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Full Length Research Paper

Optimization of callus induction and plant regeneration from germinating seeds of sweet sorghum (*Sorghum bicolor* Moench)

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An efficient regeneration system was developed using germinating seeds of two cultivars of sweet sorghum, *Sorghum bicolor* 'Yuantian No.1' and 'M81E', as explants. We tested different media supplements effects on callus induction. The effects of combinations of 2,4-D, KT, sucrose, agar and proline at different concentrations on callus induction were compared. While 'Yuantian No.1' seeds showed the highest embryogenic callus induction frequency (57%) on MS medium supplemented with 4 mg l^{-1} 2,4-D, 0.2 mg l^{-1} KT, 0.6 g l^{-1} proline, 45 g l^{-1} sucrose and 0.8 g l^{-1} agar, 'M81E' seeds showed highest embryogenic callus induction frequency (74%) on MS medium supplemented with 4 mg l^{-1} 2,4-D, 0.2 mg l^{-1} KT, 45 g l^{-1} sucrose and 1.2 g l^{-1} agar. The best shoot induction was observed when the explants were cultured on MS medium supplemented with 1 mg l^{-1} IAA and 3 mg l^{-1} 6-BA, and on MS medium supplemented with 2 mg l^{-1} 6-BA and 0.5 mg l^{-1} KT for 'Yuantian No.1' and 'M81E', respectively. The regenerated shoots were transferred onto MS medium supplemented with 3 mg l^{-1} IBA for rooting. After 2 weeks of culture, the percentage of root production was more than 85%. The plantlets were hardened on 1/2 MS medium and then transplanted to pots, where they exhibited morphologically normal growth.

Key words: Embryogenic callus, optimal media, orthogonal test, plant regeneration, range analysis, *Sorghum bicolor* Moench.

INTRODUCTION

Sweet sorghum (*Sorghum bicolor* Moench), apart from being used as food, feed and fiber, is also an excellent source for production of alcohol biofuel due to its high content of soluble sugars in the plant stalk sap (Kargi et al., 1985; Gnansounou et al., 2005; Laopaiboon et al., 2007; Rooney et al., 2007). In addition, sweet sorghum is a C_4 plant that is characterized by a high photosynthetic efficiency, which can yield a high biomass (Antonopoulou et al., 2008; Liu et al., 2009). It has been reported that the tolerance of sweet sorghum to drought and high temperature is higher than that of corn, wheat and other crops (Gnansounou et al., 2005) and sweet sorghum has the unique ability to grow under a wide array of harsh environmental conditions (House, 1995). Taken together, sweet sorghum is one of the most promising energy plants.

Genetic transformation is a powerful and essential method for molecular study; however, little research has been done on sweet sorghum at the molecular level (Peacock, 1982). There were reports on genetic transformation of grain sorghum using gene gun (Casas et al., 1993; Casas et al., 1997; Zhu et al., 1998; Tadesse et al., 2003; Girijashankar et al., 2005) and *Agrobacterium*-mediated methods (Zhao et al., 2000; Carvalho et al., 2004; Gao et al., 2005; Hiei et al., 2006), but not of sweet

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Abbreviations: 6-BA, 6-Benzyladenine; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **IAA,** indole-3-acetic acid; **IBA,** indole-3-butyric acid; **KT,** kinetin; **MS,** Murashige and Skoog; **NAA,** naphthaleneacetic acid; **PVP,** polyvinylpyrrolidone.

sorghum. Therefore, it is necessary to establish a high efficiency regeneration system for genetic transformation of sweet sorghum. In previous work, immature embryo, mature embryo and immature caryopses of sweet sorghum were used as explants with the induction percentage of embryogenic calli lower than 10% (MacKinnon et al., 1986) and 30% (Rao et al., 1995), respectively. Compared with immature embryos and immature inflorescences, mature seeds are preferred explants for *in vitro* culture as they can be stored and are available throughout the year and can be handled easily. Zhao et al. (2008) used mature seeds and embryos from sweet sorghum 'Cowley' and 'M81E' as explants to investigate callus induction and plant regeneration. Although a higher induction frequency of calli was achieved, the embryogenic callus induction frequency was lower than 40%. The callus induction and regeneration from mature seeds must be further improved.

