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ORIGINAL PAPER

Effect of growth regulators on microspore embryogenesis in coconut anthers

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Abstract The effect of growth regulators on induction of androgenesis in coconut was investigated using seven different growth regulators at various concentrations and combinations. Three auxins (1-naphthalene acetic acid—NAA, indoleacetic acid—IAA, picloram) and three cytokinins (2-isopentyl adenine-2-iP, kinetin, zeatin) were tested either alone or in combination with 2,4-dichlorophenoxyacetic acid (2,4-D), using modified Eeuwens Y3 liquid medium as the basal medium. Among the tested auxins, 100 μM NAA in combination with 100 μM 2,4-D enhanced the production of calli/embryos (123) whereas IAA and picloram showed negative and detrimental effects, respectively, for androgenesis induction over 100 μM 2,4-D alone. Kinetin and 2-iP enhanced the production of calli/embryos when 100 μM 2,4-D was present in the culture medium. Both cytokinins at 10 μM yielded the highest frequencies of embryos (113 and 93, respectively) whereas zeatin (1 or 2.5 μM) had no impact on microspore embryogenesis. When calli/embryos (produced from different treatments in different experiments) were subcultured in somatic embryo induction medium (Y_3 medium containing 66 μM 2,4-D), followed by maturation medium (Y_3 medium without growth regulators) and germination medium (Y_3 medium containing 5 μM-6-benzyladenine—BA and 0.35 μM gibberellic acid—GA_3), plantlets were regenerated at low frequencies (in most treatments ranging from 0% to 7%).

Keywords Androgenesis · Anther culture · Auxin · Cytokinin · Callus · *Cocos nucifera* · Embryo

Abbreviations

BA	Benzyladenine
2,4-D	2,4-Dichlorophenoxyacetic acid
GA_3	Gibberellic acid
IAA	Indoleacetic acid
2-iP	2-Isopentyl adenine
NAA	Naphthalene acetic acid

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Introduction

Tall coconut palm (*Cocos nucifera* L.), an allogamous and variable species, is highly heterozygous which is a major constraint in conventional breeding. Availability of homozygous lines would improve the efficiency of generating new cultivars through breeding programs. Gametic embryogenesis allows single-step development of completely homozygous lines from heterozygous parents (Dunwell 1986; Germanà 2006). Although the production of haploids by microspore embryogenesis has been reported for many

crop species, haploid plant production from anthers, or isolated microspores of woody species has had a low success rate (Peixe et al. 2004). The formation of callus from cultured anthers has been reported in some fruit species but with limited plant regeneration (Hammerschlag 1983; Todorovic et al. 1992; Peixe et al. 2004; Germanà 2006).

Even though the studies on coconut anther culture have been initiated since the 1970s, complete plant regeneration has not been reported (Iyer and Raina 1972; Iyer 1981; Kovoor 1981; Thanh-Tuyen and De Guzman 1983; Monfort 1985). Only a few anther-derived multi-celled proembryos have been obtained without further development. We reported for the first time that there is a feasibility of developing homozygous lines in coconut via anther culture (Perera et al. 2008a, b).

Growth regulators are critical for controlling the androgenic response (Dieu and Dunwell 1988; Peixe et al. 2004). Auxins have been essential for induction of callus from cultured anthers of wheat (*Triticum aestivum*; Ball et al. 1993). The type and concentration of auxin has affected the frequency of callus yield, the proportion of albino plants regenerated from calli and the frequencies of dihaploids among green plants recovered (Ball et al. 1993; Trejo-Tapia et al. 2002). The optimum yield of embryogenic structures has required different auxins in different crop species (Zhou and Konzak 1989; Ishizaka 1998; Han et al. 2000). Among the auxins, 2,4-D has been employed in many anther culture systems (Ball et al. 1993) and its optimum concentration for induction of androgenesis has been species dependent (Custódio et al. 2005). Short-term exposure to high 2,4-D concentrations followed by reduced levels was more effective in wheat (Zheng and Konzak 1999). In wheat, IAA and NAA may induce direct androgenesis whereas 2,4-D promoted rapid cell proliferation and formation of non-embryogenic callus (Ball et al. 1993).

