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BRIEF COMMUNICATION

Effect of different saccharides on viability of isolated microspores and androgenic induction in *Zea mays*

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Abstract

Sucrose, glucose, fructose, and melibiose in different concentrations and combinations in the induction media influenced the viability of the isolated maize microspores and the formation of multinuclear structures. The induction of multinuclear structures on media containing combination of sucrose, fructose and glucose was lower than on media only with sucrose. In media containing melibiose alone or in combination with sucrose, no induction of multinuclear structures was found, however, microspore viability was improved.

Additional key words: gametic embryogenesis, haploid, maize.

The production of haploid and doubled haploid plants from anther or isolated microspore culture represents a powerful tool for numerous biotechnological applications (*e.g.* Jähne and Lörz 1995). Although the *in vitro* production of haploids and doubled haploids has been realised in many agricultural crops, its use is limited in maize anther and isolated microspore culture due to the genotype dependence (Kuo *et al.* 1978, Dieu and Beckert 1986, Barloy *et al.* 1989), and the low response rates (Genovesi and Collins 1982, Petolino and Jones 1986). Successful plant regeneration from microspore cultures was firstly reported by Coumans *et al.* (1989) and Pescitelli *et al.* (1990). The isolated microspore culture system has several advantages when compared to anther culture, *e.g.*, manipulation with single cells, absence of undesirable interactions between anther wall and microspores and a better entry to biotechnological applications (Preťová *et al.* 1993). Several factors are known to be of crucial importance concerning anther or isolated microspore culture response: *e.g.* genotype, quality of the donor plant, microspore developmental stage, media composition, cold pretreatments (Pescitelli *et al.* 1990, Büter 1997).

Importance of sugars and osmotic potential of cultivation media for isolated microspore culture was shown by Pescitelli *et al.* (1990). These factors influencing initiation of embryogenesis were studied in more details in barley microspore cultures (Hoekstra *et al.* 1993, Scott and Lyne 1994). Not only the choice of sugar, but also the method of media sterilisation (filter sterilisation or autoclaving), was found to have an effect on anther culture. Filter sterilisation of media was reported to provide increased production of embryo like structures (ELS) compared with autoclaved media (MacDonald 1992, Büter *et al.* 1993). Even better effect have been obtained when activated charcoal was added to the induction media prior to autoclaving (Büter *et al.* 1993).

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The early abortion of microspores generally observed during the first days of culture might be related to a suboptimal sugar supply and high osmotic potential in the culture. Therefore, in our experiments the effect of different concentrations and combinations of sugars and the influence of changing osmotic potential in course of microspore cultivation on the viability of microspores, and the induction of multinuclear structures was investigated. The influence of the sterilisation technique and the effect of activated charcoal was also tested.

Zea mays L. (genotype ETH-M 24, a hybrid produced at the Institute of Plant Sciences of ETH, Zürich, Switzerland) donor plants were grown in greenhouse at the day/night temperature 25/18 °C and 18-h photoperiod. Tassels were harvested prior to their emergency from the leaf whorl. Harvested tassels were slightly moistened with sterile water, wrapped in paper towels and aluminium foil and kept for 14 d at 7 °C. Treated tassels contained microspores predominantly in the mid- to late uninucleate developmental stages. Subsequently the tassels were cut into 5 - 6 cm segments and surface sterilised in sodium hypochlorite (0.6 % v/v) for 15 min and rinsed with sterile distilled water three times. Spikelets taken from tassel segments were cultured for 3 d at 14 °C in a preculture medium containing 0.6 M mannitol, 50 mg dm^{-3} ascorbic acid, and 125 mg dm^{-3} L-proline, pH 5.8. Then the anthers were removed from the spikelets and placed into Petri dishes containing induction media supplemented with addition of 0.1 mg dm^{-3} 2,3,5-tri-iodobenzoic acid (TIBA), 0.25 mg dm^{-3} L-thiamine HCl, 1.3 mg dm^{-3} nicotinic acid, 15 mg dm^{-3} L-asparagine, and different sugars (Table 1).

For microspore isolation a blending procedure was used (Pescitelli *et al.* 1990). Homogenate was then passed through a 113 μm sieve. The microspores were collected by centrifugation (170 *g*, 5 min). In order to eliminate the nonviable microspores a Percoll gradient (20 % Percoll in induction medium and centrifugation at 380 *g* for 3 min) was used. Fraction of viable microspores were washed and suspended in induction media and cultivated in Petri dishes (Falcon grade, diameter 50 mm) in the dark at temperature 28 °C. Volume of microspore suspension in Petri dish was 2 cm^3. Culture density of microspores (determined by hemocytometer) was in the range from 30 000 to 40 000 microspores per cm^3. Osmotic potential of the induction medium was measured with a *Vapor Osmometer* (*Wescor 8 000*, Logan, USA). Viability of cultured microspores was determined by fluorescein diacetate (FDA) staining according to Widholm (1972). Multinuclear structures were observed after DAPI staining (Vergne 1987) in 10 × 100 microspores.

