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EFFECTS OF ALCOHOL SUGARS ON *IN VITRO* POTATO MICROTUBERIZATION

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Abstract. *This study was conducted to evaluate the effects of alcohol sugars induced via; mannitol, sorbitol on in vitro microtuber production of potato. The single-node explants of in vitro derived plantlets were cultured onto Microtuberization medium including MS basal medium supplemented with different concentrations (0.0, 0.05, 0.11, 0.17 and 0.23 mol/l) of either alcohol sugars (mannitol and sorbitol). After 5 weeks of incubation (at 18±2°C in dark condition), the microtuber initiation and formation percentage and microtubers fresh weight, length and diameter were recorded. The resulted showed that mannitol was more effective on microtuber related traits than sorbitol. Microtubers length and diameter were minimum with different concentrations of sorbitol. In medium containing mannitol, microtuber fresh weight was significantly decreased with adding mannitol above than 0.11 mol/l. For mannitol, increasing concentration up to 0.11 mol/l had raising effect on eyes number per tuber but further increase in mannitol concentration negatively affected this trait. Eyes growth was decreased when nodal explants were cultured in higher concentrations of either mannitol or sorbitol and this trend shows that alcohol sugars had inductive effects on microtubers dormancy. In total, suitable amounts of sugar alcohols improved the microtuberization and its related traits and may be a feasible practical approach in microtuber production industry.*

Key words: *Potato, Microtuberization, Mannitol, Sorbitol.*

INTRODUCTION

Microtuberization in potato (*Solanum tuberosum* L.) is a complex developmental process which has been shown to be influenced by photoperiod (Seabrook *et al.* 1993), temperature (Leclerc *et al.* 1994), carbon source (Simko 1994), inorganic nitrogen nutrition (Sarkar and Naik 1998) and even physiological age of the mother tuber (Villafranca *et al.* 1998). Those factors, either directly or indirectly, influence *in vitro* tuberization process by regulating the effects of exogenously applied growth substances and/or al-

terations in endogenous hormonal balance (Ewing and Struik 1992). The effect of carbon source is, however, more influential than other factors in promoting microtuber induction. During the past two decades several attempts have been made to develop hormone-free microtuberization methods (Yu *et al*. 2000). More attention in this case has been devoted on the inductive effects of carbon source and photoperiod (Paiva Neto and Campos Otoni 2003). Dodds *et al.* (1992) reported that the optimal sucrose concentration for tuber initiation ranged from 60 to 80 g/l. The higher or lower sugar content in the medium led to reduced tuberization and smaller microtubers (Yu *et al*. 2000). Apart from being a suitable carbon source for consumption by the plantlets, excess sucrose may be converted to starch in developing microtubers. Moreover, sucrose act as a favorable osmoticum for microtubers development (Yu *et al*. 2000). The substitution of the carbon source *in vitro* by osmatically active solutes instead of sucrose has been shown to provide a rich carbon source and act as an osmotic regulator (Paiva Neto and Campos Otoni 2003). In this case, the most frequently employed biomolecules are the two alcohol sugars mannitol and sorbitol (George 1993). Meanwhile, sugars such as mannitol and sorbitol have been shown to induce *in vitro* regeneration, further studies revealed that mannitol mainly acts as an osmoticum rather than be uptaked or metabolized as an energy or carbon source (Altindal and Karado 2010). The combinations of sucrose and mannitol reinforces their nutrition and osmolarity activities (George 1993). In consequence, many of the growth and developmental traits of the explants showed positive responses to the combinations of mannitol and sucrose compared to the medium containing only sucrose. Concomitantly, this hypothesis has been confirmed by positive control treatment with sucrose as carbon source but without osmotic contribution (Paiva Neto and Campos Otoni 2003). In contrast, Ondo Ovono *et al*. (2009) reported that the use of 1% glucose and/or 1% fructose as carbon source in the presence of 2% sorbitol led to a low rate of tuberization in yam (*Dioscorea cayenensis*) and when osmolarity was re-established by the addition of sorbitol, the delay in the tuber formation was increased.

