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Microspore cultures as donor tissue for the initiation of embryogenic cell suspensions in barley

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Abstract

We have initiated embryogenic cell suspension cultures of barley (*Hordeum vulgare* L.) 'Igri' from isolated microspore cultures. Data were obtained on the time required for establishment, frequency of establishment, i.e. number of calluses out of the total number of initiations giving rise to suspensions, and embryogenic capacity of the suspension cultures. For comparison, establishment of embryogenic cell suspensions from callus derived from immature zygotic embryos of 'Igri', 'Dissa' and 'Golden Promise' was also carried out. The results revealed that embryogenic suspension cultures were established in half the time and with a seven-fold higher frequency from microspore cultures than from zygotic embryo-derived calluses. The suspension cultures were still capable of embryo formation after two years. However, only albino plantlets were regenerated. For comparison, long term callus cultures derived from microspores, anthers and zygotic embryos were established. From the anther and zygotic embryo-derived callus cultures green plants were continuously regenerated, whereas the microspore-derived callus cultures lost this ability after the second subculture.

Introduction

In gramineous species, the major pathway for plant regeneration from cultured tissues and cells is somatic embryogenesis. For this reason, embryogenic cell suspension cultures provide a system for studies of cell differentiation and embryo formation from totipotent cells of grasses. Embryogenic cell suspensions have been established from most of the cereal crops, including rice (Kyozuka et al. 1987; Datta et al. 1990), maize (Prioli &Söndahl 1989; Shillito et al. 1989; Sun et al. 1989; Mitchell & Petolino 1991), wheat (Vasil et al. 1990) and barley (Lazzeri & Lörz 1990).

In barley, zygotic embryo-derived callus has been used for the establishment of morphogenic suspensions (Lührs & Lörz 1988; Lazzeri & Lörz 1990; Yan et al. 1990). However, the initiation phase was approximately six months long and suspensions were established successfully in only one out of ten initiations, presumably due to problems with adaptation of the calluses to growth in liquid medium (Lührs & Lörz 1988). Recently, Jähne et al. (1991a) reported cell suspensions of barley established from anther cultures for the isolation of totipotent protoplasts. An efficient regeneration system from isolated microspores of 'Igri' is now available (Hunter 1988), which also includes culture in liquid medium.

In this study, we have investigated whether microspore cultures adapt more easily to the culture conditions in liquid medium. We com-

pare here the production of embryogenic suspension cultures from two different donor materials, i.e. microspore cultures of 'Igri' and callus derived from immature zygotic embryos of 'Igri', 'Dissa' and 'Golden Promise'.

Materials and methods

Plant material for initiation of cell suspensions

Microspore cultures

Microspore cultures of 'Igri' were provided by Dr. R.B. Jørgensen, Risø National Laboratory. Briefly, spikes containing microspores in mid- to late-uninucleate stage were harvested from growth-chamber-grown plants that had been vernalised for 8 weeks at 4°C in the dark immediately after germination, then the plants were grown in 16-h photoperiod with light intensity of 400 μmol m^{-2} s^{-1} (400–700 nm) at a constant temperature, 12°C. The spikes were disinfested and anthers were isolated and incubated for 3–4 days in 0.3 M mannitol in petri dishes at 24°C in the dark. Microspores were then isolated by squashing the anthers and filtering the homogenate through a stainless steel sieve (pore size $\approx$ 100 μm). Microspores were rinsed twice by centrifugation (680 rpm; 2 min) and cultured in liquid FHG medium as described by Hunter (1988). Microspore-derived proembryos developed after 7–14 days of culture. Microspore-derived embryogenic calluses used for initiation of cell suspensions were established by plating 14-day-old microspore cultures on FHG medium (solidified with 8 g l^{-1} Sea Plaque agarose) without growth regulators and subcultured monthly over a period of three months.

Callus cultures derived from zygotic embryos

Greenhouse-grown plants [16-h photoperiod, 400 μmol m^{-2} s^{-1} (400–700 nm); temperature 18°C day/13°C night] of the spring-type barley cultivars 'Dissa' and 'Golden Promise' and the winter-type 'Igri', which had been vernalised and grown as described above, were used. Immature embryos (0.5–1 mm in size were isolated and cultured on modified CC medium (Potrykus et al. 1979) with 30 g l^{-1} sucrose as described by Lührs & Lörz (1987). The composition of the medium was modified further by the addition of 20 ml l^{-1} coconut water instead of 100 ml l^{-1} and by using the amino-acid mixture given by Biester-Miel et al. (1985) instead of 1 g l^{-1} casein hydrolysate. The medium (CC-S) was solidified with 6 g l^{-1} Sigma agarose type 1.

