

# Simple hormonal regulation of somatic embryogenesis and/or shoot organogenesis in caryopsis cultures of *Pogonatherum paniceum* (Poaceae)

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**Abstract** *Pogonatherum paniceum* (Poaceae) is a perennial plant with good potential for eco-recovery and ornamental function. This study presents in vitro culture systems of simple hormonal regulation of somatic embryogenesis and shoot organogenesis from mature caryopses. Mature caryopses of *P. paniceum* were grown on Murashige and Skoog medium with 3% sucrose (w/v) and various concentrations or combinations of 2,4-dichlorophenoxyacetic acid (2,4-D),  $\alpha$ -naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP). Morphological development was analyzed by light microscope after histological sectioning. Four types of callus were induced by different concentrations of 2,4-D. Type I callus was regenerated via somatic embryogenesis; type II callus failed to produce any regeneration; type III callus had both somatic embryogenesis and shoot organogenesis capacities; and type IV callus only displayed shoot organogenesis capacity. Regarding hormone combinations used in this study, NAA only induced type IV callus and BAP only induced direct multiple shoot formation. The combinations of 2,4-D and NAA induced type III callus. Several of the regeneration pathways were simply controlled by one or two kinds of plant hormones. The established systems will be helpful

for further research on the developmental mechanism of switch between somatic embryogenesis and shoot organogenesis.

**Keywords** *Pogonatherum paniceum* · Auxin · Cytokinin · Somatic embryogenesis · Shoot organogenesis

## Abbreviations

BAP	6-Benzylaminopurine
2, 4-D	2,4-Dichlorophenoxyacetic acid
NAA	$\alpha$ -Naphthaleneacetic acid
MS	Murashige and Skoog

## Introduction

The capacity of cultured plant cells, tissues and organs to undergo somatic embryogenesis or shoot organogenesis has provided opportunities for numerous applications of plant development and transformation (Zimmerman 1993; Hicks 1994; Sugiyama 1999; Phillips 2004). Although these two processes have been known for 50 years, plant scientists still understand only a little about what actually controls them. The search for answers might shed light on how cells' fates become determined during the development process (Vogel 2005). Plant hormones are most likely the candidates in the

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regulation of developmental switches (Bhaskaran and Smith 1990; Fehér et al. 2003). Studying the relationships between plant hormones and explants leading to morphogenesis will be a practicable approach for this issue.

Auxins and cytokinins are the two main growth regulators in plants involved in the regulation of division and differentiation (Fehér et al. 2003) and have been proved to play an important role in the induction of somatic embryogenesis and shoot organogenesis (Gaspar et al. 1996). Generally, in dicots, for the same kinds of explants, auxins with or without low levels of cytokinins have often been reported to induce somatic embryogenesis. Elevated cytokinin levels or cytokinin alone are usually required for the induction of shoot organogenesis (Matsuoka and Hinata 1979; Tabei et al. 1991; Tang et al. 2000; Nikolić et al. 2006). In some studies, the potential mechanisms of these two types of plant hormones controlling the switch between embryogenic and organogenic pathways have been further studied (Charrière et al. 1999; Thomas et al. 2004). However, in monocots, especially in graminaceous species, the plantlet regeneration was considered mainly performed via somatic embryogenesis (Vasil 1982). Evidences of morphogenic pathways in the same kind of explants under the control of auxins and/or cytokinins were only observed in few species, such as sorghum (Bhaskaran and Smith 1989), *Bouteloua gracilis* (Aguado-Santacruz et al. 2001), minor millet (*Paspalum scrobiculatum*) (Vikrant 2002, 2003), sugarcane (Lakshmanan et al. 2006), *Neyraudia arundinacea* (Ma et al. 2007). Although combinations of plant hormones required for different morphogenic pathway were similar to dicots in those studies, the regulation processes were more complicated and the studies on regulation mechanisms were much fewer.

*Pogonatherum paniceum* (Poaceae) is a perennial plant distributed in temperate, tropical and subtropical regions of Asia, Oceania and Africa (Clayton et al. 2002). Due its attractive appearance and strong adaptation to various ecosystems, it has being exploited as an eco-recovery and ornamental pot plant (Wang et al. 2007). Hassan and Debergh's study (1987) and our (Wang et al. 2006) previous works have proved that *P. paniceum* explants can regenerate plantlets via somatic embryogenesis or direct shoots formation. This paper further presents

somatic embryogenesis and/or shoot organogenesis from four types of callus and direct shoot organogenesis from mature caryopsis in vitro culture of *P. paniceum* and their simple regulation by 2,4-dichlorophenoxyacetic acid (2,4-D),  $\alpha$ -naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP).

