration to work out the highest rooting percentage of 86.67%. Sucrose showed its propensity to stimulate the rooting of both genotypes but it was not much appealing in comparison to sorbitol. Quite unfair results were yielded by glucose followed by highly meager outcome, which was given by mannitol. Within rootstocks the most supercilious outcome was given by M 26 which gained a cut above M 9 regarding rooting percentage (44.17 %), root number (2.02) and root length (1.59 cm).

Introduction

Root formation is a difficult step in micropropagation of many woody plants (Custodio *et al*., 2004) and is regulated by a number of physiological, biochemical and genetic factors (Pawlicki & Welander, 1995). It is an important aspect for enhancing survival and growth during acclimatization and losses at this stage have considerable economic value from practical point of view (Ahmad *et al*., 2003; Custodio *et al*., 2004). Moreover, root initiation and growth are high energy requiring processes, entailing the availability of metabolic substrates, mainly carbohydrates (Custodio *et al*., 2004). Carbon sources also have a direct bearing on the frequency and quality of roots, as reported by Kumar *et al*., (1999). It is well established that carbohydrate requirements depend upon the stage of culture and may show differences according to the species (Thompson & Thorpe, 1987). Consequently, the quality of established plants for *In vivo* transfer can be improved by amending different types and concentrations of carbohydrates in the culture medium (Moncousin *et al*., 1992). Therefore, the present study was formulated to evaluate the effect of different carbon sources for *In vitro* rooting of Malling 9 (M 9) and Malling 26 (M 26) to achieve subsequent success during transfer to autotrophic conditions. These rootstocks of Malling series are good substitute to Crab apple for high economic returns. M 9 (dwarf) and M 26 (semi dwarf) are commercially recommended apple rootstocks due to their suitability in terms of dwarfness, high productivity, precocity and tolerance to biotic and abiotic stresses (Atkinson & Else, 2003).

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[*]Corresponding author E-mail: nadeemabbasi65@yahoo.com

Materials and Methods

Stock cultures of apple rootstocks M 9 and M 26 were maintained on MS (Murashige & Skoog, 1962) medium consisting of MS macro & micro elements and supplemented with MS vitamins, 1.5 mg l^{-1} BAP, 0.4 mg l^{-1} IAA, 6.5 g l^{-1} agar and 30 g l^{-1} sucrose. For rooting study, proliferated shoots about 2 cm in size from stock cultures of M 9 and M 26 were transferred to MS media (MS macro, micro elements and vitamins) supplemented with 1 mg l^{-1} IBA, 6.5 g l^{-1} agar and with different concentrations of carbon sources. Sucrose, sorbitol, mannitol and glucose were used @ 0, 5, 15, 25, 35 and 45 g l^{-1} to evaluate the effect of these carbon sources on root development. The pH of media was adjusted to 5.8 before autoclaving. It was a bifactorial experiment (Rootstocks × Carbon sources) randomized in CRD (Completely Randomized Design) with three replications per treatments and five shoots per replication. Data was recorded after four weeks on rooting % age, mean root number and root length (cm). Cultures were incubated at 25 ± 1˚C under 16-h light (2,000 lux) with white fluorescent tubes (Philips TL 40 W/54). Statistical analysis of the data was carried out by using analysis of variance (ANOVA) technique and means were compared by using Least Significance Difference (LSD) Test at 5% probability level (Steel *et al*., 1997).