In the present experiment we aimed to analyze the efficiency of alcohol sugars (mannitol and sorbitol) at a wide range of concentrations, on the microtuberization induction of *solanum tuberosum* L. explants under standard *in vitro* conditions. In addition, we try to find a relationship between microtuberization parameters and osmotic potential of the medium in relation to the application of alcohol sugars *in vitro*.

MATERIALS AND METHODS

Plant material

Meristem culture derived disease-free *Solanum tuberosum* L. cv. Agria was employed as explants in the present experiment. The plantlets were maintained and multiplied through single-node cuttings on a growth regulator-free medium at 30-days interval under a 16 h photoperiod at 25±2^{o}C.

Microtuberization experiment

The experiment was continued with single-node explants. The explants were excised essentially from the middle parts of the microplant shoots for maintaining the explants homogeneity, and were cultured in 80 mm Petri dishes containing 20 ml of microtuberization medium which was based on MS supplemented with 80 g/l sucrose and different concentrations (0.0, 0.05, 0.11, 0.17 and 0.23 mol/l) of mannitol and sorbitol. The pH of the medium was adjusted to 5.8 before autoclaving at 121^{o}C for 20 min. The Petri dishes were sealed with Parafilm, and the microtuberization cultures were incubated in the dark at 18±2^{o}C. The experiment was arranged as factorial based on completely randomized design with five replications (each Petri dish considered as a replicate containing six single-node explants). After 5 weeks of incubation, percentage of microtubers initiation [cells growth in node location (diameter less than 0.5 mm)] and formation (diameter more than 0.5 mm), microtubers fresh weight, length and diameter, eyes number per microtuber and mean of eyes growth were recorded. Data were analyzed using SPSS software Ver.16. The means of treatments were compared using Duncan's Multiple Range Tests at 5 % probability level.

RESULTS AND DISCUSSION

In the present experiment, in all culture media, microtuber formation from axillary bud was initiated 5-6 days after culture. Many of the microtubers were globular and a few of them were oval-shaped, their size varied from 3-11 mm during the experiment. Skin color of microtubers was usually cream, sometimes with purple spots. Microtuber eyes numbers were varied from 2 to 6. In some cases, the eyes were grown and their shoot length was ranged from 0.5 to 20 cm. Under some sorbitol and mannitol concentrations no microtubers were developed. In those treatments axillary buds were grown and only shoots were elongated (0.5-10 cm). Moreover, all nodal segments (explants) were rooted with all treatments exploited. The number and length of roots per explant were 5-36 and between 0.5-12 cm, respectively. Microtubers were harvested from axillary buds. *In vitro* derived microtubers were germinated in pots containing compost, perlite and vermiculate (1:1:1) under mist conditions during 4-5 days. Then, the hardened plantlets

were transferred to the greenhouse (Fig. 1). Simultaneously, some microtubers were also cultured in growth regulator free MS medium, their germination occurred during 5-7 days and the resulting plants had normal growth and development (Fig. 2).

Figure 1. Planting of microtubers of *Solanum tuberosum* cv. Agria in pots under mist conditions and their subsequent growth.

Microtuber initiation

Analysis of variance showed that microtuber initiation percentage was significantly ($p \leq 0.01$) affected by the studied factors. The results showed that osmotic and nutrient sources (mannitol and sorbitol) were suitable (71%

and 67%) regarding microtuber initiation percentage. Microtuber initiation percentage was increased by adding mannitol up to 0.05 mol/l. About sorbitol, microtuber initiation process was completely retained by any addition of sorbitol into the microtuberization medium (Fig. 3).