## Materials and methods

### Plant material

The spikes of *P. paniceum* were collected from the field in Renshou, Sichuan Province, China and air dried for a week at room temperature ( $25 \pm 2^\circ\text{C}$ ). After husking, relatively large and healthy-looking caryopses were selected for experiments.

After surface sterilized with 1% solution of sodium hypochlorite (v/v) for 5 min and five times washed in sterile deionized water, the caryopses were cultured on induction medium.

### Media and induction

Explants of sterilized caryopses were cultured on solid Murashige and Skoog (1962) basal medium (MS) containing 3% sucrose (w/v) and 0.8% agar (w/v) with different plant hormone combinations. The medium was adjusted to pH 5.8 before autoclaving at  $121^\circ\text{C}$  for 20 min. The culture protocols are presented in Fig. 1.

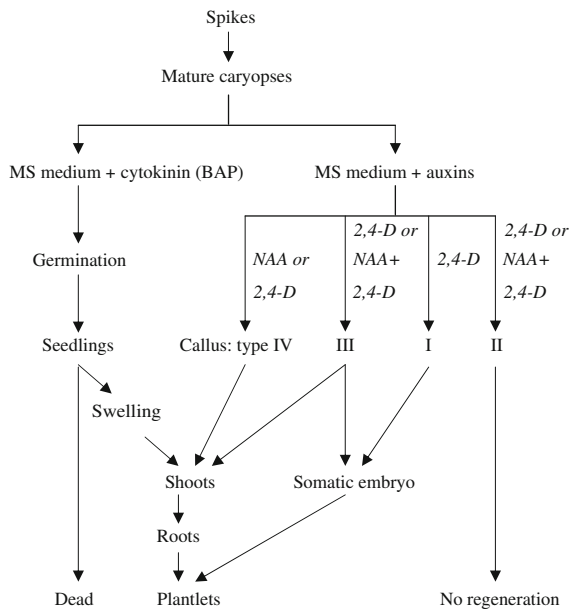
For callus induction, various concentrations of 2,4-D, NAA were used alone and in combinations (Tables 1, 3).

For direct shoot formation, various concentrations of BAP were used (Table 4).

### Plant regeneration

For the plant regeneration, the callus induced by high levels of 2,4-D and the combinations of 2,4-D and NAA were put on the MS basal medium without any plant hormones.

The shoots induced by BAP, NAA or low levels of 2,4-D were separated and rooted on MS or half strength MS basal medium with different concentrations of NAA when they were about 1 cm in height.



**Fig. 1** Flow chart of protocols for callus induction, direct shoot formation and plantlet regeneration from *Pogonatherum paniceum* mature caryopses cultures

Plants reaching about 10 cm in height were acclimatized in greenhouse conditions for 1 week. Then they were washed in water to remove agar and transferred to pots containing garden soil and vermiculite (2:1).

**Culture conditions**

Cultures were incubated in the dark at  $26 \pm 1^\circ\text{C}$  for callus induction and under 12/12 h (light/dark) photoperiod conditions with a light intensity of approximately  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  provided by cool, white fluorescent tubes (40 W, Phillips, China) for shoots induction and plant regeneration and growth. The cultures incubated with NAA alone and low concentrations of 2,4-D (less than  $0.1 \text{ mg l}^{-1}$ ) were under dark conditions for callus induction, and when the callus developed into shoots, they were transferred to 12/12 h (light/dark) photoperiod conditions.

**Histological observation**

The calli were vacuum-infiltrated for 20 min with FAA (formalin:acetic acid:ethanol:water in a ratio of 5:5:45:45 v/v) and fixed for 7 days at room temperature ( $25 \pm 2^\circ\text{C}$ ). Fixed tissues were washed in running water for 10 min and dehydrated in a gradual ethanol/xylene series of solutions (ethanol: 70, 85, 95, 100%, and ethanol: xylene in a ratio of 50:50 and 100% xylene). Sections ( $10 \mu\text{m}$ ) of paraffin wax embedded material were obtained using a sliding microtome, affixed to glass slides with albumin, stained with hematoxylin, mounted in Canada balsam, and covered with a cover glass for microscopic observations.