In the present study, germinating sweet sorghum 'Yuantian No.1' and 'M81E' seeds were used as explants to investigate the factors affecting callus induction, subculture and shoot formation. The orthogonal test design (L_{16} (4^5)) was employed to investigate the effects of 2,4-D, KT, proline, sucrose and agar on callus induction. The medium supplements of embryogenic callus production were optimized and the effects of different concentrations and combinations of phyto-hormones on plantlet regeneration were studied.

MATERIALS AND METHODS

Experimental materials

The mature seeds of sweet sorghum (*S. bicolor* Moench) 'Yuantian No.1' and 'M81E' were harvested in October 2007, dried to 0.100 ± 0.005 g H_2O (g dry weight (DW))$^{-1}$ at 15°C and 50% relative humidity (RH), and then stored in darkness at 15°C before use in the experiments.

Callus induction and subculture

The mature seeds were washed in running tap water for 30 min, sterilized in 75% ethanol for 3 min and in 0.2% $HgCl_2$ for 15-20 min, and then washed 3-5 times in sterilized water. Surface-sterilized seeds were planted onto Petri dishes (90 × 15 mm) containing 25 ml MS media (Murashige and Skoog, 1962) supplemented with 500 mg l^{-1} casein hydrolysate, 2-6% sucrose, 0.7-1.2% agar and different concentrations and combinations of plant growth regulators (pH 5.8) for seed germination and callus induction in darkness at 25 ± 2°C. For each treatment, 30-40 seeds were planted onto 3 Petri dishes as one replicate, with 3 replicates performed. The orthogonal experimental design of L_{16} (4^5) was used to investigate the effects of 2,4-D, KT, proline, sucrose and agar on callus induction. The percentage of primary callus induction (callus number/non-contaminated germinating seed number) was scored after 4 weeks of incubation and the rhizogenic calli were not counted.

The calli induced were planted on the same media for subculture. Four weeks later, the percentage of embryogenic callus (embryogenic callus number/non-contaminated germinating seed number) was scored. The calli which showed a watery and friable appearance was identified as non-embryogenic and the shiny yellow compact nodular calli as embryogenic. All the cultures mentioned above were transferred onto the fresh media at two weeks intervals.

Plant regeneration

The embryogenic calli were placed on different regeneration media, which consisted of MS basal medium supplemented with 500 mg l^{-1} casein hydrolysate, 600 mg l^{-1} proline, 10 mg l^{-1} PVP, 10 mg l^{-1} vitamin C, 30 g l^{-1} sucrose, 0.8% agar and growth regulators (pH 5.8). For each treatment, 15-20 embryogenic calli were planted on regenerate medium in 3 conical flasks (100 ml) as one replicate, with 3 replicates performed. The cultures for shoot regeneration were incubated at 25°C under an alternating photoperiod scheme (16 h light/8h dark, 40-50 µmol m^{-2} s^{-1}). After 3-4 weeks, the regenerated shoots were transferred onto MS medium supplemented with 3 mg l^{-1} indole-3-butyric acid (IBA) for rooting under the same conditions. Following the incubation for 2 weeks, the plantlets were transferred onto 1/2 MS medium and they were finally transplanted into pots.

Data analysis

All data were analyzed using one-way ANOVA, general linear model procedure and Student-Newman-Keuls at $\alpha = 0.05$ by SPSS 13.0. The range of factors at different levels in the orthogonal design of L_{16} (4^5) were calculated, which reflects the importance of the different factors on callus induction.

RESULTS

Callus induction and subculture

After 'Yuantian No.1' and 'M81E' seeds were germinated on the callus-induction medium for 2 weeks, the mesocotyl and radicle region of the seedlings firstly swelled and then gradually developed into primary callus (Figure 1a). At the same time, rhizogenic calli (Figure 1b) which were non-embryogenic and uncounted in our experiments were also observed. After 4 weeks incubation, the calli were too small to be detected and their quality was poor, but the embryogenic and non-embryogenic calli could be distinguished after a further 4 weeks subculture. Embryogenic calli could differentiate into plantlets, non-embryogenic calli would turn brown and die during subculture and regeneration and rhizogenic calli could not differentiate into plantlets, so were discarded in subsequent culture.

