IN VITRO CULTURE STUDIES ON COCONUT: PROGRESS AND PROBLEM

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Embryo Culture

Cutter and Wilson (1954) undertook the *in vitro* culture of coconut embryos to elucidate on the role of the endosperm during the post embryonic growth of the embryo. From their work which covered mainly the phase prior to visible germination they concluded that as the endosperm matures it develops substances that are inhibitory to the growth of the embryo. Subsequent attempts to grow excised coconut embryos in vitro succeeded in obtaining germination and seedling growth thus permitting observations on cultural requirements at later developmental stages.

The root was found to require more specialized conditions. In solid medium germinating embryos generally produced leaves but not roots (Abraham and Thomas, 1962; de Guzman and del Rosario, 1964). Root development in seedlings without or with rudimentarily developed roots could be induced by transfer to liquid culture (de Guzman, 1969). Concurrent and faster growth of both organs occurred when a reverse sequence of culture, that is from liquid to solid was employed (Balaga and de Guzman, 1971). With the liquid-solid sequence of culture the germination process could be separated into two phases: general enlargement which occurs in liquid culture and visible germination and seedling growth which take place in solid culture. Apparently histological and physiological changes that favor root growth occur during the liquid culture. In vitro seedlings without roots or where root growth has ceased responded to transfer in a liquid medium by initiation or reactivation of root growth.

During the initial liquid culture the physical state rather than composition of the medium is the primary consideration. The same general effect was obtained with two types of mineral formulation although improved results could be obtained by modifying the composition of the medium such as by addition of KCl and NaCl to White's liquid medium (Miniano and de Guzman, 1978). On the other hand, growth during the solid culture was greatly influenced by media composition. The concentration of dextrose during the solid culture exerted a great effect on the growth of the root and shoot. Root growth was greatly promoted and shoot growth inhibited by a high sugar supply (de Guzman, et

al., 1971). That the root has a higher sugar optima compared with the shoot was observed in embryo cultures of other species (Yates and Curtis, 1949; Reitsema et al., 1953; Ameniya et al., 1956). These results show that sugar plays a role not only in root formation (Gautheret, 1966) but also in the control of root growth. The promotive effect of sugar is dependent on its quantity and on the composition and concentration of the mineral salts in the culture medium. As reported by del Rosario and de Guzman (1978) high sugar greatly enhanced root growth when the mineral formulation used was that of Murashige and Skoog's (MS) but not with that of White's. There was no enhancement when MS was combined with low sugar. For maximum root development both high sugar and MS salts should be present; the two factors must be acting in a synergistic manner to produce the stimulation. The superiority of MS over White's medium in promoting growth and differentiation has been shown in other in vitro systems such as carrot root tissue culture for somatic embryogenesis (Reinert, 1968), Singapore orchid tissue culture (Payawal and de Guzman, 1972) and oil palm embryo culture (Jones and Dethan, 1973). This superiority of MS has been attributed to its high salt content especially nitrogen. MS has also high potassium and chloride contents and these elements are especially beneficial to the growth of coconuts. KCl fertilization of coconut palms resulted in shorter pre-flowering stages and greater yield (Magat, 1978).

Addition of growth regulators or of natural supplements is not necessary for the culture of mature coconut embryos into viable seedlings. However, results of preliminary experiments indicate that treatment with auxins increases percentage germination and faster seedling growth. In the germination of excised mature oil palm embryos better seedlings were obtained in media supplemented with auxins (Jones and Dethan, 1973). The beneficial effect of exogenous auxin applications does not necessarily imply that the mature and germinating embryos have not yet developed autotrophy with respect to growth regulators. In the case of auxins, the limiting endogenous level might be due to an increased degradation under *in vitro* conditions. As shown for the oil palm, addition of ferulic acid, a phenolic compound inhibitory to IAA oxidase, promoted root growth.

The in-vitro germinated coconut seedlings like those developed in *situ* exhibit leaf heterophylly producing the different types of leaves in the normal sequence. A marked difference in morphology is the non-development of the haustorium in the former. In coconut the in vitro conditions that enable development of the axial organs are still inadequate for the development of the cotyledon. This observation dramatizes the difference in the growth requirements of the different parts of the

embryo which in turn results from localized biosynthesis. A similar observation was made with *Zizania aquatica* L. (La Rue and Avery, 1938) with which regular growth of the cotyledon was not obtained even in cultures of the mature embryo although growth of the primary root was normal.

