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Relative importance of maltose and sucrose supplied during a 2-step potato microtuberization process

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Abstract

A 2-stage *in vitro* tuberization process comprising first micropropagation *via* nodal explants and then tuber induction in the resultant *in vitro* plantlets was studied using 2 cultivars of potato, Iwa and Daeji. In particular, the effects on both plantlet growth and subsequent *in vitro* tuberization of Murashige and Skoog (1962) basal medium containing either sucrose or maltose, each at 3 % (w/v), used for micropropagation were investigated. Sucrose and maltose were found to be equally effective in supporting development of vigorous plantlets from the nodal explants of both potato cultivars. Upon transfer to a medium with an optimised level of sucrose (*i.e*. 8 %, w/v) for *in vitro* tuberization, only the plantlets previously grown in the sucrose-containing medium were capable of forming more microtubers of the larger size category (greater than 0.5 g). The relative importance of sucrose supply at the mircropropagation stage was further confirmed when the resultant plantlets grown in the 3 % sucrose-containing medium were transferred to an *in vitro* tuberization medium containing either sucrose or maltose, each at 8 % (w/v). In this experiment, maltose and sucrose had indistingushable effects on *in vitro* tuberization.

Introduction

Sucrose has been shown to be a very important factor for potato *in vitro* tuberization. For instance, in a two-step *in vitro* tuberization process comprising first growing *in vitro* potato plantlets from nodal explants and then microtuber formation by the resultant plantlets, 8 % (w/v) sucrose alone in the tuberization medium was found to be sufficient without the need for any exogenous plant growth regulators (Leclerc *et al.* 1994, Yu *et al.* 2000).

The effect of substituting sucrose with maltose on *in vitro* tuberization has been studied (Khuri and Moorby 1995). It was found that a mixture of 4 % (w/v) sucrose and 4 % (w/v) maltose was inferior to 8 % (w/v) sucrose for *in vitro* tuberization. However, the effect of maltose alone in the tuberization medium was not established. Furthermore, in this study the nodal expants were placed right from the beginning of the 2-step tuberization process on a medium containing the mixture of dissacharides. Like most other studies on a two-step process of microtuber formation, there was no due regard to the distinct nature of the two steps involved. As a consequence, it is not possible to determine how

much direct influence the medium had on plantlet growth and microtuberization.

Zarrabeita *et al*. (1997) demonstrated that a 'carry-over' effect of a lower level of nitrogen supply in the micropropagation medium leading to the development of more vigorous plantlets which formed microtubers earlier than those plantlets grown in a higher level of nitrogen during the micropropagation step. Here we investigated the effect of varying the carbohydrate source in either the micropropagation or tuberization medium on *in vitro* tuberization. In particular, our objective was to determine if the use of maltose-containing medium can lead to the production of commercially *via*ble microtuber yields, *i.e*. more microtubers of fresh weights greater than 0.5 g (Yu *et al.* 2000). This should distinguish it from most published microtuber production methods which have little significance for successful direct field planting of potato microtubers.

Materials and Methods

Stock plantlet culture and maintenance

I*n vitro* stock potato plantlets (*Solanum tuberosum* L. cvs. Iwa and Daeji) were regularly propagated using single nodal segments every 6-8 weeks as follows. A single nodal segment of a previously propagated stock plantlet was placed in a clear polycarbonate jar (64 mm ID x 80 mm with a total capacity of 250 cm^3) containing 40 cm^3 MS basal salts and vitamin mixture (Murashige and Skoog 1962), supplemented with 30 $g \cdot dm^{-3}$ sucrose and 7 g/dm3 agar (Germantown Company, New Zealand). This and other media used in this work were all autoclaved for 20 min at 121 °C and 103 KPa after their pH was adjusted to 6.0. The cultures were kept in a growth room at 23 °C with constant illumination of 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the top of the tissue culture jars from white fluorescent lamps (Philips).

Standard experimental regimes

A sequential two-stage process was used for *in vitro* tuberization unless indicated otherwise. First, the plantlet development stage began when three stem segments, each comprising 2 nodes excised from the previously propagated stock plantlet culture were transferred to the same type of polycarbonate jars containing 25 cm^3 MS salts and vitamin mixture supplemented with 30 $g \cdot dm^{-3}$ sucrose. The culture was maintained in a growth room with the same conditions as described for stock plantlet growth for 4 weeks. At the end of the plantlet development stage, the medium in each culture jar (now with 3 potato plantlets) was drained off and replaced with 50 cm^3 of *in vitro* tuberization medium comprising MS salts and vitamin mixture supplemented with 8 $g \cdot dm^{-3}$ sucrose. The culture was maintained at 20 °C in darkness for 10 weeks.