Induction frequency of primary calli and embryogenic calli were markedly influenced by different treatments (Table 1). Their induction frequency was the highest in treatment 10 and was zero in treatments 3 and 4. A lower induction frequency of primary calli was recorded, but embryogenic calli did not get, by treatment 8. Treatments 1 and 2 induced primary calli from germinating 'M81E' Seeds (27.1 and 11.3%, respectively), but after subculture, the calli became non-embryogenic. In most of the treatments, the induction frequency of primary calli and embryogenic calli from germinating 'M81E' seeds

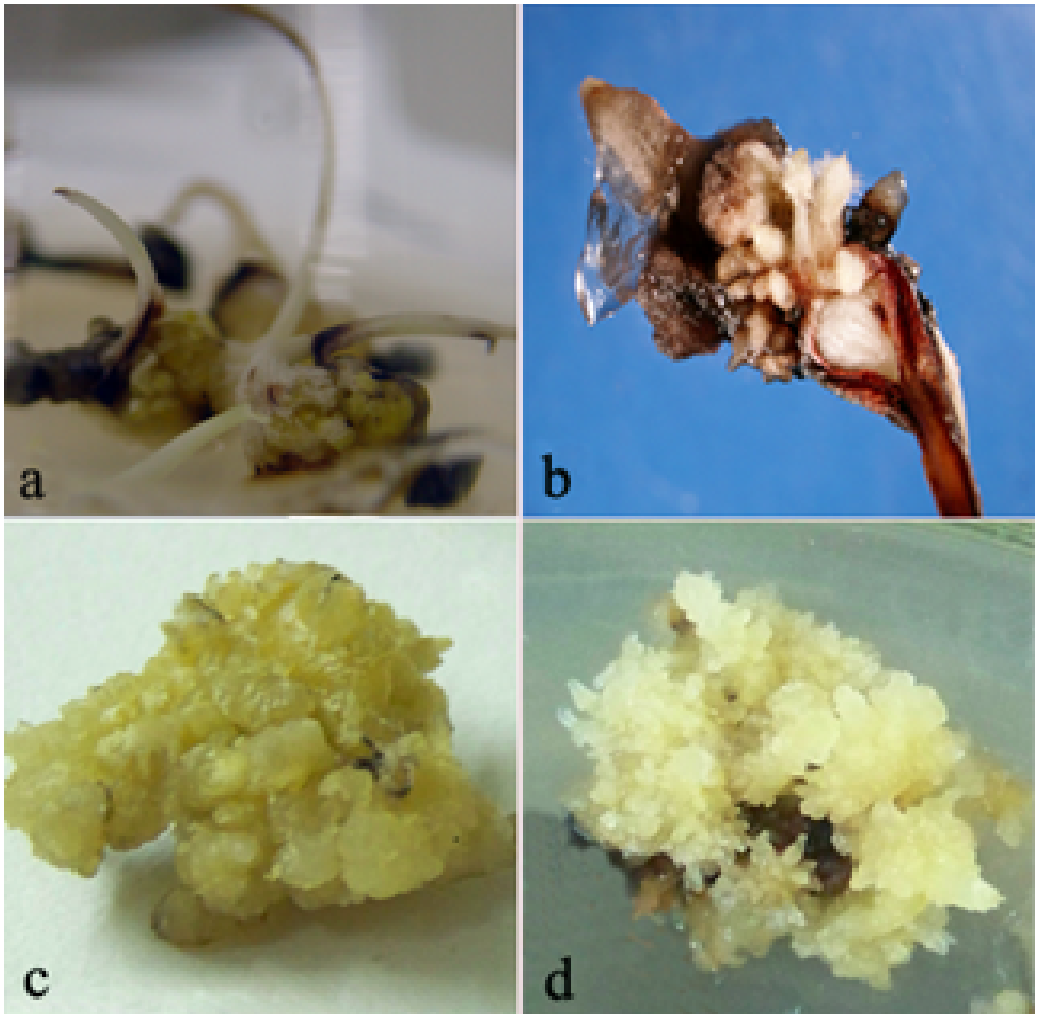

Figure 1. The different calli induced from germinating seeds of sweet sorghum 'Yuantian No.1'. (a) Primary callus, (b) the rhizogenic callus, (c) compact and nodular shining yellow embryogenic callus and (d) watery and friable non-embryogenic callus.

was higher than from 'Yuantian No.1'.