Initiation of suspension cultures

Microspore-derived suspension cultures

In all 15 microspore-derived cultures at different stages were used for the initiation of cell suspensions. Tissue derived from microspores from a single spike were used in each initiation. Initiations were made from microsprore cultures that had been cultured in liquid FHG medium for 5, 9, 14 and 31 days, respectively, and the number of initiations were 1, 6, 4 and 1, respectively. At each initiation the donor material was divided into six samples that were incubated in Nunclon Multidishes (Nunc catl. 152795). Each sample were supplemented with 2 ml of one of the following five media (for details see Table 1): N6 medium (Chu et al. 1975) with 30 g l^{-1} sucrose (N6-S); Kao8p (Kao & Michayluk 1975) with 60 g l^{-1} glucose (Kao8p-G) or 60 g l^{-1} maltose (Kao8p-M) or 40 g l^{-1} cellobiose (Kao8p-C); LM medium (Lazzeri & Lörz 1990) with 60 g l^{-1} glucose (LM-G). All media were supplemented with 9.0 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and the amino-acid mixture instead of 1 g l^{-1} casein hydrolysate, as described above. Also microspore-derived embryogenic calluses cultured for 44 and 90 days, respectively, were used as initiation material. The number of initiations were 2 and 1, respectively. The initiation media are given in Table 1. In each initiation, 2.5 g fresh tissue divided into six samples were incubated.

Zygotic embryo-derived suspension cultures

The friable, embryogenic callus that emerged upon callus induction from the zygotic embryos was selected as donor material. A total of 126 initiations each subdivided into six samples with three different cultivars were carried out. Initiations were carried out from both primary callus and from callus that had been subcultured 1–5 times (for details see Table 2). The calluses were incubated as described previously (Lührs & Lörz

1988). Each initiation used 2.5 g fresh weight tissue. The following three media were used, Kao8p-G, Kao8p-M, FHG medium with 40 g l^{-1} maltose (FHG-M) (for details see Table 2). All media were supplemented with 9.0 μM 2,4-D and amino-acid mixture of Biester-Miel et al. (1985) instead of 1 g l^{-1} casein hydrolysate.

Establishment of long-term callus cultures

Callus cultures derived from microspores
Microspores cultured in liquid FHG medium for 5, 9, 14 and 31 days, respectively, were transferred to solidified CC-S medium. The calluses were cultured as described previously by Lührs & Lörz (1987) and subcultured monthly over a period of eight months.

Callus cultures derived from anther cultures
Anthers of 'Igri' were cultured as described by Olsen (1987). Well-formed embryos from these cultures were selected and transferred to solidified CC-S medium. The calluses were cultured according to Lührs & Lörz (1987) and subcultured monthly over a period of eight months. Ten lines were established.

Callus cultures derived from zygotic embryos
Callus induced on CC-S medium from immature, zygotic embryos of 'Dissa', and 'Igri' were cultured according to Lührs & Lörz (1987) and subcultured monthly for eight months. Seven lines of 'Dissa' and 13 lines of 'Igri' were established.

Establishment and maintenance of suspension cultures

The procedure for establishment of suspension cultures followed the protocol described previously (Lührs & Lörz 1988). At the first subculture, 14–28 days after initiation, the induction media in all responding cultures were replaced by Kao8p-M medium supplemented with 9.0 μM 2,4-D. This medium was used throughout the investigation period. Suspension cultures showing stable growth with doubling time of 4–5 days were subcultured weekly: 0.5 g cell material was subcultured into 2 ml fresh medium.

