

RESPONSES OF NON-MAKAPUNO EMBRYOS IN VITRO TO CHLORIDE SUPPLEMENTATION¹

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ABSTRACT

Coconut embryos were cultured following a liquid-solid sequence. The media for the liquid culture were modified to provide increasing levels of chloride using NaCl, KCl and NH₄Cl. The effect of the chloride was also tested by modifying the chlorine content of the medium for both the liquid and solid cultures by substituting MgCl₂ for MgSO₄. Growth of the embryos during the liquid culture was significantly enhanced by KCl at 4x level but highly inhibited by NH₄Cl at 8x level. All levels of KCl pretreatments significantly improved root development during the solid culture. Applied during the initial phase, MgCl₂ enhanced growth of the embryos in liquid culture and promoted leaf formation during the subsequent solid culture.

Treatments during the liquid phase influenced growth and development during the subsequent solid culture. The lack of significant promotion of growth with NaCl and NH₄Cl was attributed to the adverse effects of the accompanying cations.

INTRODUCTION

Cutter and Wilson (1954) pioneered the culture of coconut embryos in vitro. Although only a meager amount of growth was obtained, they were able to establish the viability of the embryos in aseptic culture and had provided a basis for subsequent researches along this line. Abraham and Thomas (1962) recognized the potential of embryo culture as a possible tool in coconut breeding and this motivated them to improve the technique.

In the Philippines, in vitro culture of coconut "makapuno" embryos was initiated as a solution to the decided non-germinability of this seed in situ. That the "makapuno" embryo is viable and develops in vitro just like that of ordinary coconut was shown by de Guzman and del Rosario (1964). Successful cultures were finally obtained by further modifications of the technique and of the media (de Guzman, 1969; Balaga and de Guzman, 1970; de Guzman, del Rosario and Eusebio, 1970). The in vitro seedlings were successfully established in pot cultures and finally in open field (de

Guzman and del Rosario, 1974). Some of these are now at the bearing stage.

With this breakthrough in agriculture, a more extensive production of "makapuno" is now possible. In spite of this success, however, embryo culture technique of coconut still has room for improvement. For optimum benefits, some limitations to be overcome are the variable response and slow rate of growth during the laboratory phase.

To formulate improved media for faster growth, it was decided to consider the findings on the mineral nutrition of the intact coconut plant. Coconut had been reported to have a special requirement for chlorine (von Uexkull, 1972; Mendoza and Prudente, 1972; Premier and Brunin, 1974; Brunin, Coomans and Ouvier, 1975; and Magat and Prudente, 1975). Coconut cultured in vitro may likewise be benefited by additional supply of the element. This study, therefore, aims to establish the favorable effects of additional chlorine supply on the growth of coconut embryos and seedlings in vitro, its optimum concentration and the best salt as source.

MATERIALS AND METHODS

Experimental Set-up

This study is consisted of four experiments using different chlorinated salts. Experiments I to III dealt only with the initial liquid culture. In experiment I chlorine supplementation was done with sodium chloride (NaCl). The treatments are as follows: (1) Control - Modified White's (Wo); (2) Wo plus 50.95 mg/l NaCl (1x); (3) Wo plus 101.9 mg/l NaCl (2x); (4) Wo plus 203.8 mg/l NaCl (4x); and (5) Wo plus 407.6 mg/l NaCl (8x). Experiment II used potassium chloride (KCl) with the following treatments: (1) 1x KCl or the standard amount (control); (2) 2x KCl; (3) 4x KCl; (4) 8x KCl. The treatments for Experiment III using ammonium chloride (NH₄Cl) are as follows: (1) Control - Wo, (2) Wo plus 46.66 mg/l NH₄Cl, (3) Wo plus 93.32 mg/l NH₄Cl, (4) Wo plus 186.64 mg/l NH₄Cl and (5) Wo plus 573.28 mg/l NH₄Cl. For experiment IV-A, magnesium sulfate (MgSO₄) and magnesium chloride (MgCl₂) were compared as Mg source during the initial culture. The treatments are control (Wo) and Wo minus MgSO₄ plus MgCl₂ and Na₂SO₄. In Experiment IV-B, the comparison between MgSO₄ and MgCl₂ as Mg source was done during the first solid transfer. The treatments are control - modi-

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fied Murashige and Skoog's medium (Mo) and Mo minus $MgSO_4$ plus $MgCl_2$ and Na_2SO_4 .

Modified White's medium and modified Murashige and Skoog's mineral formulations were used as the standard basal liquid and solid medium, respectively. Dextrose at 4% and vitamins were added, as formulated by Nitsch and Nitsch (1967). For the solid medium, 0.7% agar (Japanese agar-agar) was incorporated. Activated charcoal was added at 1%. The freshly prepared media were autoclaved for 20 minutes at 15 psi with temperature maintained at 250°F.

Embryos of mature non-makapuno nuts collected from a desiccated coconut factory were used. These were sterilized and prepared for inoculation as described in previous papers on the "makapuno" embryo cultures. For statistical analysis of data the RCB design was used.

RESULTS

Effects of Chlorine Supplementation on the Growth of Embryos During the Liquid Culture.

The response of embryos to chlorine supplementation during the liquid culture varied with the kind of salt used.

The addition of NaCl resulted in a slight promotion of growth with 203.8 mg/l giving the best result (Table 1). The increase in weight however was found to be statistically insignificant.

