World Applied Sciences Journal 6 (11): 1513-1517, 2009
ISSN 1818-4952

Influence of Carbon Sources and Their Concentrations on Rooting and Hyperhydricity of Apple Rootstock MM.106

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Abstract: Influence of fructose, sucrose, glucose, sorbitol and maltose carbon sources at various concentrations on rooting and hyperhydricity apple rootstock MM106 was investigated. Type and concentration of sugars had a significant effect on rooting percentage, mean root number, mean root length, hyperhydricity, as well as survival rate. The *in vitro* cultures produced the higher rooting percentage, mean root number, mean root length, when grown on media containing 90 mM sucrose. Shoots failed to root when fructose and maltose were used. The percentage of hyperhydric shoots was significantly higher at 60 mM maltose and 30 mM sorbitol and was less at 90 or 120 mM sorbitol, 90 mM sucrose and fructose and 120 mM maltose. Regenerated plantlets were acclimatized and successfully transplanted to soil with 90% survival.

Key words: Apple · Rooting · Hyperhydricity · Sucrose · Sorbitol · Glucose

INTRODUCTION

Micropropagation of apples for producing self-rooted plants will open up new areas of research and allow changes in traditional fruit tree propagation. The MM.106 apple rootstock has been extensively used in many countries to produce semi-dwarf trees [1,2]. Therefore, *in vitro* micropropagation is very important for commercial practices.

Cultured plant tissues need a continuous supply of carbohydrates from the medium to encourage growth and to survive in vitro; photosynthetic activity of cultured tissues is reduced by the use of suboptimal light intensity, limited gas change and high relative humidity [3]. Therefore, sugars, such as sucrose, glucose and sorbitol, are generally added as a carbon source. Thorpe [4] indicated that root initiation and growth were high energy requiring processes that could only occur at the expense of available metabolic substrates, which were mainly carbohydrates. The type of carbon source and its concentration affects of rooting [5-8] in many plant species. Calamar and De Klerk [9] has been reported influence of sucrose concentrations on rooting of apple rootstock 'Jork 9', but the effect of carbon sources other on rooting of apple rootstock MM. 106 has not yet been investigated.

The environment inside culture vessels normally used in plant micropropagation is characterized by high relative humidity, poor gaseous exchange between the internal atmosphere of the culture vessels and its surrounding environment and the accumulation of ethylene, conditions that may induce physiological disorders and accelerate senescence. In vitro cultured plants frequently show abnormalities, described as water-soaked, translucent or glassy leaves or shoots. These morphological symptoms were formerly described as vitrification or glassiness and have now been redefined as hyperhydricity [10,11]. Losses of up to %60 of cultured shoots or plantlets have been reported due to hyperhydricity in commercial plant micropropagation [12], which reflects the importance of this problem. Pasqualetto *et al*. [13] found that vitrification was a problem when apple proliferation medium was gelled with phytagar. Pasqualetto *et al*. [13] has been reported influence of carbon and gelling agent concentrations on vitrification of apple cultivars *in vitro*, but the effect of different carbon sources on hyperhydricity of apple rootstock MM. 106 has not yet been investigated.

This investigation was designed to determine the effects of different carbohydrate sources and concentrations on rooting and hyperhydricity of apple.

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MATERIALS AND METHODS

Plant Material and Culture Condition: The explants employed were shoots of the apple (*Malus domestica* Borkh) rootstock MM106 of about 25 mm in length preserved from previous in vitro cultures and maintained in growth room. Shoots of apple rootstock MM106 subcultured monthly in 250 ml polypropylene containers containing 40 ml of the MS [14] culture medium with 0.5 µM IBA, 4.43 µM BAP, 0.6% agar and 87.6 mM sucrose. The pH of the media was adjusted to 5.8 before autoclaving at 121° C for 15 min. shoots were illuminated by cool- white florescent light (50 µMol m^{-2}. S^{-1} per 16-h photoperiod) at 25±1°C.