**Table 1** Effects of 2,4-D on callus induction and in vitro morphogenesis from mature caryopses of *Pogonatherum paniceum*

2,4-D ( $\text{mg l}^{-1}$ )	Morphogenesis <sup>a</sup>	Frequency of NS (%)	Frequency of callus induction (%)			
			Type I	Type II	Type III	Type IV
0.02	NS or SO	40.24	–	–	–	11.60
0.05	NS or SO	20.38	–	–	–	29.82
0.1	NS or SE & SO from callus or SO from callus	3.77	–	–	32.36	13.03
0.2	SE & SO from callus	–	5.24	22.32	32.01	–
0.5	SE & SO from callus	–	9.65	18.04	30.93	–
1	SE & SO from callus	–	21.61	20.07	20.84	–
2	SE from callus	–	45.50	17.23	–	–
5	SE from callus	–	41.86	15.00	–	–
8	SE from callus	–	36.12	21.60	–	–
12	SE from callus	–	32.11	28.93	–	–
15	SE from callus	–	28.14	28.41	–	–
20	SE from callus	–	2.41	4.11	–	–

<sup>a</sup> NS, Normal seedlings; SE, Somatic embryogenesis; SO, Shoot organogenesis

## Statistical analysis

All the experiments were laid out in completely randomized design with at least five repetitions; in each replicate 20–30 caryopses were used.

Induction percentage is expressed as the average percentage of caryopses that developed into callus, shoots or normal seedlings divided by the number of total caryopsis explants. Rooting percentage is expressed as the average percentage of shoots that rooted divided by the number of total shoots tested. Germination percentage is expressed as the average percentage of caryopses that germinated divided by the number of total caryopses tested. All percentage values were arcsine-transformed prior to analysis to normalize the data and reduce heteroscedasticity, while untransformed values are presented in the tables.

Counts of shoots per explants and length of shoots are presented as the mean  $\pm$  standard error. The data were subjected to a standard analysis of variance (ANOVA). The LSD test was used to estimate significant differences among mean values of the treatments.

## Results

After inoculation on hormone-free MS medium for about 5 days, caryopses germinated readily and eventually developed into normal plants. The germination rate was of 50% (Tables 2, 4). One caryopsis only resulted in one seedling.

## Callus induction

After 9–11 days of incubation, the primary callus appeared on the micropylar end of the caryopsis on MS medium with various combinations of plant hormones. One month later, all of the calli were between 0.5 and 0.8 mm in diameter. The calli were then classified under the following four types (Fig. 2a–f):

The first type of callus (embryogenic callus, type I; Fig. 2a, b) was compact, highly nodular and white or yellow-white in appearance. Histological studies showed that there were a lot of fuscous meristemoid in the callus, which could develop into bipolar mature somatic embryo through globule stage, pear-shaped stage and scutiform stage. This bipolar somatic embryo had no vascular connection with maternal tissue (Fig. 3b–e).

The second type of callus (non-regeneration callus, type II; Fig. 2c) looked non-nodular, soft, friable, mucilaginous, and yellow or brown-yellow, which occurred along with the embryogenic callus or the third type (type III) described below in most of the explants. The type II callus did not give rise to any organogenesis.