Slight swelling of the cotyledon in coconut has been observed in some cultures grown in media supplemented with cytokinins (tables 1 and 2). Cytokinin promotion of haustorial growth was also observed in oil palm cultures (Jones and Dethan, 1973). These observation indicate that among the conditions limiting growth of the cotyledon *in vitro* is cytokinin deficiency.

Table 1. Effect of cytokinins on the enlargement of haustorium of coconut embryos cultured in vitro. Observations were taken 16 weeks after initial inoculation.

Treatment[1]	Duration (weeks) liquid	solid	% enlargement
1.0 Ki	1	7	7.69
	2	6	14.64
2.0 Ki	1	7	0
4.0 Ki	1	7	8.39
2.5 BA	1	7	10.0
5.0 BA	1	7	23.07
10.0 BA	1	7	0
	1	7	23.64
2,4-D, GA, Ki	2	6	34.29
(Eeuweens, 1976)	2	6	33.16

[1]Growth regulator treatments were the same for liquid and solid cultures. Modified White's minerals were used for all liquid media. The solid media contains 1% Carbon and MS minerals except the last treatment which used Eeuween's. Embryos were precultured for 8 weeks in modified White's basal medium, then transferred to experimental medium.

Incidentally, the use of cotyledons as a bioassay material for cytokinins indicates the dependency of growth of this tissue on cytokinins of which it has a low endogenous content. During germination within the coconut seednut the cotyledon develops into a large bulbous structure. The cytokinin requirement for this extensive development could very well be directly provided by the endosperm. During germination the embryo proper emerges outside the shell whereas the cotyledon retains its intimate contact

Table 2. Effect of cytokinins given during the initial liquid culture on the enlargement of haustorium of coconut embryos. Observations were taken 16 weeks after initial inoculation.

Treatment	Duration in liquid culture (weeks)		% enlargement
	Wo	Expt'l.	
1.0 Ki	8	0	23.64
	6	2	19.60
	4	4	25.49
	2	6	27.24
	4[1]	4	16.67
	2[1]	6	0
2.5 BA	8	0	33.33
	6	2	6.25
	4	4	0
	2	6	0
	4[1]	4	14.29
	2[1]	6	14.29

[1]Wo (without cytokinins) before experimental medium; for all others, experimental media (with cytokinins) were used before Wo.

Embryos were transferred to solid media using MS + 0.1 2,4-D + Ki or MS + 0.1 2,4-D + 2.5 BA, both with 1% C.

with the endosperm. Coconut endosperm especially the liquid portion is a rich source of cytokinins. Two of the naturally occurring cytokinins, zeatin and zeatin riboside, have been isolated in pure state from coconut water (Letham, 1968; van Staden and Drewes, 1975).

Callus Induction

Callus growth can be induced in cultures of plant parts consisting of tissues that may vary from the meristematic to the fully differentiated. In the case of dividing cells the pattern of meristematic activity and cellular development is so altered such that the original specialized structure of the explants is lost and instead a mass of unorganized tissue (callus) is formed. With mature or dormant tissues callus formation requires first the reversion (dedifferentiation) of the non-dividing cells to the meristematic condition. Yeoman (1970) divided callus development based mainly on storage parenchyma tissue cultures into 3 phases namely: induction, division and differentiation. The

initiation of cell division is preceded by profound metabolic and cytological changes in the cell. During the divison phase there is a great increase in cell number, a decrease in cell size and progressive return to the meristematic ground state. The callus may be kept in a continued proliferating state forming additional growth centers deeper in the tissue. However, considerable cyto-differentiation may occur at the end of the division phase producing vascular tissues and sometimes growth centers for the organization of apical meristems.

It has been recognized that different tissues vary in their growth regulator requirement for dedifferentiation (Gautheret, 1966). Except for a few cases callus growth in excised tissues or organs requires the exogenous application of growth regulators. For some tissues, the addition of auxins alone suffices to promote callus growth. However, there are those tissues where callus formation depends upon the addition of both an auxin and a cytokinin. A positive interaction between auxin and gibberellin was shown with excised citron fruit tissues cultured *in vitro* (Schroeder and Specter, 1975). Finally there are tissues in which the growth regulator requirement is satisfied by cytokinin application alone.

As a mechanism for dedifferentiation Oostron et al. (1975) proposed that the first event in the reversion to cell division is the coupling of the auxin with a cellular binding site or receptor. They found in pith explants cultured in medium with IAA a receptor protein which specifically binds IAA. Bogers et al. hypothesized that the hormone-receptor complex migrates to the nucleus where it binds with chromatin, thus making new DNA regions available for transcription. The first result of this binding was speculated to be the synthesis of new m-RNA species.