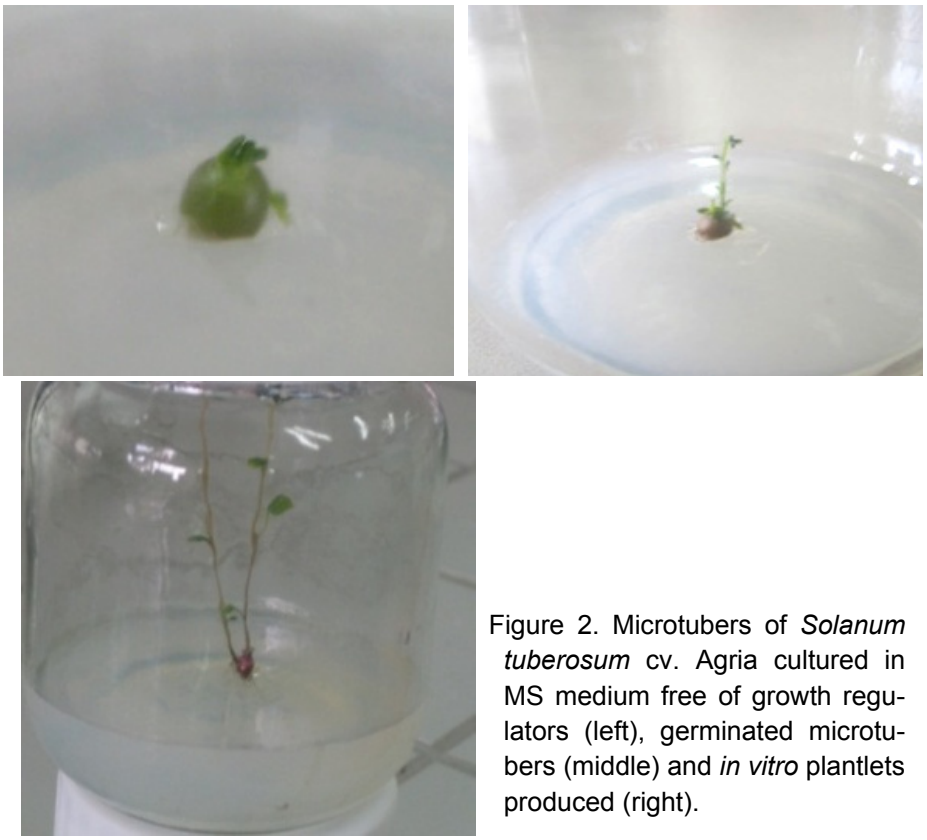

Figure 2. Microtubers of *Solanum tuberosum* cv. Agria cultured in MS medium free of growth regulators (left), germinated microtubers (middle) and *in vitro* plantlets produced (right).

Microtuber formation

Analysis of variance showed that microtuber formation percentage was meaningfully ($p \leq 0.01$) affected by the treatments. The microtuber formation percentage was 35% and 18% in media containing different concentrations of mannitol and sorbitol, respectively. The microtuber formation percentage was increased when explants were cultured in media containing 0.0, 0.05 and 0.11 mol/l mannitol. However, the use of sorbitol led to decreased microtuber formation percentage (Fig. 4).

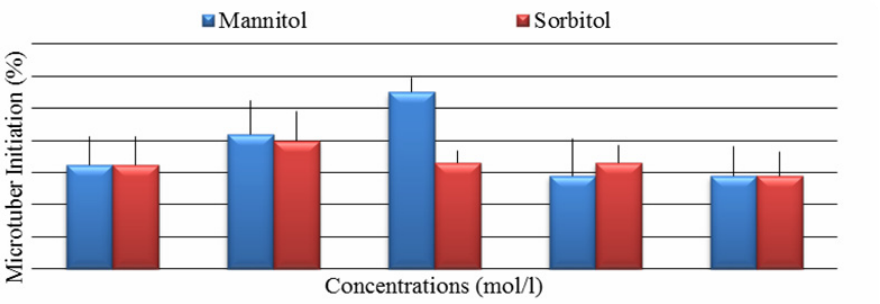

Figure 3. Microtuber initiation percentage in *Solanum tuberosum* cv. Agria explants with different concentrations of sorbitol and mannitol.