Single or combinations of growth regulators have been necessary for embryo formation in broccoli (*Brassica oleracea*; Arnison et al. 1990). Both auxins and cytokinins were required for callus production from cultured anthers in apricot (*Prunus armeniaca*; Peixe et al. 2004). However, media without growth regulators yielded a higher frequency of anther-derived callus in oat (*Avena sativa*; Rines 1983), rice (*Oryza sativa*; Chaleff and Stolarz 1981), and triticale (X *Triticosecale*; Pauk et al. 2000).

Perera et al. (2008a) showed that 100 μM 2,4-D was effective for inducing androgenesis in cultured coconut anthers. However, the frequency of calli or embryo production (8%) and their regeneration were low. Induction of embryos is preferred to callus induction due to their greater genetic stability and regeneration frequency. Thus, the present study was undertaken to test the effect of different auxins and cytokinins either individually or in combination with 2,4-D on calli/embryo yield and regeneration potential in coconut.

Materials and methods

Culture media preparation and in vitro culture conditions

Anthers containing microspores at the uninucleate stage were excised from male flowers at 3 weeks before splitting (WBS) (Perera et al. 2008a) of an adult coconut palm (cv. Sri Lanka Tall) cultivated under in the field at Bandirippuwa Estate, Lunuwila, Sri Lanka. The mother palm was about 15 years old. A newly opened inflorescence in the crown was designated as a stage 0 inflorescence and the next younger one as a stage 1 inflorescence. The palms bearing stage 0 inflorescences were selected and the rachillae (bearing male flowers) collected from stage 1 inflorescence after 1 week were designated as anthers at 3 weeks before splitting (WBS) stage. Anther culture has been performed by taking the samples in every 2 months, however, for the present study samples were collected in different seasons. The excised anthers were surface sterilized by immersion in 2% (w/v) calcium hypochlorite (with a few drops of liquid soap) under aseptic conditions for 12 min followed by four rinses (each at 2 min) with sterile distilled water.

Modified Eeuwens Y_3 liquid medium (Karunaratne et al. 1985) was used as the basal medium and it was supplemented with 9% (w/v) sucrose and 0.1% (w/v) activated charcoal (BDH Laboratory, UK). Different concentrations of each growth regulator were added to the media as described in the following experiments. After adjusting the pH to 5.8, we autoclaved the media at 121°C for 20 min. Ten anthers were cultured per Petri plate (100 × 10 mm), each containing 25 ml of culture medium. Four replicate plates were used per treatment of each experiment. The Petri plates were sealed with Parafilm and incubated in the dark at 28°C for 8 months. The following combinations of auxins and cytokinins were tested for induction and optimization of androgenic response.

Combinations of auxins

2,4-D and NAA

Nine treatments that consisted of basal medium without growth regulators, 10 μM NAA or 2,4-D, 100 μM NAA or 2,4-D, 10 μM of both NAA and 2,4-D, 100 μM of both 2,4-D and NAA, 10 μM 2,4-D and 100 μM NAA, 100 μM 2,4-D and 10 μM NAA were tested.

2,4-D and IAA

Nine treatments were tested: basal medium without growth regulators; 10 µM IAA or 2,4-D; 100 µM IAA or 2,4-D; 10 µM of both IAA and 2,4-D; 100 µM of both IAA and 2,4-D; 10 µM 2,4-D, and 100 µM IAA; 100 µM 2,4-D and 10 µM IAA.

2,4-D and picloram

The basal culture medium with 100 µM 2,4-D supplemented with 5 or 10 µM picloram was compared with the medium devoid of picloram.

Combinations of auxin and cytokinin

2,4-D and 2-iP

Three treatments that consisted of basal medium with 100 µM 2,4-D and basal medium with 5 or 10 µM 2-iP in combination with 100 µM 2,4-D were tested.

2,4-D and kinetin

Three treatments that consisted of basal medium with 100 µM 2,4-D, basal medium with 100 µM 2,4-D in combination with 5 or 10 µM kinetin were tested.

2,4-D and zeatin

Three treatments that consisted of basal medium containing only 100 µM 2,4-D and basal medium with 1 or 2.5 µM zeatin in combination with 100 µM 2,4-D were tested.

