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IN VITRO SHOOT PROLIFERATION COMPETENCE OF APPLE ROOTSTOCKS M. 9 AND M. 26 ON DIFFERENT CARBON SOURCES

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Abstract

Competence of two apple rootstocks M. 9 and M. 26 for *in vitro* shoot proliferation was appraised using a miscellany of carbon sources i.e., sorbitol, sucrose, glucose and mannitol which were employed @ 0, 5, 15, 25, 35 and 45 g l^{-1}. The most auspicious outcome was achieved by sorbitol @ 35 g l^{-1} (T_9) being the optimal carbon source for both the genotypes. M. 26 had a positive interaction with sorbitol at this concentration to produce the best caulogenic response in terms of a paramount shoot length (3.01 cm) and an overriding fresh weight increment (402 mg) whereas M. 9 at the same concentration gave an eminent shoot number (9.8). Sucrose and glucose also had a positive carryover effect on apple shoots to some extent but proved to be inferior to sorbitol. Results yielded by mannitol were highly indigent in comparison to other carbon sources. Rootstocks exhibited an inconsistency regarding their aptitude for shoot proliferation. M. 26 was recognized as a better rootstock with an acquisition of 1.05 cm shoot length and 154.6 mg fresh weight while M. 9 stood better with maximum shoot number of 2.3.

Introduction

Efficient micropropagation depends on rapid, extensive and uniform shoot proliferation (Chen & Ziv, 2003). Growth and multiplication of shoots under *in vitro* conditions depend upon a number of factors (Haque *et al.*, 2003) one of which is the type and concentration of exogenously supplied carbon sources in the medium (De Neto & Otoni, 2003). Carbohydrates partly exert their effect on growth and morphogenesis by their nutritional value, and partly through their varying osmotic potential, which influences the rate of cell division or the degree of morphogenesis of the cells (Sotiropoulos *et al*., 2006). In addition, carbon sources perform function in synthetic pathway of many compounds, build blocks of macromolecules and may control several developmental processes in the cell (Karami *et al*., 2006). Hence, carbohydrates are of prime importance for *In vitro* shoot proliferation, a high energy requiring process (Thorpe, 1980; Jain & Babbar, 2003). Being most common carbohydrate in the phloem sap of many plants (Ahmad *et al*., 2007) and due to its cheap and easy availability, sucrose is often assumed to be the sugar of choice in cell and tissue culture media (Jain & Babbar, 2003; Faria *et al*., 2004). However, it is not always the best carbohydrate to achieve shoot proliferation (Blanc *et al*., 1999) because a number of carbon sources besides sucrose are also translocated in plants (Moing *et al*., 1992). Therefore, present study was conducted to determine the comparative influence of different carbon sources for *In vitro* shoot proliferation of apple rootstocks M. 9 and M. 26. 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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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. To compare the influence of different carbon sources on shoot proliferation potential of these rootstocks, uniform sized shoot tips (25 mm) were transferred to above mentioned media with different concentrations of sucrose, sorbitol, mannitol and glucose @ 0, 5, 15, 25, 35 and 45 g l^{-1}. The pH of media was adjusted to 5.8 before autoclaving at 121°C for 15 min. It was a bifactorial experiment (Rootstocks × Carbon sources) randomized in CRD (Completely Randomized Design) with 3 replications per treatments and 5 shoots per replication. Data was recorded after 4 weeks on total number of shoots per proliferating explant, shoot length (cm) and fresh weight (mg) of shoots as well. 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

