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A new concept about carbon source roles on *in vitro* microtuberization of potato (*Solanum tuberosum* L.)

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Abstract. The present investigation was conducted to understand carbon source roles on rapid *in vitro* microtuber production of potato. The single-node explants from *in vitro* plantlets were cultured into microtuberization medium including MS basal medium supplemented with different concentrations (0.0, 0.05, 0.11, 0.17 and 0.23 mol.l^{-1}) of sorbitol (nutrient or osmotic effect), sucrose or PEG (osmotic effect). After 5 weeks of incubation (at 18±2^{o}C in dark condition), the microtuber initiation and formation percentage, microtubers fresh weight, length and diameter as well as eye number on microtuber were evaluated. The results showed that microtuberization traits were significantly influenced by carbon sources and their concentrations. Comparison showed that the sucrose was more suitable and effective for microtuberization than PEG and sorbitol. Microtuberization decreased when nodal explants were cultured on medium containing different sorbitol concentrations. The PEG treatments conducted to a decrease of microtuber production process. Also it is a well established fact that usage of PEG in microtuberization media had osmotic role. Our research showed that sorbitol treatments, on all microtuber traits had only osmotic role. We suggest that microtuberization is related to the nutrient/osmotic pressure ratio, where increasing of nutrient source by high concentrations of sucrose reduce the negative effect of osmotic pressure.

Key Words: Microtuberization, PEG, sucrose, *Solanum tuberosum* L., sorbitol.

Introduction. Microtubers generally originate as aerial structures on the stem, occasionally a few microtubers may be formed in medium. Microtubers are convenient for handling, storage and transport of germplasm (Thieme 1992). Microtubers also provide more flexible planting options and reduce the risk of disease infection in the field (Yu et al 2000).

Induction and growth of microtubers are influenced by some factors such as genotype (Ahloowalia 1994), type of explants (Khuri & Moorby 1996), light quality (Pelacho & Mingo-Castel 1991), photoperiod (Hussey & Stacey 1984; Pelacho & Mingo-Castel 1991), temperature (Akita & Takayama 1994), carbon source (Garner & Blake 1989) and growth regulators (Ortiz-Montiel & Lozoya-Saldana 1987).

Several reports have evaluated the effects of carbon source on microtuberization (Dodd's et al 1992; Garner & Blake 1989; Hussey & Stacey 1984).

Dodd's et al (1992) showed that optimal concentration of sucrose ranged from 60 to 80 g.l^{-1}. Higher or lower sucrose concentrations than 80 g.l^{-1} leads to slower tuberization, and the obtained microtubers are fewer and smaller (Yu et al 2000). Sucrose may play a dual role in microtubers development. First of all may be a suitable carbon source that is easily assimilated by the microplants and converted to starch in microtubers developing. Sucrose at 80 g.l^{-1} concentration also provides a favorable osmolarity for microtubers development (Khuri & Moorby 1995; Yu et al 2000). The substitution of the medium carbon source with osmotically active solutions has shown that sugars act as a carbon source and as osmotic regulator. In this case, the most frequently used solutions are the mannitol (sugar alcohol) and sorbitol (George 1993), which are permeating unusually metabolized solutes by plants, and long chain non-

permeating solutes, such as polyethylene glycol (PEG) (George 1993; Ramarosandratana et al 2001).

Important and not enough studied aspect is the probable non-uptake and or non-metabolization of mannitol and sorbitol, a fact which makes it suitable for use in osmotic studies.

Ondo Ovono et al (2009) and Paiva Neto & Otoni (2003) reported that the use of 1% glucose or 1% fructose as carbon source in presence of 2% sorbitol also led to a low rate of tuberization in yam and when osmolarity was reestablished by the addition of sorbitol, the delay in the tuber formation was increased.

Polyethylene glycol (PEG) is a polymer produced in a range of molecular weights (Blum 2008). Several authors reported the beneficial effect of PEG supplementation on *Pinus* somatic embryo maturation (Ramarosandratana et al 2001).

