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Efficient in vitro plant regeneration from immature zygotic embryos of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and *Sorghum bicolor* (L.) Moench

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Abstract We report here an in vitro culture system that provides reliable, highly efficient regeneration from immature embryos of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and sorghum [*Sorghum bicolor* (L.) Moench]. Immature embryos were isolated 10–20 days after pollination and cultured on various L3 media. The influence of different parameters during the callus induction phase was examined with respect to the regeneration rate: (1) the concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and various cytokinins; (2) the addition of $AgNO_3$; (3) the use of maltose or sucrose as a carbon source. Modifications in the phytohormones alone resulted in the regeneration of fertile sorghum plants at high efficiency. Significant increases in the regeneration rates of pearl millet genotypes were achieved by the combination of sucrose as a carbon source and silver nitrate as a potential ethylene inhibitor.

Keywords *Pennisetum glaucum* (L.) R. Br. (pearl millet) · *Sorghum bicolor* (L.) Moench (sorghum) · In vitro culture · Immature embryos · Somatic embryogenesis

Abbreviations *BAP*: 6-Benzylaminopurine · *2,4-D*: 2,4-Dichlorophenoxyacetic acid

Introduction

Genetic engineering having the aim of improving traits of important crops requires efficient and reproducible in vitro culture systems. In pearl millet and sorghum, plant regeneration has been described using immature inflorescences (Brettell et al. 1980; Vasil and Vasil 1981), shoot apices (Devi et al. 2000; Lambé et al. 1999; Zhong et al. 1998) and mature (Botti and Vasil 1983; MacKinnon et al. 1987; Thomas et al. 1977) and immature embryos (Gamborg et al. 1977; Lambé et al. 1999; Ma et al. 1987; Vasil and Vasil 1981) as explants.

In previous reports we have described efficient transformation systems for important monocotyledonous species – for example maize, wheat and triticale – all based on an in vitro culture system initiating from immature embryos (Becker et al. 1994; Brettschneider et al. 1997; Zimny et al. 1995). Likewise, Casas and colleagues (1993) used immature embryos for the transformation of sorghum. The low transformation rate (2 transgenic plants per 600 bombarded embryos) obtained with sorghum (Casas et al. 1993) and the absence of reports of stably transformed pearl millet plants may be a consequence of inefficient in vitro culture protocols.

The purpose of the investigation reported here was to provide a simple, reproducible and efficient in vitro culture system for both pearl millet [*Pennisetum glaucum* (L.) R. Br.] and sorghum [*Sorghum bicolor* (L.) Moench]. The use of immature embryos enabled us to isolate several hundred homogenous explants from single donor plants at the same time. Consequently, the requirement for a controlled environment space in which to grow donor plants was limited. This positive property of the system and the promising results obtained in other cereals make immature embryos attractive as explants for embryogenic cultures of pearl millet and sorghum. Previous reports using immature sorghum or pearl millet embryos rarely quantified the callus induction or regeneration ability of their systems. In those cases where a comparison is possible with respect to callus induction (Lambé et al. 1999; Vasil and Vasil 1981) or regeneration rate (Vasil and Vasil 1981), our system shows a several-fold higher efficiency and thereby may increase the probability of obtaining transgenic sorghum plants at

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higher frequencies or of successfully transforming pearl millet plants for the first time.

Materials and methods

Four pearl millet genotypes [Manga Nara and Bongo Nara from the Savannah African Research Institute (SARI), Ghana; 7402 and 841B from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), India] and 11 sorghum genotypes (Aralba provided by the company Semences de Provence, Arles, France; White Martin supplied by Dr. A. Boyat, Station d'Amélioration des Plantes, INRA, France; IRAT 204, IS 2807, 1630, 69-20, E35-1, 56-60, IS 9549, F2-20 and Sooner Milo from the Centre de Coopération Internationale en Recherche Agronomique pour le Developement (CIRAD), France] were examined. Donor plants were cultivated in the greenhouse under a 16-h (day)/8-h (night) photoperiod (220±20 $\mu E/m^2/s^1$) and changing day/night temperatures (24°C/20°C). Cross-pollination was prevented by placing bags over the flowers.