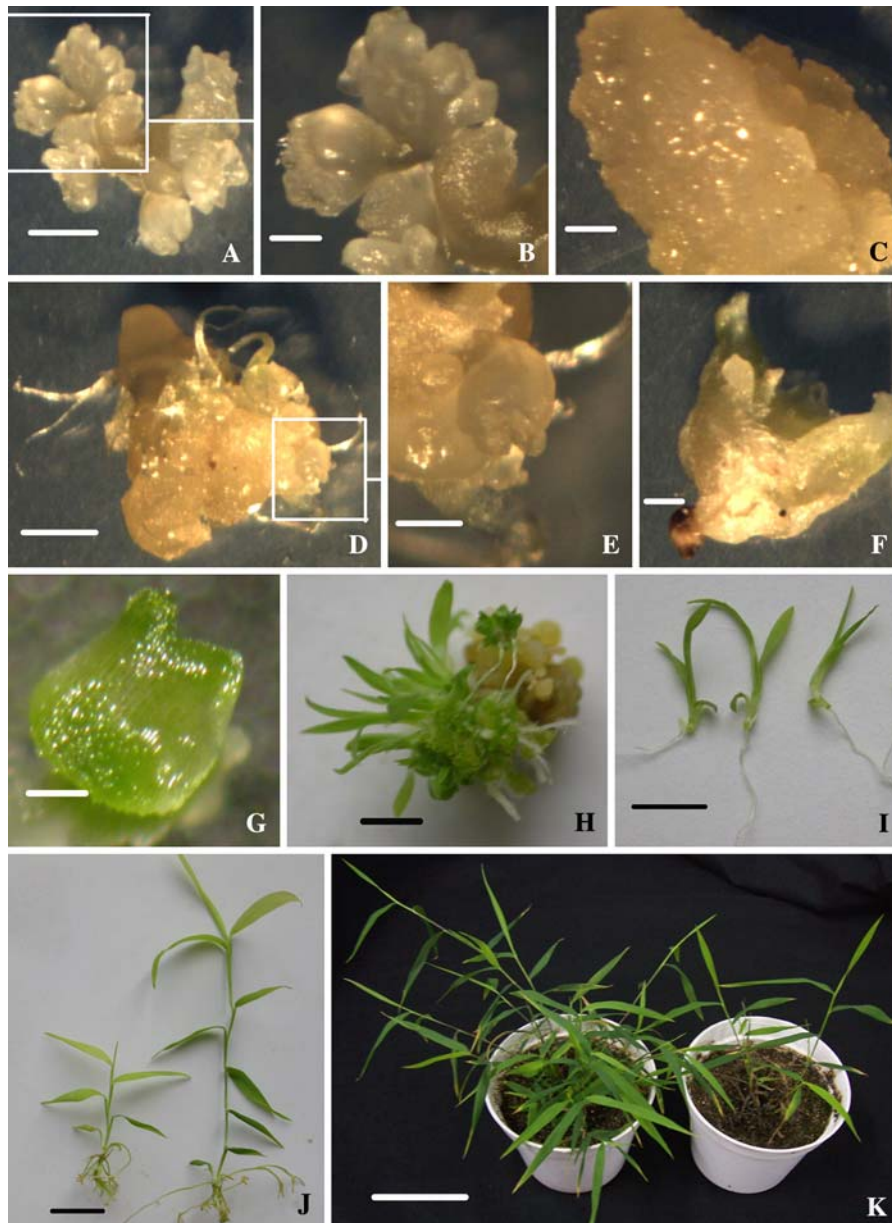
Type III callus looked hard and pale yellow, with both nodules and bud-like structures on the surface (Fig. 2d, e). The meristemoid in the outer layer of callus developed into unipolar shoot rudimental organ without root apical meristem which connected to callus tissue through vascular strains. Subsequently,

**Table 2** Effects of NAA on callus and multiple shoots induction from mature caryopses of *Pogonatherum panicum*

NAA (mg l <sup>-1</sup> )	Morphogenesis <sup>a</sup>	Frequency of normal seedlings (%)	Frequency of callus induction (type IV, %)	Number of shoots/Explants <sup>b</sup>
0	NS	50.88	–	1a
0.1	Multiple shoots or NS	22.37	27.64	3.36 $\pm$ 1.85b
0.2	Multiple shoots or NS	14.62	35.27	4.65 $\pm$ 1.24b
0.5	Multiple shoots or NS	8.00	40.39	18.17 $\pm$ 7.40c
1	Multiple shoots or NS	2.351	49.23	73.76 $\pm$ 27.22d
2	Multiple shoots	–	49.49	58.59 $\pm$ 19.64e
3	Multiple shoots	–	48.77	56.82 $\pm$ 24.45e
5	Multiple shoots	–	50.99	39.88 $\pm$ 16.18f
10	Multiple shoots	–	49.73	28.48 $\pm$ 12.18g
20	Multiple shoots	–	49.89	3.60 $\pm$ 1.16a

<sup>a</sup> NS means normal seedlings

<sup>b</sup> Values followed by the same letters are not significantly different from each other by LSD ( $P = 0.05$ ) test