Results and Discussion

Percentage of rooting (%): Data regarding rooting percentage is exhibited in Table 1 which reveals significant differences among carbon sources regarding their effect on rooting frequency at $p<0.05$. Biochemical, molecular, and genetic experiments have supported a central role of carbohydrates in the control of plant metabolism, growth and development (Sheen *et al*., 1999). Carbon sources are indispensable for rooting as these photosynthates are transported to meristematic cells in lower stem sections, where they regulate root initiation by a coordinated modulation of gene expression and enzyme activities in these carbohydrate-importing (sink) tissues. This ensures optimal synthesis and use of carbon and energy resources and also allows the availability of other nutrients including production of growth hormones (auxins), involved in rooting phenomenon (Grossman & Takahashi, 2001). Carbohydrate gradients in root developing regions have been reported to correlate spatially with mitotic activity/cell division and differentiation (Rolland *et al*., 2002). Data reveal that highest rooting percentage (96.67%) was worked out by sorbitol in M 26 at 45 g l^{-1}(T_{10}) which is quite discernible than the bearing of other carbon sources. Contrarily, in M 9 the optimum concentration of sorbitol was 35 g l^{-1}(T_9) to achieve maximum rooting of 86.67%. This good conduct of sorbitol in rooting percentage is probably due to the high mobility of boron in *Malus* which is otherwise immobile in higher plants and forms boron-sorbitol complexes only in sorbitol rich species (Brown & Hu, 1996). This supposition is in agreement with Weaver (1972) who reported that application of boron promotes root growth due to its role as one of the rooting cofactors. Moreover, sorbitol may influence the root initiation indirectly, as the HXK (Hexokinase) mediated signaling pathway of carbohydrates is connected not only to the ethylene pathway but also to the ABA pathway and ultimately the high endogenous ABA levels act as a signal for initiation of regulatory processes and results in increased root: shoot growth ratio (Creelman *et al*., 1990). Sucrose followed sorbitol with top score of 83.33% in M 26 and 76.67% in M 9 at 45 g l^{-1}(T_5). Weber *et al*., (1997) reports that high sucrose concentration is accompanied by biosynthesis of storage reserves i.e., starch which in turn is associated with meristmoid formation during root development

Table 1. Effect of different concentrations of carbon sources on rooting percentage (%) of apple rootstocks M 26 and M 9.

Treatments (Carbon sources g l^{-1})	**Rooting percentage (%)**		**Mean**
	M 26	**M 9**	
(Control) T_o (0)	0.00 p	0.00 p	0.00 J
Sucrose T_1 (5)	26.67 mno	20.00 o	23.33 I
T_2 (15)	40.00 ijk	30.00 lmn	35.00 G
T_3 (25)	56.67 ef	46.67 ghi	51.67 E
T_4 (35)	76.67 c	63.33 de	70.00 C
T_5 (45)	83.33 bc	76.67 c	80.00 B
Sorbitol T_6 (5)	36.67 jkl	30.00 lmn	33.33 GH
T_7 (15)	63.33 de	50.00 fgh	56.67 DE
T_8 (25)	80.00 bc	66.67 d	73.33 C
T_9 (35)	83.33 bc	86.67 b	85.00 AB
T_{10} (45)	96.67 a	76.67 c	86.67 A
Mannitol T_{11} (5)	0.00 p	0.00 p	0.00 J
T_{12} (15)	0.00 p	0.00 p	0.00 J
T_{13} (25)	0.00 p	0.00 p	0.00 J
T_{14} (35)	0.00 p	0.00 p	0.00 J
T_{15} (45)	0.00 p	0.00 p	0.00 J
Glucose T_{16} (5)	33.33 klm	23.33 no	28.33 HI
T_{17} (15)	40.00 ijk	26.67 mno	33.33 GH
T_{18} (25)	46.67 ghi	40.00 ijk	43.33 F
T_{19} (35)	63.33 de	53.33 fg	58.33 D
T_{20} (45)	74.00 c	69.67 de	71.67 C
Mean	**44.17 A**	**36.87 B**	

$LSD_{0.05}$, Varieties = 1.55, Interaction (V×T) = 7.08, Treatments = 5.01

Any two means not sharing a letter differ significantly at $p<0.05$

phenomenon (Thorpe & Meier, 1972). Root primordia formation is a high-energy requiring process and starch serves to act as a readily available reserve source of energy by continuing supplying free sugars for “glycolysis” and “pentose phosphate pathway” in sink tissues, related with high respiration rates (Thorpe, 2004). As mannitol did not yield roots in any of the cultured shoots, therefore rooting percentage is naught for M 9 and M 26 at all its concentrations. Vitova *et al*., (2002) articulate that mannitol is a powerful osmoprotectant; hence, it is proposed that mannitol presence in the medium means substantial lowering of medium osmotic potential leading to down regulation of its degradation and utilization which consequently results in its accumulation within plant tissues. Therefore, mannitol cannot contribute in developmental process as a carbon and energy source. Further, glucose was not much fascinating in interaction with both rootstocks and resulted in an outcome of maximum 74.00% in M 26 at 45 g l^{-1}(T_{20}) while 69.67% in M 9 at the same concentration.