Immature seeds were collected between 10 and 20 days after pollination, rinsed for 1 min with 70% ethanol, incubated for 30 min in 2% sodium hypochlorite, 0.5% Mucasol (Brand, Wertheim, Germany) and washed three times with sterile, distilled water. Immature embryos were excised under aseptic conditions and placed on callus induction media (Table 1) with the embryo axis facing the medium.

L3 medium (Jähne et al. 1991), solidified with 0.3% Gelrite (Roth, Karlsruhe, Germany), served as a basal nutrient medium for all experiments. Various concentrations of 2,4-D and cytokinins were added to the nutrient medium for the culture of sorghum. For pearl millet, maltose was compared with sucrose (3%) as a carbon source, and the addition of silver nitrate (5 mg/l) was examined.

After a callus induction phase in the dark at 26°C, calli were transferred to regeneration medium R or L3 (Table 1) and cultured under light conditions (70±5 $\mu E/m^2/s^1$) for 16 h per day. Rooting was supported on half-strength, hormone-free L3 medium containing 1.5% sucrose. Subculturing was carried out weekly on callus induction medium and every 2 weeks on regeneration medium. The callus induction rate represents the proportion of immature embryos that formed an embryogenic callus. The regeneration rate reflects the number (mean value ± standard deviation) of plated immature embryos that not only formed callus but also led to the regeneration of plantlets. Regenerated plantlets with a shoot length of 3–10 cm were planted in soil for further growth in the greenhouse. Seed set and the production of progeny confirmed the fertility of the regenerated plants.

The data were analysed using the Student's *t*-test for the comparison of the means of two populations with equal standard deviations and the ANOVA test was used when the means from three or more populations were compared.

Results and discussion

L3 medium served as the basal medium for the in vitro culture of *Sorghum bicolor* and *Pennisetum glaucum*. L3 basal medium, which differs from MS basal medium (Murashige and Skoog l962) mainly with respect to ammonium nitrate, vitamin and amino acid content, has previously been shown to be suitable for the callus induction and regeneration of recalcitrant cultures, for example, the microspores of cereals (Mordhorst and Lörz 1993). The effects of phytohormones (2,4-D and BAP), carbon source (maltose and sucrose) and silver nitrate on the regeneration from immature embryos were studied (Table 1).

Between 50 and 120 zygotic sorghum embryos with a length of 1.0–3.0 mm (Fig. 1b) from genotypes Aralba and White Martin were plated on 13 callus induction media (A–E, I–P; Table 1) containing different auxin and cytokinin concentrations. The highest callus induction rates (81–90%) for both genotypes were achieved when culture media either without BAP or with a low BAP content (A–E) were used (Table 2). Although described and recommended for the culture of immature sorghum embryos (Ma et al. 1987; MacKinnon et al. 1986), increased concentrations of the different cytokinins, such as in media I–P, drastically reduced the callus induction of the immature sorghum embryos in our system (Table 2).

To determine the regeneration rate with respect to the callus induction media, we transferred all embryogenic calli that were produced on medium I–P and at least 20

Table 1 Composition of culture media

Culture medium[a]	2,4-D (mg/l)	BAP (mg/l)	Kinetin (mg/l)	Zeatin (mg/l)	Maltose (M) or sucrose (S) (30 g/l)	$AgNO_3$ (mg/l)
L3	0	0			S	0
A	1.0	0			S	0
B	1.5	0			S	0
C	2.0	0			S	0
D	2.5	0			S	0
E	2.5	0.1			S	0
F	2.5	0.1			M	0
G	2.5	0.1			S	5.0
I	2.5	0.5			S	0
J	2.5		0.5		S	0
K	2.5			0.5	S	0
L	0.5	1.0			S	0
M	1.5	1.0			S	0
N	1.0	1.0			S	0
O	1.0		1.0		S	0
P	1.0			1.0	S	0
R	0	1.0			S	0