Shoot Hyperhydricity Experiment: Shoot tips (4 to 5 mm long) were cut from the normal explants and were transferred to 25 × 100 mm culture tubes containing 40 ml of the MS culture medium with 0, 30, 60, 90, or 120 mM of sucrose, sorbitol, fructose, glucose, or maltose supplemented with 0.5 µM IBA, 4.43 µM BAP. Shoot cultures were grown on these media for three subculture cycles; results were recorded at the end of the third cycle. Shoot tips were placed upright in the medium with five shoot tips in each vessel. Each treatment included six replications. Experiment was conducted and repeated twice. After 6 weeks on proliferation media, shoot hyperhydricity was established on the basis of shorter and thicker stems, with leaves that were light green in color, thick, wrinkled, brittle and larger than those of normal shoots. Those longer than 0.5 cm were considered usable for further rooting.

Rooting Experiment: Shoots (10 mm long) were transferred to 25 ×100 mm tubes containing 40 ml of half-strength MS media with 5 µM IBA, 0.6% (W/V) powder agar (Becton and Dickinson granulated) and 0, 30, 60, 90, or 120 mM each of either sorbitol, sucrose, fructose, glucose, or maltose. Shoots were incubated in the dark for five days. After 5 days, the shoots were transferred to auxin-free medium and to the light. Rooting frequency, survival rate, root number and root length were recorded after 4 weeks.

The data were statistically analyzed using analysis of variance (ANOVA) for the factorial experiment in the completely randomized design according to [15]. Average of the main effects and their interactions were compared using the Duncan's multiple range test at $P< 0.05$. All statistical analyses were performed using facility of computer and SAS software package [16].

Plant Acclimatization: Rooted shoots were transferred into plastic pots containing an autoclaved mixture of prelate and coco peat (1:3 v/v) and kept for 4 weeks, then transplanted into plastic pots containing garden soil and grown in the growth room (20 ± 2 °C, 16-h photoperiod, 40 µmol/m²/s illumination). Plants were finally acclimatizated in a greenhouse at 28 °C for 5 weeks before they were moved to a greenhouse without temperature control.

RESULTS AND DISCUSSION

Hyperhydricity in regenerated shoots was observed to be significantly affected by the type and concentration of sugars applied.. Overall, hyperhydricity was most prevalent among explants incubated 30 and 60 mM with fewer hyperhydric explants at 90 and 120 mM in all sugars. The percentage of hyperhydric shoots was significantly higher at 60 mM maltose (11.33%) or 30 mM sorbitol (10%), while it was less at 90 or 120 mM sorbitol, 90 mM sucrose and fructose, or 120 mM maltose (Fig.1). Adding 30 mM sorbitol and sucrose and 60 mM maltose and sucrose to the medium caused formation of hyperhydric explants at higher percentages. The percentage of hyperhydric shoots decreased significantly with increased carbon sources concentration. Shoots grown on 90 mM sorbitol and fructose did not show hyperhydricity (Fig.1). These results are in accordance with those obtained by [17]. It is well established that *in vitro* cultured plants have a disturbed carbon metabolism [18]. Some authors found that hyperhydricity was reduced or eliminated by replacing sucrose with reducing sugars, e.g. fructose, glucose or galactose [19,20]. Druart [20] suggested that these carbohydrates could affect the redox potential by decreasing the oxidative potential found in hyperhydric leaves. In addition, many studies have been shown that some sugars added to the medium may inhibit chlorophyll synthesis, photosynthesis and the Calvin cycle [21].

Rooting only occurred when media contained sucrose, glucose, or sorbitol. Media devoid of sugar did not produce roots indicating the importance of sugar in root formation. Figures (2, 3, 4 and 5) showed that type and concentration interaction of carbon sources had significant effects on rooting percentage, root length, root number and survival rate. Shoots grown on medium containing sucrose had the highest percentage of root formation. By increasing the concentration of sucrose in the medium from 0 to 90 mM, an increase of percentage rooting, mean length of roots and mean number of roots

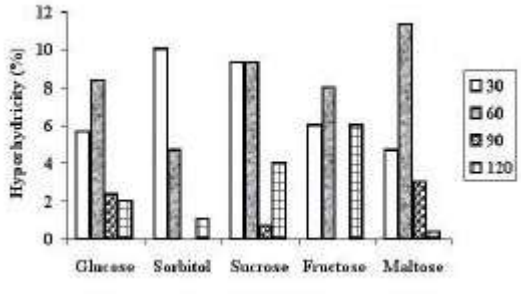

Fig. 1: Influence of different concentrations (30, 60, 90 and 120 mM) of glucose, sorbitol, sucrose, fructose and maltose on hyperhydricity of apple rootstock MM106.