Among treatments 45 g l^{-1} sorbitol (T_{10}) was outstanding with a superb rooting of 86.67%. In addition, an outstanding outcome of 80.0 and 85.0% was obtained at 45 g l^{-1}sucrose (T_5) and 35 g l^{-1} sorbitol (T_9), which were also superior statistically. Results yielded by sucrose and sorbitol are rationally similar certainly because of their equivalent roles in terms of quantity of translocated carbon (Moing *et al*., 1992). Furthermore, according to Hilae & Te Chato (2005) phenolic compounds are accumulated in media at higher concentration of sorbitol and sucrose, and there is also evidence that phenolic compounds interact with auxin to induce root initiation (Tomaszewski, 1964). Similar to sorbitol and sucrose, glucose treatments too gave the utmost rooting percentage (71.67%) at 45 g l^{-1} (T_{20}). Hence, it is evident that percentage of rooting has direct relation with carbon source concentration, being higher at increased concentration of sucrose, sorbitol and glucose. This direct relation between carbon source concentration and rooting percentage imply that rooting phenomenon is regulated by carbohydrates to a great extent, to provide sufficient energy for stimulation of cambial activity and ultimate root primordia formation (Pawlicki & Welander, 1995). Another evidence for the positive influence of carbon sources on root initiation is provided by Van Overbeek *et al*., (1946) who stated that carbohydrates produced in leaves are rooting cofactors which in combination with auxins enable cuttings to root. Therefore, by exogenously applying carbon sources promotory effect of leaves can be replaced.

With reference to percentage of rooting M 26 proved itself propitious with a consequence of 44.17% while M 9 gave the mere rooting percentage of 36.87%. A discrepancy in rooting response, between M 9 and M 26 was also documented by Lane & McDougald (1982) who reported M 9 to be substandard in rooting than M 26 and M 27 and explicate that although these rootstocks are members of same genus *Malus* but there are some possible reasons for different response of genetically related cultivars. Some of those factors are; differential rate of nutrient uptake from medium, efficiency of transport through cultures and metabolism of media components.

Number of roots per explant: Results in Table 2 show that root formation crop up in the apple shoots at various frequencies according to the type and concentration of carbon source used. It is also evident that there are significant differences among carbohydrates at $p<0.05$ in terms of their interaction with apple rootstocks. When no carbon source was added to the rooting media (T_o), stems of both genotypes M 9 and M 26 remained green throughout the culture period, but did not form any roots. This response of complete root inhibition is probably due to limited activity in the cambium as described by Pawlicki & Welander (1995) after anatomical study of stem sections of apple rootstock Jork 9. Eventually, these scientists report that a continuous supply of carbohydrates from the medium is necessary for normal root primordia formation and root development. Khateeb (1999) also stated that media devoid of carbon sources did not produce any roots in date palm indicating the importance of carbohydrates in root formation particularly for the energy supply and/or for the indirect activation of some genes during the rooting process. It is also evident from data that all carbon sources did not sustain rooting equally. With increasing concentration of all the carbon sources number of roots per shoot increased for both M 9 and M 26. However, the optimum concentration of carbohydrates varied for the two genotypes. Sorbitol proved to be an ideal carbon source to produce more number of roots (6.01) in M 26 at 35 g l^{-1} (T_9). Rooted shoots on sorbitol medium were relatively healthier, with comparatively large sized callus and quite thick roots in diameter (Fig. 1a) than other treatments. Pawlicki & Welander (1995) stated that the presence of callus on the stem discs increased the number of roots formed. This statement provides a confirmation of the present results where the development of large callus with sorbitol greatly increased the root number in M 26. Sorbitol contributes in morphogenesis of apple both nutritionally and osmotically as in apple phloem it is found to comprise 65-70% of the total carbon forms (McQueen & Minchin, 2005). Hence, it is effectively utilized as an energy source in apple (Pua & Chong, 1984). In M 9 the same concentration of 35 g l^{-1} (T_9) resulted in utmost root number of 4.00 which is somewhat

Table 2. Effect of different concentrations of carbon sources on number of roots per explant of apple rootstocks M 9 and M 26.