Number of shoots per explant: The data *vis-à-vis* number of shoots per explant indicate significant interaction between carbon sources and apple rootstocks at $p<0.05$ (Table 1). The best caulogenic response was afforded by sorbitol among the various carbohydrates tested. Apple rootstock M. 9 produced the highest number of shoots (9.8) with 35 g l^{-1} sorbitol (T_9) while M. 26 produced the highest shoot number (4.9) with 45 g l^{-1} sorbitol (T_{10}) as compared to other carbon sources (Figs. 1 & 2). M. 26 showed ascending trend in shoot number with increasing sorbitol concentration while M. 9 displayed a sudden decrease in shoot number from 9.8 to 2.6 with increasing concentration from 35 g l^{-1} to 45 g l^{-1}. The promotive influence of sorbitol on growth and proliferation of apple shoots, could be ascribed to the fact that apple plants produce sorbitol as a primary photosynthetic product; translocate it *via* the phloem and metabolize it in the sinks (Moing *et al*., 1992). Similar findings were observed by Kadota *et al*., (2001) for Japanese Pear, which gave the highest shoot number with sorbitol. Ahmad *et al*., (2007) also reported the highest shoot number per proliferated explant for Peach rootstock GF 677 with sorbitol. This preeminent response with sorbitol may be associated with the availability of one or more enzymes. These enzymes are sorbitol dehydrogenase (SDH), sorbitol-6-phosphate dehydrogenase and sorbitol oxidase, responsible for the metabolism and assimilation of sorbitol in the sink tissues (Moing *et al*., 1992; Swedlund & Locy, 1993; Ahmad *et al*., 2007). Stoop & Pharr (1993) have also confirmed the activity of these enzymes in sorbitol translocating plants. Sucrose at 35 g l^{-1} (T_4) also had a positive interaction with both rootstocks. The highest shoot number obtained with this treatment was 4.0 and 7.9 for M. 26 and M. 9 (Fig. 3) respectively. This response towards sucrose may be related to its hydrolysis, which leads to an increase in the endogenous content of glucose and fructose of cultured tissues. These reducing sugars ultimately increase the osmotic potential and positively influence the organogenesis (Khuri & Moorby, 1995; Lipavska & Konradova, 2004; Debnath, 2005, Ahmad *et al*., 2007). The availability of invertase enzyme required for the efficient conversion of sucrose into glucose and fructose is less in sorbitol translocating plants (Ahmad *et al*., 2007). However, according to Thorpe (1980), a shift in metabolism can lead to the synthesis of new enzymes originally absent, or enzymes present (invertases) show increased synthesis. Highly meager results were given up by mannitol at all the concentrations (Fig. 4). Mannitol is

Table 1. Effect of different concentrations of carbon sources on number of shoots in apple rootstocks M. 26 and M. 9.

Treatments (Carbon sources g l^{-1})		Number of shoots per explant		Mean
		M. 26	M. 9	
(Control)	T_0	0.0 s	0.0 s	0.0N
Sucrose	T_1 (5)	0.52 q	1.1 no	0.8L
	T_2 (15)	1.2 n	1.6 l	1.4J
	T_3 (25)	2.0 k	2.6 hi	2.3G
	T_4 (35)	4.0 d	7.9 b	5.9B
	T_5 (45)	2.6 hi	4.0 d	3.3D
Sorbitol	T_6 (5)	0.9 no	1.4 m	1.2K
	T_7 (15)	1.7 l	2.5 hi	2.0H
	T_8 (25)	2.4 i	3.0 f	2.7F
	T_9 (35)	4.7 c	9.8 a	7.4A
	T_{10} (45)	4.9 c	2.6 g	3.8C
Mannitol	T_{11} (5)	0.0 s	0.0 s	0.0N
	T_{12} (15)	0.2 r	0.0 s	0.1N
	T_{13} (25)	0.0 s	0.0 s	0.0N
	T_{14} (35)	0.0 s	0.0 s	0.0N
	T_{15} (45)	0.0 s	0.0 s	0.0N
Glucose	T_{16} (5)	0.0 s	1.0 o	0.5M
	T_{17} (15)	0.7 p	1.1 no	0.8L
	T_{18} (25)	2.1 k	2.3 j	2.0H
	T_{19} (35)	2.6 h	3.4 e	3.0E
	T_{20} (45)	1.6 l	2.1 k	1.8I
Mean		**1.5B**	**2.3A**	

$LSD_{5\%}$ Varieties = 0.03, Interaction (V×T) = 0.16, Treatments = 0.12
Any two means not sharing a letter differ significantly at $p<0.05$

an osmotically active solute (George, 1993) and according to Vitova *et al*., (2002), it creates an osmotic stress which strongly inhibits the plant cell, tissue and organ growth mainly by impairing the gain of photoassimilates e.g. by inducing stomata closure or lowering the activity of photosynthetic enzymes. Moreover, poor growth responses with mannitol might be due to its accumulation in plant tissues and inability of the apple shoots to metabolize it (De Nato & Otoni, 2003) probably due to unavailability of mannitol dehydrogenase (MDH), present only within mannitol translocating plants e.g. celery (Stoop & Pharr, 1993). Glucose was poor as compared to sorbitol and sucrose with maximum shoot number of 3.4 in M. 9 and 2.6 in M. 26. Lower concentrations of sucrose and sorbitol yielded very deprived results particularly at 5 and 15 g l^{-1}. Distorted stems with symptoms of necrosis and vitrification, characterized by water soaked, translucent and brittle stems, thick and curly leaves and stunted growth were produced at these concentrations (Fig. 5a & b, 6a & b, 7a & b and 8a & b). Similar to this outcome, low concentration of carbohydrates proved to be depressing in *Prunus* (Morini *et al*., 1992). No shoot proliferation was observed in media without carbon source as cultured tissues are completely heterotrophic and need a continuous supply of an exogenous energy source for morphogenesis (Debnath, 2005).