Testing of morphogenic capacity of suspension cultures

As soon as the suspension cultures were established, i.e. the donor material had been removed and the suspension consisted of aggregates that were uniform in size, released only cytoplasm-rich cell types during culture and showing stable growth (Table 3), their embryogenic capacity was tested by plating 200 mg cell material onto solid CC-S medium supplemented with 9.0 μM 2,4-D according to Lührs & Lörz (1988). After 2 and 4 weeks the cultures were examined for developing globular structures and somatic embryos (embryogenic capacity). These embryos were transferred to seven different regeneration media: CC medium with 30 g l^{-1} sucrose (CC-S), CC-S medium supplemented with 5.7 μM indole-3-acetic acid (IAA) and 0.23 μM zeatin (CC-S-IAA-Zea), FHG medium supplemented with 30 g l^{-1} sucrose (FHG-S), FHG-S medium supplemented with 1.8 μM 6-benzyladenine BA (FHG-S-BA), FHG medium supplemented with 30 g l^{-1} maltose (FHG-M), FHG-M supplemented with 1.8 μM BA (FHG-M-BA), J25-8 medium with 20 g l^{-1} sucrose and 7 g l^{-1} glucose (J25-8-SG) (Jørgensen et al. 1986). The embryos were cultured as described previously (Lührs & Lörz 1987) and examined for plantlet formation after five weeks.

Testing morphogenic capacity of long term callus cultures

Microspore, anther and zygotic embryo-derived callus cultures were tested monthly for morphogenic capacity. Embryogenic structures induced on the maintenance medium CC-S, were selected three weeks after the last subculture and transferred to CC-RI medium (Lührs & Lörz 1987). The embryogenic structures were cultured as described previously (Lührs & Lörz 1987) and examined for plantlet formation after five weeks.

Results

Establishment of suspension cultures

All initiation attempts with microspore-derived tissue yielded well-established and homogeneous

suspension cultures consisting of cytoplasm-rich aggregates each 50–100 cells in size. These aggregates released continuously cytoplasm-rich single cells that formed new aggregates during culture. Fifteen suspension cultures were established from experiments with microspore-derived embryoids. The donor cultures had been grown for different lengths of time (between 5 and 90 days) in FHG medium (Table 1).

Upon incubation of the microspore-derived tissue in cell suspension media, some of the embryogenic structures produced plantlets. This was observed in all experiments and was independent of the age of the donor material and the induction medium. These structures were removed. Other parts of the incubated cell material plasmolysed and died. However, a high proportion of the tissue proliferated within two weeks into yellowish callus clumps from which cells were released into the suspension medium. Both regular-shaped, cytoplasm-rich cells and irregular, highly vacuolated cells were observed (Fig. 1a). Only the cytoplasm-rich cells appeared to divide and form cell aggregates. Weekly subculture of these aggregates resulted in uniform, fastgrowing, i.e. showing a doubling time on fresh weight basis in 4–5 days, suspension cultures (Fig. 1b) within two months (Table 3). In several suspensions of these microspore-derived cultures, cell aggregates with a smooth surface similar to proembryogenic structures were occasionally seen (not shown).

From zygotic embryo-derived calluses of the three cultivars tested, 'Dissa', 'Golden Promise' and 'Igri' suspension cultures were established. However, in all cultivars, only one out of eight initiations was successful. In all, 18 suspensions were established from 126 initiation attempts (Table 2). This low frequency of suspension establishment was due to the fact that most of the calluses incubated in liquid medium turned brown, ceased to grow and died. From 'Dissa' and 'Igri', homogeneous suspension cultures, i.e. similar in composition to the microspore-derived ones, were obtained four months after initiation. In 'Golden Promise', the suspension cultures consisted of different cell types until six months after initiation (Table 3). The calluses that responded to initiation grew and released cytoplasm-rich cells into the suspension medium, as described by Lührs & Lörz (1988).

The medium composition had an influence on the growth of the donor tissue after initiation of the suspensions. In microspore-derived tissue, a

Table 1. Origin of 15 microspore-derived suspension cultures of 'Igri'. Age of donor material, medium used for initiation of cell suspensions, embryogenic capacity, and number of plants regenerated are given.