Plant regeneration

The embryos/calli produced in the above experiments were subcultured into flasks (125 ml) containing somatic embryo induction medium [with reduced 2,4-D (66 µM)], followed by maturation medium (devoid of growth regulators) and germination medium (containing 5 µM BA, 0.1 µM 2,4-D, and 0.35 µM GA_3) (Perera et al. 2008a). Each sub-culture was done at 4 weeks interval. All the cultures were maintained in the dark at 28°C until conversion of embryos and then the cultures were exposed to light (16 h photoperiod; PAR; 25 µmol m^{-2} s^{-1}).

Statistical analysis

After 3 months of culture initiation, the number of anther-derived structures (calli/embryos) were recorded. The androgenic frequency was calculated as the number of calli or embryos produced per 100 anthers cultured. The performance of these structures at each subculture (converted embryos, unconverted embryos, vitrification, and browning) was recorded. The data were analyzed using SAS statistical package (SAS Institute Inc 1999). Chi-square or maximum likelihood ANOVA was conducted using the Proc CatMod procedures of PC-SAS. Treatment means were compared using SE, 95% confidence intervals or orthogonal contrast coefficients, where appropriate (Compton 1994).

Results and discussion

Microspore embryogenesis was induced in coconut both directly and indirectly through callus intermediate under the same culture conditions. Induction of calli/embryos (Fig. 1a) could be observed after 3 months of culture initiation and continued up to 8 months indicating that the potential for pollen embryogenesis lasts for a considerable period of time (Perera et al. 2008a). After 8 months, the embryogenic potential of the anthers diminished. The peak response of anthers was observed 5 months after culture initiation (Perera et al. 2008a). Upon subculture to somatic embryo induction medium, calli, consisting of translucent masses of globules gave rise to somatic embryos, but they had a low regeneration capacity. In contrast, embryos converted into shoots (germinated) directly (Fig. 1b).

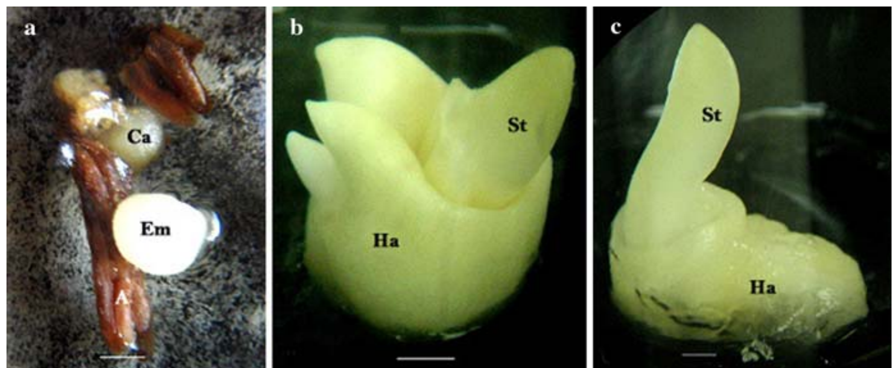

Fig. 1 Androgenesis in coconut (*Cocos nucifera*). **a** An anther (*A*) bearing both embryo (*Em*) and callus (*Ca*) (Bar = 2.5 mm). **b** Converted embryo. Note the emerging shoot (*St*) through the haustorium (*Ha*) (Bar = 2 mm). **c** Vitrified converted embryo (Bar = 1.4 mm)

Table 1 Effect of different combinations of NAA and 2,4-D on induction of androgenesis in cultured anthers of coconut

Combinations of 2,4-D and NAA (μM)		Treatment number	Number of calli or embryos per 100 anthers[a]
2,4-D	NAA		
0	0	T_1	15
0	10	T_2	13
0	100	T_3	21
10	0	T_4	31
10	10	T_5	33
10	100	T_6	81
100	0	T_7	42
100	10	T_8	65
100	100	T_9	123

[a] Mean frequency of four replications

Vitrification of the embryos, calli, and converted embryos occurred both in maturation medium and germination medium (Fig. 1c). Vitrified cultures did not regenerate.