The morphology and quality of calli were medium-dependent. In addition to the embryogenic calli (Figure 1c), rhizogenic calli and watery calli were also observed.

In treatments 1, 2, 7 and 8, there appeared many rhizogenic calli (Figure 1b), which did not differentiate into plantlets. In treatments 13, 14, 15 and 16, although a high callus induction frequency was obtained, most of the calli were non-embryogenic, turning watery and brown during subculture (Figure 1d).

The range analysis of the embryogenic callus induction frequency at different levels in the orthogonal test showed that for both germinating 'Yuantian No.1' and 'M81E' seeds, 2,4-dichlorophenoxyacetic acid (2,4-D) had the greatest effect on callus induction, followed by sucrose and kinetin (KT), whereas proline and agar had the least effect (Figure 2). The higher level of 2,4-D induced a high frequency of callus formation from germinating 'Yuantian No.1' and 'M81E' seeds (Table 1), but the embryogenic callus formation frequency was lower. It was noted that during subculture, the calli turned watery and brown by the addition of 6 mg l^{-1} 2,4-D (treatments 13 and 14). KT at the 0.2 mg l^{-1} was helpful to embryogenic callus formation. For germinating 'Yuantian No.1' seeds, the optimal combination for embryogenic callus induction was 4 mg l^{-1} 2,4-D, 0.2 mg l^{-1} KT, 0.6 g l^{-1} proline, 45 g l^{-1} sucrose and 0.8 g l^{-1} agar (Figure 2). For germinating 'M81E' seeds, the optimal combination for embryogenic callus induction was 4 mg l^{-1} 2,4-D, 0.2 mg l^{-1} KT, 0 g l^{-1} proline, 45 g l^{-1} sucrose and 1.2 g l^{-1} agar (Figure 2).

After the seeds were cultured on the optimal medium in the dark at 25 ± 2°C for 4 weeks, the primary callus induction frequency reached 80% for 'Yuantian No.1' seeds and 87% for 'M81E' seeds. After subculture for a further 4 weeks, the production frequency of embryogenic calli was 57 and 74% for 'Yuantian No.1' and 'M81E' seeds, respectively. It has been observed that the non-embryogenic calli, without regeneration ability, would turn

Table 1. The orthogonal design (L_{16} (4^5)) for callus induction and embryogenic callus formation from germinating seeds of sweet sorghum 'Yuantian No.1' and 'M81E'.

Treatment (Number)	2,4-D (mg l^{-1})	KT (mg l^{-1})	Proline (g l^{-1})	Sucrose (g l^{-1})	Agar (g l^{-1})	'Yuantian No.1' (%)		'M81E' (%)	
						C*	EC*	C*	EC*
1	1①	0 ①	0 ①	20	7 ①	22.8 ± 2.3 de	4.1 ± 2.3 gh	27.1 ± 1.9 d	0.0 ± 0.0 e
2	1	0.2 ①	0.6 ①	30 ①	8 ①	26.8 ± 0.6 de	5.3 ± 1.4 fgh	11.3 ± 3.0 ef	0.0 ± 0.0 e
3	1	0.6 ①	1.2 ①	45 ①	10 ①	0.0 ± 0.0 f	0.0 ± 0.0 h	0.0 ± 0.0 f	0.0 ± 0.0 e
4	1	1.8 ①	2.4 ①	60 ①	12 ①	0.0 ± 0.0 f	0.0 ± 0.0 h	0.0 ± 0.0 f	0.0 ± 0.0 e
5	2 ①	0	0.6	45	12	68.4 ± 2.6 a	28.9 ± 3.1 c	79.6 ± 5.3 ab	57.4 ± 6.6 ab
6	2	0.2	0	60	10	39.1 ± 2.7 c	9.5 ± 1.3 efg	65.5 ± 2.5 b	44.5 ± 3.3 bc
7	2	0.6	2.4	20	8	43.3 ± 1.7 c	13.0 ± 1.9 e	24.1 ± 0.2 de	4.8 ± 0.5 e
8	2	1.8	1.2	30	7	19.8 ± 2.0 e	0.0 ± 0.0 h	17.9 ± 3.4 de	0.0 ± 0.0 e
9	4 ①	0	1.2	60	8	73.0 ± 2.5 a	34.7 ± 2.7 b	89.0 ± 1.5 a	59.0 ± 3.6 ab
10	4	0.2	2.4	45	7	78.3 ± 3.8 a	46.0 ± 0.1 a	87.6 ± 2.0 a	68.4 ± 4.2 a
11	4	0.6	0	30	12	30.2 ± 1.0 d	7.6 ± 1.5 efg	63.5 ± 2.8 b	38.9 ± 3.1 c
12	4	1.8	0.6	20	10	56.3 ± 3.1 b	11.2 ± 1.1 ef	41.1 ± 4.8 c	11.5 ± 1.8 e
13	6 ①	0	2.4	30	10	48.0 ± 2.3 c	9.6 ± 1.2 efg	70.7 ± 2.0 b	24.1 ± 2.1 d
14	6	0.2	1.2	20	12	73.7 ± 4.5 a	23.0 ± 1.5 d	63.0 ± 8.6 b	27.0 ± 2.5 d
15	6	0.6	0.6	60	7	77.0 ± 1.8 a	29.7 ± 0.9 c	79.5 ± 4.8 ab	44.7 ± 6.9 bc
16	6	1.8	0	45	8	57.0 ± 4.6 b	21.2 ± 2.2 d	75.2 ± 6.9 ab	43.6 ± 8.4 bc