Fig. 1. High proliferation rate of M 9 with 35g l^{-1} sorbitol (T_9).

Fig. 2. Good shoot proliferation rate of M 26 with 45g l^{-1} sorbitol (T_{10}).

Fig. 3. Good rate of shoot proliferation in M 9 with 35 g l^{-1} Sucrose (T_4).

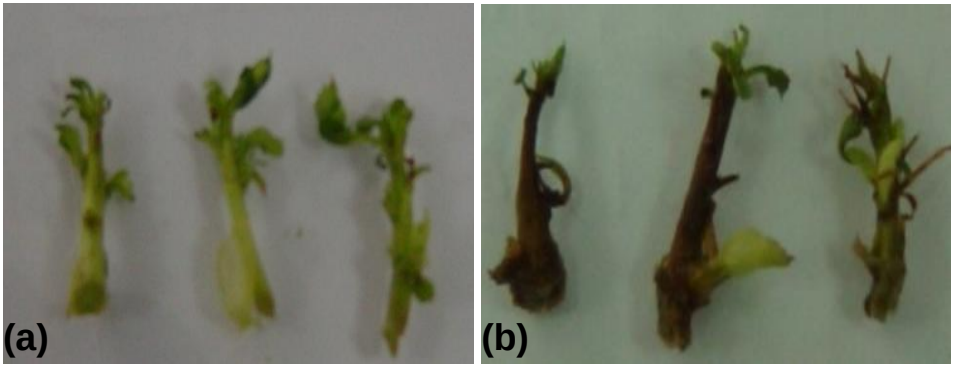

Fig. 4. Deprived results for shoot proliferation in (a) M 26 and (b) M 9 with mannitol.

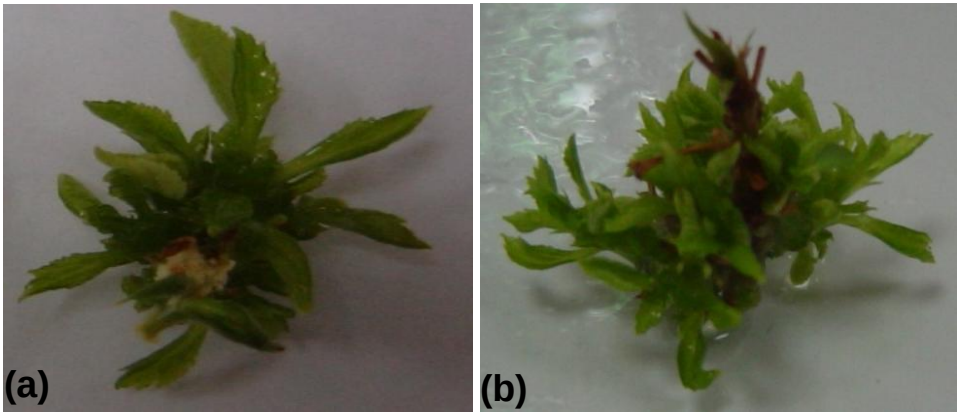

Fig. 5. Symptoms of vitrification in (a) M 9 and (b) M 26 with 5 g l^{-1} sorbitol (T_6).