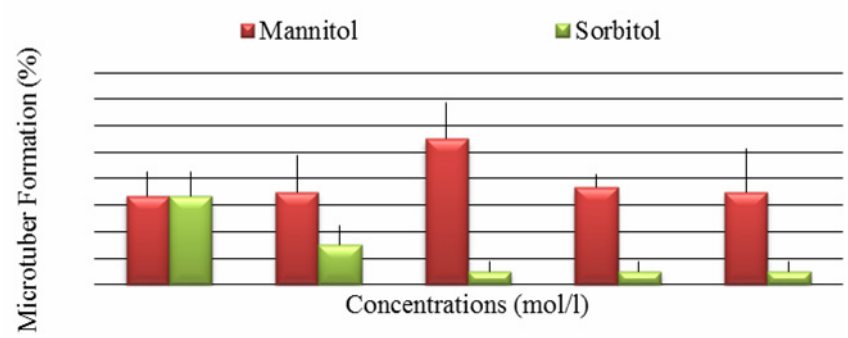

Figure 4. Microtuber formation percentage in *Solanum tuberosum* cv. Agria explants with different concentrations of sorbitol and mannitol.

Microtuber size (length and diameter)

The highest data for microtubers length and diameter were observed in mannitol treatments. Contrarily, microtubers length and diameter were minimum with different concentrations of sorbitol (Fig. 5 and 6). Any increase in mannitol concentration up to 0.05 mol/l went to profound increase in microtubers length and diameter. Decreasing the microtuber length and diameter was fixed when the concentration of sorbitol increased in microtuberization media (Fig. 5 and 6).

Microtuber fresh weight

Fresh weight of microtuber was significantly ($p \leq 0.01$) affected by treatments. Microtubers fresh weight was ranged from 10 to 1000 mg. The

lightest microtubers were less than 6 mg, so they were excluded from the experiment. In medium containing mannitol, microtuber fresh weight was significantly decreased with adding mannitol above than 0.11 mol/l. Fresh weight of microtuber was decreased when nodal explants were cultured in media containing different concentrations of sorbitol (Fig. 7).

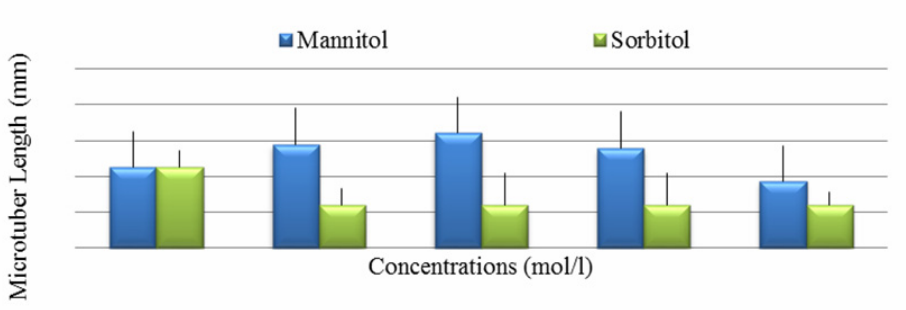

Figure 5. Microtuber length mean in *Solanum tuberosum* cv. Agria explants with different concentrations of sorbitol and mannitol.

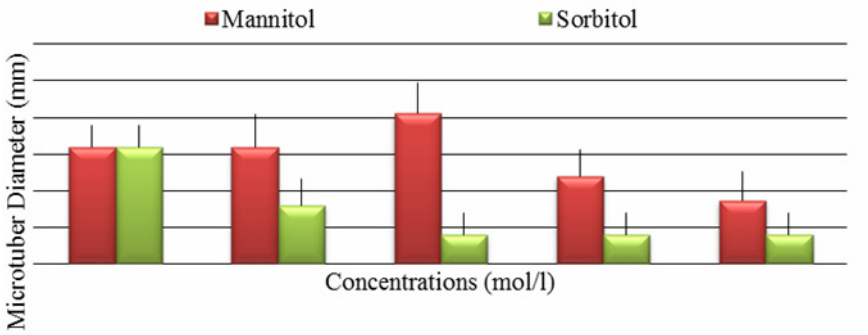

Figure 6. Microtuber diameter mean in *Solanum tuberosum* cv. Agria explants with different concentrations of sorbitol and mannitol.