C, callus induction frequency (not including rhizogenic calli), calculated as callus number/non-contaminated germinating seed number; EC, embryogenic callus induction frequency, calculated as embryogenic callus number/non-contaminated germinating seed number. *Values are means ± SD of three replicates of 30 – 40 seeds each. Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$, according to Student-Newman-Keul (SNK) multiple-range test. ①, ①, ①, and ① represent different levels.

brown and die during subsequent culture and regeneration. Also, the non-embryogenic calli often exuded a reddish-orange pigment which could inhibit callus growth and the addition of 10 mg l^{-1} vitamin C and polyvinylpyrrolidone (PVP) could decrease the pigment secretion.

Plant regeneration

The embryogenic calli (Figure 1c) were transferred onto regeneration media for shooting (Table 2) and incubated at 25°C under an alternating photoperiod scheme (16 h light /8 h dark). Multiple shoot points were observed after 2-week incubation (Figure 3a) and they grew into multiple shoots after a further 2-week culture (Figure 3b). When 3 – 4 cm long, the shoots were transferred onto rooting media and cultured at 25°C under the same alternating photoperiod scheme. After incubation for about 2 weeks, the percentage of root (plantlet) production was more than 85% (Figure 3c). In addition, some embryogenic calli produced green regions and roots on the regeneration medium for shooting, but these green regions could not grow and turned brown and died with increasing time of culture; a few embryogenic calli only produced roots (data not shown).

Induction frequency of shoots was markedly influenced by different concentrations and combinations of phyto-hormones and was cultivar-dependent (Table 2). A higher percentage of shoot induction was achieved on regeneration medium 4 for 'Yuantian No.1' embryogenic calli and on regeneration medium 6 for 'M81E' (Table 2). Induction percentage of shoots of 'M81E' embryogenic calli was always higher than that of 'Yuantian No.1' (Table 2).

Except for differentiation into plantlets, embryogenic calli had three other fates during shoot induction: (1) Root production, which subsequently could not produce shoots (Cai et al., 1987), (2) re-formation of watery calli, which lost the differentiation capacity and (3) turning brown and subsequently dying. Addition of vitamin C and PVP to culture media could decrease occurrence of these three phenomena.

The plantlets were transferred to 1/2 MS flasks and then transferred to pots for acclimatization. The plantlets survived and grew well.