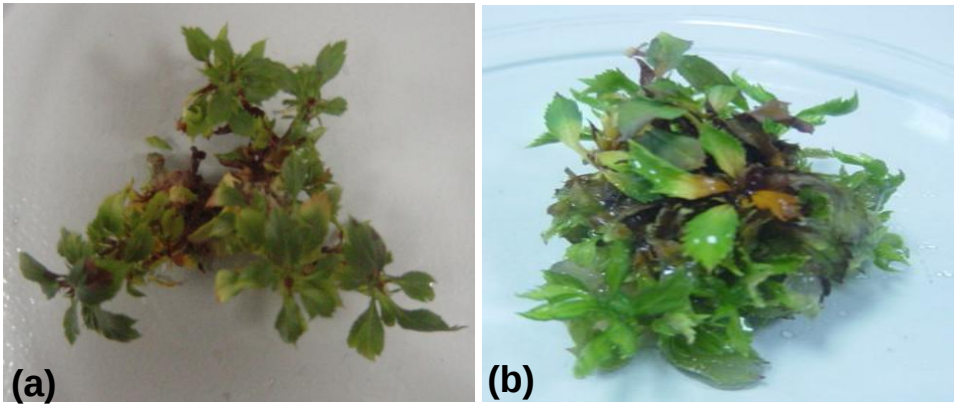

Fig. 6. Necrosis in (a) M 9 and (b) M 26 with 15 g l^{-1}sorbitol (T_7).

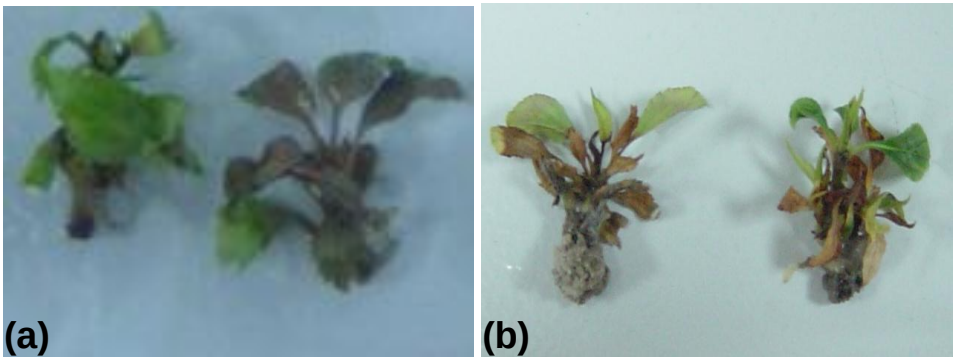

Fig. 7. Stunted growth with symptoms of necrosis in (a) M 26 and (b) M 9 with 5 g l^{-1} sucrose (T_1).

Fig. 8. An abysmal outcome in (a) M 26 and (b) M 9 with 15 g l^{-1} sucrose (T_2).

A gaze at treatments proves that 35 g l^{-1} sorbitol (T_9) was paramount in terms of shoot number (7.4) followed by 35 g l^{-1}sucrose (T_4) with shoot number of 5.9. The assenting response of sorbitol confirms that apple rootstocks are able to utilize sorbitol in a better way as it is highly exportable within the plants (Moing *et al*., 1992) and readily metabolized with the sufficient availability of enzymes that help in the hydrolysis of sorbitol (Ahmad *et al*., 2007). T_4 (35 g l^{-1} sucrose) implies that sucrose may facilitate growth and development due to its impact on the adjustment of cell osmolarity as reported by Khuri & Moorby (1995). There was a sudden decrease in shoot number with higher concentration of 45 g l^{-1} of sucrose, sorbitol and glucose. It might be related to a decline in osmotic potential associated with higher concentration of carbohydrates as narrated by Jain & Babbar (2003). This observation was also consistent with the findings of Kadota *et al*., (2001) who found high concentrations to be detrimental in Pear. The differential morphogenic response by the plants to different carbohydrates could be probably due to their differential role in vascular differentiation, differences in the endogenous content of reducing sugars (glucose and fructose) in cultured tissues and differential sensitivity of the tissues to the breakdown products such as furfural and hydroxyl furfurol (Romano *et al*., 1995; Jain & Babbar, 2003).

Table 2. Effect of different concentrations of carbon sources on shoot length (cm) of apple rootstocks M. 26 and M. 9.