In the present studies we used nodal explants of Agria cultivar to test the effect of sorbitol and PEG as stress source, on medium including 80 g L^{-1} sucrose for microtubers formation and growth.

Material and Method. In the current studies we used as biological material Agria cultivar (*Solanum tuberosum* L. cv. Agria). These were made disease free following the standard meristem culture. Disease free plantlets were maintained and multiplied through single node cuttings on medium free of growth regulators at 30-days interval under a 16 h photoperiod at 25±2°C.

The experiment was carried out with subcultured single-node explants. The single-node explants were excised essentially from middle nodes of the microplants for maintaining explants homogeneity, and cultured in 80 mm Petri dishes containing 20 ml of microtuberization medium, based on MS medium supplemented with 80 $g.l^{-1}$ sucrose and different concentrations of sorbitol and PEG. The pH was adjusted to 5.8 before autoclaving at 121°C for 20 min. The Petri dishes were sealed with Parafilm, and the microtuberization cultures were incubated in the dark at 18±2°C.

The studies included two sugars, sucrose and sorbitol as nutrient. Also an osmotic pressure source (PEG) was tested in microtuberization medium at varying concentrations (0.0, 0.05, 0.11, 0.17 and 0.23 $mol.l^{-1}$).

The experiment was carried out in a completely randomized factorial design involving three source, five different concentrations with five replicate Petri dishes each containing six single-node explants. After 5 week's incubation were recorded the percentage of initiation and microtubers formation, microtuber fresh weight (mg), microtuber length and diameter (mm), eyes number and eyes sprouting. Data obtained from this study were processed using SPSS software Ver.16. The averages of treatments were compared using Duncan's Multiple Range Tests at 5% level.

Results and Discussion. Microtuber formation started after 5-6 days when medium contained sucrose, sorbitol and high PEG concentrations in dark condition. These microtubers become mature after 4-5 weeks of initiation and were harvested when the microtuber size was more than 3 mm (Figure 1). Microtubers were grown on pots containing compost, perlite and vermiculate (1:1:1) and there germinated on mist conditions during 4-5 days. Then the hardened plantlets were transferred in a greenhouse. Microtubers also can be cultured in MS medium free of growth regulators, there germination during 5-7 days, suitable for *in vitro* plants production (Figure 2).

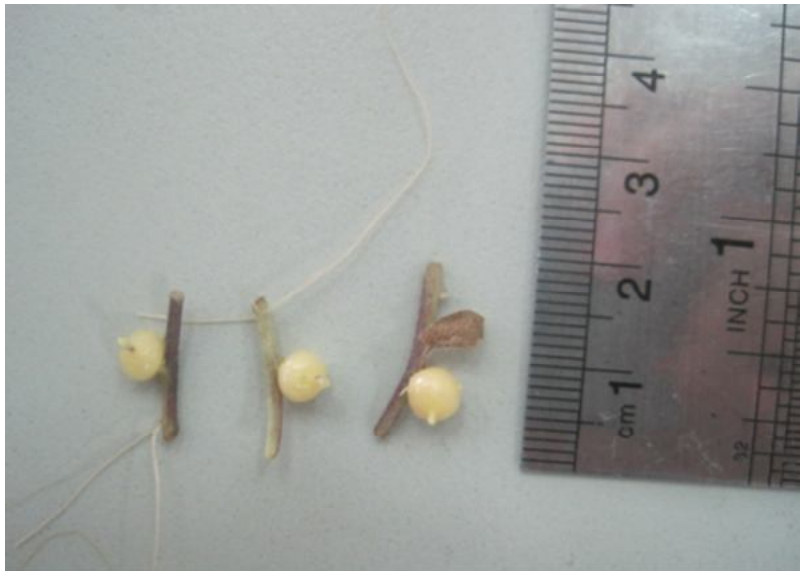

Figure 1. Harvested microtubers size was more than 3mm.

Figure 2. Microtubers were grown on pots containing compost, perlite and vermiculate (1:1:1) and they germinated on mist conditions during 4-5 days (left). Microtubers also can be cultured on MS medium, free of growth regulators, there germination during 5-7 days, desirable for plants production *in vitro* (right).

