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THE EFFECT OF SUGAR TYPE, SOURCE AND CONCENTRATION ON CAULIFLOWER (BRASSICA OLERACEA VAR. BOTRYTIS) MICROSHOOT PRODUCTION

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The effect of sugar type, source and concentration on *Brassica oleraceae* var *botrytis* microproshoot production

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ABSTRACT

An effective protocol for the mass production of cauliflower microshoots was refined using the meristematic layer of cauliflower curd. The meristimatic layer was excised, homogenized using a commercial blender and separated into desirable size classes and cultured in liquid culture media containing 2 mg/L kinetin, 1 mg/L IBA (indole butyric acid) and different types and concentrations of sugars. Among several concentrations of sucrose derived from sugar beet, the use of 3 % concentration was found to be the optimal. Fructose, glucose and maltose were also tested at 1.5, 3, and 4.5 % concentrations and compared with the use of 3 % of sucrose which was considered as a standard (control). The best explants response was obtained using maltose but without a significant difference compared with the control. The effect of the source of sucrose on the development of cauliflower culture was also investigated using different concentrations of sucrose derived from both sugar cane and sugar beet. The use of 4.4 % sugar cane sucrose was found to be the best in terms of the number of developing microshoots. The results reported in this study helps to increase the effectiveness of the cauliflower micropropagation system and to reduce the cost of micropropagule per unit of production.

INTRODUCTION

Kieffer et al (2001) designed an effective protocol for cauliflower micropropagation involving the use of a commercial blender and sieves for the production of explants and this has recently been revised by Rihan et al (2011). As the meristematic tissue derived from cauliflower curd has no chlorophyll, all the required elements necessary for growth should be provided by the culture medium especially in the early stages of explant development. Carbohydrates (sugars) are essential as a source of carbon for energy for biosynthesis (Amiri and Kazemitabar, 2011) and also have an stabilizing osmotic effect *in-vitro*. Sugars can have a large effect on the development of plant cultures (Gibson, 2001). The type and the concentration of sugar used with the culture medium should always be considered to obtain optimal results in micropropagation systems (Mendoza and Kaeppler, 2002). Sucrose has

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frequently been used as an essential carbohydrate source in plant culture media. Chen (1978) reported that the use of a high level of sucrose induced the growth of callus. However, they also demonstrated that the callus derived from 9 % sucrose containing medium differentiated into more albino plants than those from low concentrations. The use of 2-5% sucrose was suggested to be suitable for anther culture in rice. Usually the growth and development of cultures increases with sugar concentration until it reaches an optimum level and then decreases when higher concentrations are used (Thapa et al., 2007).

Although sucrose has been chosen for use with the vast majority of plant micropropagation culture media in the past, recent work suggests that it is not always the most effective sugar to be used (Amiri and Kazemitabar, 2011). Michel et al (2008) reported that the percentage of induction and dry weight of callus in cotton are more efficient with glucose followed by fructose and sucrose. Maltose was also indicated to be a superior source of carbohydrate than sucrose in several other plant species (Last and Brettell, 1990; Pande and Bhojwani, 1999; Shahnewaz and Bari, 2004). Furthermore, t has been demonstrated that morphogenesis was attributable to the concentration and type of carbohydrate used with the culture media (Romano et al., 1995). Glucose also has been used as the preferred source of carbon in culture media used for the micropropagation of several plant species (Michel et al., 2008; Salvi et al., 2002).

This study aimed to investigate the effect of several types and concentrations of sugars used with culture media on the development and growth of cauliflower microshoots. Moreover, the effect of the sucrose source (sugar beet and sugar cane) on the cauliflower micropropagation system used was tested.

MATERIAL AND METHODS

Plant material

Curds of three varieties of cauliflower, DIWAN, CENDIS and GALICIA, were obtained from Sainsbury supermarket (variety names were obtained from courtesy of Simmonds & Sons Ltd (Cornwall)). The use of 3 varieties gave a continuous supply of cauliflower heads over the experimental period.