Although there was no significant stimulation of growth, the addition of NaCl resulted in a lesser degree of browning. Unlike those of the control, there was no marked discoloration at the distal region of the embryos. Instead, they were whitish to creamy in color all over except for a few which had acquired a straw-brown coloration at the distal region.

With KCl, increasing the supply to two and four times that of the control promoted growth of the embryos with the 4x treatment giving a significant difference (Table 2). Further increasing the KCl level up to eight times no longer effected any additional stimulation of growth. Unlike in Experiment I, embryos subjected to KCl treatment showed a more or less similar degree of browning as those of the control.

As seen in Table 3 higher weights were likewise exhibited by embryos when subjected to additional chloride supplied by NH_4Cl . Statistical analysis however, showed that the promotion was not significant. On the other hand Table 3 shows that NH_4Cl was significantly inhibitory at the highest level used. This case is unlike that of NaCl in KCl where within the range of concentration tested no inhibitory level was reached compared with the control. Aside from the marked reduction of weight, high NH_4Cl intensified discoloration of the embryos. The extent of browning in the control was comparable with the other treatments.

In Experiment II, the level of potassium was not held constant for both the control and the experimental media. Hence, whether the significant promotion of growth in treatment 3 (Table 2) was actually due to potassium rather than to improved chlorine nutrition can not be determined. Experiment IV was done to complement Experiment II. In this new experiment $MgSO_4$ was replaced by $MgCl_2$. To restore the original amount of sulfate, Na_2SO_4 was added. Therefore, chloride was increased without necessitating an accompanying increase of the potassium level.

It was found out that $MgCl_2$ proved to be a better source of magnesium than $MgSO_4$. The use of $MgCl_2$ instead of $MgSO_4$ resulted in a significant increase in weight of the embryos (Table 4). This points to the favorable effect of chlorine since the treatment did not alter the level of potassium.

Also as observed in Experiment IV-A, germination was significantly enhanced in the $MgCl_2$ medium during the initial liquid culture (Table 4a). As noted by Balaga and de Guzman (1970), growth during the liquid phase was mainly an enlargement of the embryos. Germination can normally occur but in very low percentage. Although germination occurs during the liquid culture majority of the embryos remained ungerminated until they are transferred to solid media.

Table 1a shows that some embryos germinated during the liquid phase and that this ability was greatly reduced at the highest level of NaCl. Increasing the level of KCl in treatments 2 and 3 stimulated germination (Table 2a). Chloride supplementation with NH_4Cl greatly depressed germination (Table 3a).

Effect of Previous Treatments on Subsequent Growth in Solid Culture

The precultured embryos in chlorine-supplemented liquid media together with the control were transferred to solid standard media for further growth and development. No striking feature of development suggesting a further approximation of the pattern of growth in situ such as the bulbous haustorium has been noted so far. However, there are several beneficial growth responses associated with high chlorine pretreatment.

There was slight promotion of growth with seedlings developed from embryos precultured in liquid media supplemented with varying levels of NaCl (Tables 5 and 5a). However, definite enhancement of growth particularly of the root was obtained by preculturing in high KCl media (Tables 6 and 6a). All KCl treatments produced a significant increase in root weight. A more frequent occurrence of twin and triple main roots was observed (Figs. 1a and 1b).

Increased concentration of KCl tended to stimulate the growth of the leaf primordia. The maximum number of true leaves produced was three which was observed in seedlings developed from embryos pretreated with 4x KCl. In the other treatments, a number

of seedlings produced two true leaves. In contrast, the maximum leaf number produced in the control was only up to the first true leaf.

Previous treatment with NH_4Cl also resulted in certain effects on the overall growth of the seedlings. At higher levels NH_4Cl treatment produces an inhibitory effect on shoot growth but not on root growth (Tables 7 and 7a). In fact there was slight stimulation of better root development when NH_4Cl was added.

Improvement of growth was also observed when MgCl_2 was used as magnesium source (Table 8). Shoot formation was favored as manifested by a significantly higher leaf count over the control. Shoot and root length and weight, respectively, were higher too but not statistically different from the control (Tables 8 and 8a).

Magnesium chloride seems to predispose the embryos to greater root initiation. The general tendency of embryos subjected to high chlorine pretreatment to produce seedlings with more extensive laterals both in the form of pneumatophore-like laterals and rootlets and primary adventitious roots were again observed (Figs. 2a and 2b).

Preculturing in high chlorine media did not alter the percentage rooting (Tables 5, 6a, 7, 8 and 9). Failure of root formation occurred rarely. Germination of the embryos in solid culture was not enhanced by the treatments during the liquid culture with the exception of KCl where a slight stimulation was observed (Table 6a). Furthermore, inhibition of germination due to high NaCl and NH_4Cl pretreatments was not observed in the solid medium. As previously mentioned, these last two treatments tended to suppress germination during the liquid culture. The variability of response of coconut embryos in vitro as reported in previous works with the *makapuno* embryo was likewise noted. Some embryos failed to germinate even after eight weeks in solid media in spite of a very desired size acquired after the initial liquid culture. Still others may germinate but only towards the end of the first solid culture.

Effect of MgCl_2 Treatments in Solid Medium.