Maximium percentage of microtuber initiation was obtained when MS medium was supplemented with higher concentration of sucrose. Percentage of microtuber initiation decreased with the increase in concentration of sucrose (0.23 mol.l^{-1}). Its main reason may be that osmotic pressure increased in media containing 0.23 mol.l^{-1} sucrose and this increasing leads to a slower tuberization. When sorbitol was used in combination with 80 g.l^{-1} sucrose, no significant decreased in percentage of microtuber initiation was observed. The microtuber initiation percentage decreased by adding PEG from 0.05 to 0.23 mol.l^{-1} (Figure 3).

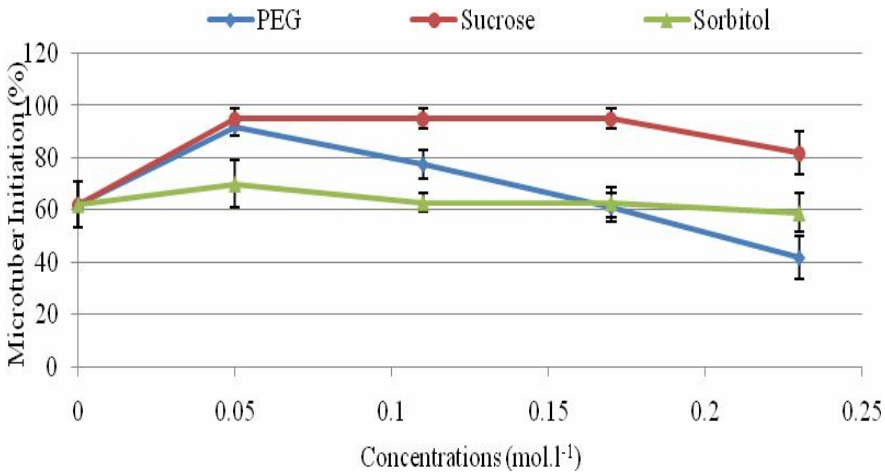

Figure 3. Microtuber initiation percentage at different PEG, sucrose and sorbitol concentration.

When medium contained higher concentrations of sucrose, the percentage of microtuber formation was maximal. As the concentration of PEG was increased over optimum, the percentage of microtuber formation decreased, indicating that PEG played a vital role in microtuber formation, but the use of sorbitol in medium will lead to decreased microtuber formation percentage. Present of higher concentrations of PEG and sorbitol in the medium reduces osmotic pressure and increase stress in medium, with decreases of the microtuber formation (Figure 4). Microtuberization limiting caused by osmotic concentration is due to the reduction of the absorbed water and nutrients from the medium. So, at high concentrations, sucrose act as osmotic agent and it is highly metabolized. PEG and sorbitol are more effective than sucrose in culture microtuberization limiting.

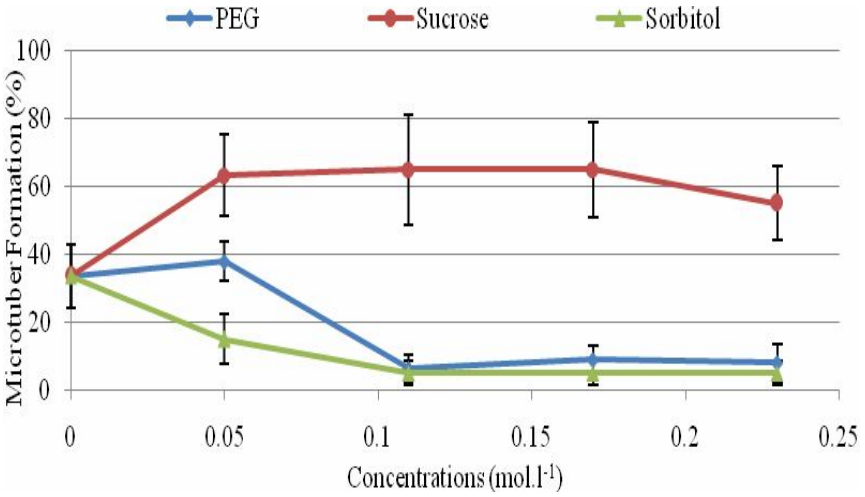

Figure 4. Microtuber formation percentage at different PEG, sucrose and sorbitol concentration.