Treatments (Carbon sources g l^{-1})		Shoot length (cm)		Mean
		M. 26	M. 9	
(Control)	T_0	0.00 r	0.00 r	0.0 O
Sucrose	T_1 (5)	0.83 lm	0.58 o	0.70 K
	T_2 (15)	1.02 j	0.70 n	0.85 J
	T_3 (25)	1.55 f	1.0 ij	1.28 FG
	T_4 (35)	1.95 d	0.94 jk	1.38 CD
	T_5 (45)	1.69 e	0.80 m	1.31 EF
Sorbitol	T_6 (5)	0.26 q	0.41 p	0.34 M
	T_7 (15)	1.38 g	0.96 jk	1.18 H
	T_8 (25)	1.41 g	1.26 h	1.34 DEF
	T_9 (35)	3.01 a	2.41 b	2.72 A
	T_{10} (45)	2.06 c	0.70 n	1.42 BC
Mannitol	T_{11} (5)	0.00 r	0.00 r	0.00 O
	T_{12} (15)	0.48 p	0.00 r	0.24 N
	T_{13} (25)	0.00 r	0.00 r	0.00 O
	T_{14} (35)	0.00 r	0.00 r	0.00 O
	T_{15} (45)	0.00 r	0.00 r	0.00 O
Glucose	T_{16} (5)	0.00 r	0.90 kl	0.45 L
	T_{17} (15)	1.00 j	0.87 lm	0.94 I
	T_{18} (25)	1.29 h	1.17 i	1.23 G
	T_{19} (35)	1.38 g	1.30 h	1.34 DE
	T_{20} (45)	2.14 c	0.80 m	1.43 B
Mean		**1.05A**	**0.78B**	

$LSD_{5\%}$ Varieties = 0.015, Interaction (V×T) = 0.07, Treatments = 0.05
Any two means not sharing a letter differ significantly at $p<0.05$

Apple rootstock M. 9 is far much superior to M. 26 regarding shoot proliferation potential with mean shoot number of 2.3 as compared to 1.5 for M. 26 (Table 1). The discrepancy in the response of apple rootstocks M. 9 and M. 26 in terms of optimum concentration of carbohydrates indicates a genotypic effect as reported by Sotiropoulos *et al*., (2006) that the ability to utilize carbon source is variety dependent.

Shoot length (cm): Results indicate that carbon sources and their different concentrations interacted significantly with both the apple rootstocks M. 9 and M. 26 at $p<0.05$ in terms of shoot length achievement (Table 2). The most superior interaction was observed in sorbitol treatments with M. 26 which gained the maximum length of 3.01 cm at 35 g l^{-1} (T_9) while M. 9 on the same treatment achieved 2.41 cm shoot length (Fig. 9). So it is clearly visible from the data that sorbitol produced continuous best results for shoot elongation as it yielded in case of shoot number. However, relatively reduced length in M. 9 than M. 26 on this treatment is probably due to competition limitation, which may be interpreted as diversion of assimilates towards shoot proliferation instead of elongation. Shoot elongation is a very critical step of the micropropagation system varying considerably with the nutritional composition of the media (Chen *et al*., 2003). Increase in shoot length with sorbitol may be attributed to its effective role in cell expansion, which is driven by turgor

pressure, and sorbitol is one of the major osmolytes used to generate turgor (Bianco & Rieger, 2002a). Furthermore, according to Bianco & Rieger (2002a) growth rate is correlated with rate of respiration at all the developmental stages and oxidation of sorbitol at the sink yields a net production of NADH (Nicotinamide Adenine Dinucleotide), which may partly contribute to the increase in respiration efficiency. Results of the present study in fact support this view and demonstrate a differential requirement for sorbitol as a key carbon source both for shoot proliferation and shoot elongation of two different genotypes i.e., M. 9 and M. 26. Interaction of sucrose was comparatively weak with both M. 9 and M. 26. The highest shoot length achieved by M. 26 was 1.95 cm at 35 g l^{-1} (T_4) whereas maximum length acquired by M. 9 with this treatment was only 0.94 cm (Fig. 10). Sucrose is a better carbon source for shoot proliferation of apple rootstocks as exhibited by the results of number of shoots per explant. However, poor response with sucrose for shoot length may be due to the competition for nutrient and osmotic components, aforementioned under the context of sorbitol. Moreover, cleavage of sucrose at the sink does not produce NADH leading to relatively low respiration efficiency and growth rate than sorbitol (Bianco & Rieger, 2002a). This may also be one of the possible reasons for limited growth associated with sucrose. Glucose resulted in moderately fair shoot length development as compared to sucrose and mannitol. However, it is noticeable that this carbon source proved to be good at an elevated concentration of 45 g l^{-1} than sorbitol and sucrose, which normally produced the best results at 35 g l^{-1}. Higher concentration of 45 g l^{-1} (T_{20}) was effective for M 26 with an upshot of 2.14 cm (Fig. 11) and 35 g l^{-1} (T_{19}) resulted in passable outcome of 1.30 cm for M. 9. Likewise, Romano *et al*., (1995) reported the promotory effect of glucose in *Quercus spp*. It is assumed that being nonreducing in nature; glucose has a great advantage of its direct entry into metabolism to fulfill the need for energy and carbon availability (Lipavska & Konradova, 2004). Weber *et al*., (1997) also stated that a metabolically active sink is characterized by high endogenous levels of hexoses (glucose and fructose), which eventually stimulate cell division and rapid growth. Mannitol yielded the poorest results both for shoot formation as well as for consequent elongation. Shoot tips of M. 9 and M. 26 cultured on this sugar alcohol expressed complete inhibition of morphogenesis with poor leaf: stem ratio and browning of leaves as well as stems (Fig. 12). These symptoms were more dominant at higher concentration of mannitol. Likewise, Hilae & Te Chato (2005) reports that a high concentration of osmoticum promotes leaf blight similar to the effect of water stress. Sairam *et al*., (2003) also demonstrates the analogous results that continuous incubation of Soybean (*Glycin max* L.) explants on mannitol for 28 days resulted in cell death as measured by the complete loss of morphogenetic potential. Vitova *et al*., (2002) states that osmotic stress caused by mannitol is more severe therefore, the growth inhibition could be more pronounced. Moreover, under high mannitol concentration, osmotic stress causes restriction of mannitol utilization and lowering availability of energy and carbon source.