Treatment with MgCl_2 during the first solid phase more or less gave a similar effect as when the treatment was introduced during the liquid culture. The treatment with MgCl_2 during the solid culture brought about a consistent although statistically insignificant increase in leaf count and weight of shoot (Tables 9 and 9a). The tendency to favor root growth is shown in Figure. 3.

DISCUSSION

Of the three salts used to raise the level of chloride, only KCl effected a significant promotion of growth during the liquid phase. The failure of NaCl and

NH_4Cl to stimulate growth significantly, apparently indicates that the chloride supplied in the standard medium was sufficient whereas potassium was still limiting. However, the consistent though slight stimulation of growth particularly of the root in NaCl and NH_4Cl treatments is also an indication that some partial effect of chlorine may be involved in the growth stimulations. The general improvement of growth with KCl was due to the enhancement or manifestation of the effect of chlorine when combined with potassium. The lower stimulations with NaCl and NH_4Cl could be attributed to the unfavorable effects of their cations especially NH_4^+ at high levels.

The importance of chloride in the growth stimulations is further supported by the observations with MgCl_2 treatment where there was significant stimulation on the weight gains of the embryos during the liquid culture followed by the significant increase in leaf count and frequent occurrence of twin and triple roots in solid media.

The better response of coconut embryo to MgCl_2 over MgSO_4 is consistent with the findings of Brunin, Coomans and Ouvrier (1975) and Daniel and Ochs (1975) that young coconuts and oil palm respond better to MgCl_2 fertilization than to the sulfate form.

Of the salts tested, NH_4Cl is the least desirable form to increase the chlorine content of the medium. At high NH_4Cl supply ammonia will accumulate inside the tissues to a toxic level. Regarding the use of NH_4Cl in embryo culture, it had been reported that immature globular embryos of *cattleya* supplied with NH_4Cl (primarily as N source) produced seedlings of poor performance in subsequent media high in NaNO_3 (Raghavan and Torrey, 1963). This is attributable to the toxicity of the ammonium ion.

The observations of Murashige and Skoog (1962) on the response of tobacco pith in vitro to chloride supplementation could be considered to further explain the failure of additional chlorine to enhance the general growth of coconut particularly when NaCl was used as source. According to them, the stimulatory effect of increasing the chlorine level was only obtained when there was a corresponding increase in the level of Mg , Ca and S , with the level of N and K already established near optimum.

The tendency of suppressed germination during the liquid culture in the presence of higher NaCl and the addition of NH_4Cl at all levels could be explained as an effect of high osmotic potential brought about by high Na and/or Cl . This explanation is not supported by some enhancement of germination in liquid with high KCl . Enhancement due to KCl is not understood due to the fact that osmotic potential was likewise raised. The results of these experiments show that although development of the embryos occurs mainly during the solid culture the conditions during the liquid phase are nevertheless very critical to subsequent growth.

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Table 1. The effect of NaCl on the weight of normal coconut embryos during the initial liquid culture after eight weeks of incubation.*

Level of NaCl	Average weight of embryos (g)			
	I	II	III	General Average (Total)
1. 0	0.8001 (15)	0.8276 (15)	0.8274 (17)	0.8183 (47)
2. 1x	0.9134 (19)	0.9363 (15)	0.9003 (16)	0.9166 (50)
3. 2x	0.9905 (17)	0.8076 (14)	0.8821 (14)	0.8934 (45)
4. 4x	1.0132 (16)	0.9904 (18)	1.0255 (16)	1.0097 (50)
5. 8x	0.7579 (16)	0.9769 (17)	0.9875 (21)	0.9074 (54)

*Figures in parentheses refer to the number of embryos per treatment.

Table 1a. Germination of embryos during the initial liquid culture with chlorine supplementation.

Level of NaCl	% Germination (based on embryo with shoot and root)			
	I	II	III	General Average
1. 0	20.00	13.33	6.66	13.33
2. 1x	5.26	6.66	6.35	6.09
3. 2x	11.76	7.14	0.00	6.30
4. 4x	12.50	16.66	6.25	11.80
5. 8x	00.00	00.00	4.76	1.58

Table 2. The effect of KCl during the initial liquid culture on the weight of normal coconut embryos after eight weeks of incubation.¹

Treatment	Average weight of embryos (g) for the different trials			
	I	II	III	General Average (Total)
1. Control	0.8343 (17)	0.8274 (15)	0.8570 (28)	0.8395 (60)
2. 2x KCl	1.0671 (18)	0.9174 (19)	0.9991 (33)	0.9945 (70)
3. 4x KCl	1.1590 (17)	1.1502 (18)	1.0238 (27)	1.1110 (62)*
4. 8x KCl	0.8337 (17)	0.9665 (17)	0.8986 (26)	0.8996 (60)

¹Figures in parentheses refer to the number of embryos per treatment.

*HSD (.05) = 0.2007

Table 2a. Germination of embryos during the initial liquid culture with chlorine supplementation using KCl after eight weeks of incubation.