In coconut, attempts to produce callus cultures were carried out with embryos, inflorescence, endosperm tissues and sub-apical stem explants, using powerful auxins as the major growth substances. Apavatjrut and Blake (1977) used 2,4-D and kinetin as the growth regulators for the culture of sub-apical stem explants from mature palms. Cell divisions were observed throughout the explants producing a cambial-like tissue. At the periphery cells in a pro-embryo like arrangement can be identified. Eeuweens (1978) obtained a marked stimulation of growth of coconut inflorescence tissues with the use of NAA or 2,4-D alone. However, even at near optimal concentration of the auxin, growth could be further stimulated with the addition of a cytokinin. In our laboratory the standard media for callus induction contains 2,4-D and kinetin although the proliferative growth responses described below can be obtained with 2,4-D alone.

Growth and Morphogenesis using Embryo Explants

Addition of the appropriate concentration of auxins to the culture medium for embryos during either the liquid or solid culture disrupted normal germination growth. Indolebutyric acid (IBA) (Table 3) was less adverse to germination and seedling development but was also less potent in callus induction. The herbicidal auxins, 2,4-D, picloram (Table 4) and MCPB (Table 5) were much more effective in inducing cell and tissue proliferation.

Characteristic effect of 2,4-D treatment on embryos though variable was the rupture of the intact embryo at the base of the root and on the upper haustorial region. In the first site the exposed tissue showed surface callusing whereas in the latter, there occurred a proliferation of elongated bodies (root-like) with a callused surface. The root-like structures originated from the procambium at the upper haustorial regions especially at the shoot-root primordial-haustorial tissue junction. During seedling development the procambial cells differentiated into vascular tissues but in media supplemented with 2,4-D or NAA they instead became actively dividing giving rise to broad bands of meristematic tissues (Sajise and de Guzman, 1972). With lower concentrations of NAA the meristematic bands could be differentiated into roots resulting in the formation of a profusion of adventitious roots. With NAA at higher concentration and more potent auxins the meristematic bands could undergo partial differentiation forming root-like bodies. As described by Ubalde (1974) on the second week in solid culture the inner portion of the procambial meristematic bands contained mature cells whereas the outer cells retained their meristematic appearance. In rice the callus initiated from seedling nodes and cotyledonary nodes were observed to arise from the procambium whereas those initiated from the leaf sheaths and coleoptile originated from cells close to the immature vascular bundles (Wu and Li, 1971). The procambial tissues around the shoot-root junction and at the upper haustorium proper portion in the coconut embryo therefore make a good target for callus induction. Up to the present, however, all attempts to induce the root-like bodies in coconut embryos to proliferate into a continuously growing unorganized mass have failed. The use of higher auxin concentration reduced instead of increased the extent of proliferation of the root-like bodies (Table 6). Although suitable auxins could bring about cell division it seems that the support of other factors are required to sustain it and to bring about the varied changes associated with the formation of a vigorous callus. The formation of nests of meristematic cells serving as additional growth centers which is a feature of actively developing callus cultures has not yet been observed.

Table 3. The comparative effects of 2,4-D and IBA on the growth of intact embryos and different tissue explants in first transfer and subculture, respectively.

	MS + 0.25 ppm 2,4-D + 1.00 ppm Ki				MS + 20 ppm IBA			
Cultured Explant	Intact Embryo	Shoot	Cotyledonary Sections	Leaf	Intact Embryo	Shoot	Cotyledonary Sections	Leaf
Percent (%) Survival	40.4 ,	31.2	42.1	66.7	85.4	66.7	78.0	82.4
Fresh weight (g)	0.532	0.478	0.793	0.251	1.173	0.302	1.245	0.507
Percent (%) Germination	31.6	—	—	—	82.9	—	—	—
Percent Root Formation	5.26	—	—	—	41.5	—	—	—
Percent Root-like Callus Formation	5.26	—	—	—	2.4	—	—	—
Percent with Surface-callus	—	40.0	62.5	0	—	6.25	31.25	0

Table 4. The effect of different levels of picloram* on the callusing response of normal coconut embryos during the first (I) and second (II) transfer

ppm picloram	% Survival[1]		% Germination[2]		% Collusing[2]	
	I	II	I	II	I	II
0.1	100	100	0	0	31.8	100
0.25	100	100	0	0	30	100
0.5	86	90	0	0	42	100
1.0	77	88	0	0	41	100