Among treatments, sorbitol at 35 g l^{-1} (T_9) showed the best result of 2.72 cm shoot length. Glucose at 45 g l^{-1} (T_{20}) was second best treatment and ascertained to be better with shoot length of 1.43 cm. As sorbitol is the main translocatable form of carbon in *Rosaceae* (Marino *et al*., 1993), the magnificent results of the present study support the assumption of Welander *et al*., (1989) that sugar compounds normally found in the sieve-tube exudates can be used as an indicator of a suitable *In vitro* carbon source. According to Rolland *et al*., (2002) hexose sugars including glucose not only fuel cellular carbon and energy metabolism but also play pivotal role as signaling molecules. Rolland *et al*., (2002) further state that diverse signals from hexoses activate multiple HXK (Hexokinase, a glucose sensor) dependent and independent pathways and also control transcription, translation, protein stability and enzyme activity by using different

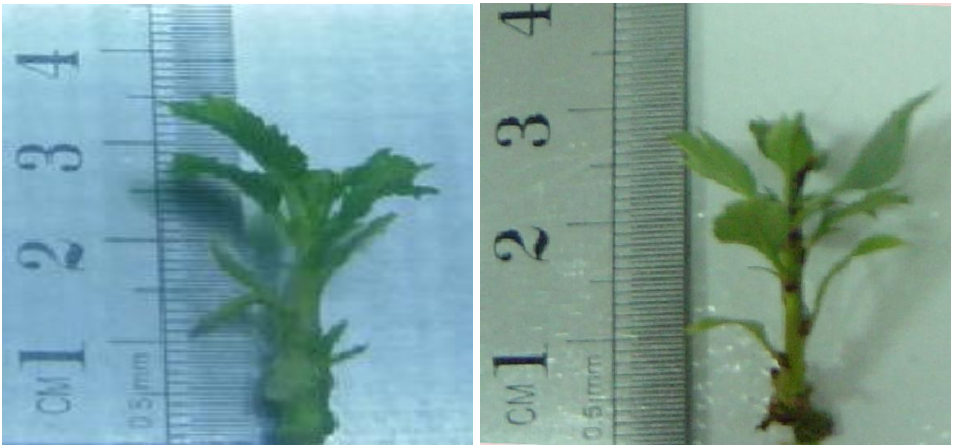

Fig. 9. Good length development in (a) M 26 and (b) M 9 at 35g l^{-1} sorbitol (T_9).

Fig. 10. Poor shoot length in M 9 with 35g l^{-1} sucrose (T_9).

Fig. 11. Fair shoot length achieved by M 26 at 45g l^{-1} glucose (T_{20}).

molecular mechanisms. All the treatments of mannitol were highly indigent and statistically alike to each other except T_{12} (15 g l^{-1}mannitol) which also had a very meager outcome of 0.24 cm. There was an increasing trend in shoot length from T_{16} (5 g l^{-1} glucose) to T_{20} (45 g l^{-1} glucose). On the other hand higher concentration of sucrose and sorbitol (45 g l^{-1}) showed depressing effect on shoot length development, probably due to the accumulation of phenolic compounds in the medium at supra optimal concentration; exerting negative effects on growth and development (Hilae & Te Chato, 2005).