Production of micro-explants

Large pieces (1-5 cm) of curds were surface sterilized by immersion in 10% (by volume) un-thickened domestic bleach (0.06% sodium hypochlorite) for 15 min followed by a double wash with sterile distilled water. Explants were produced in a laminar flow hood by mechanically eliminating the mass of non-responsive tissue (stem branches) and shaving off the upper meristematic layer using a sterilized scalpel. The meristimatic clusters were then homogenized using a commercial blender (Waring model 800) at approximately 1700 rev/min in maintenance S23 liquid medium (4.4 g/L MS (Murashige and Skoog, 1962) (Sigma, M5519-50L) + sucrose 30 g/L) for 30 s. The liquid containing the microexplants was then sieved through precision sieves (212, 300 and 600 µm) (Endecotts Ltd., London, UK). The microexplants were collected off the sieves, weighed and converted to aliquots of explants using small precision volumetric measures (74 or 240 ± 2 µL). 100 mL containers, each containing 30 mL S23 medium supplemented with 2 mg/L Kinetin, 1 mg/L IBA, 1 mL/L PPM (plant preservative mixture) to control contamination and various concentration and types of sugars depending on the experiment were cultured with a constant volume of explants (74 µL). The pots were constantly shaken (150 rev/min) during culture at 20 oC and exposed to 16 h photoperiod provided by fluorescent lighting (150 mol mol m2) until the number and average weights of microshoots were obtained.

The effect of sucrose concentration

Six concentrations of sucrose derived from sugar beet (Silver Spoon^{TM}, Tesco) (0, 1.5% (43 mM) , 3% (86 mM), 4.5% (131 mM) , 6% (172 mM) and 7.5% (215 mM) were used with

the culture media. 10 culture pots (replicates) were used with each treatment. The number and average weights of microshoots were recorded after 25 days of the culture.

The effect of sugar type and concentration

The culture media were supplemented with three types of sugars Glucose (1.5% (83.26 mM), 3% (166.52 mM) and 4.5 % (249.78 mM)), Fructose (1.5 % (83.26 mM), 3% (166.52 mM) and 4.5% (249.78 mM)) and Maltose (1.5% (22.81mM), 3% (45.63 mM) and 4.5% (68.45 mM). The effects of these sugars were investigated and compared with effect of using 3% sucrose (sugar beet) which was considered as a standard. Five culture pots (replicates) were used with each treatment. The number and average weights of microshoots were recorded after 25 days of the culture.

The effect of sucrose source

Five concentrations of sucrose derived from sugar cane (Tate & Lyle^{TM}, Tesco) (0, 1.5, 3, 4.5 and 6%) were used with culture media and their effects were compared with use of 3% of sucrose derived from sugar beet (silver spoon, Tesco).). Seven culture pots (replicates) were used with each treatment. The number and average weights of microshoots were recorded after 25 days of the culture.

Results are presented as means + standard error (S.E.). All data were subjected to analysis of variance (ANOVA) using Minitab software (version 15) and comparisons of means were made with least significant difference test (LSD) at 5 % level of probability.

RESULTS

The effect of sucrose concentration

The use of sugar with culture media was shown to be an essential requirement for cauliflower explants growth since none of them grew using sugar free culture media (Fig 1). The optimal microshoot number was obtained using 3% sucrose at high significant difference compared with other treatments ($P < 0.001$). The highest microshoot average weight was obtained using 4.5% of sucrose at high significant differences compared with the other treatments ($P < 0.001$). The use of relatively high concentration of sucrose affected the colour of microshoots and led to an increase in the level of anthocyanin (Fig 2). Therefore, the use of 3% of sucrose was recommended to produce highest number of good quality (colour) of cauliflower microshoots.