Type of donor material	No. initiating	Age (days) of donor material (no. of subcultures)	Initiation medium*	No. of suspension cultures	Embryogenic capacity**	No. of plants regenerated from 200 mg cell material in one experiment***
Cell colonies	1	5	Kao8p-G	1	non-embryogenic	0
Proembryos	6	9	Kao8p-G	5	albino plantlets	78
			Kao8p-G	1	embryos	0
Embryos	4	14	LM-G	1	embryos	0
			LM-G	1	plantlets	8
			N6-S	1	plantlets	12
			Kao8p-M	1	non-embryogenic	0
Embryos	1	31	Kao8p-C	1	plantlets	1
Embryogenic callus	2	44 (1)	N6-S	1	embryos	0
			Kao8p-M	1	albino plantlets	13
Embryogenic callus	1	90 (2)	Kao8p-M	1	embryos	0
Total	15			15	13 embryogenic suspensions	

* In the first subculture, 14–20 days after initiation, the initiation medium was replaced by Kao8pM in all cultures.
** Embryogenic capacity was determined by plating 200 mg of suspension cells on CC-S medium as soon as suspensions consist of uniform cell aggregates with stable growth.
*** The number of plants represents the sum obtained on all seven regeneration media in one experiment.

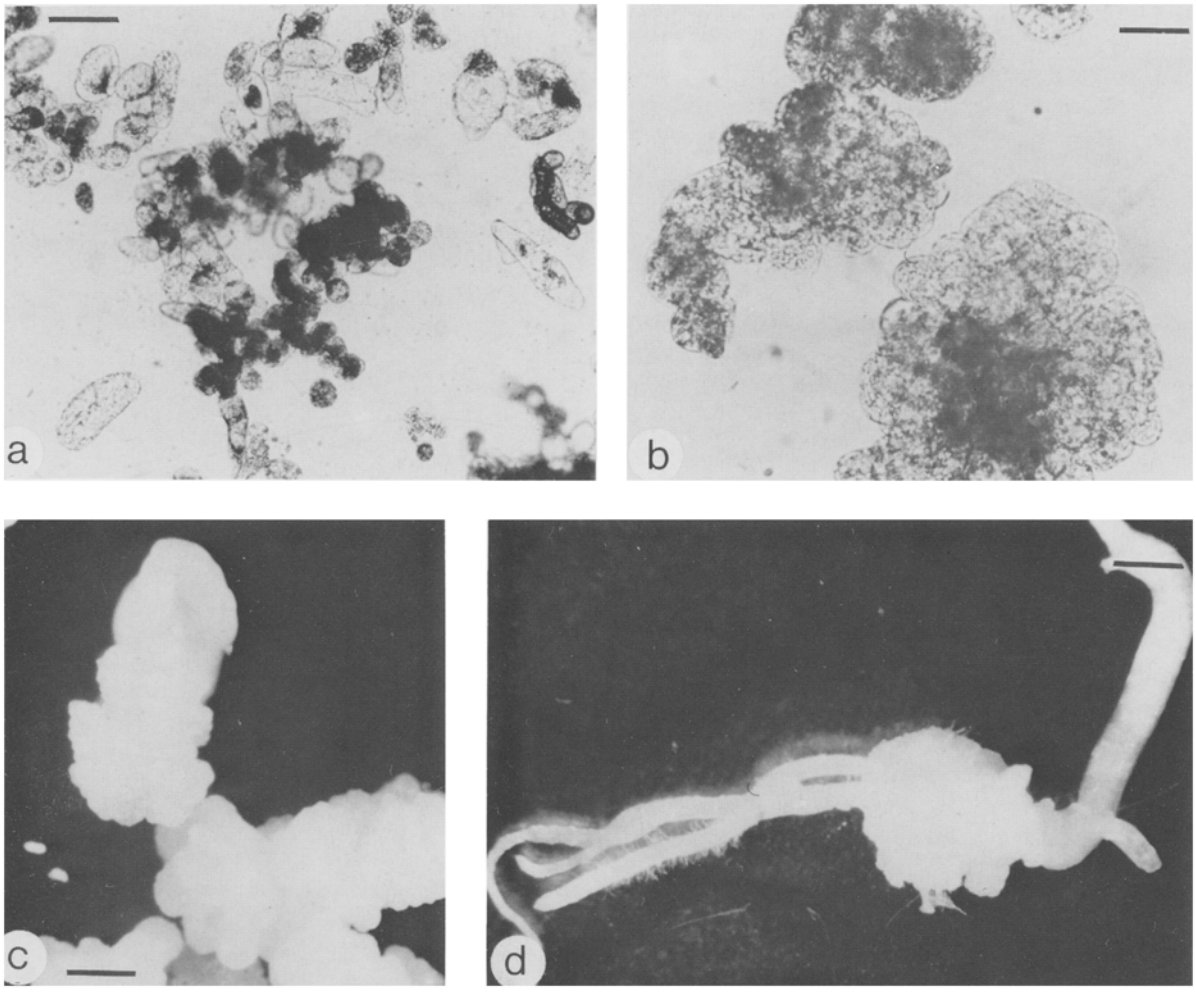

Fig. 1. Initiation of cell suspension cultures from microspore-derived cultures. (*a*) Cells released from callus, bar = 100 μm. (*b*) Small cell aggregates in a homogeneous cell suspension three months after initiation, bar = 50 μm. (*c*) Globular structures and somatic embryos, bar = 3 mm. (*d*) Plantlet development, bar = 3 mm.

major part of the donor material formed aggregates of white, soft callus upon incubation in N6-S medium. Rescue of the non-responding tissue by incubation in Kao8p-M medium resulted in yellowish callus clumps followed by release of single cells into the suspension medium. The Kao8p-M medium also supported growth of the donor tissue more than the Kao8p-G and LM-G media did. The Kao8p-C and Kao8p-M media were equally effective, but only one initiation was carried out with Kao8p-C. From the calluses of zygotic embryos, suspension cultures were obtained only in experiments with initiation in Kao8p-M medium. Experiments in which the FHG-M and Kao8p-G media were replaced with Kao8p-M medium did not result in suspension cultures (data not shown).

Morphogenic capacity of the suspension cultures

Thirteen of the 15 suspension cultures obtained from microspore-derived tissue formed globular structures within two weeks of culture on solid CC-S medium. Within two further weeks, well-