Variations to the standard experimental regimes

In some experiments, the standard medium for plantlet development stage was modified as follows: 3 % (w/v) sucrose was substituted with 3 % (w/v) maltose (plant tissue culture tested; Sigma Chemical Co., St. Louis, USA). After the plantlet development stage (4 weeks) in this modified medium, the standard *in vitro* tuberization regime was used.

In other experiments, after the standard plantlet development stage, *in vitro* tuberization of the resulting plantlets was studied using the standard tuberization medium as well as a modified tuberization medium containing 8 % (w/v) maltose instead of sucrose.

In the above modified media, maltose solution was filter-sterilized through 0.22 µm membrane filters (type GS, Millipore Corporation, USA) before it was added to the autoclaved MS basal medium.

Determination of plantlet growth

The effects 3 % (w/v) sucrose or maltose on fresh and dry weight changes during the plantlet development stage were determined. Each treatment consisted of a total of 20 tissue culture jars. Three stem segments of the appropriate potato cultivar were placed in each of the jars. All the jars in the 2 treatments were arranged randomly in the growth room. Every week over a 4-week period, 5 jars from each treatment were chosen randomly and the plant materials were pooled, surface dried and weighed. This experiment was repeated at least twice with similar results.

Evaluation of in vitro tuberization

For all the treatments, microtubers were harvested after 10 weeks from the start of the *in vitro* tuberization stage. The number of microtubers formed by plantlets within a tissue culture jar was counted, but the fresh weight of the microtubers found in all replicate jars of a treatment were determined and recorded individually. Each jar was a replicate and there were 20 jars in each treatment. The jars from different treatments were arranged randomly. All experiments were repeated at least twice with similar results.

Data analysis

The data from a typical replicate experiment on microtuber number and average microtuber weight were evaluated using Analysis of Variance (ANOVA) and when required, other post ANOVA procedures for comparison of means in Statistix for Windows (Version 7.0) at a significance level of p=0.05 unless stated otherwise. Microtuber fresh weight distributions were classified according to 5 categories (less than 0.25, 0.25-0.50, 0.50-0.75, 0.75-1.0, and greater than 1.0 g) and the data were converted to percentages for comparison.

Results

Development of *in vitro* potato plantlets each comprising an upright shoot and a long green root occurred equally well, within 4 weeks of culture of the 2 nodal stem segments of cvs. Iwa and Daeji under continuous illumination, when the liquid MS basal medium was supplemented either with 3 % (w/v) sucrose (*i.e.* the standard plantlet multiplication medium) or 3 % (w/v) maltose (a modified plantlet multiplication medium). The time courses of fresh weight changes over 4 weeks also revealed that overall there was little difference in the rate of plantlet development of both cultivars (Iwa: Fig. 1; Daeji: data not shown) in the sucrose or maltose-containing medium.

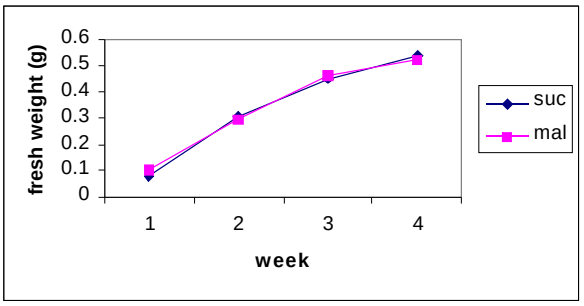


Fig. 1. Time course of potato (cv. Iwa) plantlet fresh weight increase in response to medium containing either 3 % (w/v) maltose (mal) or sucrose (suc).

The effects of prior culturing of the potato plantlets of cvs. Iwa and Daeji in media containing 3 % (w/v) sucrose or maltose on the number of microtubers formed, their average fresh weights and relaive size distribution following 10 weeks of the standard *in vitro* tuberization protocol were investigated. The number of microtubers formed by Iwa or Daeji plantlets was found to be similar (about one per plantlet) in the standard 8 % (w/v) sucrose-containing *in vitro* tuberization medium regardless of whether the plantlets were transferred from 3 % (w/v) maltose or 3 % (w/v) sucrose-containing plantlet multiplication medium.