[a] Media A-R are L3-based with modifications as indicated

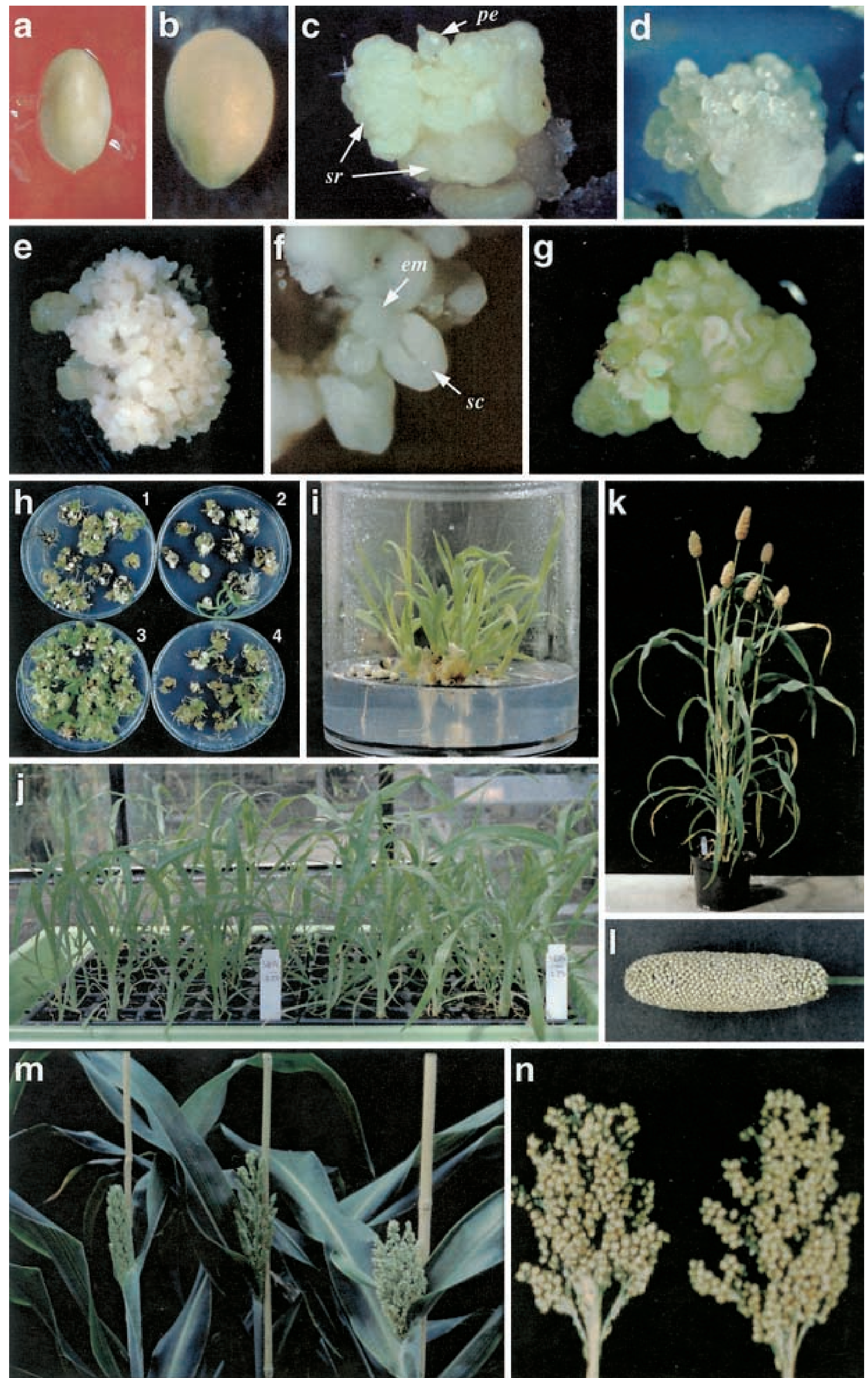

Fig. 1a–n Differentiation of somatic embryos and regeneration from immature embryos (scutella) of *Pennisetum glaucum* (L.) R. Br. and *Sorghum bicolor* (L.) Moench. **a, b** Isolated immature embryo with scutellum side upwards from **a** pearl millet (1.0 mm in length) and **b** sorghum (1.5 mm in length). **c** Scutellar ridges (*sr*) are well-developed, and the first proembryoids (*pe*) are formed, 9 days after isolation (pearl millet explant). **d** Numerous embryoids are visible 12 days after the start of callus induction (sorghum explant). **e** Pearl millet callus containing numerous, fully developed somatic embryos after 4 weeks of culture. **f** Somatic embryogenesis is shown by the formation of scutellum (*sc*) and embryo (*em*) from sorghum callus after 3 weeks of culture. **g** Multiple embryos showing chlorophyll synthesis after 2 weeks of culture under light conditions (pearl millet explant). **h** Clearly improved regeneration rate of sorghum calli induced on medium E and regenerated on medium R (*petri dish 3*) compared to calli induced similarly on medium E but regenerated on medium L3 (*petri dish 1*). Low regeneration frequencies were observed when calli were induced on medium D and regenerated on medium L3 (*petri dish 2*) or R (*petri dish 4*). **i** Formation of multiple green shoots and roots 4 months after isolation of an immature pearl millet embryo. **j** Regenerants were further cultured in the greenhouse. **k–n** Pearl millet **(k, l)** and sorghum **(m, n)** regenerants flowering and showing normal seed set

embryogenic calli from each of the genotypes Aralba and White Martin, which were produced on media A–E, to medium R or to hormone-free L3 medium (Table 2). Regeneration rates were evaluated after 10 weeks of culture. Nearly no plants regenerated when embryos were induced on media I–P, containing 0.5 and 1 mg/l cytokinin. The highest numbers of regenerated plantlets per plated sorghum embryo (12.5±2.3 for Aralba and White