Table 2. Origin of suspension cultures derived from embryogenic callus from zygotic embryos: genotype, initiation medium, age of embryogenic callus, embryogenic capacity and number of plants regenerated are given.

Cultivar	No. of initiations	Initiation medium*	No. of suspension cultures	Age (days) of donor callus (no. of subculture)	Embryogenic capacity**	No. of plants regenerated from 200 mg cell material in one experiment***
Igri	6	Kao8p-G	0			
	8	FHG-M	0			
	6	Kao8p-M	4	15 –	somatic embryos	0
				82 (2)	somatic embryos	0
				97 (3)	embryogenic callus	0
				97 (3)	non-embryogenic callus	0
Dissa	23	Kao8p-G	0			
	16	FHG-M	0			
	37	Kao8p-M	9	13 –	non-embryogenic callus	0
				13 –	plantlets	1
				14 –	embryogenic callus	0
				49 (1)	plantlets	10
				51 (1)	embryogenic callus	0
				106 (3)	somatic embryos	0
				108 (4)	plantlets	2
				119 (4)	plantlets	4
				135 (5)	embryogenic callus	0
Golden	6	Kao8p-G	0			
Promise	4	FHG-M	0			
	10	Kao8p-M	5	12 –	embryogenic callus	0
				14 –	non-embryogenic callus	0
				14 –	embryogenic callus, roots	0
				20 –	non-embryogenic callus	0
				41 (1)	non-embryogenic callus	0
Total	126		18		13 embryogenic suspensions	

* In the first subculture, 28 days after initiation, the initiation medium was replaced by Kao8pM in all cultures.
** Embryogenic capacity was determined by plating 200 mg of suspension cells on CC-S medium as soon as suspensions consist of uniform cell aggregates with stable growth.
*** The number of plants represents the sum obtained on all seven regeneration media in one experiment.

formed embryos were observed (Fig. 1c and Table 1).

In the suspension cultures derived from zygotic embryos, 13 of the 18 cultures showed embryogenic capacity. Six of the embryogenic cultures formed only globular structures, while seven cultures formed well-developed somatic embryos (Table 2). None of the 'Golden Promise' cultures produced well-formed embryos; only globular structures were observed in two

Table 3. Comparison of the production of suspension cultures from microspore-derived tissue and from zygotic embryo-derived callus.

Donor material:	Microspore cultures	Zygotic embryo callus cultures		
Cultivar	Igri	Igri	Dissa	Golden Promise
Frequency of establishment:	100%	20%	12%	25%
Time of establishment:	2 months	4 months	4 months	6 months
Embryonic capacity:	87%	75%	89%	40%
Regeneration capacity*:	60%	none	50%	none

* Frequency calculated as the number of suspensions that yielded plantlets as described in Materials and methods as percent of the total number of established cultures.