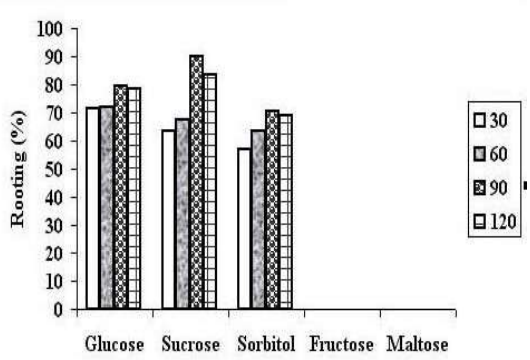

Fig. 2: Influence of different concentrations (30, 60, 90 and 120 mM) of glucose, sorbitol, sucrose, fructose and maltose on rooting percentage of apple rootstock MM106.

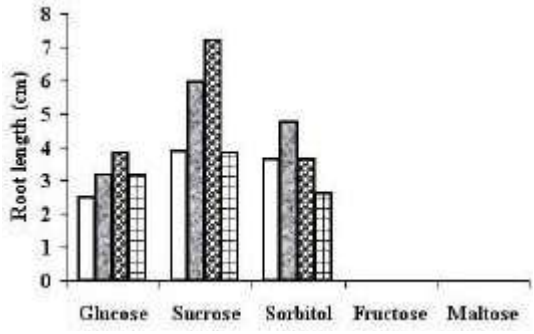

Fig. 3: Influence of different concentrations (30, 60, 90 and 120 mM) of glucose, sorbitol, sucrose, fructose and maltose on root length (cm) of apple rootstock MM106.

was observed (Figs. 2, 3 and 4). On the contrary, by increasing sucrose concentration from 90 to 120 mM, previous parameters decreased gradually. Studies by [9] on the influence of sucrose on adventitious root regeneration in apple rootstock 'Jork 9', demonstrated that by increasing the sucrose concentration up to ca. 7%

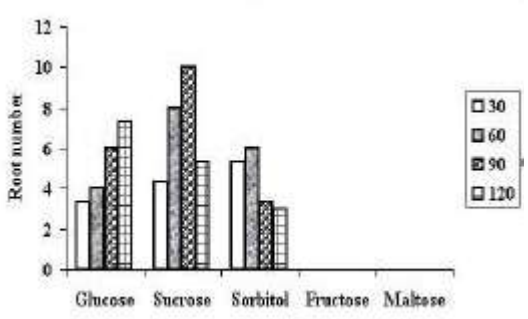

Fig. 4: Influence of different concentrations (30, 60, 90 and 120 mM) of glucose, sorbitol, sucrose, fructose and maltose on root number of apple rootstock MM106.

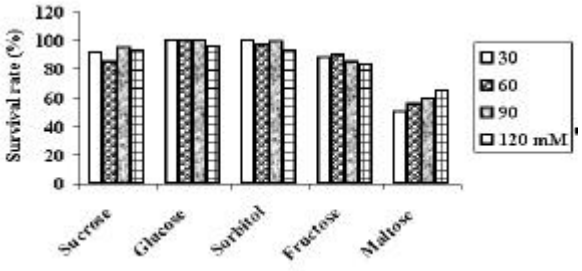

Fig. 5: Influence of different concentrations (30, 60, 90 and 120 mM) of glucose, sorbitol, sucrose, fructose and maltose on rate survival of apple rootstock MM106.

results in increased rooting. On the contrary, we observed the highest root number and rooting percentage (90%) on 3 % sucrose (90 mM) in apple rootstock MM.106. It has been reported that different cultivars do not respond in the same way during establishment, proliferation and rooting in vitro [22]. In many plant species has been shown that the response of shoot cultures to different carbohydrate treatments appears to be genotype dependent to some extent [23].