	Degree of Root-like proliferation[3]					
	Slight		Moderate		Profuse	
	I	II	I	II	I	II
0.1	100	30	0	40	0	30
0.25	35	10	65	30	0	60
0.5	28	22	72	11	0	66
1.0	17	11	11	44	0	55

[1]Based on the number of uncontaminated cultures
[2]Based on the number of surviving cultures
[3]Based on the number of cultures showing proliferative growth
*Picloram: 4-amino — 3,5,6-trichloropicolinic acid

Another region of the embryo that was stimulated to proliferative growth was the cotyledonary sheath (CS) (de Guzman et al., 1978). In seedling development the CS develops as a thin non-fleshy structure. In media supplemented with 2,4-D it could undergo hyperdevelopment becoming thick and collar-like or producing masses of nodules. High NAA (20-40 ppm) favored the occurrence of CS proliferation over that of the root-like bodies (Table 6). In this respect it can be said that CS proliferation has a higher auxin threshold. Fragments of highly proliferating nodular tissues could be severed and sub-cultured a number of times to produce more nodular masses. The nodules appeared to be discrete or conjoined bodies consisting of cells that were small, prominently nucleated and compactly arranged. Some showed shoot-primodia-like forms. It is possible that these discrete bodies are units of organization which may be manipulated to produce organs or embryos. Thus in coconut tissues in addition to

Table 5. The effect of different levels of MCPB* on the callusing response of normal coconut embryos during the first (I) and second (II) solid transfer.

ppm MCPB	% Survival[1]		% Germination[2]		% Callusing[2]	
	I	II	I	II	I	II
0.1	93	86	0	0	20	58
0.25	92	86	0	0	54	92
0.5	80	92	0	0	77	92
1.0	81	86	0	0	82	100

ppm MCPB	Degree of Root-like proliferation[3]					
	Slight		Moderate		Profuse	
	I	II	I	II	I	II
0.1	100	57	0	29	0	14
0.25	20	16.6	80	33.3	0	50
0.5	15	11	85	9	0	72
1.0	36	4	64	4	0	92

[1]Based on the number of uncontaminated cultures
[2]Based on the number of surviving cultures
[3]Based on the number of cultures showing proliferative growth
*MCPB: 4-(4-chloro-o-tolyl) oxy) butyric acid

organogenesis via callus formation direct organ regeneration also takes place.

On further subculture, however, the nodular mass may change over to a single smooth-surfaced lump of tissue. In this case cell division has been superseded by cell enlargement. The cells became large and highly vacuolated.

Growth and Morphogenesis Using Inflorescence Explants

The developing coconut inflorescence has been shown to be a promising source of explants either for organ or callus culture (Blake et al., 1975 and Eeuweens, 1976, 1978). In the following

Table 6. The effect of previous treatment with NAA on the percentage and degree of proliferation of coconut emryos[1]

Previous treatment ppm NAA	Cotyledonary Sheath Proliferation: Percentage (Total cultures)				Percentage of culture showing different degree of proliferation: Slight				Moderate				Profuse			
	I	II	III	Ave.	I	II	III	Ave.	I	II	III	Ave.	I	II	III	Ave.
2.5	12.5	16.7	10	13.1	50	100	100	83.3	50	0	0	16.7	0	0	0	0
5.0	12.5	0	72	6.57	50	0	100	50.0	50	0	0	16.7	0	0	0	0
10.0	23.1	50	9.1	27.4	66.7	100	100	88.9	33.3	0	0	11.1	0	0	0	0
20.0	30.8	33.3	55.6	39.9	75	0	40	38.3	25	100	40	55.0	0	0	20	6.67
40.0	18.2	83.3	11.1	37.5	0	100	0	33.3	50	0	0	16.7	50	0	100	50.0

Previous treatment ppm NAA	Percent showing root-like Prolifera-tion			
	I	II	III	Ave.
2.5	87.5	50	40	59.2
5.0	87.5	0	35.7	41.1
10.0	84.6	0	27.3	37.3
20.0	84.6	0	0	28.2
40.0	81.8	0	0	27.3

[1]Observations were taken after 8 weeks in 3rd transfer culture using MS + NAA + 2,4-D as culture medium.

discussion will be described our observations on the growth responses of explants from very immature inflorescence.