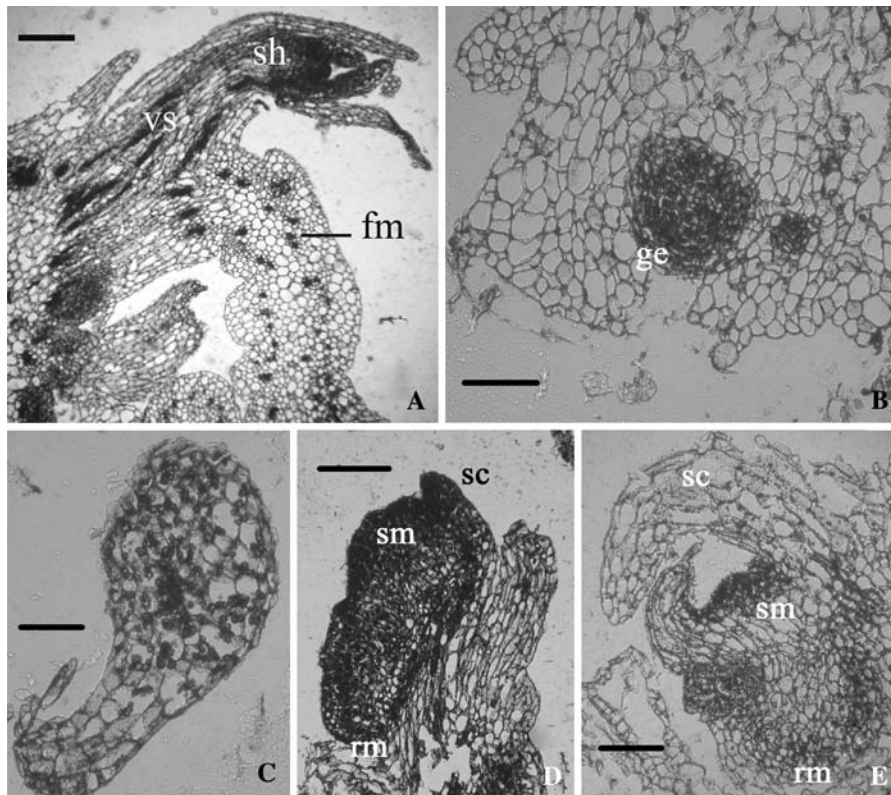


**Fig. 2** Callus induction and plantlet regeneration from mature caryopses of *Pogonatherum paniceum*. (**a–f**) The four types of callus induced by auxin: (**a, b**) Type I; (**c**) Type II; (**d, e**) Type III; (**f**) Type IV. (**g**) A developing somatic embryo on hormone-free MS medium. (**h**) Plantlets with shoots and roots from type

they regenerated into shoots after being transferred to hormone-free MS medium (Fig. 3a). At the same time, lots of fuscous meristemoid occurred in inner layer of callus and could develop into the bipolar somatic embryo as type I callus.

I callus. (**i**) Detached plantlets from somatic embryo with roots on base of shoots. (**j**) Rooted-shoots from type IV. (**k**) Regenerated plants in pots. Bars in **a, d** = 1 mm; **b, c, f, G** = 0.5 mm; **e** = 0.2 mm; **h** = 5 mm; **i, j** = 1 cm; **k** = 5 cm

The fourth type of callus (organogenic callus, type IV) was yellowish and hard (Fig. 2f). Because the callus could directly differentiate into multiple shoots on the induction medium immediately, the period of callus phase (3–5 days) was short.



**Fig. 3** Histological observation of somatic embryo development of *Pogonatherum paniceum*. (a) Shoots (sh) with vascular strands (vs) connected to the type III callus and fuscous meristemoid (fm). (b) Global embryo (ge) in type I callus. (c)

Somatic embryo in pear-shaped stage. (d, e) Somatic embryo with scutellum (sc), shoot meristem (sm) and root meristem (rm). Bars in a, d = 200  $\mu\text{m}$ ; b, c, e = 100  $\mu\text{m}$

#### Effects of 2,4-D on callus types and in vitro morphogenesis

The four types of callus described above from *P. paniceum* mature caryopses were induced by 2,4-D as the sole plant hormone. The callus type changed from type I to type IV with the decreasing concentrations of 2,4-D. Concentrations of 2,4-D higher than 1  $\text{mg l}^{-1}$  favoured type I and type II callus, but inhibited type III and IV callus; when the concentrations were over 2  $\text{mg l}^{-1}$ , the induction rate of type I callus decreased with the increasing concentrations of 2,4-D. The optimal concentration of 2,4-D for somatic embryogenesis was 2  $\text{mg l}^{-1}$ . When 2,4-D concentrations went down from 1 to 0.1  $\text{mg l}^{-1}$ , the rate of type III callus increased from 20.84 to 32.36%. But when 2,4-D concentrations were less

than 0.05  $\text{mg l}^{-1}$ , only type IV callus occurred (Table 1).