Combination of auxins

2,4-D and NAA

NAA in combination with 2,4-D promoted callus/embryo formation (Tables 1, 2). The greatest frequency of calli/embryos (123) was observed in medium supplemented with 100 μM 2,4-D and 100 μM NAA (Table 1). However, NAA alone (at 10 or 100 μM) was ineffective in enhancing calli/embryo formation and there was no significant difference among the two treatments (T_2 and T_3) compared to medium without auxins. The frequency of callus/embryo production was increased with the increase in concentration of NAA from 10 to 100 μM in combination with either concentration of 2,4-D (10 μM or 100 μM) (T_5 vs T_6 $G^2 = 9.01$; $P < 0.01$; T_8 vs T_9 $G^2 = 8.42$; $P < 0.01$)

Table 2 Maximum likelihood analysis of variance and significance of the contrasts

Source	DF	Maximum likelihood analysis of variance
Treatment	8	87.6***
Contrasts		Chi-square
T_2 vs T_5	1	4.2*
T_2 vs T_8	1	13.6***
T_3 vs T_6	1	14.8***
T_3 vs T_9	1	27.0***
T_5 vs T_6	1	9.0**
T_5 vs T_8	1	4.6*
T_6 vs T_9	1	4.0*
T_7 vs T_9	1	15.6***
T_8 vs T_9	1	8.4**

Only required significant contrasts were listed in the table

*, **, *** significance at probability levels of 0.05, 0.01 and 0.001

(Table 2). With increasing concentrations of 2,4-D from 10 to 100 μM, the production of calli or embryos also significantly increased (Table 1).

More embryos than calli were produced in all the treatments except the medium without growth regulators (Fig. 2a). The greatest embryo yield was obtained in the medium containing 100 μM 2,4-D and 10 μM NAA, in which 3% embryo conversion was recorded (Fig. 2b). Of the calli/embryos produced in the medium without growth regulators, 29% gave rise to shoots. Most of the calli or embryos were vitrified and browning was slightly higher in NAA-containing media (Fig. 2b).

Chaleff and Stolarz (1981) observed that NAA at 10.7 μM or greater concentrations was more effective in promoting callus formation in cultured rice anthers. Zhao et al. (2006) indicated that a combination of BA and NAA was more effective than 2,4-D alone in purple coneflower (*Echinacea purpurea*). Medium supplemented with BA and NAA was best for embryo induction in cucumber (*Cucumis sativus*; Song et al. 2007). In contrast to our result, the presence of 2,4-D with NAA resulted in the lowest percentage of callus yield in asparagus (*Asparagus officinalis*) whereas no callus production was observed with 2,4-D alone (Peng and Wolyn 1999).

2,4-D and IAA

2,4-D at 100 μM (T_7) more than doubled the yield of calli or embryos per 100 anthers compared to control (T_1) whereas IAA at 10 or 100 μM reduced the production of calli or embryos, either alone (T_2 and T_3) or in the presence of 2,4-D (T_4, T_5, T_8, T_9; Table 3). Compared to the medium without growth regulators, the frequency of calli or embryos was reduced by 50% and 75% when 10 μM and 100 μM IAA was incorporated into the media, respectively (Tables 3, 4). With 10 μM 2,4-D in the culture medium, IAA at 10 μM and 100 μM decreased the frequency of the calli or embryos by 9% and 50%, respectively (Tables 3, 4). Similarly, when 10 μM and 100 μM IAA were added to

