EFFECT OF PRETREATMENT AND AGE OF EXPLANTS ON CALLOID INITIATION OF COCONUT INFLORESCENCE

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Browning of explants and calloid initiation on immature coconut inflorescences of various ages collected from *Catigan x Baybay* hybrid were studied. The effect of duration of exposure to liquid pre-culture media supplemented with varying levels of 2,4-D was also evaluated. Among the inflorescences tested, inflorescence no. 4 exhibited least browning and the highest incidence of calloid formation when pre-cultured in 2,4-D-free liquid medium. Pre-culture duration of up to 3 days significantly reduced browning of the explants.

Key words: *Coconut, calloid, pretreatment, pre-culture media, browning of explants, 2,4-D*

Researchers around the world have been applying the techniques of tissue culture leading to an ultimate goal of increasing food production. The development of an asexual propagation method for oil palm (Corley et al. 1979) has greatly encouraged other workers to apply the technology to coconut. Plantlets have been regenerated from inflorescence, leaf tissues, and immature embryos of coconut. However, only sporadic, hardly repeatable successes have been reported (Aboga et al. 1990; Branton and Blake 1984; del Rosario et al. 1989; Karunaratne and Periyapperuma 1989; Raju et al. 1984; Thanh Tuyen and Apurillo 1992; Verdeil et al. 1991) by various groups working on the same aspects of coconut research. Progress is very slow since there are many difficulties to be surmounted. Several factors have to be considered before a clonal propagation system for commercial exploitation is attained (Brackpool et al. 1986).

In many crops, soaking explants in water or pre-culturing them in unsupplemented media for several days is not only effective in minimizing browning of tissues but may also have a promotive effect on growth and morphogenesis of explants in culture.

In addition to palm maturity, the physiological maturity of the explant is likewise considered to be another critical factor in obtaining embryogenic cultures. Previous findings showed that leaf explants from young coconut palms responded better to *in vitro* techniques than those from older plants (Verdeil et al. 1989). Moreover, immature embryos have been shown to respond better than the mature zygotic embryos (Karunaratne and Periyapperuma 1989).

In Albay Research Center, the immature inflorescence no. 4, corresponding to the fourth frond from the youngest open frond (spear leaf is considered no. 0), has been found most responsive to callus induction. Coconut has only one growing point and there are no axillary branches. The very young inflorescence contains numerous meristematic points making it an ideal explant for vegetative propagation. However, collection of this particular inflorescence exposes the palm to too much stress since significant damage is inflicted to the palm before the inflorescence could be collected. Although a technique has been developed to collect inflorescence of this age without killing the trees (Rillo 1989), it would be a lot more convenient if older inflorescences could be induced to form callus by way of using various auxin concentrations in the pretreatment media. Besides, more explants would be available from an older inflorescence because of its bigger size.

This paper reports on the interaction of liquid pre-culture media with varying levels of 2,4-Dichlorophenoxyacetic acid (2,4-D) at different durations of pre-culture of the explants obtained from different ages of *CAT x BAY* inflorescences. The experiment was conducted to determine the combination of these factors which will minimize browning incidence and induce more and earlier calloid formation.

MATERIALS AND METHODS

Inflorescences corresponding to frond numbers 4, 5 and 6 (designated as inflorescence numbers 4, 5 and 6, respectively) from *CAT x BAY* hybrid population were used in this study. The inflorescences were collected using the non-lethal technique as described by Rillo (1989). For disinfection, the inflorescences were swabbed with 80% ethanol and soaked in 100% commercial bleach (5.25% sodium hypochlorite) for 30 min while the outer spathe was still intact. After thorough rinsing, the outer and inner spathes were removed aseptically to expose the rachis. The rachillae were severed from the rachis and incubated whole in various pre-culture media. The cultures were incubated in the dark at various durations.

After pretreatment, the middle portion of the rachillae was transversely cut to pieces of about 0.5 mm thickness and inoculated onto callus initiation or Blake's medium (Ebert and Taylor 1990).

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The following variables were considered: physiological age of inflorescence (I_4 = inflorescence no. 4, I_5 = inflorescence no. 5 and I_6 = inflorescence no. 6); duration of exposure to pretreatment media (D_0 = 0 day, D_3 = 3 days, D_7 = 7 days, D_{14} = 14 days, D_{21} = 21 days and D_{28} = 28 days); pre-treatment media (C_0 = basic Blake's media [Blk], C_1 = Blk + $1x10^{-4}$ M 2,4-D, C_2 = Blk + $2x10^{-4}$ M 2,4-D, and C_3 = Blk + $4x10^{-4}$ M 2,4-D).

The explants were sub-cultured every 4 wk. The frequencies of browning and calloid formation were recorded every 4 wk for each treatment and replicate.

The treatments were applied in a 3-factorial experiment and replicated 3 times. The data were analyzed statistically using ANOVA and DMRT.