The Effect of sugar types in concentrations

Sugar type had a highly significant effect on the number of microshoots produced ($P< 0.001$). The use of maltose was found to be the best comparing with other types of sugars used. However, no significant difference was observed between the use of maltose and sucrose in terms of the number of microshoots. A significant effect of the sugar concentration was observed ($P< 0.01$) and the use of 1.5 % was recommended. No significant interaction was found between sugar type and concentration ($P=0.099$) (Fig 3).

Concerning the effect of sugar type and concentration on the average weights of microshoots produced, while no significant effect of the sugar type was observed ($P=0.09$), a highly significant effect of sugar concentration was found ($P<0.01$) and the optimal concentration was found to be 1.5%. A highly significant effect of the interaction between sugar type and concentration ($P<0.001$) was observed and the optimal treatment was found to be 1.5% fructose (Fig 3). However, this treatment could not be recommended considering the very low number of microshoots that developed using this treatment (Fig 4).

Overall in this experiment, the use of 1.5% maltose gave optimal number of microshoots but without a significant difference compared with the use of 3% of sucrose (control). The low average microshoot weight observed using these treatments could be caused by the big number of growing microshoots and the competition for limitingnutrients in the culture media.

The use of 3% sucrose was recommended because the cheaper cost of this sugar and the positive influence on the economics of the protocol efficiency.

The effect of sucrose source

A highly significant effect of the sucrose source was observed and the highest microshoot number was obtained using 4.5% sugar cane ($P< 0.001$) (Fig 5). However, although the use of sugar cane significantly reduced the average weight of microshoots ($P<0.001$), this could be caused by the big number of growing microshoots and the competition for the availability of other nutrient elements from the culture media. The use of 4.5% of sugar cane was recommended to be used with cauliflower culture media

DISCUSSION

Plant, cell and tissue culture usually requires a carbohydrate source in order to satisfy energy demands (Amiri and Kazemitabar, 2011) and sucrose has been used widely as a main carbohydrate source in plant tissue culture (Shahnewaz and Bari, 2004). The use of sucrose significantly increased the growth of micropropagated potatoes (Mohamed and Alsadon, 2010; Pruski et al., 2002). Mohamed and Alsadon (2010) reported that the use of higher concentration than 30 g/L resulted in a significant lower content of chlorophyll in the plantlet of micropropagated potato. However, the use of a higher than specific concentration of carbon source with the culture media could result in negative effects on the plant materials caused by an excessive osmotic contribution or by the toxicity of the carbon source (Ślesak et al., 2004) and this could explain the colour change observed in this study when higher than 3% sucrose was used in the culture media. The optimal concentration of sucrose in a medium should be enable to satisfy the energy requirements for cell division and differentiation without having any negative osmotic effect on shoot formation (Javed and Ikram, 2008). In agreement with the current results, the use of 2 to 3% sucrose in micropropagation media has been widely reported (Hazarika et al., 2004).

Although, sucrose has been used in the most of the work done in the field of *in vitro* micropropagation (Ahmad et al., 2007), it has not been always the best option. Michel et al (2008) indicated that glucose was sugar that induced the best respond of cotton callogensis. Salvi et al (2002) demonstrated that the shoot length and number and length of roots was significantly increased when glucose was used with the medium. Maltose has been also reported as a superior sugar compared with sucrose in many species such as cereals (Last and Brettell, 1990; Pande and Bhojwani, 1999). Ren et al (2010) reported that the use of maltose significantly increased the frequency of callus formation in wheat. However, in agreement with our results, Baskaran and Jayabalan (2005) demonstrated that sucrose was a better option than glucose and fructose for the regeneration of Eclipta alba shoot. The use of sucrose was reported to be the optimal compared with fructose and glucose for the regeneration of Vigna radiate (Amutha et al., 2003). Nowak et al (2004) demonstrated that the use of sucrose was better than glucose for organogenesis of *Prunus domestica*. Indeed, it seems to be that the type and concentration of carbon source used with plant tissue culture depends on the species, type and age of growth material (Sul and Korban, 1998).