The standard protocols, *i.e.* the transfer of Iwa plantlets from the standard 3 % surcrose-containing plantlet multiplication medium to the standard 8 % sucrose-containing *in vitro* tuberization medium, resulted in higher microtuber fresh weight when compared to the transfer from the modified multiplication medium containing 3 % (w/v) maltose to the standard *in vitro* tuberization medium (Fig. 2; Bonferoni's com-

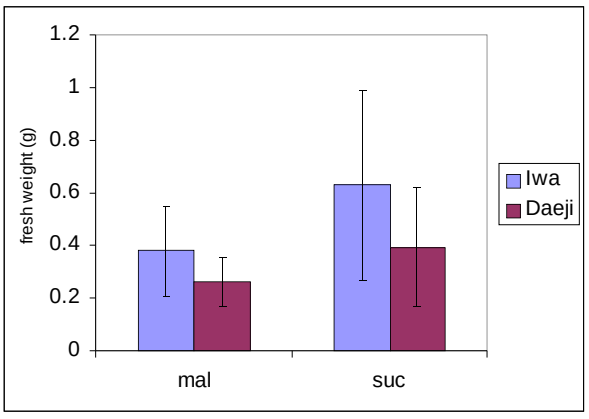
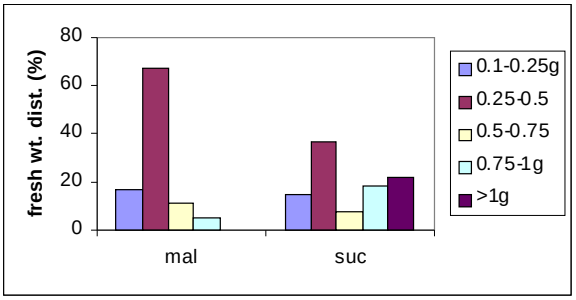
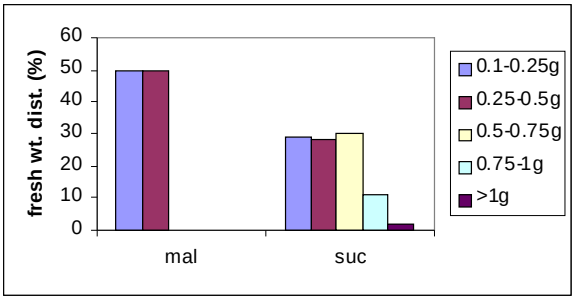


Fig. 2. Effect of plantlet multiplication medium containing either 3 % (w/v) maltose (mal) or sucrose (suc) on average fresh weights of microtubers harvested at the end of the standard microtuberization step using potato cultivars 'Iwa' and 'Daeji'. Only the Iwa results are significantly different from each other (Bonferoni's comparison of means, P=0.01 level, Statistix for Windows, version 7.0).





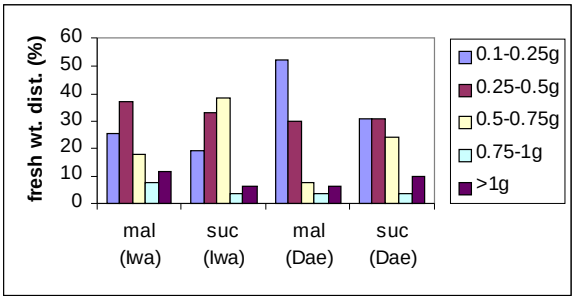


Fig. 3. Effect of plantlet multiplication medium containing either 3 % (w/v) maltose (mal) or sucrose (suc) on fresh weight distribution of microtubers harvested at the end of microtuberization of potato cultivar Iwa in a standard *in vitro* tuberization medium.

Fig. 4. Effect of plantlet multiplication medium containing either 3 % (w/v) maltose (mal) or sucrose (suc) on fresh weight distribution of microtubers harvested at the end of microtuberization of potato cultivar Daeji in a standard *in vitro* tuberization medium.

Fig. 5. Fresh weight distribution of microtubers formed by potato plantlets of cultivars 'Iwa' and 'Daeji' (Dae) in response to *in vitro* tuberization media containing 8 % (w/v) maltose (mal) or sucrose (suc). The microtubers were harvested after 10 weeks of culture on these media.

parison of means, Statistix for Windows, P=0.01). In the case of the same experiments with Daeji, statistical analysis of the results reavealed no significant differences in average microtuber weight (Fig. 2; ANOVA P=0.05).