Table 4. Morphogenic capacity of long-term callus cultures derived from microspores, anthers and immature, zygotic embryos.

	Microspore cultures	Anther cultures	Zygotic embryos	
	'Igri'	'Igri'	'Igri'	'Dissa'
No. of callus lines	4	10	13	7
No. of lines producing green plantlets	0	3	3	6
No. of lines producing albinos	4*	6	8	1
No. of lines producing both green and albino plantlets	0	1	2	0

* After the first subculture green plants were recovered.

suspensions. Among the cultures of 'Igri' and 'Dissa', well-formed embryos were observed in two and five suspensions, respectively, while the formation of globular structures (embryogenic callus) were observed in one and three cultures, respectively (Table 2).

From nine of the 13 microspore-derived embryogenic cultures, shoots and plantlets (Fig. 1d) were obtained, although all of these were albinos (Table 1). Four of the 13 embryogenic suspension cultures from embryo-derived calluses showed plant regeneration. These four cultures were of 'Dissa' (Table 2). In this case, again, only albino plantlets were obtained. Plantlets were regenerated from the somatic embryos on all seven regeneration media tested. The FHG-S-BA and FHG-M-BA media mainly stimulated shoot formation, i.e. five plants and up to 40 shoots developed from the microspore-derived suspensions, whereas CC-S and CC-S-IAA-Zea media supported both shoot and root development and a higher number of plantlets, i.e. 29 plants and 11 shoots, were formed from the microspore-derived suspensions on these media. On the FHG-M, FHG-S and J25-8-56 media almost equal amounts of shoots and plantlets were formed (11 plants and 18 shoots) (data not shown).

Morphogenic capacity of the long-term callus cultures

Long-term callus cultures were tested for capacity of forming green plants over a period of eight months. The four microspore-derived callus cultures were capable of plant regeneration throughout the eight months of investigation. Green plantlets were however, recovered only after the first subculture of the microspore-derived proembryos on 2,4-D containing CC-S medium. After the second subculture on this medium only albino plantlets were regenerated (Table 4).

In contrast to this, green plantlets were obtained from the anther-derived calluses even eight months after the callus induction. The calluses, however, segregated into three lines producing green plants, six lines producing albino plantlets only, and 1 line that continued to produce green as well as albino plantlets (Table 4).

Also, the calluses derived from immature embryos showed segregation of callus lines regenerating into either green or white plantlets (Table 4). Three of the 13 'Igri' callus lines produced green plants, while eight lines produced albinos and two lines produced both green and albino plantlets. From seven lines of 'Dissa', six produced green plants, while one produced albino plantlets.

Discussion

Microspore cultures have proved to be a valuable source material for the establishment of suspension cultures of barley. Comparison between microspore-derived suspensions and suspensions derived from zygotic embryos showed that the frequency of establishment from microspore-derived tissue was seven times higher. No microspore-derived tissue turned brown or died upon suspension induction, apparently because the donor material possessed the capacity to adapt to the altered culture conditions. Two weeks elapsed between initiation of the suspensions and observation of released single cells. A major part of the friable embryogenic callus obtained from immature zygotic embryos turned brown upon initiation and only a minor part of this donor material started to release cytoplasm-

rich single cells. These cells were not observed until four weeks or more after initiation. The frequency (14%) of establishment of suspension cultures from calluses of zygotic embryos observed in the present work was approximately equal to the frequency (9%) observed by Lührs & Lörz (1988).

The time from initiation to establishment of homogeneous suspension cultures was almost halved when the donor material was microspore-derived tissue rather than zygotic embryo-derived callus. We suggest that the main reason for the faster establishment was that the adaptation phase of the donor tissue described by Lührs & Lörz (1988) was absent in the incubation of microspore-derived embryoids. The absence of an adaptation phase may have been be due to the fact that the isolated microspores were already cultured in liquid medium before they were used as donor material for the initiation of suspension cultures.