Eyes number per microtuber

Microtubers from sorbitol treatments had significantly ($p \leq 0.01$) lower number of eyes. For mannitol, increasing concentration up to 0.11 mol/l had raising effect on eyes number per tuber but further increase in mannitol concentration negatively affected this trait (Fig. 8).

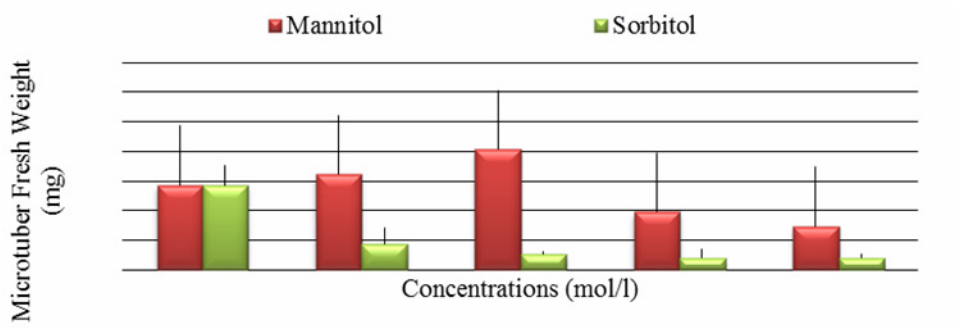

Figure 7. Microtuber fresh weight mean in *Solanum tuberosum* cv. Agria explants with different concentrations of sorbitol and mannitol

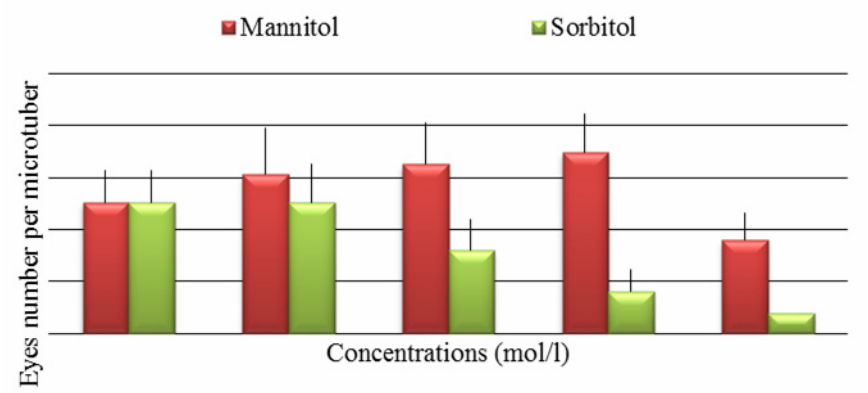

Figure 8. Microtuber eyes number mean in *Solanum tuberosum* cv. Agria explants with different concentrations of sorbitol and mannitol.

Eyes growth per microtuber

Eyes growth was decreased when nodal explants were cultured in higher concentrations of either mannitol or sorbitol. The minimum rate of eyes growth was recorded in sorbitol treatment, so that, 0.23 mol/l sorbitol showed no eye growth (Fig. 9).

Our findings showed that all microtubers related traits in mannitol treatments were higher than sorbitol. This means that this alcohol sugar had osmotic and nutritional role. But osmotic role was stronger in different concentrations of sorbitol. Lower microtuber weight under continuous darkness in the present study may be attributed to the etiolation or might be due to

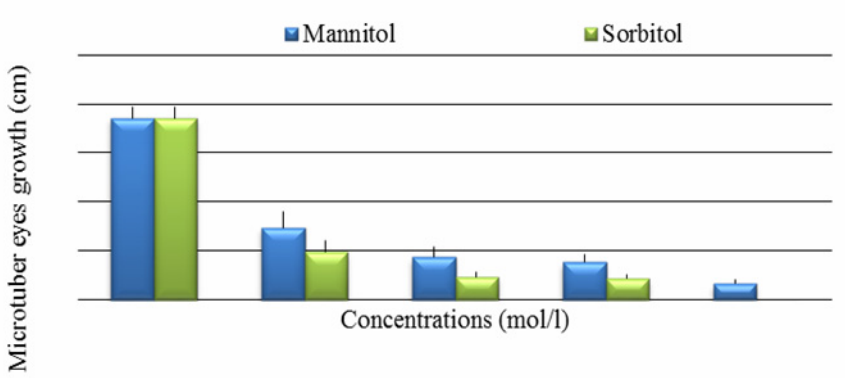

Figure 9. Microtuber eyes growth mean in *Solanum tuberosum* cv. Agria explants with different concentrations of sorbitol and mannitol.