RESULTS AND DISCUSSION

After 2 mo, cultures of I_4 exhibited the lowest browning incidence and the highest percentage of calloid formation. It took 5 mo before I_5 cultures could approximate the frequency of calloids formed on I_4 cultures incubated for 3 mo. Calloid formation on I_6 cultures started only after 3 mo in culture. The calloids formed after 5 mo in culture were just approximately equal to calloids formed in I_4 cultures after a 2-mo incubation period (Table 1). A consistent increase in calloid frequency from 2 mo to 5 mo in culture was observed on I_4 cultures. I_5 cultures exhibited less calloid formation and gradual levelling-off in frequency from 2 mo to 5 mo in culture. I_6 cultures showed the poorest performance with very low monthly increment in calloid information (Fig. 1).

Regardless of inflorescence age and concentration of 2,4-D in the pre-culture media, immediate planning (D_0) and 3-day exposure to pre-culture media (D_3) brought about the least browning. When explants were exposed for 21 days to pre-culture media, more calloid formation was observed during the first 3 mo in culture. However, further exposure of cultures to the same media resulted to insignificant differences among exposure durations tested. The effect of pre-culture treatment became negligible after 4 to 5 mo in culture (Table 2).

Minimal browning was initially observed when tissues were pre-cultured in liquid media devoid of 2,4-D (C_0). After 2 mo in culture, the effect of 2,4-D became negligible with respect to browning incidence. In terms of calloid formation, the effect of the auxin incorporated in the pre-culture media became apparent only after 5 mo in culture. Regardless of inflorescence age and duration of exposure to pre-culture media, calloid formation observed after 5 mos suggested that incorporation of 2,4-D in the liquid pre-culture media has no advantageous effect on calloid initiation (Table 3, Fig. 2).

Explants pre-cultured in 2,4-D-free (C_0) liquid media produced more calloids

TABLE 1
Browning Incidence and Calloid Formation of Inflorescence Numbers 4, 5 and 6 Observed Monthly after 1 Mo to 5 Mo in Culture[1]

TREATMENT	BROWNING INCIDENCE (%)		CALLOID FORMATION (%)			
	1 mo	2 mo	2 mo	3 mo	4 mo	5 mo
Inflorescence #4	10.34 b	22.77 c	2.27 a	10.31 a	18.08 a	25.78 a
Inflorescence #5	15.73 b	33.05 b	0.80 b	4.84 b	7.87 b	10.03 b
Inflorescence #6	24.05 a	52.86 a	0.00 b	0.89 c	1.63 c	2.46 c

[1] Means followed by the same letter in the same column, are not significantly different at 1% level.

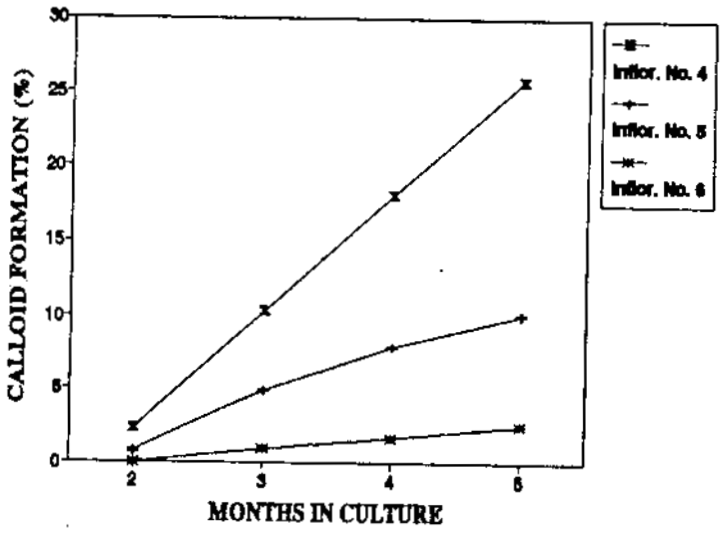

FIGURE 1
Monthly increment in calloid formation of immature inflorescence after 2-5 mo in culture

TABLE 2
Effect of Duration of Exposure to Liquid Pre-culture Media on Browning Incidence and Calloid Formation of Immature Inflorescence[1]

TREATMENT	BROWNING INCIDENCE (%)		CALLOID FORMATION (%)			
	1 mo*	2 mo**	2 mo*	3 mo**	4 mo^	5 mo^
0 day (DO)	9.13 b	18.39 c	1.48 ab	4.94 bc	9.63	10.37
3 days (D3)	10.17 b	17.45 c	0.74 ab	5.86 abc	10.31	14.69
7 days (D7)	15.56 ab	32.10 b	0.34 b	2.68 c	5.88	11.13
14 days (D14)	24.56 a	62.82 a	0.00 b	1.25 c	4.88	8.97
21 days (D21)	21.14 a	43.66 b	2.10 a	9.82 a	13.32	17.49
28 days (D28)	19.69 ab	42.94 b	1.48 ab	7.53 ab	11.13	13.89