Sugar cane has been identified as a plant which contains a high amount of sucrose and sugar cane juice has been used as a source of carbon in the micropropagation of different plant species. Sul and Korban (1998) reported that the use of sugar cane juice gave a better results than graded granular sucrose in *Pinus sylvestris*. The use of sugar cane juice was also found to be suitable for Grand Naine banana micropropagation (Kodym and Zapata-Arias, 2001). However, according to our knowledge, there is no study that has reported a comparison between the sugar types, sugar cane and sugar beet, in terms of their suitability for plant tissue culture. The current study is the first report of the superior effects of sugar cane to be used for cauliflower micropropagation. However, further analysis is still required to find out

the component differences between these two sugars and to determine the constituent that has the reported positive effect of sugar cane.

CONCLUSION

The optimal sugar type and concentration suitable for the use with cauliflower micropropagation system was determined. Although the use of maltose and sucrose gave the optimal effect on the cauliflower microshoot development, the use of sucrose was recommended as a cheaper option. Moreover, this study highlighted the effect of the source of sucrose used with the culture media on the effectiveness of the cauliflower micropropagtion. This study was the first to demonstrate the superior effect of sugar cane compared with sugar beet for the use with this culture system. The results reported in this study will play a role reducing the cost of cauliflower micro-propagule production.

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Figures

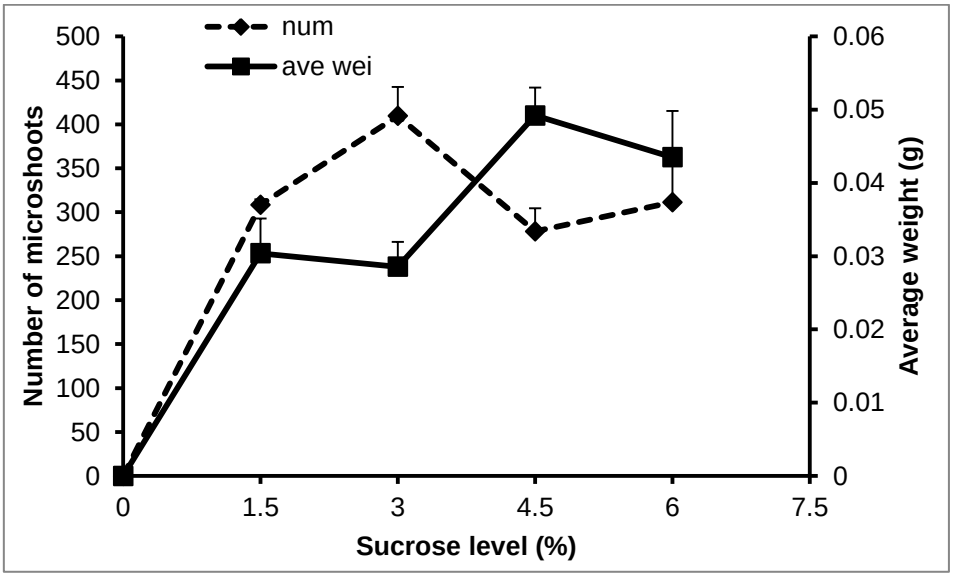

Fig.1.The effect of sucrose concentration used with the culture media on the number and average weight of cauliflower microshoots (LSD=35.43 for microshoot number, LSD=0.0051 for the micoshoots average weight)

Fig.2. A) Microshoots produced using 4.5% sucrose concentration in the culture media. B) Microshoots produced using 1.5 % sucrose in the culture media.