By increasing the glucose concentration of the medium from 0 to 120 mM, mean number of roots was increased (Fig. 4). At a concentration of 120 mM, the increase in root number was more pronounced in glucose compared with the other sugars, but by increasing glucose concentration from 90 to 120 mM, mean length of roots and percentage rooting decreased.

In this study, the number and length roots were also very low on media containing sorbitol. As described previously, sorbitol was the least effective sugar in terms of rooting frequency in apricot and related species [24], On the other hand, sorbitol was completely ineffective in stimulating the shoot proliferation and the induction of

roots in *Quercus suber* [8]. The negative results obtained with sorbitol showed that it is not efficiently metabolized by some species, or sorbitol in the medium is not used as carbon source at all, but regulates osmotic potential only.

Shoots failed to root when fructose and maltose were used. The lack of rooting by shoots in media containing fructose or maltose may be attributed to the absence or inactivation of enzymes metabolizing these carbon sources.

The survival rate of shoots was highest with sorbitol and glucose and then with sucrose and fructose. Shoot survival with all carbohydrates was >85%, except for all concentrations of maltose and 120 mM fructose treatment (Fig. 5).

In this study, 90 mM sucrose induced the highest root number and rooting percentage increase compared with all other treatments. All carbon sources do not sustain rooting equally. For most plant species including Prunus, sucrose has been used for promoting rooting of shoots [11]. Dimassi-Theriou [25] observed higher rooting percentage of GF-677 shoots on media containing 88 mM sucrose. Also [6] observed higher rooting percentage, mean root number and length of PR 204/84 peach rootstock shoots on media containing 88 mM sucrose. Starch is produced from sucrose supplied in the culture medium [26]. Starch often accumulation in the target cells just before regeneration and this starch may be a carbohydrate reserve during meristem formation. Overall, organ initiation is associated with the utilization of accumulation starch and free sugars of the medium [27]. On the basis of the above information, it appears that root formation of in vitro apple rootstock MM106 is a high energy requiring process and sugars may serve as a source of energy for this process.

High percentages (approximately 85%) of plantlets were successfully transferred into soil and they developed into normal plants in the greenhouse with 90% survival. All acclimatized plants were finally transferred to field conditions and grew normally in the natural environment. Phenotypic variability was not observed in plants in this experiment.

In conclusion, we have shown effects of different carbon sources on rooting and hyperhydricity. The results of this study show that 90 mM sucrose is most effective for rooting. It improved plant regeneration that should facilitate micropropagation and breeding this of apple rootstock. Based on our results, further investigation on the specific role of carbohydrates during the process of root formation and hyperhydricity seems imperative.

REFERENCES

1. Aklan, K., S. Centiner, Y. Aka-Kacar and Yalcin-Mend, 1997. In vitro multiplication of clonal apple rootstock M9, M-26_and MM-106 by meristem culture. Acta Hort., 441: ISSH.
2. Modgil, M., K. Mahajan, S.K. Chakrabarti, D.R. Sharma and R.C. Sobti, 2005. Molecular analysis of genetic stability in micropropagated apple rootstock MM.106. Scientia Horticulturae., 104: 151-160.
3. DePaiva, V.B. and W.C. Otoni, 2003. Carbon sources and their osmotic potential in plant tissue culture: Does it matter? Sci. Hort., 97: 193-202.
4. Thorpe, T., 1982. Carbohydrate utilization and metabolism. In: Bonga, J.M., Durzan, D.J. (Eds.), Tissue Culture in Forestry. Martinus Nijhoff Publishers, London., pp: 325-368.
5. Alkhateeb, A.A., 2001. Influence of different carbon sources and concentrations on *in vitro* root formation of date palm (*Phoenix dactylifera* L.) cv Khanezi. Zagazig J. Agric. Res., 28: 597-608.
6. Fotopoulos, S. and T.E. Sotiropoulos, 2004. *In vitro* propagation of the peach rootstock: the effect of different carbon sources and types of sealing material on rooting. Biol. Plant., 48: 629-631.
7. Li, M.Y. and C. Xu, 1992. Cotyledon culture and plantlets regeneration of Shimeichen orange (*Citrus sinensis*). Journal of Southwest Agricultural University., 14: 51-53.
8. Romano, A., C. Noronha and M.A. Martins-Loucao, 1995. Role of carbohydrate in micropropagation of Cork oak. Plant cell tissue and organ culture., 40(2): 159-167.
9. Calamar, A. and G.J. De Klerk, 2002. Effect of sucrose on adventitious root regeneration in apple. - Plant Cell Tissue Organ Cult., 70: 207-212.
10. Debergh, P., J. Aitken-Christie, D. Cohen, B. Grout, S. Von Arnold, R. Zimmerman and M. Ziv, 1992. Reconsideration of the term vitrification as used in micropropagation. Plant Cell Tiss. Org. Cult., 30: 140-165.
11. George, E.F., 1996. Plant propagation by tissue culture. Part 2, Exegetics Limited, England., pp: 654-669.
12. Paques, M., 1991. Vitrification and micropropagation: causes, remedies and prospects, Acta Hortic., 289: 283-290.