#### Effects of NAA on indirect multiple shoot formation

Unlike 2,4-D, all the NAA concentrations tested in this study only induced the type IV callus (Fig. 2f, Table 2). At low concentrations of NAA, few caryopses produced callus. The callus induction rates increased along with the increasing NAA concentrations. When the concentrations were more than 2  $\text{mg l}^{-1}$ , the induction rates maintained at 50%. But the number of shoots per explant was also affected by the NAA concentrations; the highest number happened at 1  $\text{mg l}^{-1}$ . These results suggested the optimal NAA concentration for type IV

callus induction and shoot regeneration was  $1 \text{ mg l}^{-1}$  (Table 2).

#### Effects of 2,4-D and NAA combinations on in vitro morphogenesis

No matter that the ratios of 2,4-D to NAA were low or high, in the five combinations we tested, type I and IV callus vanished and only type II and type III callus appeared. The total callus induction rates of each treatment maintained at 50%, but the rates of type III callus decreased along with increasing 2,4-D concentrations in the five combinations (Table 3). The induction rates of type III callus in the medium with the NAA and 2,4-D combinations were higher than with 2,4-D alone in corresponding concentrations, whereas the rates of type II callus were lower than 2,4-D alone (Tables 1, 3).

#### Effects of BAP on direct shoots organogenesis

After 3–5 days' culture on MS medium containing BAP as the sole plant hormone, the caryopses germinated normally (Fig. 4a). The germination rates (50%) at different concentrations were no significantly different except the ones with more than  $10 \text{ mg l}^{-1}$  (Table 4).

The seedlings were 0.5 to 0.8 cm in height after 3 weeks. Then, the basal part of some seedlings began swelling (Fig. 4b) and some bud-like structures appeared on the surface, which developed into multiple shoots (Fig. 4c–e) 1 month later. The seedlings without swelling (about 5 to 20%) did not develop further and died after 2 months (Fig. 4f).

The percentage of caryopses producing multiple shoots increased with the concentrations of BAP within a range of 0 to  $1 \text{ mg l}^{-1}$ ; but decreased along with the higher BAP concentrations (more than  $1 \text{ mg l}^{-1}$ ). Average shoot length decreased from 4.5 cm to 1 cm with the increased BAP concentration ( $0.2$  to  $15 \text{ mg l}^{-1}$ ) on MS medium (Table 4).

#### Plant regeneration and rooting of shoots

On hormone-free MS basal medium, the nodular structure on the surface of type I and III callus developed into mature embryos (Fig. 2g) and further into plantlets by elongating and rooting shoots (Fig. 2h, i). For the initiation and development of somatic embryo were not synchronous, about 100–200 shoots grew from a piece of callus between 5 mm and 8 mm in diameter after 1 month. When the plantlets reached about 1 to 2 cm after 1 month, they were transferred to fresh MS basal medium for growth. The bud-like structure on surface of the type III callus developed into shoots without roots. In contrast, no plant regeneration occurred in callus type II on hormone-free MS basal medium after more than 2 months.