We investigated the impact of different carbohydrates, different concentrations of saccharides, activated charcoal (5 g dm^{-3}) and sterilisation techniques on medium osmotic potential, microspore viability and induction of androgenesis in maize microspore cultures. As a control we used induction media without microspores. The osmotic potential of all induction media used was adjusted to the osmolality of media with 9 % of sucrose at the beginning of the experiment (Table 1).

Table 1. Concentrations [%] and combinations of saccharides, sterilization mode, and activated charcoal (5 g dm^{-3}) used in different induction media (IM).

	Sucrose	Fructose	Glucose	Melibiose	Sterilization	Charcoal
IM 1	9.0	-	-	-	filter	-
IM 2	9.0	-	-	-	autoclave	+
IM 3	9.0	-	-	-	autoclave	-
IM 4	1.5	1.5	1.5	-	filter	-
IM 5	4.5	-	-	4.65	filter	-
IM 6	2.2	-	-	6.94	filter	-
IM 7	-	-	-	9.25	filter	-

The osmotic potential in microspore cultures did not changed substantially during the first 2 d. Afterwards, the osmotic potential decreased continuously with time of cultivation in all IM used (Table 2). The rapid decrease in osmotic potential in microspore cultures occurred when the amount of viable microspores in the culture dropped below 40 % as a result of released content of aborted microspores. Osmotic potential of cultivation media has been shown to be an important parameter in microspore cultures of barley (Hoechstra *et al.* 1993) and wheat (Zhou *et al.* 1994).

The viability of microspores and the induction of androgenesis was affected by the type of sugar because the carbon source is believed to represent a key factor in tissue culture system. The induction of multinuclear structures on IM 4 containing combination of sucrose, fructose and glucose was lower than on media only with sucrose. In media containing melibiose alone or in combination with sucrose, no induction of multinuclear structures was found. The presence of melibioase, however, improved microspore viability (Table 2).

Scott and Lyne (1994) presented data on incubation of barley microspores in the presence of sucrose, glucose, or fructose resulting in death of cells whereas maltose could sustain development of embryoids and calli. Results obtained with isolated maize microspore culture showed, that attempts to replace sucrose by maltose, raffinose, cellobiose, lactose, trehalose led to decrease of androgenic response (Pescitelli *et al.* 1994). As our results indicate, sucrose in the concentration 9 % revealed to be suitable for microspore cultivation and initiation of androgenesis for genotype used in our experiment. Similar results were obtained in maize anther culture where addition of sucrose resulted in significantly higher overall plant production than all other sugars tested (Pescitelli *et al.* 1994, Büter 1997).

Number of multinuclear structures was higher on filter sterilised media or on autoclaved media with activated charcoal (Table 2). Earlier results of embryo like structures (ELS) production from maize anther cultures also showed that the method of media sterilisation had an effect on anther culture (MacDonald 1992, Büter *et al.* 1993). This effect is probably due to sucrose hydrolysis by high pressure and temperature during autoclaving (*e.g.* Hsiao and Bornman 1991).

Table 2. Osmotic potentials [mOs kg^{-1}] of media, viability of microspores [%], and induction frequency [%] of multinuclear structures in microspore cultures at the beginning of cultivation and after 4 and 7 d of cultivation.

	Osmotic potential			Viability		Induction frequency
	0 d	4 d	7 d	0 d	4 d	7 d
IM 1	284	326	334	78.2 ± 4.9	5.5 ± 0.8	1.4 ± 0.6
IM 2	277	325	332	80.6 ± 5.2	8.2 ± 1.6	1.5 ± 0.9
IM 3	283	330	335	77.6 ± 4.1	6.3 ± 1.3	1.1 ± 0.6
IM 4	283	315	340	81.6 ± 4.7	5.7 ± 1.1	0.8 ± 0.4
IM 5	274	338	360	95.0 ± 5.2	36.0 ± 4.1	0
IM 6	275	355	365	98.0 ± 6.1	34.4 ± 3.8	0
IM 7	281	319	335	93.8 ± 7.7	35.2 ± 3.1	0

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