DISCUSSION

Immature embryos and immature inflorescence have been frequently used as explant sources in sorghum tissue culture and transformation (Rao et al., 1995; Hagio, 2002; Jogeswar et al., 2007; Gurel et al., 2009), but it is usually difficult to obtain materials throughout the year and the suitable stages for tissue culture are also strictly limited. This is in contrast to the ready availability and abundance of mature seeds. However, mature seeds are considered difficult for tissue culture because of their low frequency regeneration. Zhao et al. (2008) used

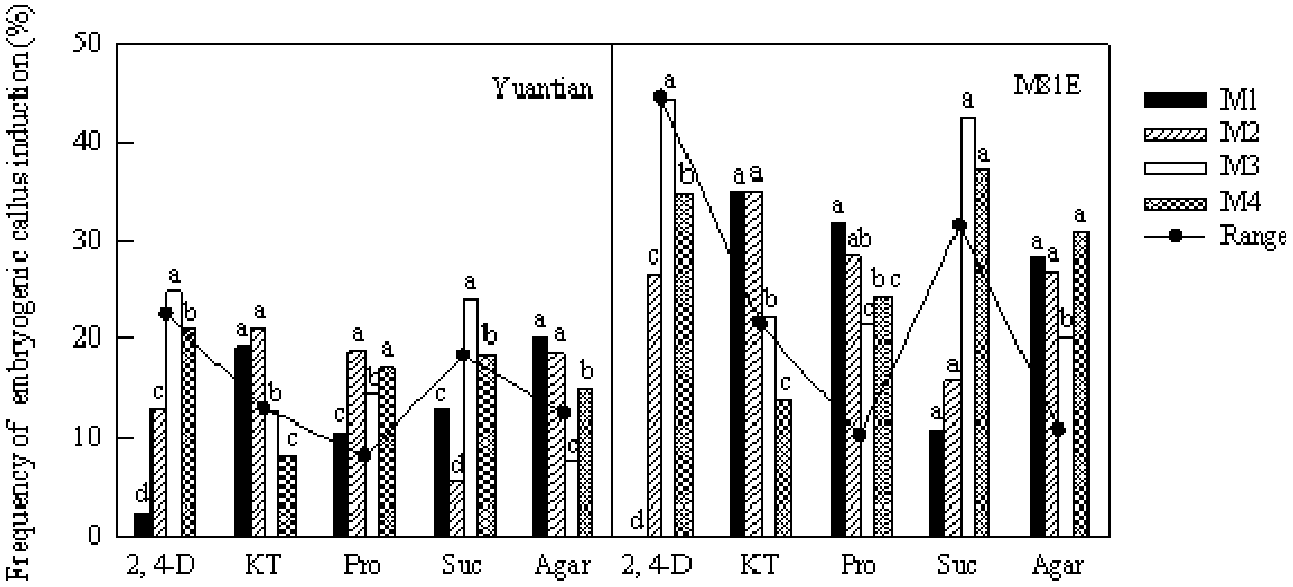

Figure 2. The range analysis of embryogenic callus induction frequency from germinating seeds of sweet sorghum 'Yuantian No.1' and 'M81E' in the orthogonal test. M1, M2, M3 and M4 are the means of 4 levels of different factors.

seeds of sweet sorghum 'Cowley' and 'M81E' as explants to investigate the callus induction and plant regeneration and found that the callus of germinating seeds could regenerate into plantlets and that the supplements of the media were important for callus induction and plant regeneration. In the present study, the orthogonal experimental design was used to optimize the media for callus induction and embryogenic callus formation of germinating seeds of sweet sorghum 'Yuantian No.1' and 'M81E'. Embryogenic, non-embryogenic and rhizogenic calli were observed in the cultures. These results were similar to those for callus induction of mature rice embryos by Rueb et al. (1994). By the optimized protocol, the frequency of embryogenic callus formation of 'Yuantian No.1' and 'M81E' were about 57 and 74%, and regeneration frequency of 'Yuantian No.1' and 'M81E' were about 21 and 38%, which significantly improved than former reports. Therefore, germinating seeds, which are readily available throughout the year, can be used as an alternative explant source in sweet sorghum tissue culture.

Sorghum has been considered one of the most difficult crops for tissue culture and transformation (Shrawat and Lörz, 2006). Also, Casas et al. (1993, 1997) obtained transgenic grain sorghum plantlets after microprojectile bombardment of immature zygotic embryos of a drought-resistant sorghum cultivar, 'P898012'. Subsequently, the *Agrobacterium*-mediated system was successfully applied to the genetic transformation of sorghum.