[1] Means followed by the same letter in the same column are not significantly different.
* = significant at 5% level; ** = significant at 1% level; ^ = not significant

TABLE 3
Effect of Various 2,4-D Levels in the Pre-culture Media on Browning Incidence and Calloid Formation of Immature Inflorescence[1]

TREATMENT	BROWNING INCIDENCE (%)		CALLOID FORMATION (%)			
	1 mo*	2 mo^	2 mo^	3 mo^	4 mo^	5 mo*
No 2, 4-D (C0)	10.69 b	31.99	0.95	4.43	11.34	17.96 a
1 x (10)-4M 2,4-D (C1)	18.86 ab	39.18	1.15	5.68	8.07	10.78 b
2 x (10)-4M 2,4-D (C2)	22.04 a	39.17	1.05	5.46	8.30	9.91 b
4 x (10)-4M 2,4-D (C4)	15.23 ab	34.56	0.95	5.81	9.05	13.39 ab

[1] Means followed by the same letter in the same column are not significantly different.
* = significant at 5% level; ^ = not significant

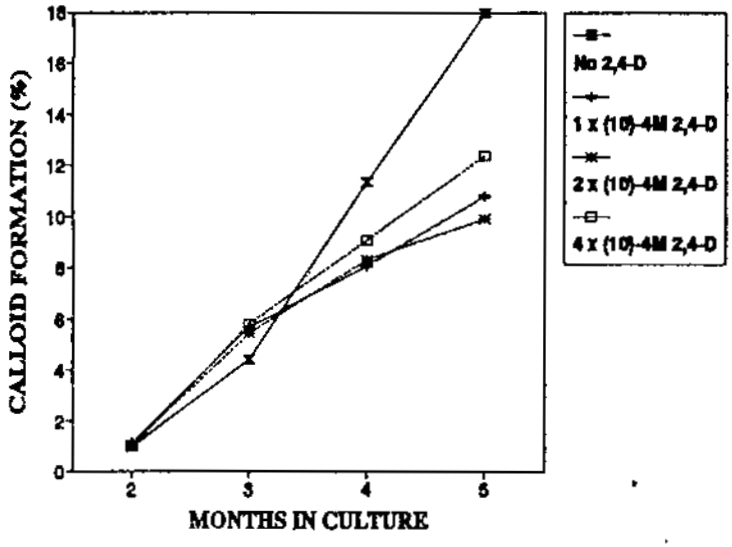

FIGURE 2
Calloid formation on immature inflorescences as influenced by 2,4-D levels in the pre-culture media

compared to those pre-cultured in 2,4-D supplemented liquid media (C_1, C_2 and C_4). Positive interaction between inflorescence age and concentration of 2,4-D in the pre-culture media was obvious after 5 mo. I_4 proved superior thereby exhibiting least browning of cultures and the highest incidence of calloid formation when pre-cultured in 2,4-D-free liquid medium (Table 4).

CONCLUSIONS

The results of the study indicated that inflorescence no. 4 is the best explant for calloid initiation. Moreover, browning incidence can be minimized by culturing the explants in auxin-free liquid pre-culture medium not exceeding 3 days.

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TABLE 4
Interaction Effect of Age of Inflorescence and 2,4-D Levels in the Pre-culture Media on Browning Incidence and Calloid Formation of Immature Inflorescence[1]

TREATMENT	BROWNING INCIDENCE (%)		CALLOID FORMATION (%)				
	1 mo^	2 mo^	2 mo^	3 mo^	4 mo^	5 mo*	
I4 x C0	4.31	17.01	2.10	10.95	25.91	39.59	a
I4 x C1	11.73	27.98	2.22	11.05	14.75	19.81	ab
I4 x C2	14.56	26.94	2.04	8.70	15.07	18.65	ab
I4 x C4	10.74	19.15	2.72	10.52	16.59	25.06	ab
I5 x C0	9.13	28.29	0.74	2.20	7.87	11.94	ab
I5 x C1	13.93	34.40	1.23	5.63	8.10	10.55	ab
I5 x C2	22.45	33.64	1.11	6.09	7.56	8.67	ab
I5 x C4	17.42	35.86	0.12	5.43	7.92	8.96	ab
I6x C0	18.64	50.68	0.00	0.12	0.25	2.35	b
I6 x C1	30.92	55.15	0.00	0.37	1.36	1.97	b
I6 x C2	29.12	56.94	0.00	1.57	2.27	2.40	b
I6 x C4	17.53	48.68	0.00	1.48	2.64	3.14	b

[1] Means followed by the same letter in the same column are not significantly different.
* = significant at 5% level; ^ = not significant