In media supplemented with 2,4-D and kinetin and other growth regulators substantial increase in size and fresh weights of floral explants were obtained (Table 7). A few cultures provide convincing evidence of reversion from reproductive to vegetative. Some of the resulting vegetative shoots have elongated, rolled scale leaves reminiscent of the leaves of in-vitro seedlings treated with gibberellic acid (Figure 1). In one culture the leaves have advanced from the scale to the true-leaf type (Figure 2). When this shoot was transferred to a medium without auxin a spike-like structure emerged instead of leaves. This rereversion from foliar to floral development was observed in several other cultures (Figure 3). Evidently in the cultures the direction of development of the shoot apex has not yet been firmly determined.

Table 7. Growth of coconut rachillae explants in different culture media. Observations (average fresh weight) were taken after 4 weeks in experimental media[1]. (Total age of culture — 8 weeks)..

Supplement to Experimental media (ppm)[2]	Fresh weight of all cultures (gm)	% alive	Fresh wt. of alive cultures (gm)
100 CH	0.6697(65)	35.38	1.1333(23)
500 CH	0.5655(78)	32.05	0.9821(25)
1000 CH	0.4165(78)	18.18	0.5851(8)
2000 CH	0.4008(35)	8.57	0.8731(3)
2.5 BA	0.4252(78)	25.64	0.9119(20)
5.0 BA	0.3078(66)	15.15	0.5376(10)
10.0 BA	0.6062(73)	53.42	1.0979(39)
1 NAA	0.3117(69)	15.94	0.7867(11)
2.5 NAA	0.3412(44)	15.90	0.8832(7)
5.0 NAA	0.2106(67)	0	—
1.0 NAA	0.3068(75)	8.0	0.7419(6)

[1]Explants were initially cultured in MS (4D) + 0.25 ppm 2,4-D + 0.02% C for 4 weeks and then transferred to experimental media. Average fresh weight of freshly excised explant was 0.050 gm.

CH = casein hydrolysate

[2]MS (4D) + 0.25 ppm 2,4—D + 0.02% C is common to all experimental media.

Likewise preliminary results indicate that the floral meristems have varied morphogenetic potentials. In some cultures

Figure 1. Coconut cultures showing development of floral meristems into vegetative shoots. Note the elongated rolled scale leaf in the left most culture.

Figure 2a. Shoot derived from a coconut floral meristem cultured in vitro. Note the presence of true leaves and the emergence of spike-like structure.

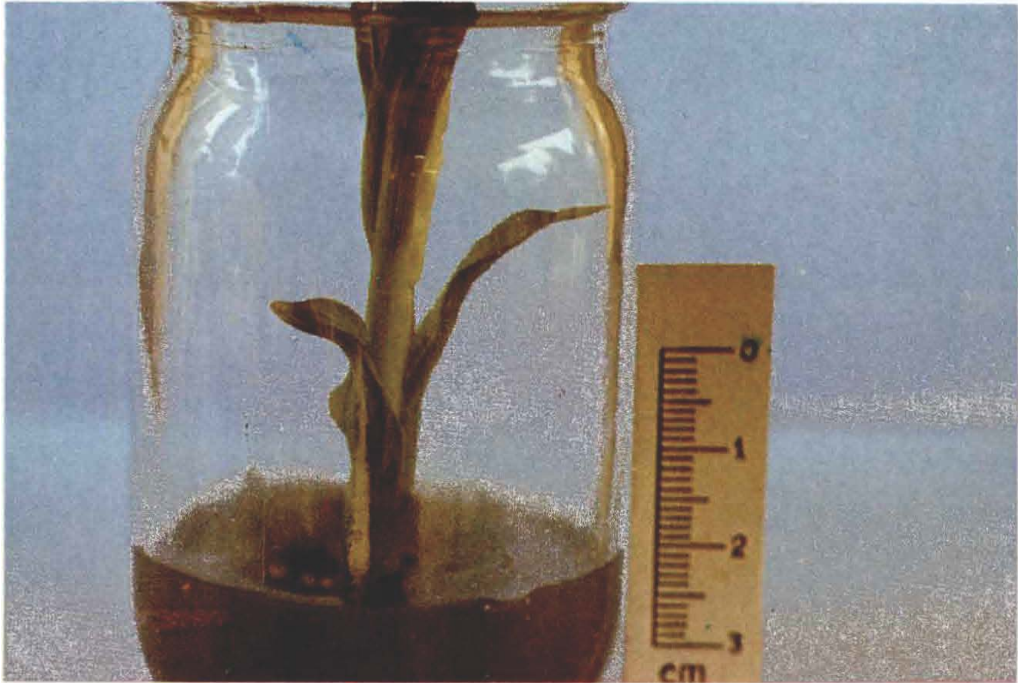

Figure 2b. Same shoot seen in Fig. 2a showing a better view of the true leaves.