In MS media containing different concentration of sucrose, the maximum length and diameter of microtuber was produced when 0.05, 0.11 and 0.17 mol.l^{-1} sucrose was added. At higher level of sucrose (0.23 mol.l^{-1}) the osmolarity of the medium increased and plants underwent stress. Due to this stress, size of microtuber decreased. Microtuber length and diameter was increased at 0.05 mol.l^{-1} PEG. Microtuber lenhgt and diameter

decreasing was fixed when concentration of sorbitol increased in microtuberization media (Figure 5 and 6).

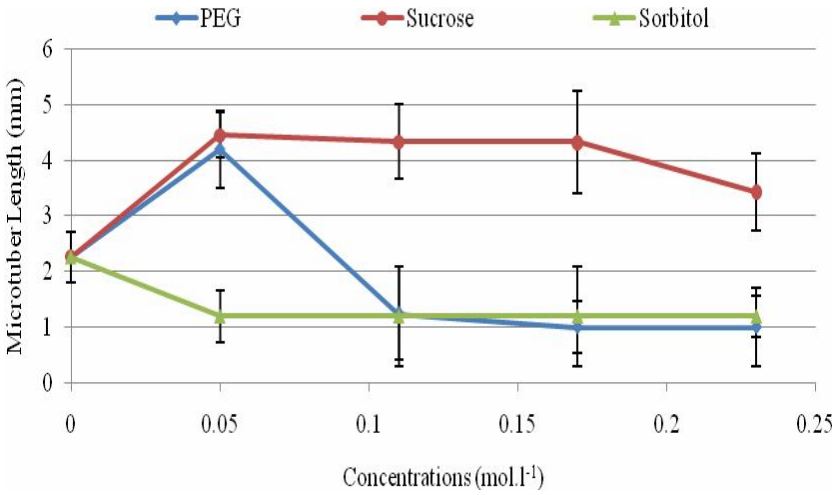

Figure 5. Average length of microtuber at different PEG, sucrose and sorbitol concentration.

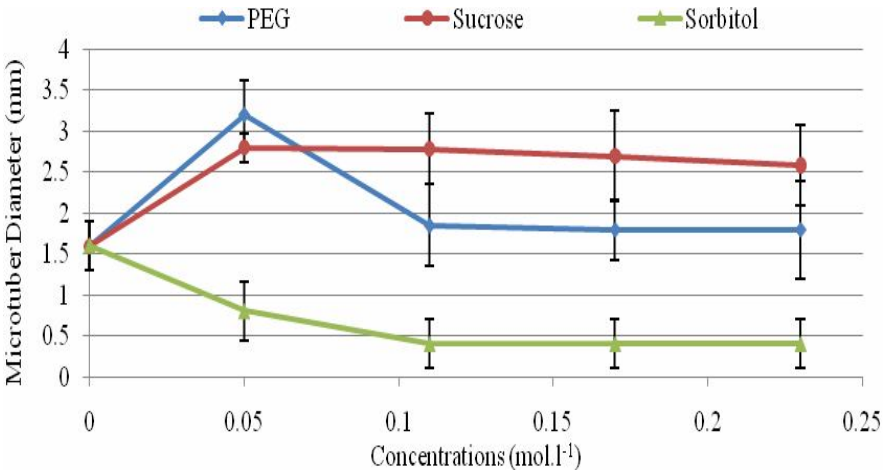

Figure 6. Average diameter of microtuber at different PEG, sucrose and sorbitol concentration.