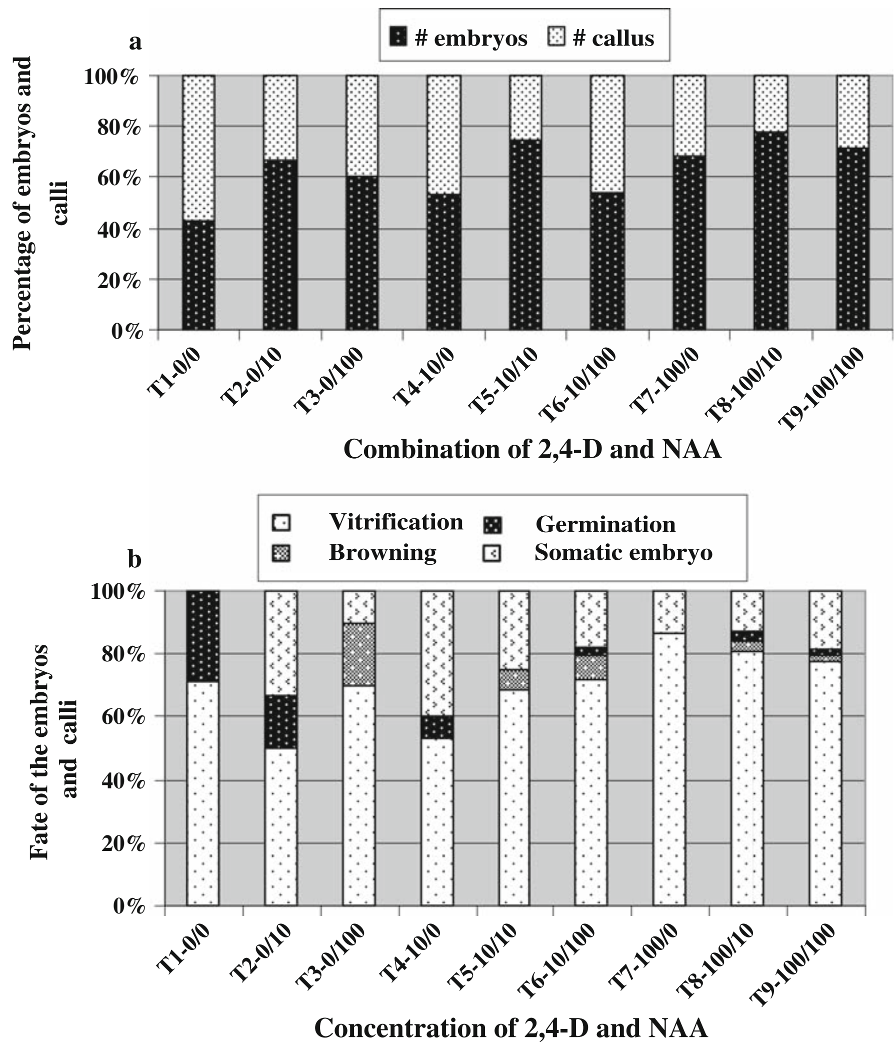

Fig. 2 **a** Percentage production of embryos and calli at each combination of 2,4-D and NAA. **b** Fate of anther derived embryos and calli at each combination of 2,4-D and NAA, after subculture into somatic embryo induction, maturation, and germination media

the media supplemented with 100 µM 2,4-D, the frequencies were reduced by 50% and 75%, respectively. A negative effect of IAA on androgenesis has also been observed in cultured anthers of rice (Chaleff and Stolarz 1981). In contrast, IAA has been used to enhance the induction of androgenesis in buckwheat (*Fagopyrum esculentum*; Bohanec et al. 1993). The intermediate level of 2,4-D (10 µM) had little influence on yield of calli or embryos.

Although the percentage of embryos among anther derived structures ranged from 50% to 89% depending on the auxin treatment (Fig. 3a) conversion of embryos occurred only in T_2 (10 µM IAA) and T_7 (100 µM 2,4-D; Fig. 3b). In medium without growth regulators, 78%

Table 3 Effect of different combinations of IAA and 2,4-D on induction of androgenesis in cultured anthers of coconut

Combinations of 2,4-D and IAA (µM)		Treatment number	Number of calli or embryos per 100 anthers[a]
2,4-D	IAA		
0	0	T_1	45
0	10	T_2	23
0	100	T_3	18
10	0	T_4	50
10	10	T_5	45
10	100	T_6	23
100	0	T_7	128
100	10	T_8	63
100	100	T_9	30

[a] Mean frequency of four replications

Table 4 Maximum likelihood analysis of variance and significance of the contrasts

Source	DF	Chi-square
Treatment	8	67.8***
Contrasts		Chi-square
T_1 vs T_3	1	4.5*
T_4 vs T_6	1	3.1*
T_4 vs T_7	1	12.6***
T_7 vs T_8	1	5.3*
T_7 vs T_9	1	20.0***
T_8 vs T_9	1	7.2**

Only the significant contrasts were listed in the table

*, **, *** significance at probability levels of 0.05, 0.01 and 0.001

embryo formation was observed but none converted (Fig. 3a, b). Production of calli in auxin-free medium has been reported in barley (*Hordeum vulgare*; Cai et al. 1992), rice (*Oryza sativa*; Chaleff and Stolarz 1981), and triticale (X *Triticosecale*; Pauk et al. 2000) but the quality and regeneration ability of such calli were poor. In wheat, anthers produced little calli without exogenous auxin added to the culture medium and most of the calli induced under low auxin concentrations were not regenerated into plants (Zheng and Konzak 1999). Low 2,4-D concentration (10 μM) gave rise to a greater embryo yield than calli indicating that the level of auxin may have been insufficient for callus proliferation (Fig. 3a). Comparable results were obtained in carob (*Ceratonia siliqua*; Custódio et al. 2005). Browning was generally greater in the cultures containing IAA (Fig. 3b).