Figure 3. Shoots developed from coconut floral meristems showing rereversion to reproductive state.

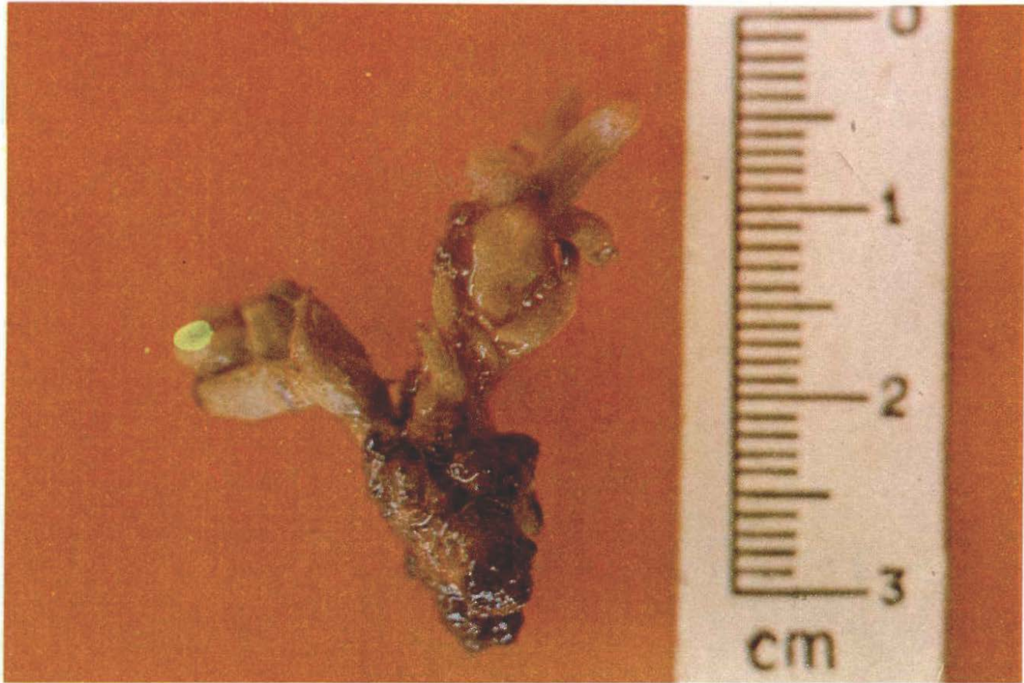

Figure 4a. A branched shoot developed from a single floral meristem.

Figure 4b. A culture derived from a single floral meristem. Note that the leaves are elongated and rolled; four shoots can be identified.

Figure 5. Branching in coconut seedling derived from an excised embryo cultured in vitro. Note that the branches are on the same node.

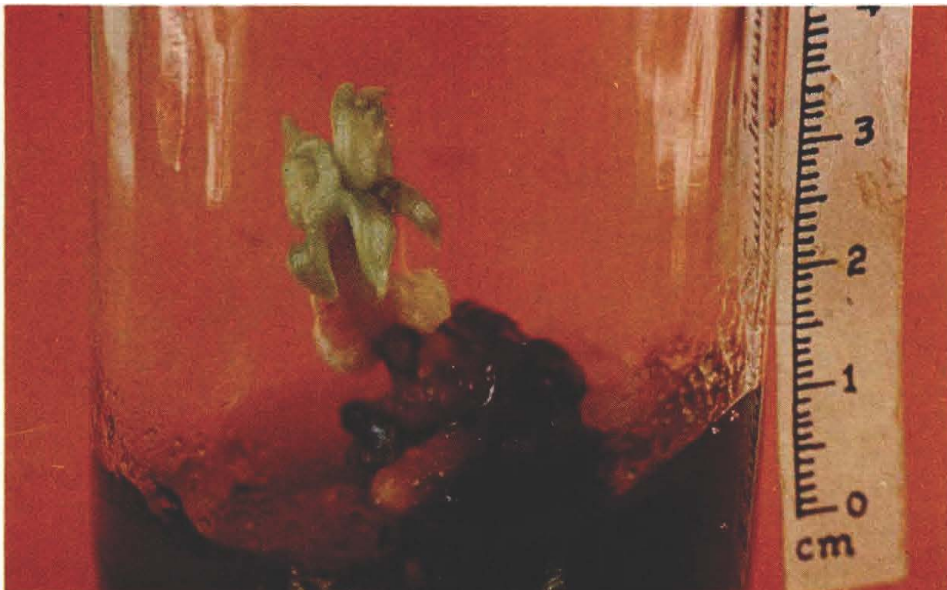

Figure 6. Culture derived from a single floral meristem with 2 shoots and a root. Root and shoot are not connected.