Table 2 The influence of callus induction and regeneration media on regeneration rates from immature sorghum and pearl millet embryos (*SD* standard deviation)

Sorghum genotypes	Callus induction media	Plated embryos [a]	Callus induction rate[b]	Regeneration media (transferred calli)	Regeneration rate[c] on L3 or R medium ± SD	
Aralba, White Martin	A	212	81%	L3 (40)/R (24)	$1.0^{A}\pm0.6/8.0^{C}\pm2.5$	
	B	209	87%	L3 (44)/R (46)	$3.8^{B}\pm1.2/4.8^{B}\pm1.7$	
	C	220	89%	L3 (43)/R (50)	$1.8^{A}\pm1.0/8.5^{C}\pm2.9$	
	D	205	90%	L3 (46)/R (44)	$2.0^{A}\pm1.0/2.3^{A}\pm1.1$	
	E	240	86%	L3 (47)/R (49)	$3.5^{B}\pm1.5/12.5^{D}\pm2.3$	
	I	100	<10%	R (5)	0	
	J	100	<10%	R (7)	0	
	K	109	<10%	R (3)	0	
	L	113	<10%	R (9)	<1.0	
	M	106	<10%	R (9)	0	
	N	118	<10%	R (7)	0	
	O	111	<10%	R (8)	0	
	P	114	<10%	R (2)	0	
Pearl millet genotypes					Proportion of plated embryos showing regeneration	
7042	E	568	84%	R (96)	72%	$6.5^{E}\pm1.7$
	F	655	83%	R (99)		$5.7^{E}\pm1.6$
	G	596	85%	R (199)		$8.9^{C}\pm2.1$
Manga Nara	E	320	70%	R (61)	50%	$5.9^{E}\pm2.6$
	F	367	87%	R (58)		$4.1^{B}\pm1.8$
	G	186	81%	R (49)		$6.8^{E}\pm2.3$
Bongo Nara	E	272	69%	R (41)	68%	$4.3^{B}\pm1.9$
	F	228	91%	R (43)		$3.8^{B}\pm1.3$
	G	150	84%	R (31)		$4.9^{B}\pm2.0$
841B	E	382	89%	R (56)	34%	$1.5^{A}\pm0.7$
	F	225	89%	R (20)		$1.0^{A}\pm0.6$
	G	166	82%	R (81)		$2.8^{F}\pm0.8$

[a] Number of plated embryos per medium
[b] Percentage of plated embryos forming embryogenic callus
[c] Average number of regenerated plants per plated embryo ± SD (means followed by the same uppercase letters are not significantly different at P=0.05 according to the ANOVA test)

Martin) were obtained by callus induction on medium E and subsequent regeneration on medium R. Therefore, a low concentration (0.1 mg/l) of BAP during the callus induction phase and a subsequent increased concentration (1.0 mg/l) in the regeneration medium were necessary and sufficient to clearly improve the regeneration from immature embryos (Fig. 1h, plates 1–4).