From an examination of the frequencies of microtubers formed in different fresh weight categories (Figs. 3 & 4), it is clear that the response to the standard *in vitro* tuberization medium is influenced by the carry-over effects of the plantlet multiplication medium. Both the Iwa and Daeji plantlets transferred from the standard multiplication medium containing 3 % (w/v)sucrose tended to form more larger microtubers (greater than 0.5 g fresh weight) and fewer smaller ones when compared to the transfer from the modified plantlet multiplication medium containing 3 % (w/v) maltose. The Daeji results are particularly contrasting as only microtubers smaller than 0.5 g fresh weight were formed (Fig. 4).

The converse experiments were also performed for comparison. Potato plantlets of cvs. Iwa and Daeji were transferred after the standard plantlet development stage, *i.e.* on medium containing 3 % (w/v) sucrose to the standard *in vitro* tuberization medium containing 8 % (w/v) sucrose or that modified to contain 8 % maltose instead of sucrose. The number (about 1 microtuber per plantlet in both cultivars), average fresh weight (Iwa: about 0.5 g; Daeji: about 0.4 g), and relative size distribution of microtubers (Fig. 5) formed by both cultivars were very similar in both sucrose- and maltose-containing *in vitro* tuberization mediuim.

When maltose was supplied both during stage 1 and the *in vitro* tuberization step, *in vitro* tuberization was inferior to the standard experimental regimes (data not provided). This is similar to the finding of Khuri and Moorby (1995).

Discussion

The *in vitro* experiments here on the 2 cultivars of potato support our contention that it is important to study the effects of variations in culture media with due regard to the plantlet development stage and the ensuing *in vitro* tuberization step. In particular, it is clear that the influence of sucrose or maltose in this 2-step *in vitro* tuberization process without addition of any exogenous plant growth regulators is largely different in the 2 component steps.

While the resultant potato plantlets from the sucrose or maltose-containing medium appeared similarly vigorous, it was noted here that only the sucrose-grown plantlets produced more of the larger,

commercially desirable size microtubers in response to the standard *in vitro* tuberization medium. This suggests that the 'carry-over' influence of plantlet vigour, if any, cannot fully explain the inferior performance of maltose-grown plantlets as far as forming microtubers in the standard *in vitro* tuberization medium containing 8 % (w/v) sucrose is concerned.

There is a general agreement in the literature that sucrose is required in the medium for *in vitro* potato tuberization (*e.g*. Fung *et al.* 1972, Estrada *et al.* 1986, Khuri and Moorby 1995, Gopal *et al*. 1998, Xu *et al.* 1998, Yu *et al.* 2000). Here, the data indicate that sucrose can be substituted with maltose to produce commercially *via*ble microtuber yields (*i.e*. fresh weight greater than 0.5 g), provided sucrose is used in stage 1 of the *in vitro* tuberization regime.

Sucrose or maltose at the equivalent concentration of 3 % (w/v) in the medium appears to be interchangeable as far as potato micropropagation is concerned, *i.e*. during stage 1. In *Albizzia* root explants, however, it has been found that maltose rather than sucrose favors the regeneration and development of healthy and vigorous plantlets (El Maataouri *et al.* 1998). In some anther cultures and somatic embryogenesis systems, maltose has also been shown to be more preferrable to sucrose in the medium. For example, maltose has been found to improve embryo induction and development in anther culture of petunia (Raquin 1983), potato (Batty and Dunwell 1989), and in the formation of mature somatic embryos in *Albies nordmanniana* (Norgaard 1997), and in *Medicago sativa* (Strickland *et al*. 1987).

The mechanism as to how maltose from the medium can support these 2 processes in potato plantlets and the other processes in other plants as reported in the literature is largely not known. Khuri and Moorby (1995) have already shown that a medium containing 4 % (w/v) maltose supplemented with 4 % sucrose or glucose led to lower tuberization performance compared to 8 % sucrose. It is therefore unlikely that a small amount (less than 0.05 %) of glucose or sucrose that might be present in the commercially available maltose used in our experiments might be responsible for the results obtained. Furthermore, our preliminary unpublished data suggest that maltose in the plantlet multiplication medium or *in vitro* tuberization medium remained largely intact, *i.e*. not hydrolyzed. This is in sharp contrast to the known fate of sucrose in the *in vitro* tuberization medium, i.e. rapid and complete hydrolysis (Yu *et al*. 2000, Yoon and Leung, unpublished data). Further research is needed to clarify the role of maltose in potato microtuberization.

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