more than one vegetative shoots were produced from a single floral meristem (Figure 4). Since in coconut axillary buds are not produced except reproductive ones the additional shoot(s) may be presumed to have originated from terminal rather than lateral branching. Coconut appears to have a predisposition to terminal branching, a striking example of which is a palm with double dichotomy (Velasquez, 1975). Another case is the production of twin shoots from what appears to be a single embryo cultured in vitro (Figure 5). Terminal branching by subdivision of the vegetative shoot apex has been described in *Nypha* and is believed to occur also in other palms (Tomlinson, 1973). In the case of the vegetative shoots derived from floral meristems the induced growth in culture may have resulted in disturbance of apical integration leading to the organization of new apices.

Differentiation of roots from tissues of floral meristem origin has been reported by Eeuweens (1978) and observed in our laboratory in one culture (Figure 6). These demonstrate the ability of the floral meristem tissues to regenerate roots. Callus growth has been observed inconsistently in some cultures. Induction of cell division leading to cellular proliferations have been induced but not sustained to produce a vigorously growing callus.

General Discussion

With our present method of culture excised embryos can be grown into viable seedlings that can grow to maturity. It has enabled the propagation of the makapuno seednut which under natural conditions is non-germinating. It can be seen from the growth responses that an interplay of nutritional, hormonal and physical factors influence the extent and direction of growth of the embryo which in turn determine the morphology of the seedling. The present technique needs improvement to achieve more rapid and an in situ-like pattern of development. Further understanding of the relationship between the embryo and the endosperm and of the role of the special environment existing within the coconut seednut during germination will provide much needed directions in future embryo culture studies.

A major difficulty with efforts to induce callus and other forms of proliferative growth in both embryos and inflorescence tissues is the cessation of growth, erratic behavior, browning and eventual death of the tissues. In the subcultured proliferating CS tissues the older tissues turn brown and die. This restricts overall growth and further development by reducing the quantity of viable tissue and eliminating older tissues which are "ripe" for differentiation. In addition, the products of browning and tissue senescence may be self-inhibitory to the growth of the cultures.

The addition of activated charcoal to presumably absorb these inhibitory substances has greatly improved root development in seedling cultures and made possible continued growth in subculture of the CS proliferations for sometime. However, it has not completely eliminated the problem of browning and senescence and brought about the problem of reversion of type of growth from nodular to smooth. In a comparative culture of coconut, oil and date palm tissues Eeuweens and Blake obtained genuine callus formation only with the oil palm. This shows that coconut tissues pose greater resistance to growth manipulations *in vitro.* On an optimistic note, however, it can be said that the different morphogenetic responses that have already been elicited *in vitro* are positive signs that this resistance will eventually be overcome.

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Ramon C. Barba, Ph. D., Discussant

Most of the recent breakthroughs in research on agricultural production recognized here and in international forums are those on plant breeding — varieties that are resistant to diseases, better producers, earlier maturity, etc. However, the very few significant researches in the regulation of plant growth to improve production can stand on their own compared to similar efforts in plant breeding. Some of these include the induction of flowering in mango or pineapple to control time of production.

While these breeding efforts are important, and should in fact be intensified, I would like to see more researches on physiology to control plant growth to increase production like the efforts of Dr. de Guzman on the coconut.

One of the significant researches on the coconut is that on macapuno. Dr. de Guzman has single handedly worked with concentrated effort and successfully, to say the least. There are other areas wherein tissue culture can be a significant tool with respect to the coconut including objectives related to breeding or objectives being pursued in breeding researches. Let me elaborate. The breeders recognize the importance of improving coconut production through breeding. It has so many problems — variations in stand, low production, late maturity, etc. But there are problems in breeding the coconut. One is the lengthly juvenile phase so that the breeding cycle will easily outlast the life span of the researcher. It has a low multiplication rate and the area needed for breeding is extensive. It is often cross pollinated and heterozygocity is widespread. There is also a wide geographical spread of the coconut and the nuts are not easy to transport because of bulk and loss of viability.