M. 9 and M. 26 differ significantly at $p<0.05$ in terms of their shoot length achievement (Table 2). M. 26 comparatively afforded a better shoot length of 1.05 cm than M. 9 which gained the maximum length of only 0.78 cm. Welander *et al*., (1989) reported that it is also well known that the capability to metabolize different types of carbohydrates differ among different plant species which is in agreement with outcome of this study. Tornero *et al*., (2000) also documented genotype variability in apricot, apple and peach for shoot bud regeneration and stated that differences between genotypes suggest the individualized study of each cultivar.

Fresh weight of shoots (mg): Data presented in Table 3 substantiates a significant interaction ($p<0.05$) between carbon sources and fresh weight of cultured shoots in M. 9 and M. 26. Overall results achieved with sorbitol were auspicious in comparison to sucrose, glucose and mannitol and concentration of 35 g l^{-1} (T_9) was most propitious as it gave the unsurpassed fresh weight of 402 mg in interaction with M. 26. Conversely, M 9 barely had the highest fresh weight of 218.7 mg at the same concentration. The superseding demeanor of sorbitol, regarding fresh weight of apple shoots, is in consistency with previous parameters i.e., number of shoots per explant and shoots length where it also yielded the most overriding outcome (Fig. 13). Moreover, the highest fresh weight on sorbitol containing media suggests that apple shoots contain the enzymology necessary to metabolize sorbitol (Swedlund & locy, 1993). Coffin *et al*., (1976) provides an affirmation in this respect and states that sorbitol has been shown to support the growth of shoot tip cultures in *Rosaceae* family. Sucrose too was found highly beneficial for accumulation of biomass and attained the fresh weight of 345.3 mg in M. 9 and 275.7 mg in M. 26 at 35 g l^{-1} (T_4). Although shoot length achieved with sucrose media was abysmal but this highest score of fresh weight is correlated with good quality of shoot which were quite healthy and had an excellent leaf: stem ratio in sucrose treatments (Fig. 14). According to Gurel & Gulsen (1998) cleavage of sucrose in the culture medium results in the production of high levels of reducing sugars (glucose and fructose), which may speed up cell division consequently leading to an increase in the weight and volume of cultured tissues. De Faria *et al*., (2004) report that sucrose in the culture medium influenced the growth and accumulation of biomass of *Dendrobium* plantlets. Comparatively highest fresh weight at 5 g l^{-1} sucrose (T_1) both in M. 26 (198.0 mg) and M. 9 (184.0 mg) than 15 and 25 g l^{-1} (T_2 and T_3) is attributed to vitrification which resulted in development of a cluster of thick, curly and large sized leaves. Subsequent to sorbitol and sucrose, glucose also showed a positive carryover effect on the fresh weight gain of cultures. Predominantly, M. 26 gave a better response (274.8 mg) at 35 g l^{-1} (T_{19}). Glucose followed the same trend as pursued by sorbitol that up to 35 g l^{-1} (T_{19}) there was a gradual increase in fresh weight which decreased abruptly up to 45 g l^{-1} (T_{20}). Walender *et al*., (1989) advocates that reducing sugars (glucose) are not normally transported in sieve tubes as sucrose and sorbitol; but can be taken up by the cells as cell membranes are permeable to these solutes. Hence they are generally accumulated in tissues and then metabolized. This phenomenon might be responsible for the moderately fair results acquired with glucose. It was observed that mannitol had poor interaction with M. 9 and

Fig. 12. Shoot tips of (a) M 26 and (b) M 9 cultured on mannitol exhibiting stunted growth and browning of leaves and stems.

Fig. 13. Good shoot quality of M 26 with 35 g l^{-1} sorbitol (T_9) in terms of length and number of leaves associated with higher fresh weight.

Fig. 14. Excellent leaf: stem ratio with sucrose 35 g l^{-1} (T_4) in (a) M 26 and (b) M 9 leading to high fresh weight.

Table 3. Effect of different concentrations of carbon sources on fresh weight of shoots (mg) of apple rootstocks M 26 and M 9.