Treatments (Carbon sources g l^{-1})	Number of roots per explant		Mean
	M 26	M 9	
(Control) T_o	0.00 r	0.00 r	0.00 L
Sucrose T_1 (5)	0.47 p	0.20 q	0.33 K
T_2 (15)	1.7 m	0.9 o	1.30 J
T_3 (25)	2.37 j	2.83 h	2.60 H
T_4 (35)	4.90 b	4.57 c	4.73 B
T_5 (45)	3.47 f	3.03 g	3.25 F
Sorbitol T_6 (5)	2.00 kl	1.47 n	1.73 I
T_7 (15)	4.03 e	2.13 k	3.08 G
T_8 (25)	4.56 c	2.60 i	3.58 E
T_9 (35)	6.01 a	4.00 e	5.00 A
T_{10} (45)	4.93 b	3.13 g	4.03 C
Mannitol T_{11} (5)	0.0 r	0.0 r	0.0 L
T_{12} (15)	0.0 r	0.0 r	0.0 L
T_{13} (25)	0.0 r	0.0 r	0.0 L
T_{14} (35)	0.0 r	0.0 r	0.0 L
T_{15} (45)	0.0 r	0.0 r	0.0 L
Glucose T_{16} (5)	0.20 q	0.30 q	0.25 K
T_{17} (15)	0.53 p	1.97 l	1.25 J
T_{18} (25)	0.87 o	2.56 i	1.72 I
T_{19} (35)	4.27 d	3.13 g	3.70 D
T_{20} (45)	3.50 f	2.83 h	3.16 FG
Mean	**2.07 A**	**1.71 B**	

LSD $_{0.05}$, Varieties = 0.32, Interaction (V×T) = 0.145, Treatments = 0.103

Any two means not sharing a letter differ significantly at $p<0.05$

reduced than M 26 (Fig. 1b). These results regarding the effect of sorbitol on rooting of M 9 and M 26 imply that although sorbitol is the major photosynthetic product in *Malus*, but capability to utilize it efficiently for growth and development is variable within species of this genus. Furthermore, there is probably a change in the carbohydrate metabolism in M 9 during the process of root initiation, responsible for slightly stumpy rooting in this rootstock. This assumption is supported by Pawlicki & Welander (1995) who report that a spontaneous change in carbohydrate metabolism during rooting phase which is associated with the growth regulator pool; can be an explanation for reduced rooting with sorbitol. Sucrose was mutually found constructive for root formation as sorbitol for M 9 and M 26. Data recorded showed that optimum sucrose concentration was 35 g l^{-1} (T_4) for both rootstocks which resulted in maximum root number of 4.57 and 4.90 corresponding to M 9 and M 26 (Fig. 2a, b). Beneficial effects of sucrose on rooting have also been demonstrated by Romano *et al*., (1995) and De Klerk & Calamar (2002) for cork oak and apple. Kumar *et al*., (1999) detected high levels of endogenous IAA and polyamines in shoot cultures of *Gladiolus hybridus* grown on sucrose media, both of which are reported to be involved in the process of adventitious root formation (Kumar *et al*., 1999). Moreover, according to Weaver (1972) auxins lead to the development of “root initials” by the stimulation of cell division in the meristmatic cells at the base of stems, which further develop into recognizable root primordia. Hence, sucrose might be responsible to promote rooting in this study due to the production of these substances i.e., IAA and polyamines. It is important to mention here that in sucrose treatments there was development of root hairs, quite visible and large in number i.e., aerobic roots which were not observed with sorbitol (Fig. 3). It is a positive aspect from practical point of view during acclimatization. Mannitol did not cause rooting at any concentration; however, there was development of very small sized callus (Fig. 4a, b). It was in accordance with the results of Kumar *et al*., (1999) who also reported complete inhibition of rooting in gladiolus with mannitol**.** Inability of the apple shoots to form roots on mannitol rooting media can be attributed to the absence of NAD-dependent MDH (mannitol 1-oxidoreductase); an enzyme responsible for utilization of mannitol in the sink tissues (Stoop & Pharr, 1993). Pawlicki & Walender (1995) also demonstrated that mannitol can be taken up by the apple cells but not metabolized. Stoop and Pharr (1993) further clarify it, that ability of the cultured cells to grow on mannitol is restricted to species that form and translocate this polyol to sinks where its utilization may occur by the MDH. Glucose was least effective in terms of rooting frequency of both M 9 and M 26 in comparison to sucrose and sorbitol. It resulted in an acquisition of 3.13 and 4.27 roots per explant in M 9 and M 26 correspondingly at 35 g l^{-1} (T_{19}). Nevertheless, it is reported as an efficient carbon source for some other species viz., *Ficus lyrata* (Custodio *et al*., 2004), *Alnus* spp., (Tremblay & Lalonde, 1984) and *Quercus suber* (Romano *et al*., 1995). Blanc *et al*., (1999) depicts that glucose and fructose are six carbon (6-C) sugars; hence media containing these carbohydrates contains half as many hexose equivalents as the media containing sucrose (disaccharide) which can be one of the reasons, responsible for intimidating outcome with glucose. It was noticed that at low concentration of 5 g l^{-1}glucose (T_{16}), roots formed were very thin, fragile and devoid of root hairs (Fig. 5).