In an independent experiment, these conditions (medium E and R) were used to compare the regeneration ability of 11 sorghum genotypes (Aralba, White Martin, IRAT 204, IS 2807, 1630, 69-20, E35-1, 56-60, IS 9549, F2-20 and Sooner Milo; Table 3) under culture conditions as listed in Table 4. The regeneration rates varied greatly among the genotypes, thereby enabling us to separate them into categories of relatively high (11.1–13.4), medium (4.1–7.2) and low regeneration rates (1.2–2.3) (Table 3).

Based on the positive results with immature sorghum embryos, the hormone combination of medium E was used for callus induction of immature pearl millet embryos ranging from 0.5–1.2 mm in length (Fig. 1a). The callus induction rate of pearl millet genotypes was between 70% and 91% (Table 2) and reflects the data from two to six independent experiments. In each experiment,

Table 3 Genotypes of *Pennisetum glaucum* and *Sorghum bicolor* (regeneration rate ± SD/number of plated embryos) (*SD* standard deviation)

Pennisetum glaucum[a]	*Sorghum bicolor*[a]
7042 (8.9C±2.5)	Aralba ($11.9^{D}\pm3.6$/24)
Manga Nara (6.8E±0.8)	White Martin ($12.4^{D}\pm3.6$/22)
Bongo Nara (4.9B±2.0)	IRAT 204 ($13.4^{D}\pm3.1$/21)
841B (2.8F±0.8)	IS 2807 ($11.1^{D}\pm2.9$/24)
	1630 ($7.2^{E}\pm1.9$/21)
	69-20 ($5.4^{B}\pm1.3$/22)
	E35-1 ($4.5^{B}\pm1.8$/21)
	56-60 ($4.1^{B}\pm1.2$/23)
	IS 9549 ($2.3^{A}\pm1.1$/21)
	F2-20 ($1.5^{A}\pm0.8$/22)
	Sooner Milo ($1.2^{A}\pm0.9$/21)

[a] Means followed by the same letters are not significantly different at P=0.05 according to the ANOVA test

at least 50 immature embryos were isolated per genotype and callus induction medium. The formation of scutellar ridges (Fig. 1c) and the development of numerous somatic embryoids (Fig. 1d) and embryos (Fig. 1e, f) took place within 3–4 weeks of in vitro culture and is in ac-

Table 4 Optimised culture conditions (*SD* standard deviation)

	Sorghum bicolor	*Pennisetum glaucum*
Callus induction medium	E (Table 1)	G (Table 1)
Callus induction period	3–4 weeks	3–4 weeks
Regeneration medium	R (Table 1)	R (Table 1)
Regeneration period	4–6 weeks	4–12 weeks
Rooting medium	Half-strength L3	Half-strength L3
Rooting period	2–4 weeks	2–4 weeks
Genotypes with highest regeneration rates (average number of regenerated plants per plated embryo ± SD)	Aralba[a] (12.1±3.6) White Martin[a] (12.6±3.4) IRAT 204 (13.4±3.1) IS 2807(10.7±3.2)	7042 (8.9±2.5) Manga Nara (6.8±0.8)

[a] Regeneration rates for these genotypes include the data from Tables 2 and 3

cordance with the detailed analysis of Taylor and Vasil (1996). In a few cases, a translucent non-embryogenic callus consisting of hyperhydritic cells as described by others (Lambé et al. 1999; Vasil and Vasil 1981) developed, which never showed regeneration.

The average regeneration rates were determined 10 weeks after the transfer of at least 20 calli to regeneration medium R (Table 2). The results in Table 2 summarise the data from two to six experiments with the exception of genotypes 841B and Bongo Nara, which were induced on media F and G, respectively. On medium E, the highest regeneration rate for pearl millet (mean value of 6.5 for genotype 7042) was significantly lower than the highest rate achieved with the sorghum genotypes (mean values of 11.1–13.4 for genotypes Aralba, White Martin, IRAT 204 and IS 2807) (Table 2 and 3).