When the concentration of sucrose was increased, fresh weight of microtubers raise again indicating that up to certain level sucrose (80 $g.l^{-1}$) may enhance microtuber weight. With the increase of microtuber formation percentage, also increased size and weight of microtubers. This indicates that raising the sucrose concentrations are efficient to improved *in vitro* microtuber production without negative side effects. Fresh weight of microtuber decreased when PEG was added, and when nodal explants were cultured in different concentrations of sorbitol (Figure 7). When we increased osmolarity adding sorbitol, the delay in the microtuber formation raised. Only a few microtubers were observed at the 35th day. So, in medium containing sorbitol, fresh weight and microtubers size were lower than in the PEG medium.

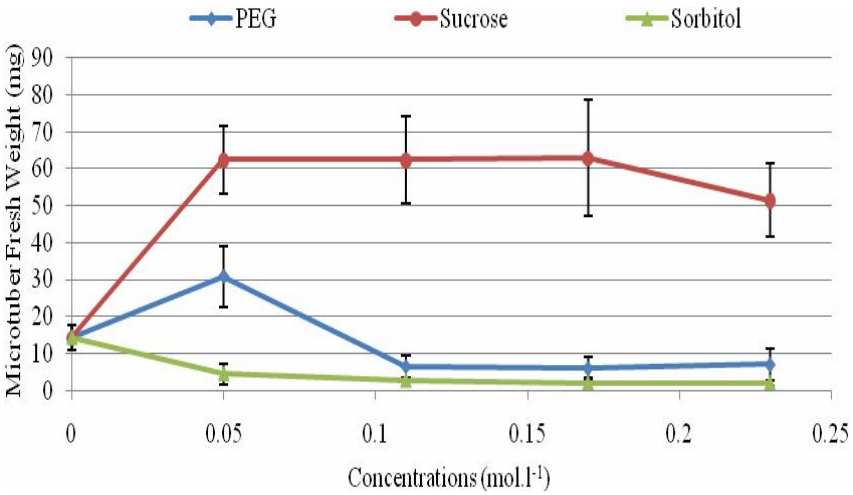

Figure 7. Average weight of fresh microtuber at different PEG, sucrose and sorbitol concentration.

In the case when medium contained high concentration of sucrose, the number of eyes was maximal. Eyes number was significantly increased when concentrations of sucrose was higher in microtuberization media. Number of eyes increased when concentration of PEG was 0.05 mol.l^{-1}. Furthermore, adding further amount of PEG (above 0.05 mol.l^{-1}) in microtuberization medium led to the decline of eyes number. Eyes number was decreased in media containing sorbitol (Figure 8).

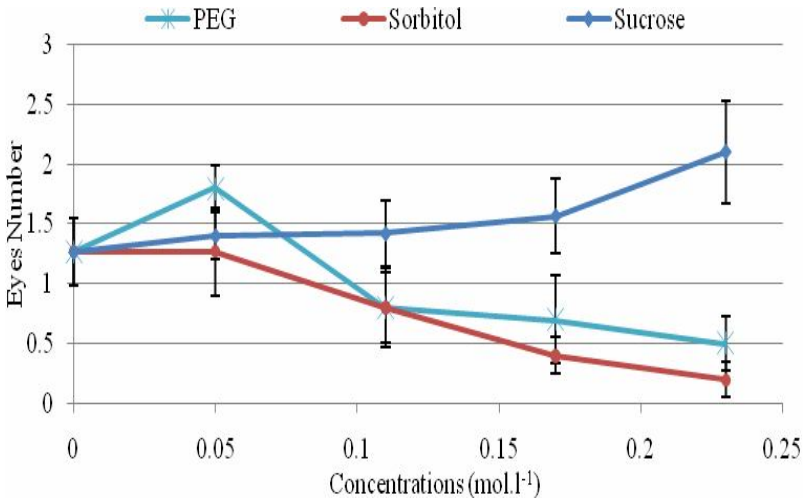

Figure 8. Average number of microtuber eyes at different PEG, sucrose and sorbitol concentration.