Level of KCl	% Germination (based on embryos with root and shoot)			
	I	II	III	General Average
1. Control	5.88	13.33	3.57	7.59
2. 2x	16.66	10.52	27.27	18.15
3. 4x	17.64	16.66	22.22	18.84
4. 8x	11.76	5.88	15.38	11.01

Table 3. The effect of chlorine supplementation with NH_4Cl during the initial liquid culture on the weight of normal embryos.¹

Level of NH_4Cl	Average weight of embryos (g) for the different trials					
	I	II	III	General Average (Total)		
1. Control	0.8763 (15)	0.7315 (15)	0.8189 (18)	0.8089 (48)		
2. 1x	0.8639 (15)	0.9951 (16)	0.8918 (20)	0.9169 (51)		
3. 2x	0.8842 (15)	1.0348 (14)	0.9509 (25)	0.9566 (54)		
4. 4x	0.8586 (15)	0.9364 (15)	0.9433 (20)	0.9127 (50)		
5. 8x	0.6906 (14)	0.6398 (17)	0.6742 (23)	0.6682 (54)**		

¹Figures in parentheses refer to the number of samples per treatment.

**HSD (.01) = 0.2180

Table 3a. Germination response of coconut embryos during the initial liquid culture with chlorine supplementation using NH_4Cl .

Level of NH_4Cl	% Germination (based on embryos with shoot and root)			
	I	II	III	General Average
1. Control	13.33	12.00	12.00	12.44
2. 1x	0.00	0.00	0.00	0.00
3. 2x	0.00	6.65	5.55	4.07
4. 4x	0.00	0.00	0.00	0.00
5. 8x	0.00	0.00	0.00	0.00

Table 4. The effect of MgCl_2 as magnesium source during the initial liquid culture of coconut embryos.¹

Treatment	Average weight of embryos for the different trials (g)				
	I	II	III	General Average (Total)	
Control	0.8274 (17)	0.9428 (20)	0.8189 (18)	0.8630 (55)	
MgCl_2	1.057 (20)	1.0977 (24)	0.9920 (20)	1.0489 (64)*	

¹Figures in parentheses refer to the number of embryos per treatment.

*Significant

Table 4a. Germination of coconut embryos during the initial liquid culture with $MgCl_2$ as magnesium source.

Treatment	% Germination (based on embryos with shoot and root)			
	I	II	III	General Average
Control	5.80	15.00	5.55	8.78
$MgCl_2$	20.00	25.00	25.00	23.33*

*Significant

Table 5. Germination of growth in solid medium of coconut embryos precultured in liquid medium containing varying levels of NaCl. (Observations taken after 8 weeks in standard solid medium).

Treatment	Trial No.	S h o o t		Root length (mm) X_3	Percentage	
		Length (mm) X_1	No. of leaves X_2		Rooting ^a X_4	Germination ^b X_5
1	I	29.00 (7)	3.66	54.00	100.0	77.77
	II	38.62 (8)	2.37	37.62	100.0	81.81
	III	49.83 (6)	3.57	50.21	100.0	66.66
	Ave.	39.15	3.20	47.30	100.0	75.41
2	I	55.25 (10)	3.87	48.00	100.0	92.30
	II	42.50 (4)	4.00	72.75	100.0	66.66
	III	29.80 (7)	2.86	52.12	100.0	60.00
	Ave.	42.51	3.57	57.62	100.0	72.98
3	I	42.57 (7)	3.42	23.00	100.0	70.00
	II	38.16 (6)	3.66	71.66	100.0	78.57
	III	33.00 (5)	2.73	40.00	100.0	69.23
	Ave.	38.29	3.27	44.88	100.0	72.60
4	I	41.88 (8)	3.22	31.87	87.5	92.30
	II	42.00 (8)	3.25	51.00	100.0	66.66
	III	26.37 (8)	3.44	66.62	100.0	76.92
	Ave.	36.75	3.30	49.83	95.8	78.63
5	I	37.14 (7)	2.85	52.42	100.0	77.77
	II	41.83 (6)	2.60	49.00	100.0	61.53
	III	42.60 (10)	2.90	57.90	100.0	76.97
	Ave.	40.19	2.78	53.10	100.0	71.92

^aBased on number of germinated embryos.^bBased on number of viable samples.

Table 5a. Growth in solid medium of coconut embryos precultured in liquid medium containing varying levels of NaCl. (Observations taken after 8 weeks in standard solid medium).¹

Treatment	Trial No.	Average weight of seedling (g)			
		Shoot X ₁	Root X ₂	Cotyledon X ₃	Total X ₄
1	I	0.5049	0.5276	1.0993	2.1318 (7)
	II	0.4854	1.3285	1.3276	2.6562 (8)
	III	1.2814	0.5702	2.0254	3.8770 (6)
	Ave.	0.7572	0.8088	1.4841	3.0501
2	I	1.0941	0.5704	1.4445	3.1090 (10)
	II	0.8978	0.9942	1.3134	3.2054 (4)
	III	0.5751	0.4842	1.6204	3.6797 (7)
	Ave.	0.8556	0.6829	1.4594	2.9980
3	I	0.9475	0.2721	1.7551	2.9747 (7)
	II	1.2550	1.0307	2.0243	4.3100 (6)
	III	0.6662	0.4515	1.4093	2.5270 (5)
	Ave.	0.9562	0.5847	1.7295	3.2705
4	I	0.6444	0.3769	1.1172	2.1385 (8)
	II	1.0546	0.5975	1.5102	3.1623 (8)
	III	0.6243	0.7641	1.9869	3.3753 (8)
	Ave.	0.7744	0.5795	1.5381	2.8616
5	I	1.0473	0.6321	1.6065	3.2859 (7)
	II	0.9242	0.6930	1.5309	3.1481 (6)
	III	1.0839	0.5974	1.4559	3.1372 (10)
	Ave.	1.0184	0.6408	1.5311	3.1904

¹Figures in parentheses refer to the number of seedlings observed.