2,4-D and picloram

Picloram (in combination with 100 μM 2,4-D) was not effective in inducing the formation of calli or embryos at any of the levels tested. Twenty-five calli or embryos per 100

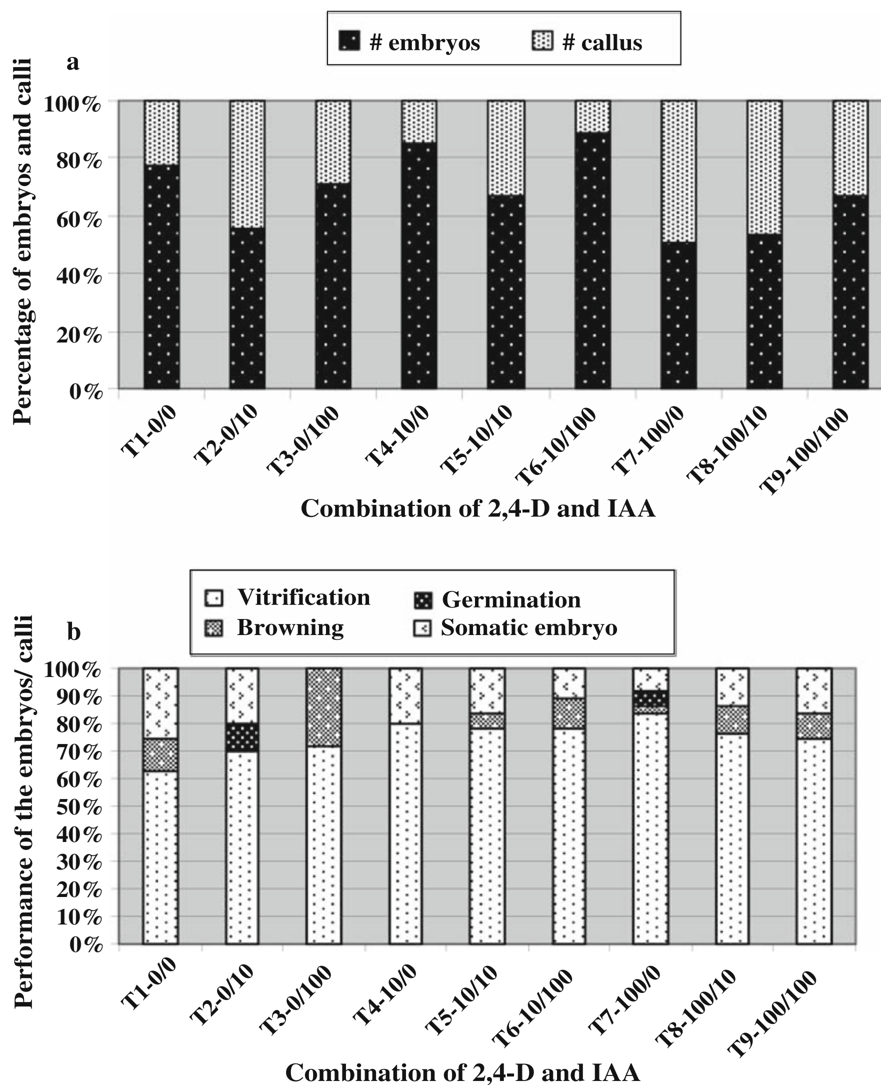

Fig. 3 **a** Percentage production of embryos and calli at each combination of 2,4-D and IAA. **b** Fate of anther derived embryos and calli for each combination of 2,4-D and IAA, after subsequent subculture into somatic embryo induction, maturation, and germination media