Treatments followed an ascending order for root number up to 35 g l^{-1} rise in the concentration of carbon source. Among treatments sorbitol capitulates with an alluring root number of 5.00 at 35 g l^{-1} (T_9). From Bianco & Rieger (2002) stand point, preference of sorbitol over sucrose in *Rosaceae* could be due in part to the fact that about half of the weight of the 6-C sorbitol is needed to generate an osmotic potential equal to that generated by the 12-C sucrose. These authors further explicate that with respect to their function as osmolytes, sorbitol ties up less carbon per unit osmotic potential decrease than an equimolar concentration of sucrose. It was established that statistically sucrose was second rate in terms of root number but in general it produced considerable good results with an outcome of 4.73 at 35 g l^{-1} (T_4). Propensity of sucrose to facilitate rooting is most certainly due to the accumulation of reducing carbohydrates (fructose and glucose) at the base of stem sections (Kumar *et al*., 1999). These reducing carbohydrates are produced from sucrose cleavage, by invertases (both cell wall and vacuolar invertases) and sucrose synthetase (SS), and are known to stimulate rooting and callusing (Pua & Chong, 1984; Kumar *et al*., 1999; Ahmad *et al*., 2007). Glucose treatments were inferior to sucrose and sorbitol from statistical point of view with highest root number of 3.70 at the same concentration of 35 g l^{-1} (T_{19}). Low rooting frequency with glucose refers to an initial amount of carbon that was insufficient for stronger growth (Blanc *et al*., 1999). Averaged across all the treatment maximum root number was obtained at 35 g l^{-1}. Best rooting response at 35 g l^{-1} of sucrose, sorbitol and glucose do not confirm the concept that the reduction in sugar content improves rooting as described by Kooi *et al*., (1999). On the other hand poor results with regards to rooting at low concentration of 5 g l^{-1} of these carbohydrates are supported by Mc Cown (1998) who stated that *In vitro* root formation did not occur when photosynthetic products were supplied in insufficient quantities.

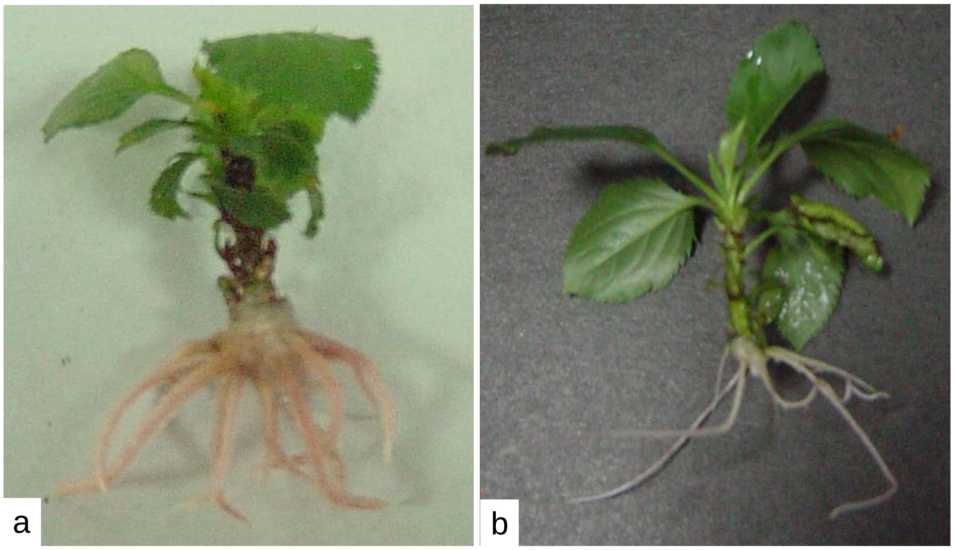

Fig. 1. (a) 35g l^{-1} sorbitol (T_9) resulting in comparatively highest root number in M 26 with large sized callus and quite thick roots. (b) Relatively reduced root number in M 9 at 35g l^{-1} sorbitol (T_9).