The effect of maltose and $AgN0_3$ during the callus induction phase was examined with respect to regeneration from immature pearl millet embryos. Both components markedly influenced the regeneration frequencies of all four pearl millet genotypes. Whereas the use of maltose (medium F, Table 1) instead of sucrose led to a general reduction in regeneration frequencies (12–33%), the addition of silver nitrate (medium G, Table 1) significantly increased average regeneration rates by 14–87% depending on the genotype. Maltose, which has been reported to be an advantageous carbon source for in vitro culture systems of anthers and microspores from cereals (Cai et al. 1992; Jähne et al. 1991; Last and Brettell 1990; Mordhorst and Lörz 1993; Orshinski et al. 1990), proved to be unsuitable for the in vitro culture of immature pearl millet embryos. The beneficial effect on regeneration of the potential ethylene inhibitor silver nitrate supports results obtained with immature maize embryos (Vain et al. 1989) and pearl millet inflorescences (Pius et al. 1993).

The highest regeneration rate, with an average of 8.9 plantlets per plated immature pearl millet embryo, was obtained with genotype 7042 on medium G (Table 2). Single immature embryos resulted in the regeneration of more than 20 plants (Fig. 1i) within 10 weeks after transfer of the former to regeneration medium R. When calli were divided and subcultured for an additional 2–3 weeks, in excess of 100 plants were regenerated in some cases. These regeneration rates show the potential of pearl millet immature embryo culture but were not taken into account in Table 2 because of the extended subculture time.

Our system thus provides a clear increase in the average number of regenerating plantlets per plated embryo (about 9 for genotype 7042) and an essentially higher percentage of calli showing regeneration relative to levels reported in the literature on the culture of immature pearl millet embryos. The percentage of plated embryos forming calli and showing regeneration was calculated for each genotype, taking into account all three different callus induction media (E, F and G). Dependent on the genotype, the percentage of regenerating explants ranged from 34% (841 B) to 72% (7042) (Table 2), with a mean value of 56% when averaged over all four pearl millet genotypes.

Vasil and Vasil (1981), who were the first to describe somatic embryogenesis of pearl millet, achieved an average number of four to five plants developing from an explant and a percentage of regenerating calli of about 30%. Between 20% and 26% of calli showed plant regeneration in the report of Lambé and co-workers (1999), but the mean number of regenerated plants was calculated per 100 mg fresh weight of calli, which allows no direct comparison to our results.

For sorghum as well as for pearl millet, a lack of regeneration could be correlated with the production of a brown pigment. This observation has also been described by others (Dunstan et al. 1978; Gamborg et al. 1977; Vasil and Vasil 1981) and can be reduced by weekly subculturing after isolation of the immature embryos.

The optimised culture conditions for immature embryos are summarised in Table 4. In contrast to immature pearl millet embryos, modifications in phytohormone concentrations (2,4-D and BAP) during the induction of callus from immature sorghum embryos were sufficient to achieve a clear improvement in regeneration rates. Altogether, somatic embryos of pearl millet require a longer period than somatic embryos from sorghum before they start to regenerate, but both species produce healthy and fertile plants with normal seed set (Fig. 1j–n).

The in vitro culture system described here provides a highly efficient regeneration of *Pennisetum glaucum* and *Sorghum bicolor*, both of which have an important eco-

nomic role in African and Asian agriculture. The regenerable embryogenic calli, produced at reliable and high efficiencies from immature embryos, will serve as a suitable target tissue for genetic transformation.

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References

Becker D, Brettschneider R, Lörz H (1994) Fertile transgenic wheat from microprojectile bombardment of scutellar tissue. Plant J 5 2:299–307

Botti C, Vasil IK (1983) Plant regeneration by somatic embryogenesis from parts of cultured mature embryos of *Pennisetum americanum* (L.) K. Schum. Z Pflanzenphysiol 111:319–325

Brettell RIS, Wernicke W, Thomas E (1980) Embryogenesis from cultured immature inflorescences of *Sorghum bicolor*. Protoplasma 104:141–148

Brettschneider R, Becker D, Lörz H (1997) Efficient transformation of scutellar tissue of immature maize embryos. Theor Appl Genet 94:737–748