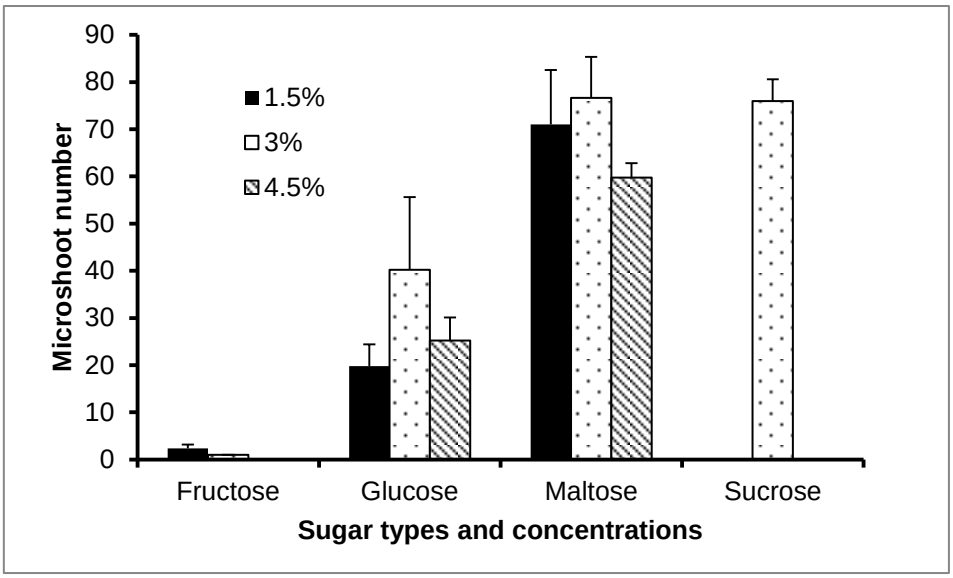

Fig. 3. The effect of sugar types and concentrations on the number of microshoots produced (LSD= 8.18 for both sugar types and concentrations)

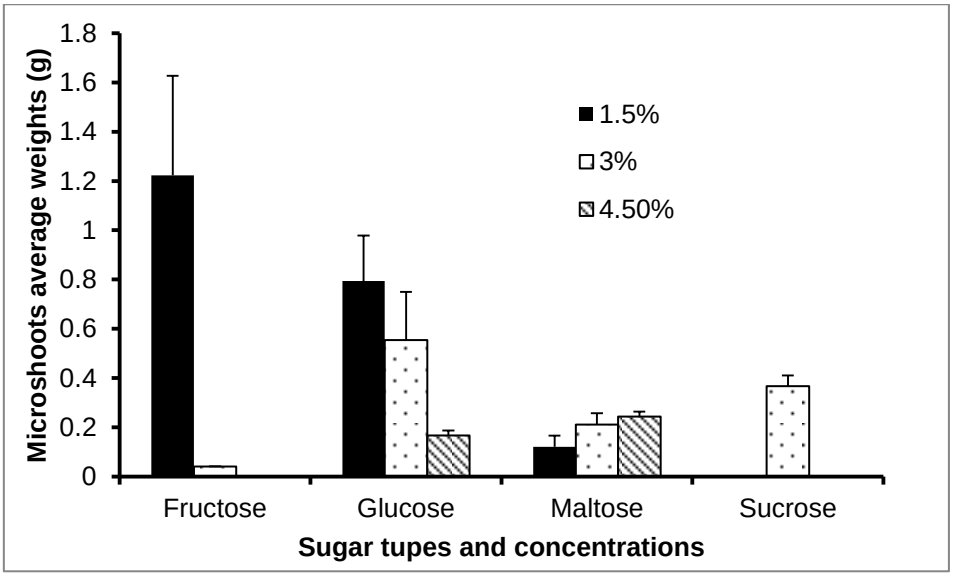

Fig. 4. The effect of sugar types and concentrations on the average weights of microshoots produced (LSD=0.216 for the sugar concentration and LSD=0.376 for the interaction between the sugar type and concentration).