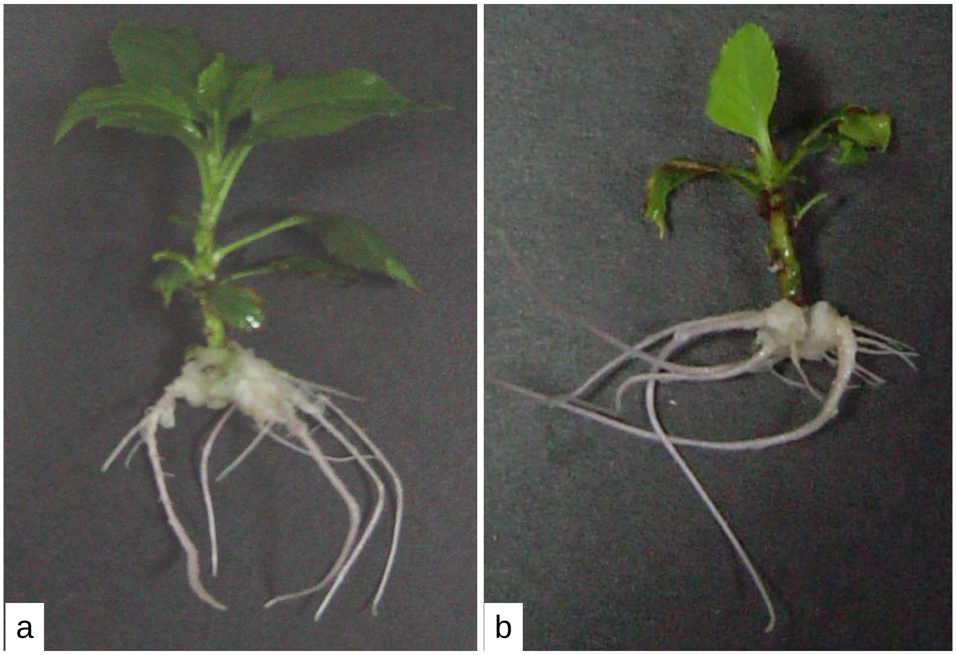

Fig. 2. Results for interaction of sucrose at 35 g l^{-1} (T_4) with (a) M 26 and (b) M 9.

Fig. 3. Aerobic roots (development of root hairs) in M 26 at 35 g l^{-1}sucrose (T_4).

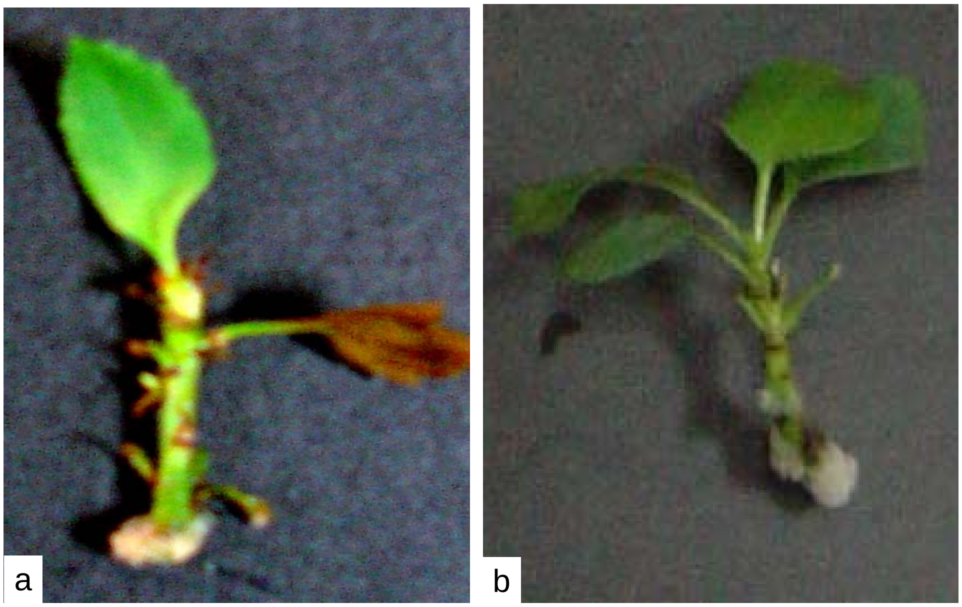

Fig. 4. Development of very small sized callus in (a) M 9 and (b) M 26 with complete inhibition of root formation in mannitol rooting media.