Table 6. Growth in solid medium of coconut embryos precultured in liquid medium containing varying levels of KCl (observations taken after 8 weeks in standard solid medium).

Treatment	Trial No.	Average weight of seedling (g)			Total X ₄
		Shoot X ₁	Root X ₂	Cotyledon X ₃	
1	I	0.8041	0.4358	1.9231	3.1603 (8)
	II	1.2814	0.4399	1.7176	3.4389 (7)
	III	1.1655	0.5701	1.9425	3.6781 (7)
	Ave.	1.0828	0.4819	1.8611	3.4258
2	I	0.7196	0.7159	2.4624	3.8979 (10)
	II	1.2698	0.8097	2.0254	4.1049 (10)
	III	1.1468	0.6465	1.2879	3.0812 (10)
	Ave.	1.0454	0.7240	1.9252	3.6947
3	I	0.8965	0.7358	1.6531	3.2854 (10)
	II	1.3124	0.6837	2.4646	4.4607 (8)
	III	0.9138	0.7027	1.3761	2.9926 (6)
	Ave.	1.0409	0.7075*	1.8312	3.5797
4	I	1.0479	0.6858	1.4529	3.1866 (10)
	II	1.3954	0.6827	2.4536	4.5317 (7)
	III	0.8591	0.7528	1.8399	3.4619 (9)
	Ave.	1.1041	0.7071*	1.9154	3.7267

Figures in parentheses refer to the number of seedlings observed.

*X₂ : HSD (.05) = 0.0972

Table 6a. Germination and growth in solid medium of coconut embryos precultured in liquid medium containing varying levels of KC1 (Observations taken after 8 weeks in standard solid medium).

Treatment	Trial No.	S h o o t		Root length (mm) X ₃	Percentage	
		Length (mm) X ₁	No. of leaves X ₂		Rooting ^a X ₄	Germination ^b X ₅
1	I	34.63 (8)	3.87	37.75	100.0	81.8
	II	49.83 (7)	3.83	34.50	100.0	66.6
	III	49.85 (7)	3.57	50.28	100.0	86.6
	Ave.	44.77	3.75	40.84	100.0	78.3
2	I	30.10 (10)	3.10	60.10	100.0	83.3
	II	53.40 (10)	4.22	52.60	100.0	92.3
	III	56.36 (10)	4.09	51.00	100.0	75.0
	Ave.	46.62	3.80	54.56	100.0	83.5
3	I	49.22 (10)	4.22	58.55	100.0	84.6
	II	42.00 (8)	4.00	40.77	100.0	75.0
	III	43.33 (6)	3.80	54.60	100.0	90.9
	Ave.	44.85	4.01	51.31	100.0	83.5
4	I	50.80 (10)	4.00	49.70	100.0	90.9
	II	48.00 (7)	3.86	40.42	100.0	75.0
	III	28.33 (8)	3.11	62.77	100.0	87.5
	Ave.	42.37	3.65	50.96	100.0	84.4

^aBased on number of germinated embryos.

^bBased on number of viable samples.

Figures in parentheses refer to the number of seedlings observed.

Table 7. Germination and growth in solid medium of coconut embryos precultured in liquid medium containing varying amounts of NH_4Cl (observations taken after 8 weeks in standard solid medium).

Treatment	Trial No.	S h o o t		Root length (mm) X_3	Percentage		
		Length (mm) X_1	No. of leaves X_2		Rooting ^a X_4	Germination ^b X_5	
1	I	31.5	(5)	2.75	40.33	80.0	47.6
	II	47.5	(5)	3.75	60.00	100.0	80.0
	III	29.0	(9)	2.60	42.00	100.0	91.6
	Ave.	36.0		3.03	50.11	93.3	73.1
2	I	69.29	(7)	4.85	50.28	100.0	75.0
	II	34.00	(9)	3.11	61.22	100.0	68.8
	III	29.00	(9)	2.75	41.57	100.0	50.0
	Ave.	44.00		3.51	51.02	100.0	64.5
3	I	42.54	(11)	4.00	54.45	100.0	80.0
	II	34.64	(11)	4.11	52.45	100.0	76.4
	III	25.55	(9)	2.44	56.66	100.0	66.6
	Ave.	34.21		3.51	54.52	100.0	74.3
4	I	28.66	(5)	3.00	38.66	100.0	55.8
	II	24.87	(8)	2.22	50.37	100.0	82.3
	III	23.40	(7)	2.57	61.42	100.0	50.0
	Ave.	25.65		2.59	50.15	100.0	62.6
5	I	26.1	(10)	2.50	53.33	90.0	80.0
	II	28.5	(6)	2.83	60.50	100.0	50.0
	III	21.0	(8)	2.42	46.00	100.0	84.6
	Ave.	25.2		2.58	53.27	96.1	71.5

^aBased on number of germinated embryos^bBased on number of viable samples

Figures in parentheses refer to the number of seedlings observed.

Table 7a. Growth in solid medium of coconut embryos precultured in liquid medium containing varying levels of NH_4Cl (observations taken after 8 weeks in standard solid medium).