The multiple shoots (about 2–3 cm) induced by NAA, BAP (Fig. 4e) or from type III callus were excised and transferred to the rooting medium. The shoots rooted after 1 week and the roots were about 3 to 5 cm in length 2–3 weeks later. The regenerated roots were from the nodes of shoots (Fig. 2j), unlike the roots of normal seedlings from caryopses germination or plantlets derived from somatic embryo, which are from the root apical meristem (Fig. 2i). All

**Table 3** Effects of 2,4-D and NAA combinations on callus induction and in vitro morphogenesis from mature caryopses of *Pogonatherum panicum*

Hormones ( $\text{mg l}^{-1}$ )		Morphogenesis <sup>a</sup>	Frequency of callus induction (%)		Number of shoots/ Explants <sup>b, c</sup>
NAA	2,4-D		Type II	Type III	
1	5	SE & SO from callus	6.00	44.89	$12.53 \pm 3.11a$
1	2	SE & SO from callus	6.88	45.37	$18.93 \pm 4.38b$
2	1	SE & SO from callus	8.29	48.00	$28.89 \pm 3.33c$
5	1	SE & SO from callus	10.85	42.03	$30.64 \pm 3.79c$
10	1	SE & SO from callus	7.55	42.57	$42.77 \pm 8.26d$

<sup>a</sup> SE, somatic embryogenesis; SO, shoot organogenesis

<sup>b</sup> Values followed by the same letters are not significantly different from each other by LSD ( $P = 0.05$ ) test

<sup>c</sup> The data were collected after 2 months



**Fig. 4** Direct multiple shoots induction of *Pogonatherum paniceum*. (a) Mature caryopsis germination. (b) Swollen seedling node. (c) Multiple shoots. (d) Multiple shoots globule.

(e) Detached shoots. (f) Non-swollen seedling. Bars in a, b = 1 mm; c, d, e, f = 2 mm

**Table 4** Effect of BAP on shoot formation from mature caryopses of *Pogonatherum paniceum*

BAP (mg l <sup>-1</sup> )	Germination rate (%)	Percentage of explant producing shoot		Length of shoots (mm) <sup>a</sup>
		Total (%)	>10 shoots (%)	
0	50.32	–	–	45.62 ± 8.86a
0.2	50.59	37.30	13.97	38.21 ± 4.79b
0.5	50.79	43.46	30.12	35.04 ± 7.84c
1	50.72	43.09	36.84	35.97 ± 2.59c
3	50.02	40.33	27.02	33.85 ± 2.80d
5	49.63	33.06	30.08	28.27 ± 6.51e
10	43.20	24.41	14.31	17.99 ± 3.92f
15	36.86	19.65	10.57	12.23 ± 5.73g

<sup>a</sup> Values followed by the same letters are not significantly different from each other by LSD ( $P = 0.05$ ) test

the media tested induced rooting, among which the half-strength basal MS media with 0.5 mg l<sup>-1</sup> NAA induced more than 90% of rooting (Table 5).

About 90.25% of the regenerated plants survived when they were acclimatized for 7 days and transferred to soil (Fig. 2k).

## Discussion

The high frequency plantlets regeneration has been reported from different vegetative parts (leaves, seeds, seedlings, and stems) of *P. paniceum* in previous papers (Hassan and Debergh 1987; Wang



**Table 5** Percentage of rooted regenerated shoots in MS and half strength MS supplemented with NAA

NAA (mg l <sup>-1</sup> )	MS	1/2MS
0	71.37	81.73
0.5	70.16	93.71
1	60.97	82.34
2	54.08	86.48

et al. 2006, 2007). In this study, we further observed that somatic embryogenesis and/or shoot organogenesis occurred not only in the same piece of callus but also in different types of callus from mature caryopses explants and the direct shoots organogenesis form seedlings germinated on induction medium.

Caryopses explants of *P. paniceum* in our culture conditions produced four types of callus with different appearances and inner structures. These calli developed obeying three basic developmental patterns. First, the type I callus conducted somatic embryogenesis program. In this developmental pattern somatic embryo has two meristems, viz. shoot meristem and root meristem. Second, the type IV callus exerted unipolar organ redifferentiation (shoot organogenesis); in this pattern only shoot rudimental organ appeared. And the third, the type II callus only proliferated but did not further regenerate into plantlets, viz. development-termination. The type III callus displayed mixed developmental pathways with both somatic embryogenesis and shoot organogenesis. Although, each of the three programs have been observed in other graminaceous species, however, each of those researches only presented one or two pattern from same kind of explants in one species (Hassan and Debergh 1987; Kothari and Varshney 1998; Yemets et al. 2003; Ncanana et al. 2005). Therefore, we can suggest that diversity of developmental programs exist in cultured plant explants and that the developmental orientation in *P. paniceum* mature caryopsis explants is more easily reprogrammed than in other graminaceous species studied previously.