Fig. 5. Development of thin and fragile roots in M 26 at low concentration of 5 g l^{-1} glucose (T $_{16}$).

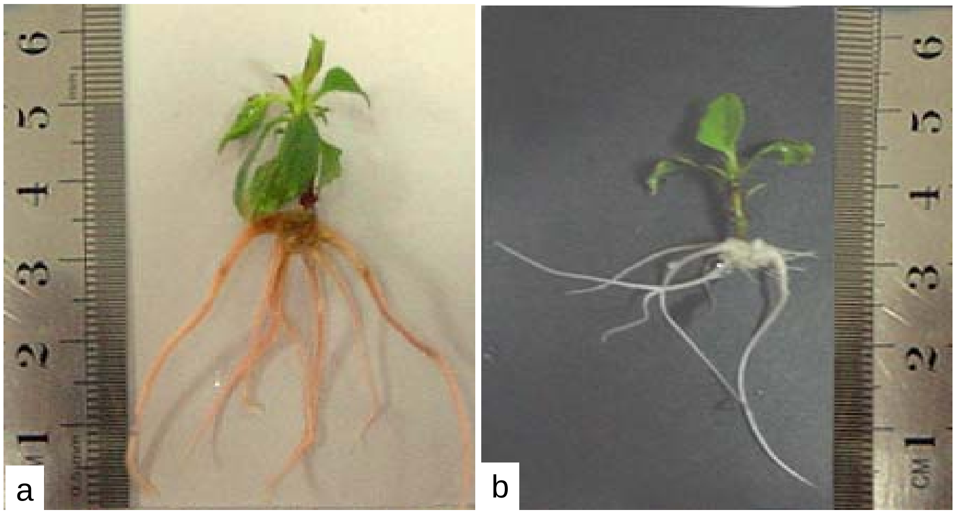

Fig. 6. Formation of longest roots in (a) M 26 and (b) M 9 at 35g l^{-1} sorbitol (T $_{9}$).

It can be concluded that rooting potential is highly genotypic dependent feature and M 26 is significantly (p<0.05) superior to M 9 in terms of rooting frequency with an average root number of 2.07 in contrast to 1.71 for M 9. This demonstration is supported by Zimmerman (1983), who accounts that different apple genotypes are known to respond differently to the same medium during establishment, proliferation and rooting *In vitro*. It is also confirmed by Alvarez *et al*., (1989) who depicts that differences between M 26 and M 9 *In vitro* rooting response may be related to differences in free IAA levels in basal sections. These differences in free IAA levels between M 26 and M 9 basal sections may reflect differences in IBA metabolism and/or IAA conjugation.

Table 3. Effect of different concentrations of carbon sources on root length (cm) of apple rootstocks M 26 and M 9.

Treatments (Carbon sources g l^{-1})	**Root length (cm)**		**Mean**
	M 26	**M 9**	
(Control) T_o	0.0 p	0.0 p	0.00 J
Sucrose T_1 (5)	0.33 o	0.56 mn	0.45 I
T_2 (15)	0.90 l	0.73 lm	0.82 G
T_3 (25)	2.23 hi	1.35 k	1.79 E
T_4 (35)	2.37 gh	2.35 gh	2.36 D
T_5 (45)	4.01 b	3.04 c	3.52 B
Sorbitol T_6 (5)	1.24 k	1.25 k	1.25 F
T_7 (15)	2.92 cd	2.03 J	2.48 D
T_8 (25)	3.12 c	2.60 ef	2.86 C
T_9 (35)	4.09 a	3.58 b	3.84 A
T_{10} (45)	2.59 ef	2.92 cd	2.76 C
Mannitol T_{11} (5)	0.0 p	0.0 p	0.0 J
T_{12} (15)	0.0 p	0.0 p	0.0 J
T_{13} (25)	0.0 p	0.0 p	0.0 J
T_{14} (35)	0.0 p	0.0 p	0.0 J
T_{15} (45)	0.0 p	0.0 p	0.0 J
Glucose T_{16} (5)	0.80 l	0.40 no	0.60 H
T_{17} (15)	0.90 l	0.73 lm	0.82 G
T_{18} (25)	2.52 fg	1.25 k	1.89 E
T_{19} (35)	2.79 de	2.09 ij	2.44 D
T_{20} (45)	2.66 ef	2.32 gh	2.49 D
Mean	**1.59 A**	**1.24 B**	