Hairy-root-mediated transformed plant regeneration using the natural genetic transformant, the soil bacteria *Agrobacterium rhizogenes*, currently occupies an important sector of cereals and other medicinal and aromatic plant transformation (Sugimura et al., 2005; Shrawat and Lörz, 2006; Gangopadhyay et al., 2008). The *Agrobacterium*-mediated methods have been used in sorghum transformation and got the transplantlets by use of immature embryos (Zhao et al., 2000; Carvalho et al., 2004; Gao et al., 2005; Howe et al., 2006). Recently, Gurel et al. (2009) established an efficient transformation protocol from immature embryos of sorghum by *Agrobacterium*-mediated infection. In comparison with immature embryo transformation, the report on sorghum transformation by geminating seeds was rare, because the frequency of plant regeneration from embryogenic callus was low. However, for rice, using the callus from mature seeds to get the transplant is very common (Bai et al., 2008; Hiei and Komari, 2008). Considering the ready availability and abundance of mature seeds, this protocol may be useful for the genetic transformation of sweet sorghum.

The plant growth regulators play important roles in cereal tissue culture (Bhaskaran and Smith 1990). Generally, the auxin, 2,4-D, is critical in the induction of primary calli and embryogenic calli, which was in accordance with the results of some monocotyledon plants (Rueb et al., 1994; Vikrant and Rashid 2003; Jogeswar et al., 2007). The results of this paper has also shown that the presence of 2,4-D could induce callus formation from germinating seeds. However, high concentrations of 2,4-D made callus subculture difficulty and decreased regeneration frequency (Mendoza and Kaeppler, 2002). Although induction frequency of calli from germinating seeds of 'Yuantian No.1' and 'M81E' increased with increasing concentrations of 2,4-D in the range of 1-4 mg l^{-1}, the induction percentage of embryogenic calli was

Table 2. Effects of different concentrations and combinations of phytohormones on shoot induction from embryogenic callus.

Treatment	Concentration of phytohormone (mg l^{-1})				Frequency of shoot induction[#] (%)	
	IAA	6-BA	NAA	KT	'Yuantian No.1'	'M81E'
RM 1	1	0.5	0	0	0.0 ± 0.0 c	16.0 ± 1.4 c
RM 2	1	1	0	0	0.0 ± 0.0 c	18.4 ± 2.0 b
RM 3	1	2	0	0	13.7 ± 2.8 b	20.1 ± 1.4 b
RM 4	1	3	0	0	21.9 ± 2.5 a	25.5 ± 1.6 b
RM 5	0.5	0.5	0	0.5	0.0 ± 0.0 c	22.7 ± 1.5 b
RM 6	0.5	2	0	0.5	11.0 ± 2.4 b	38.4 ± 4.2 a
RM 7	0	2	1	0	0.0 ± 0.0 c	0.0 ± 0.0 b

RM, regeneration medium, which consists of MS supplemented with 500 mg l^{-1} casein hydrolysate, 600 mg l^{-1} proline, 10 mg l^{-1} vitamin C, 10 mg l^{-1} PVP, 30 g l^{-1} sucrose, 0.8% agar and phytohormone of indicated concentrations and combinations (pH 5.8). [#]Values are means ± SD of three replicates of 15 – 20 embryogenic calli each. Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$, according to Student-Newman-Keul (SNK) multiple-range test.