Treatment	Trial No.	Average weight of seedling (g)			Total
		Shoot X_1	Root X_2	Cotyledon X_3	
1	I	0.5728	0.5597	1.2308	2.3633 (5)
	II	1.1042	0.6453	1.5246	3.2741 (5)
	III	0.4974	0.4145	1.1193	2.0312 (9)
	Ave.	0.7248	0.5398	1.2916	2.5562
2	I	1.4022	0.5834	1.6424	3.6280 (7)
	II	0.6511	0.6894	1.3946	2.7351 (9)
	III	0.2975	0.4167	1.2614	1.9756 (9)
	Ave.	0.7836	0.5632	1.4328	2.7796
3	I	0.7779	0.5303	1.4179	2.7261 (11)
	II	0.6799	0.5996	1.6933	2.9728 (11)
	III	0.3266	0.7638	1.2318	2.3222 (9)
	Ave.	0.5948	0.6312	1.4476	2.6737
4	I	0.3718	0.3903	1.0324	1.7945 (5)
	II	0.5758	0.6479	1.5799	2.8036 (8)
	III	0.8885	0.5230	1.3882	2.3087 (7)
	Ave.	0.4454	0.5234	1.3335	2.7908
5	I	0.4644	0.5198	1.3625	2.3467 (10)
	II	0.5844	0.6638	1.2904	2.5386 (6)
	III	0.3559	0.4937	0.9321	1.7817 (8)
	Ave.	0.4682	0.5591	1.1950	2.2890

Figures in parentheses refer to the number of seedlings observed.

Table 8. Germination and growth in the standard solid medium of coconut embryos initially cultured in a liquid medium with and without $MgCl_2$ (observations taken after 8 weeks in standard solid medium).

Treatment	Trial No.	S h o o t		Root length (mm) X_3	Percentage	
		Length (mm) X_1	No. of leaves X_2		Rooting ^a X_4	Germination ^b X_5
1	I	38.63 (8)	2.38	37.63	100	81.81
	II	29.00 (10)	2.60	42.00	100	91.66
	Ave.	33.81	2.49	39.81	100	86.73
2	I	39.90 (9)	3.00	37.70	100	86.66
	II	32.15 (13)	3.15	50.54	100	82.35
	Ave.	36.03	3.08*	44.12	100	84.51

^aBased on number of germinated embryos^bBased on number of viable samples

Figures in parentheses refer to the number of samples per treatment.

* x_2 = significantTable 8a. Growth in standard solid medium of coconut embryos precultured in liquid medium containing $MgCl_2$ as Mg source (observations taken 8 weeks in standard solid medium).

Treatment	Trial No.	Average weight of seedling (g)			Total X_4
		Shoot X_1	Root X_2	Cotyledon X_3	
1	I	0.4854	0.3483	1.3270	2.1613 (8)
	II	0.4974	0.4145	1.1193	2.0312 (10)
	Ave.	0.4914	0.3814	1.2235	2.0963
2	I	0.8301	0.4896	1.3249	2.6446 (9)
	II	0.5414	0.4766	1.2086	2.2266 (13)
	Ave.	0.6858	0.4831	1.2267	2.4356

Figures in parentheses refer to the number of seedlings observed.

Table 9. Germination and growth of coconut embryos precultured in standard liquid culture and transferred to solid medium with and without $MgCl_2$ (observations taken after 8 weeks in solid medium).

Treatment	Trial No.	S h o o t		Root length (mm) X_3	Percentage	
		Length (mm) X_1	No. of leaves X_2		Rooting X_4	Germination X_5
1	I	29.00 (10)	2.60	42.00	100	91.66
	II	30.50 (14)	2.92	53.64	100	84.91
	Ave.	29.75	2.76	47.82	100	88.28
2	I	27.20 (10)	3.30	37.70	100	81.25
	II	51.67 (14)	3.78	57.64	100	93.75
	Ave.	39.13	3.54	42.67	100	87.50

Figures in parentheses refer to the number of samples per treatment.

Table 9a. Growth of coconut embryos precultured in standard liquid media and transferred to solid medium containing different magnesium source (observations taken after 8 weeks in solid medium).

Treatment	Trial No.	Average weight of seedling (g)			Total X_4
		Shoot X_1	Root X_2	Cotyledon X_3	
1	I	0.4974	0.4145	1.1193	2.0312 (10)
	II	0.6075	0.5516	1.2467	2.4058 (14)
	Ave.	0.5524	0.4831	1.1830	1.2185
2	I	0.7599	0.4665	1.8468	3.0732 (10)
	II	0.7236	0.5856	1.0191	2.3283 (14)
	Ave.	0.7418	0.5261	1.4329	2.7008

Figures in parentheses refer to the number of seedlings observed.