Treatments (Carbon sources g l^{-1})		Fresh weight (mg)		Mean
		M. 26	M. 9	
(Control)	T_0	60.00 uv	81.30 rstu	70.65 LM
Sucrose	T_1 (5)	198.0 efg	184.0 fgh	191.0 D
	T_2 (15)	135.0 klmn	112.7 nopq	123.8 GH
	T_3 (25)	176.5 ghi	134.7 klmn	155.6 EF
	T_4 (35)	275.7 c	345.3 b	310.5 A
	T_5 (45)	108.0 pqrs	100.7O pqrs	104.3 IJ
Sorbitol	T_6 (5)	95.0 pqrs	88.67 qrs	91.83 JK
	T_7 (15)	148.0 jkl	126.0 lmno	137.0 FG
	T_8 (25)	226.0 d	207.0 de	222.3 BC
	T_9 (35)	402.0 a	218.7 def	304.8 A
	T_{10} (45)	262.7 c	169.0 hij	215.8 C
Mannitol	T_{11} (5)	91.43 pqrs	53.00 uv	72.22 LM
	T_{12} (15)	57.47 uv	56.00 uv	56.73 LM
	T_{13} (25)	69.67 tuv	80.13 stu	74.90 KL
	T_{14} (35)	57.17 uv	91.67 pqrs	74.42 KLM
	T_{15} (45)	60.17 uv	49.33 v	54.75 M
Glucose	T_{16} (5)	118.7 mnop	114.3 mnopq	116.5 HI
	T_{17} (15)	142J klm	153.0 ijkl	147.5 F
	T_{18} (25)	178.3 ghi	158.8 hijk	168.6 E
	T_{19} (35)	274.8 c	202.7 defg	238.8 B
	T_{20} (45)	110.7 pqr	104.7 pqrs	107.7 HIJ
Mean		**154.6A**	**134.9B**	

$LSD_{5\%}$ Varieties = 5.49, Interaction (V×T) = 25.18, Treatments = 17.81
Any two means not sharing a letter differ significantly at $p<0.05$

M. 26 as there was no increase in fresh weight measured after 4 weeks. The reduction in the value of assessed parameter with mannitol can be caused by an excessive osmotic stress or by toxicity of the carbohydrates (De Neto & Otoni, 2003).

An examination of treatments elucidates that 35 g l^{-1} sucrose (T_4) and 35 g l^{-1} sorbitol (T_9) produced the robust outcome of 301.5 and 310.4 mg fresh weight respectively. Parallel response of these two carbon sources for fresh weight suggests that sucrose and sorbitol are equally valuable for biomass accumulation of apple cultures. In accordance with Bianco & Rieger (2002b), both sorbitol and sucrose are exportable carbohydrates and are synthesized in *Rosaceae,* present at the ratios of about 4: 1 respectively They further depict that growth rate in the members of this family is associated with the activity of sorbitol and sucrose catabolic enzymes in the sink tissues which is also demonstrated by Wilson (1972). Second best carbon source was glucose at the same concentration (T_{19}) with fresh weight of 238.8 mg. Persistence of this passable score of fresh weight in treatments of glucose in continuity with its interaction means proves the assumption of Walander *et al*., (1989) that this sugar is diffusible through the cell membranes and further metabolized to support growth. Mannitol produced very condensed riposte of apple rootstocks. Stoop & Pharr (1993) assayed that internal

carbohydrate pool of the tissues vary with respect to the carbon source used in culture medium. Mannitol as an osmoticum is very slowly taken up by the plant cells and is not utilized in vascular plants; being metabolically inert, except in few species where it is produced as a major photosynthetic assimilate. Hence internal carbohydrate pool of mannitol grown cells consist entirely of mannitol and is extremely low in hexoses due to its accumulation in cells, which leads to poor morphogenic response (Yuri, 1988). Within treatments there was a general trend of increase in fresh weight of apple rootstocks by increasing carbon source concentration up to 35 g l^{-1}. On the other hand, a decline in the fresh weight of both M. 26 and M. 9 with elevated concentration of 45 g l^{-1} of all the four carbon sources can be referred to stress caused by this higher concentration. According to Ahmad *et al*., (2007) sugars are perceived by cells as chemical signals *In vitro*, with very high concentration acting as stressing agents.

M. 26 proved to be better than M. 9, with regards to biomass accumulation as it showed an exuberant gain in fresh weight (154.6 mg) in comparison to M. 9 which merely had a subsequent outcome of 134.9 mg. This disparity might be explained by clonal diversity, which is in accordance with the results of Duart (1995) who compared the shoots of M. 9 and M. 26 for growth consideration and verified that shoot proliferation of these rootstocks was subordinate to the genotype.

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