inappropriate osmotic pressure in medium consistent with the findings of Gopal *et al.* 1998 and Kubo *et al.* (2005), respectively. The results revealed that the increase in microtuberization upon supplementing with mannitol was mainly due to availability of nutrient sources in the medium as there was little change in microtuberization media. Furthermore, our observation showed that the promotion of microtuberization was more pronounced by mannitol than sorbitol. The results are similar with the findings of Garner and Blake (1989), and Ranalli *et al. (*1994) who reported that microtubers might be produced even without the use of growth regulators. The use of media without growth regulators would be desirable where the objective is to judge the innate capacity of genotypes to produce microtubers and to remove the possibility of any undesirable carry-over effects of growth regulators on morphology, dormancy or sprouting. In our samples, lack of microtubers dormancy may be attributed to the using of media without growth regulators similar with the findings of Gopal *et al.* (1998). Sorbitol may work as a primary carbon source to improve the dormancy frequency of microtubers in potato for the conservation of germplasm. Ondo Ovono *et al.* (2009) reported that sprouting of yam (*Dioscorea cayenensis*) tubers obtained from media with reduced sucrose levels and supplemented with sorbitol was also delayed as observed with reduced sucrose alone but the final sprouting rate was 100% after 11 weeks. The results presented here indicate that supplementing sorbitol, mannitol in the culture media could enhance the microtuberization and also alcohol sugars may induce dormancy in microtubers for the conservation of potato reservoirs. As mentioned before, mannitol supplementation positively enhanced microtuberization po-

tential. Sorbitol, a six-carbon sugar alcohol, with mannitol are commonly regarded as osmotic regulators. Some results noted that sorbitol and mannitol can only act as an osmotic regulator (Wang *et al*. 1999), while others regarded sorbitol and mannitol as energy or carbohydrate sources (Masayoshi and Takayasu 1992). So, we concluded that the microtuberization was greatly enhanced by the using of appropriate concentrations of sorbitol and mannitol in the medium. In addition, the accumulation of sugars in response to applied stress conditions is also quite well documented (Wang *et al*. 1999).

In this study, sorbitol and mannitol concentrations up to an optimal range were parallel with the growing of axillary buds and hence increased microtuberization. Reduced microtuber growth and production caused by osmotic pressure might be due to the reduced availability of water and nutrients from media.

Accordingly, the suitable concentrations of mannitol and sorbitol are required for obtaining the desired microtuber number and weight. Although, it is reported that high carbon sources stimulate tuber formation (Welander and Pawlicki 1994; Khuri and Moorby 1996), possible functions of carbon sources in microtuberization are still vague. In other words, microtuberization observed in the different concentrations of sorbitol and mannitol, in the present experiment may be due to the use of 80 g/l sucrose in all treatments. The discrepancy of microtuberization rate among the treatments can be assigned to the differences in alcohol sugars related nutrient and osmotic roles.

CONCLUSION

In conclusion, the alcohol sugars should be investigated in case of any culture for their efficiency. Evidence showed that alcohol sugars (mannitol and sorbitol) are able to initiate the microtuberization but cooperation of 80 g/l or likely variable amounts of sucrose is also essential for developing tuber and reaching to a certain result. Later, still may be other factors that directly or indirectly influence tuber formation and its further development. The results implies the possible interaction between sorbitol and sucrose and/or mannitol and sucrose, and that sorbitol and mannitol play a similar role to sucrose, regarding the dual role of sugar as carbon and osmotic source in microtuberization.

In total, inclusion of microtuberization medium with suitable amounts of sugar alcohols improved the microtuberization and its related traits and may be a feasible practical approach in microtuber production industry.

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