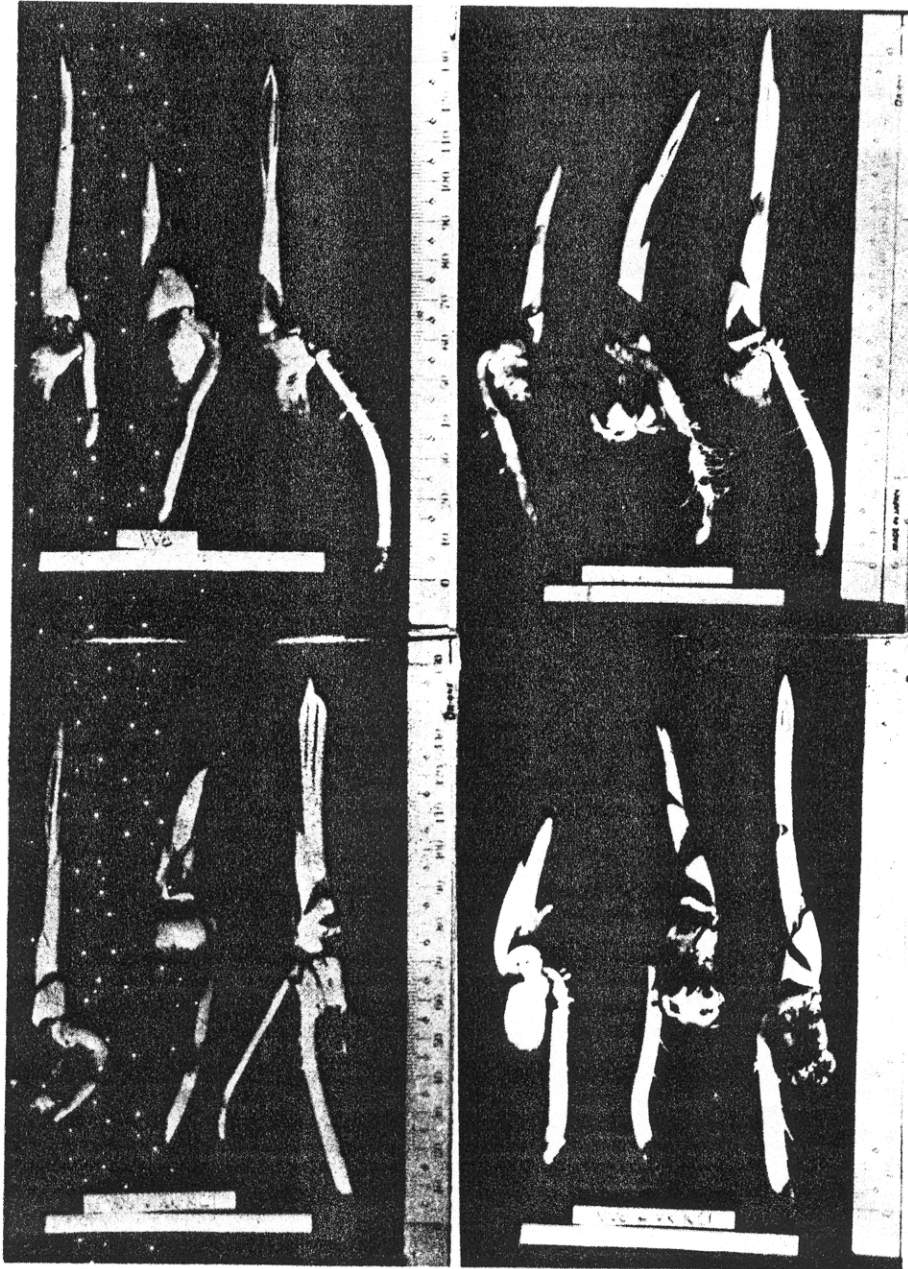


Fig. 1a. Representative seedlings developed from KCl precultured embryos after 8 weeks in solid culture (Second trial) Experiment III.