LSD $_{0.05}$, Varieties = 0.04, Interaction (V×T) = 0.19, Treatments = 0.14

Any two means not sharing a letter differ significantly at $p<0.05$

Root length (cm): Data with regards to root length of M 9 and M 26 is presented in Table 3. A significant interaction ($p<0.05$) was observed between these apple rootstocks and carbon sources which lead to variable responses at different concentrations. Khateeb (1999) working with date palm (*Phoenix dactylifera* L.) cv. Khanezi also reported that carbohydrate types, concentrations and their interactions had significant effects on root elongation. Results authenticate that proliferated shoots of apple rootstocks M 9 and M 26 are able to utilize sorbitol more efficiently than sucrose, glucose and mannitol. Sorbitol at 35 g l^{-1} (T_9) gained a cut above other carbon sources and their concentrations for M 26 which scored an eminent root length of 4.09 cm while M 9 achieved 3.58 cm root length at the same concentration (Fig. 6a, b). This distinguished outcome might be referred to an increase in the reducing/phosphorylated carbohydrate (glucose and fructose) content in the basal portion of proliferated shoots with sorbitol as compared to sucrose which react non enzymatically with nuclear proteins and cause modifications in about 10% of the proteins (Kumar *et al*., 1999). It is therefore possible that this beneficial aspect of reducing sugars is responsible for shifting the morphogenic pathway in tissues (Kumar *et al*., 1999; Ahmad *et al*., 2007). Sucrose provided a relatively prominent response at 45 g l^{-1} (T_5) that is not much different from sorbitol but it bears good results at higher concentration than sorbitol. Sucrose at this concentration generated an average root length of 3.04 and 4.01 cm in M 9 and M 26 respectively. Fair root length, developed with sucrose might be due to its positive role in cell expansion. Carpita & Vergara (1998) reported that cellulose is a component of cell wall and reduction in the amount of incorporated cellulose in cell wall, resulting from a drop in SS activity with maturity and consequent decrease in UDP-glucose (Uridine diphosphate glucose) availability, ultimately enhance the cell expansion (Bianco & Rieger, 2002), where UDP-glucose, a nucleotide sugar; is a direct precursor of cellulose. Mannitol had much impecunious outcome in terms of rooting response as no roots were formed in this rooting media both for M 9 as well as for M 26. De Neto & Otoni (2003) stated that mannitol yields poor results probably because it is an osmotically active solute and is inert from morphological point of view. Interaction of glucose was not much appealing with both genotypes and resulted in very ordinary outcome with maximum root length of 2.79 cm in M 26 (Fig. 7) at 35 g l^{-1} (T_{19}) while M 9 gained maximum length of 2.32 cm at 45 g l^{-1} (T_{20}).

As far as treatments are concerned, 35 g l^{-1} sorbitol (T_9) was dominant to other treatments and resulted in an exceptional root length of 3.84 cm. Likewise, at the same concentration sorbitol also resulted in highest root number. Sucrose appeared to be good at 45 g l^{-1} (T_5) and bears out 3.52 cm root length. This observation that both sorbitol and sucrose yielded better results in apple rootstocks side by side might be rationalized by the statement of Moing *et al*., (1992). He accounts that both sorbitol and sucrose are synthesized in the leaves of *Rosaceae*. Furthermore, synthesis of these two assimilates is correlated with each other as glucose-6-P, which is an activator of sucrose phosphate synthetase, is also a substrate of aldose-6-P reductase i.e., the precursor for sorbitol. In this study glucose did not have a positive influence on rooting response and gave quite short length of maximum 2.49 cm at 45 g l^{-1} (T_{20}). Poor root length at low concentration of sugars particularly at 5 g l^{-1} of all the carbohydrates is due to the unavailability of sufficient energy to carry out metabolic processes (De Klerk & Calamar, 2002).

Response of M 26 pertaining to root length was better similar to the number of roots and acquired upshot of 1.59 cm root length in comparison to M 9 (1.24 cm). The present results lead to the assumption that apple genotypes M 9 and M 26 are highly selective in their carbohydrate requirements and variation in the concentration of most suitable carbon source radically confine the swiftness of morphogenic process.

Fig. 7. Exhibiting root length development with glucose in M 26 at 35 g l^{-1} (T $_{19}$).

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