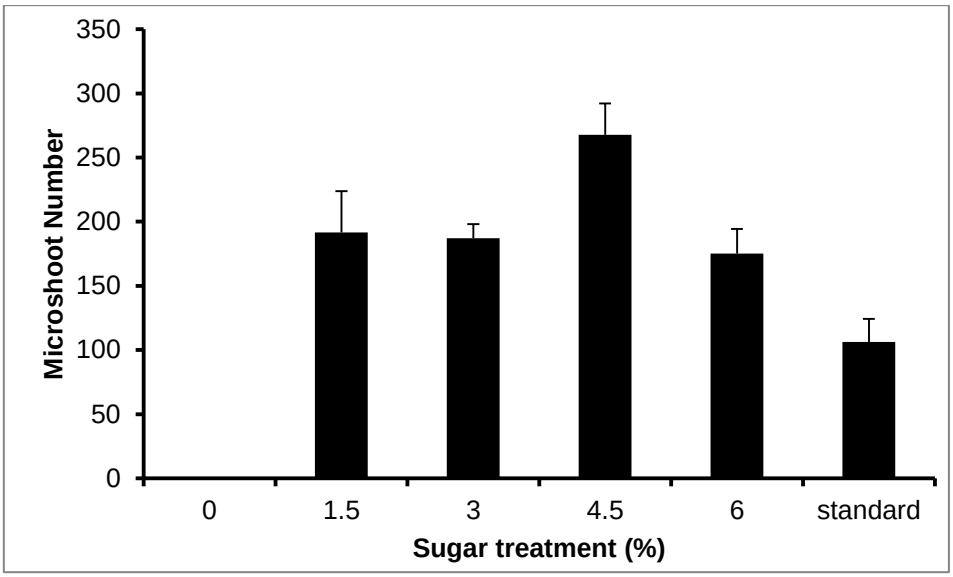

Fig. 5. The effect of sugar (sucrose) source used with culture media on the number of growing microshoots (LSD=14.83)

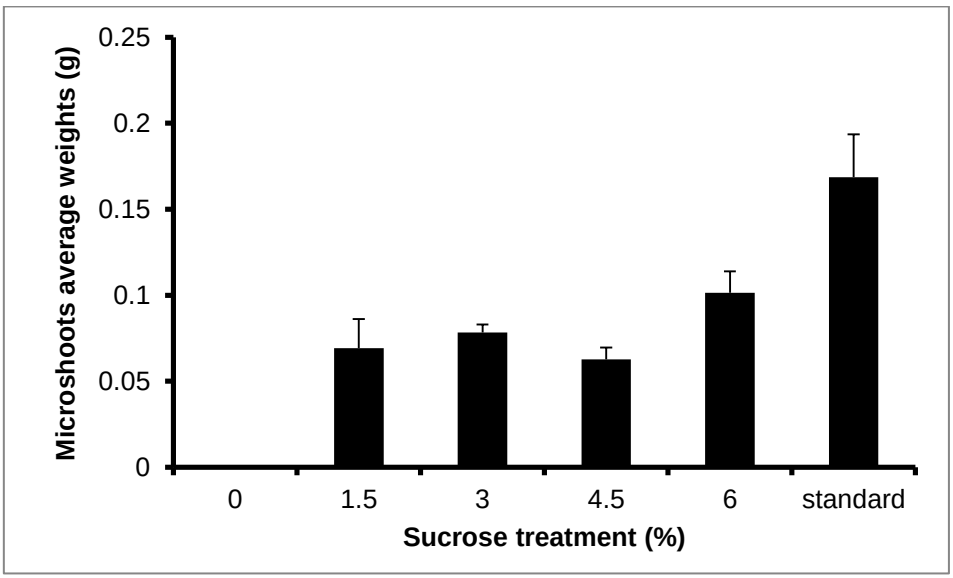

Fig. 6. The effect of sugar cane concentration used with culture media compared with the use of 3% of sugar beet (standard) on the average weights of microshoot produced (LSD=0.028).