Table 5 Effect of 2-iP, kinetin, and zeatin in combination with 2,4-D on the formation of calli/embryos in cultured coconut anthers and significance of the contrasts

Combination of auxine/cytokinin	2-iP			Kinetin			Zeatin		
	0[a] (T_1)	5[a] (T_2)	10[a] (T_3)	0[b] (T_1)	5[b] (T_2)	10[b] (T_3)	0[c] (T_1)	1[c] (T_2)	2.5[c] (T_3)
100 μM 2,4-D[d]	55	23	93	38	90	113	55	48	45
Significant contrasts[e]	T_1 vs T_2 (5.1*) T_1 vs T_3 (3.7*) T_2 vs T_3 (14.5***)			T_1 vs T_2 (8.1*) T_1 vs T_3 (13.6***)			NS		

[a] 2-iP concentrations (μM)

[b] Kinetin concentrations (μM)

[c] Zeatin concentrations (μM)

[d] Each observation is the mean of four replicates

[e] Only significant contrasts are listed and the Chi-square values and their significant levels in parenthesis

*, **, *** significance at probability levels of 0.05, 0.01 and 0.001

anthers were produced only in the medium containing 100 μM 2,4-D and 4% of them gave rise to shoots. This is in contrast to the results obtained by Han et al. (2000) in which picloram (4 μM) was found to be essential for induction and maintenance of calli in cultured anthers of *Lilium* species.

Auxin was not required for callus or embryo induction in coconut; our control cultures without auxin yielded 15 and 45 calli or embryos in different experiments (Tables 1, 3); however, androgenic response was significantly improved with auxin. Based on the results of the above three experiments, 2,4-D was the most suitable auxin that can be used alone, whereas picloram and IAA had a detrimental and negative effect, respectively. Furthermore, the effect of 2,4-D can be enhanced by incorporating NAA into the anther culture medium. According to Parrott et al. (1991), on exposure to auxin, substantial DNA methylation occurs that may stop or hinder the expression of existing developmental programmes within the cell that in turn may cause the cells to enter the callogenesis and somatic embryogenesis pathway. However, Devaux et al. (1993) reported that there was no significant difference between IAA and NAA on embryo formation in anther culture of recalcitrant barley genotypes.

Combinations of auxins and cytokinins

A series of experiments was conducted to determine the influence of three cytokinins (2-iP, kinetin, and zeatin) on embryo/callus production from coconut anthers, in combination with 100 μM 2,4-D.

2,4-D and 2-iP

Although 5 μM 2-iP was inhibitory in combination with 100 μM 2,4-D, 10 μM 2-iP had a positive effect on induction of androgenesis (Table 5). In this experiment, the highest frequency of calli or embryo production (93) was observed with 10 μM 2-iP in combination with 100 μM 2,4-D (T_3), significantly improving yield over the control (T_1) (Table 5).

Table 6 Fate of anther derived embryos/calli at each level of 2-iP, kinetin, and zeatin in combination with 100 μM 2,4-D, after subculture into somatic embryo induction, maturation, and germination media

Type and level of cytokinin	2-iP			Kinetin			Zeatin		
	0[a] (T_1)	5[a] (T_2)	10[a] (T_3)	0[b] (T_1)	5[b] (T_2)	10[b] (T_3)	0[c] (T_1)	1[c] (T_2)	2.5[c] (T_3)
Converted embryo/calli	0	0	5	7	0	2	0	0	0
Unconverted embryos/calli	14	11	25	13	6	16	10	11	11
Vitrification	86	67	65	80	86	82	90	84	78
Browning	0	22	5	0	8	0	0	5	11
Percentage production of embryos and calli									
Embryos	68	56	54	44	62	36	71	74	67
Calli	32	44	46	56	38	64	29	26	33

[a] 2-iP concentrations (μM)

[b] Kinetin concentrations (μM)

[c] Zeatin concentrations (μM)

The greatest percentage of embryos was obtained in the medium supplemented with 100 μM 2,4-D (Table 6). It was two times greater than the percentage of calli however, none of the total calli/embryos converted (Table 6). 2-iP in combination with 2,4-D resulted in a reduction in the percentage of embryo formation. Shoot formation was only observed in the treatment where 10 μM 2-iP was used (T_3, 5%, Table 6). Tissue browning was comparatively greater in medium containing 2-iP. Most calli/embryos (65–86%) were discarded due to vitrification.