Auxin and cytokinin, the very important plant hormones that regulate many aspects of growth and development (Mok 1994; Woodward and Bartel 2005; Sakakibara 2006), are usually employed for dedifferentiation and redifferentiation of explants during plant tissue cultures. Generally, auxin is used

for callus induction and proliferation, and both cytokinin and auxin are required for redifferentiation of callus (Gaspar et al. 1996). Shoots were produced at high cytokinin to auxin ratios, while roots were formed when the ratios were reversed (Che et al. 2006). However, *P. paniceum* caryopses explants here presented some distinct characters, all four types of callus with different developmental fates could be induced by synthetic auxin 2,4-D as the sole plant hormone. High concentrations of 2,4-D induced type I callus and low levels of 2,4-D stimulated type IV callus formation for shoot development (Table 1). On the other hand, NAA, another important synthetic auxin used as the sole hormone in induction medium only induced type IV callus. If 2,4-D supplemented with NAA was added into callus induction medium, mid-fated development pathways appeared, viz. type III and type II callus were induced.

Therefore, from the experiments described here we deduce the following hypotheses: (1) 2,4-D concentrations controlled the developmental pathways. Low levels of 2,4-D initiate shoot development, and only 2,4-D rising to an opportune concentration, cause root development programs to be initiated. (2) Except for the positive effect on stimulating development of explants, 2,4-D is a two-edged regulator since it inhibits further embryonic development (Steward et al. 1958; Charrière et al. 1999; Che et al. 2006). It is necessary that the type I and type III callus should be transferred to auxin-free MS medium for regeneration. And much higher levels of 2,4-D inhibited the embryonic callus formation, induced more type II callus without plantlets regeneration. (3) Compared with 2,4-D, NAA is less active on embryogenic callus induction, in this study it only stimulated shoot development. Similar results were reported in pennisetum (Talwar and Rashid 1990) and carrot (Ribnicky et al. 1996). (4) There may exist negative interactions in some extent between 2,4-D and NAA in morphogenesis of *P. paniceum* callus. 2,4-D weakens or erases the functions of NAA, and vice versa.

Many studies have confirmed that auxins like 2,4-D and NAA can activate auxin response factors (ARF) (Inukai et al. 2005) and auxin signal pathways such as Rac-like GTPases (Tao et al. 2002) and regulate a lot of genes related to growth and development (Che et al. 2002, 2006; Pischke et al. 2006), such as the *LEC1* gene (Yazawa et al. 2004), *prha* gene (Plesch et al. 1997).

On the other hand, the cytokinin usually used in plant tissue culture for plant regeneration from callus (Rout et al. 2006) was not necessary for the *in vitro* regeneration of *P. paniceum*, in which callus could directly regenerate on plant hormone-free MS basal medium. If cytokinin BAP replaced 2,4-D or NAA in callus induction medium, it markedly stimulated multiple shoot formation of *P. paniceum* caryopses cultures (no visible callusgenesis) while not affecting the caryopses germination (except concentration of BAP more than 10 mg l<sup>-1</sup>). Also, BAP inhibited shoot elongation (Table 4, Fig. 4f), which is in accordance with the previous research result in plant *Dendrocalamus asper* (Arya et al. 1999) and could be understood with decreasing apical dominance or lateral bud dormancy (Gaspar et al. 1996; Mok and Mok 2001).

As a conclusion, indirect somatic embryogenesis and/or indirect shoot organogenesis and direct organogenesis were simply controlled by one or two kinds of plant hormones. As the sole plant hormone in induction medium, the most suitable concentration of 2,4-D for indirect shoot organogenesis was 0.05 mg l<sup>-1</sup> and for somatic embryogenesis was 2 mg l<sup>-1</sup>; the optimal concentration for type III callus with both indirect somatic embryogenesis and for indirect shoot organogenesis was 0.1 mg l<sup>-1</sup>. But in the whole studies, 1 mg l<sup>-1</sup> NAA was the optimal concentration for inducing indirect shoot organogenesis and 1 mg l<sup>-1</sup> BAP was best for direct multiple shoot formation. The combination of 2 mg l<sup>-1</sup> NAA and 1 mg l<sup>-1</sup> 2,4-D was most suitable for type III callus. The simple regulation system of morphogenesis pathways will be helpful for further research works to study the relationship between plant hormones and *in vitro* explants development.

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