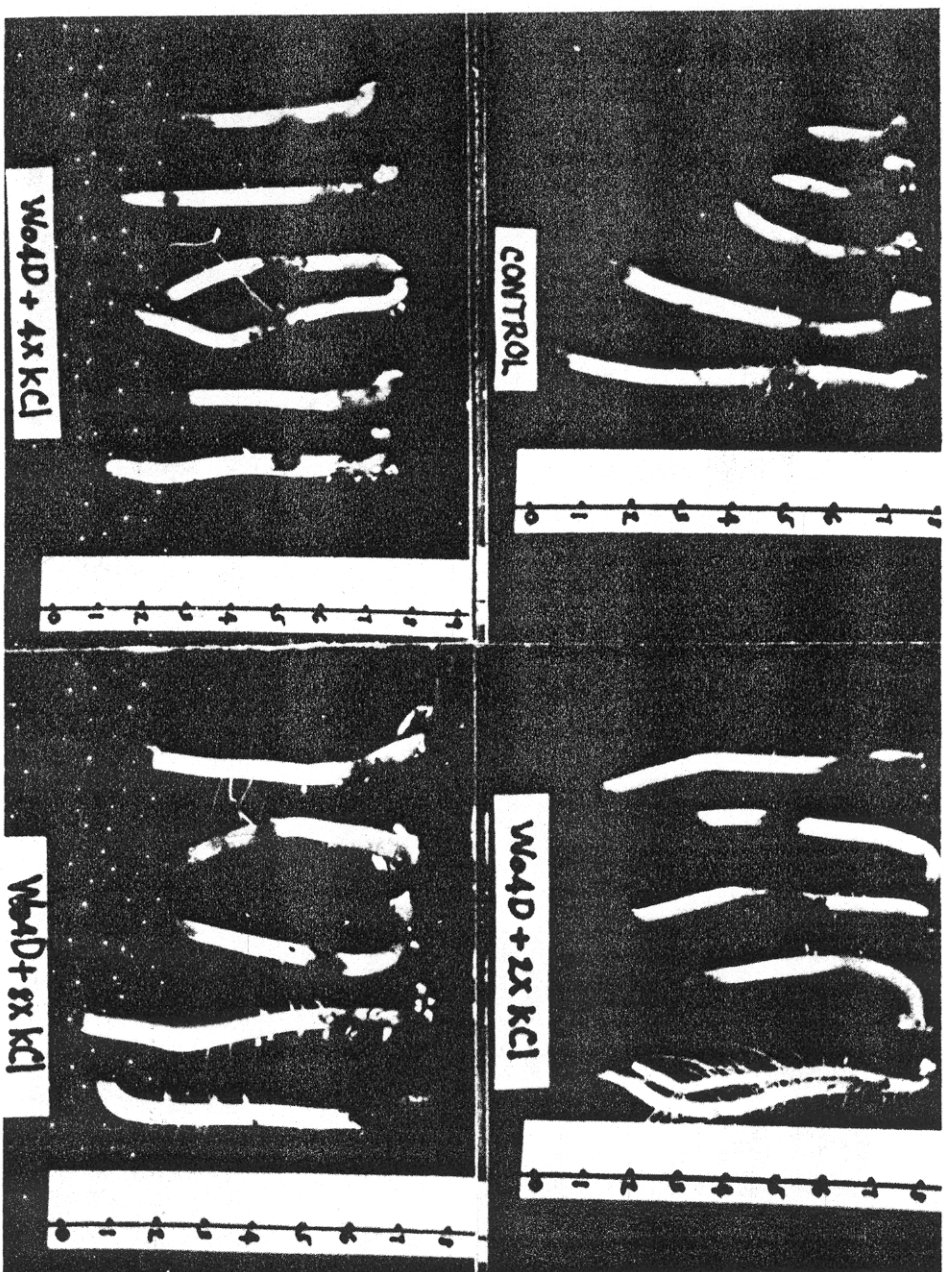


Fig. 1b. Root samples of seedlings developed from KCl precultured embryos after 8 weeks in solid culture (First trial). Note the twin roots of almost the same sizes (2x and 4x CK1).

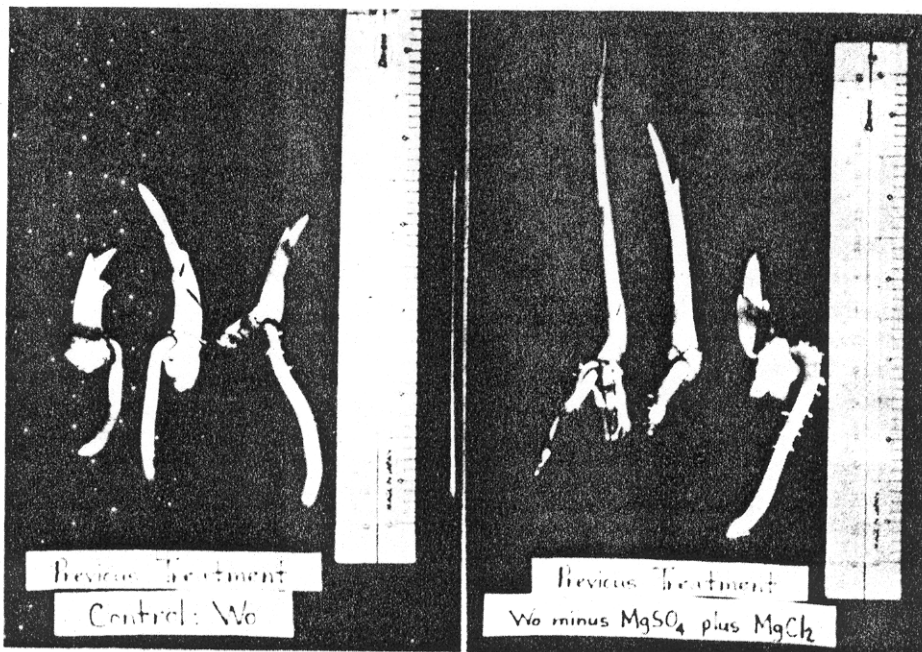


Fig. 2a. Representative seedlings from experiment IV-A. Note the better root and shoot growth with MgCl₂ treatment.

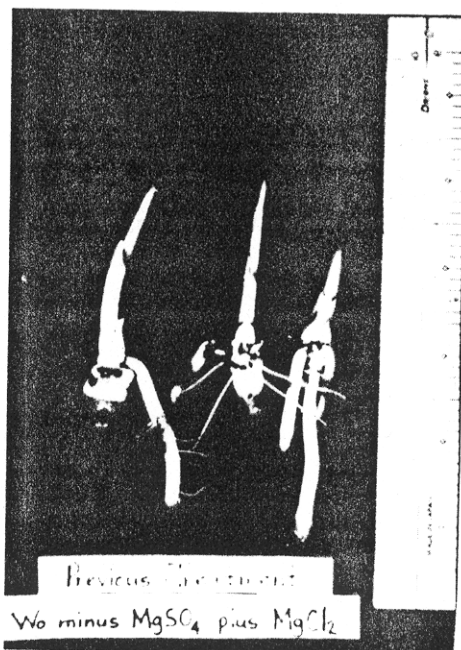


Fig. 2b. Note the seedlings with fine roots arising at almost the same point as the main root (center seedling) and with triple roots respectively.



Fig. 3. Representative seedlings grown in first solid culture with different Mg source. Note the occurrence of primary adventitious roots with $MgCl_2$ treatment.