2,4-D and kinetin

The frequency of calli or embryo production was significantly greater when kinetin either at 5 or 10 μM was incorporated into the androgenesis induction medium containing 100 μM 2,4-D (Table 5). The greater concentration of kinetin (10 μM) yielded the greatest frequency of embryos/calli (113).

More embryos than callus were observed in the treatment with 5 μM kinetin in combination with 100 μM 2,4-D (T_2) compared the medium without kinetin (T_1) or that with 10 μM kinetin (T_3) (Table 6). Comparable to this result, medium supplemented with 2,4-D and kinetin favored the production of embryogenic calli in niger (*Guizotia abyssinica*; Sarvesh et al. 1993). No response was obtained when 2,4-D was used alone and kinetin was shown to be essential for triggering cell division in niger. Song et al. (2007) reported that medium supplemented with BA in combination with kinetin was best for embryogenic callus induction in cucumber.

In this experiment, the conversion of embryos was greater in the control treatment with 100 μM 2,4-D (T_1) compared to medium supplemented with 10 μM kinetin and 100 μM 2,4-D (Table 6). In oat, plant regeneration was achieved by culturing anthers initially in a medium containing 2,4-D and kinetin (Rines 1983). Similar to the other experiments in the present study, the rate of vitrification was high (80–82%). Browning was observed only in the medium containing 5 μM kinetin (T_2).

2,4-D and zeatin

In combination with 100 μM 2,4-D, zeatin (1 μM, 2.5 μM) had no significant influence on the frequency of embryos/calli induced from anthers of coconut (Table 5). Zeatin in combination with 2,4-D, a high frequency of callus formation was recorded from peach anthers (*Prunus persica*; Todorovic et al. 1992). Despite a high ratio of embryos to callus in most treatments with zeatin (Table 6), none of these embryos converted. As in other experiments, most anther-derived structures became vitrified (78–90%) in these cultures too.

There was considerable variation in androgenesis frequencies among the five experiments in the same control treatment (100 μM 2,4-D). This could have been due to variation in the pollen developmental stage at sample collection times. Induction of androgenesis is developmentally regulated (Zheng 2003; Pechan and Smykal 2001). Even though samples were collected at 3 WBS stage, minor changes of the sporophytic pathway could occur based on the prevailing environmental conditions at different seasons of the year.

Even though greater frequencies of calli/embryos could be obtained under several of our experimental treatments, plant regeneration was infrequent. Both embryos/calli gave rise to shoots as indicated in each experiment. Out of hundreds of calli/embryos produced in this study, only 15 converted and developed into plantlets. Plantlets with a single, double, or multiple shoots were produced. Flow cytometric analysis and SSR marker analysis (Perera et al. 2008b) revealed that all the plantlets were diploid and homozygous for the loci tested.

High vitrification of cultures was a common problem in all the experiments conducted in this study. It may be attributed to a prolonged period of culture in liquid media. According to Parrott et al. (1991), when an embryogenic cell is present in a tissue, the stimulation of cell division may be all that is necessary to perpetuate the embryogenic state and form somatic embryos. However, the regeneration capacity of the anther-derived structures diminished with time. Although auxin is needed to induce somatic embryogenesis, the continued presence of auxin in the medium could be detrimental to normal development (Parrott et al. 1988), especially on the development of the apical meristem of somatic embryos (Halperin and Wetherell 1964) that could lower the regeneration capacity. Zhao et al. (2006) also indicated that the regenerated shoots had a prominent symptom of vitrification.

Results of the present study indicated that the response of anther culture in coconut mainly depends on the type and concentration of growth regulators in the culture medium. Greatest frequencies of calli or embryo production were obtained in media supplemented with 100 μM 2,4-D. The yield of embryos of calli in this treatment varied from 37 to 127 structures per 100 cultured anthers. In some experiments, the yield was improved by supplementing the medium containing 100 μM 2,4-D with 100 μM NAA, 10 μM 2-iP, or 10 μM kinetin. Further studies are important for development of an efficient protocol for embryo conversion in order to establish androgenesis as part of a breeding program to generate true coconut hybrids.

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