Cai Q, Szarejko I, Polok K, Maluszynski M (1992) The effect of sugars and growth regulators on embryoid formation and plant regeneration from barley anther culture. Plant Breed 109:218–226

Casas AM, Kononowicz AK, Zehr UB, Tomes DT, Axtell JD, Butler LG, Bressan RA, Hasegawa PM (1993) Transgenic sorghum plants via microprojectile bombardment. Proc Natl Acad Sci USA 90:11212–11216

Devi P, Zhong H, Sticklen MB (200) In vitro morphogenesis of pearl millet (*Pennisetum glaucum* (L.) R. Br.): efficient production of multiple shoots and inflorescences from shoot apices. Plant Cell Rep 19:546–550

Dunstan DI, Short KC, Thomas E (1978) The anatomy of secondary morphogenesis in cultured scutellum tissues of *Sorghum bicolor*. Protoplasma 97:251–260

Gamborg OL, Shyluk JP, Brar DS, Constabel F (1977) Morphogenesis and plant regeneration from callus of immature embryos of sorghum. Plant Sci Lett 10:67–74

Jähne A, Lazzeri, PA, Jäger-Gussen M, Lörz H (1991) Plant regeneration from embryogenic cell suspensions derived from anther cultures of barley (*Hordeum vulgare* L.). Theor Appl Genet 82:74–80

Lambé P, Mutambel HSN, Deltour R, Dinant M (1999) Somatic embryogenesis in pearl millet (*Pennisetum glaucum*): strategies to reduce genotype limitation to maintain long-term totipotency. Plant Cell Tissue Organ Cult 55:23–29

Last DL, Brettell IS (1990) Embryo yield in wheat anther culture is influenced by the choice of sugar in the culture medium. Plant Cell Rep 9:14–16

Ma H, Gu M, Liang GH (1987) Plant regeneration from cultured immature embryos of *Sorghum bicolor* (L.) Moench. Theor Appl Genet 73:389–394

MacKinnon C, Gunderson G, Nabors MN (1987) High efficiency plant regeneration by somatic embryogenesis from callus cultures of mature embryo explants of bread wheat (*Triticum aestivum*) and grain sorghum (*Sorghum bicolor*). In Vitro Cell Dev Biol 23:443–448

Mordhorst AP, Lörz H (1993) Embryogenesis and development of isolated barley (*Hordeum vulgare* L.) microspores are influenced by the amount and composition of nitrogen sources in culture media. J Plant Physiol 142:485–492

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Orshinski BR, McGregor LJ, Johnson GIE, Hucl P, Kartha KK (1990) Improved embryoid induction and green shoot regeneration from wheat anthers cultured in medium with maltose. Plant Cell Rep 9:365–369

Pius J, George L, Eapen S, Rao PS (1993) Enhanced plant regeneration in pearl millet (*Pennisetum americanum*) by ethylene inhibitors and cefotaxime. Plant Cell Tissue Organ Cult 32: 91–96

Taylor MG, Vasil, IK (1996) The ultrastructure of somatic embryo development in pearl millet (*Pennisetum glaucum*; Poaceae). Am J Bot 83:28–44

Thomas E, King PJ, Potrykus I (1977) Shoot and embryo-like structure formation from cultured tissues of *Sorghum bicolor*. Naturwissenschaften 64:587

Vain P, Yean H, Flament P (1989) Enhancement of production and regeneration of embryogenic type II callus in *Zea mays* L. by $AgNO_3$. Plant Cell Tissue Organ Cult 18:143–152

Vasil V, Vasil IK (1981) Somatic embryogenesis and plant regeneration from tissue cultures of *Pennisetum americanum* and *P. americanum* × *P. purpureum* hybrid. Am J Bot 68:864–872

Zhong H, Wang W, Sticklen M (1998) In vitro regeneration of *Sorghum bicolor* (L.) Moench: efficient plant regeneration from shoot apices. J Plant Physiol 153:719–726

Zimny J, Becker D, Brettschneider R, Lörz H (1995) Fertile, transgenic *Triticale* (× *Triticosecale* Wittmack). Mol Breed 1:155–164