The Kao8p-M and Kao8p-C media were found to be superior to the other suspension media tested, and all suspension cultures initiated from calluses of zygotic embryos came from incubation in Kao8p-M medium. The improvement of the growth of *in vitro* cultures by providing specific carbohydrates is well-known, e.g. improved growth and embryo formation are observed in isolated microspores of barley when these are cultured in the presence of maltose instead of sucrose (Hunter 1987). Not surprisingly, we observed improved growth of the microspore-derived tissue upon suspension induction when maltose was used as carbon source. However, the initiation experiments with zygotic embryo-derived callus showed that the growth of this tissue too was supported better in the maltose-containing medium Kao8p-M than in the medium Kao8p-G, i.e. the calluses adapted faster to the liquid conditions.

From 15 initiations with microspore-derived cultures, 13 embryogenic suspensions were established. Using zygotic embryo-derived calluses from 126 initiations, 13 embryogenic suspensions were obtained. The frequency of establishment of embryogenic cultures from zygotic embryo-derived callus was higher (72%) than in the former work (Lührs & Lörz 1988) where two out of seven suspensions (28%) had morphogenic capacity. This higher frequency may have resulted from very strict selection of the embryogenic callus and incubation of this callus when it was developing globular structures, i.e. 12–20 days after subculture. The establishment of embryogenic suspension cultures from embryogenic callus has been reported to be difficult (Vasil & Vasil 1986; Göbel & Lörz 1988; Lazzeri & Lörz 1990). The important step was found to be the selection of the friable embryogenic callus type (Vasil & Vasil 1986; Lührs & Lörz 1988).

All of the embryogenic cultures initiated from microspore-derived embryoids possessed the capacity for formation of well-developed somatic embryos, while one-half of the embryogenic cultures from zygotic embryo-derived calluses were capable only of development of globular structures. This could be due to the fact that the microspore-derived suspensions were cultured in maltose-containing medium throughout the entire *in vitro* period. The time of establishment may also influence the embryogenic capacity. The microspore-derived suspensions were established in half the time required for the suspensions from zygotic embryos. The capacity of the embryogenic suspensions to produce these embryos has so far been maintained for one-and-a-half years after establishment.

Embryogenic suspension cultures did not in all cases possess the capacity to regenerate plants. In the microspore-derived cultures, 70% of the embryogenic cultures possessed this capacity, while in zygotic embryo-derived cultures, only 50% of the embryogenic 'Dissa' suspensions and none of the embryogenic cultures of 'Golden Promise' and 'Igri' showed plantlet formation.

The analysis of plantlet formation was performed using seven different regeneration media, and when plantlets did not develop on any medium tested, it is possible that the embryos were unable to germinate into plantlets. The reason for the regeneration of albino plantlets only may have been the high ammonium concentration in the culture medium, e.g. in anther culture media (Olsen 1987), the concentration of ammonium ions was lowered and substituted with glutamine in order to prevent formation of albino plantlets. Also the successful regeneration of green plants from anther-derived suspensions of barley was carried out in medium

with lowered concentration of ammonium nitrate (150 mg as opposed to 650 mg in the present work) (Jähne et al. 1991b). It should, however, be noted that the microspore-derived callus cultures lost their capability of regenerating green plants after the second subculture, whereas anther-derived callus lines kept this capability.

The efficient formation of well-differentiated embryos from the microspore-derived suspension cultures provides a valuable system for studies of embryogenesis in *in vitro* cultures of barley. Most of the basic studies concerning molecular and biochemical aspects of embryogenesis have been performed with embryogenic carrot suspension cultures (Borkird et al. 1988; de Vries et al. 1988; Slay et al. 1989). Hitherto, only a few reports on biochemical events taking place during *in vitro* culture of gramineous species exist, e.g. Ramagopal (1989), Rao et al. (1990), Moreira et al. (1990) and Coppens & Dewitte (1990) and in only one report had embryogenic suspension cultures been employed as the experimental system (Hahne et al. 1988). The method for initiation of embryogenic suspension cultures of barley described in the present report provides an easy and efficient method for establishment of cultures for studies on somatic embryogenesis. The present cultures have been used in studies on extracellular proteins and their regulation during embryogenesis (Nielsen & Hansen, unpubl. data). Also three isoforms of chitinase and one isoform of β-1,3-glucanase present among the extracellular proteins from these suspensions have been purified and characterized as isoforms identical to the ones present in barley grains during maturation (Kragh et al. 1991).

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