13. Pasqualetto, P.L., R.H. Zimmerman and I. Fordham, 1988. The influence of cation and gelling agent concentrations on vitrification of apple cultivars *in vitro*. Plant Cell Tissue Organ Cult., 14: 31-40.
14. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 478-497.
15. Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for Agricultural Research, second ed. John Wiley & Sons.
16. SAS Institute, 2001. SAS for Windows, SAS User's Guide: statistics. Version 8.0 e. SAS Inst., Inc., Cary, North Carolina.
17. Kadota, M. and Y. Niimi, 2004. Influences of carbon sources and their concentrations on shoot proliferation and rooting of 'Hosui' Japanese pear. HortScience., 39: 1681-1683.
18. Bisbis, B., F. Le Dily,, C. Kevers, J.P. Billard, C. Huault and T.H. Gaspar, 1993. Disturbed sugar metabolism in a fully habituated monorganogenic callus of *Beta vulgaris* (L.). Plant Growth Regul., 13: 257-261.
19. Rugini, E., P. Tarini and M.E. Rossodivata, 1987. Control of shoot vitrification of almond and olive grown *in vitro*. Acta Hort., 212: 177-183.
20. Druart, P., 1998. Regulation of axillary branching in micropropagation of woody fruit species. Acta Hort., 227: 369-380.
21. Van Huylenbroeck, J.M. and P.C. Debergh, 1996. Impact of sugar concentration *in vitro* on photosynthesis and carbon metabolism during *ex vitro* acclimatization of *Spatiphyllum* plantlets. Physiol. Plant., 96: 298-304.
22. Karhu, S.T. and R.H. Zimmerman, 1993. Effect of light and coumarin during root initiation on rooting apple cultivars *in vitro*. Adv. Hort. Sci., 7: 33-36.
23. Cuenca, B. and A.M. Vieitez, 2000. Influence of carbon source on shoot multiplication and adventitious bud regeneration in *in vitro* beech cultures. Plant Growth Regul., 32: 1-12.
24. Marino, G., G. Bertazza, E. Magnanini and A. Altan, 1993. Comparative effects of sorbitol and sucrose as main carbon energy sources in micropropagation of apricot. - Plant Cell Tissue Organ Cult., 34: 235-244.
25. Dimassi-Theriou, K., 1989. Factors affecting the *in vitro* culture of peach- almond hybrid GF-677 in the stage of multiplication and rooting. - Ph.D. Thesis. Aristotle University of Thessaloniki, Thessaloniki.
26. Jasik, J. and G.J. De Klerk, 1997. Anatomical and ultrastructural examination of adventitious root formation in stem slices of apple. Biol. Plant., 39: 79-90.
27. Thompson, M. and T. Thorpe, 1987. Metabolic and non-metabolic roles of carbohydrates. In: Bonga, J.M., Durzan, D.J. (Eds.), Cell and Tissue Culture in Forestry. Martinus Nijhoff Publishers, Dordrecht., pp: 89-112.