There are superior existing varieties that don't come true to type from seeds, the only present means of coconut propagation. If only it can be propagated vegetatively like the camote or mango, then all that is needed is to cut and root or graft branches, respectively, to get plants with identical characteristics. Effective vegetative propagation of the coconut seems possible only by tissue culture although a lot of problems exist. For instance, it

seems that the ability to regenerate by tissue culture (cloning) is related to the ability of the plant to produce shoots and roots in their natural habitat. For example, the camote is theoretically easy to propagate by tissue culture because it is easy to regenerate camote plants by cutting stems and sticking these in the ground. Seldom does the coconut produce branches. If you cut a coconut trunk midway you do not produce new branches as in many trees but produce a dead trunk.

Perhaps it is possible to convince the coconut, as Dr. de Guzman said, to form adventitious shoots and reproduce by itself. But communicating with the coconut is in itself a problem. If I were a communications expert I might try that approach but being a plant physiologist I will put my money on tissue culture.

In spite of the apparent difficulty and cost of coconut tissue culture research, because of its importance, this specific area of research should be intensified by bigger grants and involving more experts whether in the same or separate laboratories. The investment of say, a million-peso worth of research, if successful, would easily reap dividends in a few years.

In other countries like The Netherlands, no less than a whole institute is created to solve specific problems. An "Institute for Potato Blight Research" is not far fetched, if the blight becomes severe enough to affect their potato production. While I do not advocate creating an Institute for Tissue Culture of Coconut, much more effort than presently exist should be channeled in this area of research.

Enrique P. Pacardo, Ph. D., Discussant

There are two interrelated points of the paper which I would like to focus my comments. One is related to the basic phenomenon of morphogenesis and the other pertains to the practical application of the knowledge of morphogenesis. Since the time a free somatic cell of carrot root was successfully cultured and grown into a full-grown plant in Steward's laboratory, several studies on different species have been reported. This should be a welcome development for indeed, tissue culture technique has enabled the experimenters to investigate both internal and external factors that regulate growth and differentiation of cells.

By observing painstakingly the various responses of coconut embryo under various combinations of these factors, Dr. de Guzman has uncovered a few secrets of coconut embryo. It has been shown that proper balance of salts and sugars in the medium and the physical state of the medium itself are necessary and may be indispensable for embryonic development to full-grown seedlings. The paper has provided evidence for the potential potency of coconut embryo, particularly macapuno seed nut which seemed to have lost its potency for further growth when being germinated *in situ.*

Under natural field condition, all physiological requirements of a germinating coconut embryo except water perhaps seemed to be supplied by the endosperm. However, in the present work there seemed to have been no attempt, at least no mention was made in the paper, to culture embryo in a medium containing coconut milk or a medium of coconut milk only. This approach might provide additional clues as to the growth requirements of the embryo since coconut milk could readily lend itself to chemical analysis.

One of the foremost questions in morphogenesis is that concerning the developmental potency of individual cells. Earlier experiments indicated that the potential towards organized growth is present in individual cells which are either somatic or reproductive. Their potentials are realized only under a proper balance of factors that promote growth and differentiation. In the present paper of Dr. de Guzman, no mention has been made

on whether or not experiments along this line, that is using individual free cell from embryo has ever been attempted. This may be due to some difficulties in isolating single cell but once this is done successfully, it would mean greater efficiency in the commercial propagation of macapuno, that is for one embryo, several potential free cultures could be made.

The role of growth regulators in a germinating embryo does not appear to be clear as yet. In one case, the application of 2, 4-D or NAA changed the mode of growth from differentiation to cell division.

The paper of Dr. de Guzman more or less summarizes the enormous work, time and effort, not to mention tons of coconut nuts used, spent during the last one and a half decades in her small tissue culture laboratory. And no doubt we are now in a better position than before to understand how a few basic processes in morphogenesis take place.

However, fundamental questions as to why such processes occur at a certain points of time remain as elusive as before. Why does an embryo undergoing differentiation suddenly revert back to meristimatic activity with high dosage of hormone? Why does root development take place only when a germinating embryo is transferred from solid to liquid medium?

In other words, why do these phenomena happen the way they do? These are fundamental questions in which right answers do not seem to be in sight yet. Perhaps new methodologies and approaches are needed. But with most preliminary works already done, Dr. de Guzman and her active school of tissue culture might provide us with some tentative answers to these questions during the next decade.