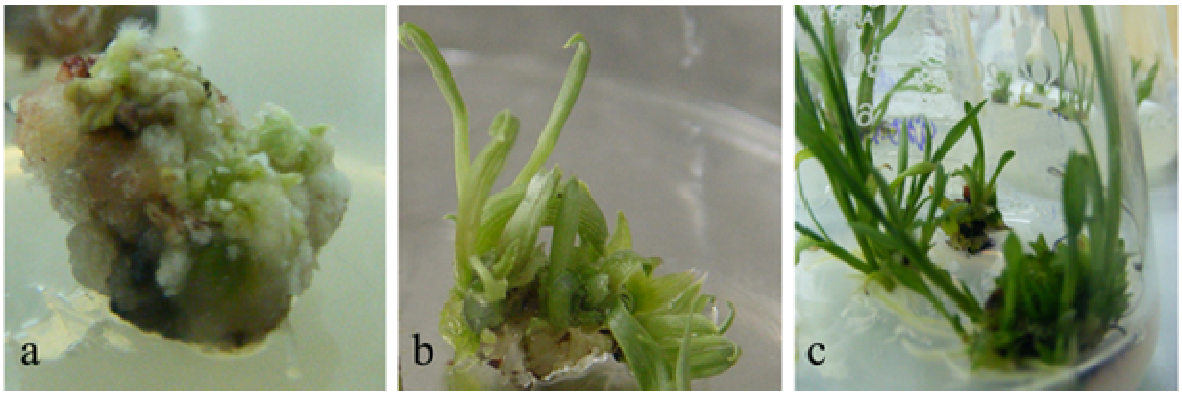

Figure 3. The different stages of plant regeneration from the germinating seeds of sweet sorghum 'M81E'. (a) The multiple shoot points, (b) the multiple shoots and (c) the plantlets.

relatively low. When 2,4-D concentration was 6 mg l^{-1}, some embryogenic calli became watery and non-embryogenic during subculture and lost differentiation capacity (data not shown).

The negative effect of KT on callus culture was noted in sorghum and wheat (Wernicke and Brettell, 1982; Mastellar and Holden, 1970; Lazar et al., 1983). The results showed that the addition of 2,4-D alone was sufficient to induce callus formation and the additional KT in high concentrations (≥ 0.6 mg l^{-1}) decreased the callus induction frequency. However, addition of a low concentration of KT (0.2 mg l^{-1}) was beneficial for keeping the calli embryogenic. The effects of other supplements, agar, sucrose and proline, on callus and embryogenic callus induction were also investigated. As a carbon source and osmotic regulator, sucrose played an important role in callus formation. A range of sucrose concentrations from 20 to 60 g l^{-1} was tested, and it was found that the sucrose level of 45 g l^{-1} was effective in promoting embryogenic callus formation for germinating seeds of 'Yuantian No.1' and 'M81E'. The calli tended to become watery and non-embryogenic at lower concentrations of sucrose. It is likely that the sucrose affects the formation of embryogenic calli by osmoregulation. It was reported that proline promoted the embryogenic callus production and enhanced the plant regeneration capacity during culture of maize and sorghum (Armstrong and Green, 1985; Rao et al., 1995). In our experiments, the influence of proline on callus induction of both 'Yuantian No.1' and 'M81E' seeds was also detected, but it was not significant at the range of 0-2.4 g l^{-1} during the two-month callus culture; this probably meant that factors other than proline have a bigger contribution to callus culture. As a consolidation regulator, agar had a minor function on callus induction of germinating seeds of sweet sorghum 'Yuantian No.1' and 'M81E' in comparison with other factors.

It has been observed that the callus production and plant regeneration of sorghum were influenced by indole-3-acetic acid (IAA), 6-benzyladenine (6-BA) and KT (Rao et al., 1995; Jogeswar et al., 2007). IAA and naphthalene-acetic acid (NAA) were supplemented during regeneration

of sorghum calli culture (MacKinnon et al., 1986; Hagio, 2002). It was reported that the induction frequency of shoots was affected by different concentrations and combinations of phytohormones and was cultivar-dependent. A higher induction percentage of shoots was obtained on MS media supplemented with 1 mg l^{-1} IAA and 3 mg l^{-1} 6-BA for 'Yuantian No.1' embryogenic calli and on MS media supplemented with 2 mg l^{-1} 6-BA and 0.5 mg l^{-1} KT for 'M81E' embryogenic calli. It appeared that the shoot induction from 'Yuantian No.1' embryogenic calli was inhibited by a higher proportion of IAA to 6-BA (Table 2, treatments RM 1, RM 2 and RM 5). IBA was known to induce roots in many cereals such as rice and sorghum (MacKinnon et al., 1986; Kishore and Reddy, 1986; Jogeswar et al., 2007). 3 mg l^{-1} IBA was supplemented into MS media and it was found that more than 85% shoots generated roots within 2 weeks, but the plantlets could not survive unless being transferred into flasks containing 1/2 MS.

In conclusion, we have optimized a protocol for sweet sorghum regeneration from germinating seeds. This report shows that it might be possible to improve regeneration from germinating seeds by optimizing the compositions of callus induction and plant regeneration media for specific genotypes. Because of the reproducibility and the easy accessibility of mature seeds, the plant regeneration system provides a foundation for genetic transformation of sweet sorghum, which is of significant importance for improving important traits such as biomass, sucrose content.

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