Eyes sprouting were decreased when nodal explants were cultured in high sucrose, sorbitol and PEG concentrations. Sprouting of eyes increased when concentration of sucrose was 0.05 mol.l^{-1}. Furthermore, adding further amount of sucrose (above 0.05 mol.l^{-1}) in microtuberization medium led to the decline of eyes sprouting. Minimum eyes sprouting were observed in different concentrations of sorbitol. Eyes sprouting were zero in 0.23 mol.l^{-1} sorbitol. At sorbitol treatment, decreasing of eyes sprouting were more pronounced than at different PEG and sucrose concentrations, this showed that alcohol sugars significantly left its mark on dormancy microtubers (Figure 9).

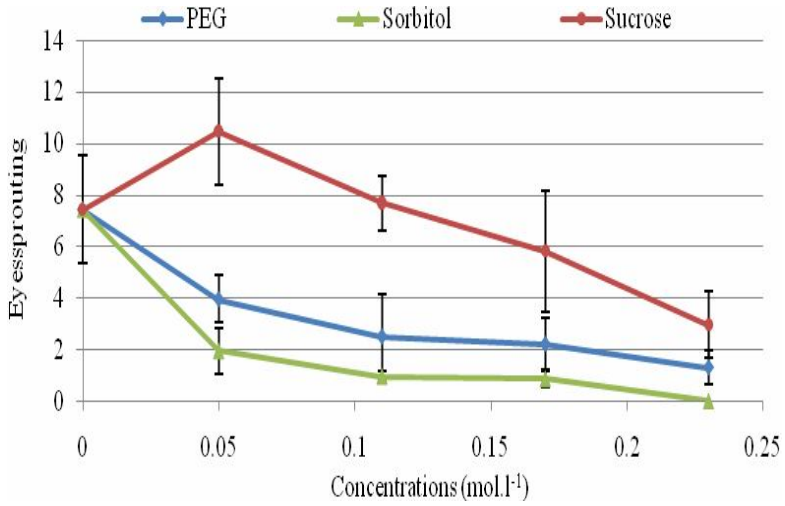

Figure 9. Average number of microtuber eyes sprouting at different PEG, sucrose and sorbitol concentration.

The results showed that sucrose, sorbitol, and PEG played a significant role in *in vitro* microtuberization. Microtuber production was influenced by increasing osmotic pressure in medium. Sucrose seems to be the most critical stimulus for microtuber formation. It may be essential as an osmotic agent. As energy source and higher concentration may have a role as a signal for microtuber formation as reported by Wang & Hu (1982) and Khuri & Moorby (1995). Our findings showed that sometimes opposite conditions might be required for the maximal expression of two different characters. For example, length of microtuber was greater in medium containing sorbitol than PEG, whereas diameter of microtuber was bigger in medium containing PEG. Microtubers sprouting decreased in medium with high sucrose, sorbitol and PEG level, but in media supplemented with 0.23 mol.l $^{-1}$ sorbitol was also delayed as observed with reduced sprouting but the final sprouting was 0.0 after 5 weeks. It signifies that sorbitol as an addition material is a very important and positive factor to enhance and maintenance microtubers. Thus, depending upon the requirement, protocols need to be optimized for different characters.

Conclusions. Microtuberization in potato is influenced by many factors including carbohydrate supply, nitrogen status, carbon-nitrogen ratio (C:N), day length, temperature, genotype, and endogenous and exogenous balance of growth regulators. Among these, carbon sources have been suggested to play a prominent role.

Sucrose is known as a microtuberization promoter. We suggest that microtuberization is related to the nutrient/osmotic pressure ratio where increasing of nutrient source reduced the negative effect of osmotic pressure on tuberization. Our results showed that exogenous supply of sucrose increased microtuber production. The present study showed that high sucrose concentration also increased microtuber production, size and weight. We suggest that, for germplasm conservation, microtubers should be induced in medium supplemented with high concentration of sucrose (80 g.l^{-1}) and appropriate concentration of osmotic material, as this enhances microtuberization without eyes sprouting. We also suggest that microtubers, after their maximal possible storage period determined by their visual condition, can also be used as explants for medium-term in *in vitro* conservation of potato germplasm. The present report perhaps is the first study of the sucrose, sorbitol and PEG effect on microtuber production.

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